



Ruminal responses, digestibility, and blood parameters of beef cattle fed diets with different oilseeds

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Abstract

It aimed to evaluate the effects of different whole oilseeds in lipid-rich diets on nutrient intake, apparent digestibility, ingestive behavior, and ruminal and blood parameters of steers. A control diet (without oilseed) and four diets containing whole oilseeds (cotton, canola, sunflower, and soybean) were tested. All diets used the whole-plant corn silage at 400 g/kg as roughage. Five diets, being a control diet (without oilseed) and four diets containing whole oilseeds (cotton, canola, sunflower, and soybean), were tested. All diets used the whole-plant corn silage at 400 g/kg as roughage. Five rumen fistulated crossbred steers, in a 5 × 5 Latin square design were distributed using five periods of 21 days. The steers fed cottonseed and canola diets had lower dry matter intakes (6.6 kg/day). Steers showed higher averages of time in rumination for treatments with sunflower, soybean, and cottonseed (406, 362, and 361 min/day, respectively). There was no treatment effect for the ruminal pH and ammonia (NH₃) variables. There was an effect of the treatment on the volatile fatty acid concentrations. The animals that received soybean showed a higher plasma urea concentration (50.7 mg/dL). Animals fed the control diet showed lower serum cholesterol levels (111.8 mg/dL) than those fed diets containing whole cottonseed, canola, sunflower, and soybean (152.7, 137.1, 146.9, and 138.2 mg/dL, respectively). We recommended using whole soybean or sunflower seeds to formulate lipid-rich diets with 70 g/kg of ether extract for crossbred steers in the feedlot.

Keywords Canola · Cottonseed · Rumen fermentation · Sunflower · Soybean

Introduction

Generally, ruminant diets have a low ether extract content, approximately 1 to 4% of dry matter (DM), and low energy content (Van Soest 1994). Lipid supplementation can increase the energy density of the diet (Plata-Pérez et al. 2022). This act may result in better efficiency and productive

performance, improving the quality of the products generated (Ítavo et al. 2021; Brito et al. 2022; Rodrigues et al. 2022; Wanderley et al. 2022) and reducing the risk of ruminal disorders (Yamamoto et al. 2005; Manso et al. 2006; Bassi et al. 2012).

A lipid-rich diet can reduce nutrient intake (Bassi et al. 2012; Rennó et al. 2015; Palangi et al. 2022). In addition, unsaturated fatty acids can cover food particles, acting as a physical barrier and reducing adherence, colonization, and degradation by ruminal microorganisms (Meale et al. 2012). Due to reduced activity and toxic effects on bacteria, especially cellulolytic ones, lipids can affect fermentation, reducing ruminal digestibility and consequently the consumption of nutrients (Jenkins et al. 2008; Oliveira et al. 2009). This effect was attributed to the longer digest stay in the rumen-reticulum compartments due to less fermentation and digestibility, mainly from the fibrous fraction of the diet (National Research Council - NRC 2000).

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Given the adverse effects, it is recommended that the lipid content not exceed 6 to 7% DM, since values above these would have the effect of depressing the digestibility of the fiber (Machado et al. 2011). The lipid content in a ruminant diet greater than 6–7% can reduce nutrient intake (Palangi et al. 2022). However, lipid sources such as whole oilseeds could allow high lipid content in the diet, avoiding unfavorable effects on the rumen environment. The fatty acids are partially protected by the grain matrix and released slowly according to the rumination of the food (Dhiman et al. 2000; Oliveira et al. 2011). Thus, the lipid quantity and fatty acid profile in the oilseed have an interactive effect (Plata-Pérez et al. 2022).

We hypothesized that lipid-rich diets using different whole oilseeds could have better ruminal responses, digestibility, and blood parameters of steers confined. Thus, it was aimed to evaluate the effects of the inclusion of whole oilseed in a crossbred steer's diet on nutrient intake, apparent digestibility, ingestive behavior, and ruminal and blood parameters.

Material and methods

This work followed the ethical principles adopted by the National Council for Animal Experimentation Control and is approved by the Ethics Committee on the use of animals at the Federal University of Mato Grosso do Sul (CEUA/UFMS—Protocol no 654/2015). The experiment was carried out at the Experimental Farm of the Catholic University Dom Bosco. Chemical composition analyses were performed at the Applied Animal Nutrition Laboratory, and blood variables were analyzed at the Clinical Analysis Laboratory of the Faculty of Veterinary Medicine and Animal Science at the Federal University of Mato Grosso do Sul in Campo Grande, Brazil.

Animals, experimental design

Five rumen fistulated crossbred steers with average body weight (BW) of 416 ± 9.7 kg in a 5×5 Latin Square were distributed in five treatments. The treatments consisted of five diets (Table 1). A control diet without the addition of oilseeds and four diets containing different whole oilseeds (cotton, canola, sunflower, and soybean) in order to obtain 70 g/kg of ether extract in DM basis (Table 2).

The animals were vaccinated, wormed, and placed in individual pens (3×6 m) with cover and free access to water and food. A pre-experimental period of 14 days was adopted to better adapt the animals to management and environment. After the pre-experimental period, all animals were allocated to the respective treatment, and, after 16 days of adaptation to each diet, 5-day sampling period was carried out. Thus,

Table 1 Ingredients and participation in experimental diets

Ingredients	Diets (g/kg DM)				
	Control	Cotton	Canola	Sunflower	Soybean
Corn silage	400	400	400	400	400
Cottonseed	–	350	–	–	–
Canola grain	–	–	187	–	–
Sunflower grain	–	–	–	132	–
Soybean	–	–	–	–	220
Ground corn	383	170	288	290	305
Soybean meal	167	30	75	128	–
Starea	20	20	20	20	20
Mineral mix ¹	30	30	30	30	30

¹Mineral mix: Na, 100 g/kg; P, 88 g/kg; Ca, 188 g/kg; S, 22 g/kg; Mg, 8000 mg/kg; Zn, 3000 mg/kg; Cu, 1000 mg/kg; Co, 80 mg/kg; I, 60 mg/kg; Se, 20 mg/kg; F, 880 mg/kg

five experimental periods with 21 days totaling 105 experimental days were carried out.

Characterization of diets

The diets were formulated in a roughage: concentrate ratio of 400:600 based on dry matter, according to National Research Council - NRC (2000), for average gains of 1.25 kg/day for crossbred steers with approximately 400 kg of BW. The diet was supplied once a day, at 8 am, to keep the leftovers around 50 g/kg of food provided. Feed ingredients, diets, leftovers, and feces were dried in an oven with forced air circulation at 55°C and also milled with 2- and 1-mm sieves to be submitted to analysis, including analyses for determination of dry matter (AOAC, Association of Official Analytical Chemists 2000; method 930.15), ash content was determined by incineration in a muffle furnace (AOAC, Association of Official Analytical Chemists 2000; method 942.05), and the organic matter (OM) content was calculated by the difference between 100 and the percentage of ash.

Total N was determined with a Tecnal TE-036/1 (Tecnal, Piracicaba, Brazil) (AOAC, Association of Official Analytical Chemists 2000, method 976.05); ether extract (EE) was determined using an Ankom XT10 extractor (Ankom Technology, NY, USA) in XT4® bags (AOAC, Association of Official Analytical Chemists 2000, Method 920.39). Heat stable α -amylase (Termamyl 120 L® Sigma–Aldrich, 3050 Spruce Street, Saint Louis, MO, USA) was used to determine neutral detergent fiber (NDF) (Mertens 2003) without sodium sulfite and was considered in the ash residue. At the same time, the NFCs of the samples containing urea were calculated using the equation proposed by Hall (2000): $NFC (g/kg) = 1000 - (CP - CPU + U) + Ash + EE + NDF$, where CPU = crude protein content derived from urea and U = urea content.

Table 2 Chemical composition of concentrates, oilseeds, and experimental diets

	DM	OM	CP	NFC	NDF	ADF	EE
Corn silage	252.2	935.6	71.5	211.8	629.7	425.9	22.6
Concentrates							
Control	882	966	253	490	226	56	32
Cotton	887	827	268	464	15	43	24
Canola	885	899	246	460	216	52	28
Sunflower	884	909	273	454	199	55	29
Soybean	885	938	189	593	176	35	26
Oilseeds							
Cotton	857	958	250	31	471	369	205
Canola	903	957	251	40	380	332	285
Sunflower	919	969	142	146	484	339	197
Soybean	891	946	388	30	333	207	219
Diets							
Control	492	956	191	341	389	209	35
Cotton	488	920	175	173	497	317	74
Canola	496	931	180	256	422	238	73
Sunflower	495	930	180	244	434	226	72
Soybean	493	944	200	301	375	211	70

DM dry matter (g/kg of fresh matter), OM organic matter (g/kg of DM), CP crude protein (g/kg of DM), NFC non-fibrous carbohydrates (g/kg of DM), NDF neutral detergent fiber (g/kg of DM); acid detergent fiber (g/kg of DM); EE ethereal extract (g/kg of DM)

Nutrient intake

The assessment of nutrient consumption was determined from the 17th to the 21st day of each experimental period. Diets and leftovers were weighed and sampled daily to determine daily consumption, and samples composed of animals per period were prepared. Nutrient intakes (DM, OM, CP, NDF, EE, and NFC) were calculated on a DM basis following this equation:

Nutrients intake (kg/day) = kg of nutrient supplied – kg of nutrient in leftovers.

Apparent digestibility

The apparent digestibility of nutrients was assessed using feces collections that followed the 2-day scheme proposed by Ítavo et al. (2002a), being performed at 8:00 am on the 13th day and at 4:00 pm on the 20th day of each experimental period. To determine fecal production, the external indicator titanium dioxide was used. The external indicator was weighed (10 g/animal/day), packed in filter paper, and infused in the rumen for 11 days, with the first 10 days for adaptation and homogenization of the indicator throughout the digestive tract.

The determination of titanium dioxide was done according to a procedure adapted from Detmann et al. (2012) (INCT-CA M 007/1). A sample of 0.2 g of feces, 10 ml of sulfuric acid, and 5 g of catalytic mixture was digested

for 2 h at 400°C in tubes for protein determination. After digestion, 10 mL of H₂O₂ (30% hydrogen peroxide) was added slowly, the tube material was transferred to 50-mL flasks, and the volume was made up with distilled water. A standard curve was prepared with 2, 4, 6, 8, and 10 mg of titanium dioxide using a spectrophotometer with a 410-nm wavelength to evaluate the titanium dioxide concentration.

The provided nutrients (DM, OM, CP, NFD, AFD, and EE), contained in leftovers and feces based on dry matter, were considered. The apparent digestibility coefficients of nutrients were obtained using this equation: Apparent digestibility (g/kg) = ((kg nutrient supplied – kg nutrient in leftovers) – kg nutrient in feces)/(kg nutrient provided – kg nutrient in leftovers).

Ingestive behavior

On the 20th day of each experimental period, the ingestive behavior of steers was evaluated. Ingestive behavior was assessed over 24 h, beginning at 8 am, after food supply until the next morning, totaling 120 h of observation per animal. The behavior information was based on instant scans and continuous sampling (Altmann 1974) including 1-min scans at 5-min intervals, using an ethogram characterized by three basic categories: consumption, leisure, and rumination. Linked to this assessment, it was also recorded: total chewing time (hours/day), the total number of ruminal bolus (n/day), the number of chewing

per rumination (n/day), number of chewing per ruminal bolus, and time spent chewing the ruminal bolus that was obtained in four periods of 6 h, totaling 12 information per animal, per period. The rates of feeding (kg DM/h), rumination (kg DM/h), and fiber rumination (kg NDF/h) were calculated using the DM and NDF intake divided by the time of ingestion and total rumination time, respectively.

Ruminal pH and ammonia (NH₃)

The pH values, collected on the 21st day of each period, were measured using a digital potentiometer inserted directly into the rumen at seven different times, before supplying the diet (0), and at 2, 4, 6, 8, 10, and 12 h post-feeding. Samples of 50 mL of ruminal fluid were collected and a 1:1 sulfuric acid solution (H₂SO₄) was added for ammonia (NH₃) analysis (Fenner 1965).

Volatile fatty acids

The sampling and analysis were carried out according to Moraes et al. (2019). The ruminal fluid collection was 50 mL of acidified samples with 2 mL of 25% metaphosphoric acid, conditioned in polyethylene bottles and frozen at -20°C for volatile fatty acid (VFA) determinations by gas chromatography methodology with flame ionization detection-CG/FID (Trace GC ultra, Thermo - Finnigan Electron Corporation) according to Da Mata et al. (2023), with Nukol chromatography column bound to free fatty acids, 30 m long, 0.25-mm internal diameter, and 0.25-µm film thickness of the stationary phase (Supelco Analytical). VFAs were determined by internal standard curve method. Standard solution with VFA mixture (Volatile Acid Standard Mix, Supelco Analytical, with concentration of VFA at 10 mM) was prepared and trimethylacetic acid, 99% purity (Sigma Aldrich®), was used as internal standard. Samples were thawed to room temperature, centrifuged at 3500 rpm for 5 min, and 100 µL of samples were transferred to tubes containing 800 µL of distilled water and 100 µL of internal standard (trimethylacetic acid) at 10 mM, homogenized for 30 s and filtered. Column temperature programming started at 110°C, raised to 136°C at a rate of 12°C/min, remained for 3 min at the same temperature. Temperature raised to 142°C at the rate of 1.5°C/min and the final temperature was 200°C reached at a rate of 30°C/min remaining for 4 min. Manual injection was performed with a volume of 1.0 µL in the split mode, the split ratio was 1:12, and the split flow was 10 mL/min. Injector maintenance temperature was 230°C and helium gas was used as the drag gas (mobile phase) with a flow rate of 0.8 mL/min.

Blood variables

Blood samples were collected on the 21st day of each experimental period, 6 h after the ration was supplied, through puncture of the jugular vein with BD Vacutainer® Fluoride/EDTA tubes for glucose analysis and BD Vacutainer® SST® II Advance® tubes with clot activator and separating gel to obtain serum for biochemical blood analysis. After collection, the tubes were taken, under refrigeration, to the clinical analysis laboratory of the Faculty of Veterinary Medicine and Animal Science of UFMS, where they were centrifuged at 3000 rpm for 15 min.

Analyses of total protein (kit ref. 04657586), albumin (kit ref. 04657357), creatinine (kit ref. 10886874), cholesterol (kit ref. 10745065), plasma urea concentration (kit ref. 11200666), triglycerides (kit ref. 04657594), alanine aminotransferase (kit ref. 10745138), alkaline phosphatase (kit ref. 11622773), aspartate aminotransferase (kit ref. 10745120), and glutamyltransferase range (kit ref. 05401461) in blood serum, and glucose (kit ref. 04657527) analyzed in blood plasma. The readings were performed in an automatic biochemical analyzer (Immunoanalyzer Cobas C111-Roche Diagnostics, Indianapolis, USA).

Statistical analysis

The data were analyzed as a Latin square design using the MIXED procedure of SAS, release 9.1 according to the model $Y_{ijk} = \mu + T_i + P_j + A_k + e_{ijk}$, where Y_{ijk} is the dependent variable, μ = general average, T_i is the treatment fixed effect, P_j is the period fixed effect, A_k is the random animal effect, and e_{ijk} is the residue random error. The Tukey test was used at 5% significance. Differences were declared significant when $P \leq 0.05$. Before comparisons, all ingestive behavior variables were submitted to the Shapiro-Wilk test.

The ruminal pH, ammonia (NH₃), and VFAs data were evaluated as subdivided plots (time) according to the model $Y_{ijkl} = \mu + T_i + P_j + A_k + H_l + e_{ijkl}$, where Y_{ijkl} is dependent variable, μ is the general average, T_i is the treatment fixed effect, P_j is the period fixed effect, A_k is the random animal effect, H_l is the hour of sampling effect, and e_{ijkl} is the residue random error. A multiple regression model was with effect of treatment and time at a 5% level of significance. The treatments were allocated in the plot and the time of collection (hour) in the subplot. Furthermore, the experimental error was split in two. Part of the error attributed to the plot factor and part of the subplot.

Results

The dry matter intake (DMI) of the control diet was 9.5 kg/day (Table 3), similar to diets containing soybean (9.0 kg/day) and sunflower (8.6 kg/day) grains. The steers-fed cotton and

canola diet presented an average dry matter intake of 6.6 kg/day. The average of organic matter intake of steers fed control treatment was 9.1 kg/day, not differing from diets containing whole soybean (8.5 kg/day) and sunflower (8.0 kg/day). The animals fed diets containing cottonseed (6.1 kg/day) and canola (6.2 kg/day) showed lower organic matter intake than diets containing soybean and sunflower and the control diet. The animals fed a diet containing soybean (1.8 kg/day) and the control diet (1.8 kg/day) presented the highest averages of protein intake (Table 3).

The steers-fed diet containing sunflower (3.7 kg/day) presented an average fiber in neutral detergent intake similar to the average of the diets containing soybean (3.3 kg/day) and cottonseed (3.3 kg/day) and control diet (3.7 kg/day). The neutral detergent fiber intake of the treatment containing canola seed (2.7 kg/day) was lower than that in the control and sunflower diets, without differing from cottonseed and

soybean treatments (Table 3). The ether extract intakes from sunflower (0.6 kg/day) and soybean (0.6 kg/day) treatments were higher than diets containing cotton (0.42 kg/day) and canola (0.4 kg/day) and the control (0.3 kg/day).

The oilseed inclusion to obtain lipid-rich diets did not influence the apparent digestibility coefficients (Table 3). There was a treatment effect for behavioral variables (Table 3). The animals in the sunflower, soybean, and cottonseed treatment showed higher averages of time spent with rumination, while the animals in the treatment containing canola presented the lowest average for this activity. However, there was no treatment effect for the feeding time with an average of 240 min/day.

There were also no significant effects on feeding efficiency (1549 g DM/h), dry matter rumination efficiency (1089 g MS/h), and the number of ruminated bolus per day (410 cakes/day). The rumination efficiency in g of NDF/hour of the steers

Table 3 Consumption of nutrients, apparent digestibility, and ingestive behavior of beef steers fed diets with high content of ether extract using oilseed grains

	Experimental diets					SEM	<i>P</i> -value
	Control	Cotton	Canola	Sunflower	Soybean		
Nutrient intake (kg/day)							
DM	9.5 a	6.6 b	6.6 b	8.6 a	9.0 a	1.22	0.0004
OM	9.1 a	6.1 b	6.1 b	8.0 a	8.5 a	1.13	0.0002
CP	1.8 a	1.2 b	1.2 b	1.5 a	1.8 a	0.23	0.0001
NFC	3.2 a	1.1 e	1.7 b	2.1 c	2.7 b	0.34	0.0001
NDF	3.7 a	3.3 ab	2.8 d	3.7 a	3.4 ab	0.55	0.0386
ADF	2.0 a	2.1	1.6	1.9	1.9	0.30	0.0743
EE	0.3 c	0.5 b	0.5 b	0.6 a	0.6 a	0.10	0.0001
Apparent digestibility (g/kg)							
DM	783.0	638.2	673.0	748.0	711.6	114.7	0.3177
OM	804.9	671.0	713.0	765.2	750.5	105.1	0.3486
CP	822.1	754.2	764.4	858.4	838.0	91.3	0.3129
NFC	918.2	848.7	897.1	882.2	830.0	82.5	0.4614
NDF	670.9	518.8	560.6	665.8	541.4	156.2	0.4050
ADF	594.1	474.5	484.9	577.3	487.7	179.2	0.7304
EE	813.2	693.8	771.0	767.6	622.7	101.3	0.0554
TDN	815.2	670.6	750.2	801.9	734.4	106.3	0.2460
Ingestive behavior							
Feeding (min/day)	204	237	232	229	247	46.5	0.5896
Rumination (min/day)	292 bc	361 ab	250 c	406 a	362 ab	72.2	0.0078
Leisure (min/day)	949 a	947 b	963 a	809 b	835 b	74.2	0.0031
Feeding efficiency (g DM/hour)	1894	1417	1416	1763	1545	562.8	0.4979
Rumination efficiency (g DM/hour)	1301	922	1381	980	1012	345.4	0.1051
Rumination efficiency (g NDF/hour)	640 ab	471 b	701 a	506 b	528 b	133.8	0.0317
Total chewing time (hour/day)	8 b	10 a	8 b	11 a	10 a	1.2	0.0031
Number of cakes per day	338	421	326	492	403	99.6	0.0504
Number of healthy chews per day	15,291 bc	20,088 ab	12,961 c	22,127 a	19,361 ab	4928.4	0.0229

Averages followed by different lower case letters differ by the Tukey test ($P < 0.05$)

SEM standard error of the mean

fed control and canola diets (640 and 701 g of NDF/h) was higher than that of the soybean, sunflower, and cotton treatments (528, 506, and 471 g NDF/h). The total daily chewing time of animals fed diets with sunflower, cottonseed, and soybean was higher than that of the control and canola diets. The number of meristic chews underwent treatment effect, with the sunflower and cotton treatments being superior to the canola treatments. The treatment containing soybeans and the control treatment showed intermediate results (Table 3).

There was no treatment effect on ruminal pH (Table 4). There was an effect of sampling time only for treatments containing oilseeds. The minimum ruminal pH values estimated using the time-adjusted regression equation were 6.2 to 7.2 h for the animals that consumed the diet containing cottonseed; 6.4 to 7.2 h for the animals that consumed the canola diet; 6.1 to 8.7 h for the animals that consumed the sunflower diet; and 6.1 to 7.9 h for the animals that consumed the diet containing soy (Table 4).

There was an effect of the treatment on the production of volatile fatty acids (Table 5). For acetate at 4 h after feeding, the control, canola, sunflower, and soybean treatments did not differ among themselves. Also control, cottonseed, and canola did not differ among themselves. However, it is worth mentioning that there was a peak of production 4 h after the feeding (84.4 mMol/L), in the diet containing sunflower. Regarding butyrate, a difference was observed between treatments at 2, 4, 6, 8, and 12 h after the meal with peak production of 25.1 mMol/L after 10 h for the diet containing sunflower. The production of total VFA shows that the treatment with a sunflower at 0, 2, and 4 h after the feeding showed the production peaks, and the treatment containing soybean at 6, 8, 10, and 12 h after the feeding was the biggest producer of gases. In contrast, the treatment containing canola at 0 and 2 h and the treatment containing cottonseed at 4, 6, 8, 10, and 12 h after the feeding proved to be the smallest VFA producers.

Table 4 Ruminal variables (pH and NH₃) of beef steers fed diets with a high content of ether extract using whole oilseeds

Hour	Experimental diets					SEM*	P-value
	Control ¹	Cotton ²	Canola ³	Sunflower ⁴	Soybean ⁵		
Ruminal pH							
0	6.8	7.0	6.9	7.0	6.8	0.07	0.8408
2	6.5	6.7	6.7	6.6	6.7	0.07	0.8545
4	6.3	6.3	6.4	6.3	6.2	0.07	0.9502
6	6.4	6.1	6.4	6.3	6.1	0.09	0.7042
8	5.8	6.2	6.4	6.0	6.1	0.06	0.0684
10	6.2	6.4	6.6	6.2	6.2	0.08	0.5348
12	6.3	6.5	6.6	6.2	6.3	0.08	0.6156
P	0.2729	0.0001	0.0247	0.0157	0.0024		
Ammonia (NH₃) (mg/100 mL)							
0	19.1	16.8	14.6	19.6	25.0	1.34	0.1224
2	37.3	31.9	22.2	27.1	28.3	2.09	0.1721
4	28.0	22.6	19.8	21.6	22.5	1.81	0.6018
6	21.6	18.0	17.0	23.5	22.6	1.24	0.3067
8	21.9	17.2	16.1	22.2	20.1	1.35	0.4296
10	17.6	16.2	12.4	18.0	18.6	1.26	0.4641
12	15.2	16.6	11.5	17.7	20.7	1.32	0.2189
P	<0.0001	<0.0001	<0.0001	0.0131	0.0099		

*Averages followed by different lower case letters differ by the Tukey test ($P < 0.05$); SEM = Standard error of the mean

¹Y pH control = 6.35238

¹Y NH₃ control = 169.196 + 35.5888*t^{0.5} - 23.8269*t + 3.92669*t^{1.5} ($R^2 = 0.66$);

²Y pH cotton = 7.02063 - 0.229464*t + 0.0158234*t² ($R^2 = 0.95$);

²Y NH₃ cotton = 19.2684 + 36.9988*t^{0.5} - 22.7080*t + 3.40162*t^{1.5} ($R^2 = 0.73$);

³Y pH canola = 6.87540 - 0.124702*t + 0.0086056*t² ($R^2 = 0.87$);

³Y NH₃ canola = 14.6053 + 13.9392*t^{0.5} - 7.44577*t + 0.911208*t^{1.5} ($R^2 = 0.91$);

⁴Y pH sunflower = 6.94127 - 0.186607*t + 0.0106647*t² ($R^2 = 0.93$);

⁴Y NH₃ sunflower = 19.7480 + 10.8732*t^{0.5} - 5.60323*t + 0.661363*t^{1.5} ($R^2 = 0.67$);

⁵Y pH soybean = 6.88135 - 0.191071*t + 0.0121032*t² ($R^2 = 0.92$)

⁵Y NH₃ soybean = 25.0590 + 12.2915*t^{0.5} - 9.73926*t + 1.67461*t^{1.5} ($R^2 = 0.85$)

Table 5 Production of volatile fatty acids (VFA, mmol/L) from beef steers fed diets with a high content of ether extract using oilseed grains

Hour	Experimental diets					SEM*	<i>P</i> -value
	Control ¹	Cotton ²	Canola ³	Sunflower ⁴	Soybean ⁵		
Acetate (mmol/L)							
0	45.0	63.8	48.5	69.0	51.9	3.42	0.0576
2	65.9	68.7	64.4	79.3	67.8	2.11	0.1042
4	72.1 ab	63.3 b	76.8 ab	84.4 a	80.2 a	2.30	0.0084
6	75.0	66.9	77.2	79.1	81.7	2.76	0.4254
8	71.7 ab	62.1 b	83.3 a	78.4 ab	80.7 ab	2.57	0.0187
10	70.9	69.0	74.1	69.5	78.6	3.13	0.5578
12	69.4	60.2	67.1	74.3	79.7	2.81	0.1507
SEM	2.55	1.81	2.80	2.66	2.43		
<i>P</i> -Linear	0.0048	0.6360	0.0088	0.7886	0.0001		
<i>P</i> -Quadratic	0.0019	0.5664	0.0002	0.1467	0.0001		
Propionate (mmol/L)							
0	20.6	19.3	21.9	22.9	22.0	1.46	0.6683
2	32.8	32.3	31.1	31.2	31.7	1.64	0.8431
4	45.4 a	28.1 b	42.2 ab	30.7 b	41.7 ab	2.10	0.0045
6	44.9 a	31.8 b	42.0 ab	29.8 b	47.4 a	2.51	0.0346
8	46.2 a	30.1 b	45.5 a	30.5 b	47.5 a	2.69	0.0217
10	42.07	29.9	47.18	33.6	48.5	3.11	0.1492
12	39.8 a	23.4 b	34.1 ab	28.4 ab	39.8 a	2.02	0.0072
SEM	2.58	1.50	1.98	1.54	2.32		
<i>P</i> -Linear	0.0134	0.6086	0.0006	0.2975	0.0004		
<i>P</i> -Quadratic	0.0050	0.0074	0.0001	0.1904	0.0010		
Butyrate (mmol/L)							
0	6.3	11.7	9.6	17.4	8.9	1.44	0.0779
2	9.7 b	15.4 ab	12.0 ab	22.6 a	11.2 b	1.49	0.0126
4	11.1 b	11.6 b	15.6 ab	22.4 a	14.6 b	1.15	0.0011
6	11.9 b	12.7 b	15.3 ab	22.5 a	17.1 ab	1.21	0.0096
8	12.0 b	12.9 b	17.0 ab	23.3 a	17.3 ab	1.34	0.0190
10	13.8	12.8	18.1	25.1	17.7	1.67	0.0719
12	10.0 b	10.8 b	13.0 ab	22.5 a	15.4 ab	1.40	0.0070
SEM	0.73	0.77	1.01	1.53	0.84		
<i>P</i> -Linear	0.0285	0.5374	0.0675	0.3094	0.0005		
<i>P</i> -Quadratic	0.0263	0.5072	0.0391	0.4453	0.0101		
Total (mmol/L)							
0	77.3	100.5	86.0	117.5	90.7	6.16	0.1986
2	114.5	123.5	113.7	141.3	118.0	4.70	0.2371
4	134.5 ab	107.8 b	140.7 a	143.8 a	143.7 a	4.30	0.0081
6	137.6	115.8	140.2	137.7	153.5	5.48	0.2103
8	135.5 ab	109.5 b	151.6 a	138.7 ab	152.7 a	5.54	0.0356
10	132.1	116.0	145.7	135.1	152.5	6.17	0.3063
12	125.3	98.3	119.7	131.8	142.5	5.48	0.0566
SEM	5.67	3.59	5.50	4.61	5.01		
<i>P</i> -Linear	0.0069	0.6664	0.0038	0.6800	0.0001		
<i>P</i> -Quadratic	0.0030	0.1347	0.0002	0.1580	0.0002		

*Averages followed by distinct lower case letters, differ by the Tukey test ($P < 0.05$). SEM = standard error of the mean

$${}^1Y_{\text{Acetate}} = 48.8188 + 7.17219.t - 0.474593.t^2 \quad (R^2 = 0.89); \quad {}^1Y_{\text{Propionate}} = 21.5315 + 6.80981.t - 0.453573.t^2 \quad (R^2 = 0.95);$$

$${}^1Y_{\text{Butyrate}} = 6.44908 + 1.61942.t - 0.105067.t^2 \quad (R^2 = 0.89); \quad {}^1Y_{\text{Total}} = 82.4002 + 15.6288.t - 0.03380.t^2 \quad (R^2 = 0.94);$$

Table 5 (continued)

$${}^2Y_{\text{Propionate}} = 21.6237 + 3.30995.t - 0.262077.t^2 \quad (R^2 = 0.69);$$

$${}^3Y_{\text{Acetate}} = 57.6843 + 5.42964.t - 0.378294.t^2 \quad (R^2 = 0.97); \quad {}^3Y_{\text{Propionate}} = 21.9511 + 5.15775.t - 0.348952.t^2 \quad (R^2 = 0.92);$$

$${}^3Y_{\text{Butirate}} = 10.9400 + 1.50395.t - 0.097476.t^2 \quad (R^2 = 0.85); \quad {}^3Y_{\text{Total}} = 97.4079 + 11.8894.t - 0.813545.t^2 \quad (R^2 = 0.96);$$

$${}^5Y_{\text{Acetate}} = 51.0602 + 7.29070.t - 0.450484.t^2 \quad (R^2 = 0.93); \quad {}^5Y_{\text{Propionate}} = 21.0264 + 6.94884.t - 0.440675.t^2 \quad (R^2 = 0.98);$$

$${}^5Y_{\text{Butirate}} = 8.26728 + 2.16091.t - 0.127801.t^2 \quad (R^2 = 0.97); \quad {}^5Y_{\text{Total}} = 91.1311 + 16.2089.t - 1.00313.t^2 \quad (R^2 = 0.99)$$

There was no effect of the lipid-rich diets intake on the serum concentrations (Table 6) of total protein (66.1 g/L), albumin (31.4 g/L), creatinine (1.2 mg/dL), alanine aminotransferase (15.3 U/L), alkaline phosphatase (121.5 U/L), aspartate aminotransferase (62.3 U/L), and gamma-glutamyltransferase (16.2 U/L). In addition to not showing differences between treatments, the concentrations of these variables met the serum reference values found in the literature (Table 6). There was no effect of the lipid-rich diets intake on the serum concentrations (Table 6) of total protein (66.1 g/L), albumin (31.4 g/L), creatinine (1.2 mg/dL), alanine aminotransferase (15.3 U/L), alkaline phosphatase (121.5 U/L), aspartate aminotransferase (62.3 U/L), and gamma-glutamyltransferase (16.2 U/L). Significant effects were detected on plasma urea concentration ($P < 0.05$) and cholesterol. The animals fed the diet containing soybean showed the highest plasma urea concentration (50.7 mg/dL). Six hours after feeding, the plasma urea concentration average of steers fed with the control treatment was 37.8 mg/dL. The plasma urea concentration was within the reference values. The average cholesterol concentration of animals that consumed diets containing cottonseed (152.7 mg/L), canola (137.1 mg/dL), sunflower (146.9 mg/dL), and soybean (138.2 mg/dL) grains was higher than that of animals that consumed the control diet (111.8 mg/dL).

Discussion

Since the diets had similar apparent digestibility coefficients (Table 3), the lower consumption of dry matter and organic matter in the diet containing cottonseed can be attributed to two factors. The first factor is that, when the cottonseed was included in the formulation of the diet in order to obtain a high content of ether extract, there was less participation of the soybean and corn meal, where such changes provided that the NDF and ADF content of the diet increased. In this regard, Costa et al. (2011), in evaluating the effect of adding 0, 14.35, 27.51, and 34.09% DM of cottonseed to the diet of confined cattle (50% roughage), observed a linear reduction in DM intake and weight gain. The authors associated these results with the increase in the EE content in the diets and the high NDF and ADF content provided by replacing soybean and corn with cottonseed.

Another factor that could have influenced the dry matter intake in diets containing cottonseed or canola is the presence of anti-nutritional factors in the oilseeds. Canola contains tannin as an anti-nutritional factor. Although this substance is usually present in a relatively low amount in canola (10 to 30 g/kg DM), it has a bitter or astringent taste, which can influence the

Table 6 Blood parameters of beef steers fed diets with a high content of ether extract using oilseed grains

	Experimental diets					Reference values ¹	SEM*	P-value
	Control	Cotton	Canola	Sunflower	Soybean			
Total protein (g/L)	67.4	68.4	62.9	62.6	67.9	66–75	10.29	0.7689
Albumin (g/L)	31.5	33.2	29.2	30.6	31.5	27–38	3.52	0.4144
Creatinine (mg/dL)	1.0	1.3	1.3	1.1	1.2	1–2	0.28	0.6824
Cholesterol (mg/dL)	111.8 b	152.7 a	137.1 a	146.9 a	138.2 a	80–120	18.89	0.0111
Glucose (mg/dL)	83.3	78.9	77.4	77.9	78.8	45–75	9.24	0.8213
Urea (mg/dL)	37.8 b	40.3 b	37.3 b	40.0 b	50.7 a	23–58	7.05	0.0193
Triglycerides (mg/dL)	17.7	26.9	20.3	20.0	15.0	0–14	6.86	0.0734
Alanine aminotransferase (U/L)	15.3	15.5	13.5	15.6	14.7	0–38	2.67	0.6433
Alkaline phosphatase (U/L)	108.6	112.3	169.9	116.7	108.0	0–196	56.45	0.2935
Aspartate aminotransferase (U/L)	73.5	79.2	52.4	63.6	63.6	0–132	25.76	0.4454
Glutamyltransferase range (U/L)	14.2	19.1	14.6	14.3	15.9	0–39	5.06	0.4213

*Averages followed by different lower case letters, differ by the Tukey test ($P < 0.05$); * SEM = Standard error of the mean

¹Kaneko et al. (2008)

palatability of the food and, consequently, the total ration, exerting a negative effect on DM intake (Cooper and Owen-Smith 1985; Landau et al. 2000). In addition, tannin is responsible for reducing food intake, growth rate, food efficiency, and digestibility of dietary protein (Tadele 2015; Caldas et al. 2021; Silva et al. 2021). Therefore, although it was not quantified in this study, the lower consumption of dry matter from the canola-containing diet can be attributed to the effect of the presence of tannin on palatability, since this diet showed *in vitro* digestibility and apparent digestibility results similar to those other treatments containing oilseeds and the control.

Likewise, the cottonseed contains gossypol as an anti-nutritional factor, which is a polyphenolic pigment that can be toxic if consumed in high doses for a prolonged period (Risco et al. 2002). Although adult ruminants have mechanisms to protect against the toxicity of many secondary chemical compounds, high doses of gossypol can overload the mechanisms that neutralize intoxication (Risco et al. 2002). Among the main effects of gossypol observed in ruminants is the decrease in dry matter consumption (Rogers et al. 2002). It should be noted that the intake of cottonseed was 2.7 kg/day (350 g/kg in the total DM of the diet), which can be considered high. This corroborates with the studies carried out by Bullock et al. (2010) who observed a reduction in the dry matter intake when the cottonseed was increased.

Regarding apparent digestibility, the oilseed inclusion did not have negative effects on the rumen environment, since the fatty acids would be partially protected from the grain matrix (Oliveira et al. 2011). The effects of lipid supplementation on ruminal digestibility are still controversial and may have negative effects (Bassi et al. 2012; Rennó et al. 2015) or absence of damage (Avila et al. 2000). However, despite the absence of significant effects of oilseeds on apparent digestibility, its negative effect on nutrient intake was evident. Wanderley et al. (2022) observed a diet containing cottonseed reduced the DMI by reducing the NDF digestibility in dairy cows fed lipid-rich diets (70 g/kg EE).

Considering that for cattle the rumination time is highly correlated with the neutral detergent fiber intake (Welch and Hooper 1988), the treatments that presented the highest averages for this activity were among the treatments that consumed the most fiber. When using whole oilseeds to obtain lipid-rich diets, there was less participation of non-fibrous carbohydrates in the diet (Table 2). Therefore, the use of whole oilseeds can be an alternative in the formulation of diets to avoid fermentative disorders such as acidosis. It is observed that the diets were formulated with a roughage:concentrate 400:600 ratio (Table 1), so the neutral detergent fiber content of the diets showed averages above 375 g/kg (Table 2), which probably sufficiently maintained rumination and salivation to maintain the ruminal pH above 6.0 for almost the entire day (Table 4).

Despite the lipid supplementation making it possible to reduce the concentration of ammonia (NH₃) in the rumen liquid

(Lin et al. 1995; Nagaraja et al. 1997), the use of oilseeds did not provide such an effect at all times evaluated (Table 4). According to Van Soest (1994), the concentration of rumen ammonia (NH₃) plays a fundamental role in microbial growth and efficiency. In order for the microbial fermentation to not be limited, the minimum concentration of ammonia should be approximately 5 mg/100 mL of rumen liquid (Satter and Roffler 1975) and 23 mg of NH₃/mL for maximum microbial synthesis (Mehrez et al. 1977). Therefore, it is noteworthy that the values observed in all treatments are in accordance with the values indicated and do not limit fermentation and maximum microbial synthesis.

The amount of organic matter and fiber digested in the rumen can cause changes in the concentration and proportion of VFA produced (Ítavo et al. 2002b; Beauchemin et al. 2022; Plata-Pérez et al. 2022). The higher production of VFA by treatments containing whole soybean and sunflower and the control can be justified by the higher OM intake (Table 3); in addition, these diets have a greater participation of NFC (Table 2). According to Mota et al. (2010), acetate production is greater from cellulose and hemicellulose degradation, while NFC degradation increases propionate production, decreasing the relationship between acetate and propionate.

The lower production of total fatty acids by steers-fed cottonseed or canola diet can be explained by lower dry matter and organic matter intakes. In addition, the treatment containing canola presented the lowest NDF intake and digestibility, in relation to the other treatments, which would justify the lower production of total fatty acids for these treatments. According to Van Soest (1994), the fermentation of starch and sugars decreases the rumen pH, as it produces a greater amount of VFA, mainly propionate via lactic acid, which can accumulate in the rumen, reducing fiber digestion, which was not observed in this experiment, where the pH remained between 6.0 and 7.0.

The increase in the serum cholesterol content has already been observed in other studies with cattle fed with lipid supplements (West and Hill 1990; Drackley and Elliott 1993; Elliott et al. 1993). According to Nestel et al. (1978), the increase in cholesterol in animals fed lipid-rich diets increases intestinal cholesterol synthesis necessary for the absorption and transport of high levels of circulating lipids from the diet.

Conclusions

Lipid-rich diets with whole oilseeds did not impair the apparent digestibility of nutrients, demonstrating the potential of using these whole oilseeds as protected sources of fatty acids. The high lipid content obtained through whole oilseed use provide an increase in the concentration of cholesterol in the bovine serum, indicating a potential for greater absorption of fatty acids. We

recommended using whole soybean and sunflower to formulate lipid-rich diets with 70 g/kg of ether extract content for crossbreed steers in the feedlot.

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Code availability Not applicable

Declarations

Ethical approval This study was conducted in strict accordance with the recommendations of the Guide for the National Council for the Control of Animal Experiments. The experimental protocol of research was approved by the Ethics Committee on Animal Use of Federal University of Mato Grosso do Sul (Protocol No. 654/2015).

Conflict of interest The authors declare no competing interests.

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