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REGULAR ARTICLES

Dietary replacement of soybean meal with heat-treated grain soybean in diets of feedlot-finished beef cattle: impacts on intake, digestibility, and ruminal parameters

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Abstract

The objective of this study was to assess the impact of increasing levels of heat-treated soybean in the diet of crossbred cattle during the finishing phase on nutrient intake and digestibility, ruminal parameters, digesta passage rate, nitrogen balance, and microbial protein synthesis. Five steers, crossbred 7/8 Jersey x Zebu, fitted with rumen cannulas and with an average weight of 350 ± 50 kg, were utilized. The experimental treatments consisted of 0, 7, 14, 21, and 28% inclusion of heat-treated soybean in the total diet dry matter. The animals were randomly allocated in a 5×5 Latin square design. Evaluation of the animals took place over five experimental periods, each lasting 20 days. During each experimental period, the first 15 days were allocated for animal adaptation to the experimental diets, followed by five days of data collection. No significant differences were observed among the diets in terms of dry matter intake (average of 6.57 kg day^{-1}; $P = 0.615$) and organic matter intake (average of 6.23 kg day^{-1}; $P = 0.832$). However, heat-treated soybean had a significant impact on the digestibility of dry matter ($P = 0.02$), organic matter ($P = 0.01$), crude protein ($P < 0.01$), and neutral detergent fiber ($P < 0.01$). There was no observed change on microbial protein synthesis (average of 409.6 g day^{-1}) in animals with the inclusion of heat-treated soybean in the diets. With each 1% inclusion of heat-treated soybean in the cattle diet, there was an increase of 0.00754 units in ruminal pH values and a reduction of 0.75839 mg dL^{-1} in ruminal ammoniacal nitrogen values. This study suggests that heat-treated soybean can be included in up to 15% of the dry matter in diets for finishing feedlot cattle.

Keywords Animal feed · Feedlot · Intake · Microbial protein synthesis · Short-chain fatty acids

Introduction

The production of ruminants in Brazil relies primarily on forage plants as the main source of nutrients for the animals. However, the low quality of tropical forages, coupled with factors such as seasonality in mass production, poses challenges in providing adequate energy intake and protein to meet the nutritional requirements of animals during the finishing phase (Vellini et al., 2020; Ferreira et al., 2021).

To address the issue of slaughtering older animals (over 36 months) that are lighter and lack proper carcass finishing, the use of intensive production systems in Brazil has been steadily increasing each year. In 2022, a total of 39.1 million cattle were slaughtered (Abiec, 2023), with approximately 15.4% of that total being finished in feedlots. In intensive production systems, the cost of feed is one of the factors

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that has the most significant impact on the overall production cost.

Soybean meal is a widely used ingredient in Brazilian feedlots as the primary source of crude protein. This nutrient represents one of the main components that drive up feed costs. Consequently, there is a search for feeds rich in crude protein to either fully or partially replace soybean meal. Among various alternatives, whole processed and heat-treated soybeans, derived from *Glycine max* (L.) Merr., are prominent sources of protein and energy in animal diets (Melo et al., 2023). Their use is advantageous due to the slow-release of fatty acids within the rumen (Bulcão et al., 2021). Soybean grains contain 384.7 g kg^{-1} DM of crude protein, 905.3 g kg^{-1} DM of total digestible nutrients, and 189.1 g kg^{-1} DM of lipids in their chemical composition (Chesini et al., 2022).

However, whole raw soyabean have inherent antinutritional factors for animals, such as proteases inhibitors, lectins (hemagglutinins), urease, and allergens (glycinin and β-conglycinin). These factors necessitate heat treatments to inactivate them before feeding them to animals (Rocha et al., 2014). After heat treatment, this feed is referred to as deactivated or heat-treated soybean (Chesini et al., 2022).

The inclusion of heat-treated soybean in ruminant diets requires careful analysis. High levels of inclusion (above 300 g kg^{-1} DM) of these ingredients can impact the voluntary intake of animals and fiber digestibility due to the high lipid content found in soybean grains (Palmquist, 1991; Ibrahim et al., 2021). Chesini et al. (2022) evaluated the inclusion of heat-treated soybean (44 g kg^{-1} DM) in the diet of Holstein cows and did not verify changesin the digestibility of the diet and milk production in the animals. There are few literature studies with heat-treated soybean compared to the use of whole raw soyabean in ruminant diets. Cônsolo et al. (2017) evaluated diets containing 80, 160, and 240 g kg^{-1} DM of whole raw soyabean in the diet of Nelore steers. The authors concluded that whole raw soyabean inclusion into the diet of Nelore steers did not change nutrient digestibility, promoted few changes in rumen metabolism and blood parameters, and was not detrimental to digestive functions. In dairy cattle, Barletta et al. (2016) examined high levels of whole raw soyabean (240 g kg^{-1} diet DM) in the diets of Holstein dairy cows and reported a linear decrease in DM intake. However, milk production (3.5% of fat) was not affected.

To avoid exceeding 80 g kg^{-1} DM of lipids in ruminant diets (Ibrahim et al., 2021), it is essential to study the optimal proportion of soybean grain inclusion in the diet of cattle, as a partial replacement for soybean meal. We hypothesize that the partial replacement of soybean meal with heat-treated soybeans will not affect the intake and digestibility of nutrients and ruminal parameters in beef cattle during the feedlot finishing phase.

Based on the above, the aim of this study was to assess the impact of increasing levels of heat-treated soybean in the diet of crossbred cattle during the finishing phase on nutrient intake, digestibility, ruminal parameters, digesta passage rate, nitrogen balance, and microbial protein synthesis.

Materials and methods

Location of execution

This research was conducted in accordance with the ethical principles established by the National Council for the Control of Animal Experimentation (CONCEA) and received approval from the Committee on Ethics in Animal Use/CEUA/UFGD (protocol number 22/2020).

The experiment was carried out at the Federal University of Grande Dourados (UFGD), located in Dourados, Mato Grosso do Sul, Brazil, at a latitude of 22° 14´S, longitude of 54° 49´W, and an altitude of 450 m.

Animals and experimental design

Five rumen-cannulated male crossbred steers (7/8 Jersey x Zebu) were utilized, each weighing 350 ± 5 kg and aged approximately 28.0 ± 3.0 months. The experiment followed a 5×5 Latin square design, with each experimental period spanning 20 days, including 15 days for adaptation to the experimental diets and 5 days for sampling. The animals were vaccinated, dewormed, and housed in individual stalls measuring 3×5 m (15 m^2) with shelter and free access to water. Feeding occurred at 8:00 a.m. with ad libitum access to feed (with a 10% allowance for refusals on an as-fed basis).

The experimental diets were formulated to include levels of heat-treated soybean at 0, 7, 14, 21 and 28% (on a dry matter basis; Table 1). The roughage-to-concentrate ratio, based on dry matter, was 15:85. As per the NRC (2016) guidelines, all diets were designed to meet the nutrient requirements for an average daily gain of 1.5 kg per day.

Heat-treated soybeans were incorporated into the diets to achieve an ether extract (EE) concentration of 80 g kg^{-1} DM. This EE level of 80 g kg^{-1} DM in the diets was established in accordance with NRC (2016) recommendations, which specify the maximum allowable lipid content in ruminant diets. Conversely, the ether extract (EE) level in the control diet was determined according to standard feed compositions for confined ruminant animals.

Intake, chemical composition and digestibility of nutrientes

The daily feed intake was determined by weighing both provided feed and the leftovers. Samples of both the offered and

Table 1 Proportion and chemical composition of experimental diets

Item (%)	Inclusion of heat-treated soybean (% DM)				
	0	7	14	21	28
Oat hay	15.0	15.0	15.0	15.0	15.0
Ground corn	65.2	64.9	63.9	60.9	54.5
Soybean meal	16.2	9.50	3.50	0.00	0.00
Heat-treated soybean	0.00	7.00	14.0	21.0	28.0
Urea	1.00	1.00	1.30	1.40	1.30
Premix mineral[1]	2.50	2.50	2.50	2.50	2.50
	Chemical composition (g kg^{-1} DM)				
Dry matter	889	890	892	893	895
Crude protein	150	150	150	150	150
Ether Extract	34.5	46.3	59.2	69.6	79.9
Neutral detergent fiber	193	198	206	213	223
Total digestible nutrients	768	780	814	812	822

[1]Guarantee levels (kg product^{-1}): Calcium 147.00 g, Phosphorus 35.00 g, Sodium 100.00 g, Sulfur 50.00 g, Magnesium 40.00 g, Buffer 100.00 g, Cobalt 90.00 mg, Copper 700.00 mg, Iodine 90.00 mg, Manganese 1,800.00 mg, Selenium 15.00 mg, Zinc 3,000.00 mg, Iron 1,800.00 mg, Fluorine 350.00 mg, Monensin 1,250.00 mg, *Bacillus subtilis* 3.00×10^{10} CFU kg^{-1}, *Bifidobacterium bifidum* 1.00×10^{10} CFU kg^{-1}, *Enterococcus faecium* 1.00×10^{10} CFU kg^{-1}, *Lactobacillus acidophilus* 1.00×10^{10} CFU kg^{-1}, *Lactobacillus buchneri* 2.00×10^{10} CFU kg^{-1}, *Lactobacillus casei* 1.00×10^{10} CFU kg^{-1}, *Lactobacillus lactis* 1.00×10^{10} CFU kg^{-1}, *Saccharomyces cerevisiae* 2.00×10^{9} CFU kg^{-1}

refused feed were collected on a daily basis and frozen for subsequent analysis of various components, including dry matter (DM; method 930.15), organic matter (OM; method 942.05), crude protein (CP; method 976.05), and ether extract (EE; method 920.39), in accordance with AOAC (2000) procedures. Neutral detergent fiber (NDF) was assessed following the method described by Mertens (2002), utilizing α-amylase (Termamyl®; Sigma Aldrich). Acid detergent fiber (ADF) was determined as per AOAC (2012) guidelines. The total digestible nutrient content (TDN) was calculated based on NRC (2001) recommendations.

Digestibility coefficients were calculated using the following formula: DC = [(kg of the portion ingested—kg of the portion excreted) / (kg of the portion ingested)] × 100.

Ruminal parameters

On the 19th day of each experimental period, samples of ruminal digesta were collected from five different locations within the rumen (cranio-dorsal, cranio-ventral, ventral, caudo-ventral, and caudo-dorsal ruminal regions). These samples were taken through the ruminal cannula shortly before and 2, 4, 6, and 8 h after the morning feeding, totaling five collections.

Rumen pH was promptly measured after collecting 200 mL of samples using a pH meter (MB-10, Marte, Santa Rita do Sapucaí, Brazil). Subsequently, 20 mL of ruminal fluid were collected, stored in sealed plastic containers, and immediately frozen at -20 °C for later analysis. Analysis of volatile fatty acids was conducted using High-performance Liquid Chromatography (Shimadzu, Japan) equipped with an ultraviolet detector (SPD-10AVP Columbia, USA) and a column (HPX-87H—30 cm × 4.5 mm, Bio-Rad Laboratories Ltd.) according to Erwin et al. (1961). Ammonia nitrogen concentration (NH_3-N) in the ruminal fluid was determined using a spectrophotometer (Spectrophotometer Mono Beam SP-22, Curitiba, Biospectro, Brazil®) following the method outlined by Broderick and Kang (1980).

Nitrogen balance and microbial protein synthesis

Urine collection was carried out on the 19th day of each experimental period, occurring 4 h after feeding. Allantoin analysis was conducted using the colorimetric method, following the technique described by Fujihara et al. (1987), as outlined by Chen and Gomes (1992). The total excretion of purine derivatives was calculated by summing the amounts of allantoin and uric acid excreted in the urine, expressed in mmol per day.

Absorbed microbial purines (mmol per day) were calculated based on the excretion of purine derivatives in the urine (mmol per day) using the equation: $DP = 0.85 \times Pabs + 0.385 \times BW^{0.75}$, where 0.85 represents the recovery of purines absorbed as urinary purine derivatives, and $0.385 \times BW^{0.75}$ accounts for the endogenous contribution to purine excretion (Verbic et al. 1990).

To determine the nitrogen balance, the nitrogen content in urine, feces, and feed was analyzed using the Kjeldahl method in accordance with AOAC (2000) procedures. Urinary volume was calculated using the equation: UV (l per day) = (27.36 × BW) / [creatinine], where BW represents body weight, and UV stands for urinary volume. The value of 27.36 signifies the average daily excretion of creatinine, in parts per million (ppm) of body weight, as established by Rennó et al. (2014).

Digesta passage rate and ruminal dynamics

The determination of nutrient pools and flow was estimated based on the concentration of the internal indicator indigestible neutral detergent fiber (iNDF). To achieve this, pre-dried samples of feed, leftovers, ruminal content, reticular content, and feces were collected and dried in an oven with forced ventilation (55 °C/72 h). The samples were processed in a knife mill with 1 mm porosity sieves, and these were placed in non-woven fabric bags (TNT 100 g m^{-2})

measuring 4×5 cm, following a ratio of 20 mg cm^2 of dry matter (Nocek, 1988).

The samples were incubated for 288 h in the rumen of two Jersey steers, previously adapted to a concentrate diet consisting of heat-treated soybean, ground corn, and oat hay, following the methodology described by Casali et al. (2008). The iNDF passage rate (kp) in the ruminal digesta was calculated by dividing the iNDF intake per hour by the ruminal compartment size, based on the two-compartment model for cellulose digestion proposed by Waldo et al. (1972). The ruminal NDF digestion rate (kd) was calculated as the difference between the total NDF removal rate from the rumen and its passage rate (Allen and Mertens, 1988), where: Kd = (Intake per hour of digestible NDF – kp) / Size of digestible NDF ruminal compartment.

The apparent rumen digestibility of NDF as a percentage of NDF was calculated as follows: NDF digestibility (%) = kd / (kd + kp). Ruminal renovation rates, passage through, and ruminal digestion rate of each component were calculated according to Oba & Allen (2003). During the total removal of the ruminal content, 10% aliquots of the digesta were separated to allow for more accurate and representative sampling.

The aliquots were filtered through a desorating cloth sieve with 1.0 mm porosity to separate the solid content from the liquid for determining the average particle size. Fractions of ruminal and abomasal content samples in the solid and liquid phases were pre-dried and corrected for the original dry matter, and conditioned in a freezer at -20 °C until the analyses were carried out (Harvatine and Allen, 2006).

Statistical analyzes

The data obtained were analyzed using SAS (Version 9.1.3, SAS Institute, Cary, NC, 2004), with checks for the normality of residuals, and homogeneity of variances. The data were analyzed through PROC MIXED according to the following model:

$$Y_{ijl} = \mu + a_i + p_j + D_l + e_{ijl}$$

where:

Y_{ijl} is the dependent variable, μ is the overall mean, a_i is the random effect of the animal ($i = 1$ to 5), p_j is the random effect of the period ($j = 1$ to 5), D_l = is the fixed effect of diet ($l = 1$ to 5), and e_{ijl} is the residual error, normally and independently distributed, with mean zero and variance σ^2.

The degrees of freedom were adjusted using DDFM = kr, and Akaike's information criterion was used to select the best covariance structure. The data obtained underwent an analysis of variance, with a significance level of 5%, and were evaluated using linear and quadratic polynomial regression models.

Results

Intake and digestibility of nutrients

The inclusion of thermally treated soybean grain to replace soybean meal in the diet of finishing-phase cattle did not result in significant changes in the intake of dry matter ($P = 0.65$), organic matter ($P = 0.83$), crude protein ($P = 0.55$), neutral detergent fiber ($P = 0.65$), and starch ($P = 0.12$). The average intakes were 6.57, 6.23, 0.995, 1.35, and 2.18 kg per day, respectively (Table 2). However, the intake of ether extract showed a linear increase ($P < 0.01$). With each 1% inclusion of soybean, there was a corresponding increase of 0.00734 kg per day in the intake of ether extract. The dry matter intake ($P = 0.23$) and NDF intake ($P = 0.23$), expressed as a percentage of body weight, did not change with the inclusion of soybean grain in the diet.

The digestibility of dry matter ($P < 0.01$), organic matter ($P < 0.01$), crude protein ($P < 0.01$), and neutral detergent fiber ($P < 0.01$) was affected by the inclusion of soybean grain in the animal's diet. The means followed a quadratic regression model. The inclusion of soybean grain that minimized the digestibility of dry matter (12.12%), organic matter (12.12%), crude protein (14.64), and neutral detergent fiber (10.97%). On the other hand, the inclusion of soybean grain that maximized the digestibility of ether extract was 20.0%.

Ruminal parameters

The inclusion of heat-treated soybean in the diet of crossbred cattle in the finishing phase led to changes in the pH values ($P < 0.01$) and ruminal ammoniacal nitrogen ($P < 0.01$). The pH values increased by 0.00754 units for every 1% inclusion of heat-treated soybean in the diet (Table 3). Ruminal ammoniacal nitrogen and acetate concentration showed a linear decrease of 0.75839 mg dL^{-1} and 0.31122 mmol L^{-1}, respectively, for each 1% inclusion of heat-treated soybean in the diet.

There were no significant differences between diets in terms of propionic acid ($P = 0.23$), butyric acid ($P = 0.10$), isobutyrate ($P = 0.10$), valerate ($P = 0.09$), and acetate:propionate ratio ($P = 0.67$). The average concentrations were 14.33, 7.23, 0.37, 0.73 mmol L^{-1}, and 2.95, respectively.

The concentrations of isovalerate ($P < 0.01$), branched-chain fatty acids ($P < 0.01$), and total fatty acids ($P < 0.01$) linearly decreased as the inclusion of heat-treated soybean in the diet increased.

Table 2 Intake and digestibility of nutrients in cattle fed different levels of heat-treated soybean

Item	Heat-treated soybean levels (% DM)[1]					SEM[2]	P-value[3]		
	0	7	14	21	28		Diet	Linear	Quadr
Intake (kg day^{-1})									
Dry matter	6.85	7.06	6.52	6.32	6.13	0.203	0.651	0.332	0.744
Organic matter	6.49	6.70	6.18	5.99	5.81	0.191	0.832	0.402	0.341
Crude protein	1.15	0.95	0.939	1.01	0.929	0.046	0.557	0.214	0.258
Ether extract^{A}	0.132	0.264	0.284	0.31	0.366	0.019	0.003	< 0.001	0.135
Neutral detergent fiber	1.32	1.40	1.34	1.35	1.37	0.089	0.654	0.223	0.358
Starch	2.27	2.32	2.16	2.10	2.06	0.068	0.125	0.541	0.235
Intake (% BW)									
Dry matter	2.36	2.18	2.07	2.00	2.19	0.098	0.235	0.385	0.239
Neutral detergent fiber	0.46	0.43	0.43	0.43	0.49	2.02	0.235	0.456	0.523
Digestibility (g kg^{-1})									
Dry matter^{B}	789.23	836.94	772.57	764.39	808.93	10.20	0.025	0.125	0.002
Organic matter^{C}	852.37	903.89	834.38	873.65	825.54	11.016	0.045	0.225	0.011
Crude protein^{D}	788.42	759.64	698.02	704.51	795.72	17.415	0.008	0.517	0.001
Ether extract^{E}	831.44	881.63	905.63	889.42	895.14	8.58	0.028	0.234	0.038
Neutral detergent fiber^{F}	548.76	599.29	547.54	456.03	771.37	11.991	0.002	0.145	0.003
Starch	880.08	928.35	925.26	916.4	939.4	10.177	0.420	0.458	0.235

SEM Standard error of the mean. *Quadr* Quadratic Effect

^{A}Y = 0.1691 + 0.00734X; r^2 = 0.76

^{B}Y = 803.361 – 4.7370X + 0.19514X^2; r^2 = 0.78

^{C}Y = 867.6301 – 5.1159X + 0.2107X^2; r^2 = 0.85

^{D}Y = 801.3825 – 13.1543X + 0.4491X^2; r^2 = 0.84

^{E}Y = -0.1883x^2 + 7.2027x + 835.16, R^2 = 0.96

^{F}Y = 594.1849 – 15.6808X + 0.7141X^2, r^2 = 0.92

Table 3 Ruminal fermentation of confined cattle fed with heat-treated soybean

Item	Heat-treated soybean levels (% DM) [1]					SEM[2]	P-value[3]		
	0	7	14	21	28		Diet	Linear	Quad
pH ^{A}	6.03	6.33	6.38	6.11	6.40	0.034	< 0.001	0.003	0.102
Ammoniacal nitrogen (mg dL^{-1}) ^{B}	48.6	35.2	31.4	27.2	26.1	1.67	< 0.001	< 0.001	0.125
Short-chain fatty acids (mmol L^{-1})									
Acetate ^{C}	44.19	42.46	36.85	38.42	35.35	0.841	0.005	< 0.001	0.458
Propionate	15.80	15.82	13.42	13.13	13.5	0.437	0.233	0.454	0.358
Butyrate	8.13	6.88	7.52	7.11	6.53	0.223	0.104	0.235	0.855
Isoburirate	0.301	0.366	0.416	0.333	0.462	0.02	0.101	0.047	0.974
Valerate	0.863	0.694	0.617	0.69	0.826	0.028	0.099	0.646	0.003
Isovalerate ^{D}	2.16	1.55	1.70	1.53	1.57	0.055	0.001	0.004	0.015
Branched-chain fatty acid ^{E}	3.33	2.61	2.73	2.56	2.85	0.079	0.005	0.043	0.003
Total short-chain fatty acids ^{F}	74.77	70.38	63.25	63.77	61.08	1.235	0.005	< 0.001	0.296
Acetate: Propionate ratio	3.01	2.87	2.85	3.13	2.93	0.073	0.672	0.846	0.732

SEM Standard error of the mean. *Quadr* Quadratic Effect

^{A}Y = 6.152 + 0.00754X; r^2 = 0.74

^{B}Y = 44.342 – 0.75839X; r^2 = 0.82

^{C}Y = 43.810- 0.31122X; r^2 = 0.89

^{D}Y = 2.0881- 0.0568X + 0.0014X^2; r^2 = 0.82

^{E}Y = 3.2680- 0.0845X + 0.0025X^2; r^2 = 0.78

^{F}Y = 73.4490 – 0.4854 X; r^2 = 0.88

Nitrogen balance and microbial protein synthesis

The inclusion of heat-treated soybean in the diet of crossbred cattle during the finishing phase did not have a significant effect on nitrogen intake (P = 0.53), nitrogen excretion in feces (P = 0.23), absorbed nitrogen (P = 0.29), and retained nitrogen (P = 0.22). The respective means were 159.7, 40.24, 119.5, and 96.99 g per day (Table 4).

The means related to the excretion of nitrogen in urine were fitted to a quadratic regression model. It was found that including heat-treated soybean at a rate of 15.62% in the cattle's diet resulted in greater urinary nitrogen excretion. There was no difference between the diets on microbial protein synthesis (MPS) in animals (P = 0.62) and efficiency of MPS (P = 0.611). The averages were 788.20 g day^{-1} and 150.42 g MPS kg of TDN^{-1}, respectively.

Digesta passage rate and ruminal dynamics

The inclusion of heat-treated soybean in the diet of crossbred cattle did not alter the post-ruminal flow of dry matter (P = 0.84), organic matter (P = 0.83), and NDF (P = 0.63; Table 5). Additionally, the passage rate (% per hour) of dry matter (P = 0.25) and organic matter (P = 0.24) also remained unchanged. However, the passage rate of NDF and the rate of disappearance of dry matter and organic matter were affected by the inclusion of heat-treated soybean grain in the diet. The means followed a quadratic regression model.

The digestion rate of dry matter, organic matter, and NDF was not affected by the different bovine diets. The inclusion of heat-treated soybean in the cattle's diet resulted in a linear reduction in the ruminal apparent digestibility of dry matter and organic matter. The average NDF ruminal digestibility followed a quadratic regression model, with the maximum point occurring at an 18.41% inclusion of heat-treated soybean.

Discussion

Heat-treated soybean is a potential ingredient for use in ruminant feed because it serves as a source of crude protein, energy, and lipids. However, its high fat content is one of the factors limiting its inclusion in larger proportions in animal diets. In this research, it was observed that replacing soybean meal with thermally treated soybean increased the intake of ether extract. This increase can be attributed to the chemical composition of the ingredient, namely, the thermally treated soybean. Nevertheless, there was no observed effect on the intake of dry matter and crude protein. According to Bulcão et al. (2021), this lack of effect may be because the population of fibrolytic bacteria in the rumen was not adversely affected by the fats present in soybean.

The lowest digestibility of dry matter and neutral detergent fiber occurred when 12.12% and 10.97% of soybean were included in the diet, respectively. Digestibility values increased when soybean inclusion exceeded the aforementioned percentages. Ibrahim et al. (2021) discovered that heat-treated soybean and other sources of vegetable oils rich in unsaturated fatty acids are known to have a negative impact on bacteria that might inhibit fiber digestion. This is because when fat becomes available in the rumen, it forms a coating on bacterial cells and feed particles, limiting bacterial access to the feed particles and thus affecting

Table 4 Nitrogen balance in confined cattle fed different levels of heat-treated soybean

Item	Heat-treated soybean levels (% DM) [1]					SEM[2]	P-value[3]		
	0	7	14	21	28		Diet	Linear	Quad
Intake (g day^{-1})									
Nitrogen	185.41	152.11	150.35	162.12	148.69	7.509	0.536	0.254	0.413
Excretion (g day^{-1})									
Feces	40.26	37.75	45.06	47.76	30.37	3.503	0.235	0.112	0.203
Urine [A]	30.49	22.44	17.08	15.8	26.59	3.154	0.017	0.232	0.007
Nitrogen balance (g day^{-1})									
Absorbed	145.14	114.35	105.28	114.36	118.31	5.996	0.291	0.209	0.089
Retained	114.65	91.9	88.19	98.54	91.71	6.84	0.221	0.351	0.541
Microbial protein synthesis (MPS; g day^{-1})									
Nitrogen	121.49	120.81	122.30	146.00	119.95	1.93	0.622	0.574	0.594
Crude protein	759.34	755.05	764.38	912.52	749.71	12.04	0.622	0.574	0.594
Efficiency of MPS, g MPS kg^{-1} of TDN	144.34	137.11	144.02	177.81	148.79	3.18	0.501	0.493	0.611

SEM Standard error of the mean. *Quadr* Quadratic Effect

[A] $Y = 31.334 - 1.9098X + 0.0608X^2$; $r^2 = 0.82$

Table 5 Ruminal dynamics of feedlot cattle fed with different levels of heat-treated soybean

Item	Heat-treated soybean levels (% DM) [1]					SEM[2]	P-value[3]		
	0	7	14	21	28		Diet	Linear	Quad
Post-ruminal flux (kg DM day^{-1})									
Dry matter	3.09	3.24	3.63	3.55	3.62	0.222	0.842	0.318	0.7
Organic matter	2.87	3.06	3.40	3.35	3.41	0.21	0.83	0.302	0.673
Neutral detergent fiber	1.11	0.91	0.91	0.983	0.957	0.06	0.655	0.523	0.915
Passage rate (% h^{-1})									
Dry matter	4.25	3.47	4.47	3.47	5.6	0.484	0.254	0.125	0.203
Organic matter	4.37	3.76	4.52	3.54	5.91	0.523	0.241	0.185	0.221
Neutral detergent fiber [A]	4.88	3.26	3.97	2.51	4.52	0.382	0.041	0.444	0.037
Turnover—disappearance rate (% h^{-1})									
Dry matter [B]	9.37	7.68	7.73	6.79	9.31	0.775	0.012	0.718	0.039
Organic matter[C]	9.77	8.36	7.91	6.98	9.86	0.845	0.046	0.691	0.036
Neutral detergent fiber	3.98	3.59	4.51	3.25	5.83	0.47	0.106	0.404	0.163
Digestion rate (% h^{-1})									
Dry matter	5.12	4.24	3.26	3.31	3.71	0.422	0.218	0.071	0.128
Organic matter	5.39	4.59	3.39	3.44	3.95	0.454	0.2	0.067	0.13
Neutral detergent fiber	0.926	1.01	1.94	1.05	1.99	0.215	0.215	0.119	0.938
Ruminal apparent digestibility (g kg^{-1})									
Dry matter[D]	546.02	533.3	458.65	449.19	408.4	28.823	0.008	0.037	0.962
Organic matter [E]	554.29	535.24	464.62	453.53	412.25	28.871	0.005	0.036	0.965
Neutral detergent fiber[F]	262.48	279.61	424.56	497.73	341.81	32.249	0.002	0.255	0.008

SEM: Standard error of the mean. *Quadr*: Quadratic Effect

[A] $Y = 4.8497 – 0.2282X + 0.00741X^2$; $r^2 = 0.83$

[B] $Y = 9.446$ 5- $0.3183X + 0.0108X^2$; $r^2 = 0.71$

[C] $Y = 9.9725 – 0.3473X + 0.0118X^2$; $r^2 = 0.80$

[D] Y $550.9815 – 5.13358$ X; $r^2 = 0.88$

[E] $Y = 557.1477 – 5.2256X$; $r^2 = 0.76$

[F] $Y = 226.1871 + 22.4393X – 0.6091X^2$; $r^2 = 0.93$

feed degradation (Ibrahim et al., 2021). However, this behavior was not observed in this research, likely because fat levels in the diets did not exceed 80 g kg^{-1} DM.

Bulcão et al. (2021) observed that the inclusion of 16% whole raw soybean in the buffalo diet (on a dry matter basis) did not lead to changes in dry matter intake. However, the same authors reported an increase in the digestibility of ether extract. In this study, a higher digestibility of ether extract was observed with the inclusion of 20% thermally treated soybean in the diet of beef cattle. According to Palmquist (1991), there are situations in which an increase in ether extract intake tends to dilute the effect of endogenous losses of ether extract in feces, resulting in higher apparent digestibility. This phenomenon was confirmed in our research.

Ruminal pH values in crossbred cattle were affected by diets containing different proportions of heat-treated soybean grain compared to the control diet (without soybean grain). However, all values remained close to the mean, falling within the normal range of 6.0 to 7.0, which optimizes the rate of ruminal fermentation and fiber degradation.

Animals fed diets containing up to 28% soybean inclusion exhibited higher ruminal pH values. This outcome can be attributed to the higher inclusion of cornmeal and soybean meal in the control diet. These ingredients undergo faster fermentation compared to the carbohydrates contained within soybeans, providing a substrate for the ruminal fermentation process and, consequently, affecting the availability of hydrogen (Macedo et al., 2016). Furthermore, the adaptation of ruminal microorganisms to varying levels of fat in the diet may explain the higher pH in the diet with 28% soybean grain inclusion. This adaptation can favor the process of biohydrogenation of unsaturated fats, in which microorganisms use free hydrogen to saturate fatty acids, thus increasing pH values in the rumen.

The concentrations of N-NH_3 decreased linearly with increasing proportions of soybean grain in the cattle diet. This reduction can be explained by the toasting process applied to the soybean grain during processing, which reduces the ruminal degradability of protein. Additionally, the deleterious effect of lipids present in soybeans on microorganisms responsible for amino acid degradation can contribute to the decrease in

$N\text{-}NH_3$ concentrations. Microbial protein synthesis typically reduces $N\text{-}NH_3$ concentration, assuming there is an adequate presence of carbohydrates. However, there was no effect of soybean in the cattle diet on microbial protein synthesis, which averaged 788.20 g per day. The presence of nitrogenous compounds and ammonia in ruminal fluid is essential for rumen microorganisms, particularly cellulolytic bacteria, which use ammonia for protein synthesis. Nevertheless, the decrease in ammonia levels in the 28% soybean diet did not affect fiber fermentation, indicating that all diets provided sufficient ammonia for microbial fermentation.

The inclusion of soybean grain in the diet of crossbred cattle resulted in a reduction in the concentration of acetic acid and total short-chain fatty acids. These changes can be attributed to the decreased proportion of cornmeal and soybean meal in the cattle's diet and the inclusion of heat-treated soybean grain. However, there is no consensus in the literature regarding the effects of oilseeds on ruminant diets. According to Ibrahim et al. (2021), the reduction in acetic acid and short-chain fatty acids may be linked to variations in the population of cellulolytic bacteria and protozoa in the rumen, which can lead to different outcomes with diets rich in oilseeds. The unsaturated fatty acids found in soybean and other oilseeds can be detrimental to many species of ruminal bacteria, particularly those involved in fiber digestion. However, no direct effect of lipids on bacteria has been established based on the results of neutral detergent fiber digestibility.

Regarding nitrogen balance, the excretion of nitrogen through urine exhibited a quadratic effect, with a minimum point at 15.62% inclusion of heat-treated soybean in the diet. The heating of soybean during the heat treatment process reduces protein degradation by ruminal microorganisms, consequently reducing free ammonia in the rumen available for absorption. This process, in turn, decreases the excretion of free ammonia through the animal's metabolism, consistent with the observed decrease in $N\text{-}NH_3$ values in ruminal fluid with increasing inclusion of heat-treated soybean in the diet. Several factors can affect the amount of nitrogen absorbed by the duodenum and the amount of nitrogen retained, such as protein content and solubility, sources of endogenous nitrogen, the amount of digestible organic matter in the diet, pre-feeding treatment of dietary protein, and ruminal absorption (Yan et al., 2006). Proper diet balancing ensures the synchronization of carbohydrates and protein for microbial protein synthesis.

Conclusion

The substitution of soybean meal with heat-treated soybean in up to 20% of the dry matter in diets for finishing feedlot cattle does not alter dry matter intake but enhances ruminal parameters and fiber digestibility.

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Data availability The data that support this study will be shared upon reasonable request to the corresponding author.

Declarations

Conflicts of interest The author declares no conflicts of interest.

References

Allen, M. S., Mertens D. R., 1988. Evaluating constraints on fiber digestion by rúmen microbes. The Journal of Nutrition, 118(2), 261–270. https://doi.org/10.1093/jn/118.2.261

Associação Brasileira das Indústrias Exportadoras de Carnes – ABIEC., 2023. Beef Report 2022. https://www.abiec.com.br/publicacoes/beef-report-2022/

Association of Official Anaytical Chemists, AOAC., 2000. Official Methods of Analysis, (17th ed. Arlington).

Association of Official Anaytical Chemists, AOAC., 2012. Official Methods of Analysis, (19th ed. Gaithersburg).

Barletta, R.V., Gandra, J.R., Freitas Junior, J.E., Verdurico, L.C., Mingoti, R.D., Bettero, V.P., Benevento, B.C., Vilela, F.G., Rennó, F.P., 2016. High levels of whole raw soya beans in dairy cow diets: digestibility and animal performance. Journal of Animal Physiology and Animal Nutrition, 100 (6), 1179–1190. https://doi.org/10.1111/jpn.12406

Broderick, G.A. and Kang, J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. Journal of Dairy Science, 63, 64–75.

Bulcão, L.F.A., Alba, H.D.R., Carvalho, G.G.P., Araújo, M.L.G.M.L., Gandra, J.R., Ribeiro, C.V.D.M., Freitas Júnior, J.E., 2021. Digestion, ruminal metabolism, and feeding behavior of buffaloes fed diets supplemented with soybean oil, whole and raw soybean, and calcium salts of fatty acids. Tropical Animal Health and Production, 53(2), 216-225. https://doi.org/10.1007/s11250-021-02654-x

Casali, A. O., Detmann, E., Valadares Filho, S. C., Pereira, J.C., Henriques, L.T., Freitas, S.G., Paulino, M.F., 2008. Influência do tempo de incubação e do tamanho de partículas sobre os teores de compostos indigestíveis em alimentos e fezes bovinas obtidos por procedimentos in situ. Revista Brasileira de Zootecnia, 37(2), 335-342. https://doi.org/10.1590/S1516-35982008000200021

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 technical details, Aberdeen, UK: International Feed Research Unit; Rowett Research Institute, 21 p.
Chesini, R.G., Takiya, C.S., Dias, M.S.S., Silva, T.B.P., Nunes, A.T., Grigoletto, N.T.S., Silva, G.G., Vittorazzi Jr., P.C., Rennó, L.N., Rennó, F.P., 2022. Dietary replacement of soybean meal with heat-treated soybean meal or high-protein corn distillers grains on nutrient digestibility and milk composition in mid-lactation cows. Journal of Dairy Science, 106(1), 233–244. https://doi.org/10.3168/jds.2022-21904
Cônsolo, N.R.B., Gandra, J.R., Gardinal, R., Freitas Júnior, J.E., Takiya, C.S., Rennó, F.P., Pereira, A.S.C., 2017. Effect of different dietary inclusion levels of whole raw soyabean on ruminal fermentation and nutrient utilization in Nelore steers. Journal of Animal and Feed Sciences, 26 (1), 311–318. https://doi.org/10.22358/jafs/80904/2017
Erwin, E. S.; Marco, G. J.; Emery, E. M., 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. Journal of Dairy Science, 44(9), 1768-1777. https://doi.org/10.3168/jds.S0022-0302(61)89956-6
Ferreira, H.C., Carvalho, C.C.S., Monção, F.P., Rocha Júnior, V.R., Ruas, J.R.M., Costa, M.D., Jesus, M.A., Gonçalves, M.C.M., Rocha, H.C.M., Ribas, W.F.G., Delvaux Júnior, N.A., Chamone, J.M.A., Rigueira, J.P.S., 2021. Effect of shading strategies on intake, digestibility, respiratory rate, feeding behaviour, and performance of feedlot-finished Nellore bulls in the semi-arid region of Brazil. Italian Journal of Animal Science, 20(1), 1759–1769. https://doi.org/10.1080/1828051X.2021.1912662
Fujihara, T., Orckov, E.R., Reeds, P.J., Kyle, D.J., 1987. The effect of protein infusion on urinary excretion of purine derivates in ruminants nourished by intragastric nutrition. Journal of Agricultural Science, 109(1), 7–12. https://doi.org/10.1017/S0021859600080916
Harvatine, K. J., Allen, M. S., 2006. Effects of fatty acid supplements on milk yield and energy balance of lactating dairy cows. Journal of Dairy Science, 89(3), 1081-1091. https://doi.org/10.3168/jds.S0022-0302(06)72176-2
Ibrahim, N.A., Alimon, A.R., Yaakub, H., Samsudin, A.A., Candyrine, S.C.L., Mohamed, W.N.W., Noh, A.M., Fuat, M.A., Mookiah, S., 2021. Effects of vegetable oil supplementation on rumen fermentation and microbial population in ruminant: a review. Tropical Animal Health and Production, 53(4), 1-11. https://doi.org/10.1007/s11250-021-02863-4
Macedo, F.L., Batistel, F., de Souza, J., Chagas, L.J. and Santos, F.A.P., 2016. Supplementation with Ca salts of soybean oil interacts with concentrate level in grazing dairy cows: intake, ingestive behavior, and ruminal parameters. Tropical Animal Health and Production, 48(8), 1593–1598. https://doi.org/10.1007/s11250-016-1132-4
Melo, H.S.A., Ítavo, L.C.V., Castro, A.P., Ítavo, C.C.B.F., Caldas, R.A., Mateus, R.G., Niwa, M.V.G., Moraes, G.J., Zornitta, C.S., Gurgel, A.L.C., Benchaar, C., 2023. Effect of whole oilseeds in the diet on bacterial diversity in the solid fraction of the ruminal content of steers. Tropical Animal Health and Production, 55(1), 32-43. https://doi.org/10.1007/s11250-022-03442-x
Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fibre in feeds with refluxing beakers or crucibles: collaborative study. Journal of AOAC International, 85(6), 1217–1240.
National Research Council-NRC. 2016. Nutrient Requirement of dairy beef cattle, 8 ed. Washington: National Academic Press, 244 p.
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. https://doi.org/10.3168/jds.S0022-0302(88)79781-7
National Research Council-NRC. 2001. Nutrient requirements of dairy cattle, 7, ed, Washinton, D.C. National Academic Press. 381p.
Oba, M., Allen, M.S., 2003. Effects of corn grain conservation method on ruminal digestion kinetics for lactating dairy cows at two dietary starch concentrations. Journal of Dairy Science, 86(1), 184–194. https://doi.org/10.3168/jds.S0022-0302(03)73599-1
Palmquist, D. L., 1991. Influence of source and amount of dietary fat on digestibility in lactating cows. Journal of Dairy Science, 74(4), 1354–1360. https://doi.org/10.3168/jds.S0022-0302(91)78290-8
Rennó, F.P., Freitas Junior, J.E., Gandra, J.R., Maturana Filho, M., Verdurico, L.C., Rennó, L.N., Barletta, R.V., Vilela, F.G., 2014. Effect of unsaturated fatty acid supplementation on digestion, metabolism and nutrient balance in dairy cows during the transition period and early lactation. Revista Brasileira de Zootecnia, 43(4), 212-223. https://doi.org/10.1590/S1516-35982014000400008
Rocha, C., Durau, J.F., Barrilli, L.N.E., Dahlke, F., Maiorka, A., Maiorka, P., 2014. The effect of raw and roasted soybean son intestinal health, diet digestibility, and pancreas weight of broilers. Journal of Applied Poultry Research, 23(1), 71-79. https://doi.org/10.3382/japr.2013-00829
Vellini, B.L., Prados, L.F., Monção, F.P., Fireman, A.K., Resende, F.D., Siqueira, G.R., 2020. Zinc amino acid complex in association with chromium methionine improves the feed efficiency of finished Nellore cattle in the feedlot. Animal Feed Science and Technology, 262:1-15. https://doi.org/10.1016/j.anifeedsci.2020.114430
Verbic, J., Chen, X.B., MacLeod, N.A., Ørskov, E.R., 1990. Excretion of purine derivatives by ruminants. Effect of microbial nucleic acid infusion on purine derivative excretion by steers. The Journal of Agricultural Science, 114(3), 243-248. https://doi.org/10.1017/S0021859600072610
Waldo, D.R., Smith, L.W., Cox, E.L., 1972. Model of cellulose disappearance from the rumen. Journal of Dairy Science, 55(1), 125-129. https://doi.org/10.3168/jds.S0022-0302(72)85442-0
Yan T., Frost J.P., Agnew R.E., Binnie R.C., Mayne C.S., 2006. Relationships among manure nitrogen output and dietary and animal factors in lactating dairy cows. Journal of Dairy Science, 89(10), 3981–3991, https://doi.org/10.3168/jds.S0022-0302(06)72441-9