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REGULAR ARTICLES

Impact of replacing protein pellets with soybean grain on nutrient utilization and the rumen and blood parameters of feedlot cattle under tropical conditions

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Abstract
The aim of this study was to explore the effect of replacing protein pellets with soybean grain in high-concentrate diets with or without the addition of silage, on the intake, digestibility, and rumen and blood parameters of feedlot cattle in tropical regions. Four cannulated, crossbred steers were used, 4.5 ± 0.5 years old, with an average weight of 685.55 ± 111.78 kg. The steers were distributed in a 4×4 Latin square, in a 2×2 factorial scheme (two sources of protein: protein pellets or whole soybean grain, with or without added dietary bulk). There was no effect ($P \geq 0.109$) from the interaction between the source of protein and the addition of silage to the diet on dry matter (DM) and nutrient intake, or the digestibility ($P \geq 0.625$) of DM or crude protein (CP). However, both factors affected ($P \leq 0.052$) the intake of DM, neutral detergent fiber (NDF), and non-fiber carbohydrates (NFC), as well as the independent digestibility ($P \leq 0.099$) of fat, NFC, total carbohydrates (TC), and total cholesterol concentration. There was an effect ($P \leq 0.053$) from the interaction between the source of protein and the addition of silage to the diet on the digestibility of NDF and total digestible nutrients (TDN), as well as on the glycose concentration ($P = 0.003$). Blood parameters (i.e. protein, albumin, creatinine, triglycerides, aspartate aminotransferase (AST), and alanine aminotransferase (ALT)) were not affected ($P \geq 0.139$) by the source of protein, the addition of silage, or their interaction. Lastly, including 150 g/kg silage DM in a high-grain diet, and using soybean grain as a source of protein in substitution of protein pellet could be a suitable nutritional strategy to ensure adequate DM and nutrient intake and digestibility, with no detrimental effects on rumen and blood parameters of feedlot cattle in the tropics.

Keywords Blood parameter · Crossbred steer · Digestibility · High-concentrate diet · Protein replacement

Introduction

The production of ruminants can be achieved by optimizing the diet, evaluating and replacing the ingredients based on nutrient utilization and animal performance (Theodoridou and Yu 2013), through evaluating and replacing feedstuffs in the diet on the basis of nutrient utilization and performance of animals (Halmemies-Beauchet-Filleau et al. 2018). This is particularly relevant for finishing cattle in confinement systems, especially in tropical countries, where the quality (e.g. nutritional quality) and quantity (e.g. growth rate) of forage can vary due to climate changes throughout the year (Palma et al. 2023).

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Diets for feedlot cattle are mostly formulated to include a higher proportion of concentrate in relation to bulk, with the former containing a high proportion of grain (Wagner et al. 2014; Marques et al. 2024). Such diets are known as high-grain diets and are generally formulated with corn grain and protein pellets as sources of energy and protein, with the aim of promoting maximum individual weight gain, reducing slaughter time, standardizing batches, reducing labor, and with other beneficial effects on the meat production chain (Paula et al. 2019; Pouzo et al. 2023). Nevertheless, the use of protein pellets as source of protein in high-grain diets can increase the cost of producing beef cattle.

To overcome this limitation, tropical countries are exploring alternative, lower-cost sources of proteins to replace protein pellets in the diet based on their climate conditions (Almeida et al. 2018; Marques et al. 2024). Soybean grain would be a suitable substitute for protein pellets in Brazil, considering the similar nutritional characteristics of both feedstuffs. Additionally, the use of soybean grain would be advantageous from an economic point of view, considering the 50% increase in production of this feedstuff in Brazil over the last 10 years. For example, in Brazil, which produced around 150 million metric tons of soybean grain in 2023 (FAOStat 2023), 1 kg of protein pellets can be found for USD 1.033, whereas soybean grain is sold for USD 0.490 per kg (CEPEA 2023).

This suggests that replacing protein pellets with soybean grain as a source of protein in the diet of feedlot cattle may be interesting from an economic and nutritional point of view, especially in tropical countries with high soybean production. Nevertheless, the effects of replacing protein pellets with soybean grain on nutrient utilization and blood parameters in feedlot cattle are still not completely understood, and may restrict the interchange of these sources of protein in cattle production. The hypothesis of this study was that replacing protein pellets with soybean grain, regardless of additional fiber, can enhance nutrient use with no harmful effects on rumen or blood parameters in feedlot cattle. The aim of this study, therefore, was to evaluate the effect of replacing protein pellets with soybean grain in high-concentrate diets with or without the addition of bulk, on the intake, digestibility, and rumen and blood parameters of feedlot cattle in tropical regions.

Materials and methods

Location of study

The experiment was conducted at the beef-cattle station of the Federal University of Tocantins in the district of Araguaína, Tocantins, Brazil (7º12’28” S; 48º12’26” W), from September to December 2018, and lasted for 80 days. The average relative humidity and air temperature during the experiment were 74.7% and 27.4 °C, respectively.

Animals and experimental design

Four cannulated, crossbred steers were used, 4.5 ± 0.5 years old, with an average weight of 685.55 ± 111.78 kg. The steers were distributed in a 4 × 4 Latin square, in a 2 × 2 factorial scheme (two sources of protein: protein pellets or whole soybean grain, with or without added dietary bulk). The experiment consisted of four experimental periods of 20 days (15 days adaptation and 5 days of data collection) to evaluate nutrient utilization and rumen and blood parameters.

The animals were submitted to four treatments: (I) protein pellets + whole corn grain (15% and 85%, respectively), 100% concentrate diet; (II) protein pellets + whole corn grain (15% and 85%, respectively) + Mombasa grass (*Panicum maximum*) silage, diet with 85% concentrate and 15% bulk; (III) whole soybean grain + whole corn grain (15% and 85%, respectively), 100% concentrate diet; (IV) whole soybean grain + whole corn grain (15% and 85%, respectively) + Mombasa grass silage, diet with 85% concentrate and 15% bulk. The composition of the ingredients and the experimental diets is shown in Tables 1 and 2.

Commercial protein pellets, 5.5 mm in diameter, were added to the diets. The sources of protein were wheat bran, cottonseed meal, soybean meal, and urea with a maximum of 180 g/kg non-protein nitrogen. The composition of the protein pellets is shown in Table 1. Mineral supplement was used at a daily dose of 90 g per animal, while 10 g V-Max®2 (Phibro, São Paulo, Brazil) per animal per day (equal to 200 mg virginiamycin per animal per day) was added to the diets with soybean grain as the source of protein to meet mineral requirements and provide a similar quantity of supplements in each of the diets.

Data collection and recorded parameters

Before starting the experiment, the animals were weighed, treated against endo- and ectoparasites, and identified using a numbered ear tag. The diets were offered twice a day, at 8 am and 4 pm in individual feeders located in each covered pen (12 m^2) with concrete floor, and water was available ad libitum. Any leftovers were collected and weighed daily, always in the morning before feeding; the amount offered was adjusted to allow for 5% leftovers.

From day 16 to day 20, samples of the diets and leftovers, as well as the feces (200 g per animal), were collected daily to determine intake and the apparent digestibility of

Table 1 Chemical composition of the ingredients fed to the animals during the experiment

Item [1]	Ingredient (g/kg DM) [2]			
	CG	PP [3]	SBG	Silage
DM (as feed)	873.3	882.4	904.3	324.3
CP	73.8	346.4	320.0	41.5
NDFap	93.80	201.2	142.6	734.9
ADF	72.3	126.0	76.4	410.6
NDIP	3.6	5.2	22.4	15.6
ADIP	1.1	1.4	2.1	0.6
Hemicellulose	26.4	132.2	80.6	336.6
Cellulose	63.5	75.5	52.10	248.7
Fat	33.1	16.0	238.8	17.3
Lignin	13.8	14.4	49.7	91.1
Ash	14.6	35.8	53.7	84.6
TC	878.4	601.6	387.4	856.6
NFC	759.0	393.9	200.5	65.6
TDN	775.3	411.2	1014.2	471.2

[1] DM = Dry matter; CP = Crude protein; NDFap = Neutral detergent fiber corrected for ash and protein; ADF = Acid detergent fiber; NDIP = Neutral detergent insoluble protein; ADIP = Acid detergent insoluble protein; TC = Total carbohydrates; NFC = Non-fiber carbohydrates; TDN = Total digestible nutrients

[2] CG = Corn grain; PP = Protein pellets; SBG = Soybean grain

[3] Ingredients and chemical composition of PP: 165 g/kg DM wheat bran; 110 g/kg DM cotton seed meal; 320 g/kg DM soybean meal; 60 g/kg DM urea; 180 mg Virginiamycin/kg; 25 g Ca/kg; 9 g P/kg; 4.5 g S/kg; 9.5 g Na/kg; 3 g Mg/kg; 200 mg Zn/kg; 130 mg Mn/kg; 30 mg K/kg; 65 mg Cu/kg; 6 mg Co/kg; 3.5 mg I/kg; 1.4 mg Cr/kg; 0.7 mg Se/kg; 40 mg F /kg; 220 g/kg mineral matter; 15,000 IU Vitamin A; 3,000 IU Vitamin D; 120 IU Vitamin E

Table 2 Proportion of ingredients and composition of the experimental diets

Ingredient (g/kg DM) [1]	PP		SBG	
	With Silage	No Silage	With Silage	No Silage
CG	725.0	850.0	725.0	850.0
SBG	-	-	125.0	150.0
PP	125.0	150.0	-	-
Silage	150.0	-	150.0	-
	Chemical composition (g/kg DM) [2, 3]			
DM (as feed)	792.1	874.7	794.8	878.0
CP	112.3	114.7	109.0	110.7
NDFap	203.4	109.9	196.1	101.1
ADF	129.8	80.4	123.6	72.9
Fat	29.7	30.6	56.4	64.0
NDIP	6.1	4.9	8.1	18.8
ADIP	1.2	1.4	1.2	1.2
NFC	609.4	704.2	585.2	675.2
Hemicellulose	86.2	42.3	79.7	34.5
Fat	29.7	30.6	56.4	64.0
Ash	27.8	17.8	30.0	20.5
TC	840.5	836.9	813.8	804.8
TDN	684.2	720.7	759.5	811.1

[1] SP = Source of protein; PP = Protein pellets; SBG = Soybean grain; CG = Corn grain

[2] DM = Dry matter; CP = Crude protein; NDFap = Neutral detergent fiber corrected for ash and protein; ADF = Acid detergent fiber; NDIP = Neutral detergent insoluble protein; ADIP = Acid detergent insoluble protein; TC = Total carbohydrates; NFC = Non-fiber carbohydrates; TDN = Total digestible nutrients

[3] The chemical composition was analyzed as per the recommendations of the AOAC (2005) and Van Soest et al. (1991)

the diets. Rumen fluid was collected on day 19, and blood samples on day 20. The animals were then weighed.

The fecal samples were frozen and homogenized, and then pre-dried in a forced air ventilation oven (Model: TMMA035/5, Trammit Medical, Belo Horizonte, Minas Gerais, Brazil) at 55 °C, ground in a knife mill, using 1 mm sieves for the chemical analysis, and 2 mm sieves to determine digestibility. The total fecal production was determined using titanium dioxide (TiO_2) external marker at a daily dose of 10 g per animal. The marker was given over 11 consecutive days, with three days for collection, and was infused via the ruminal cannula. Feces were collected on days 16 to 18 of each period, directly from the rectum of the animal, and the samples stored in duly identified plastic bags.

Fecal production was determined using the following equation:

$$\text{Fecal production (kg/DM/day)} = (\text{marker intake}/\text{proportion of marker in feces}) \times 100$$

Apparent nutrient digestibility of was calculated as follows:

$$\text{Apparent digestibility} = 1 - [(\text{nutrient intake} - \text{excreted nutrients}) / (\text{nutrient intake})]$$

Samples of feed, leftovers, and feces were analyzed for dry matter (DM - Method: 930.15), ash (Method: 923.03), crude protein (CP - Method: 990.03), and ether extract (EE - Method: 945.38) in accordance with the AOAC (2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined as per the recommendations of Van Soest et al. (1991). Total carbohydrates (TC) and non-fiber carbohydrates (NFC) were determined using the following equations (Sniffen et al. 1992):

$$\%TC = 100 - (\%CP + \%EE + \%ash)$$

$$\%NFC = 100 - (\%CP + \%EE + \%ash + \%NDF)$$

Samples of rumen fluid were collected via the rumen cannula to determine changes in rumen pH and ammonia nitrogen (NH_3-N); four collections were made: 0, 2, 4 and 8 h after the morning feed. Fifty mL of rumen fluid were collected, filtered through a triple-layered cheesecloth, and analyzed for pH using a calibrated digital potentiometer (Model: LAB PHP, Lab Import, Piracicaba, SP, Brazil). A 50 mL aliquot was sent to the laboratory for NH_3-N analysis using the Chaney and Marbach (1962) colorimetric method.

To determine the blood parameters, blood samples were collected from each animal by puncturing the jugular vein using vacuum tubes (Vacutainer®, São Paulo, SP, Brazil) immediately before the morning feed. The blood samples were refrigerated and transported to the Biochemistry Laboratory of the Postgraduate Program in Animal Science (PPGCat) at UFT, where they were centrifuged at 4,000 × *g* for 20 min to separate the plasma and serum. They were then packed in plastic tubes (Eppendorf®, Hamburg, Germany) and frozen at -20ºC for analysis. The following blood parameters were analyzed: triglycerides, total cholesterol, total protein, urea, albumin, aspartate aminotrasferase (AST), alanine transaminase (ALT), creatinine and glucose, using a Bio-2000 IL-A automatic biochemical analyzer (Bioplus®, Barueri, SP, Brazil) and commercially available diagnostic kits (Labtest Diagnóstica® S.A., Lagoa Santa, MG, Brazil).

Cost assessment

A cost assessment of the feedlot diets was made to suggest the most economical nutritional strategy, as animal performance was not determined in the experiment. Market prices in the state of Tocantins were used to determine the price of the ingredients, with all the values expressed in USD (USD 1 = BRL 4.072). The price of corn grain per kilogram was USD 0.15, while that of protein pellets and soybean grain was USD 0.49 and USD 0.33, respectively. The cost of the mineral and V-Max supplements were USD 0.67 and USD 5.41 per kilogram. The daily cost of supplementing the diets with the minerals and V-Max was USD 0.05 and USD 0.06, respectively. The cost of the Mombasa grass silage was USD 170.00 per ton of DM. The above values were used to determine the economic indicators of the diet, as follows:
Cost of the protein pellet concentrate (USD/kg) = (0.85 × price of corn grain) + (0.15 × price of protein pellets).

Cost of the soybean-grain concentrate (USD/kg) = (0.85 × price of corn grain) + (0.15 × price of soybean grain).

Cost of the protein pellet and silage diet (USD/kg) = (0.725 × price of corn grain) + (0.125 × price of protein pellets) + (0.15 × price of silage).

Cost of the soybean grain and silage diet (USD/kg) = (0.725 × price of corn grain) + (0.125 × price of soybean grain) + (0.15 × price of silage).

Daily cost of the protein-pellet diets (with and without the addition of silage, USD/animal/day) = DM Intake × cost of the protein-pellet and silage diet (with added silage), or cost of the protein-pellet and concentrate diet (with no added silage).

Daily cost of the soybean-grain diets (with or without the addition of silage, USD/animal/day) = (DM Intake × cost of the soybean-grain and silage diet (with added silage) or cost of the soybean-grain concentrate (with no added silage) + daily cost of the mineral supplement + daily cost of the V-Max 2.

TDN cost of the diet (USD/kg TDN) = (Cost of the diet in USD/kg × 1,000 g)/TDN in the diet in g/kg.

CP cost of the diet (USD/kg CP) = (Cost of the diet in USD/kg × 1,000 g)/CP in the diet in g/kg.

Statistical analysis

The statistical analyses were carried out using the SISVAR® v5.6 software (Ferreira 2011). Intake, digestibility and blood parameters were analyzed for normality and homogeneity of variance across all the treatments using the Shapiro-Wilk and Barlett tests, respectively. The following mathematical model (Bate and Clark 2014) was used:

$$\gamma_{ijkl} = \mu + A_i + P_j + G_k + V_l + G_k \times V_1 + \varepsilon_{ijkl}$$

where γ_{ijkl} = dependent variable for animal *i*, in period *j*, receiving a treatment comprising factors *k* and *l*; μ = overall mean; A_i = effect of animal *i* (random); P_j = effect of period *j* (random); G_k = effect of factor k (fixed); V_l = effect of factor l (fixed); $G_k \times V_l$ = effect of the interaction between factors *k* and *l*; ε_{ijkl} = residual experimental error.

To determine pH and NH_3-N in the rumen fluid, the experimental plots were split based on the sampling times and analyzed using a repeated-measure design. The assumptions of normal distribution and homoscedasticity were checked. The pH and NH_3-N data did not meet the criteria for normality or homoscedasticity and were transformed by $\sqrt{x+1}$ for data analysis. The effects of the treatments, collection time after feeding (hours), and the treatment × collection time interaction were tested. The significance level was set at $P < 0.100$.

Results

Intake

There was no significant interaction between the source of protein (protein pellets or whole soybean grain) and the addition of bulk to the diet (i.e., with or with no added bulk) on DM or nutrient intake (Table 3; $P \geq 0.109$); there was, however, an independent effect from the source of protein ($P \leq 0.042$) and the addition of bulk ($P \leq 0.052$) on DM, NDF, and NFC intake, when the variables were expressed in kg/animal/day (Table 3).

The DM intake of the animals fed protein pellets (13.58 kg/animal/day) was greater than in those fed soybean grain (9.91 kg/animal/day), regardless of added bulk (Table 3). Similarly, the DM intake of animals with silage in their diet (13.47 kg/animal/day) was greater than for animals with no added silage (10.02 kg/animal/day), whether with or without a source of protein (Table 3).

Animals with added silage in their diet had a higher NDF intake (5.95 kg/animal/day) than animals with no added silage (3.10 kg/animal/day), both with and without a source of protein (Table 3). The data showed that animals fed protein pellets had a higher NFC intake (6.41 kg/animal/day) than animals fed soybean grain (4.97 kg/animal/day), regardless of added silage. Similarly, the NFC intake of animals with added silage in their diet (7.63 kg/animal/day) was greater than that in animals with no added silage (3.76 kg/animal/day), whether with or without a source of protein (Table 3). In general, neither a source of protein or added bulk, nor their interaction affected the CP, fat, or TDN intake (Table 3; $P \geq 0.156$).

Digestibility

There was an effect ($P \leq 0.053$) from the interaction between the source of protein and added bulk on the digestibility of NDF and TDN (Table 4). Animals fed protein pellets or soybean grain with added bulk in their diet (832.1 and 826.0 g/kg DM, respectively) had the highest values for NDF digestibility among the treatments under evaluation. Additionally, animals fed protein pellets with added bulk (737.5 g/kg DM), as well as animals fed soybean grain with no added bulk (946.5 g/kg DM) had the greatest values for TDN digestibility among the experimental diets.

The digestibility of fat, NFC, and TC was independently affected ($P \leq 0.099$) by the source of protein and the addition of bulk to the diet (Table 4). Animals fed protein pellets (376.9 g/kg DM) presented lower fat digestibility than did the animals fed soybean grain (511.0 g/kg DM) regardless of added silage (Table 4). Similarly, animals fed silage in their diet showed lower NFC and TC digestibility (627.5 and 579.9 g/kg DM, respectively) than did animals with no added silage (726.5 and 680.4 g/kg DM, respectively), whether with or without a source of protein (Table 4). Finally, DM and CP digestibility were not affected ($P \geq 0.504$) by the source of protein, the addition of bulk, or their interaction (Table 4).

Rumen and blood parameters

The data showed that there was no effect on the rumen parameters of the cattle from the incubation time, source of protein or the addition of bulk, nor from the independent interaction between the main factors and the period ($P \geq 0.101$; Table 5). Neither pH nor NH_3-N changed significantly for the treatments or periods under evaluation, the pH and NH_3-N ranging from 5.75 to 6.22 and 3.88 to 6.46 mg/dL, respectively, over all of the treatments and periods.

For the blood parameters, there was an effect ($P = 0.003$) on the glucose concentration from the interaction between the source of protein and the addition of bulk (Table 6). Animals fed soybean grain and protein pellets, both with added bulk, showed the greatest and lowest values for glucose concentration, respectively (71.75 and 43.62 mg/dL).

The data suggested that the source of protein ($P = 0.053$) and the addition of bulk to the diet ($P = 0.090$) had an independent effect on the total cholesterol concentration (Table 6). Animals fed protein pellets had a lower total cholesterol concentration than those fed soybean grain (102.56 and 137.12 mg/dL), regardless of added silage (Table 6). On the other hand, animals fed silage had a higher total cholesterol concentration (134.37 mg/dL) than did those with no added silage (105.31 mg/dL), with or without a source of protein (Table 6). Finally, the additional blood parameters (protein, albumin, creatinine, triglycerides, AST, and ALS) were not affected ($P \geq 0.139$) by the source of protein, the addition of bulk, or their interaction (Table 6).

Cost assessment

Replacing the protein pellets with soybean grain reduced the cost of concentrate in the diet by 10% (Table 7). The daily cost of the diet with soybean grain was lower than that with protein pellets as the source of protein (USD 1.87 vs. USD 2.72 per kg). In addition, diets with no added silage had a lower daily cost per animal than when silage was included (USD 2.00 vs. USD 2.59 per kg). When using soybean grain as the source of protein, the TDN and CP costs of the diet went down by 21% and 8%, respectively, regardless of the addition of silage.

Table 3 DM and nutrient intake in cattle fed diets containing different sources of protein (protein pellet or soybean grain) with or without added silage [1]

Variable	SP [1]				Factor mean value				SEM	*P*-value		
	PP		SBG		PP	SBG	With Silage	No Silage				
	SI											
	With Silage	**No Silage**	**With Silage**	**No Silage**						**SP**	**SI**	**SP × SI**
DM intake [2]												
kg/animal/day	14.66	12.49	12.28	7.54	13.58	9.91	13.47	10.02	1.00	0.042	0.052	0.402
g/kg BW/d	20.92	16.44	17.58	11.69	18.68	14.64	19.25	14.06	1.51	0.106	0.517	0.748
CP intake [2]												
kg/animal/day	1.55	1.46	1.25	0.94	1.51	1.10	1.40	1.20	0.18	0.156	0.452	0.688
% BW	0.22	0.19	0.18	0.15	0.21	0.17	0.20	0.17	0.02	0.285	0.445	0.956
NDF intake [2]												
kg/animal/day	6.14	3.66	5.76	2.53	4.90	4.15	5.95	3.10	0.38	0.212	0.002	0.514
% BW	0.87	0.48	0.81	0.38	0.68	0,60	0.84	0.43	0.043	0.273	0.001	0.776
NFC intake [2]												
kg/animal/day	7.94	4.88	7.31	2.63	6.41	4.97	7.63	3.76	0.30	0.016	<0.001	0.109
% BW	1.13	0.64	1.06	0.40	0.89	0.73	1.10	0.52	0.068	0.158	0.001	0.439
Fat intake [2]												
kg/animal/day	0.23	0.31	0.22	0.20	0.27	0.21	0.23	0.26	0.032	0.236	0.573	0.323
% BW	0.034	0.041	0.033	0.032	0.038	0.033	0.034	0.037	0.004	0.479	0.666	0.545
TDN intake [2]												
kg/animal/day	10.77	8.32	8.06	7.68	9.55	7.87	9.42	8.00	1.09	0.328	0.403	0.534

[1] SP = Source of protein; PP = Protein pellets; SBG = Soybean grain; SI = Silage; SP × SI = Interaction between SP and SI.

[2] DM = Dry matter; CP = Crude protein; NDF = Neutral detergent fiber; NFC = Non-fiber carbohydrates; TDN = Total digestible nutrients

Discussion

There was no effect from the interaction between the source of protein and the addition of silage on DM or nutrient intake. However, when the DM intake was expressed in kg/animal/day, it was independently affected by both factors, resulting in a DM intake 27% lower for animals fed soybean grain compared to those fed protein pellets, both with and without added silage. This might have been due to the soybeans, which had a higher fat content compared to the protein pellets, regardless of added silage, corroborating the well-known negative effects of increasing dietary fat on DM use in cattle (Patra 2013). Furthermore, similar results were found by Barletta et al. (2016) and Cônsolo et al. (2017), who also saw a reduction in DM intake for an increase in the amount of soybean grain added to the diet of lactating cows and Nellore steers, respectively.

Another possible explanation for the reduction in DM intake in animals fed soybean diets might be the higher fat content of the soybeans. This may increase the levels of energy metabolites in the blood, activating the satiety center in the hypothalamus, which inhibits hunger and reduces DM intake (Baile and Forbes 1974; Roche et al. 2008). This explains why animals offered diets that include silage have a 26% greater DM intake than those without added silage and suggests that the animals increase their DM intake to meet their nutritional requirements, considering that silage is a poor source of dietary energy (Galyean and Goetsch 2015; Vargas and Mezzomo 2023). The data therefore imply that both factors have an independent, albeit complementary, effect on DM intake.

There were no effects from the interaction between the source of protein and addition of silage or from the two factors independently on the CP intake of the cattle. It should be noted that diets were formulated to be isonitrogenous to test the effects of the protein sources; as such, the treatments were expected to have no effect on CP intake. Nevertheless, although the source of protein and addition of silage affected DM intake, it is possible that due to the animal selecting protein feedstuffs in the diet, the CP intake remains statistically similar, considering the known relationship between feed intake and dietary constituents in ruminants (Riaz et al. 2014).

The higher NDF intake of the animals with silage in their diet, regardless of the source of protein, can be explained by the greater NDF content (199.8 g/kg DM) of the diets containing silage than of the diets with no silage (105.5 g/kg DM). Here, it should be noted that the aim of exploring the addition of silage in the diet was to evaluate the effects on DM and nutrient intake of changing the physically effective fiber fraction when the two sources of protein were interchanged. The results showed that protein pellets and soybean grain could be interchanged as sources of protein regardless of changes in the physically effective fiber fraction in the diet. This may be of interest to livestock producers considering the role of that dietary component in rumen nutrient fermentation (Zebeli et al. 2012). However, to confirm this assumption, further studies are necessary to examine different levels of the physically effective fiber fraction in cattle diets.

The data showed that the source of protein and the addition of silage independently affected the NFC intake. This result was similar to that of Arroquy et al. (2004), who found no effects from the interaction between the type of dietary NFC and source of degradable rumen protein on forage intake and digestion in beef cattle; the data also showed that each factor acted independently. Furthermore, the independent effects of the source of protein and the addition of silage on NFC intake corroborate the results of various studies that have also demonstrated the effect of the NFC concentration in the diet on N metabolism (Mansfield et al. 1994; Ma et al. 2015), the activity of chewing, the rumen environment, retention time or passage rate, and performance in ruminants (Poorkasegaran and Yansari 2014).

DM and CP digestibility were not affected by the interaction between the source of protein and the addition of silage, nor by the two factors independently, showing similar DM use by the cattle for all diets. The present data were similar to those obtained by Santana et al. (2015), who saw no effects from the addition of whole corn grain with no added forage on DM digestibility in bulls in confinement. These findings are consistent with those of Bassi et al. (2012) and Naves et al. (2016), who also found no effects from the addition of soybean grain on nutrient use in cattle.

Evaluating the effects of the source of protein and addition of silage on nutrient digestibility showed a significant effect from the interaction between these factors on NDF digestibility. The responses for NDF digestibility differed from those seen by Bassi et al. (2012), who found no difference when varying levels of soybean grain were included in the diet of zebu steers. Similarly, Naves et al. (2016) found no difference in fiber digestion when whole soybeans of varying sizes were included in the diet of dairy cattle. The data suggests that each source of protein has a different effect on NDF use in feedlot beef cattle. This information could be valuable when formulating diets considering the impact of fiber on rumen fermentation in beef cattle (Sousa et al. 2017).

According to Patra and Yu (2013), the addition of dietary lipids can have a negative effect on NDF digestibility. However, this effect was not seen in the present study due to the low fat content of the diets (~45.1 g/kg DM), which is lower than the range (from 60 to 70 g fat/kg DM) suggested as having a detrimental effect on gram-positive

Table 4 Dry matter and nutrient digestibility in cattle fed diets containing different sources of protein (protein pellets or soybean grain) with or without added silage

Digestibility (g/kg)[1]	SP[2]				Factor mean value				SEM	P-value[3]		
	PP		SBG		PP	SBG	With Silage	No Silage		SP	SI	SP × SI
	SI											
	With Silage	No Silage	With Silage	No Silage								
DM	542.1	536.7	572.0	647.0	539.4	609.5	557.1	591.9	0.67	0.504	0.746	0.667
CP	503.5	433.5	491.0	538.7	468.5	514.9	497.3	486.1	0.80	0.699	0.926	0.625
NDF	832.1	614.2	826.0	738.0	723.2	782.0	829.1	676.1	0.19	0.096	0.002	0.053
Fat	413.2	340.5	441.0	581.0	376.9	511.0	427.1	460.8	0.48	0.099	0.643	0.173
NFC	552.2	702.7	570.5	882.5	627.5	726.5	561.4	792.6	0.48	0.197	0.015	0.282
TC	549.2	610.5	579.0	781.7	579.9	680.4	564.1	696.1	0.43	0.157	0.078	0.298
TDN	737.5	633.8	661.1	946.5	685.7	803.8	699.3	790.2	40.7	0.086	0.166	0.015

[1] DM = Dry matter; CP = Crude protein; NDF = Neutral detergent fiber; NFC = Non-fiber carbohydrates; TC = Total carbohydrates; TDN = Total digestible nutrients

[2] SP = Source of protein; PP = Protein pellets; SBG = Soybean grain; SI = Silage.

[3] SP × SI = Interaction between SP and SI

bacteria or the waterproof coating of the fiber (Sullivan et al. 2004). Another possible explanation for the patterns of NDF digestibility in the soybean diets could be linked to the soybeans being offered as whole grain, which may have reduced the effect of fat on NDF digestibility due to lipid degradation occurring more slowly in the rumen environment (Nagaraja 2016).

When soybeans were included in the silage-based diets, fat digestibility was greater compared to using protein pellets as the source of protein. These results are consistent with those reported by Naves et al. (2016), who also found an increase in fat digestibility in cattle fed diets that included soybean grain compared to diets with no added soybeans. Furthermore, the data on fat digestibility were consistent with those for TDN digestibility, as a higher value for fat digestibility was seen in diets containing whole soybean grain compared to those containing protein pellets. This agrees with the already known nutritional relationship between fat and TDN in ruminant diets (Barth et al. 1959).

NFC and TC digestibility were influenced by the addition of silage but not the addition of a source of protein. These results are consistent with the concentration of nutritional components in the grain being greater than in the silage, since the silage includes nutritional components that are poorly digested or non-digestible, while the grain contains starch and hemicellulose as the principal carbohydrate components (Jayanegara et al. 2019), meaning that the addition of silage or a source of protein has a different effect on nutrient use.

The data showed that there was no effect from the interaction between added protein or silage and the period, nor any effect from the period on rumen pH or NH_3-N. These results are consistent with the fact that diets rich in NFC rapidly ferment in the rumen, producing high levels of volatile fatty acids (VFAs) and lactic acid, which lower the rumen pH (Owens et al. 1998). Subacute ruminal acidosis, defined as periods of moderately depressed rumen pH of around 5.5 to 5.0, is associated with laminitis and other health problems that result in reduced production (Krause and Oetzel 2006). The use of whole grains might therefore be a suitable strategy for improving the nutritional quality of the diet, as well as reducing the availability of rapidly fermentable carbohydrates. However, diets without silage addition had levels of effective fiber that were still below those recommended for cattle (20% DM; NRC 2015). Thus, inclusion of whole grains in this type of diets should be made with caution to avoid negative effects on animal production.

The values for rumen pH in the present study ranged from 5.60 to 6.31. In general, pH values from 5.9 to 7.0 help optimize the rate of rumen fermentation (Cônsolo et al. 2017), while the rapid fermentation of sugars and starch could lead to the increased accumulation of volatile fatty

Table 5 Evaluation of pH and NH3-N in the rumen fluid of cattle fed diets containing protein pellets or soybean grain with or without added silage at different times after feeding [1]

Time [1]	SP [2]				
	PP		SBG		SEM
	SI				
	With Silage	No Silage	With Silage	No Silage	
pH					
0	6.19	5.60	6.35	5.65	0.13
2	6.31	5.81	6.25	5.87	
4	5.62	6.03	6.15	5.85	
8	6.04	5.74	6.15	5.63	
Mean	6.04	5.79	6.22	5.75	
P-value [3]	SP	SI	Time	Time × SP	Time × SI
	0.750	0.101	0.182	0.851	0.630
NH_3-N (mg/dL) [4]					
0	4.78	7.02	5.02	7.18	0.37
2	7.71	6.87	4.18	7.90	
4	4.33	4.80	3.68	6.73	
8	1.95	5.43	2.67	4.04	
Mean	4.69	6.03	3.88	6.46	
P-value	SP	SI	Time	Time × SP	Time × SP
	0.922	0.403	0.154	0.548	0.838

[1] Sampling times: 0 = Immediately before feeding; 2 = two hours after feeding; 4 = Four hours after feeding; 8 = Eight hours after feeding

[2] SP = Source of protein; PP = Protein pellets; SBG = Soybean grain; SI = Silage.

[3] Time x SP = Interaction between time and SP; Time x SI = Interaction between time and SI.

[4] NH_3-N = Ammonia nitrogen

acids (VFAs) in the rumen, resulting in a higher production of propionate (Ribeiro et al. 2015). The addition of 150 g/kg bulk DM in the diet, in addition to reducing NFC intake, as seen in the present study, possibly influenced rumination activity in the animals. Among other factors, the salivary process helps to regulate rumen pH (Dijkstra et al. 2012), as chewing promotes increased saliva with a greater production of saliva buffer (Weiss et al. 2017).

The average value for NH_3-N was 5.26 mg/dL. An adequate concentration of NH_3-N in the rumen is essential for the activity of the rumen microbiome, especially cellulolytic bacteria that use ammonia for microbial protein synthesis (Marini and Van Amburgh 2005). The values for the NH_3-N concentration found in the present study were sufficient to ensure minimal bacterial growth as they were consistent with the minimum value (5 mg/dL) for NH_3-N reported by Satter and Slyter (1974). It is important to note that NH_3-N concentrations in the rumen are also directly related to the speed of N release from the feed in the rumen (Huntington and Archibeque 2000), as well as to N conversion into microbial protein (Pisulewski et al. 1981). The data suggest that replacing protein pellets with soybean grain, with or without the addition of silage, has no harmful effect on rumen pH or the NH_3-N concentration, possibly promoting suitable conditions for microbial growth. However, further studies to explore the effects of interchanging protein sources on the ecology and metabolism of the rumen microbiome in cattle are needed to confirm these assumptions.

The blood creatinine concentration indicates the production of phosphocreatine in the muscles (Bonilha et al. 2015). High levels of blood creatinine show a potential failure in renal function (Russell and Roussel 2007). In the present study, there was no difference in creatinine concentration between the diets, which suggests a similar effect on renal function between the treatments. However, further studies are necessary to confirm this assumption, since relying solely on plasma creatinine data is insufficient for a comprehensive understanding of renal functionality. (Zaitsev et al. 2020).

Serum triglyceride, AST, and ALT concentrations were also unaffected by changes in the source of protein or the addition of silage. The results suggest that all the diets afforded the animals similar protein energy and nutritional status with no hyperactivity of the liver. Meyer et al. (1992) suggest that elevated levels of AST and ALT in cattle may indicate the heightened hepatic metabolism of nutrients and potential liver damage. However, the AST and ALT levels found were lower than those suggested by various authors for cattle (Wood 2004; Yokus and Cakir 2006; Zaitsev et al. 2020).

The inclusion of soybean grain as a protein source resulted in an increase in total plasma cholesterol levels compared to the pellet-based diet. Additionally, animals fed silage had

Table 6 Blood parameters of cattle fed diets containing protein pellets or soybean grain with or without added silage [1]

Variable [1]	SP [2] PP		SBG		Factor mean value				SEM	*P*-value [3]		
	SI				PP	SBG	With Silage	No Silage		SP	SI	SP × SI
	With Silage	No Silage	With Silage	No Silage								
Pt (g/dL)	8.79	9.10	7.30	7.82	8.95	7.56	8.05	8.46	0.57	0.139	0.623	0.900
Alb (g/dL)	4.83	4.45	4.52	4.20	4.64	4.36	4.68	4.33	0.28	0.520	0.422	0.946
Crt (mg/dL)	0.27	0.25	0.20	0.20	0.26	0.20	0.24	0.23	0.03	0.249	0.807	0.807
Glc (mg/dL)	43.62	56.12	71.75	46.25	49.87	59.00	57.69	51.19	2.76	0.058	0.147	0.003
TCl (mg/dL)	119.12	86.00	149.62	124.62	102.56	137.12	134.37	105.31	10.18	0.053	0.090	0.787
Tgl (mg/dL)	16.75	26.12	20.25	19.25	21.44	19.75	18.50	22.69	1.94	0.561	0.178	0.108
AST (IU/L)	25.50	26.00	32.50	27.86	25.75	30.18	29.00	26.93	2.61	0.275	0.597	0.514
ALT (IU/L)	8.50	6.75	8.50	7.50	7.63	8.00	8.50	7.13	0.63	0.693	0.179	0.693
Urea (mg/dL)	31.37	21.00	20.25	23.25	26.19	21.75	25.81	22.13	5.06	0.558	0.625	0.387

[1] Pt = Protein; Alb = Albumin; Crt = Creatinine; Glc = Glucose; TCl = Total cholesterol; Tgl = Triglycerides; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase

[2] SP = Source of protein; PP = Protein pellets; SBG = Soybean grain; SI = Silage.

[3] SP × SI = Interaction between SP and SI

higher total cholesterol levels than those with no added silage. These findings are consistent with those reported by Cônsolo et al. (2017), who found a linear increase in total cholesterol concentration with the addition of soybeans in cattle fed a diet with a forage to concentrate ratio of 40:60. The higher concentration of total cholesterol in animals fed soybean grain may be due to the higher fat content of the diet, which results in increased fat digestion in the small intestine, thereby increasing the production of bile acids and cholesterol-rich lipoproteins (Bauchart 1993; Arshad et al. 2023). Furthermore, plasma cholesterol is closely associated with energy metabolism and vitamin synthesis (Petrera et al. 2015; Wilkens et al. 2019), as well as endocrine functions, and serves as a precursor to various hormones that affect glucose metabolism (Gross et al. 2021).

There was no effect from the source of protein or the addition of silage on the plasma urea concentration, whose values were consistent with those reported by Gandra et al. (2011) for beef cattle fed high-grain diets. Plasma urea is an indicator of adequate protein levels in the diet, as well as of renal function and protein use (Russell and Roussel 2007). Thus, the data showed that replacing protein pellets with soybean grain, with or without the addition of silage, provides adequate protein levels to meet the dietary needs of the cattle. This could be advantageous from an economic point of view, given that optimizing the rumen conversion of feed protein into human food protein is a worldwide goal of animal production (Broderick 2018).

The glucose concentration of animals fed diets containing protein pellets was lower when silage was added compared to the diet with no added silage. The opposite result was found when soybean grain was used as the source of protein. This may be due to the known relationship between glucose and lipid metabolism in ruminants, in which slight changes in lipid intake can affect the blood glucose concentration (Hanson and Ballard 1967). It should be noted that soybean grain is an important source of dietary energy. However, the values for glucose concentration were mostly within the normal range for cattle, of 40–60 mg/dL (Mair et al. 2016). As such, exchanging protein pellets and soybean grain did not appear to have any harmful effects on animal health with respect to blood glucose levels. However, added soybeans in the diet together with added silage may increase the blood glucose concentration, albeit with no pathological result. This could be beneficial from the point of view of production due to the role of glucose in energy metabolism in cattle (Abbas et al. 2020).

The additional blood metabolites (total protein, albumin, and triglycerides) were not affected by the dietary treatments and were within the normal values for cattle (Pogliani and Birgel Junior 2007); this suggests that soybean grain can reliably replace protein pellets with no significant effects

Table 7 Costs assessment of the experimental diets containing protein pellets or soybean grain with or without added silage

Variable [1]	SP [2]				Factor mean value			
	PP		SBG		PP	SBG	With Silage	No Silage
	SI							
	With Silage	No Silage	With Silage	No Silage				
Cost of the concentrate, USD/kg	0.20	0.20	0.18	0.18	0.20	0.18	0.19	0.19
Daily cost of the diet (USD/animal/day)	2.89	2.54	2.28	1.46	2.72	1.87	2.59	2.00
TDN cost of diet, (USD/animal/day)	0.29	0.28	0.23	0.22	0.29	0.23	0.26	0.25
CP cost of the diet (USD/animal/day)	1.75	1.77	1.62	1.61	1.76	1.62	1.69	1.69

[1] TDN = Total digestible nutrients; CP = Crude protein

[2] SP = Source of protein; PP = Protein pellet; SBG = Soybean grain; SI = Silage

on these metabolites regardless of added silage. However, further studies are needed to explore the effects of replacing protein pellets with soybean grain on blood metabolites in order to provide better insights into the effects of interchanging these protein sources on energy and protein metabolism in cattle.

The lower daily cost of the diets when soybean grain was included as a source of protein was due to the lower cost of the concentrate in those diets. In addition, there was a reduction in the daily, TDN, and CP costs of the diet when protein pellets were replaced with soybean grain, regardless of added silage. These results are consistent with those of Nunes et al. (2021), who found that integrating livestock into soybean systems increases the profits from livestock production. It is important to point out that although this study did not investigate the effects of replacing protein pellets with soybean grain on weight gain, the lack of any impact on CP or TDN intake may indicate a similar response from animal performance to both sources of protein. The results presented here therefore confirm the potential of replacing protein pellets with soybeans from both a nutritional and economic point of view. This information should be useful for improving beef cattle production systems in tropical regions, given the abundance of soybean production in these areas.

Conclusion

The data shows that soybean grain could be a viable source of protein for cattle, compared to conventional protein pellets, regardless of the addition of silage. Animals fed soybean grain have similar values for CP and TDN intake as those fed protein pellets. Furthermore, including 150 g/kg silage DM in a high-grain diet, and using soybean grain as a source of protein could be a viable nutritional strategy for ensuring sufficient DM and nutrient intake, and adequate digestibility, with no adverse effects on rumen or blood parameters in feedlot cattle under tropical conditions.

Author contributions D. H. S. Tavares: Data curation, writing the original draft, writing, review and editing; J. A. C. Vargas: Investigation, writing the original draft, writing, review and editing; V. L. A. Borzog: Data curation, writing the original draft; J. N. M. Neiva: Conception and design, acquisition of funds; J. H. (A) Moura, I. (B) Freitas, and T. M. S. Pinto: data collection, analysis and interpretation; L. F. Sousa: investigation, writing the original draft, statistical analysis; F. R. CH. Miotto: conceptualization, data curation, acquisition of funds, project administration, writing and preparing the original draft.

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Data availability Data from this research can be shared upon reasonable request.

Declarations

Ethics approval All the experimental procedures involving animals were approved by the Ethics Committee on the Use of Animals of the Federal University of Tocantins (CEUA-UFT), protocol number: 23101.006703/2018-46.

Consent for publication The authors agree with the current version and submission of the manuscript.

Conflict of interest The authors declare there to be no conflicts of interest.

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