Animal 15 (2021) 100155

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Effects of supplementation with corn distillers' dried grains on animal performance, nitrogen balance, and enteric CH_4 emissions of young Nellore bulls fed a high-tropical forage diet

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ARTICLE INFO

Article history:
Received 26 March 2020
Received in revised form 16 November 2020
Accepted 17 November 2020
Available online 9 January 2021

Keywords:
Beef cattle
Brachiaria
Diet supplementation
Nitrogen efficiency use
Pasture management

ABSTRACT

The inclusion of corn-dried distillers' grains (**DDG**) could be an alternative supplement to increase animal performance, nitrogen efficiency usage (**NEU**), and decrease enteric methane (CH_4) emissions. Our goal was to determine whether DDG could replace a traditional supplement (cottonseed meal) without affecting animal performance, N balance, and CH_4 emissions. The experiment was conducted during the forage growing season (December to April), with 15 d adaptation, and a 112 d experimental period. The experimental design was completely randomized with four treatments: a mineral supplement (**MS**), cottonseed meal supplement (**CS**), 50% replacement of CS by DDG (50DDG), and 100% replacement of CS by DDG (100DDG). Cottonseed meal and DDG were used as protein supplement. A total of 12 paddocks, 3 per treatment, were used to measure forage mass: morphological and chemical composition of forage, forage allowance, and animal performance. Six animals per treatment were used to evaluate DM intake, digestibility, CH_4 emissions, microbial protein production (**MCP**), and NEU of each treatment. Eighty-one Young Nellore bulls (48 testers, 12 per treatments and 33 adjusters) with initial BW of 255 ± 5 kg (10–12 months old) were supplemented with each supplement type at a level of 0.3% of BW. Pasture management was continuous stocking with a variable stocking rate (put-and-take). Enteric CH_4 was measured using the gas tracer technique. The MCP was quantified using purine derivatives and the NEU mass balance. No differences were found in nutrient intake ($P > 0.228$). Individual animal performance and gain per area were higher in the treatments with concentrates compared with that of MS; however, there was no difference among treatments CS, 50DDG, and 100DDG. The ADG was 0.83 for MS and 1.08 kg/animal/d when supplemented ($P < 0.05$). Gain per hectare was 709 kg/ha for MS and 915 kg/ha when supplemented with concentrates ($P < 0.05$). There was no difference in CH_4 production among treatments that average 180 g/animal/d; however, CH_4 per kg of gain was reduced with CS. The CH_4 conversion factor averaged 5.91%. There was no difference in the synthesis of MCP and NEU. Corn DDG can replace 100% of cottonseed meal as a protein source for supplementation of young Nellore bulls grazing in tropical pastures without affecting animal performance, NEU, MCP, and CH_4 emissions.

Implications

Here, we show that dried distillers' grains can replace cottonseed meal as a protein source in supplements for young bulls fed high-forage diets without affecting animal daily gain, microbial protein synthesis, N balance, and enteric CH_4 emissions. Young bulls supplemented with concentrates had increased daily gain and gain per area without changes in CH_4 emissions. Policymakers and beef cattle farms can integrate these findings using dried distillers' grains as a protein source with a higher tropical forage diet and improve enteric CH_4 reports and inventories.

Introduction

Brazilian beef cattle production has an important influence on the gross domestic product, even with low productivity indexes, as reported by the Brazilian Association of Beef Exports Industry (ABIEC), of a stocking rate of 1.3 animal units (AU; 1 AU = 450 BW) per ha, in a total area of 164.96 million ha (~70% of national agricultural land), with 19.4% of

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https://doi.org/10.1016/j.animal.2020.100155

the total herd slaughtered annually (ABIEC, 2018). However, these low productivity indexes cause significant environmental issues because of the inefficient utilization of N and the enteric methane (CH_4) production. One option to improve these indexes is animal supplementation on high-forage diets. In recent years, Brazil has become the third world's corn producer and is expected to produce approximately 3 million tons of corn-dried distillers' grains (**DDG**) per year by 2030, while in 2010 the Brazilian production was zero (Araujo et al., 2018).

Distillers grains are the principal by-product of ethanol production from corn. The effect of replacing a conventional source of protein in the supplements and the supplementation with DDG in high-forage diets needs to be evaluated for an in-depth assessment, quantifying the effect on animal productivity and environmental aspects. Corn DDG have a protein content similar to that of cottonseed meal (approximately 36%), both having higher ruminal undegraded protein (**RUP**). Thus, DDG can be used as an alternative ingredient to formulate beef cattle supplements (Thomas et al., 2017). The co-product DDG is also competitively priced, especially in production regions, such as Mato Grosso State in Brazil (Freitas and Miura, 2018).

Enteric CH_4 is a huge environmental issue that is formed during fermentation in the rumen. In tropical grasslands, the amount of energy intake lost as CH_4 can reach 11% (Berndt et al., 2014). Strategies to mitigate CH_4 production are desirable. Improving diet nutritive value can reduce CH_4 production and improve both average daily gain (**ADG**) and gain per area (Boland et al., 2013). This results in lower CH_4 emission per unit of product (Barbero et al., 2015). In Canada, research has shown that substituting a mixture of 35% barley grain and 5% canola meal (DM basis) with soluble DDG in a high-forage diet decreased enteric CH_4 emissions from beef cattle from 7.8% to 6.6% of gross energy (**GE**) intake (Hünerberg et al., 2013a). However, the latter authors state that the inclusion of 40% soluble wet distillers' grains (DM basis) did not affect CH_4 production (7.3% of GE intake).

Nitrogen efficiency usage by ruminants during the rearing and finishing phases in tropical pastures is low (~11%; Detmann et al. (2014). Corn by-product DDG can increase RUP in the diet, which may be associated with increased N flow to the duodenum and increased nitrogen efficiency usage. Increased metabolizable protein can shift the route of N excretion. Urine is the main source of nitrous oxide (N_2O), a potent greenhouse gas, and ammonia (NH_3) emissions to the atmosphere (Cardoso et al., 2017). Reduction in N excretion could reduce the environmental effects of beef cattle production. However, replacing barley grain with corn by-product DDG and wet DDG dramatically increased N excretion (Hünerberg et al., 2013a and 2013b).

Most previous studies on the effects of DDG on animal performance and environmental effects of beef cattle production have been conducted in temperate regions for diets with higher levels of concentrates (e.g., Hünerberg et al., 2013a and 2013b). Different interactions between these variables and animal gain, N balance, and CH_4 enteric emissions are expected for Nellore supplemented with tropical grasslands. Additionally, there is an increasing need to develop strategies to mitigate enteric CH_4 emissions and to improve inventories for CH_4 emissions. Thus, we evaluated the effects of replacement of conventional protein sources by DDG in a beef cattle diet for young Nellore bulls in pastures where measurements were taken of nutrient intake and digestibility, enteric CH_4 emissions, N balance, and aveADG. We hypothesized that DDG can be used as a protein source without decreasing animal performance or increasing CH_4 emissions.

Material and methods

Experimental site and design

The experiment was conducted in the Forage and Grasslands Sector of São Paulo State University, "Júlio de Mesquita Filho" (Unesp) (Jaboticabal, São Paulo, Brazil) during the period of forage growth (December to April) in 2015/2016. The climate is subtropical, humid, with rainy summers and dry winters. The mean annual rainfall (1970–2010) was 1 424 mm, mean air temperature was 22.3 °C, and the soil is a Rhodic Ferralsol (IUSS, 2014). Grassland was sowed in 2001 with *Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster Marandu (Marandu grass). Mean soil chemical characteristics evaluated in September 2015 were pH 4.8; organic matter (**OM**) 32.0 mg/dm; phosphorus 11.8 mg/dm; and base saturation 46.3%. In November 2015, maintenance fertilizer was applied to all paddocks at 50 kg P_2O_5 and 70 kg K_2O per ha. Urea was applied at 180 kg N/ha, split among the three applications according to the precipitation schedule: December 2015, and January and March 2016.

The experimental design was a completely randomized design, with four treatments: mineral supplementation (**MS**), conventional supplementation with cottonseed meal (**CS**), 50% replacement of protein source of CS by DDG (50DDG), and 100% replacement of protein source of CS by DDG (100DDG) (Table 1). Each treatment had three repetitions (paddocks), totaling 12 paddocks (experimental units). In the treatments, MS used three 0.7 ha paddocks and in the remaining three treatments there were two paddocks of 1.3 ha and one 0.7 ha each. The experimental site included a reserve area of 3

Table 1
Supplement composition of each experimental treatment for young Nellore bulls: mineral supplementation (MS), conventional supplementation (CS); 50% replacement of protein source of CS by DDG – dried distillers' grain (50DDG), and 100% replacement of protein source of CS by DDG (100DDG).

Ingredients (g/kg DM)	Treatment			
	CS	50DDG	100DDG	MS
Corn ground	500	500	500	–
Cottonseed meal	308	155	–	–
DDG	–	185	372	–
Salt	33.7	33.7	33.7	–
Limestone	42.2	46	49.8	–
Monocalcium phosphate	30.6	34.5	38.4	–
Kaolin	83.8	44.4	4.6	–
Mineral premix[1]	0.9	0.9	0.9	–
Monensin 200[2]	0.4	0.4	0.4	–
Mineral mixture[3]				100.0
Chemical composition (g/kg DM)				
apNDF	180	245	311	–
iNDF	108	124	99	–
NFC	408	378	349	–
RDP (%CP)	536	513	486	–
RUP (%CP)	464	488	514	–
CP	165	159	153	–
EE	24.7	22.4	20.2	–
Protein fraction (%CP)				
Fraction A	18.0	11.4	11.4	–
Fraction B1 + B2	35.2	49.7	52.7	–
Fraction B3	42.6	27.5	20.4	–
Fraction C	4.3	11.3	15.6	–
Chemical composition of Ingredients (g/kg DM)	Corn ground	Cottonseed meal	DDG	
apNDF	131	370	661	–
iNDF	22	136	69	–
NFC	738	225	18.4	–
CP	90.4	39.1	29	–
EE	40.4	14.4	31.1	–

[1] Guarantee levels: Ca – 16 204 g/kg; Cu – 45 900 mg/kg; Mn – 28 270 mg/kg; Zn – 170 000 mg/kg; Co – 2 505 mg/kg; I – 3 400 mg/kg; Se – 884 00 mg/kg.
[2] Monensin 200: Monensin sodium (200 g/kg).
[3] Mineral mixture: Ca – 153 00 g/kg; P – 90.00 g/kg; Na – 125.00 g/kg; Mg – 10.00 g/kg; S – 40.00 g/kg; Cu – 1 670 mg/kg; F – 1 500 g/kg; Mg – 1 290 mg/kg; Zn – 6 200 mg/kg; Co – 100.00 mg/kg; I – 124.00 mg/kg; Se – 32.00 mg/kg; apNDF: neutral detergent fiber corrected for ash and protein; iNDF: indigestible neutral detergent fiber; NFC: non-fibrous carbohydrate; RDP: rumen degradable protein; RUP: rumen undegraded protein; EE: ether extract.

ha for the animals used to regulate the pasture height of the experimental units.

Animal and grazing management

The animals used in this study were cared for according to the rules of the São Paulo State University Animal Care and Use Committee and the National Council of Animal Experimentation Control. The committee reviewed and approved the experiment and all procedures carried out in the study (certificate number 12703/15). Eighty-one young Nellore bulls (10–12 months old) recently weaned, with initial BW of 255 ± 21 kg were used. The 48 young bulls (testers) were identified, weighed, and randomly distributed in groups of four animals per treatment in each paddock balanced for BW. The remaining animals (33 adjusters) were used to maintain a pre-established grazing height, using the put-and-take methodology (Mott and Lucas, 1952).

The grazing method was continuous stocking with variable stock using the put-and-take methodology. We chose a grazing height of 25 ± 2 cm. This approach was chosen after a seven-year experiment show that at this grazing height occurs the greater forage production and animal performance per area (Barbero et al., 2015; Santana et al., 2016; Koscheck et al., 2020). The grazing height of 25 ± 2 cm adopted herein was based on Barbero et al. (2015). This approach corresponds to swards heights intercepting 95% of the incident light in both rotational (Trindade et al., 2007) and continuous (Barbero et al., 2015) grazing systems. The latter authors have reported greater forage production and animal gain yielded per area when sward heights were managed at 25 cm. During the experimental period of 112 d, the bulls were weighed every 28 d (without fasting), and these weights were used to adjust the stocking rate, grazing height, and supplement level. The height/forage mass relationship (bulk density) was evaluated weekly to adjust the stocking rate by use of the put-and-take animals.

Animal supplementation was conducted in group daily in the morning (11 am), in a linear space available per animal of 0.3 m in an uncovered feeder. In the treatment, young bulls were fed 120–130 g of mineral supplements daily and in the other treatments, 0.3% of BW basis was used to supplement the animals (from 0.75 to 1.11 kg animal/d). The characteristics of the supplements are presented in Table 1. The DDG used was obtained from Libra Etanol (São José do Rio Claro, Mato Grasso do Sul, Brazil) that used the patented technology FST™ (Fiber Separation Technology^{tm}) to produce DDG.

Forage sampling and chemical analysis

Pastures were measured every week at 80 random points per paddock using a graduated rule to evaluate average sward height. To evaluate forage mass, eight samples per paddock (selected at the mean height locations) were collected from a 0.25 m^2 area using a metal frame (clipped at 5 cm residual height) every 28 d (January to April 2016). Approximately 200 g samples were collected from the collected forage for further analysis of morphological composition. Samples were then separated into dead material, green stem + leaf sheath, and green leaves, and dried at 55 ± 5 °C to a constant weight to estimate forage mass. Forage allowance was calculated as the DM forage mass per ha and the animal live BW per unit area (BW/ha) relationship (Sollenberger et al., 2005). To evaluate forage nutritive value, samples were hand-plucked in the same periods (at 20 average places for each paddock) after observing animal grazing behavior (Halls, 1954). Hand-plucked samples were dried at 55 ± 5 °C to a constant weight and ground through a 1 mm screen in a shear mill (Thomas-Wiley Laboratory Mill Model 4, H. Thomas Co.) for chemical analyses.

Dry matter (AOAC 934.01) and OM (AOAC 942.05) were quantified according to procedures described by AOAC (1990). Crude protein was quantified using LECO® FP 528 (Leco Corporation, MI, USA). Neutral detergent fiber of concentrates was measured by adding alpha-amylase without the addition of sodium sulfite, and NDF of forage was measured without sodium sulfite or alpha-amylase. Neutral detergent fiber free of ash and protein a (apNDF) and ADF analyses were performed using polyester filter bags and ANKOM® equipment (Vogel et al., 1999). Both apNDF and ADF were expressed as ash-free. The indigestible iNDF of forage, concentrates, and feces was determined by an in situ incubation procedure for 288 h (Valente et al., 2011). The GE was quantified using an adiabatic bomb calorimeter (PARR Instrument Company 6300, IL, USA), as described by Barbero et al. (2015). Forage characteristics and chemical composition are presented in Table 2.

Animal performance

Bulls were weighed at the start (0 d) of the experiment, at the end of the adaptation period of each year, and the end of the experimental period, after fasting for 14 h before each weighing event. The bulls were also weighed every 28 d (without fasting) to adjust the stocking rate to maintain pasture height. Animal performance variables were calculated as follows (Mott, 1960):

Body weight gain: BW gain (kg) = (finalBW – initialBW);

Average daily gain: ADG (kg/animal/d) = (BW gain (kg)/d);

Gain per area: GPH = (ADG of tester animals × number of animals per days × experimental period (d))/area (ha)), where the number of animal days (animal/d/ha) was calculated by dividing animal stock by the mean weight of tester animals. Animal stock was determined by the sum of weights of all animals present in each paddock divided by the area of the paddock (kg BW/ha).

Stocking rate in animal unit: (AU = 450 kg BW)/ha = (∑ BWmean/450)/area (ha);

Forage mass allowance (kg DM/kg BW) = (kg DM/ha)/(∑ BWmean/area [ha]).

Intake and digestibility

The intake and digestibility were measured in six animals per treatment. Fecal production was measured using the chromium oxide (Cr_2O_3) method as an external marker and was dosed at 9 am for 10 d. Each animal received 10 g of Cr_2O_3 packed in paper cartridges and

Table 2
Forage mass, morphological fractions, and chemical composition of hand-pulled Marandu grass grazed by young Nellore bulls during the rainy period (December to April).

Variable	Treatment				SEM
	MS	CS	50DDG	100DDG	
Forage mass (kg DM/ha[1])	6 200	5 790	6 320	6 470	133
Forage allowance (kg DM/kg LW)	2.41	2.30	2.41	2.46	2.59
Green leaf (g/kg DM)	324	374	340	338	11.5
Green stem (g/kg DM))	372	367	340	349	40.7
Dead Material (g/kg DM)	303	259	320	313	10.8
Chemical composition (g/kg DM)					
OM	910	906	906	905	10.0
pdDM	842	847	845	840	7.4
apNDF	558	565	556	561	2.0
iNDF	143	147	148	152	3.0
CP	155	165	169	169	2.0
EE	24.8	24	24.6	24.9	0.1
Protein fraction (% CP)					
Fraction A	37.3	35.6	36.4	38.5	0.09
Fraction B1 + B2	34.1	36.5	35.3	34.2	0.07
Fraction B3	20.5	19.4	19.8	19.3	0.03
Fraction C	8.09	8.50	8.50	7.98	0.02

[1] DDG: dried distiller's grain; MS: mineral supplement (offered ad libitum); CS: conventional supplement with corn as an energy source and cotton meal as a protein source; 50DDG: supplement with 50% substitution of cotton meal by DDG; 100DDG: supplement with 100% replacement of cotton meal by DDG; OM: organic matter; pdDM: potentially digestible DM; apNDF: neutral detergent fiber free of protein and ash; iNDF: indigestible neutral detergent fiber; EE: ether extract.

dosed orally with an applicator. On the 8th, 9th, and 10th d of application of chromium oxide, fecal samples were collected at 07:00 and 13:00, 09:00 and 15:00, and 11:00 and 17:00 daily, respectively. To conduct this sampling, we observed the experimental animals, and immediately after spontaneous defecation, feces were collected. On the same days, a forage hand-plucked sample was used to quantify the iNDF of forage.

The DM content of fecal samples was quantified by drying them to a constant weight in an oven at 105 °C for 48 h. The ash in the feed and fecal samples was determined by combustion in a muffle furnace at 550 °C for 12 h. A composite fecal sample for each animal was determined by bulking dry samples from the three collection days on an equal weight basis and analyzed for chemical composition and chromium by atomic absorption spectrophotometry (Kimura and Miller, 1957).

Total feed intake was estimated based on fecal output and indigestible NDF (iNDF) as an internal marker. The individual consumption of the supplement was estimated from the average supply of the supplements for each animal in the paddock (0.3% BW). Forage DMI was estimated as total DMI minus the supplement offered. The total apparent digestibility was determined by the calculation of DMD = (DMI − FE)/DMI, where DMD = apparent digestibility of DM (%); DMI = dry matter intake (kg/d), and FE = fecal excretion (kg/d).

Enteric methane

Six animals (380 kg BW) per treatment were used to measure CH_4 production. Enteric CH_4 was measured using the gas tracer methodology (sulfur hexafluoride – SF_6) method, following the manual of Global Research Alliance for Greenhouse Gases on Agriculture (Berndt et al., 2014). A calibrated SF_6 permeation tube with an average of 90 μg/h constant release was inserted orally into the rumen 8 d before the first collection. The sampling apparatus consisted of polyvinyl chloride (60 mm, class 20 pipe) collection yoke and a capillary tube extending from the collection yoke to just above the mouth and nostrils of the animals. The yoke was attached to a collar around the neck of the bull.

Animals were adapted to the sampling apparatus for 1 week. During the sampling campaign at 7:00, the attached yoke was connected to the transfer line, and a valve on the collection yoke was opened. The collection yoke was changed daily for 6 consecutive days (sampling campaign). At each change, a new yoke evacuated at −95 kPa was used. To obtain the air background CH_4 and SF_6 in the paddocks, a blank collector set (yoke + halter) was installed in the paddocks of each treatment. A gas chromatograph was used to analyze CH_4 and SF_6 concentrations (Shimadzu 2014 AF) equipped with flame ionization and micro-electron capture detectors were used to quantify the CH_4 and SF_6 concentrations, respectively. The carrier gas was N_2, and the gas flame was H_2.

Enteric CH_4 animal emissions were calculated in proportion to SF_6 emissions through the rumen capsule, subtracting air background CH_4 and SF_6 as follows:

$$CH_4 = CSF_6 \times ([CH_4]v - [CH_4]air)/([SF_6]v - [SF_6]air)$$

where CH_4 is the CH_4 emission rate by an animal, CSF_6 is the SF_6 emission from the capsule in the rumen, $[CH_4]v$ is the CH_4 concentration at collection yoke, $[CH_4]air$ is the CH_4 concentration in the air of paddocks, $[SF_6]v$ is the SF_6 concentration at collection yoke, and $[SF_6]air$ is the SF_6 concentration in the air of paddocks. The CH_4 conversion factor (Ym) was calculated by dividing the energy of emitted CH_4 by the GE intake by 0.05565 MJ/g CH_4.

Blood collection and analysis

Blood collection was conducted using the same animals used for enteric CH_4 measurements. On the 11th day after adaptation to CH_4 apparatus, blood and urine collections were performed approximately 4 h after supplementation. Samples were taken from the jugular vein using a 2-way blood collection needle (BD® Brazil, Model 18 G × 1 1/2) and transferred into two heparinized vacutainers (12 ml/tube). The tubes were gently inverted a couple of times and immediately centrifuged at 5 000 rpm for 15 min. Individual plasma samples were stored in Eppendorf tubes (3–3.5 ml/tube) at −15 °C for analysis. Plasma urea nitrogen (**PUN**) was measured by a fully enzymatic method using a commercial kit (Labtest, Lagoa Santa, MG, Brazil). Plasma urea nitrogen was determined by multiplication of urea by a factor of 0.4667.

Urine collection and analysis

Using the same 24 animals used for enteric CH_4 evaluations, urine collection was performed by taking spot samples (approximately 300 ml) at the same time that fecal samples were taken. The spot sample (approximately 300 ml) was taken by waiting for spontaneous urination with the aid of a handle equipped with a bucket. The samples were filtered in a triple layer of gauze and a 10 ml aliquot was diluted with 40 ml of sulfuric acid (0.072 M), which was used to quantify the urine concentrations of creatinine and purine derivatives. A second aliquot of pure urine was separated for determination of total nitrogen by the Dumas combustion method using Leco® equipment, model FP-528 (Leco Corporation) and was kept at −20 °C to determine the total N. Creatinine was used as an indicator of urine production (Chizzotti et al., 2015) and was measured in urine spot samples using a commercial kit (Labtest). The urine volume was estimated by the ratio between creatinine excretion and concentration in the spot sample (mg/l), assuming a daily excretion value of 27.11 (mg creatinine/kg BW) determined for Nellore by Barbosa et al. (2011). Allantoin was measured according to the method described by Young and Conway (1942), which is based on the Rimini–Schryver reaction. The determination of uric acid was conducted by its reaction with uricase using a commercial kit (Labtest).

Nitrogen balance and microbial protein production

The N balance was calculated as:

$$\text{N balance (g/d)} = \text{Intake N (g/d)} - \text{fecal} - \text{N (g/d)} - \text{urine N (g/d)}$$

Purines absorbed at (Y, mmol/d) were calculated from the excretion of purine derivatives (X, mmol/d), using the equation: Y = (X – (0.30 $BW^{0.75}$))/80, where 0.80 is the recovery of purines absorbed as purine derivatives and 0.30 $BW^{0.75}$, the endogenous contribution to purine excretion for Nellore (Barbosa et al., 2011).

Ruminal synthesis of microbial nitrogen (Y, g N/d) was calculated as a function of absorbed purines (X, mmol/d) using the equation of Barbosa et al. (2011): Y = 70X/0.93 × 0.137 × 1 000, where 70 is the purine N content (mg/mmol), 0.137 N purine:total N ratio in the bacteria, and 0.93 as the true digestibility of the microbial purines. The efficiency of microbial protein synthesis was expressed in grams of microbial nitrogen per kilogram of apparently digestible OM apparently degraded in the rumen. The OM apparently degraded in the rumen was determined by calculating the digestible OM consumption by animals multiplied by the factor 0.65 (ARC, 1984). From the microbial nitrogen (g/d), the microbial protein was calculated by multiplying it by factor 6.25 and the efficiency of microbial protein per kilogram of total digestible nutrients (**TDN**) consumed.

Data analysis

The experimental design was completely randomized. Data were analyzed using Software SAS version 9.2, and the procedure PROC MIXED, considering a mixed model. Each paddock was considered

an experimental unit, and initial BW was used as a covariate for the analysis of ADG. Supplements were considered fixed effects, variables related to forage nutritive value and animal performance paddocks were random effects, and the variables related to enteric CH_4, N balance, and microbial protein production animals were random effects. The Tukey's test was used to compare means from each treatment during this process using the PDIFF function in LSMEANS. Significant differences were declared at a 5% significance level (P value <0.05).

Results

The results presented in tables 3-6 come from Dr Alvair Hoffmann's PhD thesis (Hoffmann, 2019). Total DMI and forage intake from animals grazing during the experimental period did not differ among treatments ($P > 0.09$). Apparent digestibility did not differ for DM ($P = 0.06$) and GE ($P = 0.162$), but there were differences in digestibility of OM ($P = 0.004$), CP ($P = 0.044$), and apNDF ($P = 0.040$) (Table 3).

Average daily gain, GPH, and FBW were different ($P < 0.001$, 0.028, and 0.030, respectively) among treatments with supplemented groups being higher (Table 4). The ADG was 0.83 for MS and 1.08 kg/animal/d when concentrates were added. Gain per hectare was 709 kg/ha for MS and 915 kg/ha when supplemented with concentrates. The stocking rate did not differ among treatments ($P = 0.794$) and was higher (5.73 AU/ha) than the Brazilian National Index (1.3 AU/ha).

Inclusion of DDG to replace the cottonseed meal as a protein source in the supplement did not affect enteric CH_4 production per day ($P = 0.58$), per kg of DMI ($P = 0.29$), or per kg of digestible organic matter (**DOM**) intake ($P = 0.191$) being an average of 180 g/animal/d. However, CH_4 intensity (g CH_4/kg ADG) decreased with cottonseed meal "CS treatment" ($P < 0.049$). The CH_4 conversion factor (Ym) was similar among treatments and averaged 5.91% (Table 5).

Supplements had no effect ($P = 0.795$) on PUN concentrations. N intake, N excreted in feces, N excreted in the urine, and N retained were similar in cattle fed MS, CS, 50DDG, and 100DDG. However, the inclusion of DDG in the diet reduced the fecal:urine N ratio from 1.5 to 1.2. Microbial N, microbial protein synthesis, and MCP/kg DOM and MCP/kg TDN were not affected by supplementation strategies (Table 6).

Table 3
Intake of dry matter (DMI) of forage and apparent digestibility of complete diets for young Nellore bulls fed with mineral supplementation (MS), conventional supplementation (CS); 50% replacement of protein source of CS by DDG – dried distillers' grain (50DDG), and 100% replacement of protein source of CS by DDG (100DDG) grazing Marandu grass during the wet season (December to April).

Variable	Treatment				SEM	*P*-value
	MS	CS	50DDG	100DDG		
Intake (kg/d)						
DMI	9.29	10.2	9.76	9.00	0.59	0.511
Forage	9.29	9.15	8.68	7.96	0.57	0.406
Forage (% BW)[1]	2.57	2.29	2.17	2.06	0.13	0.090
Supplement	0.00b	1.07a	1.08a	1.04a	0.03	0.001
OM	8.44	8.96	8.61	7.96	0.53	0.629
CP	1.17	1.41	1.36	1.27	0.08	0.239
apNDF	5.18	5.31	5.02	4.72	0.32	0.610
iNDF	1.38	1.43	1.41	1.31	0.10	0.811
GE (MJ/d)	156	183	165	152	0.58	0.228
Apparent digestibility (g/kg DM)						
DM	680	684	678	654	7.7	0.060
OM	703ab	721a	714a	679b	6.4	0.007
CP	637ab	657a	644ab	609b	9.7	0.044
apNDF	606ab	640a	616ab	582b	11.4	0.040
DE (MJ/d)	106	134	114	109	4.6	0.162

DMI: dry matter intake; OM: organic matter; apNDF: neutral detergent fiber corrected for ash and protein; iNDF: indigestible neutral detergent fiber; GE: gross energy; DE: digestible energy.
[a,b]Means followed by the same letter on the line do not differ by the Tukey test ($p \leq 0.05$).

Table 4
Animal performance for young Nellore bulls fed with mineral supplementation (MS), conventional supplementation (CS); 50% replacement of protein source of CS by DDG – dried distillers' grain (50DDG), and 100% replacement of protein source of CS by DDG (100DDG) grazing Marandu grass during the wet season (December to April).

Variable	Treatment				SEM	*P*-value
	MS	CS	50DDG	100DDG		
IBW (kg)	247	247	246	252	1.35	0.891
FBW (kg)	340b	370a	368a	374a	11.3	0.030
ADG (kg/d)	0.83b	1.10a	1.09a	1.08a	0.04	<0.001
Stocking rate (AU/ha)	5.55	5.83	5.76	5.78	0.29	0.794
GPH (kg/ha)	709b	926a	893a	927a	52.2	0.028

Different uppercase letters within a row denote a significant difference by Tukey's test ($P < 0.05$). IBW: initial BW; FBW: final BW; ADG: average daily gain; AU: animal unit (450 kg BW); GPH: BW gain per hectare.

Table 5
Enteric methane (CH_4) production for young Nellore bulls fed with mineral supplementation (MS), conventional supplementation (CS); 50% replacement of protein source of CS by DDG – dried distillers' grain (50DDG), and 100% replacement of protein source of CS by DDG (100DDG) grazing Marandu grass during the wet season (December to April).

Variable	Treatment				SEM	*P* value[1]
	MS	CS	50DDG	100DDG		
CH_4 g/d	179.5	165.4	184.8	190.1	13.2	0.586
CH_4 g/kg DMI	19.45	16.67	19.57	21.35	1.58	0.291
CH_4 g/kg OMI	0.30	0.26	0.31	0.35	0.02	0.191
CH_4 kg/kg ADG	0.22b	0.15a	0.17ab	0.18ab	0.01	0.049
Ym (%)	5.97	5.12	6.00	6.55	0.02	0.281

[1] Different lowercase letters in the line differ from each other by Tukey's test ($P < 0.05$). DMI: dry matter intake; OMI: digestible organic matter intake; ADG: average daily gain; Ym: CH_4 conversion factor, that is, the percentage of gross energy intake lost as CH_4 energy using 55.22 M/kg CH_4 (Berndt et al., 2014).

Table 6
Plasma urine nitrogen, N balance, and microbial protein for young Nellore bulls fed with mineral supplementation (MS), conventional supplementation (CS); 50% replacement of protein source of CS by DDG – dried distillers' grain (50DDG), and 100% replacement of protein source of CS by DDG (100DDG) grazing Marandu grass during the wet season (December to April).

Variable	Treatment				SEM	*P* value[1]
	MS	CS	50DDG	100DDG		
Nitrogen use efficiency						
PUN (mg/dl)	11.66	12.28	12.52	12.36	0.64	0.795
N intake (g/d)	187.5	225.4	217.9	203.4	12.83	0.197
Fecal-N (g/d)	67.88	77.47	77.87	79.26	5.10	0.394
Urine-N (g/d)	45.06	50.08	64.91	59.07	6.94	0.211
Ratio fecal:urine N	1.51	1.55	1.20	1.34	–	–
N retained (g/d)	74.52	97.92	75.19	65.11	10.05	0.159
N retained (% intake)	39.50	42.94	34.09	31.92	4.08	0.240
Microbial efficiency (g/d)						
Microbial N	128.1	110.7	115.7	103.1	15.3	0.709
Microbial protein (MCP)	800.5	691.9	723.2	644.6	58.6	0.724
MCP/kg DOM[2]	136.7	110.9	115.6	118.1	14.4	0.615
MCP/kg TDN[3]	129.0	105.2	110.04	111.4	14.0	0.652
Nmic/kg OMADR[4]	33.6	27.3	28.5	29.1	3.6	0.615

[1] ANOVA probability (P-value); PUN = plasma urea nitrogen.
[2] Microbial protein kg of digestible organic matter consumption.
[3] Microbial protein/kg of total digestible nutrients (TDN).
[4] Microbial N kg consumption of organic matter apparently degraded in the rumen (OMADR).

Discussion

Intake, digestibility, and animal performance

Forage mass was adequate to promote high intake and had a green leaf percentage of 34% within the total mean forage mass of 6.2 t DM/ha, that is, three times higher than the minimal preconized for Brachiaria (de Paula et al., 2012). There was also high DM digestibility (680 g/kg DM), a high CP content (164.5 g/kg DM mean) with 37% fraction A and a small fraction C (non-degradable protein) of CP (8.3% of CP). The CP contents and forage mass contents are unlikely to limit intake (Reis et al., 2009; Detmann et al., 2014), and therefore, the pasture system provided a good model by which to examine the effect of DDG supplementation on CH_4 and N excretion.

Supplementation of any source improved nitrogen efficiency usage and reduced CH_4 excretion/unit of product over just MS of the existing pasture base. There was no difference between the concentrated sources of supplements, and therefore, DDG could replace cottonseed meal and achieve advantages for the emission of N and CH_4 in these grazing systems.

Thus, DDG is a valuable supplement in this system. The RDP and UDP content could be varied significantly by the heating and drying process of manufacture. Castillo-Lopez et al. (2013) studied DDG and reported that heat used during the drying process could decrease the RDP content. Kelzer et al. (2010) reported values of RUP equal to 33.2% CP for DDG without heat exposure before fermentation and 56.3% CP for DDG exposed to heat. These RUP values confirmed that the RUP content of DDG could be changed according to the manufacturing process.

The supplements CS, 50DDG, and 100DDG had 18.0%, 11.43%, and 11.39% CP of fraction A and 35.15%, 49.72%, and 52.66% CP of fractions B1 + B2, which confirmed the effect of the manufacturing process. The higher fractions B1 + B2 from supplement 100DDG were associated with higher RUP, which could cause ruminal problems because of a lack of soluble protein (fraction A). The manufacturing of DDG occurs at high temperatures, which increases fraction C. At higher temperatures, the Maillard reaction occurs within the residual sugars of the N compound, increasing fraction C (Sniffen et al., 1992). However, the calculations showed that for these forage and supplements, the RDP supply was adequate for the rumen microbes.

The CP, OM, and apNDF (NDF corrected for ash and protein) apparent digestibility values were lower for animals supplemented with higher DDG, which might indicate an effect of the manufacturing process. The requirements of a Nellore bull weighing 400 kg to obtain 1 kg/d of ADG is 0.98 kg/d of CP and 5.2 kg/d of TDN, with a DMI intake ranging between 8.2 and 9.0 kg DM/d (Valadares Filho et al., 2010). When calculating the requirements of the ADG from animals in this research, only animals from treatment MS (0.83 kg/d) did not reach the projected ADG previously mentioned. The higher ADG, 0.260 kg/d, measured in animals from treatments using supplements, could have resulted from higher RUP. These gains are expected in young bulls during the rearing phase when the CP requirement is normally higher.

Animals from treatments supplemented with concentrates (CS, 50DDG, and 100DDG) accomplished approximately 31% more ADG than those in the MS treatment. There was no difference between the supplements in ADG. Stocking rate values were similar among all treatments. Reis et al. (2009) reported that pasture management should allow control of the quality and quantity of forage available. They suggested that an additional ADG over 0.200 kg/d compared to the value (0.260 kg/d) determined in the current experiment in response to protein/energy supplementation. Together, a higher GPH is determined because of the higher ADG, because the stocking rate was similar between treatments.

Enteric methane

Animal supplementation significantly reduced CH_4 emissions per kg of BW gain (Table 6). According to the calculations of Cardoso et al. (2016), greenhouse gas emissions per kilogram of the carcass can be reduced by up to 49% by increasing the ADG and pregnancy rate by improving animal diet quality, pasture management, and genetic and sanitary practices. In this study, CH_4 intensity was reduced by approximately 20% by supplementing the animals with conventional supplements "CS treatment."

The differences that occurred when including concentrates in the supplement are due to the differences in the carbohydrate types. The carbohydrate source within a diet affects the methanogenic potential of the diet (Lovett et al., 2005). According to these authors, diets containing high levels of starch (concentrates) will emit less CH_4 than those composed principally of structural carbohydrates (grasses). Berça et al. (2019) studied grazing management strategies with N fertilization or legumes and did not found an effect on enteric CH_4. A minimal of concentrate inclusion in the diet may be required to reduce the methanogenic potential of the diet.

Daily CH_4 production was greater than that recorded by Barbero et al. (2015). In the non-supplemented animals, the daily CH_4 production ranged from 120 to 150 g/d, lower than the average of 165–190 g/d measured in this study (Table 6). This difference can be attributed to differences in animal weight and DMI. Dry matter intake is one of the most important driver variables regulating daily CH_4 production (Reynolds et al., 2011; van Lingen et al., 2019). Corn-based DDG reduced enteric CH_4 emissions from beef cattle in a high-forage diet from 7.8% to 6.6% of GE intake (Hünerberg et al., 2013a and 2013b). However, the amount was higher than 6% of GE intake emitted as CH_4 in the control treatment studied here.

The authors of the Intergovernmental Panel on Climate Change guidelines for national inventories based on the work of Crutzen et al. (1986) preconized an enteric CH_4 daily production of 149 g CH_4/animal/d, which is lower than that found here. Concerning the percentage of GE intake lost as CH_4, the average of 6% found here agrees with Eggleston et al. (2006), who recommended that 6.5% should be used to estimate CH_4 production when the ruminant diet has more than 90% forage. Our study confirmed the value from Ritzman and Benedict (1938), and many others that approximately 7% of GE intake is lost as CH_4 for beef cattle diets with a high proportion of forage.

Nitrogen balance

None of the supplements affected the concentrations of PUN; however, the value of 12.2 mg urea-N/dl in Nellore cattle fed grass with low levels of supplements was similar to the value (12–15 mg urea-N/dl) in animals fed similar diets (Barbero et al., 2015; Chizzotti et al., 2015; Vendramini et al., 2015). Vendramini et al. (2015) found that the maximum microbial efficiency was achieved when the PUN ranged from 15 to 19 mg/dl. Values higher than this limit suggest a loss of protein.

The N balance was greater in this study (approximately 40%; Table 6) than that reported for animals raised on tropical pastures. Detmann et al. (2014), in a meta-analysis, found an N balance of 11%. The greater nitrogen efficiency usage found by us may be related to the synchrony between the degradation of carbohydrates and N compounds in the rumen that decreased fecal-N excretion. In this study, the CP was approximately 140 g/kg DM and the average protein/g DOM was approximately 200 g/kg DOM and close to the required value (Santos et al., 2016). Howard et al. (2007) reported that a diet containing synchrony between carbohydrates and N compounds usually improves N balance.

The efficiency of microbial protein synthesis depends on the availability of fermentable carbohydrates and N in the rumen (NRC, 2016). Thus, microbial growth is maximized by the synchronization between the availability of fermentable energy and degradable N in the rumen (Dewhurst et al., 2000). There was no effect of the type of supplementation offered to the animals on microbial efficiency ($P > 0.05$). These results showed that well-managed tropical grasses with high levels of

soluble N and high-quality fiber (green leaves) and low iNDF do not require additional energy supply via supplements to increase N uptake in the rumen as microbial protein to reduce excretion of N in the feces and urine.

The mean value of microbial efficiency (29.61 g Nmic/kg OMAD) was similar to that recommended by the ARC (1984) of 30 g Nmic/kg OMAD. The mean values of microbial efficiency (113.9) expressed in g MCP/kg TDN were lower than the 130 g/kg evaluated by the 7th edition of NRC and 120 g/kg recommended by Santos et al. (2016) as a reference for tropical conditions. According to NRC (2016), the slopes for the regression of MCP synthesis on TDN were substantially less than the value of 130 g/kg TDN, and the recommendation of 130 g/kg is a generalization that does not apply to all conditions. The mean ratio of 218.1 g PB/kg DOM in supplements is higher than the critical value of 210 g CP/kg DOM, as noted by Poppi and McLennan (1995), where significant net losses of dietary protein occur in transfer across the rumen as RUP and MCP.

The absence of effects on N-balances of DDG inclusion in the diets disagrees with the studies of Hünerberg et al. (2013a and 2013b) who found a dramatic increase in N intake and excretion. Therefore, DDG can be used to replace cottonseed meal of protein in supplements without an increase in N_2O and NH_3 production, which are important sources of N pollution. Cardoso et al. (2017) showed that urine is the main source of N_2O and NH_3 production. Urine N excretion did not increase with DDG inclusion.

Concentrate supplements did not change GHG emission production per animal day or DMI, but CS treatment reduced enteric CH_4 emission intensity by 20%. Thus, concentrate supplements contribute to the reduction of CH_4 emissions during the animal life cycle. No effect was found on N intake and excretion. Corn DDG can replace 100% of cottonseed meal as a protein source for animal supplementation during the rearing phase on tropical pastures without any adverse effects on ADG, enteric CH_4 emissions, or N excretion.

Ethics approval

The Ethics, Bioethics, and Animal Welfare Committee of the Unesp, Jaboticabal approved all protocols used (protocol number 12703/15).

Data and model availability statement

Data will be made available at request.

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Declaration of interest

The authors declare that they do not have any conflicts of interest.

Acknowledgements

The work was conducted as part of the first author's thesis. We are grateful for the help of UnespFor (study group) for their assistance in conducting experiments. We would like to thank Professor Dennis Poppi from Queensland University (Australia) for the critical revision of our manuscript.

Financial support statement

This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grants 2015/16631-5, and 2017/11274-5), Coordenação de Aperfeiçoamento do Pessoal de Nível Superior (CAPES), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the scholarships.

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