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Performance and enteric methane emission of growing beef bulls from different genetic groups subjected to two supplementation strategies grazing tropical grass in the rainy season

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ABSTRACT

Improving livestock production through nutrition and breeding can increase efficiency and has the potential to mitigate methane (CH_4) emissions. Additionally, supplementing beef cattle in the rainy season balances the dietary protein:energy (P:E) ratio, which can increase animal performance and reduce energy losses from CH_4 production. Therefore, the objective of this study was to evaluate the effect of supplementation strategy (SS) and genetic group (GG) on the intake, digestibility, performance, and enteric CH_4 emissions of growing beef bulls grazing tropical grass during the rainy season. One hundred sixty-two growing beef bulls averaging (mean ± SD) 10 ± 2 months old and 262 ± 31 kg of initial body weight (BW) were distributed, according to their BW, in a randomized complete block design in a 2 × 3 factorial arrangement. Factors included (1) two SSs (mineral supplementation at 0.3 g/kg of BW per day and a corn-based supplementation at 3 g/kg of BW per day) and (2) three GGs (Nellore [NN], ½Senepol½Nellore [SN], and ½Angus½Nellore [AN]). Animals were allocated in 12 paddocks composed of *Urochloa brizantha* (A. Rich.) Stapf. cv. Xaraés for 99 days during the rainy season. Regardless of the GG, the intakes of total DM, supplement DM, OM, CP, aNDFom, EE, and NFC were increased in animals supplemented with a corn-based supplement. The SN bulls had a greater digestibility of DM, OM, and CP, and animals supplemented with a corn-based supplement had greater CP and EE digestibility. There was an interaction between GG and SS for NFC digestibility, which was decreased in AN animals fed a corn-based supplement. However, the corn-based supplementation improved the animal's performance and carcass characteristics as demonstrated by the increase of final BW

Abbreviations: ADG, average daily gain; AN, ½Angus½Nellore; aNDFom, neutral detergent fiber assayed with a heat-stable amylase and expressed exclusive of residual ash; BW, body weight; CH_4, methane; CP, crude protein; CaG, carcass gain; DM, dry matter; DMI, dry matter intake; dOM, digestible organic matter; EE, ether extract; FT, fat thickness; FE, fecal excretion; FP, fecal production; GPH, gain per hectare; GG, genetic group; iNDF, indigestible neutral detergent fiber; ME, metabolizable energy; N, nitrogen; NFC, non-fiber carbohydrate; NN, Nellore; NPN, non-protein nitrogen; OM, organic matter; REA, rib-eye area; SEM, standard error of the mean; SN, ½Senepol½Nellore; SS, supplementation strategy; and VFA, volatile fatty acids.

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(kg), ADG (kg), REA (cm^2), and FT (mm). Moreover, NN animals fed a corn-based supplement showed an increase in ADG (kg). An interaction between SS and GG was observed for GPH (kg/ha) and CaG (kg), with the greatest values observed in NN and SN animals supplemented with a corn-based supplement. Enteric CH_4 emissions (g/d, g/kg of DMI, and g/kg of dOM) were lower in animals fed a corn-based supplement. A decrease in CH_4 emissions (g/d) was observed in SN compared to NN animals. In addition, there was an interaction between SS and GG for CH_4 emissions (g/kg of CaG), with the lowest values for NN and SN animals supplemented with a corn-based supplement. Taken together, our results demonstrate that corn-based supplementation is an effective nutritional strategy for use in the rainy season, especially for NN and SN genetic groups, to improve animal's performance and carcass characteristics and to decrease enteric CH_4 emissions, per unit of product, of growing beef bulls grazing tropical grass.

1. Introduction

Brazil is one of the largest beef cattle producers in the world, and,the pasture-based system represents the bulk of its beef cattle production system (Romanzini et al., 2018). This system relies on tropical grasses that vary greatly in herbage production and nutritional value during the year. Due to the seasonality of tropical grasses, they can rarely provide a balanced diet for grazing animals, and their utilization by cattle is considered sub-optimal (Detmann et al., 2014). Therefore, supplementation has been used to optimize the grazing systems by correcting possible nutritional imbalances of the forage, resulting in improvements in pasture utilization and animal efficiency (de Oliveira et al., 2016).

The supplementation strategy used is dependent on the forage characteristics and nutritional composition. Well-managed tropical grasses such as *Urochloa brizantha* cv. Xaraés may have adequate productivity and nutritional value in the rainy season, with CP ranging from 12 % to 18 % (Neto et al., 2015; San Vito et al., 2016). However, the supplementation of non-fiber carbohydrate (NFC) and N supplementation in the rainy season can provide additional energy to grazing cattle allowing greater uptake of rapidly degrading N compounds in the rumen (Detmann et al., 2005). In addition, it is well known that cereal grain feeding decreases methane (CH_4) production (Blaxter, 1962) by increasing the synthesis of propionate in the rumen and decreasing the population of ruminal methanogens microorganisms (Granja-Salcedo et al., 2016).

For the livestock industry worldwide, the mitigation of CH_4 emissions is important considering the constant pressure to reduce their negative environmental impact (Tedeschi et al., 2015). Enteric CH_4 emissions also have a negative impact on productivity, since they may represent a loss of 5–7 % of dietary gross energy (Hristov et al., 2013). Studies have shown that CH_4 emissions decrease as the concentrate ratio in the diet increases, due to modifications in the ruminal fermentation and methanogenic activity (Christophersen et al., 2008; Moss et al., 2000; Sejian et al., 2011).

Animal genetic improvement is another strategy used to mitigate enteric CH_4 emissions (Hayes et al., 2016). *Bos indicus* animals, such as Nellore, are the major type of beef cattle used in Brazil due to their adaptability to tropical climate conditions and ability to use low-quality forages (Maciel et al., 2019). On the other hand, *Bos taurus* animals have greater potential yield; however, these animals require appropriate conditions to express their genetic potential (Ducatti et al., 2009). Maciel et al. (2019) observed greater performance and lower CH_4 emissions intensity (per kg of meat produced) in crossbred Angus × Nellore when compared to Nellore. Thus, crossbred animals represent a viable alternative to improve productivity without losing the adaptability of pure-bred cattle in tropical conditions.

Therefore, the objectives of this study were to evaluate the effects of supplementation strategies (mineral or corn-based supplement) and genetic groups (NN, AN, and SN) on the intake, apparent digestibility, performance, and CH_4 emissions of growing beef bulls grazing tropical grass during the rainy season. Our hypothesis was that an improvement in animal performance and carcass characteristics and a decrease in CH_4 emissions would result from an associative effect between supplementation strategy and genetic group.

2. Material and methods

Animal care and handling used in this experiment was in accordance with the Brazilian College of Animal Experimentation (COBEA – Colégio Brasileiro de Experimentação Animal) guidelines and was approved by the Ethics, Bioethics, and Animal Welfare Committee (CEBEA – Comissão de Ética e Bem Estar Animal) of the São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences (FCAV), Jaboticabal, SP, Brazil (protocol # 5628/15).

2.1. Animals, treatments, and experimental design

The study was carried out on land owned by the Department of Animal Science of Unesp-FCAV, Jaboticabal, SP, Brazil (21°15′22′' S and 48°18′58′' W at 595 m altitude), in the rainy season, from December 2015 to March 2016. The climate of the region is characterized by hot and rainy summers and dry winters with mild temperatures (Köppen International System: Aw). During the experiment, the average temperature was 24.4 °C, with minimum and maximum monthly average temperatures of 19.8 and 31.5 °C, respectively. Average monthly accumulated precipitation was 306, 445, 206, and 127 mm for each consecutive experimental month, respectively. The experiment lasted 99 d and consisted of 14 d for adaptation and 85 d for intake, digestibility, performance, and CH_4 emission evaluations.

One hundred sixty-two testers ($n = 54$ per genetic group, used to evaluate treatments) growing beef bulls averaging (mean ± SD) 10 ± 2 months old and 262 ± 31 kg of initial body weight (BW) and eight regulator animals (used to maintain the pasture sward height at 30 cm) were evaluated. Prior to the start of grazing, animals were weighed, with a fasting period of 16 h, for adjustment of the supplementation rate and BW gain determinations; this procedure was repeated every 28 days (without fasting). Then, the animals were subjected to endo- and ectoparasite management (Ivomec Injectable, 200 mg/kg of BW, Merial Brasil, Campinas, SP, Brazil). Animals were allocated in 12 paddocks of 1.8 ha each composed of *Urochloa brizantha* (A. Rich.) Stapf. cv. Xaraés. Each paddock was fitted with smooth wire fencing, a water trough, and a collective feed bunk. The experimental area was fertilized with 48 kg/ha of P_2O_5 and 37 kg/ha of K_2O in November 2015, and with nitrogen as urea divided into three applications of 25 kg/ha of N each in December 2015 and February and March 2016.

After 14 d of adaptation, 18 animals ($n = 3$ per treatment) were slaughtered in a commercial slaughterhouse to be used as a reference for carcass gain. Then, 144 animals were distributed according to BW in a randomized complete block design in a 2 × 3 factorial arrangement. Factors included (1) two supplementation strategies (mineral × corn-based supplementation; SS) and (2) three genetic groups (NN, SN, and AN; GG), totaling six treatments, 12 animals per paddock, and two paddocks per treatment. Animals were randomly assigned to receive one of two diets that were as follows: (1) supplementation of mineral mix at a rate of 0.3 g/kg of BW and (2) supplementation with a corn-based supplement at a rate of 3 g/kg of BW. Supplementation can modulate DM intake through associative effects such as substitutive, additive, or combined effects (Moore, 1980) and a supplement intake up to 0.3 % of BW can have an additive effect on pasture intake without causing a substitution effect on it (Herd, 1997). The mineral salt supplement provided (per kg of DM) 10 g of Na, 139 g of Ca, 80 g of P, 40 g of S, 5000 mg of Zn, 1350 mg of Cu,1040 mg of Mn, 100 mg of I, 80 mg of Co, and 26 mg of Se. The corn-based supplement was composed of 735 g/kg of ground corn, 106 g/kg of soybean, and 159 g/kg of mineral mix (Table 1). Animals were supplemented daily at 10 a.m. in a collective covered feed bunk and had free access to water.

2.2. Pasture evaluation

The grazing method used was continuous stocking with a variable (put-and-take) stocking rate (Allen et al., 2011), which was adjusted through regulator animals ($n = 8$) used to maintain the pasture sward height at 30 cm (Pedreira et al., 2007). Sward height was measured every 28 days in each paddock using a graduated stick at 80 random points (Barthram, 1985). To estimate forage mass, forage was sampled every 28 days by cutting the forage mass inside five randomly selected circular frames (0.25 m^2) to ground level (5-cm residual height) with hand shears before grazing. Forage samples were weighed, subsampled, and dried under forced air for 72 h at 55 °C.

For nutrient composition determination, forage samples were obtained every 28 days, on the same days as estimation of DMI (later described), through manual simulation of grazing by the hand-plucking method, which is a methodology designed to mimic forage

Table 1
Proportion of ingredients and chemical composition of the experimental feed.

Item	Corn-based supplement	*Urochloa brizantha* (A. Rich.) Stapf. cv. Xaraés
Proportion of ingredient, g/kg		
Ground corn	735	–
Soybean meal	106	–
Mineral mix[1]	159	–
Chemical composition[2], g/kg		
DM	860	332 + 3.67
OM	892	926 + 1.55
CP	205	128 + 3.67
aNDFom	265	575 + 7.55
EE	63	24.2 + 0.32
iNDF	–	176 + 4.11
NFC	569	198 + 6.58
Forage mass, kg/ha		
Total forage mass	–	6288 + 1175
Green leaves	–	2441 + 525
Stem+sheath	–	2429 + 528
Dead material	–	1418 + 416
Sward height, cm	–	27.73 + 0.237
Stocking rate, AU/ha[3]	–	4.75 + 0.162
Protein fraction[4], % of CP		
A	–	214 + 0.608
B1 +B2	–	371 + 0.552
B3	–	313 + 0.368
C	–	103 + 0.208

[1]Provided (per kg of DM): 80 g of Na, 153 g of Ca, 30 g of P, 30 g of S, 1925 mg of Zn, 520 mg of Cu, 400 mg of Mn, 30 mg of I, 38 mg of Co, 10 mg of Se, and 620 g of non-protein nitrogen (NNP). [2]DM = dry matter; OM = organic matter, CP = crude protein, aNDFom = neutral detergent fiber assayed with a heat-stable amylase and expressed exclusive of residual ash, EE = ether extract, iNDF = indigestible neutral detergent fiber, NFC = non fiber carbohydrates. [3]AU = animal unit (standardized to a relative body weight of 450 kg). [4]Protein fractions were determined according to Licitra et al. (1996), A = protein fraction A, B1 + B2 = protein fractions B1 + B2, B3 = protein fraction B3, and C = protein fraction C.

selection by grazing cattle (Johnson, 1978). A further subsample was collected; separated into green leaves, stems, sheaths, and dead content; and used to characterize the composition of the forage consumed by the animals. In addition, supplement samples were collected every 28 days for chemical composition determinations.

2.3. Feed intake and apparent digestibility

Thirty-six animals ($n = 6$ per treatment) were used for forage and supplement intake estimation and digestibility evaluation, which were performed by marker methods.

Fecal production was assessed using chromium oxide (Cr_2O_3) as an external marker. For that, a Cr_2O_3 capsule (10 g/animal/day) was directly introduced into the esophagus of the animals at 10 a.m. for 10 consecutive days. Fecal samples were collected in the last 3 days of the dosage period at 11 a.m. and 4 p.m., 9 a.m. and 3 p.m., and 7 a.m. and 2 p.m., on the first, second, and third days of sample collection, respectively. Fecal samples were dried at 55 °C for 72 h in forced air and ground (Wiley mill; Thomas Scientific) through a 1-mm sieve. The Cr_2O_3 concentration in fecal samples was determined by atomic absorption spectrophotometry (Williams et al., 1962). Fecal production was estimated using the following equation:

$$FP = Cr_2O_3 \text{ supplied} / (Cr_2O_3 \text{ in feces} / DM\ 105\ ^\circ C)$$

where FP = fecal production obtained by Cr_2O_3 g (DM/day), Cr_2O_3 supplied = amount of Cr_2O_3 supplied to the animals per day (10 g), Cr_2O_3 in feces = Cr_2O_3 concentration in feces (%), and DM 105 °C = feces DM at 105 °C.

Forage DMI was estimated using indigestible neutral detergent fiber (iNDF) as an internal marker. For that, feces, supplements, and forage (from manual simulation of grazing) samples were dried at 55 °C for 72 h under forced air and ground to pass through a 2-mm screen sieve in a Wiley mill (Thomas Scientific). Then, samples were weighed, placed into ANKOM bags (Filter bag F57; ANKOM Technology Corporation), and incubated in the rumen of cannulated Nellore animals for 288 h (Valente et al., 2011). The NDF concentration of the bags was determined by an Ankom^{200} Fiber Analyzer (Ankom Technology, Fairport, NY, USA), and DMI was calculated according to the equation:

$$\text{Forage DMI} = ((FP \times [iMF] - DMIS \times [iMS])) / [iMH]$$

where FP = fecal production; DMIS = DMI of the supplement; and iMF, iMS and iMH are internal marker concentration in feces, supplement, and forage, respectively. The total intake was obtained by the sum of forage intake and supplement intake.

The estimation of individual supplement DM intake was performed using titanium dioxide (T_iO_2) as an external marker (Titgemeyer et al., 2001). For that, 10 g/animal/day was added to the supplement and offered to the animals for 10 days. Fecal samples were collected in the last 3 days of the dosage period as previously described for Cr_2O_3. Additionally, fecal samples were processed as previously described for Cr_2O_3. The TiO_2 concentration was determined by atomic absorption spectrophotometry according to Myers et al. (2004). Individual supplement DMI was estimated according to the equation:

$$\text{Supplement DMI} = (FP \times [eMO / eMF]) / [eMO / eMS]$$

where FP = fecal production, eMO = g of external marker (TiO_2) offered, eMF = g of external marker (TiO_2) in the feces, and eMS = g of external marker (TiO_2) in the supplement.

The apparent total-tract digestibility of the diets was calculated according to the following equation:

$$\text{Digestibility (g/d)} = [DMI \text{ or } NI - DMe \text{ or } Ne] / DMI \text{ or } NI$$

where DMI = dry matter intake or NI = nutrient intake (g/d), and DMe = dry matter excretion or Ne = nutrient excretion (g/d).

2.4. Animal performance and carcass characteristics

Animal performance was determined by the difference between initial and final BWs taken after a16h fasting period, which were determined at the beginning and end of the rearing phase, with a total period of 85 days of evaluation. The average daily gain (ADG) was calculated as the difference between the final (FBW) and the initial BW (IBW) divided by the total number of experimental days.

Eighteen reference animals ($n = 3$ per treatment) were slaughtered after 14 d of adaptation at the beginning, and 24 animals ($n = 4$ per treatment) were slaughtered at the end of the rearing phase in a commercial abattoir following the humane procedures required by Brazilian legislation (Brazil, 2000). Hot carcass weight (HCW) was recorded immediately after the carcass was cleaned and used for carcass gain determinations. Carcasses were then refrigerated at 4 °C for approximately 24 h. The fat thickness (FT) and rib-eye area (REA) were measured at 24 h *postmortem* on the *Longissimus thoracis* (LT) from the left side of each carcass, at the 12th rib level. The REA was traced on transparencies and measured with a planimeter. The FT measurements were taken on the subcutaneous fat after a cross-section in the LT with an electronic digital caliper (799 A series, Starrett®; Athol, MA, USA).

Carcass gain (CaG) was determined via the comparative slaughter technique. Data from the reference animals were used to estimate carcass gain (CaG) for each treatment by the linear equation $Y = aX \pm b$, where body weight (BW) is the independent variable (Y) and hot carcass weight (HCW) is the dependent variable (X).

Eqs. 1, 2, and 3 refer to the first reference slaughter from NN, AN, and SN animals, respectively, and were as follows:

$$Y = 0.635\ x - 29{\cdot}580\ (R^2 = 0.971) \quad (1)$$

$$Y = 0.499\ x - 2{\cdot}554\ (R^2 = 0.879) \quad (2)$$

$$Y = 0.504\ x - 4{\cdot}246\ (R^2 = 0.959) \quad (3)$$

Eqs. 4, 5, and 6 refer to the second reference slaughter from NN, AN, and SN animals supplemented with a corn-based supplement and were as follows:

$$Y = 0.781\ x - 90{\cdot}388\ (R^2 = 0.944) \quad (4)$$

$$Y = 0.766\ x - 95{\cdot}628\ (R^2 = 0.983) \quad (5)$$

$$Y = 0.499\ x - 1{\cdot}092\ (R^2 = 0.986) \quad (6)$$

Eqs. 7, 8, and 9 refer to the second reference slaughter from NN, AN, and SN animals receiving a mineral supplement and were as follows:

$$Y = 0.371\ x + 51{\cdot}317\ (R^2 = 0.685) \quad (7)$$

$$Y = 0.543\ x - 15{\cdot}002\ (R^2 = 0.959) \quad (8)$$

$$Y = 0.404\ x + 17{\cdot}214\ (R^2 = 0.922) \quad (9)$$

2.5. Methane emissions measurements

Enteric CH_4 emissions were evaluated using the sulfur hexafluoride (SF_6) tracer method described by Johnson et al. (1994). Thirty-six animals ($n = 6$ per treatment) were evaluated, and enteric CH_4 emissions were measured during 6 consecutive days for 24 h per day, from day 79 of the grazing period.

Fourteen days prior to the beginning of the CH_4 sampling, animals were fitted with gas collection halters to allow their adaptation to the equipment. In addition, a pair of permeation capsules with constant and known SF_6 emission were introduced directly into the rumen of each animal via the esophagus.

Expired gases were continuously collected with a sampling apparatus that consisted of a halter with stainless-steel capillary tubing (0.127 mm) and an in-line filter (15 μm) placed on the animal's head, connected to a pre-evacuated collection canister (polyvinyl chloride; PVC) placed on the animal's neck. The collection canisters were pre-evacuated to create a negative internal pressure. For the CH_4 sampling, as the vacuum in the collection canister was slowly dissipated, the negative pressure steadily drew an air sample from around the animal's mouth and nose. An additional identical set of canisters (two per day) were placed near the experimental pasture to collect the background concentration of CH_4 and SF_6 at the same time canisters were collected from the animals.

During the CH_4 sampling period, animals were moved to a chute and the canisters were removed daily and replaced with evacuated canisters at 9 a.m. After the sampling period, canisters containing an internal negative pressure between 40 % and 60 % of the initial pressure had the final pressure recorded. A final pressure below or above the expected range indicated a leak in the system or a defect on the halter, and therefore, a new halter with an average absorption rate within the stipulated range was placed on the animal.

2.6. Chemical analysis and calculations

Forage, feed, and feces samples were dried at 55 °C for 72 h under forced air; ground (Wiley mill; Thomas Scientific) through a 1-mm sieve; and analyzed for dry matter (DM; method 934,01), organic matter (OM; method 942,05), and ether extract concentration (EE; method 954,02) according to AOAC (1995). Neutral detergent fiber (NDF) concentration was determined with a heat-stable amylase and expressed exclusive of residual ash (aNDFom) according to Mertens et al. (2002) with adaptations for the Ankom^{200} Fiber Analyzer. Crude protein concentration was determined in a Leco® combustion N analyzer (Leco FP-528 Carbon/Nitrogen Analyzer, Leco Instruments Inc., St. Joseph, MI; method 990.13, AOAC, 2005). Protein fractions A (non-protein N), B1, B2 and B3 with different solubilities, and C (insoluble) were determined according to Licitra et al. (1996). Non-fibrous carbohydrate (NFC) concentration was determined according to Hall (2000), and metabolizable energy (ME) was calculated as described in NRC (1996).

Concentrations of CH_4 (ppm) and SF_6 (ppt) were determined by a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with a column Porapak Q (2 m × 3 mm i.d., 80–100 mesh, Shimadzu, Kyoto, Japan), a flame ionization detector (FID) for CH_4, and electron capture detector (ECD) was used to determine SF_6 concentration. The CH_4 emission rate for each animal was calculated considering the SF_6 tracer gas flux from a permeation capsule lodged in the animal's rumen of the animals and the background CH_4 concentration in the air according to the following equation (Westberg et al., 1998):

$$CH_4 = CSF_6\ ([CH_4]c - [CH_4]amb) / [SF_6]c$$

where CH_4 = individual daily CH_4 emission, CSF_6 = known SF_6 emission from the permeation capsule present in the rumen, $[CH_4]c$ = CH_4 concentration (ppm) in the canister, $[CH_4]amb$ = CH_4 concentration in the ambient (background), and $[SF_6]c$ = SF_6 concentration (ppt) in the canister. Individual animal CH_4 emissions were expressed as CH_4 production (g/d), CH_4 yield (g/kg of DMI and g/kg of

dOM), and g of CH_4/kg of CaG.

2.7. Statistical analysis

Data were subjected to least square ANOVA using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA). Treatments were the 2 × 3 factorial arrangement of two supplementation strategies (SS = mineral mix and corn-based supplementation) and 3 genetic groups (GG = Nellore, ½Angus½Nellore, and ½Senepol½Nellore). The experimental design was a randomized block design (paddock and initial BW). There were 2 paddocks per treatment and 3 BW block-levels within each paddock. The model considered SS, GG, and their interaction as fixed effects and blocks as random effects. In the end, we had 24 replications per treatment, and 4 replications within each combination of paddock and BW. Normality and homogeneity of the data were tested using the UNIVARIATE procedure of SAS. Means were reported as least square means. Treatments were considered different when $P < 0.05$ by Tukey's test. Results are discussed as all interactions were not significant. The statistical model was as follows:

$$Y_{ijklm} = \mu + SS_i + GG_j + (SS \times GG)_{ij} + P_k + BW(P)_l + \varepsilon_{ijklm}$$

where: Y_{ijklm} = observation; μ = the overall mean; SS_i = the effect of level *i* of supplementation strategy (fixed effect); GG_j = the effect of level *j* of genetic group (fixed effect); $(SS \times GG)_{ij}$ = the effect of the interaction of level *i* of SS and the level *j* of GG (fixed effect); P_k = the effect of level *k* of paddock (random effect); $BW(P)_l$ the effect of level *I* of BW within each paddock (random effect); and ε_{ijklm} = random error with mean 0 and variance σ^2.

3. Results

3.1. Intake and digestibility

No interaction between SS and GG was observed on the intake of DM (g/kg of BW and kg/d), forage DM intake (% of BW and kg/d), supplement DM, OM, CP, OM, CP, aNDF, EE, NFC, or ME. In addition, there was no interaction between SS and GG on the apparent total-tract digestibility of DM, OM, CP, aNDFom, EE, or the CP:DOM ratio (Table 2). However, an interaction between SS and GG was observed for iNDF intake and NFC digestibility. The iNDF intake was lowest in SN animals supplemented with a corn-based supplement (Fig. 1), and NFC digestibility was decreased in AN animals fed a corn-based supplement when compared to mineral supplemented AN

Table 2
Intake and apparent digestibility of growing beef bulls from different genetic groups (GG) subjected to two supplementation strategies (SS) grazing tropical grass in the rainy season.

Item[5]	SS[1]			GG[3]				*P*-value[4]		
	Mineral	Corn-based	SEM[2]	NN	AN	SN	SEM	SS	GG	SS × GG
DM intake (g/kg of BW)	19.4[b]	23.0[a]	1.02	22.0	22.9	20.6	1.31	0.010	0.690	0.060
Forage DM intake (% of BW)	2.34	2.41	0.10	2.30	2.43	2.42	0.12	0.637	0.708	0.132
Intake, kg/d										
DM	5.55[b]	6.79[a]	0.233	6.57	6.30	5.66	0.403	0.010	0.360	0.166
Forage DM	5.45	5.89	0.205	6.02	5.77	5.21	0.411	0.152	0.420	0.141
Supplement DM	0.10[b]	0.91[a]	0.011	0.55	0.53	0.45	0.272	0.001	0.082	0.102
OM	5.05[b]	6.23[a]	0.301	5.98	5.74	5.19	0.384	0.001	0.429	0.149
CP	0.69[b]	0.95[a]	0.052	0.87	0.82	0.77	0.079	0.001	0.364	0.295
aNDF	3.14[b]	3.64[a]	0.211	3.58	3.48	3.09	0.241	0.010	0.579	0.108
EE	0.16[b]	0.23[a]	0.009	0.21	0.20	0.18	0.013	0.010	0.429	0.244
NFC	1.05[b]	1.61[a]	0.082	1.31	1.23	1.14	0.086	0.010	0.415	0.175
iNDF	0.81	0.90	0.042	0.92	0.86	0.77	1.009	0.129	0.193	0.011
Total ME, MJ/kg of DM	9.23	9.29	0.157	9.14	9.15	9.48	0.148	0.632	0.875	0.619
Digestibility, % of DM										
DM	59.70	60.44	0.512	58.87[b]	59.56[b]	61.57[a]	0.699	0.550	0.010	0.163
DOM	63.81	64.13	0.491	63.35[b]	63.21[b]	65.35[a]	0.678	0.390	0.010	0.076
CP	51.45[b]	57.57[a]	1.273	53.22[b]	53.66[b]	55.77[a]	1.701	0.010	0.010	0.241
aNDFom	65.49	63.82	0.610	63.45	64.43	66.24	0.871	0.069	0.093	0.363
EE	55.68[b]	59.29[a]	1.153	56.39	57.25	57.83	1.389	0.001	0.161	0.297
NFC	69.42	68.58	0.908	69.59	67.33	69.91	1.278	0.222	0.143	0.051
CP: DOM, gCP/kgDOM	193.81[b]	217.08[a]	8.037	208.48	203.99	203.86	18.861	0.001	0.459	0.153

[a–b]Least squares means within the same row with different superscripts are significantly different ($P < 0.05$). [1]SS = supplementation strategy (mineral supplement × corn-based supplement) in a forage-based diet (*Uruchloa brizantha* [A. Rich] Stapf. cv. Xaraés). Mineral supplement was fed at a rate of 0.3 g/kg of BW per day. Corn-based supplement was fed at a rate of 3 g/kg of BW/day and was composed of 735 g/kg of ground corn, 106 g/kg of soybean, and 159 g/kg of mineral mix. [2]SEM = standard error of the mean. [3]GG = genetic group (NN = Nellore, AN = ½Angus½Nellore, and SN = ½Senepol½Nellore). [4]SS × GG = interaction between supplementation strategy (SS) and genetic group (GG). [5]DM = dry matter, OM = organic matter, CP = crude protein, CP:DOM = ratio between CP (Crude protein content in diet (g/kg DM) and DOM (digestible organic matter content in diet (g/kg DM), aNDFom = neutral detergent fiber assayed with a heat-stable amylase and expressed exclusive of residual ash, EE = ether extract, NFC = non fiber carbohydrate, and ME = total metabolizable energy as forage + corn (estimated using the NRC, 1996).

and SN animals (Fig. 2).

The SS did not affect the intake of forage DM, iNDF, and ME and the apparent total-tract digestibility of DM, OM, iNDF, NFC, and CP:DOM. However, corn-based supplementation increased the intake of total DM (g/kg of BW and kg/d), supplement DM, OM, CP, aNDFom, EE, and NFC; CP and EE apparent total-tract digestibility; and the CP:DOM ratio (Table 2).

The GG did not affect the intake of total DM, forage DM, supplement DM, OM, CP, aNDF, EE, NFC, iNDF, or ME. In addition, no effects of GG were observed for apparent total-tract digestibility of aNDFom, EE, NFC, or the CP:DOM ratio. However, in relation to the GG, the digestibility of DM, OM, and CP ($P = 0.010$) were greater in SN animals (Table 2).

3.2. Animal performance and carcass characteristics

There was no interaction between SS and GG for initial BW, final BW, average daily gain (ADG kg), rib-eye area (REA cm^2), or fat thickness (FT mm; Table 3). However, an interaction between SS and GG was observed on the gain per hectare (GPH kg/ha) and carcass gain (CaG, kg), with greater values observed for NN and SN animals supplemented with a corn-based supplement (Fig. 3 & 4).

Corn-based supplementation increased final BW, ADG, REA, and FT. Nellore animals showed an increase in ADG and REA, and SN animals had a decrease in the final BW and FT (Table 3).

3.3. Methane emissions

There was no interaction between SS and GG for emissions of CH_4 expressed as g/d, g/kg of DMI or g/kg of dOM (Table 4). However, there was an interaction between SS and GG for the emissions of CH_4 expressed as g/kg of carcass gain (Fig. 5).

Corn-based supplementation decreased CH_4 emissions expressed as g/d, g/kg of DMI, and g/kg of dOM. In addition, no effects of GG were observed on the emissions of CH_4 expressed as g/kg of DMI or g/kg of dOM. However, in relation to GG, the CH_4 emissions expressed as g/d were decreased in SN (Table 4).

4. Discussion

In the present study, the similarity in forage DM intake observed across treatments can be explained by the similar chemical composition and structural characteristics of the pasture, and it shows that forage intake was not limited by non-nutritional factors. The pasture managed at 30 cm height resulted in an average total herbage mass of 6288 kg/ha for all paddocks and did not vary through the experiment. In addition, the pasture provided an average green leaves herbage mass of 2440 kg/ha, indicating the good nutritional value of the ingested forage (Nave et al., 2013). In the current study, the total voluntary DMI of forage was estimated by the use of markers and was in accordance with that reported by Maciel et al. (2019). These authors evaluated for 2 years the effects of breed composition on the enteric CH_4 emissions of 10 months old beef steers in tropical conditions and observed a total forage DMI of 5.9 and 6.2 kg/d for grazing Nellore (year 1: BW = 171.5 ± 19 kg; year 2: BW = 215.8 ± 32 kg) and Angus x Nellore (year 1: BW = 214.2 ± 26; year 2: BW = 242.5 ± 32 kg) animals, respectively.

Despite the comparable age (10 ± 2 months old) between the animals, the BW was greater for AN cattle' sire (286 kg) in relation to NN (268 kg) and SN (230 kg) at the beginning of the trial, which was due to the inherent properties of genetic lines. However, this difference in initial BW was not followed by differences in the intake of the animals from the 3 different GG evaluated in the present study. On the other hand, considering the similar forage chemical composition and forage DM intake among the treatments, the differences in the intakes of total DM (g/kg of BW and kg/d), OM, CP, aNDFom, EE, and NFC were a result of the increase in concentrate intake by the animals fed a corn-based supplement, regardless of GG. The positive additive effect on the intake observed in animals fed a corn-based supplement can occur in response to interactions between the forage and supplement (Moore, 1980) and may directly affect animal performance. Dixon and Stockdale (1999) reported that when animals are fed medium- or high-quality forages,

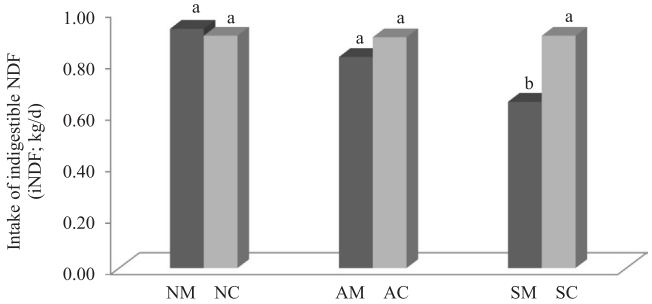

Fig. 1. Interaction between supplementation strategy (SS) and genetic group (GG) on the iNDF intake (kg/day) of growing beef bulls grazing tropical grass in the rainy season. NM = Nellore animals supplemented with minerals, NC = Nellore animals supplemented with a corn-based supplement, AM = ½Angus½Nellore animals supplemented with minerals, AC = ½Angus½Nellore animals supplemented with a corn-based supplement, SM = ½Senepol½Nellore animals supplemented with minerals, and SC = ½Senepol½Nellore animals supplemented with a corn-based supplement. Means followed by different superscripts differ ($P < 0.05$).

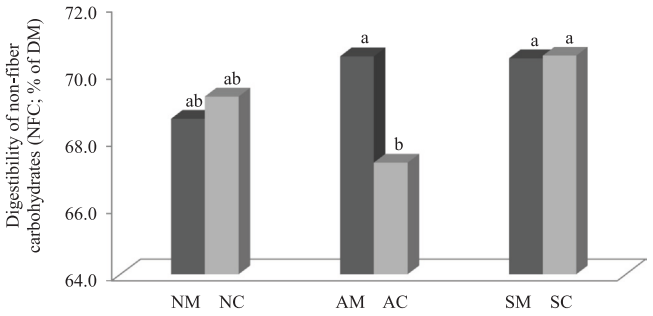

Fig. 2. Interaction between supplementation strategy (SS) and genetic group (GG) on the NFC digestibility (% of DM) of growing beef bulls grazing tropical grass in the rainy season. NM = Nellore animals supplemented with minerals, NC = Nellore animals supplemented with a corn-based supplement; AM = ½Angus½Nellore animals supplemented with minerals, AC = ½Angus½Nellore animals supplemented with a corn-based supplement, SM = ½Senepol½Nellore animals supplemented with minerals, and SC = ½Senepol½Nellore animals supplemented with a corn-based supplement. Means followed by different superscripts differ ($P < 0.05$).

Table 3
Performance and carcass characteristics of growing beef bulls from different genetic groups (GG) subjected to two supplementation strategies (SS) grazing tropical grass in the rainy season.

Item[5]	SS[1]			GG[3]				*P*-value[4]		
	Mineral	Corn-based	SEM[2]	NN	AN	SN	SEM	SS	GG	SS × GG
Initial BW, kg	263	261	15.34	268[b]	286[a]	230[c]	15.38	0.008	0.001	0.295
Final BW, kg	323[b]	334[a]	18.17	342[a]	347[a]	298[b]	18.22	0.032	0.001	0.509
ADG, kg	0.67[b]	0.85[a]	0.035	0.84[a]	0.69[b]	0.76[ab]	0.043	0.001	0.001	0.749
GPH, kg/ha	633	785	34.80	775	641	711	42.61	0.010	0.021	0.050
CaG, kg	0.25	0.38	0.009	0.35	0.28	0.32	0.016	< 0.01	< 0.01	< 0.01
REA, cm^2	61.61[b]	67.22[a]	0.757	68.44[a]	62.25[b]	62.87[b]	1.128	< 0.01	< 0.01	0.749
FT, mm	0.81[b]	1.04[a]	0.139	1.10[a]	0.91[ab]	0.77[b]	0.141	0.010	0.010	0.068

[a–b]Least squares means within the same row with different superscripts are significantly different ($P < 0.05$). [1]SS = supplementation strategy (mineral supplement × corn-based supplement) in a forage-based diet (*Uruchloa brizantha* [A. Rich] Stapf. cv. Xaraés). Mineral supplement was fed at a rate of 0.3 g/kg of BW per day. Corn-based supplement was fed at a rate of 3 g/kg of BW per day and was composed of 735 g/kg of ground corn, 106 g/kg of soybean, and 159 g/kg of mineral mix. [2]SEM = standard error of the mean. [3]GG = genetic group (NN = Nellore, AN = ½Angus½Nellore, and SN = ½Senepol½Nellore). [4]SS × GG = interaction between supplementation strategy (SS) and genetic group (GG). [5]BW = body weight, ADG = average daily gain, GPH = gain per hectare, CaG = carcass gain, REA = rib-eye area, and FT = fat thickness.

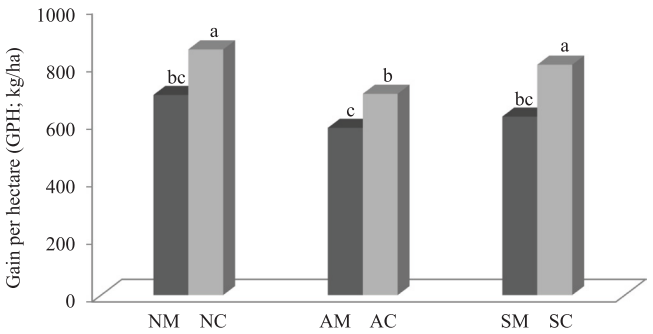

Fig. 3. Interaction between supplementation strategy (SS) and genetic group (GG) on the gain per hectare (GPH; kg/ha) of growing beef bulls grazing tropical grass in the rainy season. NM = Nellore animals supplemented with minerals, NC = Nellore animals supplemented with a corn-based supplement, AM = ½Angus½Nellore animals supplemented with minerals, AC = ½Angus½Nellore animals supplemented with a corn-based supplement, SM = ½Senepol½Nellore animals supplemented with minerals, and SC = ½Senepol½Nellore animals supplemented with a corn-based supplement. Means followed by different superscripts differ ($P < 0.05$).

supplements rich in energy can interact with ruminal microorganisms and induce a negative effect on fiber digestibility. In the present study, corn-based supplementation at a rate of 0.3 % of BW did not affect aNDFom digestibility; however, it provided additional energy to the animals and, in association with the rapidly available nitrogen (fractions A, B1, and B2), resulted in greater average daily gain (ADG) on those animals.

Additionally, improvements in voluntary intake have been associated with an adequate CP:DMO ratio, where a positive response in

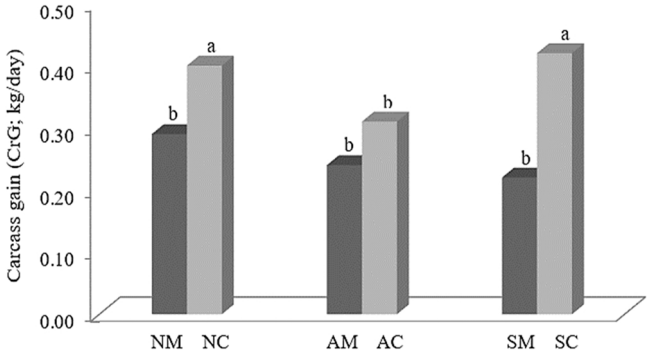

Fig. 4. Interaction between supplementation strategy (SS) and genetic group (GG) on the carcass gain (CaG; kg/day) of growing beef bulls grazing tropical grass in the rainy season. NM = Nellore animals supplemented with minerals, NC = Nellore animals supplemented with a corn-based supplement, AM = ½Angus½Nellore animals supplemented with minerals, AC = ½Angus½Nellore animals supplemented with a corn-based supplement, SM = ½Senepol½Nellore animals supplemented with minerals, and SC = ½Senepol½Nellore animals supplemented with a corn-based supplement. Means followed by different superscripts differ ($P < 0.05$).

Table 4
Enteric methane (CH4) emission of growing beef bulls from different genetic groups (GG) subjected to two supplementation strategies (SS) grazing tropical grass in the rainy season.

Item[5]	SS[1]			GG[3]				*P*-value[4]		
	Mineral	Corn-based	SEM[2]	NN	AN	SN	SEM	SS	GG	SS × GG
CH_4, g/day	130[a]	104[b]	2.052	122[a]	117[ab]	112[b]	2.899	< 0.01	0.020	0.290
CH_4, g/kg of DMI	24.51[a]	15.74[b]	0.435	19.45	19.55	21.33	0.817	< 0.01	0.534	0.141
CH_4, g/kg of dOM	41.42[a]	27.13[b]	1.128	33.72	33.51	35.40	2.036	< 0.01	0.263	0.081
CH_4, g/kg of CaG	475	281	19.35	356	403	375	21.91	< 0.01	0.030	0.001

[a–b]Least squares means within the same row with different superscripts are significantly different ($P < 0.05$). [1]SS = supplementation strategy (mineral supplement × corn-based supplement) in a forage-based diet (*Uruchloa brizantha* [A. Rich] Stapf. cv. Xaraés). Mineral supplement was fed at a rate of 0.3 g/kg of BW per day. Corn-based supplement was fed at a rate of 3 g/kg of BW per day and was composed of 735 g/kg of ground corn, 106 g/kg of soybean, and 159 g/kg of mineral mix. [2]SEM = standard error of the mean. [3]GG = genetic group (NN = Nellore, AN = ½Angus½Nellore and SN = ½Senepol½Nellore). [4]SS × GG = interaction between supplementation strategy (SS) and genetic group (GG). [5]DMI = dry matter intake, dOM = digestible organic matter, and CaG = carcass gain.°C.

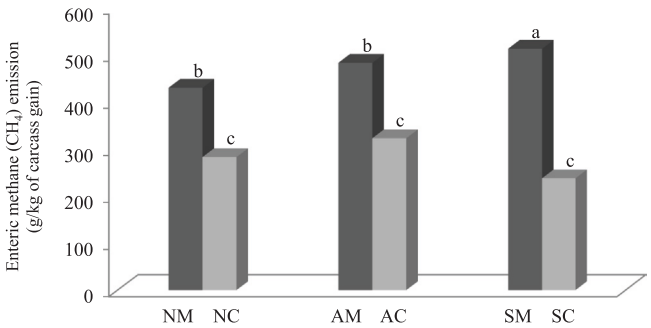

Fig. 5. Interaction between supplementation strategy (SS) and genetic group (GG) on the enteric methane (CH_4) emission (g/kg of carcass gain) of growing beef bulls grazing tropical grass in the rainy season. NM = Nellore animals supplemented with minerals, NC = Nellore animals supplemented with a corn-based supplement, AM = ½Angus½Nellore animals supplemented with minerals, AC = ½Angus½Nellore animals supplemented with a corn-based supplement, SM = ½Senepol½Nellore animals supplemented with minerals, and SC = ½Senepol½Nellore animals supplemented with a corn-based supplement. Means followed by different superscripts differ ($P < 0.05$).

intake is observed when the ratio is 210 g CP/kg DOM (Poppi and McLennan, 1995), and losses of protein will occur when the CP content of the diet exceeds this value. Detmann et al. (2014) used a meta-analytical approach to evaluate the effects of nitrogen supplementation in cattle fed tropical grass pastures in Brazil. The authors observed an increase in intake with a CP/kgDOM ratio up to 288 g. Detmann et al. (2010), evaluating data from 20 trials conducted in Brazil during the growing season of the grasses, stated that a nutritional unbalance can be observed on tropical grasses due to their seasonality. Moreover, the authors concluded that it increased heat production, which in turn, limits intake. Therefore, increasing dietary CP through supplementation can optimize intake in animals

under tropical conditions. This agrees with the present study, which observed an increase in the intake with greater CP:DMO ration in animals receiving supplementation.

Although the difference in initial BW was not followed by statistical differences in the intake of the animals from the 3 different GG evaluated in the present study, the intakes of DM (g/kg of BW and g/d), forage DM and supplement DM were numeric lower for SN animals. Additionally, the nutrient digestibility was affected by GG, with greater values of DM, DOM, and CP digestibilities showed by SN animals compared to NN and AN. Studies have demonstrated differences in digestive capacities between *Bos taurus* and *Bos indicus* cattle (Hungate et al., 1960; Hunter and Siebert, 1985). *Bos indicus* animals evolved under a challenging environment with marginal feeding conditions, which made them more adapted to those situations. Such adaptability resulted in greater digestibility and ruminal fermentation rates compared to *Bos taurus* animals (Hungate et al., 1960; Hunter and Siebert, 1985). Therefore, the increase in DM, OM, and CP digestibilities and ADG observed for SN compared to AN can be explained by the greater adaptability to tropical conditions exhibited by *Bos indicus* crossbred animals.

The relationship between CP concentration and digestible OM can alter the amount of N reaching the intestine and the transfer of ingested protein to animal protein. Additionally, immediately after rain, tropical grasses cause rapid synthesis of NH_3 in the rumen, which can result in protein loss if energy is not available (Poppi and McLennan, 1995). Therefore, this is the period when concentrate supplementation would be most effective to synchronize energy and NH_3 release in the rumen and improve animal performance. In the present study, the good nutritive value of the forage (128 g/kg of CP, DM basis) associated with the increase in CP and energy intakes and CP and EE digestibilities of animals supplemented a with corn-based supplement resulted in an increase of GPH and CaG for animals from NN and SN genetic groups. These results demonstrate the positive effect of GG and corn-based supplementation in the rainy season. It can be inferred that GG in association with supplementation may have caused an increase in N use efficiency and consequently resulted in an increase in the animal's ADG, REA and FT. Additionally, this increase in productivity makes the animal production system more profitable and may contribute to the reduction of the environmental impacts caused by beef production. Maciel et al. (2019) evaluated the effects of energetic-protein supplementation (CP = 207 g/kg of DM, containing corn gluten meal, soybean meal and mineral salt) on growing beef cattle grazing *Megathyrsus maximus* cv. Mombaça pasture. The authors observed a DMI of 1.12 kg/day for both breeds and reported an ADG of 0.67 kg/day, which is close to 0.77 kg/day observed on animals from N and AN genetic group in the current study.

Animal performance is affected not only by dietary factors but also by the genetics of the animal (Hodgson, 1990). *Bos indicus* animals are more thermotolerant and therefore are less affected by heat stress when grazing under tropical conditions compared to *Bos taurus* (Hansen, 2004). Animals outside their thermal comfort zone expend more energy to maintain their body temperature, which reduces the energy available for production (Nardone et al., 2006). In the present study, the increase in ADG and the greater REI in the tropically adapted breeds (NN and SN) may have reflected their adaptation to heat stress. Furthermore, considering that greater muscle deposition is a genetic characteristic of European-origin cattle breeds, and that AN animals started the trial with the greatest BW, a greater REA was expected for AN animals. However, our results showed the opposite and demonstrated the superiority of the NN compared to AN crossbred animals when grazing tropical grass in the rainy season.

Ribeiro et al. (2009), evaluating the heat tolerance of NN, SN, and AN heifers, observed better adaptation by SN cross heifers during the heat tolerance test. The superior heat tolerance of SN animals reported by those authors may help to explain the similar GHP and CaG between NN and SN animals observed in the current study. Additionally, although the greatest initial BW was observed in AN animals, the ADG, CaG, REA, and FT were greater to NN compared to AN, demonstrating the advantages of these genetic group in relation to AN under tropical conditions.

According to IPCC (2019), the average annual enteric CH_4 emissions from growing steers in Latin America is 129 g of CH_4/animal/day. In the present study, the enteric CH_4 emissions of concentrate-supplemented cattle were lower (104 *vs.* 129 g/day), and those of mineral-supplemented cattle were similar (130 *vs.* 129 g/day) to those reported by IPCC (2019). The difference in CH_4 emissions can be due to a different efficiency of fermentation process between animals, which is linked to changes in metabolic processes (Edwards et al., 2008) and to host-microorganism interactions (Pinares-Patiño et al., 2013). Such interactions are known to be altered as diet changes and result in modification of the composition and activity of the rumen microbiota to improve the use of the available substrates. Therefore, the different enteric CH_4 emissions observed in the current study may be partially due to differences in diet composition, especially in the amount of starch between the two SS evaluated. This difference may have caused a modification in the microbiota composition, and therefore in the efficiency of the fermentation process, resulting in lower enteric CH_4 emissions in animals supplemented with a corn-based supplement.

In the current study, NN and SN animals can be considered more efficient than AN when supplemented with corn. These animals had lower initial BW and the similar forage DM intake, yet, emitted 10 % less CH_4 per unit of product (g/kg of CaG) than AN animals. Considering animals consuming the same diet, the individual characteristics of the animal play an important role in the mitigation of CH_4 emissions (O'Hara et al., 2003). Therefore, the selection of more efficient animals can be an alternative to reduce CH_4 emissions per unit of product (Waghorn and Hegarty, 2011).

Although the literature reports divergent results related to animal efficiency and enteric CH_4 emissions (Mercadante et al., 2015; Méo-filho et al., 2020), a decrease in CH_4 production has been found in more efficient animals. Maciel et al. (2019), evaluating the effects of animal breed and production system (pasture *vs.* feedlot) on enteric CH_4 emissions, reported that growing AN crossbred animals had an increase in total CH_4 production (grazing plus feedlot) compared to NN. That finding is in line with our results of CH_4 emissions per unit of produced product (356 *vs.* 403 g/kg of CaG for NN and AN, respectively) and demonstrates that the genetic improvement in AN animals did not guarantee lower enteric CH_4 emissions under grazing conditions. However, the similar results of CH_4 emissions (g/kg of CaG) between NN and SN animals supplemented with a corn-based supplement in the current study highlights the importance of identifying more efficient cattle breeds for the livestock industry worldwide, especially due to the increasing public

concern about and constant pressure to reduce the negative environmental impact (Tedeschi et al., 2015).

The present study's results for NFC digestibility, GPH, CaG, and enteric CH_4 emissions (per unit produced product), demonstrate the importance of and dependence between the two evaluated factors (SS and GG). Animals that had greater carcass gain and lower enteric CH_4 emissions were more efficient in using the extra energy provided by the corn-based supplement, as observed in NN and SN. Taken together, our findings support the hypothesis that an improvement in animal performance and carcass characteristics and a decrease in CH_4 emissions would result from an associative effect between supplementation strategy and genetic group.

5. Conclusion

Crossbred animals may be an option to increase animal performance and reduce CH_4 emissions per kg of meat produced in beef bulls grazing tropical grass in the rainy season. Additionally, corn-based supplementation can be used in the rainy season to improve animal performance and carcass characteristics and to decrease CH_4 emissions per unit of product of growing beef bulls, especially for NN and SN animals grazing tropical grass.

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CRediT authorship contribution statement

T.A. Simioni: Data curation, Formal analysis, Investigation, Writing – original draft. **J.D. Messana:** Data curation, Formal analysis, Investigation, Supervision, Methodology, Writing – original draft, Writing – review & editing. **L.G. Silv:** Data curation, Visualization, Writing – original draft, Writing – review & editing. **L.F. Brito:** Writing – original draft, Writing – review & editing. **J.A. Torrecihas:** Investigation, Methodology. **Y.T Granja-Salcedo:** Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **E. San Vito:** Conceptualization, Data curation, Investigation, Supervision, Methodology. **J.F. Lage:** Conceptualization, Methodology. **R.A. Reis:** Conceptualization, Data curation, Methodology. **T.T. Berchielli:** Conceptualization, Funding acquisition, Supervision, Methodology.

Conflicts of interest

The authors declare no conflicts of interest associated with this project or the article.

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