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Effect of diurnal feeding times and sources of energy supplementation to optimise rearing of F1 Angus × Nellore young bulls

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ABSTRACT

Context. Energy supplementation with highly fermentable carbohydrates can be a strategy to maximise the efficiency of nitrogen use (ENU) from high-nutritional value forages. **Aims.** The study aimed to investigate the independent or associated effects of two diurnal feeding times (0900 or 1700 hours) with two sources of energy supplementation (corn or citrus pulp) in the growing of F1 Angus × Nellore young bulls on palisade grass pastures, and their implications on the forage chemical composition, nutrient intake and digestibility, ENU, microbial protein synthesis, animal performance, and gain per area. **Methods.** There were 36 bulls used as experimental animals, with initial bodyweight (BW) of 290 ± 5 kg, and 32 similar bulls were used in a put-and-take system to maintain sward characteristics. The experiment was conducted in a complete randomised design with four treatments and three replications (paddocks). The treatments consisted of energy supplementation at 0.3% BW with corn or citrus pulp fed at 0900 or 1700 hours for four periods of 28 days. For evaluation of ruminal and blood parameters, ENU, intake, and nutrient digestibility, eight ruminal cannulated 1/2 Aberdeen Angus × 1/2 Nellore young bulls were used, with 280 ± 7 kg of initial BW, distributed in a 4 × 4 double Latin square design, consisting of four periods and four treatments. **Key results.** Supplementation at 0.3% of BW with corn or citrus pulp at 0900 or 1700 hours can reach up to 1.06 kg/day of young bulls reared on palisade grass pastures fertilised with 180 kg nitrogen/ha/year and managed at 25 cm of height compared with corn-based supplements. There were no effects on microbial protein synthesis or its efficiency. **Conclusions.** Citrus pulp can be used as an alternative energy source to corn, because it has the same potential for animal performance, ENU and microbial protein synthesis in tropical pastures with a high proportion of soluble protein, and structural and non-fibrous carbohydrates with high dry matter digestibility. **Implications.** Supplementing grazing beef cattle at 0.3% BW with corn or citrus pulp in the morning or afternoon is an efficient nutritional strategy to improve animal performance.

Keywords: animal performance, beef cattle, citrus pulp, corn, microbial protein, palisade grass, ruminal parameters, supplementation time.

Introduction

In intensive cattle production systems, pastures fertilised with high doses of nitrogen (N) have high amounts of soluble protein, making energy supplementation essential to capture excess N of forage, and ensure the rumen balance between N and energy (Poppi and McLennan 1995). The energy source and the supplementation time combined with the grazing behavior of animals may directly affect the efficiency of nutrients used from the forage and, consequently, the responses in production.

The high degradability of N compounds in intensively managed tropical pastures, which can reach 14–15% of crude protein (CP), and 40–50% of soluble protein (De Oliveira *et al.* 2016; Delevatti *et al.* 2019*a*; Hoffmann *et al.* 2021; Leite *et al.* 2021), associated with the high content of slow degradation structural carbohydrates of forage, can compromise the efficiency of nitrogen use (ENU) and microbial protein synthesis (Detmann *et al.* 2014). However, this condition generates excessive ammonia (NH_3) losses in the urine, generating a protein limitation relative to energy for high weight gains (Poppi *et al.* 2018). This N loss represents economic inefficiency, and can harm the environment through N losses in the form of volatilised NH_3, nitrous oxide emission and nitrate leaching (Aboagye *et al.* 2018; Cardoso *et al.* 2019).

To minimise this problem, energy supplementation with highly fermentable carbohydrates can be a strategy to maximise the ENU from high nutritional value forages and microbial protein synthesis (Costa *et al.* 2011, 2019; Barbero *et al.* 2015; De Oliveira *et al.* 2016; Ferrari *et al.* 2021; Fonseca *et al.* 2022). The supply of metabolisable energy, combined with suitable amounts of rumen degradable protein in the forage, can capture the excess of rumen N by providing fermentable substrate containing starch, pectin, sugars or digestible fibre, depending on the energy source (Poppi and McLennan 1995; Costa *et al.* 2011; Barbero *et al.* 2015). Consequently, there will be a better balance between the availability of energy and NH_3 in the rumen and, thus, greater animal performance (Poppi and McLennan 1995; Barbero *et al.* 2015; Ferrari *et al.* 2021).

In this scenario, citrus pulp has shown promising results in animal nutrition as an alternative to corn due to its positive effects on rumen fermentation (Ariza *et al.* 2001). As it is a byproduct of the orange juice industry and other citrus fruits, citrus pulp is a less expensive ingredient and does not compete with humans as a food source, unlike corn (Mottet *et al.* 2018). It has been shown that pectin promotes greater and faster capture of NH_3 from degradation, reducing ruminal ammonia nitrogen ($N\text{-}NH_3$), in addition to maximising microbial protein synthesis when compared with the addition of starch from corn (Ariza *et al.* 2001; Costa *et al.* 2011; De Oliveira *et al.* 2016). Moreover, ruminal fermentation of pectin leads to an increase in acetic acid production, and does not affect the production of lactic and propionic acids, which is in contrast to starch fermentation that decreases the rumen pH due to lactic and propionic acid formations (Van Soest 1994; Owens and Basalan 2016). However, although citrus pulp and corn are readily available sources, further studies evaluating the associated and/or independent effects of the energy supplement source and times to grazing animals are needed to maximise microbial protein synthesis and performance.

Besides the type of energy substrate (Poppi *et al.* 1997), the responses to energy supplementation may occur due to the supplementation timing, coinciding with the end of the main grazing events during the day. Studies on ingestive behavior by ruminants and specifically Nellore cattle show that the animals' longest period of grazing occurs during the afternoon, more specifically between 1400 and 1700 hours (Thomsom *et al.* 1985; Páscoa 2009; Casagrande *et al.* 2011). Thus, the supply of energy supplements in the late afternoon after the intense intake of forage with a high amount of soluble N can be an efficient strategy to optimise animal performance, as the excess of N might be partly assimilated by the energy substrate for MCP.

This research hypothesises that energy supplementation, mainly citrus pulp supplied in the late afternoon, could optimise the use of forage with high CP and soluble N contents by capturing this N in the rumen or by independent effects of the carbohydrate being fermented. This could result in greater efficiency of MCP and, consequently, greater animal performance. Therefore, the objectives of this study were to investigate the independent or associated effects of two times of supplementation (0900 or 1700 hours) with two sources of energy supplementation (corn or citrus pulp) during the rearing of F1 Angus × Nellore young bulls on palisade grass pastures, and their implications on nutrient intake and digestibility, ENU and MCP, animal performance, and gain per area and stocking rate.

Materials and methods

The experiment was approved by the UNESP Council of Animal Experimentation and Animal Use, Campus Jaboticabal, under protocol 015234/19. The experiment was conducted at the Forage and Grasslands sector of the Sao Paulo State University 'Julio de Mesquita Filho' (UNESP), in Jaboticabal, SP, Brazil (21°15′22″S latitude, 48°18′58″W longitude and 595 m elevation). The soil of the experimental area is classified as Ferralsol (Embrapa 2013). According to the Köeppen system, the climate is Aw type (tropical, characterised by dry winters).

The experiment was conducted in the rainy season of 2019/2020, between December 2019 and April 2020, during the rearing phase of young bulls. Animals underwent a 15-day adaptation period to the experimental conditions and the diet, and subsequent 112 days of experimental evaluation.

The meteorological data were measured daily through the UNESP/FCAV Agro-Meteorological Weather Station. Over the whole experimental period, the average temperature was 24.2°C, with a minimum of 20.4°C and a maximum of 30.9°C, the average precipitation was 156.2 mm, and the insolation time was 203.0 h.

Experimental design

The experiment was conducted using a complete randomised design in a 2 × 2 factorial arrangement, with four treatments and three replications (paddocks) per treatment. Treatments consisted of energy supplementation at 0.3% dry matter (DM)

Table 1. Composition of supplements provided to F1 Angus × Nellore young bull during rearing phase.

Item	Corn	Citrus pulp
Ingredients proportion (%DM)		
Ground corn grain	95.0	–
Ground citrus pulp	–	95.0
Mineral premix[A]	5.0	5.0
Chemical composition of supplements (g/kg DM)		
Dry matter	889.8	901.3
Organic matter	914.4	863.1
Ash	85.6	137.0
Crude protein	106.0	82.9
NDF	114.1	302.6
iNDF	52.6	74.7
Ether extract	37.0	24.0
NFC	657.3	544.6
TDN	850.0	770.0
Starch	720.0	2.0
Pectin	–	235.0

[A]Guarantee levels: 16.2 g/kg Na; 45.9 g/kg Cu; 28.2 g/kg Mn; 170.0 g/kg Zn; 2.5 g/kg Co; 3.4 g/kg I; 0.8 g/kg Se.
DM, dry matter; NDF, neutral detergent fibre; iNDF, indigestible NDF; TDN, total digestible nutrients.

of bodyweight (BW) with corn or citrus pulp at 0900 or 1700 hours. The evaluated supplements are described in Table 1 with respect to ingredients and chemical composition.

Animals and pasture management

We used 36 1/2 Aberdeen Angus × 1/2 Nellore young bulls (aged 14 ± 2-months) as experimental animals, with mean initial BW (IBW) of 290 ± 5 kg, and eight 1/2 Aberdeen Angus × 1/2 Nellore young bulls (aged 14 ± 2-months), cannulated in the rumen, with an IBW of 280 ± 7 kg, distributed in a double 4 × 4 Latin square design. In addition, 32 young bulls were also used as put-and-take animals to adjust the stocking rate and maintain 25 cm of pasture height, with the same genetic pattern and IBW of 256 ± 8 kg.

Before the beginning of the experimental period, all animals were weighed, identified with numbered ear tags, and endo- and ectoparasites controlled using albendazole sulfoxide (Agebendazol 15%, 7.5 mL/head, Agener Uniao®, Sao Paulo, SP, Brazil) as a vermifuge, and fluazuron pour-on (Acatak, 30 mL/head, Novartis®, Campo Grande, MS, Brazil) for cattle tick control. Experimental animals were distributed into the treatments based on average BW, and the adjustment of stocking rate per paddock was based on the canopy height. The experiment with experimental animals had a total duration of 112 days, divided into four periods of 28 days.

Cannulated animals were kept in the same paddocks as experimental animals during the experiment, and received the same type and amount of supplement for each treatment (0.3% BW). First, these animals underwent a 15-day adaptation period to the pastures and the supplement. The total experimental period for cannulated animals was 84 days, divided into four periods of 21 days. The first 15 days were destined for diet adaptation, and the last 6 days were for collections of ruminal fluid, blood, urine and faeces. The eight cannulated animals were randomly divided into four pairs (two cannulated/treatment), rotating in all treatments throughout the four experimental periods. These two animals were allocated in separate paddocks of the same treatment; therefore, within the three paddocks of each treatment, only two received cannulated bulls.

Experimental animals were used to evaluate animal performance, whereas cannulated animals were used to assess nutrient intake and digestibility, urinary, ruminal, and blood parameters.

The experimental pastures were palisade grass (*Urochloa brizantha* R. D. Webster cv. Marandu). The total experimental area was 13 ha, of which 1 ha was reserve area, and 12 ha for the evaluations of treatments and replications. The site was divided into 12 paddocks (three paddocks/treatment), provided with an open trough with 40 linear cm per animal and a water trough, with fresh, clear water. Each paddock allocated three experimental animals (n = 9 animals/treatment). The management corral is round, and equipped with a cattle chute and a digital scale.

Maintenance fertilisation of pastures was performed before the animals' adaptation period and determined according to the soil analysis results, following the recommendation of Technical Bulletin 100 (Van Raij *et al.* 1997). Nitrogen fertilisation of all paddocks was provided by ammonium nitrate (32% N), fractionated in three applications of 60 kg N/ha/year, totalling 180 kg N/ha/year (11 December 2019; 31 January 2020; 28 February 2020).

Pastures were managed under continuous stocking with a variable stocking rate, following the put-and-take technique (Mott and Lucas 1952) to maintain 25 cm of height and at a grazing utilisation efficiency of 50%. The stocking rate was adjusted using put-and-take animals. For this assay, the height/forage mass ratio (bulk density) was evaluated weekly to estimate the forage mass, and adjust the stocking rate using the put-and-take animals by measuring 80 random points of each paddock with a graduated ruler (Barthram 1985).

Previous studies showed that under these experimental conditions, 25 cm of height promotes 95% light interception, which allows achievement of maximum net forage accumulation, with high leaf production and low senescence, resulting in high-quality forage allowance that may increase animal performance (Barbero *et al.* 2015; de Araújo *et al.* 2021; Hoffmann *et al.* 2021).

Forage mass and morphological composition of pasture

To determine forage mass, forage allowance and morphological composition, every 28 days, 80 points/ha of palisade grass height were randomly measured with a graduated ruler (Barthram 1985). From the average height, three representative samples of grass were collected per paddock by cutting at 5-cm height from the soil, all forage contained in a 0.25-m^2 metallic frame. Each forage sample was subdivided into two subsamples, one for determination of DM concentration and the other for determination of the morphological composition, fractionating them into leaf, stem + sheath and dead material. After drying in an oven with forced air circulation at 55°C for 72 h, total DM and morphological components were obtained to estimate the total forage mass. The forage allowance (kg DM/kg BW) was calculated as forage mass divided by the animal stocking rate, and multiplied by the percentage of leaves to determine the leaf allowance.

Forage mass, forage and leaf allowances, and morphological composition of pastures are presented in Table 2.

Chemical composition of the forage

To evaluate the chemical composition of forage, hand-plucked samples of each paddock were taken every 28 days by collecting the forage at average canopy height after closely observing the animal grazing behaviour, avoiding causing the animals stress, to represent all forage consumed during the day (Sollenberger and Cherney 1995). After drying in an oven with forced air circulation at 55°C for 72 h to determine DM, samples were ground in a Willey mill with a 1-mm sieve and then submitted to chemical analysis, following the methodologies of AOAC (2012). Chemical components analysed were as follows: total N (CP) by the Kjeldahl method; acid detergent fiber (ADF) and neutral detergent fiber (NDF) corrected for ash and protein (apNDF) by the ANKOM fiber analyser method (ANKOM Technology, USA); lignin by the acid hydrolysis method; ash; and ether extract (EE) by the Goldfish method. The organic matter (OM), hemicellulose and cellulose contents were calculated by the difference between previously determined fibrous components, following the Eqns 1–3:

$$\text{OM}\,(\%) = 100 - \%\text{Ash} \quad (1)$$

$$\text{Hemicellulose}\,(\%) = \%\text{NDF} - \%\text{ADF} \quad (2)$$

$$\text{Cellulose}\,(\%) = \%\text{ADF} - \%\text{Lignin} \quad (3)$$

The fractionation of N was determined following the Cornell system (Sniffen *et al.* 1992). Fraction A was obtained by analysing the hand-plucked samples in trichloroacetic acid to extract soluble N (Detmann *et al.* 2012), and later calculated by the difference between the total N content and the non-protein nitrogen (NPN). The B3 fraction was calculated by the difference between the neutral detergent-insoluble nitrogen (NDIN) and the acid detergent-insoluble nitrogen (ADIN) contents. Fraction C, in turn, was considered ADIN, described by Sniffen *et al.* (1992) and thoroughly discussed by Tedeschi and Fox (2020). Finally, Fraction B1 + B2 (true protein) was calculated as the difference between the total N and the other fractions (B1 + B2 = total N − (A + B3 + C)).

Forage carbohydrate fractions were obtained from Eqns 4–6 proposed by Sniffen *et al.* (1992), respectively, for total carbohydrates (TC), non-fibrous carbohydrates (NFC) and total digestible nutrients (TDN). The chemical composition, carbohydrate and protein fractions of forage are presented in Table 3.

$$\text{TC}\,(\%) = 100 - (\%\text{CP} + \%\text{EE} + \%\text{Ash}) \quad (4)$$

$$\text{NFC}\,(\%) = 100 - (\%\text{CP} + \%\text{EE} + \%\text{apNDF} + \%\text{Ash}) \quad (5)$$

$$\text{TDN}\,(\%) = \text{CPad} + \text{NFCad} + \text{NDFd} + 2.25 \times \text{EEad} \quad (6)$$

where 'ad' means apparently digestible fractions and 'd' means digestible fractions.

Nutrients intake and digestibility

Intake by animals was determined based on faecal production of cannulated bulls, using indigestible neutral detergent fibre (iNDF) as an internal marker and chromium oxide (Cr_2O_3) as an external marker, which is the standard used for investigative purposes in Brazil, and approved by the local and national Ethics Committees on the Use of Animals.

Table 2. Forage mass, forage and leaf allowances, stocking rate, and morphological composition of palisade grass pastures fertilised with 180 kg N/ha/year during the rearing phase of F1 Angus × Nellore young bulls, supplemented with 0.3% BW with corn or citrus pulp at 0900 or 1700 hours.

	0900 hours		1700 hours	
	Corn	Citrus pulp	Corn	Citrus pulp
Forage mass (kg DM/ha)	7720	7870	8360	8660
Forage allowance (kg DM/kg BW)	3.7	4.1	4.0	4.1
Leaf allowance (kg DM/kg BW)	0.8	1.0	1.1	1.0
Leaves (%)	25.7	23.4	25.5	22.9
Stem + sheath (%)	27.8	27.6	31.4	29.0
Dead material (%)	46.5	49.0	43.1	48.1
Leaf/stem	1.0	0.9	0.9	0.9

DM, dry matter; BW, body weight.

Table 3. Chemical composition, and carbohydrate and protein fractions of palisade grass pastures fertilised with 180 kg N/ha/year during the rearing phase of F1 Angus × Nellore young bulls, supplemented with 0.3% BW with corn or citrus pulp at 0900 or 1700 hours.

	0900 hours		1700 hours	
	Corn	Citrus pulp	Corn	Citrus pulp
Dry matter (g/kg)	941	941	945	942
Organic matter (g/kg DM)	913	918	915	914
Crude protein (g/kg DM)	142	140	142	138
Ether extract (g/kg DM)	24	23	24	23
NDF (g/kg DM)	682	693	675	683
apNDF (g/kg DM)	574	573	601	558
pdNDF (g/kg DM)	392	391	398	354
Hemicellulose (g/kg DM)	360	357	352	358
ADF (g/kg DM)	323	337	323	325
Cellulose (g/kg DM)	126	128	128	121
Lignin (g/kg DM)	18	19	21	16
TDN (g/kg DM)	570	576	595	606
Carbohydrate fractionation (g/kg DM)				
TC	747	754	749	753
NFC	173	179	149	197
iNDF	188	197	186	187
Protein fractionation (g/kg CP)				
Fraction A	355	332	294	268
Fraction B1 + B2	180	215	217	245
Fraction B3	223	228	238	234
Fraction C	242	225	251	255

DM, dry matter; NDF, neutral detergent fibre; apNDF, NDF corrected for ash and protein; pdNDF, potentially digestible NDF; ADF, acid detergent fibre; CP, crude protein; TC, total carbohydrates; NFC, non-fibrous carbohydrates; TDN, total digestible nutrients; iNDF, indigestible NDF.

For this assay, 10 g of Cr_2O_3/animal/day were wrapped in paper and allocated directly in the rumen of each cannulated animal for 10 days at the same time, of which the first 7 days for adaptation and the last 3 days for faeces collection (Hopper *et al.* 1978). The supply of Cr_2O_3 began on the eighth day of each cannulated experimental period, and collections started on the 15th day. Faecal collections were performed twice a day, according to the schedule: first day – 0700 and 1900 hours; second day – 1100 and 2300 hours; 3rd day – 1500 and 0300 hours.

After collection, faeces samples were dried in an oven with forced air circulation at 55°C for 72 h, weighed, and constituted a composited sample of 3 days of collection per animal and period.

Faecal recovery of Cr_2O_3 was determined following the methodology of Williams *et al.* (1962), and, from these data, faecal excretion (FE) was calculated through the equation proposed by Detmann *et al.* (2001; Eqn 7). Forage intake was calculated based on iNDF concentrations of forage and faeces (Eqn 8).

$$\mathrm{FE}\left(\frac{\mathrm{kg}}{\mathrm{day}}\right)=\frac{\mathrm{Cr_2O_3\ provided\ (g/day)}}{\mathrm{Cr_2O_3\ concentration\ (g/kg\,DM)}} \quad (7)$$

$$\text{Forage intake}\left(\frac{\mathrm{kg\ DM}}{\mathrm{day}}\right)=\frac{\mathrm{FE}\left(\frac{\mathrm{kg}}{\mathrm{day}}\right)\times \mathrm{iNDF_{faeces}}(\%)}{\mathrm{iNDF_{forage}}(\%)} \quad (8)$$

To estimate supplement intake of cannulated animals, titanium dioxide (TiO_2) was used as an external marker (Titgemeyer *et al.* 2001). Approximately 10 g TiO_2/animal/day were provided for 10 days, being properly homogenised to the supplement immediately before feeding the animals from the group supplement trough. Each paddock's total amount of TiO_2 was provided according to the number of grazing animals. The indicator concentration in individual animal faeces was analysed by atomic absorption spectrophotometry, and a standard curve was established with concentrations 0, 2, 4, 6, 8 and 10 (Myers *et al.* 2004). Individual animal supplement intake was calculated following Eqn 9.

$$\text{Supplement intake}\left(\frac{\mathrm{kg}}{\mathrm{day}}\right)=\frac{\mathrm{FE}\left(\frac{\mathrm{kg}}{\mathrm{day}}\right)\times \mathrm{TiO_{2_{faeces}}}\ (\%)}{\mathrm{TiO_{2_{supplement}}}\ (\%)} \quad (9)$$

Nutrient digestibility was estimated through the quantification of the iNDF content. Samples of forage, faeces and supplements were individually placed in filter bags model F-57 from ANKOM brand, and allocated in the rumen of cannulated animals for *in situ* incubation for 288 h (Norris *et al.* 2019). After removing the bags from the rumen, they were washed until completely clear, dried in a forced circulation oven at 55°C for 72 h and then in a non-ventilated oven at 105°C for 45 min. In sequence, bags were subjected to extraction with neutral detergent in an ANKOM fibre analyser, and the entire drying procedure was repeated to quantify iNDF, according to Detmann *et al.* (2001). Nutrient intake and digestibility by cannulated animals were calculated from these data, following Eqns 10 and 11, respectively, considering the total intake of forage and supplement (diet).

$$\mathrm{TNI}\left(\frac{\mathrm{kg}}{\mathrm{day}}\right)=\frac{\mathrm{FE}\left(\frac{\mathrm{kg}}{\mathrm{day}}\right)\times \mathrm{iNDF_{faeces}}(\%)}{\mathrm{iNDF_{diet}}(\%)\times \mathrm{NC_{diet}}(\%)} \quad (10)$$

$$\text{Digestibility of nutrient}\ (\%)=\frac{\mathrm{TNI}\left(\frac{\mathrm{kg}}{\mathrm{day}}\right)-\mathrm{FE}\left(\frac{\mathrm{kg}}{\mathrm{day}}\right)}{0\mathrm{TNI}\left(\frac{\mathrm{kg}}{\mathrm{day}}\right)}\times 100 \quad (11)$$

where TNI refers to total nutrient intake, FE to fecal excretion and NC is the nutrient concentration of diet (forage + supplement).

Efficiency of N use and microbial protein synthesis

Spot samples of urine were collected from each cannulated animal simultaneously with faecal collections: first day at 0700 and 1900 hours, second day at 1100 and 2300 hours, and third day at 1500 and 0300 hours (Chizzotti *et al.* 2008; Silva Júnior *et al.* 2018). Composite samples were obtained from the six collections for each animal per period.

Urine was immediately filtered, and 10 mL aliquots were diluted with 40 mL of 0.072 M sulfuric acid (H_2SO_4) to prevent bacterial degradation of purine derivatives (PD) and precipitation of uric acid. Samples were stored at −15°C until analysis.

Concentrations PD, such as uric acid (UA) and allantoin (ALLA), were determined using the commercial Analisa kits by enzymatic-colorimetric methodology (Cat. 451/MS80022230065), and Young and Conway (1942), respectively.

The total excretion of PD was calculated by summing the amounts of ALLA and UA excreted in the urine, expressed in mmol/day. Absorbed purines (Pabs) were calculated from the excretion of PD, following Eqn 12. Then, the MCP (g/day) was calculated as a function of Pabs, using Eqn 13 (Detmann *et al.* 2014).

$$\text{Pabs}\left(\frac{\text{mmol}}{\text{day}}\right) = \frac{\text{PD} - (0.385 \times \text{BW}^{0.75})}{0.85} \quad (12)$$

$$\text{MCP}\left(\frac{\text{g}}{\text{day}}\right) = \left(\frac{70 \times \text{Pabs}}{0.93 \times 0.137 \times 1000}\right) \times 6.25 \quad (13)$$

The ENU was calculated following Eqn 14, described by Detmann *et al.* (2014).

$$\text{ENU} = \frac{\text{N balance}\left(\frac{\text{g}}{\text{day}}\right)}{\text{N ingested}\left(\frac{\text{g}}{\text{day}}\right)} \quad (14)$$

where 70 corresponds to the N concentration in the purines (mg N/mol); 0.137 to the ratio N purine:total N in the bacteria (Barbosa *et al.* 2006); and 0.85 to the digestibility of microbial purines. The N balance was calculated from the sum of the N excretions in the faeces and the urine, subtracted from the N ingestion.

Another aliquot of concentrated urine was used to estimate the total N concentration by the Kjeldahl methodology (Fenner 1965), and the urea concentration through commercial Analisa kits by the colorimetric-enzymatic method (Cat. 427/MS80022230063). Creatinine concentration in the spot sample was also determined through commercial Analisa kits using the colorimetric method with alkaline picrate (Cat. 435/MS80022230066) and subsequently used to estimate the urinary volume. The daily creatinine excretion (CE) was calculated by the following Eqn 15, proposed by Chizzotti *et al.* (2008):

$$\text{CE}\left(\frac{\text{mg}}{\text{day}}\right) = 37.88 \times \text{BW}^{0.9315} \quad (15)$$

The nitrogen excreted via urine was obtained by multiplying the urine volume by urinary N concentration (Chizzotti *et al.* 2008).

Ruminal and blood parameters

To evaluate the pH, N-NH_3 and short-chain fatty acids (SCFA), ruminal fluid samples were collected from the cannulated animals every three alternate hours in the last 2 days of each experimental period. The ruminal fluid was collected at the solid-liquid interface and immediately filtered in a triple layer of gauze for pH measurement with a digital pH analyser, calibrated with pH 7.0 and 4.0 buffers.

One millilitre of sulfuric acid (1:1) was added to a 50-mL sample of ruminal fluid and stored at −20°C for subsequent analysis of N-NH_3 using the Kjeldahl methodology (Fenner 1965). Analysis of SCFA was performed using concentrated ruminal fluid samples, following Serafim *et al.* (2021) methodology on a Shimadzu high-performance liquid chromatography system (model Prominence) equipped with an ultraviolet detector model SPD-20 and programmed to operate at a wavelength of 210 nm. The internal standard for the high-performance liquid chromatography was the crotonic acid. The ruminal fluid samples were centrifuged at 6026*g* for 5 min and filtered through a 13-mm diameter nylon filter (0.22-μm pore size). The injection volume was 20 μL. The quantified SCFAs were formic, lactic, acetic, propionic, butyric, isovaleric and valeric acids.

Total gas production and *in vitro* dry matter degradability

The *in vitro* dry matter degradability (IVDMD) and total gas production trials were conducted at Premix Company Research Center Laboratory, located in Patrocinio Paulista, SP, Brazil.

The analysis was performed on samples of hand-plucked forage, supplement and composite samples of forage + supplement in the real proportion of animal DM intake (88% and 12%, respectively), following the methodology of Bueno *et al.* (2005, 2008). For this assay, 1 g of the substrate (forage and/or supplement samples) was weighed and incubated in previously weighed and identified glass bottles. The composite sample substrate was formulated by mixing 0.88 g (DM) of forage and 0.12 g (DM) of supplement (corn or citrus pulp).

In addition to the substrate, 90 mL of aqueous culture media were added to each bottle, consisting of a solution of microminerals, macrominerals, buffer solution, resazurin solution, medium B and distilled water. After inserting the

culture medium, 10 mL of ruminal fluid was added to each bottle, which was collected on the day of incubation from two cannulated bulls, immediately capped, homogenised and incubated in a forced air circulation oven at 39°C with constant movement and shaker type. Those animals were kept in Marandu grass pastures and adapted to a 0.3% BW energy supplement (810 g/kg TDN, 95 g/kg CP), and the average BW was 310 ± 7 kg.

The IVDMD was determined through the recovery of three bottles of the non-degraded fraction of each treatment, using filtration of the residue in a glass crucible previously prepared with glass wool (Bueno *et al.* 2005, 2008).

For forage samples incubation, the IVDMD was measured at seven different timepoints: 0, 3, 6, 12, 24, 48 and 72 h after incubation. The treatments were Marandu grass pastures where animals were supplement with corn at 0900 hours, citrus pulp at 0900 hours, corn at 1700 hours and citrus pulp at 1700 hours (n = 4). We used two forage samples/treatment, and the IVDMD was evaluated by recovering two bottles of the non-degraded fraction of each treatment at each timepoint, totalling 14 bottles/treatment.

For supplement and forage + supplement incubations, the IVDMD was measured at six different timepoints: 0, 3, 6, 12, 24 and 48 h after incubation. For supplements only, the treatments were corn or citrus pulp (n = 2), and we used three forage samples/treatment, so the IVDMD was evaluated through the recovery of three bottles of the non-degraded fraction of each treatment at each timepoint totalling 18 bottles/treatment.

For the forage + supplement incubation, the treatments were Marandu grass + corn or Marandu grass + citrus pulp (n = 2), and four samples/treatments were used, so the IVDMD was evaluated through the recovery of four bottles of the non-degraded fraction of each treatment in each timepoint, totalling 24 bottles/treatment.

The potential degradability was calculated following the equation of Ørskov and McDonald (1979) (Eqns 16, 17).

$$\mathrm{DE} = A + B(1 - \mathrm{e}^{-CT}) \tag{16}$$

where potential degradability is the potential degradability (%) in incubation time T; A is the fraction of feed that degrades instantly; B is the fraction insoluble in water, but potentially degradable in the rumen; C is a constant rate of degradation of fraction B.

The effective degradability (DE) of feed, allowing for rate of passage, was calculated by the equation below.

$$\mathrm{DE} = A + \left[\frac{B \times C}{C + \mathrm{kd}}\right] \times (1 - \mathrm{e}^{-(C+\mathrm{kd})T}) \tag{17}$$

where DE is the effective degradability (%); kd is the degradation rate, estimated by regression analysis.

Total gas production was measured on all remaining bottles throughout the timepoints before recovering the bottles to determine IVDMD, using a transducer and a data logger, the total gas volume was determined in psi and later converted to mL. For forage samples, measurements were taken on all bottles at times 0, 6, 12, 18, 24, 32, 40, 48, 60 and 72 h after incubation. For supplement and forage + supplement samples, gas production was measured at times 0, 3, 6, 12, 24, 32, 40 and 48 h after incubation.

Animal performance

To determine the ADG of experimental animals, weighing was performed at the beginning (IBW), after the adaptation period and the end (final BW (FBW)) of the total experimental period, after a 14-h feed and water fasting. The ADG was calculated by dividing the difference between FBW and IBW by the number of days (112 days). In addition, every 28 days after the adaptation period, we conducted an intermediate weighing, with no previous fasting, to adjust the stocking rate, grazing height and supplement quantity.

The days of the occupation of the put-and-take animals in each paddock were recorded for further calculation of stocking rate and gain per area. The gain per area was calculated according to the experimental ADG, and the number of put-and-take animals and experimental animals of each paddock during the evaluation period (Mott and Lucas 1952). The stocking rate in animal units (AU) per hectare (450 kg BW/ha), forage and leaf allowances were determined from the total BW of the animals in each paddock in each period.

Statistical analysis

Data were analysed for the homoscedasticity and normality of the residues, using the Box–Cox and Cramer–von Mises tests, respectively, using the Nortest package of the R program (R Core Team 2018). For animal performance evaluation, the paddock was assumed as the experimental unit (three paddocks/treatment), considering the mean composed of three animals in each paddock fed the same supplement and forage. The IBW was added as a covariate on the FBW and ADG, and the interactions between the main factors and the IBW were removed if not significant. The ANOVA was conducted using a complete randomised design procedure, in a 2 × 2 factorial arrange, using the Nortest package of the R program (A × B; Factor A – two energy supplementation sources: corn and citrus pulp; Factor B – two supplementation times: 0900 and 1700 hours). Treatments were considered fixed effects, whereas paddocks and periods were random effects.

For variables including nutrient intake and digestibility, ENU, urine, ruminal and blood parameters, we considered cannulated animals and periods as experimental units (two cannulated animals/treatment). ANOVA was conducted in a double 4 × 4 Latin square design, with repeated measures over time (*lme*), considering four experimental periods, to

assess collection times (h), using the Agricolae package of the R program (R Core Team 2018). Latin square, the animal within a square and collection times were considered fixed effects. The period was considered random effects. The best covariance structure used for repeated-measures analyses was chosen as the one that achieved the lowest corrected Akaike and Bayesian information, significant effects for treatment were declared at $P < 0.05$. When a significant effect was found, the means of the four treatments were compared by the Tukey honest significance test test at 5% probability. When the period was significant, orthogonal polynomial contrasts were used to identify the effect of periods.

Results

Total intake and digestibility of nutrients

The sources and times of energy supplementation did not interfere with the intake and digestibility of nutrients from forage and supplement ($P > 0.05$) (Table 4). However, the ratio g CP/kg DOM was lower in animals that received corn compared with citrus pulp both times ($P = 0.002$), with no difference among times for the same source ($P > 0.05$), both for corn and citrus pulp, neither between the sources at 0900 hours ($P > 0.05$).

Efficiency of N use and microbial protein synthesis

The intake, excretion, retention of N, as well as the excretion of urea by cannulated animals were similar among treatments ($P > 0.05$; Table 5). In addition, the microbial protein synthesis, the g MCP/kg DOM ratio and the ENU by the animals were not affected by the sources and/or time of energy supplementation ($P > 0.05$).

Ruminal parameters

The N-NH_3 of the animals supplemented with corn was higher than citrus pulp ($P = 0.005$), with means of 14.92 and 13.03 mg/dL, respectively, and the supplementation at 0900 hours promoted lower N-NH_3 compared with 1700 hours ($P = 0.052$). During collection times, N-NH_3 values differed both between sources and between supplementation times ($P < 0.001$); however, there were no interactions between sources, supplementation times and collection times ($P = 0.712$; Table 6). The peak of N-NH_3 occurred at approximately 1800 hours in animals supplemented with corn at 0900 and 1700 hours, and with citrus pulp at 0900 hours.

The pH of animals supplemented with citrus pulp was, on average, higher than those supplemented with corn

Table 4. Total intake and digestibility of nutrients (forage + supplement) in cannulated F1 Angus × Nellore young bulls grazing palisade grass pastures and supplemented with 0.3% BW with corn or citrus pulp at 0900 or 1700 hours.

	0900 hours		1700 hours		s.e.m.	*P*-value*		
	Corn	Citrus pulp	Corn	Citrus pulp		Source	Time	Source × time
Total intake								
DMI (kg/day)	7.0	6.7	7.3	6.7	0.61	0.342	0.686	0.776
DMI (% BW)	2.2	2.2	2.3	2.2	0.13	0.111	0.680	0.683
OMI (kg/day)	6.5	6.1	6.8	6.2	0.47	0.246	0.650	0.744
DOMI (kg/day)	4.8	4.4	5.1	4.5	1.48	0.340	0.595	0.746
CPI (kg/day)	0.9	1.0	0.9	1.0	0.09	0.431	0.903	0.867
g CP/kg DOM	197.8b	224.7a	183.4b	218.1a	8.65	0.002	0.183	0.624
NDFI (kg/day)	4.5	4.5	4.5	4.2	0.36	0.699	0.733	0.702
EEI (kg/day)	0.2	0.2	0.2	0.2	0.01	0.147	0.863	0.666
TDNI (kg/day)	4.6	4.1	4.8	4.2	0.35	0.171	0.517	0.756
Digestibility								
DMD (%)	70.0	71.3	66.5	68.5	3.31	0.275	0.144	0.856
OMD (%)	72.2	71.1	73.9	71.1	2.97	0.308	0.199	0.510
CPD (%)	55.2	56.0	55.7	60.7	4.25	0.595	0.299	0.749
NDFD (%)	69.0	69.0	66.9	66.0	4.20	0.881	0.197	0.909

Source: energy source (corn/citrus pulp); time: supplementation time (0900/1700 hours).
*Within a row, different lowercase letters indicate significance at 0.05.
DMI, dry matter intake; BW, body weight; OMI, organic matter intake; DOMI, digestible organic matter intake; CPI, crude protein intake; CP, crude protein; DOM, digestible organic matter; NDFI, neutral detergent fibre intake; EEI, ether extract intake; TDNI, total digestible nutrients intake; DMD, dry matter digestibility; OMD, organic matter digestibility; CPD, crude protein digestibility; NDFD, neutral detergent fibre digestibility; TDND, total digestible nutrients digestibility; s.e.m., standard error of mean.

Table 5. Efficiency of nitrogen use and microbial protein synthesis of F1 Angus × Nellore young bulls reared in palisade grass pastures and supplemented with 0.3% BW with corn or citrus pulp at 0900 or 1700 hours.

	0900 hours		1700 hours		s.e.m.	*P*-value*		
	Corn	Citrus pulp	Corn	Citrus pulp		Source	Time	Source × time
Urea (mmol/day)	483	645	684	558	82.91	0.810	0.515	0.093
N ingested (g/day)	144	157	145	153	14.57	0.435	0.900	0.871
N excreted (g/day)	69	67	67	63	5.15	0.318	0.473	0.808
N retention (g/day)	74	77	78	90	11.46	0.476	0.393	0.671
ENU (%)	49	55	52	57	3.02	0.143	0.339	0.828
MCP (g/day)	737.4	551.0	591.8	784.0	191.50	0.997	0.647	0.127
g MCP/kg DOM	158	150	124	176	8.16	0.615	0.911	0.156

Source: energy source (corn/citrus pulp); time: supplementation time (0900/1700 hours).
N, nitrogen; Nmic, ruminal synthesis of N compounds; DOM, digestible organic matter; ENU, efficiency of N use; s.e.m., standard error of the mean.

(P = 0.021), but similar between animals supplemented at 0900 and 1700 hours (P = 0.969; Table 6). There was a difference between collection times (P < 0.001); however, there was no interaction between sources and/or supplementation times and collection times (P > 0.05).

In general, animals supplemented with corn in the morning kept the pH consistently lower than those receiving citrus pulp at 0900 hours. In addition, young bulls supplemented with citrus pulp at 1700 hours had a pH range higher than those that received corn at 1700 hours. The lowest pH value occurred around 2100 hours in all supplementation strategies, and animals supplemented in the afternoon had a ruminal pH peak at midday.

Among the SCFAs evaluated, only formic and valeric acids showed differences between sources of energy supplementation (P = 0.005 and P = 0.042, respectively), being lower when the animals were supplemented with corn in relation to the citrus pulp (Table 6). Furthermore, there was an interaction between energy sources and collection times in the concentration of formic acid (P = 0.042). The concentrations of lactic, propionic, butyric and isovaleric acids differed between collection times (P < 0.05). There was no difference in SCFAs concentrations between supplementation times (P > 0.05).

Dry matter *in vitro* degradability

Fraction A of citrus pulp isolated or associated with forage was higher than that of corn, isolated or associated, unlike Fraction B, which was lower in citrus pulp in both cases (Fig. 1). In contrast, the indigestible and unavailable Fraction (C) of citrus pulp isolated was higher than corn, but when associated with forage, both ingredients presented the same amount of Fraction C. The degradation rate (kd) of citrus pulp separated or associated with forage was higher than corn in both situations. The potential degradability of isolated corn was higher than citrus pulp; however, when associated with forage, the forage + corn mixture had lower potential degradability than the forage + citrus pulp mixture. Estimates of degradability at 2, 5 and 8 h revealed that both citrus pulp and the combination forage + citrus pulp had greater potential for DM degradation than corn separated or combined with pasture.

When comparing the *in vitro* degradability profile of corn and citrus pulp isolated, we observed that up to 24 h after incubation, the citrus pulp was greater than corn (P < 0.0001) and, consequently, presented higher gas production (P = 0.0002; Fig. 2). However, between 48 and 72 h of incubation, DM degradation of corn was greater than citrus pulp (P = 0.0001), as well as its gas production (P < 0.0001).

By associating forage with the supplement in the actual proportions of animal intake (88% of forage and 12% of supplement), we found that in the first 3 h of incubation, the DM degradation was the same between forage + corn and forage + citrus pulp (P = 0.499), whereas between 6 and 24 h, the association of pasture with citrus pulp showed greater degradation (P = 0.001; Fig. 2). At 48 h after incubation, however, both associations showed the same degradation (P = 0.785). At the same time, gas production remained similar in the first 3 h (P = 0.227), being higher in the forage + citrus pulp association between 6 and 12 h (P = 0.014), and between 24 and 48 h it was similar between forage + corn and forage + citrus pulp (P = 0.244).

Animal performance and gain per area

The F1 Angus × Nellore young bulls supplemented with corn or citrus pulp at 0900 or 1700 hours had similar IBW, FBW and ADG (P > 0.05; Table 7). The gain per area and stocking rate were also similar between sources and times of energy supplementation (P > 0.05).

Table 6. Ruminal parameters of cannulated F1 Angus × Nellore young bulls reared in palisade grass pastures and supplemented with 0.3% BW with corn or citrus pulp at 0900 or 1700 hours.

Ruminal parameter	0900 hours		1700 hours		Collection time[A]								s.e.m.	*P*-value*					
	Corn	Citrus pulp	Corn	Citrus pulp	0300 hours	0600 hours	0900 hours	1200 hours	1500 hours	1800 hours	2100 hours	2400 hours		A	B	CT	A × B	A × CT	B × CT	A × B × CT
N-NH_3 (mg/dL)	14.1a	12.8b	15.7a	13.2b	15.2	10.5	15.8	14.5	15.3	18.7	11.7	10.2	0.23	0.005	0.052	<0.001	0.246	<0.001	<0.001	0.712
pH	6.05b	6.21a	6.06b	6.20a	6.17	6.31	6.17	6.30	6.11	6.18	5.84	5.95	0.0204	0.021	0.969	<0.0001	0.992	0.999	0.200	0.9546
SCFA (mmol/L)																				
Formic acid	4.3b	5.1a	4.0b	4.5a	4.6	4	4	4.2	4.2	5.2	4.8	4.8	0.1	0.005	0.189	<0.0001	0.548	0.042	0.142	0.056
Lactic acid	9.7	11.2	10.5	12.1	5.1	5	9.6	8.1	16.3	10.2	17.8	15.1	1.0	0.236	0.716	0.003	0.468	0.276	0.985	0.479
Acetic acid	49.5	43.0	46.8	47.0	48.1	52.8	48.4	47.1	45.6	44.3	43.8	42.8	1.9	0.272	0.743	0.449	0.372	0.312	0.804	0.793
Propionic acid	17.6	15.1	17.9	15.2	14.8	12.5	9.5	11.1	16.8	21.3	23.3	22.2	1.0	0.784	0.871	0.001	0.735	0.613	1.000	0.914
Butyric acid	10.5	11.0	11.5	11.7	8.3	7.3	7.4	7.9	13.5	15.5	16.7	13.2	0.6	0.628	0.597	<0.0001	0.254	0.414	0.572	0.731
Isovaleric acid	2.4	2.7	2.4	4.0	1.8	1.6	1.5	2.3	4.5	6.6	2.8	1.7	0.4	0.964	0.530	0.006	0.832	0.327	0.345	0.185
Valeric acid	11.0b	12.3a	10.9b	13.4a	13.5	13.9	11.3	11.3	11	10.3	11.1	12.7	0.5	0.042	0.720	0.125	0.848	0.311	0.671	0.625
(% SCFA)																				
Formic acid	4.10b	5.08a	3.85b	4.17a	4.78	4.12	4.36	4.57	3.75	4.59	3.99	4.27	0.1	0.005	0.189	<0.0001	0.548	0.042	0.142	0.056
Lactic acid	9.24	11.16	10.10	11.21	5.30	5.15	10.47	8.80	14.57	8.99	14.80	13.42	1.0	0.236	0.716	0.003	0.468	0.276	0.985	0.479
Acetic acid	47.14	42.83	45.00	43.56	50.00	54.38	52.78	51.20	40.75	39.07	36.41	38.04	1.9	0.272	0.743	0.449	0.372	0.312	0.804	0.793
Propionic acid	16.76	15.04	17.21	14.09	15.38	12.87	10.36	12.07	15.01	18.78	19.37	19.73	1.0	0.784	0.871	0.001	0.735	0.613	1.000	0.914
Butyric acid	10.00	10.96	11.06	10.84	8.63	7.52	8.07	8.59	12.06	13.67	13.88	11.73	0.6	0.628	0.597	<0.0001	0.254	0.414	0.572	0.731
Isovaleric acid	2.29	2.69	2.31	3.71	1.87	1.65	1.64	2.50	4.02	5.82	2.33	1.51	0.4	0.964	0.530	0.006	0.832	0.327	0.345	0.185
Valeric acid	10.48b	12.25a	10.48b	12.42a	14.03	14.32	12.32	12.28	9.83	9.08	9.23	11.29	0.5	0.042	0.720	0.125	0.848	0.311	0.671	0.625

[A]Means of the four treatments.

*Within a row, different lowercase letters indicate significance at 0.05.

N-NH_3, ammoniacal nitrogen; SCFAs, short-chain fatty acids; s.e.m., standard error of mean; Factor A, energy source (corn/citrus pulp); Factor B, supplementation time (0900/1700 hours); CT, collection time.

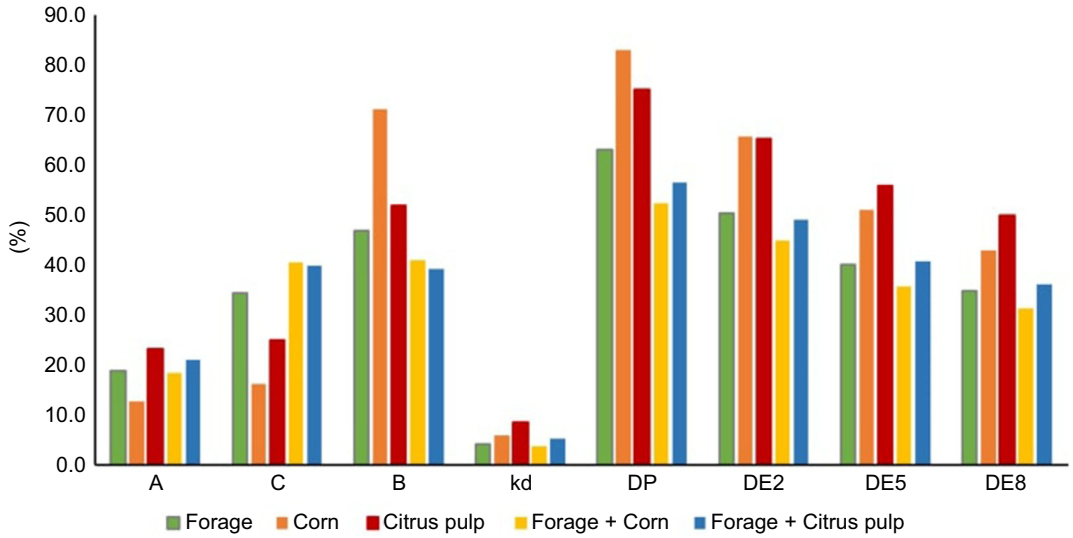

Fig. 1. Ruminal kinetics of forage, corn, citrus pulp and forage associated with energy supplement sources. A, fraction A of carbohydrate, with a rapid rate of ruminal degradation (organic acids and sugars); B, fractions B1 and B2 of carbohydrate, with intermediate-slow degradation rate (starch, pectin, cellulose and hemicellulose); C, fraction C of indigestible and unavailable carbohydrate (lignin); kd, degradation rate; DP, potential degradability; DE2, estimated degradability in 2 h; DE5, estimated degradability in 5 h; DE8, estimated degradability in 8 h.

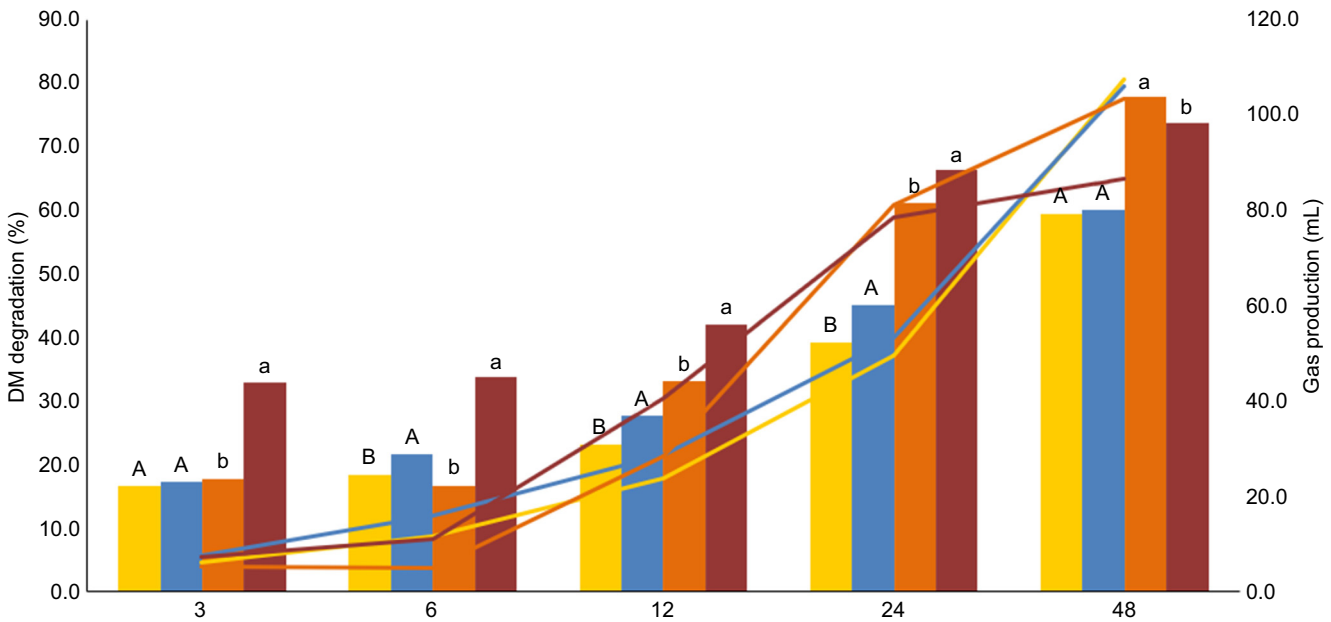

Fig. 2. Dry matter *in vitro* degradability and total gas production of the corn, citrus pulp, and its association with forage. Different uppercase letters indicate significance at 0.05 between forage + corn and forage + citrus pulp. Different lowercase letters indicate significance at 0.05 between corn and citrus pulp.

Discussion

Animal performance and gain per area

The average gain per area in this study, 743.3 kg BW/ha, is higher than the average reported by Ruggieri *et al.* (2020), 525 kg BW/ha, when evaluating cattle reared in palisade grass pastures fertilised with N and managed at 25 cm of height, and supplemented with mineral salt *ad libitum*. Furthermore, the results of this study are above those recorded by Casagrande *et al.* (2011), of 621 kg BW/ha, for Nellore cattle reared in palisade grass pastures managed at

Table 7. Performance, gain per area and stocking rate of F1 Angus × Nellore young bulls reared in palisade grass pastures and supplemented with 0.3% BW with corn or citrus pulp at 0900 or 1700 hours.

	0900 hours		1700 hours		s.e.m.	*P*-value*		
	Corn	Citrus pulp	Corn	Citrus pulp		Source	Time	Source × time
IBW (kg)	312	300	292	320	34.25	0.509	0.996	0.082
FBW (kg)	412	408	403	425	21.17	0.209	0.607	0.099
ADG (kg/day)	0.95	0.91	0.86	1.06	0.35	0.193	0.606	0.076
GPH (kg BW/ha)	775.5	730.5	741.0	726.0	27.0	0.891	0.777	0.792
Stocking rate (AU/ha)	4.6	4.3	4.7	4.7	0.01	0.214	0.764	0.162

Source: energy source (corn/citrus pulp); time: supplementation time (0900/1700 hours).
*Within a row, different letters indicate significance at 0.05 using the Tukey test.
IBW, initial body weight; FBW, final body weight; ADG, average daily gain; GPH, gain per hectare; BW, body weight; AU, animal unit (1 AU = 450 kg BW); s.e.m., standard error of the mean.

25 cm and supplemented at 0.3% with protein-energy based on citrus pulp and cottonseed meal. Petty *et al.* (1998) recorded values even higher (1570–2110 kg BW/ha), with a pangola grass–leucaena-based pasture with or without supplements of corn.

It was expected that 0.3% BW supplementation would not interfere with the stocking rate and, consequently, in the forage mass, because the effect of replacing forage with supplement (substitution) and the consequent increase in the carrying capacity of pastures under supplementation level occurs from 0.8% BW (Euclides *et al.* 2001). Additionally, under the adopted management criteria, even if the animals stopped consuming forage to consume the supplement, other animals would be added into the system to maintain the proposed pasture height and, thus, the canopy structure. The average stocking rate in this study, 4.6 AU/ha, was 4.3 times higher than the national livestock average of 1.06 AU/ha (ABIEC 2020), emphasising that fertilisation with high N doses and better management of sward structure when using the put-and-take technique can increase the stocking rate.

The ADG of bulls ranged from 0.86 to 1.06 kg/day, and did not differ among treatments. As the palisade grass pastures were equally fertilised with 180 kg N/ha/year, and managed at 25 cm of height in a continuous stocking system and variable stocking rate, the pastures presented similar chemical and morphological composition. Additionally, even though supplements had different chemical compositions, the total nutrient intake by animals was similar among treatments. Consequently, there was no effect of treatments on ADG.

Hoffmann *et al.* (2021) showed that low levels (0.3% BW) of a corn-based supplement increases ADG by approximately 33% when compared with mineral salt *ad libitum*, the means were 0.83 kg/day and 1.10 kg/day, respectively. The ADG values recorded here are higher than these values, and demonstrate what high-quality pastures and supplements can achieve, and citrus pulp appears to be a suitable replacement for corn as a supplement on these pasture types. Compared with other studies, the ADG obtained in this experiment is close to that recorded by Hoffmann *et al.* (2021), at 1.1 kg/day, by Nellore bulls reared on palisade grass pastures managed at 25 cm and supplemented at 0.3% BW with concentrate based on cottonseed meal and/or DDG. Furthermore, in the same experimental area, Koscheck *et al.* (2020) found an average of 0.94 kg/day by Nellore bulls supplemented at 0.3% BW with energy and protein based on citrus pulp, close to that study.

Considering the protein and energy requirements determined by BR-Corte (2016), under the experimental conditions of pasture and supplements provided, which generated an average intake of 0.98 kg CP/day and 4.10 kg TDN/day by forage, added to 0.10 kg CP/day and 0.87 kg TDN/day by the supplement, the predicted ADG for the animals would be 0.98 kg/day, which corresponds to the actual ADG of animals in the experiment, of 0.97 kg/day.

Our ADG results also corroborate with the NASEM (2016), which predicts an average ADG of 0.96 kg/day, considering an intake of 2.2% BW of a diet composed of 88% forage and 12% of energy supplement (corn or citrus pulp), with 61.6% of TDN that provided metabolisable energy approximately 9.33 MJ/kg of DM.

Total intake and digestibility of nutrients

Despite differences in the chemical composition of the supplements, the total intake of nutrients from forage and supplements was similar between the animals that received corn and citrus pulp. However, the g CP/kg DOM ratio was higher in the treatment of citrus pulp compared with corn in both supplementation times.

Poppi and McLennan (1995) suggested that the maximum efficiency in the microbial protein synthesis and transfer of ingested protein to the intestine is reached when amounts <160 g CP/kg DOM are observed, whereas values >210 g CP/kg DOM result in losses of net CP transfer from ingested CP to the passage of CP to the small intestine. In our study, however, it was expected that the g CP/kg DOM

ratio was high due to the high soluble protein fraction from pastures fertilised with high doses of N, which justified the adoption of energy supplementation as a way to capture excess N from the forage in the rumen by providing fermentable substrate containing starch or pectin, depending on the source.

Thus, considering forage and supplement intakes, the g CP/kg DOM ratio is above the proposed limit of 160 g CP/kg DOM in all treatments to obtain maximum use of forage nutrients and microbial protein synthesis. Supplementation with corn generated the lowest quotients compared with citrus pulp (197.8 and 183.4 g CP/kg DOM, respectively, to corn at 0900 hours and corn at 1700 hours).

As digestibility is determined as a function of the difference between what was consumed through forage and supplement, and excreted in the faeces of animals using iNDF as an internal marker (Detmann *et al.* 2001), in this study, nutrient digestibility was similar in all treatments, mainly due to similarity in the intake of forage and supplement of grazing animals and iNDF content.

From a nutritional point of view, DMD exerts an important influence on the animals' forage intake, so the higher the DMD, the greater the amount of DM that can be ingested, especially if the CP contents are >7% (Van Soest 1994) and if the ADF values are <40%, as it is the component with the greatest influence on digestibility (Figueira *et al.* 2015). Thus, the digestibility values found in this study can be considered within the expected and adequate range not to decrease the animals' intake.

Digestibility and CP content was high for these pastures, and total tract digestibility was also high for a tropical pasture supplemented at these low levels with fermentable energy sources. This is a consequence of pasture management, and reinforces why pasture forage mass and structure, and fertilisation are important agronomic tools to present a high-quality pasture to grazing animals. Both energy supplement sources are high in digestibility and fermentable sources of starch or pectin. These values result in the high ADG recorded for a tropical pasture system.

Efficiency of N use and microbial protein synthesis

As there were no differences in the total intake of DM and CP in the four supplementation strategies, the amount of N ingested by the animals was also similar between treatments, as well as the amount of N excreted, which was determined from the multiplication of concentration of N in the urine by the urinary volume of each animal. Consequently, the retention of N by the animals was similar between treatments, as it is primarily due to the excretion of N in the urine (Atkinson *et al.* 2013).

The ENU by ruminants indicates how much available N is being used by microorganisms and whole-body metabolism, calculated by the difference between ingested and excreted N in faeces (N balance), divided by ingested N (Detmann *et al.* 2014). As there was no difference in N excreted and N ingested, ENU was similar between treatments. ENU is also affected by the efficiency of absorbed amino acids, which is the function of the metabolisable protein/metabolisable energy ratio (Poppi 1990).

The efficiency of MCP depends on the availability of fermentable carbohydrates and N in the rumen. The microbial growth is maximised when there is synchronism between the degradation of carbohydrates and protein in the rumen environment (NASEM 2016). The absence of differences between sources and times of energy supplementation in MCP suggests that both corn and citrus pulp supplied in the morning or the afternoon have the same potential to increase the uptake of N in the rumen as a microbial protein to reduce the excretion of N in faeces and urine. It is worth emphasising the importance of well-managed tropical pastures on the efficiency of microbial synthesis due to the high content of soluble N of forage and high-quality fibre, in addition to the low content of iNDF.

In general, citrus pulp can be an alternative energy source to corn without causing an increase in N excretions by the animal and, consequently, an increase in nitrous oxide production, nitrate leaching and NH_3 emission volatilised, which can be harmful to the environment (Cardoso *et al.* 2016). In addition, citrus pulp is not directly consumed by humans, as it is a byproduct of the agroindustry and is less expensive than corn, which makes its use advantageous.

Ruminal parameters

As expected, supplementation with citrus pulp showed lower concentrations of N-NH_3 during the day compared with supplementation with corn. These results corroborate other studies, which reported that citrus pulp could promote greater and faster capture of NH_3 for its degradation, reducing ruminal N-NH_3 and protein degradation (Costa *et al.* 2011; Alvarez Almora *et al.* 2012; De Oliveira *et al.* 2016). The results of our study and Alvarez Almora *et al.* (2012) support the hypothesis on the effects of synchronisation of fermentable energy and N in the rumen in decreasing post-feeding concentrations of N-NH_3.

According to Detmann *et al.* (2014), minimum concentrations of 8 mg/dL of N-NH_3 in rumen fluid are ideal for ruminal microorganisms to degrade fibre from forage efficiently. In our study, all treatments presented concentrations of N-NH_3 above the minimum value for adequate rumen function and animal intake throughout the day.

The concentration of N-NH_3 in the rumen varies depending on the amount and rate of degradation of the protein source and uptake by microbes, and absorption across the rumen wall (Poirier *et al.* 2017). In this sense, the N-NH_3 peak obtained in the period between 1200 and 1800 hours coincides with the peak of animals grazing, observed by Páscoa (2009) and Casagrande *et al.* (2011). Thomsom *et al.* (1985) showed

that the highest proportion of daily intake occurred in this period in grazing sheep. That is, the animals spent the afternoon consuming forage with a high amount of soluble N, the excess N of which was in part captured by the energy substrate from the supplement for microbial protein synthesis, mainly when citrus pulp was supplied, as it presented the lower N-NH_3 peaks when compared with corn.

Thus, supplementation with citrus pulp in the morning or the afternoon can maximise the use of N from high-nutritive value forage, as it reduces N-NH_3 concentrations in the rumen by promoting a greater capture of the forage N, which is usually in excess due to the fertilisation.

The replacement of starch sources in the diet with sugars increases the ruminal pH (Chamberlain *et al.* 1993; Penner *et al.* 2009), and in the current study, supplement with a pectin source (citrus pulp) similarly maintained a higher rumen pH than corn.

The critical pH level of 6.2 was established as the minimum value to avoid the inhibition of cellulolytic bacteria and, thus, the reduction in forage cell wall degradation (Hoover 1986). In our study, however, citrus pulp promoted an average pH of 6.2 throughout the day, whereas corn maintained an average pH of 6.05. The animals were not at risk of acidosis due to the low inclusion of starch in the diet, and were above the critical range to cause animal disorders; that is, <5.6 for >3 h (Gozho *et al.* 2005). The pH values found in this study were close to the means reported by Ferrari *et al.* (2021) and Delevatti *et al.* (2019*b*), of 6.3, by beef cattle reared on palisade grass pastures managed at 25 cm height and fed with corn-based supplement at 0.3% BW.

The intense forage intake in the afternoon contributed to the reduction in the ruminal pH, especially between 1800 and 2100 hours, when the lowest pH peak occurred, most likely due to an extensive fermentation and SCFA production in the rumen, which intensifies up to 6 h after ingestion (Owens and Goetsch 1986). However, the pH values were not low enough to reduce NDF digestibility and degradability (Delevatti *et al.* 2019*b*).

The molar proportion of SCFA in the rumen may differ due to microbial species, microbial growth rates, substrate type and availability, level of DM intake, and ruminal pH (Schären *et al.* 2016). In this study, however, there was no effect of sources (starch or pectin) and times on the concentration of the main SCFA, with the average acetate: propionate:butyrate ratio being 63:21:16, close to that stipulated by Wanapat *et al.* (2014) for forage-based diets, from 65:25:10. The low level of supplement would not be expected to affect SCFA concentration (Van Soest *et al.* 1991).

Pectin fermentation increases acetate production, and generally does not increase lactic and propionic acid production during fermentation, in addition to maintaining a higher rumen pH than starch fermentation, which increases propionate production (Owens and Basalan 2016). There was no difference in the concentration of acetic and lactic acid between sources or supplementation timing. However, it was found that citrus pulp promoted a higher pH than corn throughout the day at both times. As Yang *et al.* (2004) reported, the lower pH of corn-supplemented bulls was due to the production of lactate after starch digestion.

The formic acid concentration was higher in animals that received citrus pulp than in corn. However, contrary to what is generally observed, the pH remained higher in this source throughout the day, and there was no influence of formic acid in the production of lactic acid, as reported by Ítavo *et al.* (2000). Citrus pulp provided a greater concentration of valeric acid compared with corn. Nevertheless, as it makes up a small proportion of the total SCFA produced, it usually does not generate critical information for studies, as it is often not evaluated in routine diagnoses, which makes it of secondary importance in evaluating ruminal fermentation (Filípek and Dvořák 2009).

Dry matter *in vitro* degradability

Experimental pastures had similar DM degradability profiles among treatments, considering their similarity in chemical and structural compositions. When evaluating corn and citrus pulp separately, however, it was noted that ruminal parameters associated with the ingredients were different. The potential degradability, which does not consider the passage rate, was higher in corn compared with citrus pulp when analysed separately. This suggests that corn has a more degradable carbohydrate fraction in the rumen, but may not affect microbial efficiency (Santos and Mendonça 2006).

Pectin is approximately 95% degraded in the rumen, whereas ruminal starch degradation varies from 50 to 85%, depending on the starch source and degree of processing (Santos and Mendonça 2006). In our study, similarly to the forage + citrus pulp, citrus pulp alone had greater effective degradability than corn, especially in the first 24 h after incubation. Such behaviour was probably due to the readily fermentable status of pectin, as reported by Moreira *et al.* (2009), characterised as a structural carbohydrate with high and rapid rumen degradation (Van Soest *et al.* 1991).

In general, the total gas production (mL) of citrus pulp, isolated or associated with pasture, was greater than that of corn in both circumstances. As reported by Miron *et al.* (2001) and Tedeschi *et al.* (2009), gas production is directly related to the rate of ruminal fermentation, probably due to the higher NFC concentration that ferments faster than fibre. In this sense, despite being a fibrous carbohydrate, pectin has a fermentative behaviour similar to NFC (Homem Junior *et al.* 2017).

Supplementation at 0.3% BW with corn or citrus pulp at 0900 or 1700 hours can optimise the performance of F1 Angus × Nellore young bulls reared in palisade grass pastures fertilised with 180 kg N/ha/year and managed at 25 cm of height. Citrus pulp can be used as an alternative

energy source to corn, and it has the same potential for animal performance, ENU and microbial protein synthesis in tropical pastures with a high proportion of soluble protein. Because citrus pulp is a byproduct of agroindustry and inedible for humans, it is less expensive than corn and does not show large price fluctuations throughout the year. The management of palisade grass pastures at 25 cm of height, with fertilisation of 180 kg N/ha/year, grazing efficiency of 50% and energy supplementation at 0.3% BW during the rainy season, promotes high production and allowance of high-quality forage, and allows a high stocking rate and production per animal and per hectare, in addition to avoiding long-term degradation of pastures.

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

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