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Emissão de metano, produção de leite e comportamento de ovelhas mantidas em pastagem ou suplementadas com concentrado à base de milho grão ou caroço de algodão

[*Methane emission, milk yield and behavior of ewes kept on pastures or supplemented with concentrate based on corn grain or whole cottonseed*]

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ABSTRACT

This study aimed to evaluate methane emission, milk yield and behavior of ewes kept exclusively on irrigated pasture of Tifton 85 grass (*Cynodon* spp.) or supplemented with ground corn or whole cottonseed (WCS) based concentrates. Twelve Lacaune x Santa Ines ewes (43.07±0.83 kg of body weight, 77±24 days after parturition, on average) were distributed in replicated 3x3 Latin square. Treatments consisted of three diets: pasture (no concentrate supplementation); corn (pasture + corn-based supplement); whole cottonseed (pasture + whole cottonseed-based supplement), offering 0.5 kg/ewe/day. The WCS group showed the highest concentrate dry matter intake (DMI) (*p*=0.049) and crude protein (CP) intake (*p*=0.001) compared to the others. There was no difference on total DMI (*p*=0.115) for the tested diets. Animals exclusively kept on pasture had the greatest forage DMI (*p*=0.004), lowest CP digestibility (*p*=0.015), showed longer grazing time (*p*=0.01) and shorter idle time (*p*=0.01) compared to the supplemented groups. Milk yield (0.36 kg/ewe/day) (*p*=0.15) and methane emission (33.12 g/ewe/day) (*p*=0.95) were similar for all three evaluated groups. Supplementation with concentrate based on corn or whole cottonseed does not improve productive performance nor decrease methane emission. However, lactating ewes kept exclusively in pasture show longer grazing time, without changes in milk yield and methane emission.

Keywords: concentrate supplementation, greenhouse gas emission, tropical pasture

RESUMO

Objetivou-se avaliar a emissão de metano, a produção de leite e o comportamento de ovelhas mantidas exclusivamente em pastagem irrigada de Tifton 85 (Cynodon spp.*) ou suplementadas com concentrados à base de milho grão ou caroço de algodão. Doze ovelhas Lacaune x Santa Inês (43,07±0,83 kg de peso corporal e 77±24 dias após a parição, em média) foram distribuídas em quadrados latinos 3x3 replicados. Os tratamentos consistiram em três dietas: pastagem (sem suplementação concentrada); milho (pastagem + suplemento à base de milho); caroço de algodão integral (pastagem + suplemento à base de caroço de algodão), oferecendo 0,5 kg/ovelha/dia. O grupo caroço de algodão apresentou maior consumo de matéria seca (CMS) (P=0,049) e consumo de proteína bruta (PB) (P=0,001) em relação aos demais. Não houve diferença no CMS total (P=0,115) para as dietas testadas. Os animais exclusivamente em pastagem apresentaram maior consumo de forragem (P=0,004), menor digestibilidade da PB (P=0,015), maior tempo em pastejo (P=0,01) e menor tempo em ócio (P=0,01), em relação aos grupos suplementados. A produção de leite (0,36 kg/ovelha/dia) (P=0,15) e a emissão de metano (33,12 g/ovelha/dia) (P=0,95) foram semelhantes nos três grupos avaliados. A suplementação com concentrado à base de milho grão ou caroço de algodão não melhorou o desempenho produtivo e não reduziu a emissão de metano. Entretanto, as ovelhas lactantes mantidas exclusivamente em pastagem apresentaram maior tempo em pastejo, sem alterações na produção de leite e na emissão de metano.*

Palavras-chave: suplementação concentrada, emissão de gases do efeito estufa, pastagem tropical

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INTRODUCTION

Pasture represents an important feed source for ruminants worldwide (Distel *et al*., 2020), however, its exclusive use may not be enough to supply the total amount of nutrients required by the animals (Souza *et al*., 2010; Wang *et al*., 2015). Ewes during the lactation need greater nutritional demands compared to other physiological stages, requiring a greater nutrient supply (Santos and Godoy, 2017).

Concentration supplementation can contribute to the supply of nutrients for grazing animals. The increase in the concentrate supplementation (5 to 15 g/ewe/day) reduced body weight (BW) and body condition score (BCS) losses of ewes kept on *Andropogon gayanus* pasture, during the lactation (Torreão *et al.*, 2014). Concentrate supplementation enhanced milk yield of ewes kept on thinned caatinga or having access (2 hours/day) during dry season in irrigated Tifton 85 pasture (Vasconcelos *et al.*, 2017), indicating the supplementation can be a strategic tool for grazing ewes, which can positively reflect on growth and productive performance of their lambs.

Livestock farming is considered a source of greenhouse gas emission (GHG). According to Gerber *et al*. (2013), GHG emission from livestock accounts for 14.5% of global anthropogenic emissions, including the contribution from sheep systems based on pastures. Additionally, Zubieta *et al*. (2021) reported that grazing sheep could produce 3.98 to 15.2kg of methane (CH_4) annually.

High quality forage can reduce CH_4 production (Haque, 2018), as well as the concentrate supplementation can mitigate the methane emission of grazing animals. Hristov *et al*. (2013) reported that the increase in the proportion of concentrate in ruminant diets reduces the methane emission per unit of feed intake and animal product, considering that diet composition may alter the production of methane by ruminants.

In Brazil, corn grain is the most available and the main energy source for supplements to grazing ruminants, and the whole cottonseed (WCS) is also an available source to feed animals, considered as an alternative ingredient for supplements, as it contains high levels of crude protein (CP) and total digestible nutrients (TDN), especially when the price of the corn grain is high.

Diets containing high starch levels, provided by the inclusion of corn grain, favor the production of propionic acid and, consequently, lead to a reduction in methane production (Moss *et al*., 2000). Furthermore, Patra (2013) and Beck *et al*. (2018) reported that supplementation using lipid sources is an option for reducing the methane emission of ruminants, in which several modes of action reported that lipids influence the ruminal fermentation (Muñoz *et al*., 2021).

We formulated two hypotheses. First, concentrate supplementation improves the productive performance and reduces methane emission of lactating grazing ewes. Second, supplementation based on WCS decreases methane emission from lactating ewes on pasture, compared to corn grain.

The study aimed to evaluate the animal behavior, milk production, and methane emission of ewes in lactation stage kept exclusively on pasture or supplemented with corn grain or WCS based concentrates.

MATERIAL AND METHODS

This research was approved by the Committee for Ethics in the Use of Animals (CEUA) of Embrapa Semiárido, under protocol number 11/2017.

The study was carried out at the Campo Experimental do Bebedouro (9°09‘S, 40°22‘W, 365.5 m altitude), Embrapa Semiárido (Brazilian Agricultural Research Corporation), located in the municipality of Petrolina, Pernambuco, Brazil. The average temperature during the experimental period was 25.46 ºC, ranging from 19.5 ºC to 32.3 ºC, and the relative humidity ranged from 39.5% to 87.7%, presenting on average 71.1%.

Twelve crossbred Lacaune x Santa Ines ewes were evaluated, showing on average 77±24 days after parturition, aged 2.1±0.9 years, 2.94±0.44 of BCS and 43.07±0.83 kg of BW at the beginning of the study. Initial BW, on average per group were 43.97, 42.35 and 42.89kg, respectively for pasture, corn and WCS groups, while final BW were 43.15, 42.87 and 43.50 for pasture, corn and WCS, respectively. Animals were previously identified with ear tags and received anthelmintics.

Four ewes were distributed in each group, in a replicated 3x3 Latin square design, with 12 animals. To perform the methane emission evaluation, six ewes were used in replicated 3x3 Latin square.

The experimental period lasted 63 days, divided into three periods of 21 days each, being 16 days of adaptation and five days of sampling for each period.

All ewes were kept in the same paddock, with three different diets. 1. Pasture and mineral mix, no concentrate supplementation. 2. Pasture plus corn-based supplement, 3. Pasture plus WCS based supplement. The amount of concentrate offered daily was 0.5kg/ewe.

The corn-based concentrate consisted of ground corn, soybean meal, urea, and mineral mix, while the WCS based concentrate was composed of whole cottonseed, ground corn, soybean meal, urea, and mineral mix (Table 1).

Table 1. Ingredient proportion (% dry matter) of concentrates offered to Lacaune x Santa Ines ewes grazed on irrigated Tifton 85 pasture

Ingredient	Ground corn	Whole cottonseed (WCS)
Ground corn	88.0	47.9
Whole cottonseed	-	50.0
Soybean meal	9.5	1.0
Urea	1.5	0.1
Mineral mix*	1.0	1.0
Total	100	100

*Centesimal composition of commercial mineral mix for sheep: calcium (maximal) (g/kg): 202.77; phosphorus (g/kg): 45.0; magnesium (g/kg): 10,0; sulfur (mg/kg): 10.00; chlorine (g/kg): 240.0; sodium (g/kg): 156.0; cobalt (mg/kg): 35.0; copper (mg/kg): 150.0; iodine (mg/kg): 40.0; manganese (mg/kg): 2,000; selenium (mg/kg): 15.0; zinc (mg/kg): 2,500; iron (mg/kg): 1,300; fluorine (maximal) (mg/kg): 450.0.

The ewes received mineral mix *ad libitum*. The animals of all groups were brought together from the paddocks to perform the milking at 9 am. Immediately after milking the ewes from the pasture group were taken to the paddocks.

Supplemented ewes received concentrate after milking, in individual pens (1.5m x 2.0m), with feeding troughs and drinkers. The concentrate was offered from the end of the milking up to 1 pm, and then, the ewes from the supplemented groups were taken to the grazing area, joining the animals of the pasture group, in the same paddock. All ewes remained in the paddock until the milking of the next day. The water was offered *ad libitum*.

There were three paddocks of irrigated Tifton 85 bermudagrass (*Cynodon* spp.) pasture already established for approximately 15 years, measuring 0.3 ha each. The stocking rate was fixed in 12 ewes/paddock, and the fertilization was 40kg of nitrogen (N) per ha, applied as urea. Each paddock was grazed for 21 days, and after this period the ewes were taken to another paddock.

Forage mass and sward height pre-grazing was measured before the ewes entered the paddock, while the post-grazing forage mass and sward height was determined immediately after the animals left the area.

On average, the paddocks presented 4,250kg DM (dry matter)/ha and 27.6cm, respectively, in the pre-grazing condition, while in the post-grazing they showed 2,750kg DM/ha and 14.8cm, respectively. Each paddock was grazed for 21 days, corresponding to every experimental period.

Forage mass was determined at five points in the paddock using a square frame measuring $0.25m^2$, performing the cut of the forage at the ground level. Sward height was measured in 30 points in the paddock, using a ruler graduated in centimeters.

Samples of forage for chemical analyses were obtained by hand-plucking, harvesting the forage during the last five days in every period. Forage samples were dehydrated in a forced ventilation oven at 55°C until constant weight, ground through a 1mm screen in a mill and then stored at freezing temperature (-20°C).

To estimate the total dry matter intake (DMI) for the pasture group the following formula was used: Forage DMI = FP/1-forage IVDMD*100; being: DMI = dry matter intake; FP = fecal production; IVDMD = *in vitro* dry matter digestibility.

The forage intake was estimated using titanium dioxide (TiO_2) as external marker for the estimation of fecal production. The TiO_2 was orally supplied (2.0g/day) in cellulose capsules for 15 consecutive days, collecting the fecal samples directly from the rectal ampulla during the last five days of application, in the morning, before offering the supplement. Fecal samples were pre-dried (55 °C until constant weight), and they constituted a composite sample by treatment, being stored at -20^{o}C.

Fecal production was estimated by the equation: FPtit (g DM/day) = supplied TiO_2 (g/day) / (% TiO_2 in feces/DM 105^{o}C); being: Fptit = fecal production obtained through titanium dioxide; supplied Tit. = quantity of TiO_2 administrated orally; DM = dry matter 105^{o}C.

The total DMI for supplemented groups was estimated considering the fecal production from concentrate and forage intakes, through the amount of ingested concentrate, and determining the digestibility of the supplement.

Concentrate DMI was determined by subtracting the amount of concentrate offered daily and the leftovers. Fecal production from concentrate was estimated as follows: FP = concentrate DMI – (concentrate DMI x concentrate IVDMD (*in vitro* dry matter digestibility), while the estimative of FP from the forage was performed as follow: FP from forage = total FP – FP from concentrate).

The IVDMD of the concentrates and forage was performed following the adapted methodology of Tilley and Terry (1963). The intake of nutrients (CP, NDF) was determined as the difference between total nutrients in the consumed feed and the total nutrients in the leftovers.

The digestibility of DM, CP and NDF was calculated using the equation: DC = [(kg of ingested fraction – kg of excreted fraction)] / (kg of the ingested fraction) x 100, while the total digestible nutrients (TDN) were calculated according to Sniffen *et al*. (1992).

Milking was performed every day in the morning using a mechanical milking machine. For complete milk ejection, 3.0 IU oxytocin was intramuscularly applied 10 minutes before the milking. To quantify the milk yield, the ewes were milked individually during the last five days of each period. After every milking, the milk was weighed individually.

Animal behavior was evaluated on the 16th day of each period, totaling three days of observations. All animals were identified by numbering both sides of their bodies, using different paint colors depending on the color of the animal. They were observed for 24 hours.

All observations were reported at intervals of 10 minutes, registering the grazing, rumination, and idle times for each ewe. Idle time corresponded to rest and other activities, except grazing or rumination. Data were collected by trained observers, which were changed every six hours of observation. From after the milking until 1pm there were two groups of observers, one to record the behavior of the supplemented ewes and another to register the behavior of the ewes in the paddock.

Concentrate samples were sampled before being offered to the animals. The samples of leftover concentrates were individually collected per ewe, during the last five days of every period. Fecal samples were also collected in the last five days of each period and pre-dried at a forced ventilation oven at 55°C until constant weight. Samples were ground through a 1 mm screen in a mill, and they were stored at freezing temperature (-20°C).

Laboratory analyses were performed using methods described by the *Association of Official Analytical Chemists* (Official…, 2016) for dry matter (DM; method 967.03), ash (MM; method 942.05), crude protein (CP; method 981.10), and ethereal extract (EE; method 920.29). The contents of neutral detergent fiber (NDF) and of acid detergent fiber (ADF) were determined as described by Van Soest *et al*. (1991). Chemical composition of hand-plucked forage samples, and corn and WCS based concentrates are shown in Table 2.

Table 2. Chemical composition of Tifton 85 grass obtained by hand-plucking, and concentrates based on ground corn grain and whole cottonseed (WCS) for lactating ewes

Nutrients, % DM	Experimental groups		
	Forage	Corn	WCS
Dry matter[a]	28.97	91.60	91.38
Ash	6.26	1.51	3.20
Crude protein	14.76	16.49	16.55
Neutral detergent fiber	74.83	25.94	34.66
Acid detergent fiber	35.54	7.28	12.38
Lignin	4.52	1.18	8.80
Ether extract	1.92	4.14	11.26
In vitro dry matter digestibility	55.90	70.86	65.62

[a]in % of fresh matter. DM = dry matter.

Sulfur hexafluoride (SF6) tracer gas technique was used to determine methane emission (Johnson and Johnson, 1995). Six ewes (two for each group) received orally SF6 tracer gas capsule at the beginning of the study.

Methane devices (adapted halters and saddles) were adjusted to the animals, during 10 days in each period before the collection period. Gas collections were performed during the last five days of each period, exchanging the cylinder with known negative pressure every 24 hours. Five “white” environmental samples were collected, placing the device in the grazing area to consider the levels of gases present in the environment.

At the end of each collection period, 30 samples (6 animals x 5 days) and five “white” samples (one per day) were obtained, totalizing 35 samples. Every day, after exchanging the cylinders, they were submitted to the admeasurement of the final negative pressure and to promote the injection of nitrogen until a positive pressure (approximately 2.9 psi). Subsequently, the gas samples were transferred to vials previously submitted to vacuum.

The analyzes to determine the concentrations of SF6 and CH_4 were performed in a 6890N gas chromatograph equipped with a flame ionization detector and an electron capture detector (Agilent Technologies Inc., Santa Clara, CA, USA), and in a 7820A gas chromatograph, (Agilent Technologies Inc., Santa Clara, CA, USA), respectively. Methane (CH_4) emission was shown as: g CH_4/animal/day; g CH_4/kg DMI; kg CH_4/kg NDF intake.

The data were first submitted to the Kolmogorov-Smirnov test for normality. The analysis of variance (ANOVA) was applied, followed by the Tukey test. The software SAS University (Statistical…, 2016) was used, and the differences were considered significant when $p < 0.05$.

RESULTS

Total DMI was similar for supplemented and non-supplemented groups (p = 0.115). Forage DMI was greater for pasture group compared to the others (p = 0.004), representing a forage intake 36% higher for the group exclusively grazing, compared to the supplemented groups (Table 3).

Lactating ewes fed WCS showed a higher concentrate DMI compared to corn (p = 0.049) (Table 3). Concentrate consisted in 26.9% and 30.1% of Total DMI for the grazing lactating ewes.

The greatest CP intake was observed for WCS (p = 0.001), while no differences were observed for NDF intake (p = 0.28) and TDN (p = 0.35) (Table 3). The digestibility of CP was different for the evaluated groups (p = 0.015), with the lowest value for animals of the pasture group. Regarding the coefficient of digestibility of DM and NDF and TDN no differences were observed between the evaluated groups (Table 3).

Table 3. Intake and digestibility of dry matter and nutrients, milk yield and methane emission by Lacaune x Santa Ines ewes kept exclusively on irrigated Tifton 85 pasture or receiving concentrate supplementation based on corn ground or whole cottonseed (WCS)

Variable	Experimental groups			SEM	p-value
	Pasture	Corn	WCS		
	Intake, kg/ewe/day				
Concentrate DMI	-	0.29[b]	0.37[a]	0.03	0.049
Forage DMI	1.12[a]	0.79[b]	0.86b	0.08	0.004
Total DMI	1.12	1.08	1.23	0.07	0.115
Crude protein intake	0.17[b]	0.18[b]	0.22[a]	0.01	0.001
NDF intake	0.83	0.75	0.85	0.04	0.28
	Digestibility, % DM				
Dry matter	57.61	60.33	59.48	1.89	0.593
Crude protein	69.52[b]	71.04[a]	73.84[a]	1.38	0.015
Neutral detergent fiber	54.65	46.26	45.48	2.31	0.114
Total digestible nutrient	55.70	59.44	56.56	2.14	0.354
	Milk yield, kg/ewe/day				
Milk yield	0.33	0.36	0.38	20.69	0.15
	Methane emission				
CH_4 (g/animal/day)	33.89	32.18	33.29	0.03	0.95
CH_4 (g/kg DMI)	25.28	25.17	22.11	0.07	0.66
CH_4 (g/kg NDFI)	40.83	42.91	39.16	0.03	0.25

Means followed by different lowercase letters differ among themselves according to the Tukey test ($p < 0.05$). SEM = Standard error of the mean; p-value = probability value. DMI = dry matter intake. NDF = neutral detergent fiber. NDFI = neutral detergent fiber intake.

No differences were observed for milk yield considering the evaluated groups and diets (p = 0.15) (Table 3). Methane emissions were not influenced by diets (Table 3), presenting, on average 32.76g/animal/day, 24.19g/kg DMI and 40.97g/kg NDFI).

Rumination time was not influenced by diets (p = 0.88). Ewes exclusively grazing on pasture showed longer grazing times in comparison to supplemented animals (p = 0.01) (Table 4), representing an increase of 29.41% for pasture in relation to the supplemented groups. Supplemented animals spent greater idle time compared to the pasture group (p = 0.01) (Table 4).

Table 4. Animal behavior of Lacaune x Santa Ines ewes grazed on Tifton 85 pasture and supplemented with concentrates based on ground corn or whole cottonseed (WCS)

Variable	Experimental groups			SEM	p-value
	Pasture	Corn*	WCS*		
Grazing time (h/day)	8.8a	6.7b	6.9b	0.25	0.01
Rumination time (h/day)	6.4	6.4	6.2	0.44	0.88
Idle time (h/day)	8.8b	10.0a	10.1a	23.43	0.01

Means followed by different lowercase letters differ among themselves according to the Tukey test ($p < 0.05$). SEM = Standard error of the mean. *Difference to 24h represents the feeding time (concentrate intake)

DISCUSSION

Total DMI was not increased by concentrate supplementation, since the supplemented groups ingested less forage compared to the pasture group, promoting a substitution effect, demonstrating a reduction on 23% to 30% on forage DMI for supplemented compared to non-supplemented ewes, which is attributed to the forage allowance and to the nutritive value of the forage.

In this study, the pre- and post-grazing forage mass were, on average, 4,250kg DM/ha and 2,750kg DM/ha, respectively, indicating the amount of forage was not restrictive. According to Bargo *et al.* (2003), high forage allowance can provide the opportunity to a greater forage selectivity by the animals.

Additionally, the forage offered to the ewes presented 14.76% of CP, indicating a medium to high-quality forage, which may have favored the supply of nutrients, and led to the observed substitution rates.

In the present research, the concentrate substitution rate was lower than the one found by Wang *et al*. (2019) (0.56kg/kg), who offered 0.5kg/day of concentrate to ewes grazing fresh perennial ryegrass, justifying this substitution rate with the high forage quality.

Whole cottonseed-based supplementation promoted greater concentrate intake compared to the corn-based supplement. Dixon and Stockdale (1999) reported that digestive and metabolic interactions occur when the intake of energy is changed by the inclusion of supplements in diets. Although the corn-based supplement represented 26.9% of the ewes' diet, as it is more rapidly degraded in the rumen, it may promote changes such as reducing pH, which can reduce the intake of supplement, compared to the WCS based supplement. Even with the greater supplement intake for WCS compared to the corn, the two supplemented groups had similar forage and total DMI.

The higher intake of CP for WCS may be explained by the greater intake of this supplement, and by its higher CP levels compared to corn concentrate. While the TDN and digestibility of DM and NDF were not influenced by the diets, averaging 59.14%, 48.73%, and 57.23%, respectively, the lower CP digestibility for the pasture group is possibly due to the greater number of nitrogenous compounds bound to the cell of the forage.

The lower protein digestibility for the pasture group is also related to the CP content, which was 14.76% for the forage, while the supplements showed 16.49% and 16.55% of CP for both concentrates, respectively, in which whole cottonseed and soybean meal are considered digestible protein sources.

Although there were no significant differences among the evaluated groups on milk yield, the ewes of the pasture group lost BW (43.97 *vs.* 43.15kg), while the supplemented animals maintained their BW or showed a moderate weight gain (42.35 to 42.89 *vs.* 42.87 to 43.50kg), suggesting the concentrate supplementation reduced the negative energy balance during lactation.

Similar total DMI, TDN and digestibility of DM and NDF presented by the ewes kept exclusively on pasture or receiving concentrate supplementation can explain the absence of difference in milk production, indicating that the nutrient supply was similar for the animals.

The longer grazing time contributed to the supply of nutrients to the non-supplemented group. The absence of difference in milk yield between the supplemented groups (corn *vs*. WCS) is also due to the total DMI and digestibility of nutrients, in which, according to Bargo *et al*. (2003) the increase in total DMI, nutrient and energy intake is the main objective of the supplementation for grazing animals.

Starch based concentrates for grazing ewes, such as the corn supplement used in this research, may favor body weight gain. Molle *et al*. (2008) reported based on dairy lactating ewes that increasing the supply of starch concentrates during the mid to late lactation phases may not enhance the milk response because the sheep being more prone than lactating cows to drive energy to body reserves, due to the stimuli for gluconeogenesis and insulin action.

Although the WCS based supplement provided higher supplement intake and greater protein digestibility, it was not enough to lead to a better response in milk production. This supplement had in its composition 50.0% of WCS, 1.0% of soybean meal and 47.9% of dry ground corn grain, suggesting that the levels of corn grain included in the concentrate may have contributed to the substitution rates observed, and consequently to the observed milk yield responses.

The observed milk yield, considering all evaluated groups, can be related to the lactation stage, which reached, on average, 140 days after parturition at the end of the study. As the ewes were in mid to late lactation phases, the greater supply of nutrients provided by supplementation may have been directed to the synthesis of body tissues.

Supplemented ewes spent less time grazing in comparison to the ewes receiving corn or WCS based concentrates, which represented 36.7% considering 24h for non-supplemented ewes, while it corresponded to 27.9% or 28.8%/24h for supplemented animals, indicating the ewes kept exclusively on pasture compensated by increasing grazing time, the intake and supply of nutrients that the supplemented group received from the concentrates.

Grazing times observed in this research are in accordance with Jochims *et al*. (2010) who observed a longer grazing time for sheep kept exclusively on pearl millet pasture (*Pennisetum americanum* (L.) Leeke) compared to animals supplemented with concentrate based on cassava meal or corn gluten meal, receiving 1% BW daily. The same authors observed that non-supplemented sheep grazed for 8.2 h/day, while supplemented animals grazed for 6.6 or 7.0 h/day, and the supplementation increased the idle time (9.4 h/day) with cassava meal, compared to non-supplemented (8.1 h/day).

According to Krysl and Hess (1993), the efficiency to obtain nutrients per unit of time is greater when animals receive supplementation, which may explain the reduction in grazing time. In addition, the longer grazing time spent by non-supplemented ewes was needed to provide more nutrients from forage to meet their nutritional requirements.

Rumination time is one parameter of the animal behavior that can be influenced by the nutritional characteristics of the diet. However, in this research, no differences on rumination times may be due to the similar total DMI and intake of NDF by the evaluated groups (Table 3).

Rumination is also affected by the physically effective fiber, and even for the supplemented groups the proportion of forage in the diets was high, reaching 69.9% and 73.1% for corn and WCS based supplements, respectively. The supply of physically effective fiber may promote similar rumination time, as forage from pasture group was an important source of this component.

Greater idle time for supplemented groups in comparison to non-supplemented ones can be explained by the supply of nutrients via concentrate which can promote lower dependence of the pasture, reducing the grazing time and consequently increasing the idle time. Jochims *et al*. (2010) reported that concentrate supplementation may reduce the competition among the animals in search of forage, increase the selectivity of grazing patches in the pasture and improve the efficiency of obtaining nutrients from the pasture.

Methane emissions observed for lactating ewes exclusively on pasture or supplemented with concentrate ranged from 32.18 to 33.89g/animal/day. Savian *et al*. (2014) reported methane emissions ranging from 20.7 to 41.7g/sheep/day kept on pasture, and specifically for lactating ewes the methane emission varied to 38.7 to 41.7g/day. These authors also observed 13.8 to 27.0g CH_4/kg DMI, which are compatible to the values found in this present research that ranged from 22.11g CH_4/kg DMI for WCS group to 25.28 g CH_4/kg DMI to the pasture group.

Dry matter intake is considered the main factor influencing CH_4 emissions, contributing to 81% of daily variation (Pinares-Patiño *et al*., 2013). However, Savian *et al*. (2014) verified a negative association between DMI and methane emission, considering g CH_4/kg DMI), indicating that the efficiency in converting the ingested dry matter to animal products is also important. In the present study, total DMI and the NDF intake, as well as the digestibility of DM and NDF were similar for all groups and may justify no differences observed on CH_4 emissions.

The average proportion of concentrate in the ingested diet corresponded to 26.9% to 30.1% for corn or WCS based concentrate groups, respectively, which might have been insufficient to alter the methane emission of grazing ewes.
Molle *et al*. (2008) recommend an increase in digestible fiber in supplements for mid to late lactation ewes to change the main short chain fatty acids (SCFA) as product of the ruminal fermentation, decreasing the gluconeogenesis stimuli. Likewise, Lovett *et al*. (2005) indicate the inclusion of fibrous source in the concentrate for late lactating dairy cows as a strategy to reduce the substitution rate, leading to a decrease in CH_4 production/kg of animal product.

However, in this research, the inclusion of 50.0% of WCS as fibrous and lipid source replacing part of the dry ground corn grain in the supplement, was also not enough to increase DMI or to reduce the methane emission, suggesting the possibility to enhance the inclusion of fibrous sources in the concentrate to mid to late lactating ewes on tropical pastures. Besides, Kumar *et al*. (2013) indicate a greater inclusion of concentrate into the diet, reaching up to 50%, to drive changes in ruminal fermentation.

Our results demonstrate that late lactating non-supplemented ewes used their longer grazing time to increase total DMI through greater forage DMI, compared to supplemented ewes. Although ewes supplemented with WCS presented greater CP intake and the CP digestibility was greater for supplemented ewes in comparison to non-supplemented ones, the concentrate supplementation did not alter NDF intake, TDN, and digestibility of DM and NDF, promoting similar milk yield and methane emission. The non-restrictive forage allowance and the quality of the forage may be a strategy to avoid an increasing methane emission for grazing sheep.

Additionally, Wang *et al*. (2019) did not observe a reduction in methane emission by sheep grazing fresh ryegrass and supplemented with 0.5 kg/day of concentrate, compared to sheep grazing exclusively on fresh ryegrass, due to the absence of supplementation effects on nutrient digestibility or feeding levels, indicating that the high-quality forage can improve the efficiency of nutrient use by grazing sheep, as well as Zubieta *et al*. (2021) reported that it is possible to reduce methane yield and intensity offering nutrient-dense diets to grazing animals, however it is difficult when pastures already present high quality, reinforcing that grazing management practices is an important tool to optimize the intake of the nutritive forage aiming to mitigate methane emission from grazing animals.

CONCLUSION

Under the experimental conditions, supplementation with concentrate based on corn or whole cottonseed does not improve productive performance nor decrease methane emission. However, lactating ewes kept exclusively on irrigated Tifton 85 pasture show longer grazing time, without changes in milk yield and methane emission.

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