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Nutritive value of sheep diets made up of hay from Tifton 85 grass and leucaena[1]

Valor nutritivo de dietas para ovinos constituídas pelos fenos de capim-Tifton 85 e de leucena

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ABSTRACT - The intake, digestibility, nitrogen balance and energetic balance were evaluated in sheep diets consisting of hay from Tifton 85 grass at two ages of regrowth (28 and 42 days) and hay from leucaena at 40 days of regrowth, in the proportions of 20 and 40%. Twenty Santa Inês sheep, kept in metabolic cages, were used in a randomised block design according to body weight. The hay from the Tifton 85 grass, processed at 28 days of regrowth, resulted in a higher CP intake than when processed at 42 days of regrowth. There was higher protein intake for the 40% proportion of leucaena hay in relation to that of 20%, regardless of the regrowth age of the grass. Diets containing Tifton 85 grass processed at 28 days resulted in greater values for both DE and ME. There was greater intake, absorption and urinary excretion of N, and a lower N_{faecal} to $N_{urinary}$ ratio, with diets containing Tifton 85 grass processed at 28 days, as well as with those diets containing 40% leucaena hay. When formulating sheep diets containing hay from Tifton 85 grass processed at 28 days of regrowth and combined with 20% hay from leucaena, the grass, despite showing good energetic value, is not enough to provide full use of the nitrogen available to the ruminant, making it necessary need to find combinations with concentrated food sources which are able to optimise the use of nitrogen, reduce environmental impact and maximise sheep production.

Key words: Nutrient balance. Voluntary intake. *Cynodon* spp. Digestibility. *Leucaena leucocephala*.

RESUMO - Avaliou-se o consumo, a digestibilidade e o balanço de nitrogênio e energético de dietas para ovinos constituídas por feno de capim-Tifton 85 em duas idades de rebrota (28 e 42 dias) e feno de leucena aos 40 dias de rebrota, nas proporções 20 e 40%. Foram utilizados 20 ovinos da raça Santa Inês, em gaiolas de metabolismo, em delineamento de blocos ao acaso, quanto ao peso vivo. O feno de capim-Tifton 85 processado aos 28 dias de rebrota resultou em maior consumo de PB que aos 42 dias de rebrota. Houve maior consumo de proteína quando da proporção 40% de feno de leucena em relação a 20%, independente da idade de rebrota da gramínea. As dietas contendo feno de capim-Tifton 85 processado aos 28 dias resultaram em maior valor para ED e EM. Houve maior ingestão, absorção e excreção urinária de N e menor relação $N_{fecal}/N_{urinário}$ para as dietas contendo feno de capim-Tifton 85 processado aos 28 dias, assim como para as dietas contendo 40% de feno de leucena. Quando da formulação de dietas para ovinos contendo feno de capim-Tifton 85 processado aos 28 dias de rebrotação associado a 20% feno de leucena, embora a gramínea apresente bom valor energético, este não é suficiente para propiciar o aproveitamento do nitrogênio disponível ao ruminante, do que resulta necessidade de se buscar associação com fontes de alimentos concentrados capazes de otimizar o aproveitamento do nitrogênio, reduzir o impacto ambiental e potencializar a produção de ovinos.

Palavras-chave: Balanço de nutrientes. Consumo voluntário. *Cynodon* spp. Digestibilidade. *Leucaena leucocephala*.

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INTRODUCTION

Conserved forages are an important feed supplement for ruminants whenever there is a scarcity *in natura* of bulk forage for production systems (which occurs during dry seasons). They are also important in meeting the demands of intensive production systems where feed is the main factor in production costs, or the specific demands of certain animal types, such as the importance of hay in stimulating the development and correct functioning of the pre-stomachs of young ruminants.

In Brazil, forage species of the genus *Cynodon* are the most suitable for producing hay in view of their high production potential and nutritional value (AGUIAR *et al*., 2006), although the nutritional value decreases with the advances in growth stage. Among the bulk feeds which are currently most evaluated as to their nutritional value, legumes stand out, as their nutritional characteristics are favourable for the feeding of ruminants, especially as regards their high crude-protein content (POSSENTI *et al*., 2008).

Because of the frequent nutrient deficiency of grasses for ruminants, especially in systems of low investment, mixing these legumes can be a good option for supplying nutrients. Thus, leucaena (*Leucaena leucocephala* (Lam.) de Wit) displays many agronomic and nutritional benefits and can be mixed with such grasses as Tifton 85 (*Cynodon* spp.) which is widespread in the tropics.

Forage from leucaena is a source of nitrogen for microflora in the rumen and for the ruminant itself. Moreover, according to Soltan *et al*. (2013), it is a potential supplement to tropical grasses, due to supplying extra-microbial metabolic protein and possibly bypass protein, besides reducing methane emissions without any adverse effects on degradation in the rumen or on digestibility.

This research was carried out therefore, with the aim of evaluating the nutritional value of sheep diets made up of hay from Tifton 85 grass, when harvested at two different ages and associated with two proportions of leucaena hay.

MATERIAL AND METHODS

This research was carried out at the Department of Animal Science at the Centre for Agrarian Sciences of the Federal University of Piauí, in Teresina, in the state of Piauí, Brazil, located at 05°02’28 17” S and 42°46’56.99” W, at an altitude of 71.3 m.

Data were grouped into a randomised-block design according to the weight of the sheep, in a 2 x 2 factorial scheme, where the treatments were diets made up of hay from Tifton 85 grass at two ages of regrowth (28 and 42 days) and hay from leucaena at 40 days of regrowth, in the proportions of 20 and 40%, on a DM basis (Table 1).

Forage from Tifton 85 grass was left in the sun for 24 h and baled, while the forage from leucaena, consisting of branches with stems up to 1.0 cm in diameter, was ground in a forage grinder into 2.5 cm particles before being left in the sun for 12 h. In order to standardise the diets offered, the hay from the Tifton 85 grass was also ground into particles of 2.5 cm.

Twenty Santa Inês sheep were used, each eight months old, not neutered, dewormed and in good health. They were placed into metabolic cages, 1.0 m x 0.5 m fitted with a container for salt and troughs for drinking

Table 1 - Chemical composition of the diets and of the hays from leucaena (LH) and Tifton 85 grass, processed at 28 (TH28d) and 42 (TH42d) days of regrowth

Constituents	TH28d		TH42d		LH	TH28d	TH42d
	-------------------Levels of LH (%)-------------------						
	20	20	20	20			
Dry matter (%)	84.98	84.46	83.78	83.56	82.90	85.50	84.00
as% DM							
Organic matter	93.5	93.72	93.10	93.43	94.40	93.27	92.78
Crude proteín	12.69	14.41	11.32	13.39	19.60	10.96	9.25
Neutral detergent fibre	61.11	58.88	64.30	61.28	52.20	63.34	67.33
Acid detergent fibre	36.62	38.65	37.53	39.34	44.76	34.58	35.72
NDF/CP	4.82	4.09	5.68	4.58	2.66	5.78	7.28
ADF/CP	2.89	2.68	3.32	2.94	2.28	3.16	3.86

and feeding. The experiment was for 20 days, including 14 days for the animals to adapt and 6 for taking samples.

When starting the experiment, the animals were weighed, after consuming no solid food for 14 hours, so they could be distributed across treatments, being weighed again on the first and last days of the sample-collection phase in order to obtain their weights and establish consumption parameters.

The diets were offered at 07:00 and 16:00, with the aim of producing leftover food corresponding to 15% of that supplied; the diets being adjusted based on the previous day's consumption. Each time the diets were offered, samples were taken from them in order to obtain a composite sample per animal and diet. During the collection phase, the leftovers were weighed prior to the meals being offered, and samples were collected corresponding to 20% of the weight of these leftovers. These were placed into plastic bags and stored at -5 to -10 °C, resulting in composite samples. Food and nutrient intake was calculated as the the difference between the weight of the food offered and of the leftovers.

Digestibility and nutrient balance was determined using the total collection method. The metabolic cages were fitted with separating screens, leaving samples of faeces in trays, and urine in buckets containing 20 mL of HCl (1:1). Collections were made at the time the diets were offered, and the weight of faeces and volume of urine were recorded. Samples corresponding to 20% of the weight of the faeces and 15% by volume of urine were collected, which were placed into plastic bags (faeces) and plastic bottles (urine) and stored at -5 to -10 °C. At the end of the experiment, the samples of leftover food, faeces and urine were unfrozen and homogenised in order to obtain composite samples for each animal.

Samples of diets, leftovers and faeces were pre-dried at 55 °C in a forced air circulation oven for 72 h, ground in a Willey mill having a sieve with a Ø 1.0 mm screen, and analysed for dry matter (DM), organic matter (OM), crude protein (CP), gross energy (GE) using a Parr ® adiabatic calorimeter in accordance with AOAC (2010), neutral detergent fiber (NDF) and acid detergent fiber (FDA) by the Van Soest method (VAN SOEST; ROBERTSON; LEWIS, 1991). N content and urine GE were also analysed by the methods described above.

The apparent digestibility (AD) of the DM, CP, NDF, ADF and energy, were calculated from the difference between ingested nutrients and nutrients excreted in the faeces, as a function of nutrient intake (KOZLOSKI *et al.*, 2009): AD (%) = [(nutrient intake - nutrient excreted in the faeces) / nutrient intake] x 100.

The metabolisable energy (ME) was obtained by subtracting the energy losses through the urine and methane, from the digestible energy. To estimate the loss of methane (Cm), the Blaxter and Clapperton formula (1965) was adopted (Equation 1),

$$Cm = 3{,}67 + 0{,}062D \qquad (1)$$

where: Cm = methane production in kcal $100kcal^{-1}$ of energy consumed and D = apparent digestibility of the GE of the food.

To evaluate nitrogen usage (N), N_{intake}, N_{faeces} and N_{urine} were quantified, with $N_{retained}$ (g day^{-1}) = N_{intake} - (N_{faeces} + N_{urine}). The percentage of N_{intake} which was apparently retained (BN) was calculated.

The data were analysed using the GLM procedure of the SAS statistical software (2002), with the means being compared by the Tukey test at 5% probability.

RESULTS AND DISCUSSION

The voluntary intake of dry matter (DM), acid detergent fibre (ADF) and digestible protein (DP) was on average 3.17 ± 0.30% BW, 349.39 ± 27.27 g day^{-1}, 652.32 ± 50.67 g day^{-1} respectively, and the digestibility of DM, crude protein (CP), neutral detergent fibre (NDF) and ADF, on average 63.76 ± 3.38, 71.70 ± 4.07, 60, 21 ± 3.58 and 56.43 ± 4.95% respectively. These values were not influenced (P> 0.05) by the age of regrowth of the Tifton 85 grass or by the inclusion of leucaena hay in the diet. Interaction between the regrowth age of the Tifton 85 grass and the inclusion of leucaena hay had no effect on the voluntary intake of nutrients.

DM intake was superior to that obtained by Longo *et al*. (2008) for sheep diets made up of leucaena hay and Tifton grass, this also being explained by the lower NDF content of leucaena hay (52.2%) used in this research. Despite the Tifton-85 hay being produced at 42 days of regrowth, the levels of CP (9.25%) and ADF (35.72%) of the hay resulted in an NDF to CP ratio of 7.28 and an ADF to CP ratio of 3.28 in the diets, lower than those obtained by Longo *et al. (2008)* of 20, 50 and 12,20 respectively. The results for intake and digestibility are explained by the low level of ADF and ratio of fibre to CP, since intake is directly influenced by the NDF, and digestibility by the ADF.

The inclusion in the diets of Tifton 85 hay produced at 28 days of regrowth, resulted in a greater consumption of CP than when produced at 42 days, whereas mixing 40% leucaena hay with Tifton 85 hay resulted in a higher CP intake, regardless of the regrowth age of of the grass

(Table 2). This indicates the advantage of including leucaena hay, and is in agreement with the results of Mizubuti *et al*. (2007) when giving up to 40% gandule bean (*Cajanus cajan*) mixed with hay from Coast cross grass. Any delay in the production of hay from Tifton 85 grass with a view to greater forage production can therefore be compensated for by mixing in 40% leucaena hay, as the inclusion of this legume increases the daily intake of CP and compensates for the effect of the age of the grass.

The inclusion of a lower proportion of leucaena hay (20%) resulted in a greater intake of NDF ($P < 0.05$) (Table 2), explained by the smaller fibre content of the legume (Table 1), although this did not alter with the regrowth age of the grass. This differs from the result obtained by Longo *et al*. (2008), who found no effect on the intake of NDF from mixing leucaena hay with Tifton hay in sheep diets, this being explained by the high NDF content (79.8%) compared to that obtained for hay processed at 28 and 42 days of regrowth, 63.34 and 67.33% respectively, due to a higher rate of passage increasing consumption.

The influence of fibre from the grass hay should be considered when assessing the digestibility of diets containing legumes. In this research, the inclusion of 20 and 40% of leucaena in the diet did not influence the digestibility of DM or nutrients. Accordingly, Soltan *et al*. (2013) when mixing 50% leucaena hay and 50% Tifton hay in sheep diets with 70% bulk forage, found no difference in digestibility compared to the diet containing 70% Tifton hay, with values for digestibility lower than those obtained in this study. It is noteworthy that FDA from the Tifton Hay in this study was approximately 12 percentage points lower than the results obtained by those authors. Despite this result, Soltan *et al*. (2013) highlight the reduction in methane emissions, lesser environmental impact and reduced energy losses as being the contribution of leucaena to the diets of ruminants

The production of hay from Tifton 85 grass at 28 days of regrowth resulted in an increased intake of digestible energy (DE) than at 42 days (Table 2), although this effect was not so marked when including hay produced from leucaena. This is a result of the higher values for DE (2.08 ± 0.21 Mcal kg^{-1}) and metabolisable energy (ME) (1.73 ± 0.17 Mcal kg^{-1}) in those diets containing hay from grass processed at 28 days of regrowth, with there being no effect from including hay from the legume on the energy value (Table 3). Taking into account the average DM intake of 921.97 ± 79.35 g day^{-1}, an ME intake of 1.59 Mcal day^{-1} is seen, sufficient to meet the requirements of sheep weighing 20 kg with a weight gain of 100 g day^{-1}, according to the NRC (2007). However, these animals would require only 70 g PB day^{-1}, indicating an excess of PB when fed on these diets.

When considering the intake of GE from the diets, the potential was found to meet the requirements of sheep of eight months, 30 kg BW and with a daily

Table 2 - Voluntary intake by sheep of nutrients from diets made up of the hays from Tifton 85 grass (TH) and from leucaena (LH)

Intake	--------Age of regrowth---------		--------Levels of LH (%)--------		CV (%)[1]
	TH 28 days	TH 42 days	20	40	
Crude protein (g day^{-1})	131.27 A	113.96 B	113.75 b	131.48 a	10.79
Neutral detergente fibre (g day^{-1})	547.90 A	535.57 A	562.04 a	521.42 b	7.40
Digestible energy (Mcal day^{-1})	2.14 A	1.76 B	2.04 a	1.86 a	17.81
Metabolisable Energy (Mcal day^{-1})	1.78 A	1.45 B	1.71 a	1.52 a	16.75

[1]CV = Coefficient of variation; Means followed by the same letter on the same line, uppercase for age of regrowth and lower case for levels of leucena hay, do not differ between themselves by Tukey test ($P < 0,05$)

Table 3 - Energy value of diets for sheep, made up of hay from Tifton 85 grass (TH) and from leucaena (LH)

Energy (Mcal kg^{-1})	Regrowth age		Levels of LH (%)		CV(%)[1]
	FT 28 dias	FT 42 dias	20	40	
Digestible Energy	2.21 A	1.96 B	2.14 a	2.02 a	10.30
Metabolisable Energy	1.84 A	1.62 B	1.80 a	1.66 a	10.20

[1]CV = Coefficient of variation; Means followed by the same letter on the same line, uppercase for age of regrowth and lower case for levels of leucena hay, do not differ between themselves by Tukey test ($P < 0,05$)

gain of 200 g, however, the available MS was inferior to demand, 2.46 Mcal kg^{-1}, according to the NRC (2007). This fact demonstrated the need for supplementing the diets with a source of EM having more available biomass, in order to meet the nutritional requirements of sheep in this category when using hay processed at 42 days of regrowth mixed with 20% leucaena hay, thus making the best use of the legume.

When evaluating the use of nitrogen, the N balance, the N excreted in the faeces with an impact on the stability of digestibility of the CP, and the ratio N_{faeces} / N_{intake} were not influenced by the regrowth age of the Tifton 85 grass and the included levels of leucaena hay. High positive values for the N balance suggest equilibrium between the protein and the digestible energy in the diet.

The N excreted in the faeces (36.5% of N_{intake}) and the N excreted in the urine (10.5% of N_{intake}) indicate efficiency in the use of N_{intake} by the sheep when meeting the requirements of micro-organisms in the rumen, as evidenced by the higher N_{faeces} / N_{urine} ratio. According to Alves *et al*. (2011), the inclusion of field bean pods as an energy source resulted in a reduction in the excretion of N_{urine} as a proportion of N_{intake}. Nitrogen metabolism was not influenced by the interaction of the regrowth age of the Tifton 85 Hay and the levels of leucaena hay (Table 4).

The N_{faeces} / N_{intake} ratio was lower than that obtained by Dantas Filho *et al*. (2007) at 54.77% , indicating that the N_{intake} was metabolised more efficiently to meet N requirements, helping to prevent the mobilisation of N from the tissues of the animal and resulting in less $N_{urinário}$ excretion, with a lower impact on the environment. According to Silva *et al*. (2010), the absorbed nitrogen when ingested and properly metabolised can result in less environmental contamination.

In the present research, a nitrogen balance (NB) of over 50% was seen, which is close to the value obtained by Silva *et al*. (2010) of 59.19 ± 4.68%, being explained by levels of N_{urine} close to those obtained by those authors (2.17 ± 0.71 g day^{-1}). However, Karda (2007), when supplementing diets with 200 g leucine day^{-1} for sheep fed on hay from Rhodes grass, where leucaena represented 16.9% of the DM intake, obtained retention of 6.2 g N day^{-1} and NB of 42.4%, lower than those obtained in this study. In assessing diets containing 30% concentrate, Soltan *et al*. (2013) claim that the retention of N in the body is increased and the excretion of N_{faeces} and N_{urine} is reduced when leucaena is included in the diet. Future research aiming to optimise the utilisation of dietary N, should therefore consider including energy sources which are easily degradable in the rumen.

There was higher intake, absorption and urinary excretion of N, and a lower N_{faeces} / N_{urine} ratio for diets containing Tifton 85 hay processed at 28 days compared to hay produced at 42 days, and for diets containing 40% leucaena hay in relation to 20% (Table 4). According to Longo *et al*. (2008), the loss of N_{faeces} by animals fed a diet containing 40% leucaena hay compared to 20%, may be explained by the higher consumption of acid detergent insoluble nitrogen (ADIN) and DM intake contributing to greater loss of N_{faeces}.

According to Van Soest (1994), meeting the requirements for N avoids mobilisation of N from the

Table 4 - Nitrogen balance of diets for sheep, made up of hay from Tifton 85 grass (TH) and from leucaena (LH)

Parameter	------------Regrowth age------------		------------Levels of LH%------------		CV(%)[1]
	FT 28 days	FT 42 days	20	40	
N_{intake} (N_i, g/day)	21.00 A	18.23 B	18.20 b	21.03 a	10.80
$N_{excreted}$ (N_e. g/day)					
N_{faeces} (N_f)	7.64 A	6.64 A	6.91 a	7.37 a	15.40
N_{urine} (N_u)	2.46 A	1.68 B	1.80 b	2.34 a	25.00
$N_{excreted\ total}$	10.10 A	8.32 B	8.72 a	9.71 a	15.74
Ratioo N_f/N_i *(%)*	36.39 A	36.73 A	38.20 a	34.91 a	11.70
Ratio N_u/N_i *(%)*	11.48 A	9.10 B	9.68 a	10.90 a	23.10
Ratio N_f/N_u *(g/g)*	3.30 B	4.33 A	4.28 a	3.35 b	23.30
$N_{absorbed}$ *(g/day)*	13.36 A	11.58 B	11.28 b	13.66 a	11.90
$N_{retained}$ *(g/day)*	10.90 A	9.91 A	9.42 b	11.32 a	14.90
% do N_{inpur} *(BN)*	51.90 A	54.36 A	52.28 a	53.83 a	11.30

[1]CV = Coefficient of variation; Means followed by the same letter on the same line, uppercase for age of regrowth and lower case for levels of leucena hay, do not differ between themselves by Tukey test (P < 0,05)

animal's bodily reserves and limits the excretion of N_{urine}. When there is surplus of N, the animal excretes the excess in the form of N_{faeces} and N*urine* with the expenditure of energy for synthesis of urea in the liver, in addition to excretion into the environment. Nevertheless, that author asserted that losses of N_{faeces} are less variable than those of Nurine, corresponding on average to 0.6% of the total DM intake and 3-4% of the total CP intake. In this research, the excretion of N_{faeces} was approximately 0.7% of the ingested DM, while that of N_{urine} was 1.5% of CP intake, with the low value of N_{urine} being noteworthy.

A higher total excretion of N and higher N_{urine} to N_{intake} ratio was seen in sheep fed diets containing Tifton 85 hay processed at 28 days compared to 42 days, with no difference being noted for the diets containing leucaena. This result shows that hay processed earlier results in a greater availability of N to the animal and that the nitrogen to energy imbalance in the diet associated with the good digestibility of the CP, results in excess nitrogen in the body, with the consequent excretion of urine, indicating the need to supplement the energy of forage diets consisting of early Tifton 85 hay (28 days) mixed with leucaena hay.

CONCLUSION

When formulating sheep diets containing hay from Tifton 85 grass processed at 28 days of regrowth and combined with 20% hay from leucaena, the grass, despite showing good energetic value, is not enough to allow full use of the nitrogen available to the ruminant, making it necessary to find combinations with concentrated food sources which are able to optimise the use of nitrogen, reduce environmental impact and maximise sheep production.

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