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Nutritional and metabolic parameters of sheep on deferred pasture with different strategy changes during the dry season

[*Parâmetros nutricionais e metabólicos de ovinos em pasto diferido com diferentes estratégias mudam ao longo do período seco*]

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ABSTRACT

To verify if strategies to reduce the height of Marandu grass pasture at beginning of the deferral period change intake and metabolic parameters in sheep during the dry season, 18 crossbred females, ½ Dorper + ½ Santa Inês were used, distributed in 9 Marandu grass paddock handles to 3 drawdown strategies at the beginning of deferral period: 1) maintenance of grass with 15cm for 5 months before deferral start (15/15cm); 2) maintenance of marandu grass with 25 cm for 5 months, but at the beginning of deferral it was reduced to 15cm (25/15cm); 3) maintenance of 35 cm grass for 5 months, but at the beginning of deferral it was reduced to 15cm (35/15cm). The experiment was conducted in a completely randomized design (CRD), with 6 repetitions and repeated measures over time. Strategies to reduce the height of Marandu grass pasture, at the beginning of the deferral period, do not change nutrient intake and energy and protein metabolism. However, changes occurring in the pasture during the dry season alter these variables, and deferral is an interesting strategy to be used for forage production in winter, however, to optimize its use, it is necessary to use supplementation with concentrated rich in fermentable carbohydrates.

Keywords: albumin, deferral, total protein, urea

RESUMO

Para verificar se estratégias de redução da altura do pasto de capim-marandu, no início do período de diferimento, alteram consumo e parâmetros metabólicos em ovinos durante o período seco, foram utilizadas 18 fêmeas, mestiças, ½ Dorper + ½ Santa Inês, que foram distribuídas em nove piquetes de capim-marandu, com três estratégias de rebaixamento no início do período de diferimento: 1) manutenção do capim com 15cm, por cinco meses, antes do início do diferimento (15/15cm); 2) manutenção do capim-marandu com 25cm, por cinco meses, rebaixado, no início do diferimento, para 15cm (25/15cm); e 3) manutenção do capim com 35cm durante cinco meses, rebaixado, no início do diferimento, para 15cm (35/15cm). O experimento foi conduzido em delineamento inteiramente ao acaso (DIC), com seis repetições e medidas repetidas no tempo. Estratégias de redução da altura do pasto, no início do período de diferimento, não alteram o consumo de nutrientes e metabolismo energético e proteico. Porém, as mudanças no pasto durante o período seco alteram essas variáveis, sendo o diferimento uma estratégia interessante para utilizar na produção de forragem no inverno. Para otimizar seu uso, faz-se necessária, entretanto, a utilização da suplementação com concentrados ricos em carboidratos rapidamente fermentáveis.

Palavras-chave: albumina, diferimento, proteína total, ureia

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INTRODUCTION

Sheep production on pasture under tropical conditions is limited by seasonality in forage production, with low pasture production in the dry period and constant forage demand by the animals throughout the year, which can be used as a technique for pasture production in the dry period, the deferral of pastures, which consists of selecting certain pasture areas and restricting them to animal access, at the end of the summer (Silva *et al*., 2016; Afonso *et al*., 2018). In this way, it is possible to reserve forage produced during the rainy season to be used during the shortage period.

Pasture deferral is a technique for feed production in the dry period, with several advantages (Silva *et al*., 2016). It is possible to carry out management actions to ensure that the accumulated forage has good nutritional value (Santos *et al*., 2010). In this sense, in the Cerrado region for *Urochloa brizantha* cv. Marandu (marandu grass), Afonso *et al*. (2018) determined that the pasture should be lowered to 15cm at the beginning of the deferral period to obtain better quality forage and increase sheep performance. However, it is not known how best to carry out this pasture lowering.

It is possible that keeping the pasture low until the beginning of the deferral period will result in a high growth rate of the deferred pasture (Santos *et al*., 2013) which can worsen the structure of the pasture for animal consumption, due to the greater presence of old tillers and, consequently, of worse nutritional value at the end of the deferral. On the other hand, if the sward lowering occurs immediately before the beginning of the deferral period, a large amount of leaf blades when removed could produce sward with better structure in winter, due to the greater presence of young tillers and better nutritional value. Different pasture structures can affect animal consumption, according to Carvalho *et al*. (2001) the morphological model with which the forage is available to the animal at the time of consumption is known as the structure of the pasture, which is responsible for the amount of nutrients ingested in grazing, so that pasture with a predominance of stalks and dead leaves makes it difficult to intake by the animal and decrease their performance.

The evaluation of blood metabolites allows for the evaluation of the nutritional aspects of the animal, since the ingested diet has effects on the biochemical constituents of the blood (Lima *et al*., 2012). In addition, the evaluation of these metabolites helps in the clinical diagnosis of metabolic and nutritional disorders, which can cause reduced production, fertility problems and even death of the animals. (Lopes *et al*., 2015). Thus, the use of this tool in pasture sheep production systems can provide us with important information about the nutrients that have been ingested and those that are scarce or excess in the animal's body, and with this, propose improvements in the supplementation of these animals. Metabolic profiles in beef and dairy cattle have been well studied (Fonte *et al*., 2021; Stefańska *et al*., 2021), however, information on metabolic profiles in sheep is still poorly known.

So far, it is not known how different pasture management strategies can improve the quality of deferred pasture and how this could interfere with the animal's metabolic response depending on the consumption of different nutrients. The animal's response to a given management action should not only be studied as a function of the animal's greater or lesser performance, but it is also important to know what the animals' metabolic adaptations are in the different challenges during the dry period.

In this sense, we aimed to verify if the height reduction strategies of *Urochloa brizantha* cv. Marandu (Marandu grass) at the beginning of the deferral period could change intake and metabolic parameters in sheep throughout the dry seazon.

MATERIAL AND METHODS

The experiment was conducted from June to September 2018, at the Experimental Farm Capim-branco, belonging to the Faculty of Veterinary Medicine of the Federal University of Uberlândia (UFU), in Uberlândia, MG. The experimental area used was a pasture of *Urochloa brizantha* cv. Marandu (marandu grass), which was already established without any degradation stage, consisting of nine pickets, each with 800 m^2, in addition to a reserve area, totaling approximately two hectares. The study was approved by the Ethics Committee on the

Use of Animals (CEUA) of the Federal University of Uberlândia (registration/protocol number 112/18).

The experiment was conducted in a completely randomized design (CRD) with repeated measures over time, with the treatments being the strategies of lowering the pastures allocated in the paddocks, with two animals per paddock and three replications (paddocks) per treatment, totaling nine paddocks. Evaluations were carried out throughout the grazing period, during the beginning (7th day), middle (4th day) and end (90th day), being analyzed as repeated measures over time. Three sward lowering strategies (15, 25, 35cm) were studied at the beginning of the deferral period:

1) Maintenance of marandu grass with 15cm grass for five months before the start of the deferral period (15/15cm);

2) Maintenance of marandu grass with 25cm since October/2017, but at the beginning of the deferral period the pastures were lowered to 15cm (25/15cm);

3) Maintenance of 35cm grass from October/2017 until the beginning of the deferral period, when the pasture was lowered to 15cm (35/15cm).

In October 2017, all paddocks were managed in continuous stocking with variable stocking rate, using adult sheep so that the desired heights (15, 25 or 35cm) were reached and maintained until mid-March 2018, when the deferral period for all pastures. One week before the start of the deferral period, the pastures of the 25/15 and 35/15 cm treatments were lowered to 15 cm. For this, the stocking rate was increased in these paddocks, using sheep with greater body weight. Pasture heights were monitored daily, so that, within a week, canopy lowering to 15cm was established.

After a 90-day deferral period, in June 2018 the period of use of deferred pastures began, which were managed in continuous stocking at a fixed stocking rate, using 18 females, crossbred, ½ Dorper + ½ Santa Inês, non-pregnant, aged between 4 and 5 years and average initial body weight of 57.5kg. During the pre-experimental period, the animals were kept in the experimental area, adapted to the daily routine of the experiment and used to carry out a uniform grazing of the experimental paddock.

The period of use of the deferred pastures started on June 19, 2018, and ended on September 17, 2018, and the consumption assessments and blood collections were carried out at the beginning, middle and end of this period. Blood collections took place at the end of July, August, and September, for three alternate days to obtain mean values in each month of assessment.

The sheep remained on pastures during the day and night periods, where they received protein salt with the following composition: white salt (62.5%), mineral salt (12.5%), corn meal (10%), urea (7.5%) and soybean meal (7.5%). The supplement intake was 0.03kg DM at the beginning, 0.03kg of DM in the middle and 0.02kg of DM at the end of the grazing period.

During the grazing period that occurred between 06/19/2018 and 9/17/2018, there was rainfall of 34.7mm on August 18, 3.3mm and 1.7mm on August 25 and 26, respectively. In September, the rains occurred in the period from 14 to 17 September, accounting for 29mm (Table 1).

Table 1. Monthly rainfall on the Capim Branco farm obtained by the Climatology and Environmental Meteorology Laboratory – CLIMA – UFU

Month	Rainfall (mm)
June	0.0
July	0.0
August	39.7
September	50.3

In each paddock, two animals were used to determine the simulated grazing, intake, and metabolic parameters, totaling eighteen animals, and these evaluations were done at the beginning, middle and end of the grazing period (experimental period). To carry out the simulated grazing, a forage sample was collected by paddock, trying to simulate, during grazing, the morphological composition of the forage consumed by the sheep. (Sollenberger and Cherney, 1995). Sampling was carried out by observing the forage consumption of two animals present in the paddock and collecting a sample similar to that consumed by each animal.

To estimate fecal excretion, purified and enriched lignin LIPE® was used, through the relationship between dose and fecal concentration of the external indicator. LIPE® was administered by esophageal tube, at a daily dosage of 0.5g.animal.day^{-1} in capsules given in the morning, once a day, for 6 days, with two days of adaptation and four days of collection. At the end of the collection period, a composite sampling of feces of each animal was performed. The samples were dried, ground to a size of 1mm for further analysis of the concentration of LIPE®. This determination was made by infrared spectroscopy, using the Watson Galaxy model device, FT-IR 3000 series. Fecal production was calculated as described by Saliba *et al.* (2003). Fecal collection was performed on the third day, after the supply of LIPE®, for 4 days and at the same time as the supply of the capsules. Indigestible neutral detergent fiber (iNDF) was used as an internal indicator to estimate pasture intake. The concentration of iNDF in simulated grazing and feces samples were determined by incubation in TNT bags in the rumen of cattle for 240 h for NDFi (Valente *et al*., 2011). Pasture DM intake was estimated as follows:

Intake = (FE* iNDFF)/ iNDFFor) + IntakeSup
where: Intake = Dry matter intake (g.day^{-1}); FE = fecal excretion (g.day^{-1}); iNDFF= iNDF concentration in feces (g.g^{-1}); IntakeSup = supplement DM intake (g.day^{-1}); and iNDFFor = iNDF concentration in forage (g.g^{-1}).

Samples of pasture, simulated grazing, feces and supplement ingredients were ground in a knife mill (1mm) and placed in plastic pots. The contents of dry matter, mineral matter, crude protein, insoluble fiber in neutral detergent were subsequently determined and ether extract by the methods proposed by the INCT-CA (Detmann *et al*., 2012). To determine the dry matter potentially digestible (DMpd) content of the simulated grazing, the equation proposed by Paulino *et al*. (2008):

DMpd = 0.98 (100 – NDF) + (NDF – iNDF)
where: NDF = neutral detergent fiber; and iNDF = indigestible neutral detergent fiber.

To obtain blood metabolites, blood collections were performed at the beginning, middle and end of the grazing period, at 8 am, through jugular venipuncture with Vacuntainer® coupled to a tube without anticoagulant. Soon after blood collection, the samples were centrifuged at 3000 rotations for 10 minutes, and the serum were separated into aliquots, stored in micro tubes, and kept in a freezer at -5°C for further analysis. After thawing the serum, they were processed in a semi-automatic analyzer (spectrophotometer) Bioplus® 2000, using commercial Lab Test® kits. The energy metabolites analyzed were cholesterol and triglycerides, and the protein metabolites evaluated were total protein, urea, uric acid, creatinine, and albumin.

For the inferential analysis of the data, all variables were analyzed regarding the assumptions of normality and homoscedasticity of variance. Those that met the assumptions were evaluated using analysis of variance, followed by the means test (Student's t test). The variables that did not meet the assumptions were evaluated using non-parametric analysis (Kruskal Wallis test) at the level of 10 % probability for Type I error.

RESULTS AND DISCUSSION

The different strategies to reduce the height of Marandu grass pasture at the beginning of the deferral period did not change the intake and energy and protein metabolism of animals (P<0.10;), which shows that despite the improvement in pasture quality deferred, with crude protein values in simulated grazing ranging from 3.6 to 15.4%, NDF from 57.1 to 82.8% and DMpd from 58.5 to 84%, the different strategies to reduce sward height deferred were not able to promote changes in the pasture, able to change the consumption of nutrients and protein and energy metabolism of animals.

Furthermore, there was also no interaction effect between the different lowering strategies and the grazing period on nutrient intake and metabolite parameters of sheep in deferred pasture (P<0.10). The main effects observed in the study were in relation to the grazing period, that is, changes in the plant that occurred during the dry season were predominant in modifying the consumption and metabolism of the animals.

The grazing period affected the DM intake, when expressed as percentage of body weight (%BW), the CP intake and the NDF digestibility (P<0.10; Table 2), so that the sheep had higher DM intake (%BW) at the beginning and end of the grazing period, and the CP intake and NDF digestibility were higher at the end of the grazing period (P<0.10; Table 2).

Table 2. Effect of grazing period on nutrient intake and chemical composition of samples of simulated grazing in sheep grazing *Urochloa brizantha* cv. Marandu deferred with different lowering strategies

	Grazing Period			
	Start	Middle	End	P value
DM Intake (kg.day^{-1})	0.492	0.447	0.495	0.34
DM Intake (%BW)	0.868 a	0.742 b	0.911 a	0.06
CP Intake (kg.day^{-1})	0.049 b	0.032 c	0.060 a	<0.10
NDF digestibility	32.65 b	29.24 c	37.10 a	<0.10
Simulated grazing (SG)				
Dry matter (%)	34.35 c	60.85 a	45.01 b	<0.10
Organic matter (%)	91.12 ab	91.82 a	91.48 a	<0.10
Crude protein (%)	9.14 a	5.90 b	10.06 a	<0.10
NDF (%)	68.13 b	73.50 a	68.55 b	<0.10
Indigestible NDF (%)	22.03 ab	26.86 a	24.59 a	<0.10
DMpd (%)	77.31 a	72.61 ab	74.77 a	<0.10

[a, b, c] Line means followed by different letters differ statistically (P<0.10). DM: dry matter; CP: crude protein; NDF: fiber insoluble in neutral detergent; DMpd:dry matter potentially digestible; [a,b,c] differ by Student's t test (P<0.10).

At the beginning of the grazing period (winter) it is possible that animals were consuming the upper strata of the pastures, with a greater presence of live leaves, however, with the advance of the dry season, when the climatic conditions were unfavorable to regrowth of the pasture (Table 1), there was a worsening in the quality of deferred pasture, with an increase in dead material (Silva *et al*., 2016; Pessoa *et al*., 2016). In the middle of the grazing period, the animals began to consume the most basal strata of the pasture, with a greater presence of dead material and stem. (Vendramini *et al*., 2019). However, at the end of grazing period, from late August to early September, which coincides with the beginning of the rainy season in the Cerrado region, there were some rains (Table 1), which favored the appearance of young tillers and caused greater consumption of live leaves and protein. In fact, Santos *et al*. (2011), analyzed the chemical composition of different morphological components of the pasture and found that the live leaf has higher CP content and lower NDF and iNDF.

There was no effect of grazing period on cholesterol concentrations (P>0.10). High cholesterol levels occur when there are biliary obstructions, hypothyroidism, or diets high in fat or carbohydrates (Gonzáles and Silva 2006). In the present study, the low concentrations of cholesterol (54.07 until 56.29mg. dL^{-1}), with reference values of 15 to 139.9mg. dL^{-1} by Silva *et al*. (2020) and 52 until 76mg. dL^{-1} for Kaneko *et al*. (2008), reflect the diet low in fat and rapidly fermentable carbohydrates ingested by animals.

In the middle and end of grazing period, the lowest serum concentrations of triglycerides in the animals were verified (P=0.06; Table 4). In the middle of grazing period, which was the most critical dry season, the animals had lower intake (%BW) (Table 2), with low energy intake resulting from low forage intake. In this time, the animals started to use energy present in triglycerides to meet their maintenance energy demand, thus reflecting in low concentrations blood in the middle of grazing period (Santos *et al*., 2015).

The highest concentrations of uric acid, urea, creatinine, total protein, and albumin were verified in animals at the end of grazing period (P<0.10; Table 3). In fact, at the end of grazing period, a higher consumption of crude protein by the animals was identified (P<0.10; Table 2), which was caused by the regrowth of the pasture that occurred due to rainfall at the end of the month of August and early September.

The higher concentration of urea at the end of grazing period (34.10mg.dL^{-1}) reflects the higher consumption of crude protein by the animals in the regrowth pasture, which is richer in fraction A (Non-Protein Nitrogen) and is rapidly available in the ruminal environment. Queiroz *et al*. (2011) verified for marandu grass at 30 and 60 days of regrowth age, high concentrations of CP fraction A, with values ranging from 38.3% to 45.02% of Total Nitrogen. However, as the animals did not receive supplementation rich in rapidly fermentable carbohydrates, the rapid release of nitrogen in the rumen led to increased urea synthesis in the animals' liver and blood. (Flores *et al*., 2020; Tan and Murphy, 2004; Kebreab *et al*., 2002). Xia *et al*. (2018) found in Dutch cattle fed three levels of CP in the diet (10, 12 and 14% CP) a linear increase in serum urea concentration in animals with increased protein intake.

The blood concentration of uric acid reflects the synthesis of microbial protein in the rumen, as it is a result of the degradation of the DNA of ruminal microorganisms, such as bacteria and protozoa that have undergone digestion, having their nucleic acids absorbed in the intestine. (Araújo *et al*., 2012). In this sense, animals at the beginning and end of the grazing period had greater nitrogen supply reaching the rumen, which resulted in increase of microbial protein and thus a higher level of uric acid in the blood.

The higher concentrations of total protein obtained in animals at the end of grazing period are due to better quality of ingested forage, with higher CP levels and NDF digestibility. Ribeiro *et al*. (2003) evaluating the metabolic profile in Corriedale ewes reared in native pastures in southern Brazil observed a higher concentration of total protein in the spring season, where there is better quality pasture.

Table 3. Effect of the grazing period on the blood concentration of energy and protein metabolites expressed in $mg.dL^{-1}$ in sheep using deferred pasture with different lowering strategies

	Grazing Period					
Variable	Start	Middle	End	P value	RV Kaneko *et al.* (2008)	RV Silva *et al.* (2020)
Cholesterol	54.53a	56.29a	54.07a	0.701	52-76	15-139.9
Triglycerides	20.00a	15.52b	16.96b	0.061	52-76	5-78
Uric acid	0.473a	0.236b	0.500a	P<0.001	0-1.9	0-2.9
Urea	22.15b	21.38b	34.10a	P<0.001	12.8–100	17-43
Creatinine	0.495c	0.715a	0.758b	P<0.001	0.40-1.80	1.2-1.9
Total Protein	4.23b	5.97a	5.94a	P<0.001	6-7.9	3.1-11.4
Albumin	3.00c	3.67b	3.80a	P<0.001	2.4-3.0	1.12-5.38

RV: reference values

Albumin is considered a more sensitive indicator for assessing protein nutritional status than total proteins. Persistently low values of suggest inadequate protein intake. Albumin concentrations verified in the present study were above the reference values recommended by Kaneko *et al.* (2008) and Silva *et al.* (2020). The highest concentrations of albumin were also verified at the end of grazing period (P<0.10), due to higher CP contents of deferred pasture at the beginning and end of grazing period. Saro *et al*. (2020) when offering three levels of CP in the diet of finishing sheep, they verified an increase in the concentration of serum albumin in the treatment with higher CP content (173g CP. kg DM^{-1}). Albumin is the most abundant circulating protein, and its plasma concentration is mainly modulated by dietary protein intake (Paula e Silva *et al*., 2008).

Among the nitrogenous metabolites present in the blood, creatinine is an indicator of renal function, formed as an end product of muscle metabolism and eliminated by the kidneys. High creatinine levels may indicate impaired renal function (Issi *et al.* 2016). The serum creatinine concentrations in the present study ranged from 0.495 to $0.757mg.dL^{-1}$, which shows that the animals did not present kidney problems and intense metabolism of amino acids stored in the tissues.

The metabolic parameters measured in the blood were all within the expected ranges for the sheep species considering the age and body weight of the animals, suggesting no effect of the treatments, with greater effects from the grazing period. Furthermore, changes in protein and energy metabolites throughout the dry season indicate the need for differentiated supplementation for phases this period. In other words, to reduce problems arising from the loss of nutritional value of pasture and with consumption reduction by animals during the winter grazing period, an appropriate strategy is to use feed supplements, concentrated or forage, differentiated over the grazing period (Silva *et al*., 2021).

Furthermore, the results with the main protein and energy metabolites analyzed showed the lack of readily fermentable carbohydrate sources in sheep fed on deferred pasture exclusively, requiring the inclusion of feeds rich in this nutrient so that it is possible to optimize the use of deferred forage

CONCLUSION

Strategies to reduce pasture height of *Urochloa brizantha* cv. Marandu (Marandu grass) at beginning of deferral period do not alter nutrient intake and energy and protein metabolism in sheep during the grazing period. However, changes occurring in pasture during the dry season alter these variables. The deferral is an interesting strategy to be used for forage production in winter, and to improve its use, it is necessary supplementation with concentrates, rich in rapidly fermentable carbohydrates.

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