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Metabolic profile of female lambs on annual ryegrass pasture managed under different grazing intensities and methods

[*Perfil metabólico de cordeiros fêmeas em pastagem de azevém manejada sob diferentes intensidades e métodos de pastejo*]

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ABSTRACT

The aim of this study was to evaluate the effect of grazing methods and intensities on the metabolic profile of lambs grazing ryegrass. This study was performed in Eldorado do Sul (RS). The treatments consisted of two grazing intensities with rotational and continuous stocking methods: moderate and low. The experimental design was a randomized block arranged in a factorial scheme with four replicates. To evaluate the metabolic and nutritional conditions of the lambs, blood samples were collected to evaluate phosphorus, albumin, glucose, magnesium, globulin, cortisol, urea and total proteins. The highest blood urea value was observed in the month of September in lambs kept in continuous method grazing. There was no statistical difference between the grazing intensities and grazing methods for albumin, glucose, magnesium, globulin, cortisol and total protein levels in profile lambs. The cortisol values suggested that the animals were not subjected to high stress levels. In conclusion, rearing female lambs on ryegrass, irrespective of grazing methods or intensities, allowed the maintenance of body homeostasis and did not cause any metabolic unbalances, nutritional unbalances or stress.

keywords: animal welfare, blood, continuous stocking, grazing system, rotational stocking, continuous stocking

RESUMO

O objetivo deste estudo foi avaliar o efeito dos métodos e das intensidades de pastejo no perfil metabólico de cordeiros. Este estudo foi realizado em Eldorado do Sul (RS). Os tratamentos consistiram em duas intensidades de pastejo (moderada e baixa), com métodos lotação rotativa e lotação contínua. O delineamento experimental foi em blocos ao acaso, em esquema fatorial com quatro repetições. Para avaliar as condições metabólicas e nutricionais dos cordeiros, foram coletadas amostras de sangue para avaliar fósforo, albumina, glicose, magnésio, globulina, cortisol, ureia e proteínas totais. O maior valor de ureia no sangue foi observado no mês de setembro nos cordeiros mantidos em pastejo contínuo. Não houve diferença estatística entre as intensidades de pastejo e os métodos de pastejo para albumina, glicose, magnésio, globulina, cortisol e níveis de proteína total nos cordeiros perfilados. Os valores de cortisol sugeriram que os animais não foram submetidos a altos níveis de estresse. Concluindo, a criação de cordeiros em azevém, independentemente dos métodos ou das intensidades de pastejo, permitiu a manutenção da homeostase corporal e não causou desequilíbrios metabólicos, desequilíbrios nutricionais ou estresse.

Palavras-chave: bem-estar animal, sangue, pastejo de ovinos, sistema de pastejo, lotação rotativa, lotação contínua

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INTRODUCTION

Sheep farming has grown widely in Brazil using systems based on pasture. Rearing lambs on pastures is fundamental for small extensions of lands, and it helps to keep man in the field and can be easily integrated with other farming enterprises. The number of consumers interested in information regarding how to feed animals has been increasing due to interest in healthy products. Grazing animals is considered healthier because this practice is considered a safe and natural production system that considers the well-being of the animals and environment. A healthy animal is produced with suitable feeding, and the nutritional status among other parameters can be evaluated through the determination of several serum metabolites.

Based on serum measurements, the metabolic profile can be determined, and the metabolic profile consists of a series of indicators that work as tools to evaluate the productive and reproductive index of a flock. The analysis of these biochemical blood components reliably reflects the balance between the entrance, exit and metabolism of nutrients in animal tissues (González*,* 2000).

In sheep production, the period of lamb rearing is fundamentally important for the future reproductive performance of the lamb. It is necessary to know the metabolic status of the lamb to avoid any damage to the estrus. The growth of poorly nourished females can be delayed, and puberty of these females can be late because they do not have enough energy to produce follicles with a capacity of ovulating and do not have enough hormones to begin the estrous cycle (Hafez *et al.,* 2004).

Despite the successful use of metabolic profiling in beef and milk cattle (Peixoto *et al.,* 2006, Wittwer, 2000, Alvarenga *et al.,* 2017), information regarding metabolic profiles in sheep is scarce. Therefore, the aim of this study was to evaluate the effect of grazing methods and intensities during grazing period on the metabolic profile of lambs grazing ryegrass (*Lolium multiflorum* Lam) under different intensities and grazing methods.

MATERIALS AND METHODS

The experiment was conducted in the Experimental Agronomic Station of the Federal University of Rio Grande do Sul (EEA/UFRGS) in the city of Eldorado do Sul, Rio Grande do Sul (located at the Central Depression of state). The geographical coordinates of the study area are as follows: 30º05'22" S and 51º39'08" W with an altitude of 46 m. According to the Koppen classification system**,** the climate of the region is Cfa (humid subtropical). The soil is classified as red ultisoil typical dystrophic according to the Brazilian system of soil classification (Sistema…, 1999).

Natural ryegrass reseeding (*Lolium multiflorum* Lam) was used for grazing. The fertilization was composed of 300kg ha^{-1} of fertilizer (formula N-P-K: 5-20-20) at beginning germination, and 90kg ha^{-1} of nitrogen (N) in a form of urea was applied to the study area in single application on September 26. The experimental period began with grazing in the pasture on August 2 and lasted 102 days.

The trial was conducted in an area corresponding to 16 experimental units (paddock) in which the individual area varied from 0.23 to 0.41 ha. The treatments consisted of two grazing intensities (moderate and low) and two grazing methods (continuous and rotational) with tree periods in four replications per area. The intensity of moderate grazing was defined by herbage allowance of 2.5 times the potential animal intake, and the low pasture intensity was defined by herbage allowance of 5 times the potential animal intake (Nutrient…, 1985). 147 lambs were used in the trial, an average age of nine months born in previous spring and an average weight of 28 ± 1.6kg.

A variable number of regulating animals to adjust the herbage allowance in the same physiological status as the experimental animals was used by the put-and-take technique (Mott and Lucas, 1952). Because of the need to keep the same forage allowance for both grazing methods, the period of adjustment of animal load was the same for both. In rotational grazing, the leaf life duration (LLD) was used as a tool to establish the duration of grazing cycle and adjustment date of allowances.

The number of days of pasture grazing varied during the cycle of grazing, and the occupation period was fixed lasting two days. Based on the information obtained by Pontes *et al.,* (2003), the duration of the grazing cycle was calculated through the relationship between Phyllochron (PHC) and the maximum number of live leaves per tiller (MNLL) (LLD = MNLL/PHC). The Phylochon was estimated from the following formulas: PHC = FSLB/(A. LER), being "FSLB " The final size of the leaf blade and "a" the number of growing letters growing simultaneously in the same tiller and read leaf elongation rate obtained by the difference between the lengths of the expanding green blades. The LLD value (degrees day $^{-1}$) of August (500°C leaf $^{-1}$) and the LLD value of the period from September to November (410°C leaf $^{-1}$) were divided by the average temperature from August to November. These values were obtained from climatic series data collected by the Agrometeorology Division of EEA/UFRGS. The calculations resulted in four grazing cycles as follows: 32, 26, 24 and 20 days.

The animals were weighed at the beginning and at the end of each grazing cycle after 12 hours fasting of solids and liquids. During the animal weighing process, the body condition score (BCS) was evaluated based on a scale (1 = extremely thin and 5 = extremely fat). The animals had access to mineralized salt and water *ad libitum*, and the animals had periodic sanitary control. In continuous grazing, for forage mass evaluation (FM), six samples of forage were cut aboveground in each paddock within a frame of 0.25m^2 at each cycle grazing. In the rotational grazing paddocks, forage mass was evaluated before grazing. Two cuts were made in a grazing range at the beginning of the cycle and two in the penultimate grazing range at the end of the cycle. The samples were dried in a stove at 65°C until a constant weight was reached. The forage mass was expressed as kilograms of dry matter (DM) per area unit.

The herbage allowance (HA) was calculated using the following formula:

$$HA= (FM/n + FA)*100/AL$$

Where: HA is the herbage allowance (%); FM is the average forage mass in each grazing cycle (kg ha^{-1} of DM); n is the number of days in the grazing cycle; FA is the daily forage accumulation rate (kg ha^{-1} days^{-1} of DM); and AL is the average animal load of grazing cycle (kg ha^{-1} of PV).

To evaluate the metabolic and nutritional conditions of the lambs, blood samples using "vacutainer" tubes (Becton Dickinson, Brazil) were collected from the jugular vein of lambs in tree periods of evaluation at the end of each month. In each evaluation two tubes were collected, one containing sodium fluoride and 10% EDTA for plasma use and one without serum anticoagulant with coagulation activator gel. All blood sampling was taken in the morning to avoid the influence of circadian rhythm on biochemical blood parameters. The samples were sent to the lab where they were centrifuged at 2500 rpm, and the plasma and serum aliquots were frozen at -20°C until analysis.

The representative metabolites were quantified as follows: protein and minerals by specific techniques of photocolorimetry; glucose by means of glucose oxidasis; beta-hydroxybutyrate by an ultraviolet enzyme kinetics method; total protein by the Biuret method; albumin by a green bromocresol method; urea by means of urease; phosphorus by an ammonium molybdate method; magnesium by a blue xylitol method (Kit Labtest reagent set, Brazil); and globulin by calculations (albumin subtracted from total protein). Cortisol was also used as an indicator of stress, and cortisol was measured by a solid phase radioimmunoassay using the Kit ICN reagent set (Biomedicals, Canada).

The trial was performed according to a randomized blocks experimental design arranged in a factorial scheme (2 x 2), with repeated measurements in time with on animals during three experimental periods and four field repetitions. In the statistical model the level of herbage pasture, the method of grazing, the time, as well as the corresponding interactions as fixed effects were considering fixed effect and the animals and the replicate was be included as random effects into each treatment. The obtained data were submitted to analysis of variance and F-tests at a 5% level of significance using mixed models. When detected differences between means, this was comparted using Tukey-Kramer test wish the same significance level.

RESULTS

The forage mass (FM) varied from 2187 to 2877kg ha^{-1} of dry matter (DM). The herbage allowances (HAs) in ryegrass pastures were different (P<0.05) between the grazing intensities with 9 and 24% BW for the moderate and low grazing intensities, respectively. The HA values were similar (P>0.05) for the continuous and rotational grazing methods with the moderate (10.5 and 7.5% BW, respectively) and low (27 and 20% BW, respectively) grazing intensities.

The animals subjected to the different treatments of grazing intensities and methods had statistically similar (P>0.05) body weight (BW) values and body condition (BC) scores. At the beginning of the winter grazing cycle, the lambs had average BW and BC values of 28 ± 1.6kg and 2.0 ± 0.1, respectively. At the end of the grazing cycle, the lambs had average BW and BC values of 41 ± 2.0kg and 2.7 ± 0.1, respectively. There were no differences (P>0.05) between the treatments.

The obtained values for the studied metabolites (Tables 1, 2 and 3) were similar to the values referenced by several authors (Kaneko *et al.,* 1997; Jain, 1993). With regard to the plasma phosphorus concentration, there was no interaction between the grazing method and experimental periods (P>0.05). In the experimental periods, however, the plasma phosphorus concentrations varied according to the grazing intensity with higher phosphorus concentrations found in the animals subjected to moderate grazing intensity than in the animals subjected to low grazing intensity (P<0.05).

Table 1. Metabolic profile and standard error of lambs in annual ryegrass grazing with different pasture intensities

Pasture intensity	Period			Average
	September	October	November	
		Phosphorus (mg dL^{-1})		
Moderate	6.6±0.2	6.1±0.2	7.3±0.2	6.7±0.1 A
Low	6.1±0.2	5.7±0.2	6.6±0.2	6.2±0.1 B
Average	6.4±0.1 b	5.9±0.15 c	6.9±0.1 a	
		Urea (mg dL^{-1})		
Moderate	46.1±2.2 ab	47.2±2.2 ab	43.6±2.2 ab	45.6±1.5
Low	53.4±2.2 a	43.8±2.2 b	40.0±2.4 b	45.8±1.5
Average	49.8±1.6	45.5±1.6	41.8±1.6	
		Beta-hydroxybutyrate (mmol L^{-1})		
Moderate	0.31±0.02 ab	0.33±0.02 a	0.23±0.02 b	0.29±0.02
Low	0.27±0.02 ab	0.31±0.02 ab	0.29±0.03 ab	0.29±0.02
Average	0.29±0.02	0.32±0.02	0.26±0.02	

Averages followed by different capital letters for the same characteristic differ between grazing method according to the Tukey-Kramer test (P<0.05). Different lower-case letters for the same characteristic between months differ according to the Tukey-Kramer test. Neither urea nor beta-hydroxybutyrate (BHB) were influenced (P>0.05) by the grazing intensities. The urea values in the animals subjected to moderate grazing intensity did not differ among the evaluated periods. In the low grazing intensity group (September), higher values were found at the beginning of the evaluation period than at the end of the evaluation period.

There was an interaction between the grazing method and experimental period for the blood urea concentration (P<0.05) (Table 2). The highest blood urea value was observed in the month of September in lambs kept in continuous method grazing. In the remaining months, there were no differences between grazing methods. Table 3 shows the albumin, glucose, magnesium, globulin, cortisol and total protein values as detected in the metabolic profile of lambs. There was no statistical difference between the grazing intensities and grazing methods for albumin, glucose, magnesium, globulin, cortisol and total protein levels (P>0.05). The blood concentrations of albumin, glucose and cortisol were different in the evaluated periods (P<0.05).

Table 2. Values of blood urea and standard error in lambs fed with ryegrass under different grazing methods

Grazing method	Period			Average
	September	October	November	
	Urea (mg dL^{-1})			
Continuous	58.6±2.2 Aa	45.6±2.2 Ab	41.9±2.2 Ab	48.7±1.5
Rotational	40.9±2.2 Bb	45.4±2.2 Ab	41.7±2.4 Ab	42.7±1.5
Average	49.8±1.6	45.5±1.6	41.8±1.6	

Different lower-case letters in the row and uppercase letters in the column for the same characteristic differ test (P<0.05).

Table 3. Metabolic profile and standard error in lambs fed by grazing ryegrass under different grazing intensities and methods in Eldorado do Sul in 2006

Metabolites	Period		
	September	October	November
Albumin (g L^{-1})	28.8±0.9 a	27.9±0.9 ab	25.9±0.9 b
Glucose (mg dL^{-1})	71.7±2.4 a	63.7±2.3 b	58.9±2.4 b
Magnesium (mg dL^{-1})	2.1±0.1	2.2±0.1	2.1±0.1
Globulin (g L^{-1})	40.4±1.4	39.1±1.3	42.3±1.4
Cortisol (Mcg dL^{-1})	2.8±0.2 a	2.0±0.2 b	2.8±0.2 a
Total Proteins	69.3±1.3	67.1±1.3	68.3±1.4

Different lower-case letters in the same line differ according to Tukey-Kramer test (P<0.05).

The lowest (P<0.05) albumin value was observed in the final period of evaluation. In the present study, the blood glucose concentrations in the initial period were higher (P<0.05) than the concentrations found in the other experimental periods. The lowest values of serum cortisol in the experimental animals were found in the intermediate period (P<0.05). However, the cortisol values found in the animals under different grazing intensities and methods for all of the periods were not below the values considered indicators of discomfort in animals. The levels of magnesium found in this experiment indicated that the sheep had a nutritional mineral balance.

DISCUSSION

According to the data of Amaral *et al.,* (2013) in pasture of annual ryegrass herbage intake is maximized with FM values around of 2600 at 2800kg ha^{-1}. When there is high forage mass, there is a tendency to increase the manipulating movements of apprehension and chewing~~,~~ caused by increase of stems and reduction of leaves in FM (Gonçalves *et al.,* 2018) which may result in decreased intake. However, slightly higher values did not promote any nutritional imbalance. According to Hafez *et al.* (2004), the weight reached by the lambs allowed them to reproduce because the first ovulation occurs when the female weighs from 50 to 70% of adult weight, which is an average of 80kg of BW in the studied breeds (Padrões, 2020).

Importantly, the low phosphorus concentrations in this study were not below the minimum concentration necessary to complete development, but lower than the values cited by Santos *et al.* (2018) from 8.1 to 10.02mg/dL. Phosphorus contributes to the growth of young animals and maintenance of adult animals because it is an important component of bones, soft tissues, cell membranes and other body fluids. Moreover, phosphorus participates in the generation ATP. Phosphorus is also a constituent of phospholipids and phosphoproteins, and it is an excellent buffer (Georgievskii, 1982; Breves and Schroder, 1991). Despite being an excellent predictor of nutritional status of sheep, González (2000) reported that there is no correlation between the availability of phosphorus from grazing and plasma phosphorus in grazing cattle and sheep suggesting that variations in the plasma levels of phosphorus are related more to the metabolic demand rather than to the mineral intake.

The lack of correlation may have occurred because the ryegrass was in a vegetative phase, which may have provided high values of crude protein available (average of 24.2%) (Fox *et al.,* 2003). Consumed protein is converted to ammonia by the action of bacterial enzymes in the rumen, and the ammonia together with the carbons that come from the diet carbohydrates are then used by the ruminant microbiota for the production of amino acids. The ammonia that is not used by the ruminant flora passes through the rumen wall in the blood and goes to the liver where it is processed into urea, which is not toxic and is hydro soluble. Urea circulates in the blood is excreted in the urine or milk, or it is recycled to the rumen by saliva or by diffusion through the organ wall (Church, 1988). With the progression of the phenological status of plants, there is a decrease in plant quality, which decreases the availability of digestible protein. The decrease in available digestible protein may have been responsible for the low concentrations of blood urea in the last period for the sheep kept under low grazing intensity. Low grazing intensities are related to lower defoliation and plant growth and in advance of the physiological stage. According to Santos *et al.* (2018), nutritional variation is likely due to physiological modifications in forage cycle, which causes reduction in leaves proportion, increased stem participation and lignification of plant.

BHB is one of the ketone bodies involved in the process of consuming energetic reservations, and it has been used to detect a negative energetic balance (Brito *et al.,* 2006). The BHB values in the present study did not reach the levels of high lipomobilization (greater than 1.0mmol L^{-1}) according to previous studies (Russel *et al.,* 1977), which indicated that negative energetic balances were not found in these animals despite the variations between the experimental periods. In the present study, the methods of grazing animals did not cause a negative energy balance in sheep, and even in lower forage supply indicated by BHB values. The pasture was maintained in a vegetative stage with high leaf to stem ratio, presenting high values of water-soluble carbohydrates (Ullmann, *et al.,* 2017), which contributes to maintain the nutritional status of the animal.

Plasma urea is an indicator of ingestion and short-term mobilization of protein components (González, 2000). The animals in continuous rotational grazing presented the highest concentrations of urea in the period of September, and the animals in rotational grazing did not have variation in serum urea concentrations. The maintenance of serum urea levels in rotational grazing may be explained by the continued quality of herbage consumed because the animals grazed successive regrowth. Marley *et al.* (2007) related the best herbage quality in rotational grazing with lower fiber concentrations and higher digestibility of green leaves when compared to continuous grazing.

Continuous low urea values suggest inadequate protein intake (Payne & Payne, 1987). Even the extremely low urea values in this study were not less than the values considered normal (Contreras, 2000). Even though the albumin levels were 50 to 65% of the total proteins (Payne & Payne, 1987), albumin levels did not vary according to the period. The same pattern was found for globulin, which is another important constituent of total proteins. The liver, especially with high protein and vitamin A levels and hepatic functionality (González *et al.,* 2000), synthesizes blood proteins. The blood glucose level in the present study was higher than the values reported by Ribeiro *et al.* (2003) for lambs in Rio Grande do Sul with values between 49.3 and 54.6mg dl^{-1}.

Changes in feeding or physiological state (rapid growth, pregnancy, and milking) affect the total consumption levels of glucose. Ruminants fed with high percentage of herbage in the diet depend on glucose hepatic synthesis to satisfy their metabolic demands (Huntingon, 1997), Marley *et al.* (2007) found lower concentrations of glucose in the blood of lambs subjected to rotational grazing, and this difference was not found in the present experiment.

Cortisol is the main hormone indicator of stress in lambs, and its dosing has been used to evaluate the effect of adverse factors in management procedures (e.g., castration) (Kent *et al.,* 1993). serum cortisol values of experimental animals were found in the intermediate period ($P<0.05$), but in none of the periods the values found in animals subjected to different grazing intensity and grazing methods are lower than those considered as indicators of animal discomfort. Kent *et al.* (1993) have found maximum levels of cortisol (1.0mg dl^{-1}) in lambs subjected to castration. In this sense, the cortisol is a classical indicator of stress response, however, it is a better indicator of acute stress.

The level of magnesium in the metabolic profile can indicate the subclinical status of hypomagnesemia before the problem arises (abnormal level between 2.0 and 3.0mg dl^{-1}). The homeostatic control of magnesium is not rigid, so blood magnesium concentration directly reflects the level of magnesium in the diet (González & Campos, 2003). Results found in this study are in agreement with Santos *et al.* (2018) that affirms that different systems did not promote significant changes in the profiles. Similarly, low and moderate grazing intensity did not influence the metabolic profile of sheep kept in annual ryegrass.

CONCLUSION

The grazing method does not change the metabolic profile of lambs on ryegrass pasture at the studied intensities. The annual ryegrass can be used in any method or intensity of grazing by lambs and allows the maintenance of body homeostasis without causing any nutritional unbalances, metabolic unbalances or stress during pasture occupation period.

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