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The effect of grazing intensity and supplementation on performance, stress indicators and metabolic profiles of finishing lambs

Efeito de intensidade de pastejo e suplementação no desempenho, indicadores de estresse e perfil metabólico de cordeiros em terminação

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Abstract

In the present study, the effect of grazing intensity (GI) and feed supplementation in sixty-four crossbred Santa Inês lambs was assessed based on performance, blood stress indicators, and metabolic profiles. The GIs analyzed were VH = very high, H = high, L = low, and VL = very low, and were represented by the residual leaf area index (LAIr). The lambs were divided into two groups, supplemented (SP) and non-supplemented (NSP). The dry matter intake (DMI) and average daily gain (ADG) increased linearly with decreasing GI ($P < 0.05$), and the ADG was higher for the NSP group than the SP group ($P < 0.05$). The plasma cortisol concentration was higher in the NSP group ($P < 0.05$), and it was not affected by GIs ($P > 0.05$). The neutrophil:lymphocyte (N:L) ratio decreased linearly with GI ($P < 0.05$), and the SP group had a higher N:L ratio ($P < 0.05$). None of the parameters evaluated for protein metabolism were affected by GI ($P > 0.05$), except albumin, where the SP group had a higher concentration of this metabolite ($P < 0.05$). Regarding energy metabolism indicators, glucose showed a linear increase with a decrease in GI ($P < 0.05$) and was higher in the SP group ($P < 0.05$); however, there was a decreasing linear effect ($P < 0.05$) and the NSP group had higher serum levels ($P < 0.05$) of non-esterified fatty acids (NEFA). The concentration of beta hydroxybutyrate (HBA-B) was higher in the SP group ($P < 0.05$). The concentration of calcium was affected by GI ($P < 0.05$). The phosphorus concentration was higher in the SP group ($P < 0.05$), and concentration of magnesium was not affected by any of the treatments ($P > 0.05$). It was concluded that GI and SP modified the performance, stress indicators, and metabolic profiles of finishing lambs.
Key words: Blood parameters, cortisol, energy metabolism, ovine, pasture, protein metabolism

Resumo

No presente estudo foi avaliado o efeito de intensidades de pastejo (IP) pelo índice de área foliar residual (IAFr). Onde, 0,8; 1,4; 2,0 e 2,6 de IAFr corresponde a MA= muito alto, A= alto, B= baixo e MB= muito baixo com suplemento (SP) ou não suplementado (NSP) no desempenho, indicadores de estresse e perfil

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metabólico em sessenta e quatro cordeiros mestiços Santa Inês (15,40±2,31 kg peso vivo). O consumo de matéria seca (CMS) e ganho médio diário (GMD), em resposta a IP, apresentaram efeito linear crescente (P<0,05) com a diminuição da IP e o grupo NSP foi superior (P<0,05) ao grupo SP quanto ao CMS. A concentração plasmática de cortisol foi maior no grupo NSP (P<0,05) e não apresentou efeito (P>0,05) entre as IP. A relação Neutrófilo:Linfócito apresentou efeito linear decrescente (P<0,05) com a IP e o grupo SPT apresentou maior (P<0,05) relação Neutrófilo:Linfócito. Nenhum dos parâmetros avaliados referentes ao metabolismo proteico foram afetados (P>0,05) pela IP, exceto a Albumina que apresentou efeito (P<0,05) e o grupo SP apresentou maior concentração desse metabólito. Referente aos indicadores do metabolismo energético, a glicose apresentou efeito linear crescente (P<0,05) com o a diminuição da IP e foi maior no grupo SP (P<0,05), de maneira oposta, houve efeito linear decrescente dos ácidos graxos não esterificados (AGNE) (P<0,05) e o grupo NSP apresentou maior (P<0,05) nível sérico. A concentração de betahidroxibutirato (B-HBA) foi maior (P<0,05) no grupo SPT. A concentração do cálcio foi modificada (P<0,05) pela IP. A concentração do fósforo foi maior (P<0,05) no grupo SP e o magnésio não apresentou efeito (P>0,05) de nenhum dos tratamentos. Conclui-se que a IP e SP modificou o desempenho, indicadores de estresse e perfil metabólico de cordeiros em terminação.
Palavras-chave: Cortisol, metabolismo energético, metabolismo proteico, ovino, parâmetros sanguíneos, pasto

Introduction

In Brazil, the production of grazing ruminants is the most widespread agricultural practice. Given the importance that the production system has for Brazilian livestock, grazing management strategies have been studied with the objective of manipulating canopy structure to optimize the process of growing forage, and harvesting by grazing animals (CARVALHO et al., 2001) .

The main consequence of pasture management is the generation of grazing intensities (GI) leading to different growth rates, proportions, and plant morphology (NABINGER et al., 2009). Moreover, it generates different pastoral environments regarding capacity (number of animals per area) and forage offer (INYANG et al., 2010). In response to changes in pasture structure and food availability, the animal alters its behavior in an attempt to maintain forage intake (CARVALHO et al., 2007). Since animal behavior change is the first stress response (MOBERG, 2000), this change may characterize a stressful situation. Similar situations to those found in pastures at different grazing intensities have already been observed in the production of ruminants and were characterized as stressful, including increase in stocking density (CAROPRESE et al., 2009) and food deprivation (PURCHAS, 1973), which may be potentially stressful, since in the pasture the same may occur, thus modifying animal grazing welfare.

Apart from behavioral change in stress response, the neuroendocrine system promotes the secretion of glucocorticoid hormones such as cortisol (MATTERI et al., 2000). Increased cortisol concentration in the bloodstream causes changes in metabolism (ELSASSER et al., 2000) and leads to inefficient use of dietary energy by the body (KNOTT et al., 2010). The chronic increase in cortisol secretion triggers catabolic effects in protein and lipid tissues, besides immunosuppression (ELSASSER et al., 2000; DHABHAR et al., 1996), thus possibly affecting the performance of grazing animals. As cortisol plays an important role in metabolic energy (BASSETT, 1968), supplementation (SP) with energy foods increases the energy input in the body, and it could help combat possible stressors in the pastoral environment.

This study investigated whether GI, with or without SP, can influence the performance, stress condition, and consequently the metabolism of finishing lambs. Knowing the role of stress in different grazing intensities and how it is related to supplementation, will enable preventive measures to be adopted or not according to its need.

Therefore, the aim of this study was to assess the effect of GI and SP on performance, stress indicators, and metabolic profiles of finishing lambs.

Material and Methods

The experiment was conducted at the "Julio de Mesquita Filho" São Paulo State University, Faculty of Agriculture and Veterinary Sciences, Campus Jaboticabal-São Paulo, Brazil, Department of Animal Science in the Forage and Grassland Section, located at 21° 14' 05'' S, 48° 17' 09' W, at an altitude of 615.01 m a.s.l.. Data collection was conducted from December 2010 to April 2011, characterized as the rainy season. The animals were previously adapted to the experimental conditions for 14 d, and the whole experimental period lasted 119 d. The procedures used in the experiment were approved by the Ethics Committee on Animal Use (ECAU), Protocol n° 012490/10.

The treatments consisted of four GIs: very high (VH), high (H), low (L), and very low (VL), and two feeding levels: SP and non-supplemented (NSP), in a factorial 4 x 2 design. For pasture management, GI was characterized by using the residual leaf area index (LAIr), consisting of the remaining leaf area after grazing per soil area, with VH, H, L, and VL as GI of 0.8, 1.4, 2.0, and 2.6 respectively.

The structural characteristics of the pasture were evaluated repeatedly over time (TM), with 4, 5, 5, and 6 cycles (time) of grazing for GIs of VH, H, L, and VL, respectively, in a random blocks design (n = 4) with six blocks, where four were evaluated and two were reserves.

The blood stress indicators and metabolic profiles were assessed randomly (n = 8) on days 0, 21, 42, 63, 84, and 105. The dry matter intake (DMI) was evaluated in animals (n = 4) contemporary to the *testers* so that the others were not affected by the evaluation.

Seventy-nine crossbred Santa Inês lambs were used, aged four to six months old, and 64 of these experimental animals had an average body weight of 15.4 ± 2.31 kg, and 15 regulators were used to increase stocking rate in paddocks to reach residuals. Before starting the experiment the animals were maintained in a single paddock (0.7 ha) with the same characteristics as experimental paddocks for 21 d.

Throughout the experiment the animals were kept on pasture during the day (07:00 to 18:00). Each paddock had artificial shade and enough clean and fresh water for the whole group. At dusk, the animals were collected in wooden cages equipped with individual drinkers and feeders to facilitate individual feeding. The cages were maintained inside a masonry shed with a metal roof.

The animals received 0.7% body weight of commercial supplement (Table 1), according to the manufacturer's recommendations, on arrival at the shed. The ingredients used in manufacturing the supplement were not provided by the manufacturer. The chemical composition of the food samples was determined in samples of grazable stratum of pre-grazing of four repetitions per treatment in 4, 5, 5, and 6 cycles of GI of VH, H, LV, and VL, respectively.

Every two weeks, fecal examinations for egg counts per gram of feces (EPG) were performed by the modified McMaster technique (GORDON; WHITLOCK, 1939), and the examination of conjunctival mucosa (FAMACHA), was carried out every seven days. Deworming to control nematode parasites on animals were performed in EPGs over 700, and FAMACHA degree equal to or over 3. Doramectin (1%) was used for the experiment, with 25% sodium sulfaquinoxaline for the prevention and control of coccidiosis.

We used the intermittent stocking grazing method in paddocks established with Tifton 85 (*Cynodon* spp.) measuring 100, 120, 140, and 160 m² for VH, H, L, and VL respectively. As animal entry criteria on the paddock, we used 95% luminous interception (LI) (PARSONS et al., 1988), and as exit criteria,

LAIr. These measurements were performed daily in the pastures using canopy analyzer equipment (AccuPAR Model LP, 80 PAR/LAI, Decagon devices®). The animals remained for approximately four d in each paddock. When the residue target was reached (LAIr), the group was transferred to the next paddock that showed 95% LI, so that the same group only entered the same paddock throughout the experiment.

Table 1. Chemical composition of supplement and pasture.

	Tifton-85	Supplement[a]
Dry matter (%)[b] (DM%)	27.3	89.0
PB[b]	16.7	22.7
NDF[c]	72.0	23.4
ADF[c]	32.6	9.6
Ash[b]	7.9	17.1
P	-	5
Ca	-	2.0
EE	1.5	1.4
TND[d]	63.5	80.2

[a] Commercial supplement (/kg of supplement): Mg, 3 g; S, 3 g; Na, 2.5 g; Cu, 13 mg; Mn, 51 mg; Zn, 48 mg; I, 1 mg; Co, 0.8 mg; Se, 0.4 mg; Vitamin A, 5,500 UI; Vitamin D, 825 UI; Vitamin E, 40 UI.
[b] AOAC (1990).
[c] Van Soest and Robertson (1985).
[d] NRC (2001).

The average daily weight gain (ADG) was obtained by subtracting the weight of the animals on the last day of the experiment by the initial weight and dividing by the number of days of experiment.

For the evaluation of consumption, we used contemporary animals for performance so that the procedure would not interfere in the other evaluations.

The evaluation of daily forage intake was performed by estimating fecal output:

DMI = FE / (1 – DIG) and; FE = amount of supplied indicator (g/day) / concentration of feces indicator (g/g DM). Where DMI = dry matter intake (kg/day), FE = fecal excretion (g/day), and DIG = food digestibility.

For this, an external marker of 0.25 g LIPE® (lignin isolated, purified, and enriched from *Eucalyptus grandis*), was provided in capsule form daily for six d to the animals as follows: two days of adaptation and four of collection. At the end of the collection period, feces were dried in an oven at 55° C for 72 h, and ground in a knife mill with a 1 mm mesh sieve. An aliquot of 2.5 g of each day's collection was mixed, forming a pool for each animal. The fecal dry matter production from the LIPE® reading was performed as described by Saliba (2005). The estimated *in vitro* true digestibility of dry matter (IVTDDM) was performed using *in vitro* assay with 0.5 g samples weighted in bags (ANKOM®-F57) and incubated in the apparatus "Daisy-II Fermenter" for 48 h (ANKOM® Technology Corp. Fairport, New York, USA) and subsequently analyzed for neutral detergent fiber (NDF) (TILLEY; TERRY, 1963).

Blood was collected directly from the jugular vein of all animals every 21 d from the beginning of the experiment. We collected blood using three types of vaccutainer® tubes (BD Vacutainer, Franklin Lakes, NJ) specific to each blood assessment: a tube containing EDTA (for measurement of cortisol

and neutrophil: lymphocyte (N:L) ratio), one with sodium fluoride + EDTA (for measurement of glucose, non-esterified fatty acids (NEFA), and beta-hydroxybutyrate (B-HBA)), and another without anticoagulant (for measurement of the other metabolites). After collection, serum and plasma were obtained by centrifugation at 5,000 RPM for 10 min, later they were packed in Eppendorf tubes and stored at –20 ºC until further analysis.

As stress indicators, the concentration of plasma cortisol and N:L ratio were measured. The concentration of cortisol was determined using an enzyme immunoassay kit (Monobind® Lake Forest, CA 92630, USA), and N:L was obtained by differential leukocyte counts made from fixed smears on slides and stained with May-Grunwald/ Giemsa-MGG (MATOS; MATOS, 1988).

For these evaluations, blood was collected in the late afternoon of the last day of permanence of the animals in the paddock that characterized the grazing residue of each treatment.

Protein metabolism indicators was indicated by: total proteins (colorimetric-biuret), creatinine (alkaline Picrate-Colorimetric, Labtest), albumin (Colorimetric-Green Bromcresol), urea (UV enzymatic), and hemoglobin (automatic contactor cells ABC-VET, Horiba ABX®). The concentration of globulin was obtained from the subtraction of the total protein albumin. Energy metabolism indicators was indicated by: glucose (enzymatic-colorimetric Trinder), cholesterol (enzyme-colorimetric Trinder), B-HBA (UV enzymatic), NEFA (colorimetric) using a diagnostic kit test (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom), and mineral metabolism was indicated by: calcium (Colorimetric-cresolphthalein), phosphorus (UV-Daly and modified Ertingshausen), and magnesium (Colorimetric-Magon sulfonated). The concentrations were measured by means of a diagnostic test kit (Labtest Diagnostica SA, Lagoa Santa, MG, Brazil), with the exception of B-HBA and NEFA. All indicators were determined using a semi-automatic spectrophotometer (LabQuest mod. E-225-D, Labtest Diagnostics SA, Lagoa Santa, MG, Brazil).

For the variables related to stress indicators, metabolic profiles, and pasture chemical composition, the statistical analysis was performed using repeated measures. Initially we performed the sphericity test matrix of variance and covariance between times. When the hypothesis of sphericity was rejected, we estimated the covariance structure that best fit the data. For the other variables, we performed the analysis of variance (ANOVA) without considering the time factor. For comparisons between levels of GI, we used orthogonal polynomial contrasts (LITTELL et al., 2006). The analyses were performed using the MIXED procedure of SAS (2008) (Statistical Analysis System), version 9.2.

Results and Discussion

Table 1 shows that the chemical composition of the pasture was not affected by GI, SP, or time ($P > 0.05$).

Table 2 shows the results of animal performance. The DMI and ADG, in response to GI, had a positive linear effect ($P < 0.05$), and the NSP group showed higher consumption levels ($P < 0.05$). According to these results, GI and SP modify the performance of lambs subjected to intermittent stocking.

In the present study, the results show that GI and SP changed the performance of lambs significantly. ADG increased with increasing GI and DMI decreased with decreasing GI (Table 2). This behavior is consistent with findings in the literature (BARBOSA et al., 2007; KUNRATH et al., 2014). According to Carvalho et al. (2008), grazed pastures at low GI have higher forage mass, which optimizes consumption, reflecting directly on individual performance. On the other hand, intensely grazed pastures have less forage mass, resulting in lower consumption and consequently, lower performance.

Table 2. Effect of grazing intensities (GI) and supplementation (SP) in the performance of finishing lambs.

Item	GI[a]				OPC[b]	SP		MSE[c]	P-value[d]		
	VH	H	L	VL		SP	NSP		GI	SP	GI×SP
IBW[e] (kg)	15.3	15.4	15.4	15.5	NS[f]	15.4	14.4	0.14	NS	NS	NS
DMI[g] (%PC)	3.27	3.36	3.51	3.64	L**	3.27	3.62	0.06	***	*	NS
ADG[h] (g/dia)	59.0	67.2	72.5	79.6	L**	71.6	67.6	2.42	*	NS	NS

[a] VH = very high; H = high; L = low and VL = very low; SP = supplemented; NSP = non supplemented;
[b]OPC = orthogonal polynomial contrast;
[c]MSE = mean standard error;
[d]GI = effect of intense grazing; SP = supplementation effect; GI × SP = interaction effect between GI and SP;
[e]IBW = initial body weight;
[f]NS = not significant (P > 0.05); * = P < 0.05; ** = P < 0.01; *** = P < 0.001; L= linear effect (P < 0.05);
[g]DMI = consumption of dry matter (% body weight);
[h]ADG = average daily gain.

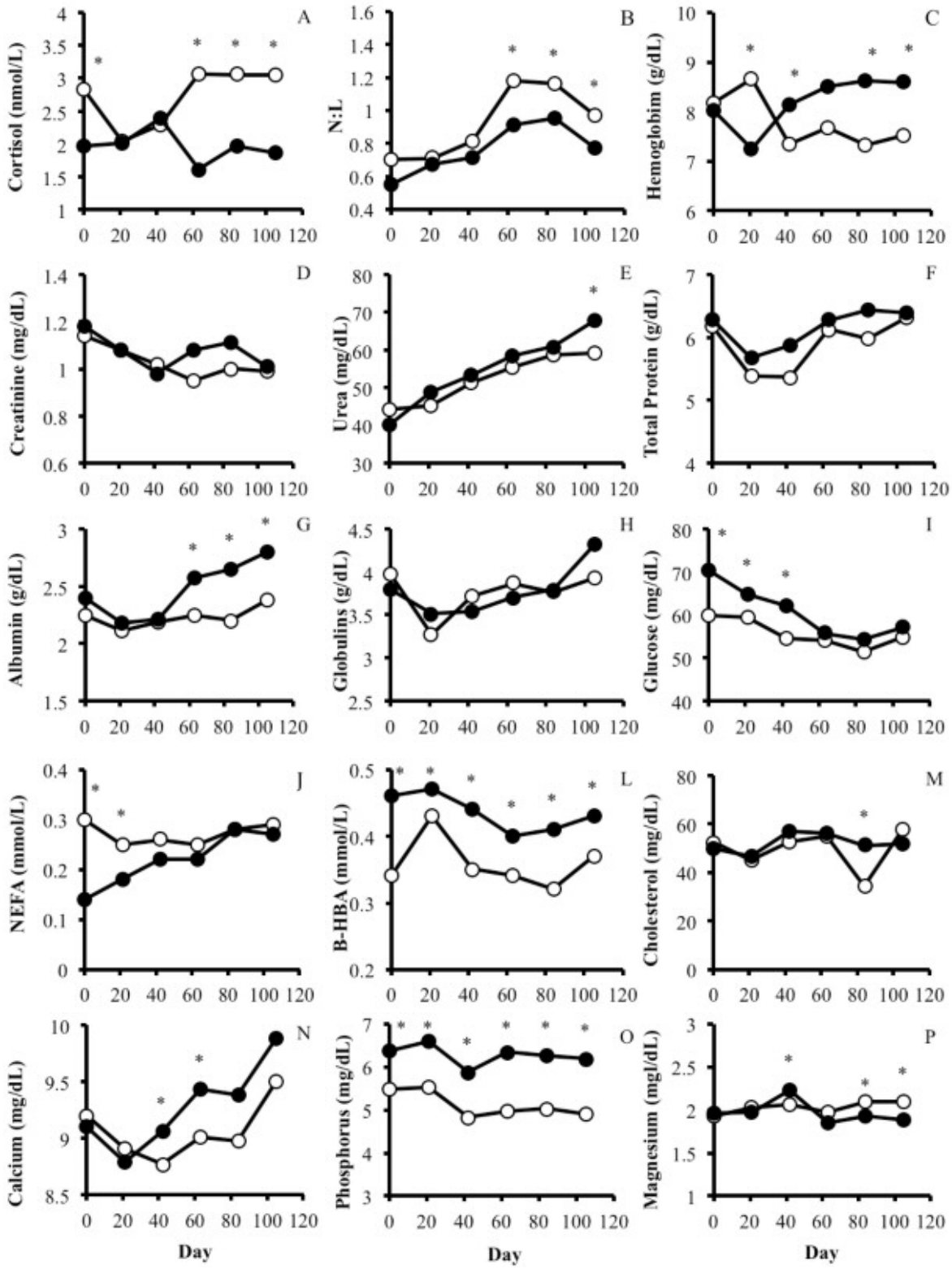
As with GI, SP affected DMI (P < 0.05), however, there was no modification of ADG (P < 0.05). Studies have shown that energy and protein feeds used in pasture supplementation at different levels of inclusion increase the performance of lambs (ARVIZU et al., 2011; SANTRA et al., 2002). As ADG is related to DMI, this behavior is expected. However, in this study DMI was decreased by protein supplementation. This effect could be related to the amount of protein present in the supplement (22.3% DM). As reported by Detmann et al. (2007), consumption of large amounts of protein increases the production of ruminal ammonia, which in excess is converted into urea in the liver resulting in higher circulating concentrations. This high concentration of circulating urea promotes negative effects on animal metabolism, resulting in voluntary intake diminishing. Therefore, this sets a negative associative effect since the total consumption was decreased.

Table 3 shows the results of the stress indicators and metabolic profiles of lambs. The plasma cortisol concentration showed higher concentrations in the NSP group (P < 0.05) and was not effected by GI (P > 0.05). The N:L ratio indicated a negative linear trend in relation to to the GI (P < 0.05), and the SP group had a higher N:L ratio (P < 0.05). Thus, GI and SP are factors that modify the concentration of stress indicators in lambs under intermittent stocking.

The results of the interactions between SP and TM (SP × TM), and GI and TM (GI × TM) are presented in Figures 1 and 2, respectively. Stress indicators showed significant differences (P < 0.05) in the last three collections, and the SP group had lower concentrations of both indicators.

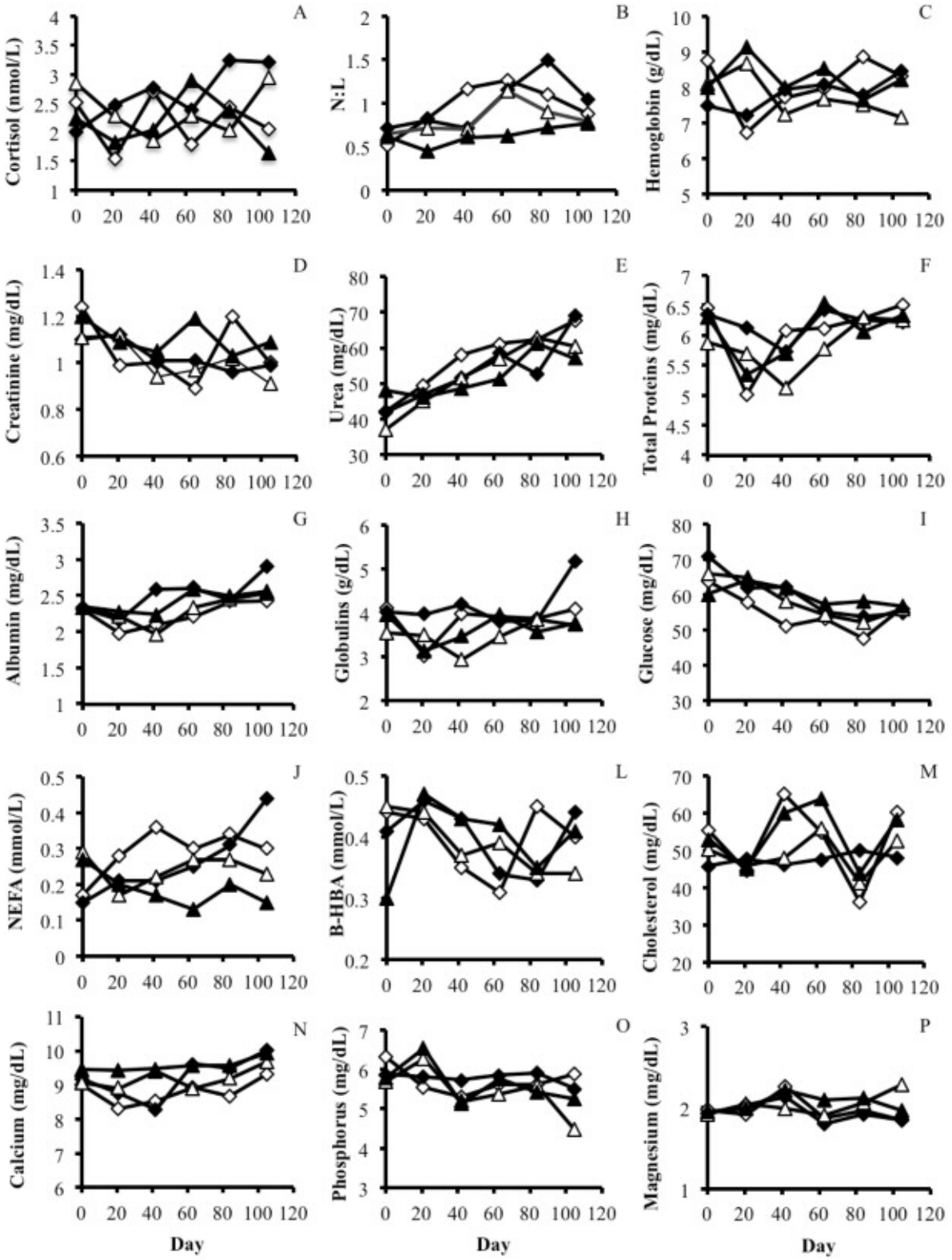
The main objective of this study was to evaluate the effect of GI and SP in stress indicators and blood indicators of animal metabolism. The GI and conditions of the pastoral environment were not enough to modify the cortisol concentration of lambs, and SP had little effect on cortisol concentration. Some studies show that the increased concentration of plasma cortisol is a neuroendocrine response to activation of the hypothalamic-pituitary-adrenal response to stress (HASHIZUME et al., 1994), therefore this hormone is related to stress in animals. However, GI had no significant effect on cortisol concentration and this effect could have been due to the fact that in the studied grazing method (intermittent stocking), the animals were kept in a single paddock for approximately 4 days, being relocated to another when grazing interruption criteria was reached (GI). Thus, they were repeatedly subjected to the same situation for every change of paddock. These results are supported by studies indicating that stress provoked by transport, when animals are accustomed to the situation, display a decreased neuroendocrine response (WICKHAM et al., 2012; STOCKMAN et al., 2011).

Figure 1. Effect of the interation of supplementation and time (SP×TM) in the blood concentration of stress indicators and metabolic profile of finishing lambs[a].



[a] N:L = Neutrophyl/Limphocytes ratio; NEFA = Non esterified fatty acids; B-HBA = Beta hydroxybutyrate; Supplemented Groups (—●—); non supplemented (—○—);* = $P < 0.05$.

Figure 2. Effect of interaction of the intensity of grazing and time (IG×TM) in blood concentration of stress indicators and metabolic profile of finishing lambs[a].



[a]N:L= Neutrophil/lymphocytes ratio; NEFA= Non esterified fatty acids; B-HBA= beta hydroxybutyrate; Very high (—◇—); High (—◆—); Low (—△—); Very low (—▲—).

Table 3. Effect of grazing intensities (GI) and supplementation (SP) in blood concentration as an indicator of stress and metabolic profile of finishing lambs.

Item	GI[a]				OPC[b]	SP		SEM[c]	P-value[d]		
	VH	H	L	VL		SP	NSP		GI	SP	GI×SP
Stress Indicator											
Cortisol (nmol/L)	58.7	74.2	65.1	64.3	NS[e]	55.2	76.1	2.92	NS	*	NS
N:L[f]	0.96	0.98	0.82	0.63	L*	0.77	0.92	0.05	*	*	NS
Protein metabolism											
Hemoglobin (g/dL)	8.07	7.84	7.73	8.27	NS	8.18	7.77	0.08	NS	NS	NS
Creatinine (mg/dL)	1.05	1.03	1.01	1.11	NS	1.07	1.03	0.01	NS	NS	NS
Urea (mg/dL)	56.6	53.4	52.3	52.1	NS	54.8	52.3	0.63	NS	NS	NS
Total protein (g/dL)	6.07	6.18	5.81	6.00	NS	6.13	5.89	0.05	NS	NS	NS
Albumin (g/dL)	2.22	2.45	2.27	2.38	NS	2.45	2.21	0.04	NS	*	NS
Globulin (g/dL)	3.83	4.12	3.47	3.63	NS	3.78	3.68	0.08	NS	NS	NS
Energy metabolism											
Glucose (mg/dL)	54.9	59.7	58.6	59.7	L*	60.8	55.7	0.84	*	*	NS
NEFA (nmol/L)[g]	0.29	0.26	0.24	0.19	L*	0.22	0.27	0.01	*	*	NS
B-HBA (nmol/L)[h]	0.39	0.40	0.39	0.39	NS	0.43	0.36	0.01	NS	*	NS
Cholesterol (mg/dL)	52.7	47.4	48.9	53.9	NS	52.1	49.4	0.89	NS	NS	NS
Mineral Metabolism											
Calcium (mg/dL)	8.78	9.08	9.17	9.53	L**	9.21	9.06	0.08	*	NS	*
Phosphorus (mg/dL)	5.71	5.77	5.61	5.65	NS	6.26	5.11	0.13	NS	*	NS
Magnesium (mg/dL)	1.97	1.94	2.03	2.05	NS	1.97	2.02	0.01	NS	NS	NS

[a] VH = very high; H = high; V = Low and VL = very low; SP = supplemented; NSP = non supplemented;
[b] OPC = Ortogonal polinomial contrast;
[c] SEM = Standard error of mean;
[d] GI = effect of grazing intensity; SE = supplementation effect; GI × SE = Interaction effect between GI and SE;
[e] NS = non significant (P > 0.05); * = P < 0.05; ** = P < 0.01; *** = P < 0.001; L = linear effect (P < 0.05);
[f] N:L = neutrophil lymphocyte ratio;
[g] NEFA = non sterified fatty acids;
[h] B-HBA = beta-hydroxybutyrate;

Like cortisol, immune function has been recognized as a reliable animal welfare indicator (MINTON, 1994; DAVIS et al., 2008), because it is affected by hypercortisolism (GRIFFIN, 1989). In this study, N:L was modified by both GI and SP. According to Dhabhar et al. (1996), in situations of stress and concomitant secretion of glucocorticoids, there is an increase in the number of neutrophils and a decrease in lymphocytes. Thus, increasing N:L is indicative of stress, which in was higher in the higher GI . As for supplementation, the NSP group showed a higher N:L, that is, supplementation was a factor that decreased stress. To reinforce this effect, cortisol and the N:L ratio showed higher concentrations in the NSP group. Some studies that quantified sheep (WICKHAM et al., 2012) and cattle (STOCKMAN et al., 2011) transport stress show that the N:L ratio varies from 1.2 to 1.3 when the animal is stressed, therefore close to the N:L ratio found in the GIs, VH and H, of this study. Therefore, both GI and SP affected the stress indicators of lambs. These findings demonstrate that SP positively influences the stress condition of finishing lambs in pasture, since there is a decrease in cortisol levels and the N:L ratio.

Regarding the metabolic profiles of lambs, the concentrations of protein metabolism parameters did not change significantly. Only albumin was effected by SP, where the SP group showed a higher concentration (P < 0.05). However, SP ×

TM showed significant interactions (Figure 1 C, E, and G). Regarding energy metabolism indicators, glucose showed a positive linear effect with GI ($P < 0.05$) and was higher in the SP group ($P < 0.05$); however, NEFA showed a negative linear effect ($P < 0.05$), and the NSP group had higher NEFA serum levels ($P < 0.05$). B-HBA concentration was higher in the SP group ($P < 0.05$). All energy metabolism indicators showed the effect of SP × TM interaction ($P < 0.05$) (Figure 1 I, J, L, and M). The calcium concentration was effected by GI ($P < 0.05$). The phosphorus concentration was higher in the SP group ($P < 0.05$) and magnesium was not effected by any of the treatments ($P > 0.05$). All mineral metabolism indicators showed an effect due to the interaction SP × TM ($P < 0.05$) (Figure 1 N, O, and P). In general the metabolic profile was modified more intensely by SP than GI itself.

Although the effect of GI and SP was observed in stress indicators, the same was not observed in protein metabolism indicators, where none of the indicators were modified, except albumin, which was higher in the SP group. This increase may be related to providing higher protein via the supplement, since the catabolic effects of cortisol on proteinaceous tissues was not observed by the concentration of urea and creatinine, which are indicators of protein catabolism.

In general, energy metabolism indicators were affected by GI and SP. The concentration of glucose increased as GI decreased, and it is directly related to DMI, because of increased consumption. Similarly, the amount of substrate that reached the rumen was higher, which may be the cause of the increase in the levels of this metabolite. The higher concentration of glucose in the SP group is related to supplementation, because in the ruminant, glucose is derived from gluconeogenesis, and it is the primary propionate precursor, which is produced by rumen bacteria that ferment non-fibrous carbohydrates found in food concentrates (FAHEY JUNIOR and BERGER, 1988). Thus, higher glucose concentrations in the SP group can be attributed to the higher inflow of fermentable carbohydrates and consequently greater production of propionate.

The concentration of NEFA decreased as GI decreased, that is, fatty acids were mobilized from adipocytes when the lambs were kept at higher GIs. According to Khoo et al. (1973), NEFA is stimulated by hormone-sensitive lipase (HSL), an enzyme present in the adipocyte. This enzyme is stimulated by several hormones, including cortisol and glucagon. Both hormons are involved in energy metabolism, however, stimulated by different factors. Glucagon is involved in glucose metabolism and as observed in this study the glucose concentration behaved in a manner contrary to NEFA regard to GI and SP. This is in accordance with Caldeira et al. (1999), whose experiment with sheep food restrictions resulted in decreased glucose concentrations and increased NEFA with less food availability. With SP, both cortisol and glucose were affected; however, the higher NEFA concentration in the NSP group cannot be attributed solely to the effect of cortisol, as a lower glucose concentration in the blood stimulates the mobilization of fatty acids by glucagon (KANEKO, 2008). As in NEFA, B-HBA was modified by SP; however, increased SP appears to be the primary contributor to increased B-HBA, since NEFA, one of the main precursors of B-HBA was not enough to increase its concentration in the NSP group, and according to Bergman (1990) diets containing higher concentrations of energy promote higher proportional production of butyrate in the rumen, which is the main precursor of B-HBA.

Among blood indicators of mineral metabolism, only calcium and phosphorus concentrations were modified by the treatments. The calcium concentration increased with decreasing GI, which may have occurred because of the largest DMI achieved by the groups at the lowest GI. Although phosphorus metabolism is regulated by the same endocrine control as calcium metabolism (GONZÁLEZ, 2000), its concentration was also affected by SP and GI, as for calcium. In the SP

group, the phosphorus concentration was higher, because the supplement contained this mineral. Studies showing the effect of stress on mineral metabolism of ovines are scarce. However, the increase in blood glucocorticoid for intravenous injections did not alter the concentration of either calcium or phosphorus (CHAVASSIEUX et al., 1993), showing little influence of stress on mineral metabolism.

Conclusion

In conclusion, GI and SP altered the performance, stress indicators, and blood metabolism of finishing lambs. These results suggest that lower GIs and SP improve stress conditions and mainly affect the energy metabolism of finishing lambs.

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References

ARVIZU, R. R.; DOMÍNGUEZ, I. A.; RUBIO, M. S.; BÓRQUEZ, J. L.; PINOS-RODRÍGUEZ, J. M.; GONZÁLEZ, M.; JARAMILLO, G. Effects of genotype, level of supplementation, and organic chromium on growth performance, carcass, and meat traits grazing lambs. *Meat Science*, Oxon, v. 88, n. 3, p. 404-408, 2011.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS – AOAC. Official methods of analysis of AOAC International. 15th ed. Washington, D. C., 1990. 1141 p.

BARBOSA, C. M. P.; CARVALHO, P. C. F.; CAUDURO, G. F.; LUNARDI, R.; KUNRATH, T. R.; GIANLUPPI, G. D. F. Terminação de cordeiros em pastagens de azevém anual manejadas em diferentes intensidades e métodos de pastejo. *Revista Brasileira de Zootecnia,* Viçosa, MG, v. 36, n. 6, p. 1953-1960, 2007.

BASSETT, J. M. The relation of fat and protein catabolic actions of cortisol to homeostasis in fasting sheep. *Metabolism*, Philadelphia, v. 17, n. 7, p. 644-652, 1968.

BERGMAN, E. N. Energy contributions of volatile fatty acids from the gastro-intestinal tract in various species. *Physiology Reviews*, Bethesda, v. 70, n. 2, p. 567-570, 1990.

CALDEIRA, R. M.; ALMEIDA, M. A.; SANTOS, C. C.; VASQUES, M. I.; VAZ PORTUGAL, A. Daily variation in blood enzymes and metabolites in ewes under three levels of feed intake. *Canadian Journal of Animal Science*, Ottawa, v. 79, n. 2, p. 157-164, 1999.

CAROPRESE, M.; ANNICCHIARICO, G.; SCHENA, L.; MUSCIO, A.; MIGLIORE, R.; SEVI, A. Influence of space allowance and housing conditions on the welfare, immune response and production performance of dairy ewes. *Journal of Dairy Research*, New York, v. 76, n. 1, p. 66-73, 2009.

CARVALHO, P. C. F.; GONDA, H. L.; WADE, M. H.; MEZZALIRA, J. C.; AMARAL, M. F.; GONÇALVES, E. N.; SANTOS, D. T.; NADIN, L.; POLI, C. H. E. C. Características estruturais do pasto e o consumo de forragem: o quê pastar, quanto pastar e como se mover para encontrar o pasto. In: PEREIRA, O. G.; OBEID, J. A.; FONSECA, D. M. (Ed.). *Manejo estratégico da pastagem.* Viçosa, MG: Editora UFV, 2008. p. 101-130.

CARVALHO, P. C. F.; KOZLOSKI, G. V.; FILHO, H. M. N. R.; REFFATTI, M. V.; GENRO, T. C. M.; EUCLIDES, V. P. B. Avanços metodológicos na determinação do consumo de ruminantes em pastejo. *Revista Brasileira de Zootecnia*. Viçosa, MG, v. 36, p. 151-170, 2007. Suplemento.

CARVALHO, P. C. F.; RIBEIRO FILHO, H. M. N.; POLI, C. H. E. C.; MORAES, A.; DELAGARDE, R. Importância da estrutura da pastagem na ingestão e seleção de dietas pelo animal em pastejo. In: A PRODUÇÃO ANIMAL NA VISÃO DOS BRASILEIROS, 38., 2001, Piracicaba. *Anais*... Piracicaba: Fealq Inc, 2001. p. 853-871.

CHAVASSIEUX, P.; PASTOUREAU, P.; CHAPUY, M. C.; DELMAS, P. D.; MEUNIER, P. J. Glucocorticoid-induced inhibition of osteoblastic bone formation in ewes: a biochemical and histomorphometric study. *Osteoporosis International*, London, v. 3, n. 2, p. 97-102, 1993.

DAVIS, A. K.; MANEY, D. L.; MAERZ, J. C. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology,* Londres, v. 22, n. 5, p. 760-772, 2008.

DETMANN, E.; PAULINO, M. F.; VALADARES FILHO, S. C. Fatores controladores de consumo em suplementos múltiplos fornecidos ad libitum para bovinos manejados a pasto. *Cadernos Técnicos de Veterinária e Zootecnia*, Belo Horizonte, v. 55, p. 73-93, 2007.

DHABHAR, F. S.; MILLER, A. H.; MCEWES, B. S.; SPENCER, R. L. Stress-induced changes in leukocyte distribution: Role of adrenal steroid hormones. *The Journal of Immunology*, Bethesda, v. 157, n. 4, p. 1638-1644, 1996.

ELSASSER, T. H.; KLASING, K. C.; FILIPOV, N.; THOMPSON, F. The metabolic consequences of stress: targets for stress and priorities of nutrient use. In: MOBERG, G. P.; MENCH, J. A. (Ed.). *The biological of animal stress*: basics principles and implications for animal welfare. New York: CABI Publishing, 2000. p. 77-110.

FAHEY JUNIOR, G. C.; BERGER, L. L. Carbohydrate nutrition of ruminants. In: CHURCH, D. C. (Ed.). *The ruminant animal:* digestive physiology and nutrition. New Jersey: Prentice Hall, 1988. p. 269-297.

GONZÁLEZ, F. H. D. Uso do perfil metabólico para determinar o status nutricional em gado de corte. In: GONZÁLEZ, H. D.; BARCELLOS, J.; PATINÕ, H. O.; RIBEIRO, L.A. *Perfil metabólico em ruminantes:* seu uso em nutrição e doenças nutricionais. Porto Alegre: Gráfica da Universidade Federal do Rio Grande do Sul, 2000. p. 63-74.

GORDON, H. M.; WHITLOCK, A. V. A new tecnique for counting nematode eggs in sheep faeces. *Journal of the Council for Scientific and Industrial Research*, New Delhi, v. 12, p. 50-52, 1939.

GRIFFIN, J. F. T. Stress and immunity: a unifying concept. *Veterinary Immunology and Immunopathology*, Amsterdan, v. 20, n. 3, p. 263-312, 1989.

HASHIZUME, T.; HAGLOF, S. A.; MALVEN, P. V. Intracerebral methionine-enkephalin, serum cortisol, and serum β-endorphin during acute exposure of sheep to physical or isolation stress. *Journal of Animal Science,* Champaign, v. 72, n. 3, p. 700-708, 1994.

INYANG, U.; VENDRAMINI, J. M. B.; SOLLENBERGER, L. E.; SELLERS, B.; ADESOGAN, A.; PAIVA, L.; LUNPHA, A. Forage species and stocking rate effects on animal performance and herbage responses of 'mulato' and bahiagrass pastures. *Crop Science*, Madison, v. 50, n. 3, p. 1079-1085, 2010.

KANEKO, J. J. Carbohydrate metabolism and its diseases. In.: KANEKO, J. J.; HARVEY, J. W.; BRUSS, M. L. *Clinical biochemistry of domestic animals*. 6th ed. San Diego: Academic Press, 2008. p. 45-80.

KHOO, J. C.; STEINBERG, D.; THOMPSON, D.; MAYER, S. E. Hormonal regulation of adipocyte enzymes: the effects of epinephrine and insulin on the control of lipase, phosphorylase kinase, phosphorylase and glycogen synthase. *The Journal of Biological Chemistry,* Bethesda, v. 248, n. 11, p. 3823-3830, 1973.

KNOTT, S. A.; CUMMINS, L. J.; DUNSHEA, F. R.; LEURY, B. J. Feed efficiency and body composition are related to cortisol response to adrenocorticotropin hormone and insulin-induced hypoglycemia in rams. *Domestic Animal Endocrinology*, New York, v. 39, n. 2, p. 137-146, 2010.

KUNRATH, T. R.; CADENAZZI, M.; BRAMBILLA, D. M.; ANGHINONI, I.; MORAES, A.; BARRO, R. S.; CARVALHO, P. C. F. Management targets for continuously stocked mixed oat × annual ryegrass pasture in a no-till integrated crop-livestock system. *European Journal of Agronomy,* Amsterdam, v. 57, n. 3, p. 71-76, 2014.

LITTELL, R. C.; MILLIKEN, G. A.; STROUP, W. W.; WOLFINGER, R. D. *SAS System for mixed models.* 2th ed. Cary, NC: SAS Institute Inc. 2006. 813 p.

MATOS, M. S.; MATOS, P. F. *Laboratório clínico médico-veterinário.* 2. ed. São Paulo: Atheneu, 1988. p. 238.

MATTERI, R. L.; CARROLL, J. A.; DYER, C. J. Neuroendocrine response to stress. In: MOBERG, G. P.; MENCH, J. A. (Ed.). *The biological of animal stress*: basics principles and implications for animal welfare. New York: CABI Publishing, 2000. p. 43-76.

MINTON, J. E. Function of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in models of acute stress in domestic farm animals. *Journal of Animal Science*, Champaign, v. 72, n. 7, p. 1891-1898, 1994.

MOBERG, G. P. Biological response to stress: Implications for animal welfare. In: MOBERG, G. P.; MENCH, J. A. (Ed.). *The biological of animal stress:* basics principles and implications for animal welfare. New York: CABI Publishing, 2000. p. 1-21.

NABINGER, C.; FERREIRA, E. T.; FREITAS, A. K.; CARVALHO, P. C. F.; SANT'ANNA, D. M. Produção animal em campo nativo: aplicações de resultados de pesquisa. In: PILLAR, V. D.; MÜLLER, S. C.; CASTILHOS, Z. M. S.; JACQUES, A. V. A. *Campos sulinos*: conservação e uso sustentável da biodiversidade. Brasília: Ministério do Meio Ambiente, 2009. p. 175-198.

NATIONAL RESEARCH COUNCIL – NRC. Nutrient requirements of dairy cattle**.** 7th ed. rev. Washington: Natl. Acad. Sci., 2001. p. 65-67.

PARSONS, A. J.; JOHNSON, I. R.; HARVEY, A. Use of a model to optimize the interaction between frequency and severity of intermittent defoliation to provide a fundamental comparison of the continuous and intermittent defoliation of grass. *Grass and Forage Science*, Malden, v. 43, n. 1, p. 49-59, 1988.

PURCHAS, R. W. The response of circulating cortisol levels in sheep to various stresses and to reserpine administration. *Australian Journal Biological Science*, Collingwood, v. 26, n. 2, p. 477-489, 1973.

SALIBA, E. O. Uso de indicadores: passado, presente e futuro. In: PROCEEDINGS OF THE CONFERENCE OF MARKERS ON ANIMAL NUTRITION, 2005, Belo Horizonte. *Anais*... Belo Horizonte: Universidade Federal de Minas Gerais, 2005. p. 4-22.

SANTRA, A.; KARIM, S. A.; CHATURVEDI, O. H. Effect of concentrate supplementation on nutrient intake and performance of lambs of two genotypes grazing a semiarid rangeland. *Small Ruminant Research,* Amsterdam, v. 44, n. 1, p. 37-45, 2002.

STATISTICAL ANALYSIS SYSTEM INSTITUTE – SAS. SAS Institute. SAS/STAT 9.2 User's Guide. SAS Institute Inc, Cary, NC, 2008.

STOCKMAN, C. A.; COLLINS, T.; BARNES, A. L.; MILLER, D.; WICKHAM, S. L.; BEATTY, D. T.; BLACHE, D.; WEMELSFELDER, F.; FLEMING, P. A. Qualitative behavioural assessment and quantitative physiological measurement of cattle naïve and habituated to road transport. *Animal Production Science,* Collingwood, v. 51, n. 3, p. 240-249, 2011.

TILLEY, J. M. A.; TERRY, R. A. A two-stage technique for the in vitro digestion of forage crops. *British Grassland Society*, Hoboken, v. 18, n. 2, p. 104-111, 1963.

VAN SOEST, P. J.; ROBERSON, J. B. *Analysis of forages and fibrous feed.* Laboratory Manual for Animal Science 613. Ithaca, New York: Cornell University, 1985. 202 p.

WICKHAM, S. L.; COLLINS, T.; BARNES, A. L.; MILLER, D. W.; BEATTY, D. T.; STOCKMAN, C.; BLACHE, D.; WEMELSFELDER, F.; FLEMING, P. A. Qualitative behavioral assessment of transport-naïve and transport-habituated sheep. *Journal of Animal Science,* Champaign, v. 90, n. 2, p. 4523-4535, 2012.