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Performance, fecal egg count and feeding behavior of lambs grazing elephant grass (*Pennisetum purpureum* Schum.) with increased levels of protein supplementation

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ABSTRACT

The current study investigated the effect of increased protein supplementation levels on fecal egg count, performance and feeding behavior of lambs grazing elephant grass (*Pennisetum purpureum Schum.*). The treatments consisted of five levels of crude protein (CP) in an isoenergetic supplement (0%, 8%, 16%, 24% and 32% CP) provided to growing lambs. Fifty lambs at initial live weight of 20.2 ± 2.94 kg were slaughtered after 75 days of protein supplementation. Ether extract intake decreased with the increase of CP in the supplement. Average daily weight gain was over 60% greater for lambs receiving supplement with 16% and 24% CP than lambs in the control group. Protein supplementation did not affect grazing and ruminating behavior but lambs in the control group spent from 82% to 88% less time eating the supplement (4.01 min; $P < 0.01$) and visited the supplement trough 3.01 times a day. Fecal egg count remained low with the increase of protein in the supplement in the beginning of the grazing period. Intake, weight gain, carcass length and rump width of the lambs enhanced as CP in the supplement increased, with optimal performance obtained with 8% CP in the supplement for lambs grazing elephant grass.

1. Introduction

As a natural feed source, pasture is the cheapest and least labor-intensive system in extensive livestock production with the ability to maximize lambs' performance by using fibrous feedstuff as an energy source. Elephant grass (*Pennisetum purpureum Schum.*) Elephant grass is originally from Africa and exhibits high production potential and quality (Deresz et al., 2006). This grass has several uses and it is cultivated in South America, Southern USA, Australia and the Pacific Islands Middle East, India, South East Asia and China; thus its protein content varies widely across regions in the world (Rusdy, 2016). Additionally, the structure and growth pattern of elephant grass (i.e., often forming large bamboo-like clumps) may limit the physical ability of the animal to harvest forage and the effects of pasture structure on feeding behavior may be critical in controlling intake (Penning et al., 1991). Also, elephant grass for sheep can be challenging of management as the rapid growth of this pasture makes it difficult to control grazing height for small ruminants, especially those at young age.

Gastrointestinal parasites (i.e., *Haemonchus*) together with anthelmintic resistance are major concerns in grazing systems worldwide due to their capacity to cause substantial losses in productivity and impaired animal health, which leads to death in some instances (Roeber et al., 2013). As a result, farmers face challenges to meet market specifications due to the flock vulnerability to gastrointestinal nematode infection. Protein and energy availability in pasture-only systems can vary significantly across seasons (Ribeiro et al., 2012; Howes et al., 2015). In this sense, poor nutrition can be another factor that limits animal wellbeing and performance with a detrimental impact on profitability of grazing systems.

Supplementation is commonly provided during the finishing period

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to enhance carcass traits of lambs under grazing systems (Arvizu et al., 2011), as well as to assist the lambs to cope with gastrointestinal nematode challenge (Sykes and Coop, 2001). Nutritional management has been widely demonstrated to be a viable non-chemical means to boosting host resilience and/or resistance to gastrointestinal nematode infection (Coop and Kyriazakis, 1999; Kyriazakis and Houdijk, 2006; Hoste and Torres-Acosta, 2011). Previous research reported that lambs supplemented with a diet containing 173 g/kg DM of metabolizable protein resulted in a 6.6-fold difference on fecal egg count (FEC) between non-supplemented and supplemented lambs and better immune response in supplemented lambs compared to those fed a diet containing 98 g/kg DM of metabolizable protein (Strain and Stear, 2001).

The aim of the current study was to evaluate, growth, fecal egg count and feeding behavior of lambs grazing elephant grass supplemented with increasing levels of protein (0%, 8%, 16%, 24% and 32% crude protein – CP). The outcomes of this study will provide an improved understanding of the optimal level of protein supplement that enhances growth and carcass characteristics of grazing lambs as well the indirect responses on animal health and feeding behavior.

2. Material and methods

The experiment was conducted in the Sheep sector of the Departamento de Zootecnia of the Universidade Federal de Lavras (latitude 21°09′52.41″S, longitude 44°55′52.40″W, 843 m above sea level) in a dystrophic red latosol from May to June 2012. According to the classification of Köppen, the climate of the region is defined as Cwa (monsoon-influenced humid subtropical climate with dry winters and hot summers) with average temperature and rainfall of 20.2 °C and 1237 mm per year. The current experiment was carried out under approved animal ethics authority (protocol no 076/11) issued by the Federal University of Lavras (UFLA, Brazil) Animal Ethics Committee.

2.1. Animal management, growth and feed intake

Fifty male lambs (½ Santa Ines ½ Dorper) at 170 ± 14.4 days of age (± standard deviation) and an average live weight of 20.2 ± 2.94 kg (± standard deviation) were used. The experimental dietary treatments were isoenergetic and consisted of five levels of CP supplementation (0%, 8%, 16%, 24% and 32% of CP in dry matter – DM basis; n = 10) with the animals from the control group (0% CP) receiving only mineral mixture salt (Table 2). Experimental feeding was undertaken for 75 days with live weight of the lambs recorded fortnightly to determine the daily weight gain, with no prior adaptation to the supplement. Prior to the experimental period, lambs were weaned at 15 kg of live weight and vaccinated against rabies and clostridial diseases. Fecal worm eggs counts were individually monitored every fortnight using the McMaster technique reported by Whitlock (1948). Lambs were dewormed with Valbazen® (albendazole 10%, Zoetis, USA) upon start and when the EPG value exceeded 500 (day 14).

Lambs were maintained in a 1.5 ha paddock comprising elephant grass (*Pennisetum purpureum Schum.*) and fenced with woven wire fence, from 7:00–10:00 am and from 3:00–6:00 pm every day. The pasture was composed of 74.5% elephant grass (25.1% leaf matter, 44.9% stems and 4.42% dead matter), 13.7% *Brachiaria*, 8.02% *Cynodon*, and 3.78% of other species. Chemical composition of the elephant grass was tested in its natural form of roughage – pasture, and as chopped roughage provided in the trough. The elephant grass in its natural form, pasture, contained on average 28.5 ± 0.53% DM, 9.32 ± 0.32 g CP/100 g DM, and 54.8 ± 3.10 g NDF /100 g DM. From 10:00 am to 3:00 pm, the lambs were separated into outside pens by protein supplement treatment groups and maintained in an open environment (approximately 3 m^2 per animal). During this period, the lambs had free-access to fresh water, shade (a 15 m^2 polyethylene screen providing 80% shade) and chopped elephant grass (~ 10 mm) supplied in a trough. From 6:00 pm to 7:00 am, the animals were kept in a covered shed (1.0 m^2 per animal) and separated into pens according to their respective treatments, one pen per supplement treatment. Each group received water and chopped elephant grass in a trough in the stall. This management was necessary during the night to protect the flock from predators. The forage and supplements were weighed and provided ad libitum, and refusals were weighed daily and adjusted to maintain a 20% surplus to avoid interference from selectivity. Although in this experiment the number of pens was equal to the number of treatments, all measurements were taken for each individual lamb including individual intake determination using external markers (chromium oxide and titanium dioxide). Thus, animals were considered the experimental unit (Freitas et al., 2017).

Pasture samples of elephant grass were collected using the simulated grazing technique described by Johnson (1978) cited by Benatti et al. (2012). From each sample (~ 2 kg), a subsample (~ 0.8 kg) was retrieved for chemical and another subsample (~ 1.2 kg) was used for plant morphology analyses (proportion of leaves, stems and dead matter) and pasture characterization (species composition).

Forage and supplement intake were measured using chromium oxide and titanium dioxide as external indicators according to the methodology described by Williams et al. (1962) cited by (Silva and Queiroz, 2002). Twelve days were used to assess the intake (i.e., seven days of adaptation and five days of feed, refusals, and feces collection). From day 1–12 the lambs were given a capsule containing two grams of chromium oxide per animal via esophagus at 7:00 am, and 2% of titanium dioxide mixed with the supplement, daily. Every batch of the supplement was sampled and subsequently bulked for chemical analysis. From day 8–12, three fecal grab samples were collected from each animal. Samples were collected on the following days and times: day 8 at 7:00 am; day 10 at 12:00 pm and day 12 at 6:00 pm. To minimize animal stress, fecal samples were not taken on days 9 and 11. The samples were stored at − 18 ºC, and then dried and processed using a grinder equipped with a 1 mm sieve for subsequent laboratory analysis.

Determination of dry matter (DM), ash, CP, ether extract (EE) and chromium in feces, feed and refusals were performed as Silva and Queiroz (2002). Titanium dioxide content in feces was determined according to Myers et al. (2004). For the determination of indigestible neutral detergent fiber (NDFi), 0.5 g of forage, feces and refusals, and 1.0 g of supplement were packaged in textile non-textile (TNT) bags (previously dried and weighed) and incubated for 264 h in a cow rumen (Casali et al., 2008). After this period, bags were removed and washed with water until they were completely clean. Then the bags were dried and boiled for 1 h in a neutral detergent solution (Van Soest and Robertson, 1985), washed with hot water and acetone, dried and weighed. The remaining residue was recorded as indigestible neutral detergent fiber (NDFi). Fecal DM output (FDMO) was determined using the following formula: FDMO = indicator intake (kg)/concentration of the indicator in the feces (%).

2.2. Feeding behavior

Individual behavior records were collected using scan sampling technique with 10 minutes interval by 11 trained observers over a period of 48 consecutive hours at both the beginning (day 6) and the end of the experiment (day 55). Behaviors are presented in minutes/day by multiplying the number of time that a specific behavior occurs throughout the day times 10 minutes. The animals were identified by handwritten numbers on different parts of the lamb body so that at least one of these were visible from any view angle. Visual observations and data records were made by an observer every 10 min for the bulk forage trough, supplement trough, rumination, grazing and resting behavior for all treatments. Two additional evaluators per treatment recorded the time spent in eating supplement by each animal.

For the animal behavior assessment, the following parameters were recorded during the period of grazing in the pasture: grazing time (time spent grazing). The following parameters were assessed during the period the animals were into the stalls or shed: feeding time (time spent

at the forage trough); supplementation time (time spent at the supplement trough); number of visits in the supplement trough and supplement trough (time spent in the supplement trough per visit). Ruminating time (time spent ruminating), resting time (time spent resting) and feeding plus grazing time were recorded in both grazing period and the confinement periods.

2.3. Biometric measurements and slaughter

At the end of the experimental period, lambs were weighed, and final body weight (FBW) was recorded. After weighing, the following biometric measurements were taken: body length (distance between the cervical-thoracic joint and the base of the tail), withers height (distance between the withers and the ground), rump height (distance between the rump and the ground), thoracic perimeter (thorax circumference, measured behind the shoulders), rump width (distance between the greater trochanters of the femur) and thorax width (distance between the sides of the scapulohumeral joints). All measurements were performed on a flat surface and taken by the same person to minimize variation according to methodology adapted from Fernandes et al. (2010). The body compactness index was calculated using slaughter body weight (SBW) divided by body length.

Lambs were transported to a commercial abattoir, 132 km from the experimental site, where they were held in solid diet fasting (16 h) prior to slaughter. The lambs were slaughtered at 245 ± 14.4 days of age by stunning with a captive bolt pistol and subsequently the jugular veins and carotid arteries were sectioned for bleeding according to abattoir procedures. During evisceration, the gastrointestinal tract was removed, weighed full, emptied and weighed again to obtain weight of gastrointestinal content and empty body weight (EBW), as follows: EBW = SBW - gastrointestinal content.

A sample (~ 3 cm^2) of rumen wall was collected from the ventral sac and then divided into two smaller fragments for morphological measurements of the rumen mucosa. One of the fragments was preserved in a Krebs-ringer bicarbonate buffer (Sigma-Aldrich®) and then used to perform macroscopic measurements in accordance with the methodology described by Daniel et al. (2006) and Resende Júnior et al. (2006). The number of rumen papillae was counted, and subsequently individual papillae was sectioned at the base using a scalpel blade. Images of the rumen papillae were digitized with a scanner, and the areas of the rumen papillae were estimated using the image analysis program UTHSCSA Image Tool. The surface area of the fragment was determined according to the methodology described by Daniel et al. (2006), and the total area of the ruminoreticulum was estimated by the regression equations proposed by the same authors. A second fragment was immersed in Bouin's solution for 18 h, preserved in a solution of 70% ethanol and processed according to routine protocols for paraffin embedding. The mitotic index were performed using microscopic analyses under a light microscope (Olympus CX31, Olympus Optical Co, Japan) of 5 μm fragment sections stained using the hematoxylin-eosin technique as proposed by Sakata and Tamate (1974).

2.4. Statistical analysis

The experiment was conducted as a completely randomized experimental design, including the fixed effect of supplement. Analysis of variance was performed on the data using the GLM procedure in the SAS statistical package (SAS® version 9.4, SAS Institute Inc., Cary, North Carolina, USA) at a 5% significance level. When significant differences were detected among treatments for one of the variables, two procedures were performed: a) comparison of means using Tukey's test among treatments (0%, 8%, 16%, 24% and 32% CP), and b) polynomial regression analysis using the REG procedure in the SAS statistical package for the supplementation treatments (8%, 16%, 24% and 32% CP). Orthogonal contrasts were used to detect the linear, quadratic or cubic effects of the increase in levels of CP in supplements. In both analyses, significance was declared at $P < 0.05$. For count variable (EPG), PROC GLIMMIX was used as its distribution is not normal.

3. Results

3.1. Nutrient intake

Dry matter intake relative to body weight did not differ ($P > 0.05$) with the increase of protein supplementation (Table 2). There was a positive linear effect ($P < 0.05$) on DM, crude protein (CP) and mineral matter (MM) intake for supplement, forage and total intake (supplement + forage) as protein level in the supplement increased (Table 2). Supplement organic matter (OM) intake did not differ across protein supplement levels ($P = 0.07$; Table 2). However, the lowest level ($P < 0.01$) of supplement intake of OM was observed in the control group, which received mineral mixture salt only (Table 2).

As CP content increased across the treatment groups, and corn content of the supplement declined (Table 1), the EE% declined in a negative linear pattern ($P < 0.01$, Table 2).

3.2. Feeding behavior

Protein supplement treatment had no effect on time eating forage at the trough, grazing, ruminating, resting, eating (forage + grazing), or average supplement trough time ($P > 0.05$, Table 3). As expected, lambs in the control group spent less time eating supplement and had a lower number of visits to the supplement trough (Table 3). On the other hand, the lambs on 8% protein treatment spent 9 times longer eating behavior at the supplement trough and visited the supplement trough 27 times more than the control group. Lambs fed 16%, 24% and 32% CP spent approximately 6 times longer eating at the supplement trough than the control group and visited the trough 12–14 times more, but were not significantly different from each other (Table 3).

3.3. Rumen wall characteristics

Values for mitotic index, papillae area, papillae number and absorptive surface were assessed to investigate rumen development as a response of dietary protein availability (Table 4). Lambs from the control group had lower ($P < 0.05$) mitotic index compared with lambs supplemented at 8% and 24% protein. The mitotic index, papillae area and number, and absorptive surface were not affected by increasing levels of protein in the supplement provided to the lambs ($P > 0.05$, Table 4).

Table 1
Ingredients and nutritional composition of the protein supplement.

Ingredient (%)	Protein level (%)				
	0	8	16	24	32
Mineral supplement[a]	100	30	30	30	30
Salt	0	5.0	5.0	5.0	5.0
Corn	0	53.5	30.2	6.8	2.0
Soybean meal	0	8.0	31.3	54.7	59.5
Lime	0	3.5	3.5	3.5	3.5
Nutrient (% DM)[2]					
CP (%)	0	8.08	16.33	24.56	31.27
NDF (%)	0	5.71	7.05	8.4	9.01
ME (MJ/kg)	0	7.74	7.57	7.41	7.67
EE (%)	0	2.3	1.70	1.11	1.02
Ca (%)	8.2	4.06	4.13	4.2	4.38
P (%)	6.0	2.15	2.23	2.32	2.43
Na (%)	13.2	6.28	6.26	6.25	6.49

[2] Estimated according NRC (2007).
[a] Levels of minimum guarantee per kg of product - Ca: 110 g (minimum) and 135 g (maximum); P: 87 g; Na: 147 g; S: 18 g; Co: 15 mg; Cu: 590 mg; I: 50 mg; Mn: 2000 mg; Se: 20 mg; Zn: 3800 mg; and F: 870 mg (maximum)

Table 2
Mean values (g/day) of nutrient intake for supplement, forage and total (supplement + forage) of lambs supplemented with increased protein levels.

	Protein level (%)					P value§	Regression Equation	P value¥	SEM
	0	8	16	24	32				
DMI, %BW	2.16	3.07	1.93	2.05	2.34	0.53	Y = 2.31	0.40	0.545
Supplement intake									
DM	41.4^{b}	126^{a}	135^{a}	131^{a}	162^{a}	< 0.01	Y = 0.112 + 0.001x	0.04	10.5
CP	0.00^{d}	9.54^{d}	22.1^{c}	37.4^{b}	49.6^{a}	< 0.01	Y = −0.00410 + 0.00200x	< 0.01	2.64
EE	0.381^{b}	1.69^{a}	1.66^{a}	1.40^{a}	1.29^{a}	< 0.01	Y = 0.002–0.00001x	< 0.01	0.105
MM	33.4^{c}	39.4^{bc}	43.8^{abc}	55.9^{a}	50.8^{ab}	< 0.01	Y = 0.037 + 0.0005x	0.03	35.3
OM	0.889^{d}	87.4^{bc}	90.9^{bc}	75.2^{c}	111^{ab}	< 0.01	Y = 0.093	0.07	7.17
Forage intake									
DM	580^{a}	407^{b}	482^{ab}	516^{ab}	557^{a}	0.02	Y = 0.376 + 0.006x	< 0.01	35.9
CP	41.5^{a}	28.8^{b}	34.2^{ab}	36.6^{ab}	39.5^{a}	0.02	Y = 0.027 + 0.0004x	< 0.01	2.55
MM	41.1^{ab}	35.6^{b}	41.9^{ab}	45.0^{ab}	48.7^{a}	< 0.01	Y = 0.033 + 0.0005x	< 0.01	2.37
OM	474^{ab}	374^{b}	449^{ab}	473^{ab}	512^{a}	< 0.01	Y = 0.343 + 0.005x	< 0.01	24.9
Total intake									
DM	622^{ab}	533^{b}	617^{ab}	647^{ab}	718^{a}	0.04	Y = 0.487 + 0.007x	< 0.01	41.7
CP	40.1^{c}	38.4^{c}	56.3^{b}	73.9^{ab}	89.0^{a}	< 0.01	Y = 0.023 + 0.002x	< 0.01	4.18
MM	78.6^{bc}	75.0^{c}	85.7^{abc}	100^{ab}	99.6^{a}	< 0.01	Y = 0.069 + 0.001x	< 0.01	4.92
OM	475^{b}	461^{b}	531^{ab}	548^{ab}	624^{a}	< 0.01	Y = 0.418 + 0.006x	< 0.01	28.4

For a single factor, means followed by different letters show significant differences according to Tukey's test at 5% significance level. SEM: Standard error of the mean. §P value for Tukey's test. ¥P value for regression analysis. DM = dry matter. CP = crude protein. EE = ether extract. MM = mineral matter. OM = organic matter.

Table 3
Mean values for time (in minutes) at the forage trough (forage), grazing, rumination resting, eating (forage + grazing), total time of the supplement intake (supplement), number of visits to the supplement trough (No. visits) and average time of the supplement intake (average trough time) of lambs supplemented with increased protein levels.

	Protein level (%)					P value§	Regression Equation	P value¥	SEM
	0	8	16	24	32				
Forage	190	204	168	167	180	0.41	Y = 179.0	0.29	15.2
Grazing	347	332	348	335	336	0.18	Y = 340	0.82	5.58
Ruminating	472	465	477	469	465	0.98	Y = 470	0.83	16.5
Resting	375	337	350	380	375	0.56	Y = 364	0.10	21.0
Eating	537	537	517	502	516	0.49	Y = 521.7	0.26	15.6
Supplement	4.01^{c}	35.9^{a}	25.5^{b}	22.3^{b}	22.3^{b}	< 0.01	Y = 1.584–0.0087x	< 0.01	2.12
No. visits	3.01^{c}	82.6^{a}	42.5^{b}	35.6^{b}	43.6^{b}	< 0.01	Y = 1.91–0.0130x	< 0.01	5.66
Average trough time	0.893	0.457	0.739	0.681	0.629	0.50	Y = 0.62	0.36	0.158

Results reported in average of a 24-h period. For a single factor, means followed by different letters show significant differences according to Tukey's test at 5% significance level. SEM: Standard error of the mean. §P value for Tukey's test. ¥P value for regression analysis.

Table 4
Mean values for mitotic index, papillae area, papillae number and absorptive surface (AS) of the rumen of lambs supplemented with increased protein levels.

	Protein level (%)					P value§	Regression Equation	P value¥	SEM
	0	8	16	24	32				
Mitotic index	0.825^{b}	1.00^{a}	0.963^{ab}	1.00^{a}	0.929^{ab}	0.02	Y = 0.940	0.21	0.0381
Papillae area, cm^2	0.143	0.217	0.151	0.212	0.201	0.05	Y = 0.180	0.79	0.0210
Papillae number	47.0	64.7	55.7	54.2	56.1	0.42	Y = 55.5	0.17	5.98
Absorptive surface	4.78^{b}	6.84^{a}	5.33^{ab}	6.09^{ab}	6.14^{ab}	0.04	Y = 5.83	0.37	0.448

For a single factor, means followed by different letters show significant differences according to Tukey's test at 5% significance level. SEM: Standard error of the mean. §P value for Tukey's test. ¥P value for regression analysis.

Table 5
Mean values for average daily gain (ADG), feed conversion (F:C), final body weight prior to fasting (FBW), slaughter body weight (SBW), fasting weight loss (FL), empty body weight (EBW) and gastrointestinal content (GC) of lambs supplemented with increased protein levels.

	Protein level (%)					P value§	Regression Equation	P value¥	SEM
	0	8	16	24	32				
ADG, kg	0.0810^{b}	0.108^{ab}	0.129^{a}	0.131^{a}	0.116^{ab}	0.02	Y = 0.100	0.61	0.00969
F:C	7.35	5.65	4.29	4.70	6.05	0.13	Y = 5.60	0.62	0.802
FBW, kg	27.3^{b}	29.4^{ab}	31.1^{ab}	31.2^{a}	30.9^{ab}	0.03	Y = 30.0	0.73	0.839
SBW, kg	23.5^{b}	25.7^{ab}	27.5^{a}	26.1^{ab}	26.5^{a}	0.02	$Y = 6.96 + 3.91x - 0.22x^2 + 0.00400x^3$	0.04	0.657
FL, kg	4.46	4.73	4.99	5.30	4.99	0.42	Y = 4.89	0.43	0.295
EBW, kg	19.0^{b}	21.1^{ab}	22.1^{a}	21.5^{ab}	22.0^{a}	0.02	Y = 21.1	0.65	0.558
GC, kg	4.47	4.58	5.34	4.63	4.51	0.54	Y = 4.70	0.48	0.411

For a single factor, means followed by different letters show significant differences according to Tukey's test at 5% significance level. SEM: Standard error of the mean. §P value for Tukey's test. ¥P value for regression analysis.

3.4. Performance and in vivo measurements

The protein supplementation provided to the lambs throughout the 75 days of experiment affected average daily gain, body weight gain and final body weight (Table 5). Lambs supplemented with 24% CP had a numerical greater ADG; however, they were statistically similar to lambs that received 8%, 16% and 32%of protein supplement. Likewise, protein supplementation at 16% and 32% significantly increased SBW and EBW compared to the control group, however, there was no significant difference in these traits between the four supplementation groups (Table 5). Feed conversion did not differ ($P > 0.05$) with the increase of protein supplementation (Table 5).

There were no significant differences ($P > 0.05$) among treatments for withers height (WH), rump height (R), thorax perimeter (TP) and thorax width (TW) (Table 6). However, lambs in the 24% protein supplement treatment had a higher rump width (RW) and body compactness index (BCI) than the control group ($P > 0.05$) but were not significantly different from the other protein treatments (8%, 16% and 32% protein supplementation) (Table 6).

3.5. Fecal egg count

At the beginning of the experiment, there was no difference ($P > 0.05$) in eggs per gram of feces (EPG) across protein supplement treatments (Fig. 1). Regardless of the protein level in the supplement, the fecal egg count, indicated by the EPG count, reduced from day 14 to 28 of the trial (effect of day, $P = 0.02$; Figure 1). After day 28 the EPG count was not affected by day. No effect of protein supplementation ($P = 0.78$) and the interaction between supplementation and day (0.09) was found..

4. Discussion

Animals supplemented with any CP percentage were equally superior in performance (final body weight prior and after fasting, ADG and EBW). Considering the cost-benefit aspect, the present study suggests that 8% of protein in the supplement for lambs raised on elephant grass is ideal for meeting growing nutritional requirements. Previous study indicated that the maximum performance for lambs supplemented with 16% CP in weaned lambs grazing native pastures (Ramos et al.,2019). Others have reported a positive linear effect on weight gain and slaughter body weight when lambs were raised on pasture Tifton-85 and supplemented increasing levels of concentrate (Carvalho et al., 2006). Geron et al. (2012) studied the effect of concentrate supplementation (0.0%, 0.5%, 1.0% and 1.5% live weight) on lambs grazed on *Brachiaria brizantha* cv. Marandu during the dry season. These authors reported a weight gain of 137 g/day for animals that consumed 260 g/day of supplement. Similarly, in the current study, a weight gain of 131 g/day was observed in animals that consumed only 130 g/day of supplement through a diet containing 24% CP, indicating that the elephant grass support a greater ADG. Although there was a difference in weight gain between control and supplemented lambs, the feed conversion rate was not influenced by protein supplementation. Feed conversion data in the current study were lower than reported by Almeida et al. (2012) in a study assessing grazing lambs on urochloa grass (*Urochloa mosambicensis* (Hack) Daudy) pasture and fed with different supplement sources (mesquite pod meal, sorghum and wheat meal) at 1% live weight.

In the current study, grazing time were not affected by protein availability in the diet. This may indicate that the use of elephant grass for lambs is an acceptable option in grazing systems. This could also be supported by grazing time which is reported to occur in an average time of 418 min in daylight time for grazing lambs (Gallardo et al., 2014), and in the current study eating time (forage trough + grazing) averaged around 520 min.

There was no evidence in this study of an effect of CP supplementation on time spent ruminating, suggesting that the digestion process was unaltered. Rumination time is influenced by diet and has been shown to be proportional to the amount of cell wall material in the forage (Van Soest, 1994). However, Souza et al. (2011) reported a longer rumination time and shorter grazing time than that reported in the present study for animals that were fed a 1.5% live weight supplement and finished by grazing on buffelgrass (*Cenchrus ciliaris* L. cv. Bioela). In the current study, it was expected that rest time would increase with the inclusion of protein in the supplement. The higher nutrient input in the supplements was expected to meet the animals' nutritional requirements, resulting in more rest time or time spent on other activities compared to control animals (no protein supplement). However, there was no observed significant effect of protein supplementation on resting time, as substitution effect was not observed. Lambs supplemented with 8% protein spend more time consuming the supplement to reach similar body weight compared to other animals receiving supplement. This was reflected by the higher number of times visiting the supplement trough and increased dry matter intake (although not statistically significant).

Lambs supplemented with 8% protein supplementation had a larger absorptive surface than the control group, which may have been influenced by greater volatile fatty acid (VFA) production in the rumen (Sutton et al., 2003). This result is in agreement with the behavioral pattern for increased supplement intake and number of trough visits for the group supplemented with 8% protein (i.e., resulting frequent influx of CP in the rumen for microbial growth). Moreover, responses in cell growth may be affected by the type of VFA present in the contents of the rumen (Costa et al., 2008), and increased production of these VFAs occurs through the fermentation of foods that are high in carbohydrates and protein (Nussio et al., 2003). Thus, the supplement provided in this study most likely caused significant changes in the proportions of volatile fatty acids in the rumen of the lambs due to the change in CP intake.

In agreement with the current study, supplementation under grazing conditions has been reported to have little impact on in vivo biometric measurements of lambs (Ribeiro et al., 2012). The lack of effect has been associated to animals with similar genotype and slaughter age (same sexual maturation phase), which was the case in the present study. In the current study rump width was higher for lambs supplemented with protein reaching maximum values for lambs fed 24% protein, which was followed by a greater body compactness index. The animals that

Table 6
In vivo biometric measurements of lambs supplemented with increased protein levels.

	Protein level (%)					P value§	Regression Equation	P value¥	SEM
	0	8	16	24	32				
Body length, cm	50.2^{ab}	51.3^{a}	48.5^{ab}	46.6^{b}	49.6^{ab}	0.03	$Y = 0.414 + 0.0137x - 0.0003x^2$	0.02	1.05
Withers height, cm	60.1	61.1	59.5	61.3	60.0	0.66	$Y = 60.5$	0.91	0.993
Rump height, cm	62.4	64.1	62.3	63.8	62.4	0.63	$Y = 63.1$	0.55	1.07
Thorax perimeter, cm	70.0	71.1	76.0	72.5	73.9	0.08	$Y = 73.4$	0.10	1.50
Rump width, cm	19.5^{b}	19.8^{ab}	20.2^{ab}	21.3^{a}	20.2^{ab}	0.04	$Y = 20.4$	0.07	0.411
Thorax width, cm	16.1	16.8	17.7	17.1	17.4	0.13	$Y = 17.2$	0.33	0.437
Body compactness index	0.485^{b}	0.500^{b}	0.557^{a}	0.553^{a}	0.522^{ab}	< 0.01	$Y = 0.414 + 0.0137x - 0.0003x^2$	0.02	0.0119

For a single factor, means followed by different letters show significant differences according to Tukey's test at 5% significance level. SEM: Standard error of the mean. §P value for Tukey's test. ¥P value for regression analysis.

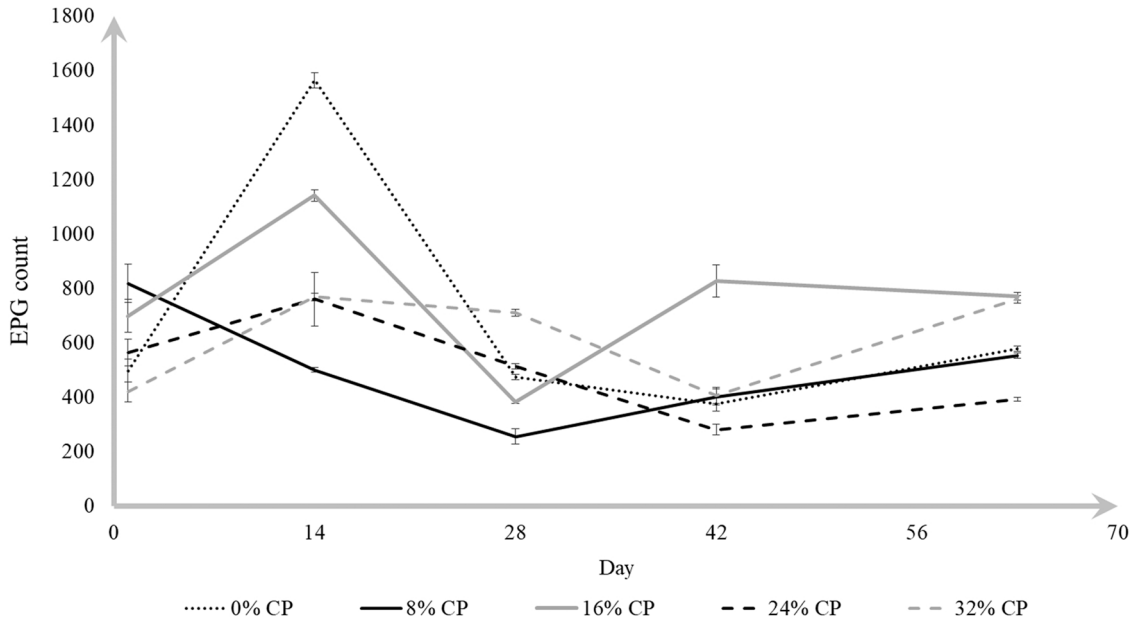

Fig. 1. Mean values for eggs per gram of feces (EPG) content monitored every fortnight for 60 days (d) in lambs supplemented with increased protein levels (0%, 8%, 16%, 24% and 32% crude protein, CP). All lambs were treated on days 0 and 14.

received 16% and 24% CP also had the highest values of FBW and lowest body length which explains the results of body compactness index. Heavier animals at slaughter at a similar age obtain a greater muscular mass per unit length, which leads to animals with more compact carcasses. Thus, the protein supplementation improved the body weight of the lambs and reflected in better body compactness index.

The increase of EPG within the first 15 days of this experiment may be explained by the pasture being grazed by sheep prior to the introduction of the lambs to the pasture. The nematodes (e.g. *Haemonchus contortus*) infective third-stage larvae has a survival time of up to 13 weeks on pasture (Banks et al., 1990, Onyali et al. 1990) depending on rainfall and temperature. This would naturally increase the infection rate in grazing sheep (Mavrot et al., 2015, Gonçalvez et al., 2018, Louvandini et al., 2006). Over time, EPG was reduced due to oral administration of anthelmintics (from day 14). Previous literature reported host resistance improvement against parasitic gastrointestinal nematodes in response to level of protein nutrition for small ruminants under temperate conditions (Houdijk, 2012; Crawford et al., 2020). It is likely that the additional CP in the diet of lambs increased their metabolizable protein supply, previous studies have reported an increase in metabolizable protein requirements in highly infected lambs (Steel, 2003; NRC, 2007). The need of protein to allow for immune expression to fight the infection as well as that required to repair damaged host tissue (Coop and Kyriazakis, 1999) are likely to be the main drivers behind the increased protein requirements. In the present study FEC was not differentiated by protein supplementation at 28, 42 and 63 days of trial. It was expected an improvement on lambs resistance to gastrointestinal parasites due to CP supplementation, however this additional CP in the diet did not increase the host resistance to gastrointestinal helminth infection.

5. Conclusion

Protein supplementation provided a positive response in the protein balance of the lambs. Supplementation at 8% crude protein for lambs grazing elephant grass showed a similar growth performance (ADG, empty body weight) compared to greater levels of CP and promoted greater absorptive area in the rumen.

Conflict of interest

We have no conflict of interest to declare.

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