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REGULAR ARTICLES

Castor bean cake for the control of parasites in pasture-finished sheep

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Abstract

The purpose of this study was to evaluate castor bean cake as alternative input in the control of gastrointestinal parasites in sheep raised on irrigated pasture under continuous stocking. The treatments consisted of sheep supplemented with standard diet and pasture fertilized with urea; sheep supplemented with alternative diet and pasture fertilized with urea; sheep supplemented with standard diet and pasture fertilized with *in natura* castor bean cake; and sheep supplemented with alternative diet and pasture fertilized with *in natura* castor bean cake. A randomized complete block design (CBD) was used, with 16 replications (sheep), with repeated measures over time, the plots being the treatments, and the subplots the collection times. Infective nematode larvae in the pasture (L3.g DM^{-1}), number of eggs per gram of feces (EPG), globular volume (GV), and total plasma protein (TPP) were evaluated. For adult gastrointestinal parasite counts, CBD was also used with six replications. Except for FAMACHA© grade, all variables had effect ($P < 0.01$) of the time factor. The average number of L3.g DM^{-1} and EPG were 126 and 841, respectively, with no effect ($P > 0.05$) of the treatment factor. The values observed for GV and TPP were higher than 25.9% and 6.4 g·dL^{-1}, respectively, which were considered normal. As organic fertilizer, the fractionated application of *in natura* castor bean cake does not reduce the contamination of pastures by nematode larvae. The evaluated feeds improve the resilience of the sheep to infection by gastrointestinal parasites. The treatments using castor bean cake reduced the adult parasites present in the abomasum of sheep.

Keywords Agro-industrial residue · *Haemonchus contortus* · Helminths · *Ricinus communis* · Total plasma protein

Introduction

The rapid and continuous population growth has increased the demand for food worldwide, forcing producers to produce larger quantities and more efficiently, mainly in the agricultural sector. The use of supplementation, application of fertilizers, mainly nitrogen (N), and irrigation techniques, has become an important option in the intensification of pastoral systems, by promoting increase in biomass production and animal performance, raising the carrying capacity and gain per area (Fagundes et al. 2011).

Among ruminants, sheep stand out for their easy adaptability to different soil and climate conditions, showing good productive performance on pasture (Oliveira et al. 2016). However, sheep farming is limited, mainly due to gastrointestinal parasitism, which causes weight loss, reduced production, low fertility, and high mortality rates during the warm, rainy season in intensively raised animals (Nogueira et al. 2011; Molento et al. 2013).

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Haemonchus contortus is the main helminth species that affects small ruminants. It is a hematophagous parasite, causing anemia, weight loss, and it can lead to death (Mavrot et al. 2015; Emery et al. 2016). Anthelmintics have been widely used to control worms. However, its use increases production costs and when used continuously, incorrectly, and indiscriminately, can promote resistance (Melo et al. 2015).

Facing this health concern, several studies have been developed to mitigate the negative effect caused by gastrointestinal nematodes, such as the use of breeds that are more resistant to worm infection, pasture management, use of energy diet, biological control, as well as the use of plants that have anthelminthic properties, and control strategies of the free-living stage of gastrointestinal nematodes in the soil (Vilela et al. 2012; Miranda 2018; Salles et al. 2019).

In addition, Brazil being an agricultural country, the coronavirus pandemic (SARS-COVID-19) generated an increase in commodity prices, mainly corn and soybean, due to the supply and demand behavior of the market, leading to an increase in these agricultural inputs. The use of by-products generated by agribusinesses can contribute to the reduction of costs of animal feeds, such as castor bean cake and meal, which are derived from the extraction of oil of the ricin-chemical chain.

Castor bean cake is a protein-energy feed that digests well. Although its use in the *in natura* form for animal feeding is limited because it contains toxic substances such as *Ricinus communis agglutinin*, ricinin, and allergen complex (Dang and Van Damme 2015), but a safe detoxification method (Andrade et al. 2019), with satisfactory responses in animal production (Santos Neto et al. 2019; Araújo et al. 2020), can be performed.

As an organic fertilizer, in its *in natura* form, this by-product presents interesting nutrient contents, such as nitrogen, phosphorus, potassium, calcium, and low C:N ratio, which favors the mineralization and availability of nutrients for plants (Silva et al. 2012), besides having a positive effect on the control of the free-living stage of gastrointestinal parasites in grazing sheep (Maranguape et al. 2020).

Therefore, the purpose of this study was to evaluate the effect of detoxified castor bean cake replacing soybean meal as well as castor bean cake as organic fertilizer replacing urea in the control of gastrointestinal parasites of Santa Inês sheep finished on irrigated pasture of *Megathyrsus maximus* cv. BRS Tamani under continuous stocking.

Material and methods

Description of the experimental area

The study was performed at Três Lagoas Farm, owned by Embrapa Goats and Sheep, and is located in the city of Sobral-CE, Brazil, at latitude 3°44′50″ South and longitude 40°21′28″ West, from October 2019 to February 2020. The climate of the region is classified as BSh, which means it is semi-arid hot, according to the classification proposed by Köppen (1936). Figure 1 shows the climatic data collected at the weather station installed in the experimental area, during the evaluation period.

Treatments and experimental design

The treatments were SMUR — sheep supplemented with standard diet (based on corn and soybean) and pasture fertilized with urea (SMUR); sheep supplemented with alternative diet (based on corn and detoxified castor bean cake) and pasture fertilized with urea (CCdUR); sheep supplemented standard diet (based on corn and soybean) and

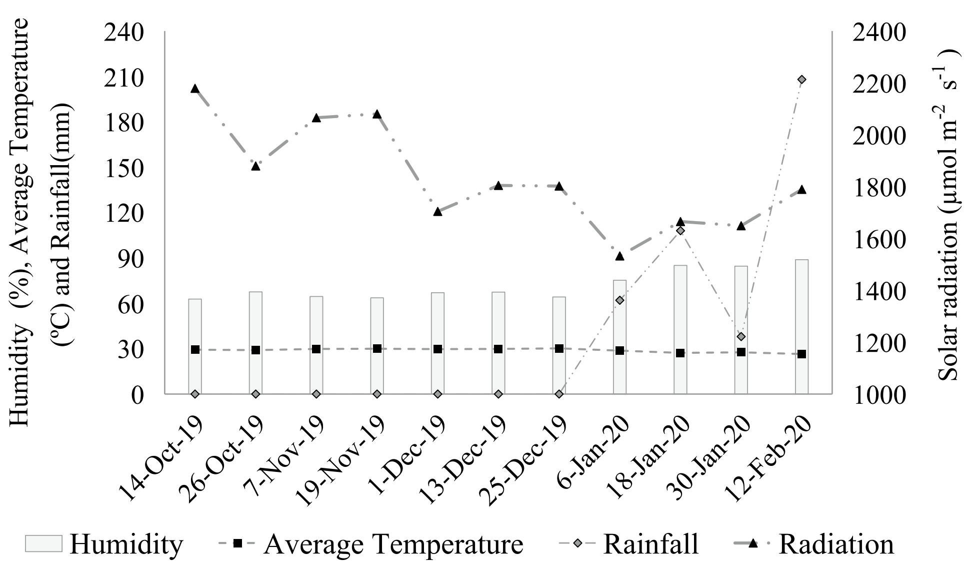

Fig. 1 Air relative humidity (%), average temperature (°C), rainfall (mm), and solar radiation (µmol m^{-2} s^{-1}) of the experimental period (Oct/2019–Feb/2020)

pasture fertilized with *in natura* castor bean cake (SMCC); and sheep supplemented with alternative diet (based on corn and detoxified castor bean cake) and pasture fertilized with *in natura* castor bean cake (CCdCC). A randomized block design (RBD) with repeated measures over time was adopted. The plots were the four treatments (SMUR, CCdUR, SMCC, and CCdCC) and the subplots were the collection times, with sixteen replications (animals). The following variables were evaluated: pasture contamination by infective nematode larvae (L_3.g DM^{-1} of forage), number of eggs per gram of feces (EPG), globular volume (GV), total plasma protein (TPP), FAMACHA© grade, and body condition score (BCS). Collection times were at 21-day intervals for the variable pasture contamination by infective nematode larvae (L3.g DM^{-1} of forage) and at 14-day intervals for the other variables. The design used to count parasites in the gastrointestinal tract of the sheep was the randomized block design with four treatments (SMUR, CCdUR, SMCC, and CCdCC) and six replications (sheep).

Sixty-four Santa Inês sheep, 32 castrated males and 32 females, averaging 3.6 ± 0.6 months of age and initial weight of 19.42 ± 3.60 kg, were distributed in four treatments formed by four replications, that is, four paddocks (500 m^2) containing 4 animals, thus totaling 16 sheep per treatment. Thirty ewes were used as balance animals, with body weight (BW) of 35.0 ± 3.53 kg and approximately 5 years old. The ewes were split into two groups, maintained in two paddocks of Tamani grass, near the experimental area, managed under continuous stocking, and receiving the same concentrate supplements as the test animals. Adult animals were used because they are more resistant to gastrointestinal nematode infection, and, consequently, interfere less in environmental contamination. Balance animals are not considered test animals and are used strategically to maintain the same average height of the pasture (22 cm) among the different treatments. It is worth mentioning that adjusting the stocking rate under grazing allows identical conditions of herbage allowance for all treatments and replications (paddock) of an experiment, reducing biases in the estimates of animal performance and production per area, according to the methodology proposed by Mott and Lucas (1952).

Pasture establishment and detoxification of the castor bean cake

With the aid of a Dutch auger, 30 single soil samples were collected from the experimental area, then mixed in a container, thus forming a composite sample. Subsequently, a 300–500-g portion of this composite sample was packed in a cardboard box, identified, and taken to the laboratory for analysis of the chemical and physical parameters of the soil. The soil of the experimental area was classified as Ortic Chromic Luvisol according to Santos et al. (2018) and presented the following characteristics: pH = 6.8; organic matter = 17.3 g·kg^{-1}; P = 23 mg·kg^{-1}; K = 0.2 $cmol_c$·kg^{-1}; Ca = 11.5 $cmol_c$·kg^{-1}; Mg = 3.4 $cmol_c$·kg^{-1}; H + Al = 2.0 $cmol_c$·kg^{-1}; Al = 0.0 $cmol_c$·kg^{-1}; sum of bases (SB) = 15.1 $cmol_c$·kg^{-1}; cation exchange capacity (CEC) = 17.1 $cmol_c$·kg^{-1}; and V = 89%. The soil presented for S, Na, Cu, Fe, Zn, Mn, and B the values of 153; 23; 40; 80; 13; 159; and 1.3 $cmol_c$·kg^{-1}, respectively, while the values obtained for clay, silt, coarse sand, and fine sand were 161, 219, 327, and 293 g·kg^{-1}, respectively.

Based on the soil analysis, the foundation fertilization was carried out with the formulation 06:28:16 to meet the recommendation of 40:70:40 kg of NPK, in addition to 40 kg·ha^{-1} of micronutrient FTE BR-12 (Cantarutti et al. 1999). Previously, the area went through mechanized crop treatments: stump removal, plowing, and harrowing. The Tamani grass pasture was implemented on July 12, 2019, with the aid of a four-row hydraulic planter. An amount of seeds equivalent to 20 kg·ha^{-1} was used, and sown at a distance of 40 cm between rows and 2.0 cm deep.

The area was provided with a low-pressure fixed sprinkler irrigation system, with service pressure < 2.5 kgf cm^2. Irrigation was carried out daily, over the night. The supplied amount of water corresponded to an average crop evapotranspiration of 6.9 mm·day^{-1}, with application efficiency of 75%. The evaluation of the uniformity of water distribution by the system was performed with the aid of rain gauges spaced at distances of 3.0 × 3.0 m, at a height of 0.5 m from the ground, in two diagonally alternated paddocks.

The *in natura* castor bean cake used as organic fertilizer had the following values for N, P, K, Ca, Mg, and S: 55; 12; 15.7; 6.5; 8.7; and 1.60 g·kg^{-1}, respectively, and C:N ratio = 5.2, while for Cu, Fe, Zn, Mn, and B, the values were 26; 532; 168; 62; and 7 mg·kg^{-1}, respectively. While the castor bean cake destined for animal supplementation was detoxified with calcium oxide, it was performed using electrophoretic characterization (SDS-PAGE) of the samples of *in natura* castor bean cake extract (*in natura* CC), detoxified castor bean cake (CCd), and of the diet containing CCd (CCdD), in addition to the analysis of the hemagglutinating activity of lectins of CC according to Andrade et al. (2019).

General pasture management

Prior to the beginning of the trial, on September 09, 2019, the presence of L3 stage larvae in the pasture was assessed (L_3.g DM^{-1}), according to Ueno and Gonçalves (1998). At the time, low contamination of the pasture was observed with a value of 1.0 L_3.g DM^{-1} of forage. The contamination of the paddocks occurred between September 09 and 24, 2019, through naturally infected sheep with average of 860 EPG. Subsequently, on September 24, 2019, 42 kg of infected feces with 90% *Haemonchus* spp (determined by coproculture) were added to the pasture per paddock, which were obtained from the institutions' goat farm. The following calculation was used to determine the quantity of feces needed to infect the paddock: four sheep with an average weight of 25 kg, defecating 2% of their BW during 21 days of stay in the pasture, which is the period corresponding to the evolutionary cycle of the parasites. The feces were obtained from naturally infected animals, presenting an average of 3,505 EPG. The test animals were introduced to the test area on October 3, 2019, and remained there until February 17, 2020.

The applications of fertilizers, chemical (urea) and organic (*in natura* castor bean cake), occurred 7 days after the end of the pasture contamination (October 1, 2019). The Tamani grass pasture was fertilized according to the treatments with urea (45% N) or *in natura* castor bean cake (5% N), respectively. For the fertilization management of the Tamani grass under intensive conditions, the recommended 450 $kg \cdot ha^{-1} \cdot year^{-1}$ was followed (Vasconcelos et al. 2020), maintaining an average height of 22 cm. Both applications were equally fractioned, at the beginning and midway of the crop production cycles, which lasted 24 days each. The pasture was managed under continuous stocking with variable stocking rate (Mott and Lucas 1952). All the paddocks were delimited by a wire mesh fence and provided with feeders, drinking troughs, salt troughs, and 2.0 × 3.0 m shading screens with 50% light transmittance.

General management of animals and diet

Before being placed on to the paddocks, the animals were treated with anti-parasitic drugs including closantel sodium 10% (10 $mg \cdot kg^{-1}$) and levamisole hydrochloride 5% (5 $mg \cdot kg^{-1}$) which were administered orally with 10-day intervals between applications. First: levamisole hydrochloride then closantel and lastly levamisole hydrochloride. The absence of gastrointestinal parasite eggs was later confirmed through EPG. The male sheep were castrated using castration pliers at the beginning of the trial, according to CFMV Resolution No. 877 of February 2008 (CFMV 2008). After fecal collection on day 42, it was found that the animals showed low number of EPG with normal GV and TPP, indicating low infection; therefore, on November 20, 2019, the animals were orally infected, with the aid of a syringe, with approximately 3,000 larvae (L3), obtained by coprocultures (Ueno and Gonçalves 1998), containing 90% *H. contortus*.

The diets based on corn, soybean, and detoxified castor bean cake were formulated for an average daily weight gain of 200 $g \cdot day^{-1}$, at a ratio of crude protein (CP) and total digestible nutrients (TDN): 14:67.9 $g \cdot kg^{-1}$ DM as recommended by Miranda (2018). These diets provide greater microbial protein synthesis to the animals which promote parasite resilience in sheep on pasture and therefore reduce the effects caused by haemonchosis (Miranda 2018). The chemical composition of the ingredients and their proportions are shown in Tables 1 and 2.

Table 1 Chemical composition of the ingredients of the experimental diets

Items ($g \cdot kg^{-1}$ DM)	Ingredients				
	TGu	TGc	GC	SM	CCd
Dry matter	954.80	956.80	889.90	902.40	931.20
Organic matter	877.50	874.30	932.10	985.10	846.40
Mineral matter	122.50	125.70	18.50	14.90	153.60
Crude protein	101.30	101.40	101.50	489.40	301.30
Neutral detergent insoluble protein	38.10	40.30	25.00	30.50	129.40
Acid detergent insoluble protein	10.00	7.30	20.60	81.30	33.10
Ether extract	36.40	31.60	58.00	43.80	78.30
Total carbohydrates	739.80	741.30	822.00	451.90	466.80
Non-fiber carbohydrates	16.80	3.80	659.60	271.40	136.60
Structural carbohydrates	720.30	737.50	163.00	180.50	330.20
Neutral detergent fiber	758.10	764.70	191.90	192.90	355.20
NDF corrected for ashes and protein	723.00	737.50	163.00	180.50	330.20
Acid detergent fiber	366.70	372.00	54.00	39.70	328.50
Lignin	13.30	15.70	6.10	1.30	36.00
Hemicelluloses	391.40	392.70	137.90	153.20	26.70
Total digestible nutrients	574.70	565.20	861.30	853.60	695.20

Source: Own research data

TGu, Tamani grass fertilized with urea; *TGc*, Tamani grass fertilized with *in natura* castor bean cake; *GC*, ground corn; *SM*, soybean meal; and *CCd*, detoxified castor bean cake

Table 2 Percentage of ingredients and chemical composition of the experimental diets

Item	Percentage of ingredients	
	Standard diet (%dry matter)	Alternative diet (%dry matter)
Tamani grass	51.13	46.95
Ground corn	39.10	33.42
Soybean meal	9.77	—
Detoxified castor bean cake	—	16.45
Soybean oil	—	3.18
Mineral premix[1]	At will	
Total	100	100
Items (g·kg^{-1} dry matter)	Chemical composition of the diet	
Dry matter	924.80	931.00
Organic matter	908.50	862.00
Mineral matter	72.10	89.70
Crude protein	139.30	131.10
Neutral detergent insoluble protein	32.80	48.00
Acid detergent insoluble protein	20.40	16.40
Ether extract	44.30	79.70
Total carbohydrates	744.20	699.20
Non-fiber carbohydrates	289.50	247.50
Structural carbohydrates	454.70	451.60
Neutral detergent fiber	483.20	480.00
NDF corrected for ashes and protein	454.70	451.60
Acid detergent fiber	213.80	245.50
Lignin	9.90	14.80
Hemicelluloses	269.30	234.50
Total digestible nutrients (NRC 2001)	711.60	735.60

Source: Own research data. Standard diet: based on soybean and ground corn and alternative diet: based on ground corn and detoxified castor bean cake. [1]Composition: phosphorus — 65.00 g; calcium — 177.50 g; sulfur — 20.00 g; magnesium — 8.00 g; sodium — 162.00 g; cobalt — 0.04 g; zinc — 1.90 g; manganese — 1.35 g; iodine — 0.071 g; selenium — 0.02 g; fluorine — 0.76 g; copper — 0.20 g; and vehicle — 1.000 g

The supplement was provided at the rate of 1.8% of BW, considering a daily dry matter intake equivalent to 3.6% of BW (NRC 2007). The mineral salt was provided at will in the morning, and the concentrated supplement was provided daily at 5:30 p.m., when African bees (*Apis mellifera*) are least active in the troughs. The lambs were weighed fortnightly, in the mornings using a digital weighing scale, model BL300pro brand Laboremus.

Data collection

The evaluation of pasture contamination by the quantification of infective L3 nematode larvae recovered occurred every 21 days, between days 0 and 126, at the outset of the experiment (October 3, 2019). With the aid of pruning shears, three random samples of Tamani grass from each paddock were collected, placed in plastic bags, identified, and taken to the laboratory for recovery of infective larvae according to the method of Ueno and Gonçalves (1998).

The average height of the pasture was recorded daily with a graduated ruler, sampling thirty-five spots per paddock. With the aid of a rectangular frame measuring 0.5×0.5 m^2, the total forage dry biomass (TFB) was estimated from the average of two samples of Tamani grass, cut close to the ground in each experimental paddock, always at the end of each 24-day crop growth cycle. In the laboratory, the samples were placed in perforated paper bags, weighed, identified, and dried in a forced ventilation oven at 55 °C until reaching constant weight and then were weighed again, obtaining the TFB, expressed as kg DM·ha^{-1}.

The collection of variables EPG, GV, TPP, BCS, and FAMACHA© degree were performed every 14 days between days 14 and 126, day 0 being the first day of feeding and beginning of the trial. The degree of anemia of the animals was analyzed by comparing the color of the conjunctiva (robust red, pinkish red, pink, white, and pale white) to the FAMACHA© grades (one, two, three, four, and five) contained in the guide card (Kaplan et al. 2004). To evaluate the BCS, a scale of one to five was considered, taking into account the body condition of the lumbar region of the lambs, where they were rated from one (very thin) to five (obese) (Russel et al. 1969). Both evaluations were always performed by the same previously trained person.

The feces were collected directly from the rectal ampullae to count the number of EPG by coproculture (Ueno and Gonçalves 1998). To quantify the EPG, the McMaster technique was used (Hansen and Perry 1994). The identification of the genus of infective larvae was performed by the technique described by Ueno and Gonçalves (1998). Also, blood samples were collected from the jugular vein of each animal in 4.5 mL Vacutainer® tubes with sodium ethylenediaminetetraacetate (EDTA) as anticoagulant, and used for the determination of GV and TPP variables.

Following Costa et al. (2020), the first group of animals were slaughtered when they reached an average body weight of 28 kg. Before slaughter, the animals were weighed, fasted for 16 h on solid diet, 12 h on liquid diet, and then weighed again after which they were transported to the abattoir plant accredited by the Ministry of Agriculture. Twenty-four animals, six of each treatment, three males and three females, were slaughtered by desensitization through electronarcosis, with electric discharge of 220 V for 8 s, followed by bleeding through carotid and jugular sections, according to RISPOA (Brasil 2017). After slaughter, the anterior and posterior ends of the abomasum, small intestine, and large intestine were ligated in order to avoid leakage of content and helminths between the sections. In the laboratory, the organs were separated, washed, and all the content was recovered in separate buckets per animal and per organ. The adult parasites were then recovered according to Ueno and Gonçalves (1998).

Description of statistical procedures

For the EPG and number of L3 infective larvae, the data were transformed in log (x + 10) to stabilize variance. The data were subjected to normality (Shapiro–Wilk) and homoscedasticity (Levene) tests and, once the assumptions were met, they were subjected to analysis of variance using the *F* test. The interaction between treatments × collection time was only unfolded when significant at 5% of probability. To evaluate the treatments, the means were compared through the Tukey's test at 5% of probability. The statistical analyses were performed through the MIXED procedure of the SAS software, version 9.4 (SAS 2005), according to the model:

For the variables (L3.g DM^{-1}, EPG, GV, TPP, FAMACHA© grade, and BCS):

$$Yijkl = \mu + \propto_j + \beta_k + (\propto \beta)_{jk} + \gamma_{jk} + \delta_l + (\propto \delta)_{jl} + (\beta\delta)_{kl} + (\propto \beta\delta)_{jkl} + \varepsilon_{ijkl},$$

where $Yijkl$ = value of the i_{th} experimental unit, which received the treatment combination referring to the j_{th} diet and k_{th} fertilization evaluated in the l_{th} repeated measure; μ = effect of general mean; α_i = effect of diet; β_j = effect of fertilization; $(\alpha\beta)_{ij}$ = effect of interaction between diet and fertilization; γ_{jk} = random effect of the error associated to the experimental units; δ_l = effect of repeated measure; $(\propto\delta)_{jl}$ = effect of interaction between diet and repeated measure; $(\beta\delta)_{kl}$ = effect of interaction between fertilization and repeated measure; $(\propto\beta\delta)_{jkl}$ = effect of interaction between diet, fertilization, and repeated measure; and ε_{ijkl} = effect of random error associated to each observation, assuming a normal distribution.

For the analysis of the variable gastrointestinal parasites count:

$$Y_{ijk} = \mu + \propto_j + \beta_k + (\propto \beta)_{jk} + \varepsilon_{ijkl},$$

where Y_{ijk} = value of the i_{th} experimental unit that received the combination of treatments referring to the j_{th} diet and k_{th} fertilization evaluated in the l_{th} repeated measure; μ = effect of the general mean; α_i = effect of the diet; β_j = effect of the fertilization; $(\alpha\beta)_{ij}$ = effect of interaction between diet and fertilization; and ε_{ijk} = effect of the random error, assuming a normal distribution.

Results

No effect of interaction ($P > 0.05$, treatments × collection times) was found on any of the evaluated variables. The adopted managements (SMUR, CCdUR, SMCC, and CCdCC), with or without the use of castor bean cake as supplement (CCd) or organic fertilizer (*in natura* CC), had no effect ($P > 0.05$) on the number of infective larvae in the pasture (L3.g DM^{-1}), EPG, and FAMACHA© grade (Fig. 2). On the other hand, effects of the adopted managements were observed ($P < 0.05$; $P < 0.01$; and $P < 0.01$) on GV, TPP, and BCS, which decreased throughout the evaluations.

The highest numbers of L3 infective larvae, which were 212 and 287 L3.g DM^{-1}, were found at days 63 and 126, respectively, of collection (Fig. 2A). The mean EPG during the experimental period was 841 EPG, which was higher on days 42, 56, and 126, with mean values of 1,172; 1,173; and 1,071 EPG, respectively (Fig. 2B). The animals presented GV ranging from 25.90 to 30.50% during the evaluated periods, with treatment CCdUR presenting a value of 27.45% while for the others, the average was 28.44% (Fig. 2D).

Sheep supplemented with detoxified castor bean cake in pasture fertilized with *in natura* CC (CCdCC) showed blood levels of TPP similar to the ones managed with CCdUR and SMUR, with SMUR being similar to SMCC (Fig. 2F). Treatment CCdUR caused the highest BCS value, which was 2.70, while the others had an average value of 2.50 (Fig. 2E). Except for the FAMACHA© grade, the other variables analyzed had significant effect ($P < 0.01$) during the trial period. Throughout the trial, the animals had a FAMACHA© score of 2 or 1 (Fig. 2C). The average body weight of the animals among treatments was 28.08 kg, which had no significant difference ($P > 0.05$).

The EPG before slaughter (EPG_{126d}) showed no difference ($P > 0.05$), averaging 900 eggs/g $feces^{-1}$. After slaughter of the animals, there was a significant difference ($P < 0.05$) in the number of adult gastrointestinal parasites from the abomasum, also, the number of helminths was higher in the animals fed with feed containing soybean meal in pasture fertilized with urea (Table 3).

As for the prevalence of adult helminth species identified in the sections of the gastrointestinal tract of the animals, regardless of the treatments adopted, 100% of the parasites in the abomasum were *H. contortus*, 100% in the small intestine were *Trichostrongylus colubriformis*, and 100% in the large intestine were *Oesophagostomum columbianum*.

Discussion

Throughout the experimental period, there was a tendency for a linear increase in the number of L3 infective larvae in the pasture, which was related to the allocation and the time the animals stayed in the pasture, associated with high temperature, humidity (daily irrigation),

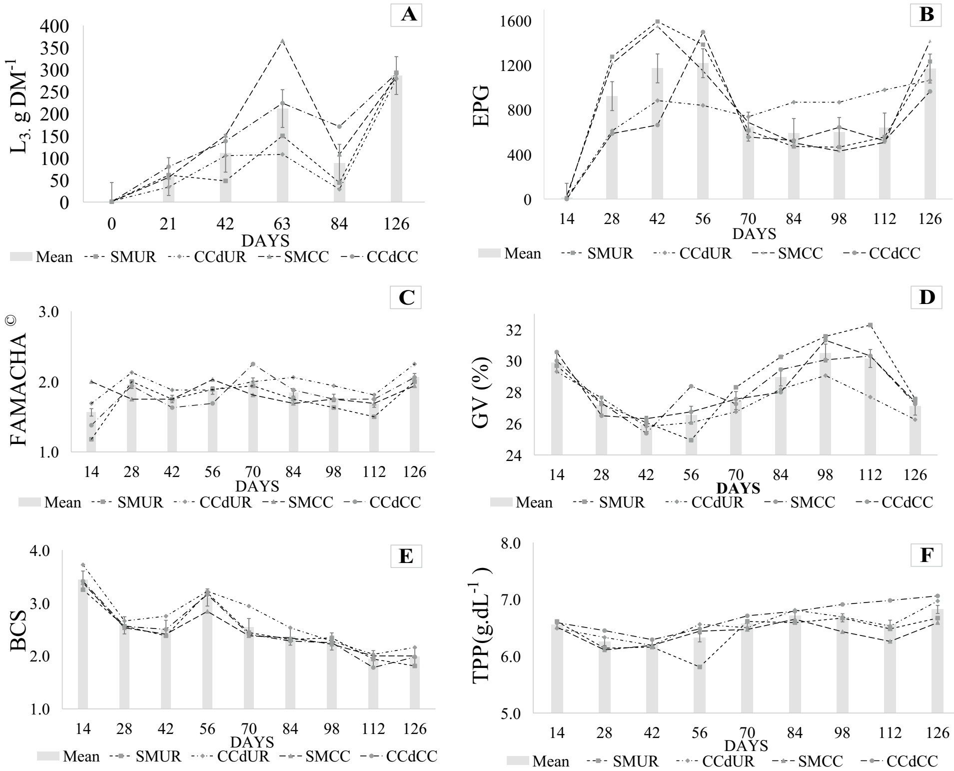

Fig. 2 Averages by treatment and periods of the variables infective larvae count (L3.g DM^{-1} forage), eggs per gram of feces (EPG), globular volume (GV), total plasma protein (TPP), body condition score (BCS), and degree of anemia by the FAMACHA© method of sheep finished on pasture under continuous stocking, using castor bean cake as a supplement or nematicide fertilizer in the control of gastrointestinal parasites. Treatments: SMUR — animals supplemented with soybean meal and pasture fertilized with urea; CCdUR — animals supplemented with detoxified castor bean cake and pasture fertilized with urea; SMCC — animals supplemented with soybean meal and pasture fertilized with in natura castor bean cake; and CCdCC — animals supplemented with detoxified castor bean cake and pasture fertilized with in natura castor bean cake. Means followed by different uppercase letters in the treatments and lowercase letters in the periods differ from each other by the Tukey's test at $P<0.05$ (*), $P<0.01$ (**), and absence of letters ($P>0.05$)

Table 3 The average eggs per gram of feces for the experimental collection period and at day 126, and number of parasites per section of the gastrointestinal tract of sheep finished on pasture under continuous stocking, using castor bean cake as a supplement or nematicide fertilizer in the control of gastrointestinal parasites

Variable	Production systems evaluated				Mean	[e]CV (%)
	[a]SMUR	[b]CCdUR	[c]SMCC	[d]CCdCC		
EPG_{period}	954	698	924	765	841	10.79
EPG_{126d}	1038	667	1165	606	900	14.64
Abomasum	644a	228ab	413a	108b	337	20.68
Small intestine	550	400	305	246	372	22.18
Large intestine	3	17	7	38	16	57.51

[a]SMUR — animals supplemented with ground corn and soybean meal, in pasture fertilized with urea;

[b]CCdUR — animals supplemented with ground corn and detoxified castor bean cake, in pasture fertilized with urea;

[c]SMCC — animals supplemented with ground corn and soybean meal, in pasture fertilized with *in natura* castor bean cake;

[d]CCdCC — animals supplemented with ground corn and detoxified castor bean cake, in pasture fertilized with *in natura* castor bean cake; [e]CV(%) — coefficient of variation

Means followed by the same lowercase letter in the row are not statistically different through the Tukey's test at 5% of probability and vegetation cover, which favored the reduction of solar radiation inside the canopy ensuring the survival and persistence of helminth larvae of stage L3 (Fig. 2A). This result was surprising, since Maranguape et al. (2020), who worked with the application of *in natura* castor bean cake in tanzânia grass pasture during the rainy season to control gastrointestinal parasites, reported that when on application of castor bean cake at a level of 500 kg·ha^{-1} every 2 months (equivalent to 100 kg·ha^{-1} of N), there was a reduction in animal infection and contamination of pasture by *H. contortus* larvae in 60 and 65%, respectively. However, fertilizer distribution every 12 days, particularly organic fertilizer (*in natura* CC), may have contributed to a reduction in the nematocidal effect of castor bean cake on nematodes in the pasture.

Fertilizer fractionation may have mitigated the concentration of active molecules such as volatile organic compounds (VOCs) generated during the organic matter decomposition process, such as phenols (Pedroso et al. 2019). VOCs have nematocidal character for phytonematoids (Freire et al. 2012) and may also have nematocidal effect on the free-living stages of gastrointestinal nematodes of small ruminants. Therefore, both organic fertilization with *in natura* CC and chemical fertilization with urea, applied in portions, were not able to efficiently reduce the contamination of pastures. In addition, the area was irrigated daily, keeping the environment favorable for the movement of larvae along the canopy.

Similar values for the number of L3 infective larvae on the pasture were observed at days 63 and 126 of collection, which are associated to the irrigation management, climatic changes favorable to rainfall, and the increase in stocking rate (equivalence of 120 sheep·ha^{-1}) in the last growth cycle of the grass, aiming to maintain pasture management to optimize the carrying capacity, observed in the last collection. At 63 days of collection, the amount of water applied to the pasture was readjusted to more than 1 mm·day^{-1} in order to meet the daily water demand required for plant growth. The adjustment was based on the crop evapotranspiration 18 days before collection. That contributed to the displacement of L3 larvae along the canopy profile, since in the L3 stage, larvae have migratory behavior, being able to move vertically and horizontally in the pasture, and this movement is more frequent in the presence of water (Van Dijk and Morgan 2011), enabling the recovery of a greater number of L3 larvae under these conditions.

The reduction observed at 84 days of collection for the variable number of L3 infective larvae in the pasture may be associated with the lowering of the grass, initiated 7 days before this collection, in order to ensure the average height of the pasture of 22 cm. Balance animals were distributed among treatments SMUR, CCdUR, SMCC, and CCdCC, with stocking rates corresponding to 85.63; 86.34; 84.60; and 85.31 sheep·ha·cycle^{-1}, respectively. The management momentarily provided unfavorable conditions for the survival of L3 larvae in the pasture, due to the removal of a greater amount of forage, especially leaves, which contributed to increased luminosity within the canopy, decreased humidity, and destabilization of the thermal condition in the microenvironment, causing larvae to migrate to the lower stratum of the pasture (Santos et al. 2012), resulting in lower recovery of infective larvae in this collection (Fig. 2A). It is also inferred that the increase in stocking rate increased the daily intake of L3 larvae by the animals, influencing the results.

The linear increase observed for EPG up to day 56 of collection is explained by the reinfection of the grazing animals, thereafter there is a reduction, and only at the beginning of the rainy season, corresponding to 126 days of collection, the number of eggs per gram of feces increases again, reaching a value similar to those observed at days 42 and 56 of collection. The decline in the EPG variable observed after 56 days of collection is associated with an immune response of the host to parasitism (Hendawy 2018). Therefore, it is deduced that the artificial infection with 3,000 L3 larvae (90% *Haemonchus* spp.), performed between 42 and 56 days of collection and, consequently, the establishment of infective larvae in the gastrointestinal tract of the animals, led to the elimination of adult parasites, causing the phenomenon of self-healing (Hendawy 2018) of the sheep, and may have influenced the higher number of infective L3 larvae recovered in the pasture on collection day 63 (Fig. 2A).

The self-healing response is more pronounced in genetically resistant animals (Allonby and Urquhart 1976). Santa Inês is identified as a breed that is less susceptible to gastrointestinal parasitism, with great ability to initiate an immune response to parasitic infection (Albuquerque et al. 2019; Amarante et al. 2004) due to the high number of eosinophils in the blood, high IgG production, normal cell volume, and low worm charge, requiring less anthelmintic treatments when compared to other breeds (Albuquerque et al. 2019). This fact, associated with the nutritional status of the animals, may have influenced the EPG values, as well as the number of adult gastrointestinal parasites observed in the present study, which was considered a mild infection (< 1000), according to Ueno and Gonçalves (1998). In addition, Embrapa promotes careful selection in its herd, adopting the culling based on the FAMACHA© grade, which allows the selection of animals with greater resistance and resilience to helminths, associated with the breed and nutritional management adopted, corroborating with the results found.

The protein (CP) and energy (TDN) ratio of 14:67.9 proposed by Miranda (2018) and adopted in the present study provided the animals with adequate nutritional support, allowing a better balance in the host-parasite relationship in all the treatments evaluated (supplemented with or without detoxified castor bean cake). The ratio between CP and TDN in the diet allows animals infected or not with *Haemonchus spp*. to maintain the same production of microbial synthesis and therefore the availability of metabolizable protein for the animal, indicating resilience of the animals to gastrointestinal parasitism (Miranda 2018). This availability of metabolizable protein can divert protein synthesis from muscles and bones to repair and react to stomach lesions caused by *H. contortus* (Amarante 2009).

Initially, there was a reduction in the globular volume and total plasma proteins between 14 and 56 days of collection, which is explained by the parasitic reinfection of young animals on pasture, confirmed by the increase in EPG in that period. According to Hendawy (2018), young animals are more susceptible to parasitic infections, but with advancing age, the immune response increases. In all treatments and periods evaluated, the GV levels were higher than 23%, indicating that the sheep were clinically healthy, with no signs of anemia (Molento et al. 2013), which is compatible to the evaluation of the FAMACHA© grade, where the color of the ocular mucosa of the animals ranged from robust red to pink red, representing a value below grade two and TPP levels within the range considered normal for the ovine species that is between 6.0 and 7.5 g·dL^{-1} (Kaneko et al. 1997).

The most evident reduction in body condition score in the last two production cycles (late January and early February) was motivated by changes in the ingestive behavior of the animals, which reduced grazing time with the onset of the rainy season and, consequently, reduced dry matter intake. The lower consumption of dry matter caused nutritional deficit, leading the animals to mobilize body reserves for their physiological maintenance, influencing the reduction in weight gain during the period. However, on average, it was possible to record values between 2.5 and 2.7, which was higher in sheep supplemented with diets containing detoxified castor bean cake and pasture fertilized with urea. The BCS values found in this study are considered median according to Russel et al. (1969), and this value is understood as ideal for determining the slaughter or mating of sheep in this category (Hashimoto et al. 2012; Castro et al. 2013).

The fewer number of parasites in the abomasum of sheep fed with detoxified castor bean cake and pastures fertilized with *in natura* castor bean cake (CCdCC) is related to the additional and associative effect of the input on the control of gastrointestinal parasites in sheep, increasing its effectiveness in control. It is worth noting that other factors such as adequate nutritional support and a breed more resilient to helminths have contributed decisively to the results found in the other treatments. In these treatments, the sheep showed mild infections, and therefore, there was no need for the application of anthelmintics throughout the experimental period. Thus, there is evidence of the nematocidal effect of castor bean cake on the control of gastrointestinal parasites in sheep, as confirmed by the reduction in the total number of adult worms in the gastrointestinal tract of the animals, which was more significant in sheep supplemented with CCd and pastures fertilized with *in natura* CC.

Fractionated fertilization with *in natura* castor bean cake does not reduce pasture contamination by nematode larvae.

Diets that promote resilience to hemoncosis with or without detoxified castor bean cake are efficient in controlling gastrointestinal parasites in Santa Inês sheep.

The association supplement-fertilization with castor bean cake is more efficient in reducing the parasites present in the abomasum of sheep.

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Author contribution AJGM: conceptualization, methodology, validation, data curation, writing — original draft, visualization. RCFFP: conceptualization, methodology, validation, formal analysis, data curation, writing — reviewing and editing. HOS: methodology, validation, data curation, writing — reviewing and editing, visualization. LdSV: methodology, writing — proofreading and editing. MT: resources, project management. MCPR: methodology. AMPS: validation. PLP: validation. MJDC: conceptualization, supervision.

Data availability The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

Declarations

Ethics approval All procedures of this study were approved by the Ethics Committee on the Use of Animals (CEUA) of Embrapa Goats and Sheep, under protocol No. 001/2017.

Conflict of interest The authors declare no competing interests.

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