ORIGINAL ARTICLE

Use of nutraceutical functional oil in the control of coccidiosis in confined lambs

Uso de óleo funcional nutracêutico no controle da coccidiose em cordeiros confinados

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Abstract

The objective of this study was to evaluate the inclusion of functional oil in the diet of lambs as a substitute for monensin in the control of coccidiosis. For this purpose, 34 lambs were distributed into three treatments: control (CONT – without additive), functional oil (FO - 495 mg of commercial powder mix composed of cashew nut oil and castor oil/kg dry matter (DM) of the concentrate), and monensin (MON - 12.5 mg/kg DM of the concentrate). The experimental period lasted 61 days, with fecal examinations of the animals carried out on days 0, 14, 28, 41, and 55 of the experimental period. Except for day 0, in all other collections, the lambs in the FO group presented a higher OPG value ($p \leq 0.05$) compared to the lambs in the MON group. In the collection on day 28, a greater difference was observed between the groups, with the animals that received FO presenting 827 OPG, while those that received MON presenting zero. For this reason, a reduction in body weight and average daily body weight gain was noted in lambs of the FO group from this collection until the end of the experiment, with a statistical difference ($p \leq 0.05$) between the two groups. These two groups also presented higher eosinophil and leukocyte counts, and alterations in the small intestine mucosa. Thus, the inclusion of 495 mg of a mix composed of cashew nut oil and castor oil/kg of DM of the concentrate was not effective in controlling coccidiosis in confined lambs.

Keywords: Additive. Production. Essential Oil. Sheep.

Resumo

O objetivo deste estudo foi avaliar a inclusão de óleo funcional na dieta de cordeiros como substituto da monensina no controle da coccidiose. Para isso, 34 cordeiros foram distribuídos em três tratamentos: controle (CONT – sem aditivo), óleo funcional (OF – 495 mg de mistura comercial em pó composta por óleo de castanha de caju e óleo de rícino/kg de matéria seca (MS) do concentrado) e monensina (MON – 12,5 mg/kg de MS do concentrado). O período experimental teve duração de 61 dias. A coleta de fezes dos animais foi realizada nos *dias 0, 14, 28, 41 e 55 do período experimental. Com exceção do dia 0, em todas as demais coletas os cordeiros do grupo OF apresentaram maior valor de OPG (p ≤ 0,05) em comparação aos cordeiros do grupo MON. Na coleta realizada no dia 28, observou-se uma diferença maior entre os grupos, com os animais que receberam FO apresentando 827 OPG, enquanto os que receberam MON apresentaram zero. Por esse motivo, notou-se uma redução no peso corporal e no ganho médio diário de peso nos cordeiros do grupo FO a partir dessa coleta até o final do experimento, com diferença estatística (p ≤ 0,05) entre os dois grupos. Esses dois grupos também apresentaram maiores contagens de eosinófilos e leucócitos, além de alterações na mucosa do intestino delgado. Assim, a inclusão de 495 mg de uma mistura composta por óleo de castanha de caju e óleo de rícino/kg de MS do concentrado não foi eficaz no controle da coccidiose em cordeiros confinados.*

Palavras-chave: *Aditivo. Produção. Óleo essencial. Ovelha.*

Introduction

Coccidiosis is caused by the ingestion of sporulated oocysts of *Eimeria* spp. present in the environment and is a common disease that affects sheep in intensive farming systems, due to the high concentration of animals and the accumulation of organic matter (Bangoura and Bardsley, 2020). Lambs from three weeks to five months of age are the most affected (Mohamaden et al., 2018) and may present significant drops in performance and high mortality, causing a great economic impact in intensive systems; therefore, infection control becomes important. Ionophores such as monensin sodium, classified as ionophore antibiotics (Vendramini et al., 2016), have been widely used as coccidiostats in ruminant production systems. In the European Union, the ban implemented in January 2006 targeted the use of antibiotics as growth promoters under Regulation No. 1831/2003 (European Comission, 2003), motivated primarily by concerns regarding antimicrobial resistance. Although certain ionophores remain authorised as coccidiostats for specific target species under strict conditions, their use has become increasingly scrutinised within the framework of antimicrobial stewardship. As a result, there is growing interest in natural plant-derived alternatives for parasite control.

The use of feed additives, such as essential oils and monensin sodium, offers a practical advantage in controlling coccidiosis in animal production systems, as these products can be administered directly via the diet. This strategy has been shown to facilitate management, thus eliminating the need for individividual treatments, which are common with conventional anticoccidial agents and require more labour. Consequently, incorporation through dietary means has the potential to reduce labour requirements, minimise animal stress arising from handling, and facilitate large-scale implementation within commercial systems.

Due to these concerns, there is a growing interest in the search for alternatives, including bioactive compounds, the so-called nutraceuticals, in the form of essential and functional oils. This control method appears to be promising, with the advantage of the low risk of emergence of microbial resistance (Maurizio et al., 2023). Oils are volatile (terpenes, hydrocarbons, simple alcohols, and aldehydes) and aromatic substances present in plants, which have antimicrobial, antioxidant, antifungal, and antiparasitic properties (Mohammed et al., 2025). Functional oils are defined as those that are not extracted from spices and have metabolic and physiological effects on the body (Matera et al., 2023). Nutraceuticals are defined as a food or part of a food that provides medical and health benefits, including the prevention and/or treatment of disease (Garza-Juárez et al., 2023).

Among the promising strategies for improving lamb performance is the use of additives containing bioactive compounds with antiparasitic potential. The castor oil (*Ricinus communis*) and cashew nut shell liquid contain phenolic constituents such as anacardic acid, cardol (40 g/kg), and cardanol (200 g/kg) (Mazzetto et al., 2009) as well as ricinoleic acid (90 g/kg), which may exhibit anthelmintic (Ademola and Eloff, 2011) and antimicrobial activities (Alves et al., 2022). To date, there are no published studies evaluating the functional oil blend of castor oil and cashew nut shell liquid for the control of coccidiosis in small ruminants. Based on these properties found in the literature and on study evaluating the effect of the blend on coccidiosis-challenged broilers (Moraes et al., 2019).

The objective of the current study was to evaluate the inclusion of functional oil from a mix of castor oil and cashew nut shell liquid in the diet of lambs, reared in a confinement system, as a substitute for monensin, in the control of coccidiosis and its effects on the average daily weight gain of the animals.

Material and methods

The experiment was carried out at the Capão da Onça School Farm (FESCON), belonging to the State University of Ponta Grossa (UEPG), located in Ponta Grossa, PR, Brazil. FESCON is located at 25° 05′ 49″ south latitude and 50° 03′ 11″ west longitude, 990 meters above sea level, with 312.11 hectares, along the Ponta Grossa – Itaiacoca highway km 07. The climate of the region is mesothermal humid subtropical (Cfb) according to the Köppen classification. The average temperature in winter is 13 °C with frequent frosts and in summer the average temperature is 21 °C. The average rainfall is 1600 to 1800 mm per year, with average annual temperatures between 17 and 18 °C, and an average annual relative humidity of 70 to 75%.

Experimental design and protocol

The project was approved by the Animal Use Ethics Committee of UEPG, protocol No. 2196/2019. Thirty-four lambs were used, 18 Ile de France (IF) and 16 Texel (Tx), with an average initial age of 29 days and an average body weight of 11.89 kg. The animals were distributed into three groups: functional oil (FO) (IF = 6; Tx = 6); control (CONT), without treatment (IF = 6; Tx = 6); and with monensin (MON) (IF = 6; Tx = 4). Animals were allocated to treatment groups by randomization (breed, initial body weight, and age).

The animals were housed in collective pens, separated according to treatments and subjected to controlled breastfeeding management. During the first week, the lambs spent the entire day with their mothers in their pens. Afterwards, the adult sheep remained grazing in the morning, returning at 11 am to feed their young, remaining there for two hours, then returned to graze again in the afternoon. At the end of the afternoon, around 4 pm, the adult sheep returned to the collective pens, with their lambs, where they spent the night.

The lambs remained confined in collective pens separated according to group throughout the evaluation. They were weighed at birth and, from 29 days of age, evaluations began, with 14-day intervals until the end of the experimental period, when they had a mean age of 85 days. At approximately 60 days of age, the lambs began to adapt to weaning. Therefore, the ewes did not return to the stalls at 11 am, remaining in the pasture. This management was maintained for five days, when the lambs were completely weaned. They were then finished in confinement for another 30 days. At the end of the experimental period, 61 days, 14 animals of the IF breedwith a mean age of 85 days were destined for slaughter in a commercial slaughterhouse, five from the FO group, five from the MON group, and four from the CONT group.

Experimental diet

Diets were formulated (Table 1) to meet the nutritional requirements of growing lambs using the Small Ruminant Nutrition System, v. 1.8.6 (Cannas et al., 2004).

Table 1 - Proportion of ingredients — g/kg dry matter (DM) — and chemical composition of experimental diets offered to lambs of the Ile de France and Texel breeds kept in feedlot

Ingredients	Treatments		
	CON	FO	MON
Ground corn	767	767	767
Soybean meal	210	210	210
Mineral salt[2]	9	9	9
Ammonium chloride	5	5	5
Limestone	7	7	7
FO (mg/kg DM)	-	495	-
MON (mg/kg DM)	-	-	12.5
Chemical composition	**Concentrate**	**Silage**	-
Dry matter	90.40	32.38	-
Crude protein	14.40	6.12	-
Neutral detergent fiber	23.15	44.53	-
Acid detergent fiber	6.08	21.81	-
Ether extract	3.75	3.39	-
Ash	5.73	4.08	-

Note: CON (control) = without addition of FO or MON; FO (functional oil) = 495 mg/kg DM; MON (monensin) = 12.5 mg/kg DM.

Quantities of concentrate were provided to allow a 10% surplus over the expected intake, ensuring that all lambs had continuous access to the concentrate throughout the experimental period. The additives were included when mixing the concentrate ingredients, following the manufacturer's instructions. Thus, the following formulations were obtained: CONT (without additive); FO (495 mg of commercial powder blend composed of cashew nut oil and castor oil/kg of dry matter (DM) of the concentrate); MON (Rumensin® 200, Elanco Saúde Animal) in the amount of 12.5 mg/kg DM of the concentrate. The functional oil blend consisted of 40 g/kg of cardol, 90 g/kg of ricinoleic acid, and 200 g/kg of cardanol, formed by a mixture of cashew oil, castor oil, and silica. In addition to the concentrate, the animals received corn silage *ad libitum* and had free access to water. The diet was offered twice a day (8 am and 1 pm), directly in the collective pens where the lambs were confined.

Diet samples and chemical analyses

Diet samples were collected weekly throughout the experimental period and a composite sample was taken at the end. The sample was pre-dried in an oven at 55 °C for 24 hours and then ground in a "Willey" type mill with a 1 mm sieve (AOAC methods No. 930.15) and ash (AOAC methods No. 942.05). The crude protein (CP) was determinated using the Leco TruMac N (Leco Corp., St Joseph, MI; AOAC, 1990; #968.06). To convert total nitrogein into CP, a conversion factor of 6.25 was used. Neutral detergent soluble fiber and acid detergent soluble fiber were determined according to the method described by Van Soest et al. (1991).

Parasitological analysis

Collections began when the animals were 30 days old. Fecal samples were collected on days 0, 14, 28, 41, and 55 directly from the rectal ampoule of the lambs for fecal examination (Figure 1). Oocyst counts per gram of feces (OPG) and eggs per gram of feces (EPG) were performed using the modified McMaster technique (Ueno and Gonçalves, 1998), with a lower limit of detection of 100 OPG/EPG. A final OPG was performed on the animals that were slaughtered, after 16 hours of fasting.

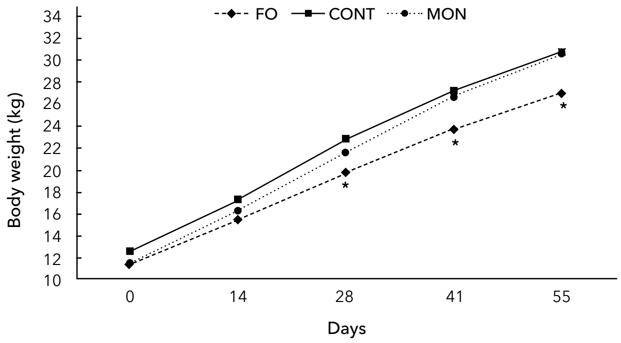

Figure 1 - Body weight of lambs confined supplemented with a diet containing functional oil (FO), monensin (MON), and without additives (CONT).

Note: *Represents a significant difference ($p < 0.05$) between treatments on different collection days.

Hematological tests

Blood samples were collected on days 0 (beginning of the experiment), 14, 28, 41, and 55 from the jugular vein in vacuum tubes (with and without EDTA). The packed cell volume (PCV) was determined by centrifugation in a microhematocrit tube. Subsequently, the tubes without EDTA were centrifuged and the serum was extracted and stored in Eppendorf tubes. Total plasma protein (TPP) (6.25 x N) and albumin were analyzed using standard commercial kits in a semi-automatic biochemical analyzer BIO – 200. Globulin was calculated through the difference between TPP and albumin.

Blood eosinophil counts were performed in a Neubauer chamber after staining in Carpentier's solution and the counts are expressed as the number of cells per microliter (μl) of blood (Dawkins et al., 1989). The total leukocyte count was also performed in a Neubauer chamber, after staining in modified Turk's liquid, according to the recommendations of Viana et al. (2002). The results were presented in thousands of cells per μl of blood.

Histological analyses

For histological examinations, samples of intestinal tissue were collected from slaughtered animals. The tissues were removed from the mid-jejunum and duodenum and fixed in Carnoy's fluid.

Subsequently, they were embedded in paraffin, then cut into 4 micrometer (μm) sections and stained with hematoxylin and eosin (HE). The slides were photo-graphed and measurements were performed using the LAS EZ provided by Leica Microsystems®. The crypts were evaluated preferably in a way that included the largest number of whole crypts in the field and in three different fields per sample. The flattening and junction of the villi were also analyzed, together. The inflammatory intensity was assessed through the cell type present, whether the inflammatory process was of the mononuclear type with the presence of lymphocytes, plasma cells, and macrophages, or of the polymorphonuclear type, with the presence mainly of neutrophils.

Statistical analysis

Data were analyzed using SAS version 9.3. Breed was included as a fixed effect in the statistical model, as its effect was found to be non-significant, data were pooled across breeds for all subsequent analyses. Parasitological and hemato-biochemical variables were subjected to analysis of variance using the MIXED procedure with repeated measures over time. Means were compared using the Tukey test at a 5% probability level. In the variables with repeated measures over time, the effects of treatment and period, and the interaction between treatment and period were tested. Data for EPG, OPG, eosinophils, and leukocytes were log-transformed [log10(x + 1)] to normalize the distribution and stabilize variances, but are presented as original, untransformed means in tables for ease of interpretation. The histopathological findings are presented descriptively.

Results

Parasitological analyses

In fecal examinations, it was possible to identify oocysts of the genus *Eimeria* (OPG) and eggs of *Strongyloides* spp. and the family Trichostrongylidae. In the first collection (day zero), no significant difference ($p > 0.05$) was observed in the OPG count between the experimental groups (Figure 1).

On day 14, animals treated with FO (66.3 ± 16.9 OPG) excreted greater amounts of *Eimeria* spp. oocysts ($p < 0.05$) compared to lambs in the CONT (18.20 ± 9.75 OPG) and MON (3.89 ± 2.61 OPG) groups (Figure 1).

From day 14, lams in the FO group showed higher OPG counts compared to those in the CONT and MON. From days 28 to 55, OPG values did not differ between lambs in the FO and CONT groups ($p > 0.05$). However, differences were found for animals supplemented with MON ($p < 0.05$), which presented the lowest OPG averages. On day 28, lambs in the FO group presented the highest OPG count of the entire experimental period (Table 2).

Table 2 - Oocyst count per gram of feces in lambs confined supplemented with a diet containing functional oil (FO), monensin (MON), and without additives (CONT)

Days	Treatments			p-value
	FO	MON	CONT	P
0	2 ± 0.11^{a}	7 ± 0.16^{a}	1 ± 0.10^{a}	0.3400
14	66 ± 0.15^{a}	2 ± 0.14^{b}	17 ± 0.22^{b}	<.0001
28	827 ± 0.28^{a}	13 ± 0.22^{b}	347 ± 0.29^{a}	<.0001
41	111 ± 0.32^{a}	3 ± 0.15^{b}	127 ± 0.25^{a}	0.0061
55	39 ± 0.22^{a}	1 ± 0.09^{b}	55 ± 0.26^{a}	0.0002

Note: FO = 495 mg/kg dry matter (DM); MON = 12.5 mg/kg DM; P = period effect. Different letters in the same line indicate significant difference ($p < 0.05$) between treatments.

In the OPG count of animals that were slaughtered, animals that received FO and control diet presented the highest OPG counts ($p < 0.05$) (57,700 ± 19,091 and 89,275 ± 47,340, respectively) when compared with lambs that received monensin (2,060 ± 1,642).

The EPG of nematodes of the Trichostrongylidae family was negligible, with no statistical difference ($p > 0.05$) between the groups (Table 3). The lambs in the FO group presented mean values of 16 ± 7.0 EPG, the animals in the MON group of 30 ± 10.6 EPG, and those in the CONT group of 7 ± 3.7 EPG. For *Strongyloides* spp. there was a significant difference ($p < 0.05$). Lambs from the MON and CONT groups presented higher counts with means of 173 ± 51.7 and 144 ± 62.0, respectively, when compared with animals from the FO group (32 ± 12.8).

Table 3 - Count of Trichostrongylidae and *Strongyloides* spp. in lambs confined supplemented with a diet containing functional oil (FO), monensin (MON), and no additives (CONT)

	Treatments			p – value		
	FO	MON	CONT	T	P	TxP
Trichostrongylidae	16 ± 7.0	30 ± 10.6	7 ± 3.7	0.11	0.0300	0.08
Strongyloides spp.	32 ± 12.8[b]	173 ± 51.7[a]	144 ± 62.0[a]	0.03	0.0008	0.09

Note: FO = 495 mg/kg dry matter (DM); MON = 12.5 mg/kg DM; T = treatment effect; P = period effect; T*P = effect between treatment and period. Different letters in the same line indicate significant difference ($p < 0.05$) between treatments.

Dry matter intake and animal performance

At the initial collection 14 days later, there were no differences in the body weight of the lambs in the different groups ($p > 0.05$) (Figure 1). However, from day 28 to 55, the animals that received FO presented lower body weight ($p < 0.05$) when compared with the CONT and MON groups.

Concentrate consumption, expressed in grams of DM per day, was 458 g/day for lambs in the FO group (approximate consumption of 0.226 g of FO/day), 516 g/day in the CONT group, and 463 g/day in the MON group (approximate consumption of 0.0058 g of MON/day).

Animals supplemented with FO showed lower performance ($p < 0.05$) compared to lambs in the other experimental groups, evidenced by the lower average daily weight gain (DWG) (Table 4).

Table 4 - Initial age, initial weight, final weight and average daily weight gain (ADWG) (g/day) of lambs confined, supplemented with diets containing functional oil (FO), monensin (MON), or without additives (CONT)

Parameters	Treatments			p – value		
	FO	MON	CONT	T	P	TxP
Initial age (days)	29 ± 1.50	32 ± 1.50	26 ± 1.65	-	-	-
Initial weight (kg)	11.40 ± 0.51	11.63 ± 0.56	12.65 ± 0.51	-	-	-
Final weight (kg)	27.05 ± 3.07[b]	30.78 ± 4.33[a]	30.60 ± 3.41[a]	0.04	-	-
ADWG (g/day)	0.275 ± 0.01[b]	0.301 ± 0.01[a]	0.316 ± 0.01[a]	0.01	< 0.0001	0.48

Note: FO = 495 mg/kg dry matter (DM); MON = 12.5 mg/kg DM; T = treatment effect; P = period effect; T*P = effect between treatment and period. Different letters in the same line indicate significant difference ($p < 0.05$) between treatments.

Hematological tests

The treatments did not alter the values of PCV, TPP, and globulin ($p > 0.05$) (Table 5), and the observed PCV values are within the reference values for the sheep species.

However, there was an effect of treatment x collection on albumin concentration ($p < 0.05$) on days 41 and 55 (Table 6). On day 41, the FO group presented a lower albumin concentration when compared with CONT, but did not differ from MON. On day 55, the FO and MON groups presented higher albumin concentrations when compared with CONT. The mean globulin concentrations were: 2.76 ± 0.10 g/dL, 2.94 ± 0.10 g/dL and 2.76 ± 0.10 g/dL, for FO, CONT, and MON, respectively (Table 5).

Throughout the experimental period, animals supplemented with monensin consistently exhibited the lowest eosinophil and leukocyte counts compared to both the FO and CONT groups ($p < 0.05$) (Table 5).

Table 5 - Mean values (± standard deviation) of packed cell volume (PCV - %), total plasma protein (TPP - dL), albumin (g/dL), globulin (g/dL), eosinophils (cells/μL), and leukocytes (μ/L) of lambs confined fed a diet containing functional oil (FO), monensin (MON), and control (CONT) without additive

Parameters	Treatments			p - value		
	FO	MON	CONT	T	P	TxP
PCV	34.81 ± 0.77	37.25 ± 0.82	36.76 ± 0.75	0.080	< 0.0001	0.57
TPP	5.38 ± 0.11	5.58 ± 0.11	5.65 ± 0.10	0.210	< 0.0001	0.11
Globulin	2.76 ± 0.10	2.76 ± 0.10	2.94 ± 0.10	0.340	< 0.0001	0.77
Eosinophils	36.43 ± 0.89^{a}	9.02 ± 0.66^{b}	38.19 ± 0.9^{1a}	0.009	< 0.0001	0.21
Leukocytes	11,727 ± 0.13^{a}	8,991 ± 0.18^{b}	12,714 ± 0.17^{a}	0.009	0.4600	0.57

Note: FO = 495 mg/kg dry matter (DM); MON = 12.5 mg/kg DM; T = treatment effect; P = period effect; T*P = effect between treatment and period. Different letters in the same line indicate significant difference ($p < 0.05$) between treatments. Reference values for PCV (27 - 45), TPP (6 - 7.5), globulin (3.1 - 5.1), eosinophils (0 - 1,000) and leukocytes (4,000 - 12,000) by Seixas et al., 2021.

Table 6 - Mean values albumin (g/dL) (±SD) of lambs confined fed a diet containing functional oil (FO), monensin (MON), and control (CONT) without additive

Days	Treatments			p-value
	FO	MON	CONT	T
0	2.44 ± 0.12	2.70 ± 0.07	2.74 ± 0.20	0.2700
14	2.84 ± 0.08	2.79 ± 0.06	2.88 ± 0.08	0.8900
28	2.63 ± 0.07	2.73 ± 0.11	2.60 ± 0.05	0.8000
41	2.48 ± 0.08^{b}	2.74 ± 0.14^{ab}	2.97 ± 0.1^{7a}	0.0400
55	2.82 ± 0.18^{a}	3.09 ± 0.2^{4a}	2.29 ± 0.13^{b}	0.0004

Note: SD = standard deviation; FO = 495 mg/kg dry matter (DM); MON = 12.5 mg/kg DM; T = treatment effect. Different letters in the same line indicate significant difference ($p < 0.05$) between treatments. Reference values for albumin: 2.4 - 3 (Seixas et al., 2021).

Histological analyses

In the jejunum and duodenum of the groups, a marked detachment of the intestinal epithelium was observed, with hyperplasia of the crypts and goblet cells. All groups presented marked inflammatory process, associated with the presence of mononuclear cells. The duodenum samples from the animals in the FO group did not show flattening and junction of the villi, however the jejunum samples presented a marked degree of flattening and junction of the villi. The duodenum of lambs in the CONT group showed slight flattening and junction of the villi and marked jejunum. Animals supplemented with MON showed flattening and junction of the villi in both the duodenum and jejunum (Figures 2 and 3).

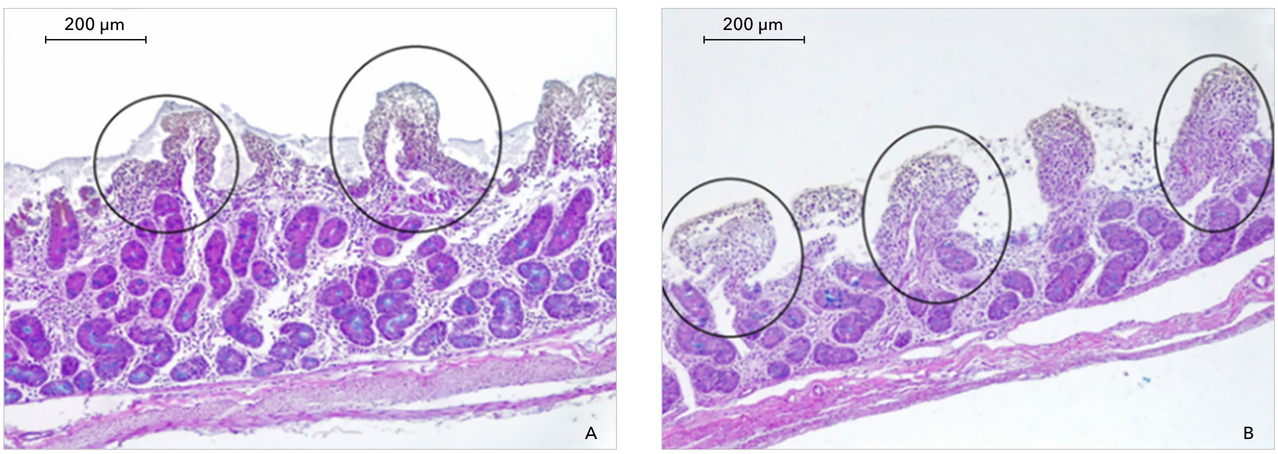

Figure 2 - Duodenum of lambs in the control group with flattening and junction of the villi, with a mild degree (A) and duodenum of lambs in the group supplemented with monensin sodium with flattening and junction of the villi, with a moderate degree (B).

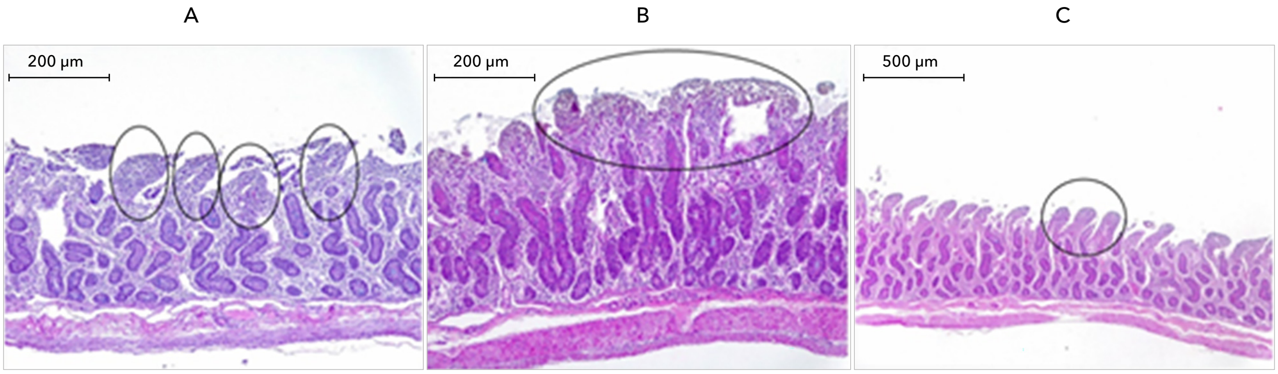

Figure 3 - Jejunum of lambs in the group supplemented with monensin with moderate flattening and junction of villi (A) and with severe flattening and junction of villi in lambs in the control group (B) and supplemented with functional oil group (C).

Discussion

The inclusion of 12.5 mg of monensin/kg of DM was effective in controlling coccidiosis in lambs, confirming the coccidiostatic capacity of this product. However, in the current study, the inclusion of 495 mg of the blend composed of cashew nut oil and castor oil/kg of DM of the concentrate did not show a positive effect in controlling coccidiosis in confined lambs. For this coccidiostatic effect to occur, it is important that the oil reaches the small intestine, the preferred location for *Eimeria* species that attack sheep. However, ruminal microorganisms have the ability to metabolize the compounds present in the oils (Benchaar and Greathead, 2011), as well as transforming some molecules into other molecules or converting some bioactive compounds into inert substances (Poulopoulou et al., 2020), hindering the coccidiostatic effect of the product.

The results of using oils to control coccidiosis in ruminants are inconsistent. Although some research with oils has been carried out to observe their effects on ruminal fermentation (Coneglian et al., 2019), little is known about how oils behave in the intestines of animals. Recent findings indicate that terpenes are rapidly degraded within the rumen of sheep and goats, resulting in only trace amounts being detected in the duodenal digesta (Poulopoulou et al., 2020). The disappearance of oils at the ruminal level includes bioconversion in the ruminal fluid, transfer to the gas phase due to high volatility (Amin et al., 2021), and absorption by the rumen wall (Poulopoulou et al., 2020).

In an *in vitro* study conducted by Remmal et al. (2011), artemisia, tea tree, thyme and clove oils demonstrated the ability to destroy *Eimeria* spp. oocysts that affect birds. The authors observed that during microscopic counting some oocysts were deformed and had damaged layers. Moraes et al. (2019) in an in vivo study reported that the use of a commercial mixture of cashew nut shell liquid and castor oil modulated the inflammatory response against *Eimeria* spp. in broiler chickens. However, in a study conducted *in vivo* by Chapman et al. (2017), no positive effect was observed with the inclusion of 1 to 2 mg of cinnamaldehyde essential oil/kg of the diet on the performance and control of coccidiosis in calves.

The lower DWG in the FO group can be attributed mainly to the higher parasite load by *Eimeria* spp., observed in this group, since coccidiosis is one of the factors that most negatively influences the zootechnical performance of lambs, directly affecting weight gain and body development (Junkuszew et al., 2015; Bangoura and Bardsley, 2020). As a consequence of the lower DWG, the animals in this group had a lower final weight, which implies the need for a longer confinement period to reach the ideal slaughter weight, directly impacting the productive efficiency of the system. Regarding gastrointestinal nematodes, although a statistical difference was detected between groups, absolute EPG values remained very low across all treatments. These results should not be interpreted as evidence of a robust anthelmintic effect of the functional oils, as variations occurred within a subclinical infection range.

Coccidiosis can cause damage to the intestinal mucosa (Bangoura and Bardsley, 2020). The epithelial loss, and crypt and goblet cell hypertrophy demonstrated in the histopathological analyses is related to the invasion of the parasites into the crypts and epithelium of the intestine. In addition, the goblet cell hyperplasia confirms that mucus was being produced to form a physical barrier against the parasites (McRae et al., 2015). Consequently, repairing damaged tissue and stimulating the immune system increases metabolic costs and reduces the animal's ability to convert nutrients into energy for weight gain (Chen et al., 2016). Although *Strongyloides* and *Trichostrongylus colubriformis* (Trichostrongylidae) can also cause lesions and inflammation in the mucosa of the small intestine (Amarante, 2015), EPG in the MON group were relatively low throughout the experimental period. Therefore, the precise etiology of the mucosal lesions observed in the MON group remains unclear and cannot be attributed primarily to gastrointestinal nematodes given the mild level of infection. The present study did not produce any evidence that the functional oil dose was responsible for the reduced performance observed in the lambs. Bezerra et al. (2020) evaluated the same oil at a comparable level (500 mg/kg DM) and reported no adverse effects on lamb growth. However, Michailoff et al. (2020) tested the inclusion of 0, 2, 4, and 6 g of FO/animal/day (commercial mix of cashew nut shell oil and castor oil) and observed no negative effects on nutrient intake, ruminal fermentation, microbial protein synthesis in the rumen, and nutrient digestibility in sheep. Thus, the inclusion of 495 mg of FO/kg of DM of the concentrate, which gave approximately 0.226 g of FO/day, may not have caused nutritional losses and, consequently, affected performance of the animals. The findings suggest that coccidiosis infection was a more probable contributor to the observed decline in growth.

Since no high incidence of hematophagous parasites was found during the study, PCV levels remained within the normal range (27 - 45%), with no indications of anemia. Low PCV values are correlated with high OPG counts, due to bloodsucking by hematophagous parasites (Amarante, 2015). In the present study, no such occurrence was observed. The TPP concentration was slightly below the limits considered normal for sheep (6 - 7.9 dL) (Seixas et al., 2021). However, according to Lins et al. (2020), healthy Ile de France lambs up to 68 days of age tend to have TPP concentrations between 5.0 and 6.2 g/dL, which may explain the low values observed. Albumin concentrations in the control group, although lower compared to treated groups, remained within the normal reference range (Seixas et al., 2021).

The higher numbers of blood eosinophils and leukocytes in the FO and CONT groups probably occurred in response to coccidian infection in the lambs of these groups. Leukocytes and eosinophils are important defense cells against parasitic infections (Huang and Appleton., 2016). After infection, they migrate towards the target with the function of regulating the immune system, fighting parasites, and healing tissues (McRae et al., 2015).

Even though the literature contains studies reporting that functional oils can present effects similar to those of monensin sodium in controlling coccidiosis, this benefit was not observed in the present study. In addition, infection by *Eimeria* spp. negatively affected the performance of lambs that received FO when compared to lambs that received monensin. Further research with different doses is needed to test the effect of functional cashew and castor oil on the control of coccidiosis and performance of confined lambs.

Conclusion

This study confirmed the effectiveness of monensin in controlling coccidiosis in lambs but showed that the functional oil based on cashew and castor oil did not present a significant coccidiostatic effect. These results highlight the need for further research with different doses and formulations, given that there are few studies that prove the practical effectiveness of essential oils against coccidiosis. Despite the negative results, this work advances scientific knowledge and contributes to improving sheep health management practices, preventing the inappropriate use of products that may not be effective.

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Authors´contributions

Conceptualization: JMG, RAR. Data curation: JMG, JI. Formal analysys: JI. Methodology: JMG, RAR, MML. Writing of the original draft: JMG, FAA. Writing, review and editing: JMG, PAB.

Data availability statement

The research data are not publicly available.

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