FULL ARTICLE

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Brazilian red pepper leaves essential oil (*Schinus terebinthifolius*) in diets for feedlot lambs

Óleo essencial das folhas de aroeira (Schinus terebinthifolius) em dietas para cordeiros confinados

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ABSTRACT

Essential oil (EO) from Brazilian red pepper leaves contains antimicrobial compounds that control Gram-positive bacteria in the rumen content, improving the efficiency of ruminal fermentation. The objectives of the present study were to evaluate the effects of the Brazilian red pepper leaves EO (*Schinus terebinthifolius*) as a substitute for monensin on performance, occurrence of coccidiosis by *Eimeria ssp.*, carcass characteristics and meat composition of feedlot lambs. Forty-four lambs, 16 males (12 ½ Dorper × ½ Santa Inês and 4 Santa Inês) and 28 females (16 ½ Dorper × ½ Santa Inês and 12 Santa Inês), with 21.4 ± 1.05 kg of initial body weight (BW), were used in a randomized complete block design. The experiment lasted 56 days, divided into 2 periods of 28 days each. The treatments were defined by the inclusion of 8 ppm of monensin (MON), and the doses 0.14% (14EO), 0.28% (28EO) and 0.42% (42EO) of red pepper leaves essential oil (EO). At the end of 56 days, 32 lambs were slaughtered for the measurement of carcass parameters and meat composition. There was no interaction among treatments and periods for average daily gain (ADG), dry matter intake (DMI), feed efficiency (FE) and oocyst of *Eimeria ssp*. in feces. The treatments did not affect the ADG, DMI and FE; however, the monensin inclusion decreased the oocyst of *Eimeria ssp.* ($P = 0.01$). There was a tendency ($P = 0.06$) of increase in hot carcass yield for lambs fed 28EO compared to 14EO. In addition, the cold carcass yield was higher ($P = 0.02$) in the animals fed 28EO and 42EO. The subcutaneous fat thickness was not affected by the experimental diets; however, there was a tendency for lambs from 28EO and 42EO treatments to present higher body wall thickness ($P = 0.07$) and Longissimus muscle area ($P = 0.07$) when compared to MON. The higher doses of red pepper leaves EO increased the percentage of crude protein ($P < 0.01$) and mineral matter ($P = 0.02$) in the chemical composition of meat. Although the performance of lambs did not change, the inclusion of 0.28 and 0.42% red pepper leaves EO improve the carcass characteristics and change the meat composition, demonstrating the potential of the use of this additive in confined lamb diets. However, the monensin has greater potential to control coccidiosis in feedlot lambs compared with red pepper leaves EO.

Keywords: Additives. Performance. Ionophore. Vegetal extracts.

RESUMO

O óleo essencial das folhas da aroeira possui compostos antimicrobianos que controlam a população de bactérias gram-positivas presentes no conteúdo ruminal, melhorando a eficiência do processo de fermentação. Os objetivos do presente estudo foram avaliar os efeitos do óleo essencial das folhas da aroeira (*Schinus terebinthifolius*) em substituição a monensina sobre o desempenho, ocorrência de coccidiose por *Eimeria ssp.*, características de carcaça e da carne de cordeiros confinados. Quarenta e quatro cordeiros, 16 machos (12 ½ Dorper × ½ Santa Inês e 4 Santa Inês) e 28 fêmeas (16 ½ Dorper × ½ Santa Inês e 12 Santa Inês), com 21,4 ± 1,05 kg de peso inicial, foram utilizados em delineamento de blocos completos ao acaso. O experimento teve duração de 56 dias, divididos em 2 períodos de 28 dias cada. Os tratamentos foram definidos pela inclusão de 8 ppm de monensina sódica (MON) e as doses 0,14% (14OE), 0,28% (28OE) e 0,42% (42OE) do óleo essencial (OE) das folhas da aroeira. Ao final dos 56 dias, 32 animais foram abatidos para

a mensuração dos parâmetros de carcaça e análise química da carne. Não houve interação entre tratamento e período experimental para ganho médio diário (GMD), consumo de matéria seca (CMS), eficiência alimentar (EA) e ocorrência de coccidiose. Não houve efeito das dietas experimentais sobre GMD, CMS e EA, entretanto, a inclusão de monensina reduziu o número de oocistos de *Eimeria ssp.* (P = 0,01). Houve tendência de aumento no rendimento de carcaça quente (P = 0,06) para os cordeiros alimentados com 28OE comparados com o tratamento 14OE. Além disso, o rendimento de carcaça fria foi maior (*P* = 0,02) para os animais alimentados com 28OE e 42OE. A espessura de gordura subcutânea não foi afetada pelas dietas experimentais, entretanto, houve tendência dos cordeiros dos tratamentos 28OE e 42OE apresentarem maior espessura de parede corporal (*P* = 0,07) e área de olho de lombo (*P* = 0,07) quando comparados a MON. As maiores doses de OE das folhas de aroeira aumentaram a porcentagem de proteína bruta (*P* < 0,01) e matéria mineral (*P* = 0,02) na composição química da carne dos cordeiros. Apesar de não alterar o desempenho dos cordeiros, a inclusão de 0,28 e 0,42% de OE das folhas de aroeira foi capaz de alterar as características de carcaça e composição química da carne, demonstrando o potencial de utilização desse aditivo em dietas para cordeiros confinados. Entretanto, a monensina possui maior capacidade de controlar a coccidiose em cordeiros confinados quando comparado ao OE das folhas da aroeira.

Palavras-chave: Aditivos. Desempenho. Ionóforos. Extratos vegetais.

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Introduction

High energy diets are provided in confined breeding system aiming to maximize animal performance. High concentrate diets for feedlot lambs provide enough energy and protein for weight gain over 250 g/d (Ferreira et al., 2011; Polizel et al., 2017). The use of feed additives that modulate ruminal fermentation is important, improving the propionate production and reducing the damage caused by some rumen microorganisms (Owens et al., 1998), since the inclusion of high levels of concentrate may hinder rumen fermentation (Nagaraja et al., 1997).

Monensin is the main ionophore used in ruminant nutrition, resulting in an increase in energy retention and dietary nitrogen use (Tedeschi et al., 2003), changes in molar concentration of short chain fatty acids (Ellis et al., 2012), decrease in dry matter intake (DMI) and increase in average daily gain (ADG) and feed efficiency (FE) (Duffield et al., 2012). Ionophores are classified as antibiotics by the Food and Drug Administration, but are not used in human medicine. The interest in exploring natural products that pose no health risks and can be used as feed additives has increased. Due to the ban on the use of ionophores as growth promoters in animal feed carried out by the European Union, there is increased interest in alternative feed additives.

Some essential oils have a similar capacity to the ionophores, acting selectively on the microbial populations of the rumen (Calsamiglia et al., 2007). The use of EO and their bioactive compounds results in a change in the fermentation pattern, reducing the acetate:propionate ratio and the methane production, making rumen more efficient (Khiaosa-ard & Zebeli, 2013). From the Brazilian red pepper (*Schinus terebinthifolius*), it is possible to extract EO from the leaves, fruits and trunk, with the main substances found including α-pinene, sabineno, β-pinene, α-felandreno, Δ-3-carene, β-felandreno, α-copaene, germacrene-D, bicyclogermacrene, β-caryophyllene and δ-cadinene (Barbosa et al., 2007; Santos et al., 2007).

Therefore, it has been hypothesized that Brazilian red pepper leaves EO could be included in diets for feedlot lambs as a feed additive, resulting in similar response compared with monensin. The objective of this study was to evaluate the effects of the inclusion of red pepper leaves EO as a substitute for monensin on performance, occurrence of coccidiosis by *Eimeria ssp.*, carcass characteristics and meat composition of lambs.

Material and Methods

Forty-four lambs were used, 16 male (12 ½ Dorper × ½ Santa Inês and 4 Santa Inês) and 28 female (16 ½ Dorper × ½ Santa Inês and 12 Santa Inês), with 21.4 ± 1.05 kg of initial body weight (BW).

The experiment consisted of a 56-d period, divided into two 28-d periods. The animals were weighed after 14 h of solid fasting period on d 0, 28 and 56 of the experiment. The experiment design used was randomized complete blocks, each block defined by sex, breed and initial BW. The lambs were kept indoors, in an individual tie-stall system, with a slatted floor, feed bunk, and waterer. On d 28 and 56, feces samples were collected to evaluate the coccidiosis oocyst *Eimeria ssp.* (COC) (Gordon & Whitlock, 1939).

The experimental diets were defined by the addition of monensin (Rumensin 200, Elanco Animal Health, São Paulo, SP, Brazil), or doses of red pepper leaves EO (Lazlo Aromaterapia, Belo Horizonte, MG, Brazil). The experimental diets were as follows: inclusion of 8 mg of monensin/kg of DM (MON); diets with 0.14% (14EO), 0.28% (28EO) and 0.42% of red pepper leaves EO (42EO; Table 1). The red pepper leaves EO composition was performed by gas chromatography coupled to mass spectrometry provided with an automatic injector, using a capillary column with 30 meters in length and 25 mm internal diameter. The total chromatographic run time was 60 min, divided into five heating cycles, as follows: 50 °C (30 min), 200 °C (4 °C/min), 240 °C (10 °C/min), 280 °C (10 °C/min), and 290 °C (5 °C/min).

The total mixed diets were offered *ad libitum* once a day. The experimental diets were prepared weekly to avoid possible changes in the compounds present in the EO. Orts were recorded daily to determine the DMI, and the refused feed did not exceed 5% of daily intake. In each experimental period (28d), ADG and feed efficiency were calculated.

The DM and ash content were determined according to Association of Official Analytical Chemists (1990). Sequential detergent fiber analyses were determined according to Van Soest et al. (1991). Total N and ether extract of the diets and meat were determined according to the Association of Official Analytical Chemists (1990). Non fiber carbohydrates of the diets were estimated according to the following equation: NFC (%) = 100% - (% NDF + %CP + % fat + % ash).

At the end of the 56 days, 32 lambs (16 male and 16 female) were slaughtered, following the norms described in the Regulation of the Industrial and Sanitary Inspection of Products of Animal Origin - RIISPOA. The lambs were weighed after 14 h fasting period to obtain the slaughter weight (SW). Carcass characteristics evaluated were hot carcass weight (HCW) and hot carcass yield (HCY), obtained at the time of slaughter. After 24 h of chilling (4 °C), the chilled carcass weight (CCW), chilled carcass yield (CCY), subcutaneous fat thickness over the 12th rib (SFT), body wall thickness (BWT), and *Longissimus dorsi* muscle area (LM area) were obtained. Approximately 15 cm samples of the *Longissimus dorsi* muscle from the right half carcass of each animal were collected and stored at -18 °C for determination of the chemical composition of the meat. The DM, ash, total N and ether extract content of the *Longissimus dorsi* meat were determined according to Association of Official Analytical Chemists (1990).

Statistical analyses were performed using the MIXED procedure of the SAS (SAS version 9.0; SAS Inst. Inc., Cary, NC, USA). All data were submitted to the Shapiro-Wilk test to check the normality of the residuals and the removal of

Table 1 – Proportion of the ingredients and chemical composition of the experimental diets containing monensin or Brazilian red pepper leaves EO (Piracicaba, 2019)

Item	Diets[1]			
	MON	14EO	28EO	42EO
Ingredient, % of DM				
Coastcross hay	10.00	10.00	10.00	10.00
Ground corn	72.00	71.86	71.72	71.58
Soybean meal	14.00	14.00	14.00	14.00
Urea	0.50	0.50	0.50	0.50
Mineral premix	1.50	1.50	1.50	1.50
Ammonium chloride	0.50	0.50	0.50	0.50
Limestone	1.50	1.50	1.50	1.50
Red pepper leaves EO	0.00	0.14	0.28	0.42
Monensin, ppm	8.00	0.00	0.00	0.00
Chemical composition[2]				
DM, as-fed basis	87.45	87.33	87.60	87.62
Ash, % DM	5.40	5.41	5.59	5.71
CP, % DM	19.26	18.71	19.11	19.11
NDF, % DM	19.05	18.05	18.39	18.61
ADF, % DM	7.75	7.40	7.38	7.66
Ether extract, % DM	4.67	3.59	3.97	3.86
NFC, % DM	51.62	54.24	52.94	52.71

[1]MON = diet containing 8 mg of monensin/kg of DM; 14EO = inclusion of 0.14% of red pepper leaves EO (DM basis); 28EO = inclusion of 0.28% of red pepper leaves EO; 42EO = inclusion of 0.42% of red pepper leaves EO; [2]DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; NFC = non-fiber carbohydrate.

"outliers" and the Levene test to verify the homogeneity of variances. The animals were considered as the experimental unit to perform statistical analyses. The treatment means were obtained by the LSMEANS command. The treatment effect was defined by Tukey test. The period effect and treatment x period interaction were defined by the *F* test. Statistical significance was declared at $P \leq 0.05$, with trends noted at $P > 0.05$ to $P < 0.10$.

Results

The main components found in the red pepper leaves EO used in the present study were Δ-3-carene (28.7%), limonene (17.5%), α-pinene (11.1%), α-felandrene (8.3%), *p*-cimene (6.1%), α-elemol (3.9%), β-cariofilene (3.5%), mircene (3.0%) and germacrene D (2.5%). In addition, the compounds sabinene, Δ-cadinene, β-pinene, α-terpinolene, δ-cadinene, δ-selinene, α-bergamotene and carvacrol were identified in the red pepper leaves EO in concentrations less than 1.5%.

There was no interaction between treatment and period and treatment effects for ADG, DMI and oocyst of *Eimeria ssp*. (Table 2). The experimental diets did not affect the ADG, DMI, FE and BW at 28 d and 56 d. Period effect was observed for DMI ($P < 0.01$) and FE ($P < 0.01$), in which the DMI increased during the experiment (P1: 878.57 ± 38.07 and P2: 1003.71 ± 40.46 g/d), and, consequently, the FE during the first period was higher than the second period (P1: 0.29 ± 0.01 and P2: 0.25 ± 0.01).

Lambs fed MON had lower oocyst ($P = 0.01$) when compared with lambs fed red pepper leaves EO. In addition, the oocyst count was higher in the first period (70.4 oocysts/g) when compared with the second period (11.4 oocysts/g).

The experimental diets did not affect slaughter weight, HCW and SFT (Table 3). However, the 28EO and 42EO diets

Table 2 – Performance and oocyst count of feedlot lambs fed high-concentrate diets containing monensin or Brazilian red pepper leaves EO (Piracicaba, 2019)

Item[4]	Diets[1]				SEM[2]	*P*-value[3]		
	MON	14EO	28EO	42EO		Diets	P	D × P
BW, kg								
Initial	21.36	21.37	21.32	21.36	1.05	-	-	-
28d	27.89	28.87	28.71	28.83	1.47	0.42	-	-
56d	35.62	36.11	35.99	36.16	1.91	0.94	-	-
ADG, g	236.20	265.50	262.99	271.92	21.71	0.17	0.50	0.24
DMI, g/dia	904.66	943.27	964.08	952.35	42.22	0.26	<0.01	0.40
FE, gain:feed	0.261	0.281	0.273	0.285	0.01	0.20	<0.01	0.12
Oocysts/g	4.11[b]	17.44[a]	19.35[a]	17.24[a]	3.47	0.01	<0.01	0.14

[1]MON = diet containing 8 mg of monensin/kg of DM; 14EO = inclusion of 0.14% of red pepper leaves EO (DM basis); 28EO = inclusion of 0.28% of red pepper leaves EO; 42EO = inclusion of 0.42% of red pepper leaves EO; [2]SEM = standard error of the means; [3]P = period effect; D × P = diets and periods interaction; [4]ADG = average daily gain; DMI = dry matter intake; FE = feed efficiency; [a-b]means in the same row with different superscripts differ ($P \leq 0.05$); [A-B]means in the same row with different superscripts tented to differ ($P > 0.05$ to $P < 0.10$).

Table 3 – Carcass characteristics and meat composition of feedlot lambs fed high-concentrate diets containing monensin or Brazilian red pepper leaves EO (Piracicaba, 2019)

Item[4]	Diets[1]				SEM[2]	*P*-value[3]
	MON	14EO	28EO	42EO		Diets
Slaughter weight, kg	37.66	39.64	38.35	38.50	1.56	0.27
Carcass characteristics						
HCW, kg	18.59	19.36	19.74	19.53	0.83	0.28
HCY, %	49.06[AB]	48.84[B]	51.53[A]	50.92[AB]	0.78	0.06
CCW, kg	17.69[B]	18.94[AB]	19.26[A]	19.41[A]	0.83	0.06
CCY, %	47.94[b]	47.76[b]	50.29[a]	50.64[a]	0.74	0.02
BWT, mm	13.78[B]	15.58[AB]	15.71[AB]	16.50[A]	0.88	0.07
SFT, mm	1.23	1.16	1.12	1.44	0.16	0.50
LM area, cm^2	12.80[B]	14.15[AB]	14.55[A]	14.71[A]	0.64	0.07
Meat composition, %						
DM	25.72	25.40	25.88	25.90	0.27	0.52
CP	23.23[b]	22.81[c]	24.25[a]	23.60[ab]	0.16	<0.01
Ash	1.27[ab]	1.25[b]	1.26[ab]	1.33[a]	0.02	0.02
Ether extract	2.97	2.84	2.62	2.98	0.23	0.66

[1]MON = diet containing 8 mg of monensin/kg of DM; 14EO = inclusion of 0.14% of red pepper leaves EO (DM basis); 28EO = inclusion of 0.28% of red pepper leaves EO; 42EO = inclusion of 0.42% of red pepper leaves EO; [2]SEM = standard error of the means; [3]Diets = fixed effect of the experimental diets; [4]HCW = hot carcass weight; HCY = hot carcass yield; CCW = chilled carcass weight; CCY = chilled carcass yield; BWT = body wall thickness; SFT = subcutaneous fat thickness; LM area = Longissimus muscle area; DM = dry matter; CP = crude protein; [a-b]means in the same row with different superscripts differ ($P \leq 0.05$); [A-B]means in the same row with different superscripts tented to differ ($P > 0.05$ to $P < 0.10$).

tended to increase the HCY ($P = 0.06$) compared with 14EO and tended to increase CCW ($P = 0.06$), BWT ($P = 0.07$) and LM area ($P = 0.07$) when compared with MON. The higher doses of red pepper leaves EO increased CCY ($P = 0.02$) compared with MON.

The experimental diets did not affect the DM and EE of the meat. However, the diets containing 0.28% of red pepper leaves EO increased CP on meat ($P < 0.01$) when compared with MON and 14EO. In addition, 42EO increased the meat ash content ($P = 0.02$) compared with 14EO.

Discussion

During the extraction process, the same raw material is subject to factors that may change its composition, such as the maturation stage of the plant, climatic and agronomic conditions, extraction process (Marco et al., 2007; Quispe Condori et al., 2008). Thus, although the literature describes the main compounds already identified in the red pepper leaves EO, there is a great variation in the data on the concentration of each one of these compounds, which may interfere in the effects of the EO use. Araújo (2010) reported that EO extracted from red pepper leaves presented 58.4% of α-pinene, 15.7% of β-pinene and 13% of limonene, while Faleiro (2015) reported the concentration of 40.2% α-pinene, 16.2% P-cymene and 11.3% β-pinene in red pepper leaves EO. In the present study, the three compounds identified in the highest concentrations were Δ-3-carene, limonene and α-pinene and it is not possible to establish the effects independent of these components on animal performance, since several compounds can act in synergy and there are no studies that report the isolated effects of these in animal performance.

When used in association with other compounds (thymol, eugenol and vanillin) as supplement for growing beef cattle fed a silage-based diet, limonene showed similar FE to monensin-supplemented animals, which were higher than control animals (Benchaar et al., 2006). In a study with juniper oil, which contained 35% α-pinene, an increase in ADG of lambs was observed when compared to control animals (Chaves et al., 2008). Some EO can manipulate the ruminal fermentation process (Araújo, 2010; Faleiro, 2015), as well as monensin (Bergen & Bates, 1984). This may explain the performance of the lambs that received EO not being different from those on MON treatment.

Some EO may decrease the total mixed ration acceptability, reducing the DMI (Calsamiglia et al., 2007), and four volatile compounds evaluated individually were related to intake, camphor, α-pinene, camphene, and caryophyllene oxide (Estell et al., 2008). Although red pepper leaves EO used in this study presented a characteristic and marked odor and high concentration of α-pinene (11.1%), the doses used did not affect DMI when compared to the diet containing monensin.

Although, in the present study, the EO could be considered possible substitute for monensin, these antibiotics have a coccidiostatic capacity, controlling the intestinal population of *Eimeria spp.*, and in many cases, coccidiosis infestation is not evaluated on the studies (Chaves et al., 2008, 2011). Lambs that received diets with red pepper leaves EO showed higher infestation by *Eimeria ssp.* when compared to lambs fed with MON. In addition, a greater number of oocysts/g of feces were observed in the first experimental period, when the lambs were recently weaned, a stressing factor, which could impair the immune response and to promote the occurrence of the disease (Chartier & Paraud, 2012). As the animals got older, they may have acquired resistance, since in healthy animals kept under adequate management conditions, the continuous intake of oocysts in a small amount causes the development of protective immune response, which limits the infection but does not eliminate it totally (Amarante, 2015). Another factor that may have contributed to the reduction of infestation of lambs over time was the type of floor on which they were kept, which was made of slats, easily drained, reducing the contact of lambs with feces. Even though oocyst infestation was higher in EO treatments, it is possible that this oil has no effect on this parasite. It is important to emphasize that the administration of monensin and lasalocid, mixed with the feed, is usually quite efficient in the prevention of coccidiosis in ruminant animals (Amarante, 2015).

The performance and carcass characteristics are directly influenced by the nutritional composition of the diet (Gonzaga et al., 2006). In the present study, the inclusion of monensin or EO in diets did not affect the slaughter weight of lambs, which may have been a consequence of similar performance among treatments during the experimental period. However, with the data obtained in this study, it is possible to suggest that the high doses of red pepper leaves EO were able to increase the energy availability of the diet for the lambs, thus increasing the CCY, as well as the deposition of fat, as observed with the increase in BWT. The data also suggest that the use of EO may have generated metabolic changes in lambs, directly affecting the carcass composition, as observed in the increase in LM area of lambs fed the highest EO doses.

The values obtained for SFT in the present study may be considered lower for Dorper x Santa Inês crossbreed (Souza et al., 2013). Nevertheless, the protein content in

the experimental diets presented high levels, which may have influenced the low-fat deposition, since the muscle growth and the deposition of adipose tissue are directly related to the protein levels ingested by the animal.

The increased in LM area and protein content in the meat of lambs fed higher doses of red pepper leaves EO can be explained by the availability of protein for the ruminant post-absorptive metabolism. Meschiatti et al. (2019) described that the blend EO did not affect the nitrogen intake compared with diets containing monensin; however, an increase in protein digestibility and nitrogen absorbed was observed when blend EO was used.

Conclusion

Although it did not affect the performance of the lambs, the use of higher doses of red pepper leaves EO resulted in greater effects on the carcass and meat characteristics, showing the potential of the use the EO in feedlot lambs fed high concentrate diets. However, it is important to consider that the monensin was more efficient in controlling the number of oocysts of *Eimeria ssp*. in feces of lambs compared with red pepper leaves EO. As a suggestion, it is necessary to study the efficiency of use of Brazilian red pepper leaves OE for feedlot lambs in relation to diets with no feed additives inclusion.

Conflict of Interest

We have no conflict of interest to declare.

Ethics Statement

The ethics committee approval certificate is attached (CEUA n° 5468211016).

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