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Effect of essential oils, monensin sodium, and calcium malate on *in vitro* gas production, *in vivo* nutrient digestibility, and growth performance of finishing lambs

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ABSTRACT

Rumen modifiers play a crucial role in minimizing dietary energy loss for finishing lambs. This study endeavors to assess nutrient digestibility, *in vitro* gas production, productive performance, and carcass characteristics in finishing lambs through the incorporation of three rumen fermentation modifiers (monensin sodium, calcium malate, and essential oils). Thirty-five four-months-old Pelibuey lambs of 23.6 kg ± 3.2 were assigned to a completely randomized block design to evaluate five diets: control (CON, without rumen modifier), monensin sodium (MON, 25 g/t); calcium malate (MAL, 2.5 kg/t), essential oils (EO, 150 g/t); and EO (150 g/t) plus MON (25 g/t). Daily feed intake, average daily weight gain, feed conversion ratio, dorsal fat thickness, rib eye area, ruminal pH, and chewing time did not differ among the diets. Notably, feed efficiency trend to be superior ($P = 0.07$) in the EO lambs, showing a 15.31 % and 17.28 % increase *versus* CON and MON diets, respectively. Dry matter intake in g/kg$^{0.75}$ was highest ($P < 0.05$) in MAL lambs by 23 % higher than lambs fed on diets added with EO and MON. The control diet (*i.e.,* CON) exhibited the lowest ($P < 0.05$) *in vivo* dry matter digestibility compared to all other diets. Additionally, there was a trend ($P = 0.056$) towards reduced crude protein digestibility in CON diet. The inclusion of EO led to a higher ($P < 0.05$) proportion of ruminal acetic acid and a decrease ($P < 0.05$) in propionic acid *versus* the CON diet. The observed effects can be attributed to the antimicrobial activity of EO, specifically their secondary metabolites, which demonstrate antimicrobial properties. This underscores their potential in addressing concerns related to antibiotic use. Compared to MON, dietary inclusion with EO improves feed efficiency, with no notable effects on average daily gain, final weight, or the investigated carcass characteristics. The EO supplementation emerges as a practical alternative to antibiotic ionophore monensin for enhancing feed efficiency in finishing feedlot lambs.

1. Introduction

Growth performance and the time required from birth to slaughter weight are crucial factors determining the productive and economic efficiency of sheep farms dedicated to lamb meat production (Galvani et al., 2014). In this context, rumen development, health, and functionality are key factors for maximizing the productive performance and profitability of sheep meat production enterprises (McGrath et al.,

Abbreviations: ADF, acid detergent fiber; ADG, daily weight gain; EO, diet with essential oils 150 g/t; EO+MON, diet with essential oils 150 g/t and monensin sodium 25 g/t; CO_2, carbon dioxide; CON, control diet; CP, crude protein; DM, dry matter; DMI, dry matter intake; EE, ether extract; EOs, essential oils; CH_4, methane; FCR, feed conversion rate; FE, feed efficiency; MAL, diet with calcium malate 2.5 kg/t; MON, diet with monensin sodium 25 g/t; N, nitrogen; NDF, neutral detergent fiber; NFC, non-fibrous carbohydrates; OM, organic matter; VFA, volatile fatty acids; Vmax, maximum volume of gas production; t, ton.

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2018). Ruminal acidosis is an economically significant condition in ruminants fed high-energy diets, with subacute ruminal acidosis affecting up to 26 % of feedlot sheep. Prevention strategies include gradual adaptation to high-concentrate feeds, maintaining adequate dietary fiber, and supplementation with buffers or other feed additives that stabilize ruminal pH and improve fermentation (Hernández et al., 2014). Rumen fermentation manipulation can be achieved through substances that alter its microbiota, such as ionophores, these substances modify metabolic activity and the proportion of specific microorganisms affecting Gram-positive bacteria (Elghandour et al., 2024). The use of new technologies, including additives, has become commonplace in fattening farms. However, feed additives do not provide specific nutrients such as energy, proteins, or minerals, but improve nutrient utilization by reducing digestive disorders, minimizing energy loss in microbial metabolic processes, and promoting efficient microbial populations (Elghandour et al., 2024).

Historically, antibiotics have been included as additives in ruminant diets to manipulate the rumen microbiome and optimize digestion and nutrient utilization (Carberry et al., 2014). However, the extended use of antibiotics, such as monensin sodium, is not recommended due to the formation of antimicrobial resistance and the generation of “superbugs” from excessive use of antibiotics in feeds for animals intended for human consumption (Gonzalez-Momita et al., 2009), and prophylactic antibiotics are being banned by more and more countries. Consequently, ongoing research must validate and provide alternative options to enhance fermentation efficiency with effects equal to or greater than antibiotics (Michalak et al., 2021). Monensin sodium is an ionophore that has been widely used to improve feed efficiency in ruminants. It acts by altering ion transport across cell membranes, leading to shifts in rumen microbial populations and fermentation patterns (Besharati et al., 2021; Rodríguez et al., 2023). Monensin supplementation typically increases propionate production, reduces methane emissions, and improves the feed conversion ratio in lambs (Zhang et al., 2021). Calcium malate is an organic acid salt that can serve as an alternative to antibiotics for manipulating rumen fermentation. It acts as an electron sink, promoting propionate production and potentially reducing methane formation (Carro et al., 2006). Supplementation with calcium malate has been shown to increase nutrient digestibility and improve growth performance in lambs fed high-concentrate diets (Mungói et al., 2012). Essential oils (EO) have emerged as a significant group of phytogenic feed additives, gaining attention as potential alternatives to antibiotics in animal production (Elghandour et al., 2018). These bioactive compounds are characterized by their strong aromatic properties and diverse biological activities. The EO have a long history of use in various applications, including traditional medicine, food preservation, and aromatherapy. In recent years, their potential as natural feed additives, growth promoters, and antimicrobials in livestock production has been extensively investigated (Asghari et al., 2021). The growing interest in EO stems from increasing consumer demand for natural products and the need to reduce antibiotic use in animal husbandry (Simitzis, 2017). Research has demonstrated that EO can positively influence animal health, performance, and product quality when used as feed additives (Hashemipour et al., 2013; Asghari et al., 2021). However, EO possess antimicrobial properties that can modulate rumen fermentation, enhance protein metabolism, decrease methane production, and improve animal performance (Salem et al., 2014; Hernandez et al., 2017). For example, supplementation with thyme essential oil has been shown to increase average daily gain and improve feed efficiency in lambs (Ribeiro et al., 2020), while the addition of thymol and carvacrol have been shown to decrease acetate:propionate ratio and ammonia production in vitro (Castillejos et al., 2006). However, the effects can vary depending on the type and dose of EO used. Additionally, some phytogenics (natural growth promoters derived from herbs, spices, or other plants) could be toxic to ruminal microbiota due to their antimicrobial and "anti-nutritional" effects at high doses (El-Gindy et al., 2021). While these rumen modifiers have shown promise individually, limited research has directly compared their effects, particularly in high-concentrate diets typical of intensive ruminant production. Additionally, potential interactions between different modifiers when used in combination warrant further investigation. It was hypothesized that the combination of monensin and essential oils would have synergistic effects on rumen fermentation and animal performance, potentially allowing for reduced monensin inclusion rates while maintaining or improving production outcomes. Therefore, this study aimed to evaluate the effects of monensin sodium, calcium malate (E352), and a commercial essential oil blend, both individually and in combination, on rumen fermentation, nutrient digestibility, and growth performance of finishing lambs fed a high-concentrate diet. Hence, this study aims to analyze nutrient digestibility, in vitro gas production, productive performance, and carcass characteristics of finishing lambs in the presence of three rumen fermentation modifiers (monensin sodium, calcium malate (E352), and essential oils) to generate information that validates their use.

2. Materials and methods

2.1. Location and climate conditions of the studied region

The study was carried out in the experimental farm of the Chapingo Autonomous University, Mexico, with a latitude 19°29’05” N, and longitude 98°53’112” O. Located 2250 m above sea level, with an annual average temperature of 17.2° C and an annual average precipitation of 598 mm concentrated from May to September. The animals used in this study were handled according to NOM-062-ZOO 1999 “Technical specifications for the production, care, and use of laboratory animals” (NOM is the national standard).

2.2. Animals and treatments

Thirty-five four-month-old Pelibuey lambs, 20 females, and 15 males, with an initial body weight of 23.6 ± 3.2 kg, were randomly assigned into five treatments (4 females and 3 males) with a randomized complete block design, the blocking criteria was the weight. The diet fed during the experimental period was formulated for a gain of 300 g per day according to the National Research Council (NRC, 2007). Diet

Table 1
Ingredients and chemical composition of the evaluated diets.

Ingredients (g/kg)	Diets[a]				
	CON	MON	MAL	EO	EO+MON
Maize forage	150.0	150.0	150.0	150.0	150.0
Ground maize	300.0	300.0	300.0	300.0	300.0
Soybean meal	54.4	54.4	58.2	58.1	58.1
Ground sorghum	282.2	282.2	293.9	291.1	291.1
Maize gluten	55.0	55.0	55.0	55.0	55.0
Wheat bran	58.4	58.4	42.9	45.8	45.8
Bypass fat	11.0	11.0	11.0	11.0	11.0
Cane molasses	51.0	51.0	51.0	51.0	51.0
Urea	6.0	6.0	6.0	6.0	6.0
Calcium carbonate	11.0	11.0	11.0	11.0	11.0
Mineral premix[b]	21.0	21.0	21.0	21.0	21.0
Chemical composition					
Crude protein, g/kg	150.0	150.0	150.0	150.0	150.0
ME, Mcal/kg[c]	2.7	2.7	2.7	2.7	2.7
Neutral detergent fiber, g/kg	221.2	221.2	213.2	215.8	215.8
Calcium, g/kg	11.5	11.5	11.5	11.8	11.8
Phosphorus, g/kg	4.0	4.0	3.8	3.8	3.8

[a] CON, control diet; MON, monensin sodium; MAL, calcium malate; EO, essential oils; EO+MON, essential oils + monensin sodium.

[b] Label composition (g or mg/kg): 240 g of Ca; 30 g of P; 20 g of Mg; 80 g of Na; 120 g of Cl; 5 g of K; 5 g of S; 5 mg of Cr; 4000 mg of Mn; 2000 mg of Fe; 5000 mg of Zn; 100 mg of I; 30 mg of Se; 60 mg of Co; 500000 IU of vitamin A; 150000 IU of vitamin D; 1000 IU of vitamin E.

[c] EM = metabolizable energy.

composition is presented in Table 1.

The assessed treatments were: (1) Control diet without additives (CON, *n*=7), (2) Diet with monensin sodium 25 g/t (MON *n*=7; Rumensin 200®; Elanco Mexico, Jalisco, Mexico), (3) Diet with calcium malate 2.5 kg/t (MAL *n*=7; Rumalato®, Norel Mexico, Queretaro, Mexico), (4) Diet with essential oils (40 %, Thymol, Carvacrol, Eucaliptol) 150 g/t (EO *n*=7; MixOil®, Nutryplus, Queretaro, Mexico), and (5) Diet with essential oils 150 g/t and monensin sodium 25 g/t (EO+MON *n*=7). Feed additives were included in the mineral premix for each diet and then then mixed with all the dietary ingredients. The level of the additives was established according to the manufacturer's label instructions.

2.3. Management and performance measurement

Lambs were individually confined in previously labeled pens, with a feeding stall and a plastic container used as a drinker. On arrival, lambs were housed in a common pen for three days to be fed an adaptation diet that consisted of *ad libitum* oat forage. They were dewormed with Ivermectine and Clorsulom (Ivomect® F, Merial Mexico, Querétaro, Mexico) with one mL of the substance per 50 kg of body weight. Additionally, lambs were vaccinated against *Clostridium* A, B, C and D (Bovimune Clostri 10®, Lapisa, Michoacan, Mexico). Before transporting them to their individual pens to begin the finishing stage, the lambs were weighed with a hanging scale (CRS® Hanging Scale, Torrey, Mexico City, Mexico) to obtain the initial weight of the animals.

Once the lambs were housed in their pens, an adaptation phase began where forage was offered along with the diet to be tested by treatment, and each day 20 % of the corresponding treatment diet was exchanged until the forage was completely removed (5 days). The finishing phase lasted 50 days and feed was offered at 7:00 am and 4:00 pm. On the first day, 1.5 kg of feed was offered per lamb, and then the amount was assigned by increasing 15 % the previously registered consumption to ensure *ad libitum* intake. Water was offered in an 18-liter capacity container with a total water change every three days, but with daily additions when required. During the finishing phase, the weight of the lambs was recorded every 14 days using a hanging scale (CRS®, Torrey, Ciudad de México, México), while the offered and rejected feed was weighed daily using an Ohaus Heavy Duty Solution Balance Scale (Ohaus, New Jersey, USA). With the aforesaid data, productive variables such as dry matter intake (DMI), average daily weight gain (ADG), feed conversion rate (FCR = DMI/ADG), and feed efficiency (FE) were calculated.

2.4. Apparent digestibility

On day 30 of the fattening period, 15 lambs from three blocks were equipped with a denim bag and plastic inner covering for fecal collection. For 9 consecutive days, the individual amount of feces produced was collected, weighed, identified, and frozen. The amount of feed offered was recorded daily, and the rejected feed (24 hours later) was placed in a bag, identified, and frozen for later analysis. On day 9 of the collection phase, samples of ruminal fluid were obtained using an oro-ruminal probe, 2 hours after feeding. This ruminal fluid served as inoculum for *in vitro* gas production analysis and volatile fatty acid analysis. The lambs were weighed one day before and one day after the sampling phase, to get the average weight, and with this weight per lamb as a reference, the DMI was calculated based on the metabolic weight ($kg^{0.75}$). Once the sampling phase finished, the lambs remained on the farm until reaching selling weight around day 50.

2.5. Chewing activity

On day 38 of the study, feed intake, and rumination activity were monitored in all 35 lambs over 24 hours. Observers recorded feeding and rumination behaviours at 5-minute intervals, assuming each activity persisted throughout the interval. To estimate the total time (min/day) spent eating or ruminating, a rumination period was defined as at least 5 consecutive minutes of ruminant activity followed by at least 5 minutes without rumination. The total chewing time was calculated as the sum of time spent eating and ruminating. Resting time was determined by subtracting the total chewing time from the 24 hours (Lu et al., 2005).

2.6. Ruminal volatile fatty acids and pH and in vitro gas production

On day 39 of the finishing period, two hours after feeding, rumen fluid was obtained via an oro-ruminal probe. The pH of the rumen was immediately measured using a Beckman model 390 pH meter. The rumen fluid samples were quickly frozen to stop fermentation. To determine the volatile fatty acids, two mL samples per treatment were mixed with 25 % metaphosphoric acid in a 4:1 ratio, centrifuged for 20 minutes at 11,000 rpm, and one µL supernatant was taken and injected into a Perkin Elmer Clarus 500 gas chromatograph (Perkin Elmer, 2015) with a capillary column (Elite PFAP) to determine acetate, propionate, and butyrate content (Erwin et al., 1961). Hydrogen was used as the carrier gas at a flow rate of 5.5 mL/min. The injector temperature was 250°C and the oven temperature was 80°C for one minute with 20 °C/min increments until reaching 140°C with an 8-minute reading time.

The *in vitro* fermentation kinetics were determined (Theodorou et al., 1994). The offered feed samples were placed in 125 mL amber glass bottles, 0.5 g of substrate, 90 mL of 1:9 (v/v) diluted rumen inoculum in a reduced mineral solution composed of K_2HPO_4 (0.45 g/L), KH_2PO_4 (0.45 g/L), $NaCO_3$ (0.6 g/L), $(NH_4)_2SO_4$ (0.45 g/L), NaCl (0.9 g/L), $MnSO_4$ (0.18 g/L), $CaCl_2$ (0.12 g/L), L-cysteine (0.25 g/L) and Na_2S (0.25 g/L), and a drop of resazurin (Cobos and Yokoyama, 1995). The bottles were placed in a water bath at 39°C and the formed gas was measured at 0, 2, 4, 6, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, and 72 hours of incubation using a manual manometer (scale of 0 to one kg/cm). The rumen fluid was obtained individually from the 15 male lambs of three blocks fed with the control diet or the different evaluated modifiers.

The following fermentation kinetics parameters were calculated with the registered accumulated gas volume from 0 to 72 h of incubation: fermentation potential (V_m; mL/g MS); fermentation rate (S; mL/h); and delay time (L; h) using a logistic model (Pitt et al., 1999). The NLIN procedure of SAS version 9.0 (SAS Institute, 2002) was used for the calculation.

2.7. Backfat thickness measurement

At day 50 of the finishing phase, backfat thickness, and rib-eye muscle area were measured in all lambs included in the study with an ultrasound (Sono Vet 600®, KeeboMed Inc, Illinois, USA) with a 7.5 MHz transductor.

2.8. Sample analysis

The offered feed, rejected feed, and collected feces were dried individually in a forced air oven at 65°C for over 96 hours until a constant weight was reached (AOAC, 1997). The samples of offered feed, rejected feed, and feces were ground using a mill (Wiley®, Thomas, New Jersey, USA) with a 2 mm diameter screen. To remove residual water and calculate the absolute dry matter (DM) as well as express nutrient composition on a dry basis, samples of offered feed, rejected feed, and feces were dried at 105°C in a forced air oven (AOAC, 1997). Ether extract (EE) was determined using the Soxhlet method (AOAC, 1997). Ash content was obtained after combustion in a muffle oven at 550°C for 3 hours.

The nitrogen (N) content was determined using the Micro-Kjeldahl method, and the crude protein content (CP) was calculated by multiplying N x 6.25 (AOAC, 1997). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the ANKOM200 FIBER

ANALYZER. The content of non-fibrous carbohydrates (NFC) available for ruminal fermentation was calculated as follows: NFC = DM - (CP + EE + NDF + Ash) (De Bellis et al., 2022). With the data from the analysis of consumed and excreted nutrients, the apparent digestibility of the DM, organic matter (OM), NDF, and CP was estimated, as well as individual nutrient intake.

2.9. Statistical analysis

Productive performance and carcass characteristics were analyzed with a randomized complete block design (with weight as the blocking criterion), using SAS 9.0 PROC MIXED (SAS Institute, 2002). The statistical model used was:

$$Y_{ijkl} = \mu + A_i + B_j + C(B)_{jk} + E_{ijkl}$$

were Y_{ijkl} = dependent variable; μ = general mean, A = treatment effect (i = 1…5); B = fixed effect of sex (j = female, male); C(B) = random effect of the block within sex (j (k) = female (1…4), male (1…3); E_{ijkl} = random error.

The data for apparent digestibility, nutrient intake, *in vitro* gas production, VFA content, and mastication activity were analyzed with a randomized complete block design using the following model:

$$Y_{ijk} = \mu + A_i + B_j + E_{ijk}$$

were Y_{ijkl} = dependent variable; μ = general mean, A = treatment effect (i = 1…5); B = random effect of the block (j = 1…3); E_{ijk} = random error. For all variables where treatment differences were detected, least squares means were compared using the Tukey test. Significant differences between treatments were declared at $P < 0.05$, while trends were noted when $0.05 < P < 0.10$.

3. Results

The inclusion of rumen fermentation modifiers did not significantly affect final live weight, total weight gain, average daily gain (ADG), dry matter intake (DMI), feed conversion ratio (FCR), rib-eye area, or backfat thickness in finishing lambs (Table 2). However, lambs receiving essential oils (*i.e.,* EO diet) showed a trend (P = 0.068) towards improved feed efficiency, with 15.3 % and 17.3 % higher values compared to the CON and MON groups, respectively.

During the 9-day digestibility trial, lambs fed calcium malate exhibited the highest dry matter intake, consuming 23 % more than those receiving the EO+MON combination ($P < 0.05$; Table 3). The CON diet resulted in the lowest ($P < 0.05$) dry matter digestibility, which was 4.8 %, 5.7 %, 4.3 %, and 2.5 % lower than the MON, MAL, EO, and EO+MON treatments, respectively. A trend (P = 0.056) towards reduced crude protein digestibility was observed in the CON group compared to the other treatments.

The addition of rumen modifiers increased the maximum volume of gas production (V_{max}) compared to the CON, with the EO group showing the highest value 10.2 % greater than the CON ($P < 0.001$; Table 4). The EO+MON combination resulted in the fastest gas production rate and shortest lag time, with the latter being 29.5 % shorter than the CON ($P < 0.05$). Regarding volatile fatty acids production, the EO group exhibited the lowest propionic acid concentration, which was 36.2 % lower than the CON ($P < 0.05$). When expressed as molar percentages, the EO and EO+MON groups showed significantly higher acetic acid (8.2 % and 9.6 % increase) and lower propionic acid (18.6 % and 28.4 % decrease) proportions compared to the CON ($P < 0.0001$). The acetate:propionate ratio was markedly higher in the EO and EO+MON groups, increasing ($P < 0.001$) by 38.2 % and 55.1 % respectively, relative to the CON.

No significant differences were observed in rumination time, consumption time, total mastication time, or the proportion of time spent on these activities among the experimental groups (Table 5). This suggests that the rumen modifiers did not substantially alter feeding behavior in the lambs.

4. Discussion

The lack of significant effects on growth performance and carcass characteristics from the rumen fermentation modifiers tested suggests that these additives did not substantially alter nutrient utilization or partitioning in a way that impacted these parameters and may be explained by several factors. The high-concentrate diet used in this study likely already provided optimal conditions for rumen fermentation and nutrient utilization, potentially masking any additional benefits from the additives (Olafadehan et al., 2018; Hernandez et al., 2017; Elghandour et al., 2015). Additionally, the dosages used, while based on manufacturer recommendations, may not have been optimal for eliciting measurable changes in a relatively short 50-day feeding period. However, the trend towards improved feed efficiency with essential oils (EO) supplementation, despite no significant differences in average daily gain or dry matter intake, suggests subtle shifts in rumen fermentation and nutrient utilization (Asghari et al., 2021). The EO have been shown to selectively inhibit certain rumen bacteria, particularly those involved in amino acid deamination (Newbold et al., 2004). This could lead to reduced ammonia production and improved nitrogen utilization, potentially explaining the observed feed efficiency (Benchaar et al., 2008). The antimicrobial effects of EO may also reduce energy losses associated with methane production, further contributing to improved feed efficiency (Cobellis et al., 2016). The increased dry matter digestibility observed with MON, MAL, and EO supplementation likely stems from different mechanisms. Monensin is known to shift rumen fermentation towards propionate production, which is energetically more efficient (Russell and Strobel, 1989; Rodríguez et al., 2023). Malate can act as an electron sink, promoting the growth of lactate-utilizing bacteria and stabilizing rumen pH (Martin, 1998). However, the EO may enhance nutrient digestibility by modulating

Table 2
Productive performance of finished lambs that received a diet with an inclusion of three different rumen fermentation modifiers.

	Diets[a]						
	CON	MON	MAL	EO	EO+MON	SEM[c]	P value
Initial weight, kg	23.85	24.11	24.17	22.34	24.35	1.28	0.173
Final live weight, kg	38.32	39.53	39.60	40.10	39.24	1.76	0.783
Total weight gain, kg	14.45	15.20	15.42	17.75	14.88	1.12	0.217
ADG 0–50 d, g[b]	289.10	303.95	308.52	355.10	297.67	22.45	0.217
DMI, g/d[b]	1309.30	1411.16	1313.16	1345.01	1242.58	59.58	0.2694
FCR[b]	4.75	4.84	4.39	4.15	4.35	0.21	0.113
Feed efficiency	213.56	209.96	230.13	246.26	233.57	9.89	0.068
Rib-eye area, cm	9.43	8.80	9.22	8.71	8.79	0.58	0.615
Backfat thickness, mm	4.0	4.14	4.14	4.14	4.42	0.38	0.904

[a] CON, control diet; MON, monensin sodium; MAL, calcium malate; EO, essential oils; EO+MON, essential oils + monensin sodium.
[b] ADG, average daily weight gain; DMI, dry-matter intake; FCR, feed conversion rate.
[c] SEM, standard error of the mean.

Table 3
Intake and nutrient digestibility during 9 days of a diet complemented with three different rumen fermentation modifiers.

Parameter[y]	Diets[a]					SEM[c]	P value
	CON	MON	MAL	EO	EO+MON		
DM Intake							
g/d	1093.6[ab]	1213.1[ab]	1258.9[a]	1148.4[ab]	1023.6[b]	44.49	0.036
g/kg$^{0.75}$	72.68[ab]	79.02[ab]	82.21[a]	74.83[ab]	67.11[b]	2.95	0.048
Nutrient intake, g/d[b]							
OM	951.5	1070.5	1103.9	989.0	893.5	45.97	0.062
CP	144.1	153.8	168.5	151.5	141.8	10.59	0.468
NDF	298.7	316.1	324.8	273.3	264.2	22.47	0.295
ADF	77.97	79.62	85.77	72.65	76.25	6.03	0.588
EE	28.07	26.64	28.18	31.63	23.07	4.68	0.667
NFC	480.5	574.0	582.4	532.7	464.5	28.02	0.057
Digestibility, g/kg[b]							
DM	780.6[b]	818.1[a]	825.1[a]	813.9[a]	800.1[a]	1.25	0.011
OM	819.8	835.7	842.0	825.7	834.4	0.89	0.482
CP	719.1	772.4	812.5	766.4	795.7	1.87	0.056
NDF	630.8	700.1	649.6	614.0	647.5	2.67	0.300
ADF	367.7	508.6	400.6	425.3	475.1	8.07	0.199
EE	753.2	779.0	805.2	820.5	771.1	6.86	0.703
NFC	935.9	928.8	957.8	944.2	923.6	0.91	0.141

a, b, different literals in the same row (P≤0.05).
[a] CON, control diet; MON, monensin sodium; MAL, calcium malate; EO, essential oils; EO+MON, essential oils + monensin sodium.
[b] DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract; NFC, non-fiber carbohydrates.
[c] SEM, standard error of the mean.

Table 4
Rumen fluid pH, *in vitro* fermentation parameters and volatile fatty acids (VFA) profile (72 h) of a diet for finishing lambs complemented with three different rumen fermentation modifiers.

	Diets[a]						
	CON	MON	MAL	EO	EO+MON	SEM[c]	P value
pH	6.54	6.67	6.61	6.56	6.57	0.289	0.998
Vmax, mL/ g DM[b]	353.6[b]	374.9[a]	381.0[a]	389.6[a]	377.8[a]	33.25	<0.001
S, mL/h	0.045[b]	0.044[b]	0.045[b]	0.045[b]	0.047[a]	0.001	0.003
L, h	2.88[a]	2.45[ab]	2.69[ab]	2.26[ab]	2.03[b]	1.01	0.012
VFA, mM							
Acetic acid	21.93	20.58	21.62	18.9	26.08	2.54	0.351
Propionic acid	12.47[a]	10.00[a]	12.22[a]	7.95[b]	9.38[a]	1.17	0.045
Butyric acid	2.13	2.16	2.31	2.07	4.11	0.54	0.065
Total VFA	36.54	32.74	36.16	28.93	39.59	3.94	0.390
VFA, molar percent							
Acetic acid	60.26[b]	62.76[ab]	59.56[b]	65.18[a]	66.04[a]	1.18	0.0001
Propionic acid	33.88[a]	30.79[a]	34.27[a]	27.57[b]	24.25[b]	1.43	0.0001
Butyric acid	5.85[b]	6.49[ab]	6.17[b]	7.26[ab]	9.7[a]	0.81	0.018
Acetate: Propionate	1.78[b]	2.04[b]	1.78[b]	2.46[a]	2.76[a]	0.15	<0.001

a, b, different literals in the same row (P≤0.05).
[a] CON, control diet; MON, monensin sodium; MAL, calcium malate; EO, essential oils; EO+MON, essential oils + monensin sodium.
[b] Vmax, maximum volume of gas production; S, gas production rate; L, lag phase.
[c] SEM, standard error of the mean.

Table 5
Consumption times and chewing activity of finishing lambs fed with three different rumen fermentation modifiers.

	Diets[a]						
	CON	MON	MAL	EO	EO+MON	SEM[c]	P value
Rumination, min/d	296.7	346.7	380.0	375.0	313.33	42.17	0.577
Consumption, min/d	188.3	160.0	216.7	158.3	163.33	23.07	0.129
Total Mastication, min/d	485.0	506.7	596.6	533.3	476.67	35.25	0.207
Proportion, %[b]	31.59	38.45	37.25	34.80	38.03	2.66	0.333

[a] CON, control diet; MON, monensin sodium; MAL, calcium malate; EO, essential oils; EO+MON, essential oils + monensin sodium.
[b] Proportion of the day invested in consumption and rumination.
[c] SEM: Standard error of the mean.

rumen microbial populations and enzyme activities (Besharati et al., 2021). These mechanisms could collectively contribute to improved nutrient extraction from the diet. The tendency towards improved crude protein digestibility in the modifier groups may be due to a reduction in protein degradation and ammonia production in the rumen, as reported by Newbold et al. (2004) for monensin and essential oils (Besharati et al., 2021; Rodríguez et al., 2023). The increased maximum volume of gas production (V_{max}) and altered fermentation kinetics in the modifier

groups can be attributed to changes in the rumen microbial ecosystem. The faster gas production rate and shorter lag time in the EO+MON group suggest a synergistic effect of these additives on the colonization and fermentation of substrates by rumen microbes (Wallace et al., 2002). The antimicrobial activity of essential oils, combined with the selective inhibition of gram-positive bacteria by monensin, may have created a more efficient fermentative environment (Benchaar et al., 2008). The rumen pH showed no variations, ranging between 6.54 and 6.67, in all diets suggesting the absence of subclinical acidosis, which usually occurs at 5.6 or less (González et al., 2012). The changes in VFA profiles, particularly the increased acetate:propionate ratio with EO and EO+MON treatments, were unexpected given the typical effects of these additives. This shift suggests that the EO used in this study may have preferentially inhibited propionate-producing bacteria or stimulated acetate producers with a potential reduction in methanogenesis, as acetate formation is associated with hydrogen production, which methanogens can use to produce methane (Moss et al., 2000). The specific composition of the essential oil blend used in this study (a blend of thymol, carvacrol, and eucalyptol) could explain this effect, as different compounds can have varying impacts on rumen microbes (Asghari et al., 2021). The increased butyrate proportion with EO+MON may indicate enhanced epithelial development and nutrient absorption capacity, as butyrate is a primary energy source for rumen epithelial cells (Górka et al., 2011). The variability in responses to essential oils observed in this study compared to previous research (Malekkhahi et al., 2015; Wu et al., 2021) highlights the complexity of rumen modulation. Factors such as the specific chemical composition of the essential oil blend, interactions with dietary components, and the adaptation of rumen microbes over time can all influence outcomes (Benchaar and Greathead, 2011; Elghandour et al., 2018). Future studies should consider longer adaptation periods and more detailed analysis of rumen microbial populations to understand these dynamics better. The lack of significant effects on chewing activity and rumination time suggests that the rumen modifiers did not substantially alter feeding behavior or physical characteristics of the diet. This is important from an animal welfare perspective, as normal rumination patterns are indicative of good rumen health and comfort (Beauchemin, 2018).

5. Conclusions

Supplementation with essential oils enhances feed efficiency and dietary net energy utilization in finishing lambs without significantly affecting average daily gain, final weight, or carcass characteristics compared to the control and monensina treatments. Additionally, EO alone or combined with MON improved nutrient digestibility. Given these findings, we recommend the use of essential oils or calcium malate as viable alternatives to monensin in finishing lamb diets to improve productive performance. The decision to use these additives should be based primarily on their effects on feed efficiency and nutrient digestibility. Further research is needed to understand better the underlying ruminal processes, particularly regarding microbial populations and greenhouse gas production when using these feed additives in lamb production systems.

CRediT authorship contribution statement

Abdelfattah Z.M. Salem: Writing – original draft, Visualization, Validation, Conceptualization. **Maria A. Ortiz H.**: Conceptualization, Formal analysis, Methodology, Writing – original draft. **Pedro A. Martinez H.**: Funding acquisition, Project administration, Supervision, Validation, Visualization, Writing – original draft. **Oscar V. Vazquez M.**: Conceptualization, Data curation, Formal analysis, Writing – review & editing. **Mona M.M.Y. Elghandour**: Writing – review & editing. **Moises Cipriano-Salazar**: Visualization, Writing – original draft, Writing – review & editing. **Edson B. Figueroa P.**: Writing – original draft, Writing – review & editing.

Ethics approval

The animals used in this study were handled according to NOM-062-ZOO 1999 “Technical specifications for the production, care, and use of laboratory animals” (NOM is the national standard).

Consent for publication

Not applicable.

Code availability

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

AOAC, 1997. Association of Official Analytical Chemists International Official Methods of Analysis, 16th. AOAC, Arlington.

Asghari, M., Abdi-Benemar, H., Maheri-Sis, N., Salamatdoust-Nobar, R., Salem, A.Z.M., Zamanloo, M., Anele, U.Y., 2021. Effects of emulsified essential oils blend on performance, blood metabolites, oxidative status and intestinal microflora of suckling calves. Anim. Feed Sci. Technol. 277, 114954 https://doi.org/10.1016/j.anifeedsci.2021.114954.

Beauchemin, K.A., 2018. Invited review: Current perspectives on eating and rumination activity in dairy cows. J. Dairy Sci. 101, 4762–4784. https://doi.org/10.3168/jds.2017-13706.

Benchaar, C., Calsamiglia, S., Chaves, A.V., Fraser, G.R., Colombatto, D., McAllister, T.A., Beauchemin, K.A., 2008. A review of plant-derived essential oils in ruminant nutrition and production. Anim. Feed Sci. Technol. 145, 209–228. https://doi.org/10.1016/j.anifeedsci.2007.04.014.

Benchaar, C., Greathead, H., 2011. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Anim. Feed Sci. Technol. 166-167, 338–355. https://doi.org/10.1016/j.anifeedsci.2011.04.024.

Besharati, M., Palangi, V., Moaddab, M., Nemati, Z., Barababosa Pliego, A., Salem, A.Z.M., 2021. Influence of cinnamon essential oil and monensin on ruminal biogas kinetics of waste pomegranate seeds as a biofriendly agriculture environment. Waste Biomass-.-. Valor 12, 2333–2342. https://doi.org/10.1007/s12649-020-01167-2.

Carberry, C.A., Kenny, D.A., Kelly, A.K., Waters, S.M., 2014. Quantitative analysis of ruminal methanogenic microbial populations in beef cattle divergent in phenotypic residual feed intake (RFI) offered contrasting diets. J. Anim. Sci. Biotechnol. 5, 41. https://doi.org/10.1186/2049-1891-5-41.

Carro, M.D., Ranilla, M.J., Giraldez, F.J., Mantecon, A.R., 2006. Effects of malate on diet digestibility, microbial protein synthesis, plasma metabolites, and performance of growing lambs fed a high-concentrate diet. J. Anim. Sci. 84, 405–410. https://doi.org/10.2527/2006.842405x.

Castillejos, L., Calsamiglia, S., Ferret, A., 2006. Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in vitro systems. J. Dairy Sci. 89, 2649–2658. https://doi.org/10.3168/jds.S0022-0302(06)72341-4.

Cobellis, G., Trabalza-Marinucci, M., Yu, Z., 2016. Critical evaluation of essential oils as rumen modifiers in ruminant nutrition: a review. Sci. Total Environ. 545-546, 556–568. https://doi.org/10.1016/j.scitotenv.2015.12.103.

Cobos, M.A., Yokoyama, M.T., 1995. Clostridium paraputrificum var ruminantum: colonization and degradation of shrimp carapaces in vitro observed by scanning electron microscopy. Rumen Ecology Research Planning. Proc. a Workshop Int. Livest. Res.

De Bellis, P., Maggiolino, A., Albano, C., De Palo, P., Blando, F., 2022. Ensiling grape pomace with and without addition of a *Lactiplantibacillus plantarum* Strain: effect on polyphenols and microbiological characteristics, in vitro nutrient apparent digestibility, and gas emission. Front. Vet. Sci. 9, 808293 https://doi.org/10.3389/fvets.2022.808293.

Elghandour, M.M.M.Y., Abu Hafsa, S.H., Cone, J.W., Salem, A.Z.M., Anele, U.Y., Alcala-Canto, Y., 2024. Prospect of yeast probiotic production enhance livestock feeds utilization and performance: an overview. Biomass-.-. Convers. Biorefin. 14, 2923–2935. https://doi.org/10.1007/s13399-022-02562-6.

Elghandour, M.M.M.Y., Kholif, A.E., Márquez-Molina, O., Vázquez-Armijo, J.F., Puniya, A.K., Salem, A.Z.M., 2015. Influence of individual or mixed cellulase and xylanase mixture on in vitro rumen gas production kinetics of total mixed rations

with different maize silage and concentrate ratios. Turk. J. Vet. Anim. Sci. 39, 435–442. https://doi.org/10.3906/vet-1410-26.
Elghandour, M.M.M.Y., Salem, M.Z.M., Greiner, R., Salem, A.Z.M., 2018. Effects of natural blends of garlic and eucalypt essential oils on biogas production of four fibrous feeds at short-term of incubation in the ruminal anaerobic biosystem. J. Sci. Food Agric. 98, 5313–5321. https://doi.org/10.1002/jsfa.9070.
El-Gindy, Y.M., Zahran, S.M., Ahmed, M.A., Salem, A.Z.M., Misbah, T.R., 2021. Influence of dietary supplementation of clove and rosemary essential oils or their combination on growth performance, immunity status, and blood antioxidant of growing rabbits. Trop. Anim. Health Prod. 53, 482. https://doi.org/10.1007/s11250-021-02906-w.
Elmer, Perkin, 2015. Operator's Manual. Clarus 500 (Gas Chromatograph).
Erwin, E.S., Marco, G.J., Emery, E.M., 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44, 1768–1771. https://doi.org/10.3168/jds.S0022-0302(61)89956-6.
Galvani, D.B., Pires, C.C., Hübner, C.H., Carvalho, S., Wommer, T.P., 2014. Growth performance and carcass traits of early-weaned lambs as affected by the nutritional regimen of lactating ewes. Small Rumin. Res. 120 (1), 5. https://doi.org/10.1016/j.smallrumres.2014.04.006.
González, L.A., Manteca, X., Calsamiglia, S., Schwartzkopf-Genswein, K.S., Ferret, A., 2012. Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). Anim. Feed Sci. Technol. 172, 66–79. https://doi.org/10.1016/j.anifeedsci.2011.12.009.
Gonzalez-Momita, M.L., Kawas, J.R., García-Castillo, R., Gonzalez-Morteo, C., Aguirre-Ortega, J., Hernandez-Vidal, G., Fimbres-Durazo, H., Picón-Rubio, F.J., Lu, C.D., 2009. Nutrient intake, digestibility, mastication and ruminal fermentation of Pelibuey lambs fed finishing diets with ionophore (monensin or lasalocid) and sodium malate. Small Rumin. Res. 83, 1–6. https://doi.org/10.1016/j.smallrumres.2008.11.008.
Górka, P., Kowalski, Z.M., Pietrzak, P., Kotunia, A., Kiljanczyk, R., Flaga, J., Holst, J.J., Guilloteau, P., Zabielski, R., 2011. Effect of sodium butyrate supplementation in milk replacer and starter diet on rumen development in calves. J. Physiol. Pharm. 62, 47–53. https://doi.org/10.1111/j.1439-0396.2010.01052.x.
Hashemipour, H., Kermanshahi, H., Golian, A., Veldkamp, T., 2013. Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. Poult. Sci. 92, 2059–2069. https://doi.org/10.3382/ps.2012-02685.
Hernandez, A., Kholif, A.E., Lugo-Coyote, R., Elghandour, M.M.Y., Cipriano, M., Rodríguez, G.B., Odongo, N.E., Salem, A.Z.M., 2017. The effect of garlic oil, xylanase enzyme and yeast on biomethane and carbon dioxide production from 60-d old Holstein dairy calves fed a high concentrate diet. J. Clean. Prod. 142, 2384–2392. https://doi.org/10.1016/j.jclepro.2016.11.036.
Hernández, J., Benedito, J.L., Abuelo, A., Castillo, C., 2014. Ruminal acidosis in feedlot: from aetiology to prevention. Sci. World J. 2014, 702572 https://doi.org/10.1155/2014/702572.
Institute, S.A.S., 2002. The SAS System for Windows. Release Version 9.0. SAS Inst, Cary.
Lu, C.D., Kawas, J.R., Mahgoub, O.G., 2005. Fibre digestion and utilization in goats. Small Rumin. Res. 60, 45–52. https://doi.org/10.1016/j.smallrumres.2005.06.035.
Malekkhahi, M., Tahmasbi, A.M., Naserian, A.A., Danesh Mesgaran, M., Kleen, J.L., Parand, A.A., 2015. Effects of essential oils, yeast culture and malate on rumen fermentation, blood metabolites, growth performance and nutrient digestibility of Baluchi lambs fed high-concentrate diets. J. Anim. Physiol. Anim. Nutr. 99, 221–229. https://doi.org/10.1111/jpn.12230.
Martin, S.A., 1998. Manipulation of ruminal fermentation with organic acids: a review. J. Anim. Sci. 76, 3123–3132. https://doi.org/10.2527/1998.76123123x.
McGrath, J., Duval, S.M., Tamassia, L.F., Kindermann, M., Stemmler, R.T., de Gouvea, V. N., Acedo, T.S., Immig, I., Williams, S.N., Celi, P., 2018. Nutritional strategies in ruminants: a lifetime approach. Res. Vet. Sci. 116, 28–39. https://doi.org/10.1016/j.rvsc.2017.09.011.
Michalak, M., Wojnarowski, K., Cholewinska, P., Szeligowska, N., Bawej, M., Pacon, J., 2021. Selected alternative feed additives used to manipulate the rumen microbiome. Animals 11. https://doi.org/10.3390/ani11061542.
Moss, A.R., Jouany, J.-P., Newbold, J., 2000. Methane production by ruminants: its contribution to global warming. Ann. Zootech. 49, 231–253. https://doi.org/10.1051/animres:2000119.
Mungói, M., Flores, C., Casals, R., Caja, G., 2012. Effect of malate and starch source on digestibility and nutrient balance of growing-fattening lambs. Anim. Feed Sci. Technol. 174, 154–162. https://doi.org/10.1016/j.anifeedsci.2012.03.016.
Newbold, C.J., McIntosh, F.M., Williams, P., Losa, R., Wallace, R.J., 2004. Effects of a specific blend of essential oil compounds on rumen fermentation. Anim. Feed Sci. Technol. 114, 105–112. https://doi.org/10.1016/j.anifeedsci.2003.12.006.
NRC (National Research Council), 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. The National Academies Press, Washington, DC. https://doi.org/10.17226/11654.
Olafadehan, O.A., Njidda, A., Okunade, S.A., Salihu, S.O., Balogun, D.O., Salem, A.Z.M., 2018. Performance and hemtochemical parameters of buck-kids fed concentrate partially replaced with tropical Piliostigma thonningii foliage. Anim. Sci. J. 89, 340–347. https://doi.org/10.1111/asj.12814.
Pitt, R.E., Cross, T.L., Pell, A.N., Schofield, P., Doane, P.H., 1999. Use of in vitro gas production models in ruminal kinetics. Math. Biosci. 159, 145–163. https://doi.org/10.1016/s0025-5564(99)00020-6.
Ribeiro, A.D.B., Ferraz Junior, M.V.C., Polizel, D.M., Miszura, A.A., Barroso, J.P.R., Cunha, A.R., Souza, T.T., Ferreira, E.M., Susin, L., Pires, A.V., 2020. Effect of thyme essential oil on rumen parameters, nutrient digestibility, and nitrogen balance in wethers fed high concentrate diets. Arq. Bras. Med. Vet. Zootec. 72, 573–580. https://doi.org/10.1590/1678-4162-11322.
Rodríguez, G.B., Elghandour, M.M.M.Y., Adegbeye, M.J., Salem, A.Z.M., 2023. Effect of propionate, monensin, and *Saccharomyces cerevisiae* or their combination on production and rumen fermentation of holstein steers. Trop. Anim. Health Prod. 55, 125. https://doi.org/10.1007/s11250-023-03540-4.
Russell, J.B., Strobel, H.J., 1989. Effect of ionophores on ruminal fermentation. Appl. Environ. Microbiol. 55, 1–6. https://doi.org/10.1128/AEM.55.1.1-6.1989.
Salem, A.Z.M., Ryena, A.C., Elghandour, M.M.Y., Camacho, L.M., Kholif, A.E., Salazar, M.C., Domínguez, I.A., Jiménez, R.M., Almaraz, E.M., Martínez, A.G.L., Mariezcurrena, M.A., 2014. Influence of *Salix babylonica* extract in combination or not with increasing levels of minerals mixture on in vitro rumen gas production kinetics of a total mixed ration. J. Anim. Sci. 13, 3110. https://doi.org/10.4081/ijas.2014.3110.
Simitzis, P.E., 2017. Enrichment of animal diets with essential oils—a great perspective on improving animal performance and quality characteristics of the derived products. Medicines 4, 35. https://doi.org/10.3390/medicines4020035.
Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Technol. 48, 185–197. https://doi.org/10.1016/0377-8401(94)90171-6.
Wallace, R.J., McEwan, N.R., McIntosh, F.M., Teferedegne, B., Newbold, C.J., 2002. Natural products as manipulators of rumen fermentation. Asian Austral J. Anim. Sci. 15, 1458–1468. https://doi.org/10.5713/ajas.2002.1458.
Wu, J.P., Zhou, R., Liu, L.S., Casper, D.P., Lang, X., Wang, C.L., Zhang, L.P., Wei, S., Liu, H.B., 2021. Growth performance, nutrient digestibility, blood parameters, and carcass characteristics by lambs fed an oregano and cobalt blend. Animal 15, 100365. https://doi.org/10.1016/j.animal.2021.100365.
Zhang, Z.W., Wang, Y.L., Chen, Y.Y., Zhang, L.T., Zhang, Y.J., Liu, Y.Q., Guo, Y.-X., Yang, H.J., 2021. The dietary supplemental effect of nitroethanol in comparison with monensin on methane emission, growth performance and carcass characteristics in female lambs. Animals 11 (2), 327. https://doi.org/10.3390/ani11020327.