agriculture

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# Effect of Oregano (*Lippia graveolens*) Essential Oil as a Phytogenic Feed Additive on Productive Performance, Ruminal Fermentation, and Antioxidant Activity in Lamb Meat

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**Abstract:** The objective of this study was to evaluate the inclusion of oregano essential oil (OEO) as a phytogenic feed additive in the diet of lambs on the productive performance, ruminal pH, ammoniacal nitrogen production, volatile fatty acids, nitrogen balance, carcass yield, backfat thickness, loin eye (*Longissimus dorsi*) area, shelf life, and antioxidant activity of the meat. Thirty-nine Suffolk × Hampshire lambs (19 ± 3.7 kg live weight) were used in a completely randomized design with three treatments ($n$ = 13) consisting of 0, 0.02, and 0.04% OEO in the diet. The inclusion of OEO in the diet did not affect ($p > 0.05$) the productive performance due to the treatments; similarly, no differences ($p > 0.05$) were observed in the ruminal variables and nitrogen balance. Backfat thickness and loin eye area had similar results ($p > 0.05$), and the antioxidant activity in meat was improved ($p < 0.05$) with the inclusion of OEO. Shelf life and crude protein content increased ($p < 0.05$) with the inclusion of OEO in the diet and with the passing of storage days. Dry matter was affected ($p < 0.05$) by the storage time, with a decrease observed on day 24. pH, ether extract, and ash were not affected ($p > 0.05$). It is concluded that the productive performance, ruminal variables, nitrogen balance, loin eye area, backfat thickness, and carcass yield were not affected by the inclusion of OEO. However, crude protein remained stable and antioxidant activity improved, increasing the shelf life of lamb meat.

**Keywords:** phytogenic additive; oxidative stability; meat quality

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**Citation:** Muñoz-Cuautle, A.; Ortega-Cerrilla, M.E.; Herrera-Haro, J.G.; Nava-Cuellar, C.; Gutiérrez-Olvera, C.; Ramírez-Bribiesca, J.E.; Zetina-Córdoba, P. Effect of Oregano (*Lippia graveolens*) Essential Oil as a Phytogenic Feed Additive on Productive Performance, Ruminal Fermentation, and Antioxidant Activity in Lamb Meat. *Agriculture* **2022**, *12*, 973. https://doi.org/10.3390/agriculture12070973

Academic Editor: Jörg R. Aschenbach

Received: 17 May 2022
Accepted: 28 June 2022
Published: 6 July 2022



## 1. Introduction

Ruminants have been fed antibiotics to prevent disease and improve performance and feed efficiency [1,2]. However, there are currently restrictions in many countries on the use of antibiotics as additives, given their risk to public health, which has made it necessary to search for safe alternatives for the consumer [3]. Phytogenic additives, mainly plant extracts (essential oils, oleoresins, and flavonoids), are used as alternatives to antibiotics because they might maintain a healthy gut environment, modulate the microbiota, and sustain rumen fermentation dynamics; they also have antioxidant properties that increase animal production and contribute to the consumer's demand for healthier foods of animal origin [4,5]. Essential oils contain bioactive compounds, mainly terpenoids and phenylpropanoids, which favor the functioning of the digestive system, reduce the growth of pathogenic bacteria, and diminish the oxidation of lipids [6]. Oregano essential oil (OEO) is

an aromatic compound containing compounds such as carvacrol, thymol, and terpinene. It is considered an alternative to antibiotics due to its antioxidant and broad-spectrum antimicrobial properties [7,8]. It can be used as a nutritional additive to improve milk yield and feed efficiency of dairy cows and meat-producing animals [9]. Antioxidants inhibit the propagation of reactive oxygen species, and their lack causes oxidative stress [10,11] related to lipid oxidation [12]. Some studies have been carried out on the use of OEO in ruminants, with a positive [9,13], negative [14], or similar effect [15,16] on productive behavior and carcass characteristics. OEO (*Lippia S. berlandieri*) has been used in diets for studies on the characteristics of lamb meat due to its antioxidant properties [11]. However, limited information has been generated on lamb's productive performance and meat characteristics using oregano essential oil (*Lippia graveolens*). Because of the potential benefits of OEO, it has been hypothesized that its inclusion in a lamb's diet can improve productivity without affecting carcass composition and meat quality. The objective of this study was to evaluate the effect of OEO as a phytogenic additive in lambs' diets on the productive performance, ruminal pH, ammoniacal nitrogen production, volatile fatty acids, nitrogen balance, carcass yield, backfat thickness, and *Longissimus dorsi* muscle area, together with shelf life and antioxidant activity of lamb meat.

## 2. Materials and Methods

### *2.1. Location*

The experiment was carried out in the sheep module of the Colegio de Postgraduados, Campus Montecillo, the State of Mexico (19°27′33″ N, 98°54′24″ W) and 2240 masl. Animal care and handling procedures were conducted according to the Technical Specifications for the Production, Care and Use of Laboratory Animals, established in the Official Mexican Standard (NOM-062-ZOO-1999).

### *2.2. Animals, Diets and Experimental Design*

Thirty-nine Suffolk × Hampshire male lambs were used, with an average age and live weight of 3 months and 19 ± 3.7 kg, respectively. They were given vitamins ADE (2 mL lambs$^{-1}$, Vigantol®, Bayer, Mexico City, Mexico) and vaccinated against *Clostridium* and *Pasteurella* (2.5 mL lambs$^{-1}$, BIOBAC® 11 tracks, BIO ZOO, Zapopan, Mexico). The animals were housed in individual pens with individual feeders and water troughs, with ad libitum access to feed and fresh water; they were fed twice a day (09:00 and 17:00 h). The experimental diets (Table 1) were formulated according to the NRC [17]. Commercial essential oregano oil (OEO) (*Lippia graveolens*) was obtained from BioAgroCert® (Uruapan, Mexico) (a-caryophyllene (2.12%), a-terpenine (3.0%), b-myrcene (3.12%), thymol (3.72%), caryophyllene (4.16%), *p*-cymene (29.13%), carvacrol (50.21%)). A completely randomized design with 3 treatments ($n$ = 13) was used: (($OEO_0$)—control diet); (($OEO_{02}$)—control diet + 0.02% OEO), (($OEO_{04}$)—control diet + 0.04% OEO). After a 20 d adaptation period to the diet, the experimental test was carried out for 60 d.

**Table 1.** Ingredients and chemical composition of the experimental diets.

| Ingredient | Treatment | | | Composition | Treatment | | |
|---|---|---|---|---|---|---|---|
| | $OEO_0$ | $OEO_{02}$ | $OEO_{04}$ | | $OEO_0$ | $OEO_{02}$ | $OEO_{04}$ |
| (g kg$^{-1}$ DM) | | | | (g kg$^{-1}$ DM) | | | |
| Ground corn | 540.0 | 539.8 | 539.6 | Dry matter (g kg$^{-1}$ as feed) | 895 | 886 | 881 |
| Cane molasses | 30.0 | 30.0 | 30.0 | Crude protein | 175.9 | 174.2 | 173.9 |
| Urea | 10.0 | 10.0 | 10.0 | Ether extract | 32.3 | 33.5 | 33.1 |
| Soybean meal | 200 | 200 | 200 | Neutral detergent fiber | 231.4 | 217.0 | 212.9 |
| Corn stubble | 200 | 200 | 200 | Acid detergent fiber | 85.2 | 78.5 | 77.4 |
| Oregano essential oil | 0 | 0.2 | 0.4 | | | | |
| Mineral premix * | 20.0 | 20.0 | 20.0 | ME (Mcal kg$^{-1}$) | 2.67 | 2.54 | 2.54 |

$OEO_0$: control diet; $OEO_{02}$: control diet plus 0.02%; $OEO_{04}$: control diet plus 0.04% oregano essential oil. DM: dry matter. * $CoSO_4$ 0.068%, $CuSo_4$ 1.04%, $FeSO_4$, 3.57%, ZnO 1.24%, $MnSO_4$ 1.07%; IK 0.052%; and NaCl 92.96%. ME: metabolizable energy.

### 2.3. Productive Performance, Loin Eye Area, and Backfat Thickness

The difference between the initial and final weights, divided by the 60 experimental days, was considered the daily weight gain (DWG). Dry matter intake (DMI) was obtained from the difference between the feed offered and rejected daily. Feed conversion ratio (FCR) was calculated as the ratio between daily feed consumption and DWG. At 55 d into the experimental period, the loin eye area of the *Longissimus dorsi* muscle and the backfat thickness were evaluated using ultrasound with a signal transducer (Model 5040, Aloka® SSD-500 V, Tokyo, Japan). The images were processed with Centralized Ultrasound Processing software, using the transactional plane between the 12th and 13th ribs [18].

### 2.4. Ruminal Variables and Nitrogen Balance

At the end of the experiment (day 60), ruminal fluid samples were obtained by esophageal probe 3 h after providing the morning feed; the pH was immediately measured with a portable potentiometer (Model Orion Star A121, Thermo Scientific®, Chelmsford, MA, USA). Subsequently, the samples were stabilized with metaphosphoric acid (25%) in a 4:1 ratio and stored at −4 °C for later analysis. Ammoniacal nitrogen ($NH_3$-N) was determined through absorbance in an ultraviolet light spectrophotometer (Model Cary 1E, Varian®, Santa Clara, CA, USA) at 630 nm [19]. The production of volatile fatty acids (VFA) was quantified according to Erwin et al. [20] with a gas chromatograph (Model 6890, Hewlett Packard, Palo Alto, Santa Clara, CA, USA) with an Elite FFAP capillary column. For nitrogen balance (NB), 8 lambs per treatment were randomly chosen, housed in metabolic cages, and fitted with feces collection bags. After 2 days of adaptation, feces were collected for 8 days, and 10% was taken individually and stored at −4 °C. In the end, the individual samples were mixed, and 10% of the total was kept at −4 °C until analysis. Individual urine collection was done by placing containers under the cage with 10 mL of 50% hydrochloric acid. At the end of the collection period, they were homogenized, and a 100 mL aliquot was taken for subsequent analysis. NB was calculated using Harris's [21] formula: NB = N consumed − N excreted.

### 2.5. Carcass Yield and Antioxidant Capacity in Meat

At the end of the collection period of feces and urine, the lambs were slaughtered after fasting for 12 h. Hot carcasses were immediately weighed to calculate hot carcass yield and hot carcass biological yield, according to Santos et al. [22]. For the pH and temperature measurements, a portable potentiometer (Model HL99163, HANNA®, Waterproof Tester, Woonsocket, RI, USA) was used with a penetration electrode in the *Longissimus dorsi* muscle between the thoracic and first lumbar vertebrae, 15 min after slaughter. *Longissimus dorsi* muscle samples were stored at −60 °C for 24 h in order to measure antioxidant activity. Subsequently, they were lyophilized, ground, and the ferric ion reducing antioxidant power (FRAP) was determined using the technique described by Benzie and Strain [23] and the thiobarbituric acid reactive substances (TBARS) analysis according to Ohkawa et al. [24].

### 2.6. Chemical Composition of the Meat

The physicochemical characteristics of the meat were analyzed in *Longissimus dorsi* muscle samples taken at the time of slaughter, which were cut into five equal portions, packaged, and stored at −4 °C for evaluation at 24 h and 3, 6, 12, and 24 d post mortem. The corresponding pH reading was done with a potentiometer (Model HL99163, HANNA®, Waterproof Tester, Woonsocket, RI, USA).

### 2.7. Chemical Analyses

Dry matter (DM), crude protein (CP), ether extract (EE), and ash [25] were determined in samples of the diets and *Longissimus dorsi*, and neutral detergent fiber (NDF) and acid detergent fiber (ADF) in the diets [26]. The total N was quantified in the diets, feces, and urine [25].

### 2.8. Statistical Analysis

An analysis of variance (ANOVA) was performed with SAS software [27]. All the parameters analyzed, except the chemical composition of the meat, were analyzed with PROC GLM. The means test was performed with the Tukey method. The statistical model was as follows:

$$Y_{ij} = \mu + \tau_i + e_{ij}$$

where: $Y_{ij}$ = dependent variable, μ: general mean; *j*th observation under the *i*th treatment, and $e_{ij}$ = experimental error.

The chemical composition of the meat was analyzed with measurements repeated through time (PROC MIXED), and the mean test was performed with the Tukey method. The general structure of the statistical model used was:

$$Y_{ij} = \mu + \tau_i + r_{j(i)} + p_k + (\tau p)_{ik} + e_{ijk}$$

where: $Y_{ij}$ = dependent variable; μ: general mean; $\tau_i$: effect of the *i*th treatment; $r_{j(i)}$: effect of the *j*th repetition under the *i*th treatment; $p_k$: effect of the *k*th time; $(\tau p)_{ik}$: interaction effect of the kth time by the ith treatment; and $e_{ijk}$: experimental error.

## 3. Results

### 3.1. Productive Performance, Loin Eye Area, and Backfat Thickness

No differences were found with the addition of 0.02 and 0.04% OEO in the final weight, DMI, DWG, and FCR ($p < 0.05$). The loin eye area and backfat thickness were unaffected ($p < 0.05$) by the addition of OEO into the diet (Table 2).

**Table 2.** Productive performance, loin eye area, and backfat thickness in lambs fed with different OEO levels in the diet.

| Variable | Treatment | | | SEM | *p*-Value |
|---|---|---|---|---|---|
| | $OEO_0$ | $OEO_{02}$ | $OEO_{04}$ | | |
| Initial weight (kg) | 29.05 | 27.93 | 29.49 | 2.95 | 0.3942 |
| Final weight (kg) | 48.83 | 48.29 | 48.94 | 3.85 | 0.8985 |
| Dry matter intake (kg) | 1.54 | 1.51 | 1.53 | 0.12 | 0.8530 |
| Daily weight gain (kg $d^{-1}$) | 0.329 | 0.339 | 0.324 | 0.04 | 0.7301 |
| Feed conversion ratio | 4.61 | 4.40 | 4.66 | 0.58 | 0.4772 |
| Backfat thickness (mm) | 3.46 | 3.58 | 3.69 | 0.50 | 0.5132 |
| Loin eye area ($mm^2$) | 1233.69 | 1194.42 | 1176.62 | 88.36 | 0.2561 |

$OEO_0$: control diet; $OEO_{02}$: control diet plus 0.02%; $OEO_{04}$: control diet plus 0.04% oregano essential oil. SEM: standard error mean.

### 3.2. Ruminal Variables and Nitrogen Balance

The values of ruminal pH, VFA, and $NH_3$-N production were not modified ($p > 0.05$) when OEO was included in the diets (Table 3), although a tendency to decrease propionic acid production was observed as the inclusion level of OEO increased. The nitrogen balance results (Table 3) were similar to the different OEO levels in the diets ($p > 0.05$).

### 3.3. Carcass Characteristics and Antioxidant Capacity in Meat

The weight of the hot carcass, hot carcass yield, true biological yield, temperature, and pH showed no differences ($p < 0.05$) due to the effect of the inclusion of OEO in the diet (Table 4). The results showed a difference ($p < 0.05$) in the antioxidant activity in the *Longissimus dorsi* muscle for the $OEO_0$, $OEO_{02}$, and $OEO_{04}$ treatments, with values of 1.49, 1.76, and 2.13 mmol Trolox $g^{-1}$ protein, respectively. The response of antioxidant activity by TBARS was influenced ($p < 0.05$) by the addition of OEO to the diet, showing a decrease of 13.26, 9.31, and 7.28 nmol malondialdehyde (MDA) $g^{-1}$ protein when 0, 0.02, and 0.04% were included, respectively (Table 4).

**Table 3.** Ruminal variables and nitrogen balance of lambs fed with different OEO levels in the diet.

| Variable | Treatment | | | SEM | *p*-Value |
|---|---|---|---|---|---|
| | $OEO_0$ | $OEO_{02}$ | $OEO_{04}$ | | |
| Rumen pH | 6.26 | 6.14 | 6.26 | 0.40 | 0.7391 |
| $NH_3$-N (mg $dL^{-1}$) | 15.09 | 12.49 | 11.70 | 7.11 | 0.4535 |
| *Volatile fatty acids* (mmol $L^{-1}$) | | | | | |
| Acetate (A) | 45.73 | 42.37 | 45.49 | 7.31 | 0.4346 |
| Propionate (P) | 28.87 | 27.42 | 26.46 | 9.07 | 0.7930 |
| Butyrate | 7.50 | 7.41 | 6.98 | 2.81 | 0.8819 |
| Others [£] | 2.14 | 2.34 | 1.88 | 0.74 | 0.3135 |
| A:P Ratio | 1.71 | 1.65 | 1.75 | 0.56 | 0.8964 |
| Total | 84.25 | 78.77 | 78.52 | 17.17 | 0.6334 |
| *Nitrogen balance* | | | | | |
| Feed N (g $d^{-1}$) | 39.47 | 36.67 | 34.94 | 4.379 | 0.1381 |
| Fecal N (g $d^{-1}$) | 1.082 | 1.073 | 1.061 | 0.177 | 0.9728 |
| Urine N (g $d^{-1}$) | 14.24 | 14.66 | 14.01 | 1.486 | 0.6858 |
| N retained (g $d^{-1}$) | 24.15 | 20.94 | 19.86 | 4.439 | 0.1579 |
| Retained N (% feed N) | 60.67 | 56.33 | 56.54 | 6.772 | 0.3691 |

$OEO_0$: control diet; $OEO_{02}$: control diet plus 0.02%; $OEO_{04}$: control diet plus 0.04% oregano essential oil. SEM: standard error mean. $NH_3$-N: ammoniacal nitrogen. N: nitrogen. [£] Sum of isobutyric, isovaleric and valeric acid.

**Table 4.** Carcass characteristics and antioxidant activity in meat from lambs fed with different oregano essential oil levels in the diet.

| Variable | Treatment | | | SEM | *p*-Value |
|---|---|---|---|---|---|
| | $OEO_0$ | $OEO_{02}$ | $OEO_{04}$ | | |
| Slaughter weight (kg) | 45.87 | 46.14 | 45.22 | 2.55 | 0.7565 |
| Hot carcass weight (kg) | 25.18 | 25.47 | 25.88 | 1.28 | 0.5533 |
| Hot carcass yield (%) | 54.99 | 56.10 | 56.41 | 2.24 | 0.4544 |
| Yield biological (%) | 59.02 | 59.26 | 60.06 | 3.19 | 0.8021 |
| pH (slaughter) | 6.08 | 5.92 | 5.97 | 0.31 | 0.6092 |
| Temperature (°C) | 23.81 | 24.26 | 24.37 | 3.48 | 0.9479 |
| *Antioxidant activity* | | | | | |
| FRAP (mmol Trolox $g^{-1}$ protein) | 1.49 [a] | 1.76 [ab] | 2.13 [b] | 0.29 | 0.0011 |
| TBARS (nmol MDA $g^{-1}$ protein) | 13.26 [a] | 9.31 [b] | 7.28 [b] | 2.93 | 0.0047 |

$OEO_0$: control diet; $OEO_{02}$: control diet plus 0.02%; $OEO_{04}$: control diet plus 0.04% oregano essential oil. SEM: standard error mean. FRAP: ferric reducing antioxidant power. TBARS: thiobarbituric acid reactive substances. MDA: malondialdehyde. [a,b] Means in the same row with different superscripts are significantly different ($p < 0.05$).

### *3.4. Chemical Composition of the Meat*

Table 5 shows the chemical composition of the meat. The inclusion of OEO and the interaction with time did not influence ($p > 0.05$) DM content. However, an increase was observed at 12 d and a decrease at 24 d ($p > 0.05$) with time. The treatments, the days of storage, and the treatment x time interaction modified the CP content ($p > 0.05$). No differences were observed regarding pH, ether extract, or ash ($p > 0.05$) from the treatments, times, or time x treatment interaction.

**Table 5.** Chemical composition of meat from lambs fed with different OEO levels in the diet.

| | | Treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Variable** | **24 h** | **3 d** | **6 d** | **12 d** | **24 d** | **SEM** | **Treat** | **Time** | ***p*-Value** |
| pH | $OEO_0$ | 5.53 | 5.34 | 5.45 | 5.44 | 5.28 | 1.19 | 0.79 | 0.16 | 0.88 |
| | $OEO_{02}$ | 5.46 | 5.38 | 5.56 | 5.39 | 5.49 | | | | |
| | $OEO_{04}$ | 5.52 | 5.41 | 5.43 | 5.45 | 5.39 | | | | |
| Dry matter (%) | $OEO_0$ | 25.54 $^{y}$ | 27.71 $^{x}$ | 27.78 $^{x}$ | 28.77 $^{x}$ | 24.13 $^{z}$ | 0.85 | 0.42 | 0.05 | 0.61 |
| | $OEO_{02}$ | 24.85 $^{y}$ | 27.93 $^{x}$ | 25.58 $^{xy}$ | 27.77 $^{x}$ | 24.78 $^{y}$ | | | | |
| | $OEO_{04}$ | 25.54 $^{y}$ | 27.32 $^{x}$ | 27.88 $^{x}$ | 28.67 $^{x}$ | 24.58 $^{y}$ | | | | |
| Crude protein (%) | $OEO_0$ | 17.03 $^{ax}$ | 15.98 $^{ay}$ | 15.29 $^{ay}$ | 14.75 $^{ayz}$ | 14.60 $^{ayz}$ | 0.38 | 0.05 | 0.05 | 0.06 |
| | $OEO_{02}$ | 17.08 $^{ax}$ | 16.25 $^{ax}$ | 16.55 $^{bx}$ | 16.83 $^{bx}$ | 16.57 $^{bx}$ | | | | |
| | $OEO_{04}$ | 16.92 $^{ax}$ | 16.57 $^{ax}$ | 16.48 $^{bx}$ | 16.31 $^{bx}$ | 16.17 $^{bx}$ | | | | |
| Ether extract (%) | $OEO_0$ | 13.93 | 16.70 | 14.84 | 16.55 | 15.47 | 0.91 | 0.44 | 0.74 | 0.78 |
| | $OEO_{02}$ | 13.77 | 13.98 | 13.11 | 14.54 | 15.73 | | | | |
| | $OEO_{04}$ | 13.17 | 12.76 | 14.82 | 13.97 | 13.20 | | | | |
| Ash (%) | $OEO_0$ | 4.25 | 4.12 | 4.15 | 4.75 | 4.10 | 0.78 | 0.38 | 0.25 | 0.20 |
| | $OEO_{02}$ | 4.39 | 4.33 | 4.36 | 4.18 | 4.65 | | | | |
| | $OEO_{04}$ | 4.32 | 4.20 | 4.25 | 4.23 | 4.30 | | | | |

$OEO_0$: control diet; $OEO_{02}$: control diet plus 0.02%; $OEO_{04}$: control diet plus 0.04% oregano essential oil. SEM: standard error mean. $^{x,y,z}$ Means in the same row with different superscripts are significantly different ($p < 0.05$). $^{a,b}$ Means in the same column with different superscripts are significantly different ($p < 0.05$).

## 4. Discussion

Essential oils improve the taste and palatability of feed, thus increasing voluntary intake, resulting in better weight gain [1]. In the present investigation, no effect on final weight, DMI, DWG, and FCR was observed; the results obtained are similar to those reported by Ünal and Kocabağli [15] and Ünlü et al. [28] when including different levels of OEO in diets for sheep. Other similar results were reported by Farghaly and Abdullah [16], who included 2.5% OEO in the diets of Saidi sheep. In contrast, Canbolat et al. [29] reported a decrease in DWG in lambs when increasing OEO (400, 800, 1200 mg $kg^{-1}$ of DM). However, Sun et al. [9] mentioned a positive effect on final weight, DWG, and DMI by including 300 mg $kg^{-1}$ of OEO in diets for lambs and a decrease in feed conversion. In the same context, Scarpa et al. [30] used a combination of flaxseed and OEO, without effect on the productive performance of lambs. The differences in the positive results when two essential oils or another compound are used can be explained by the interaction of the compounds with a synergistic effect that favors the mixture's effectiveness on the productive performance of the lambs [31]. The main compounds of OEO are carvacrol, thymol, and *p*-cymene, which have a high antioxidant capability and antimicrobial potential that might affect rumen microbial growth and development [32], affecting ruminal fermentation and thus productive performance [14]. However, their effectiveness has not been demonstrated to improve lambs' performance [33]. The results obtained in this study might be due to the amount of these compounds provided by OEO that was not enough to affect the variables evaluated. These results are similar to those of Biricik et al. [34]. It has been observed that the best results related to propionate and $NH_3$-N concentration in the rumen are seen when essential oils are given for at least 30 days, which suggests that rumen microbes might adapt to essential oils through time [33]. These results coincide with those observed in the present study because OEO was provided to lambs for 60 days. However, the effect of OEO on the productive performance of animals can be affected by its composition, processing, and conservation, which determines its biological properties. The effect of OEO on the productive performance of animals can be affected by its composition, processing, and conservation, which determine its biological properties [35].

The pH, $NH_3$-N concentration, and VFA are the leading indicators of the functioning of ruminal fermentation [36]. The pH in the ruminal fluid can vary between 5.5 and

7.5 depending on the nature of the diet time after ingestion, feeding frequencies and times, and the time from feeding to ruminal fluid collection [37,38]. In the present study, pH values between 6.14 and 6.26 were observed, close to the lower range of 6.2 to 7.0, ensuring normal ruminal fermentation [39]. The results obtained in the present study are consistent with previous research [13,40,41] in sheep fed with diets and different levels of OEO (*Oreganun vulgare* L.). However, they differ from what was reported by Ünlu et al. [28] and Passetti et al. [12]. Generally, the ruminal concentration of $NH_3$-N is in the range 10 to 50 mg $dL^{-1}$ [13]; the results obtained in the present study are within the range recommended by Hoover [42] for the initiation of microbial growth and to maximize microbial protein synthesis in sheep [43], and coincide with the research carried out by Jiao et al. [13] and Passetti et al. [12]. Although no significant differences were observed, adding 0.02 and 0.04% OEO reduced ruminal $NH_3$-N concentration by 16 and 22%, respectively. These results can be attributed to the antimicrobial activity of OEO [44] by inhibiting ammonia-producing bacteria [45], produced by thymol, one of the main components of OEO (*Lippia graveolens*) [46], acting on rumen microorganisms and by affecting protein deamination [47].

VFAs are the main products of carbohydrate degradation in diets and provide most of the energy precursors for metabolic processes in ruminants, so a variation in the acetate: propionate ratio could affect the productive performance [45,48]. Other studies have reported that the inclusion of different levels of OEO has not affected VFA production [41,49] or the acetate: propionate ratio [13]. In this study, similar results were found. A molar decrease in propionate was observed as the inclusion level of OEO increased. This effect could be caused by the inhibition of the growth of amylolytic bacteria, mainly *Streptococcus bovis*, by carvacrol and thymol [49], the main components of the OEO (*Lippia graveolens*) [46].

Nitrogen balance depends on DMI, N concentration, diet digestibility, and rumen microbial protein synthesis. Soltan et al. [50] found differences in retained nitrogen, suggesting an effect of post-ruminal digestion of the microencapsulated diet of a mixture of essential oils (cinnamaldehyde, eugenol, carvacrol, and pepper oleoresin). In the present study, the inclusion of OEO did not modify the nitrogen balance, possibly because OEO is not considered a nitrogen source and partly because the experimental diets were isonitrogenous.

Sun et al. [9] indicated that the eye of the *Longissimus dorsi* muscle area of Sewa lambs increased with the addition of OEO in the diet, mainly with 300 mg $kg^{-1}$; however, Wu et al. [2] did not detect differences in back fat or the eye of the *Longissimus dorsi* muscle area in lambs given 7 g $d^{-1}$.

In the present study, no effect was found on carcass characteristics from the inclusion of OEO, which coincides with Ünal and Kocabağli [15], who added 250 and 500 ppm kg $DM^{-1}$ of OEO to the diet for lambs. Simitzis et al. [51] reported that supplementing lambs with 1 mL per kg $DM^{-1}$ of OEO (83.10% carvacrol, 2.10% thymol) did not affect either hot carcass weight or yield. In contrast, Sun et al. [9] indicated that 300 mg $kg^{-1}$ of OEO in the diet improved carcass weight and yield in Sewa lambs. In goats, carcass weight was different from the control group by providing OEO plus cobalt in the diet, attributed to consumption stimulation [52]. In studies where a positive effect of OEO has been reported, it is considered that it improves nutrient absorption [53]. Ünal and Kocabağli [15] mention that the hot carcass yield is related to the results obtained in the productive performance, similar to the present study, where hot carcass yields were similar between treatments.

Oxidation is one of the main factors of food deterioration, which decreases the quality and causes rejection by the consumer [54]. Previous studies have shown that OEO supplementation has demonstrated antioxidant effects by delaying lipid oxidation in fresh lamb meat stored in refrigeration and freezing, extending the shelf life of the meat [11,51]. In the present study, the FRAP value was different from the control diet, indicating that adding OEO to the diet increased the antioxidant capacity of meat. The TBARS values with the inclusion of 0.02 and 0.04% OEO were lower compared to the control treatment, indicating less oxidation of meat. OEO (0.2% in the diet) had a favorable effect on the oxidative stability of Dalagh lamb meat for a longer time to be considered in the storage of

meat in refrigerators [55]. In this regard, Simitzis et al. [51] observed that the inclusion of 1 mL of OEO $kg^{-1}$ DM reduced lipid oxidation in lamb meat. At low doses of OEO (0.2 and 0.3 g $kg^{-1}$) in diets for lambs, TBARS values in meat were lower, with higher doses there is an increase in the oxidation of lipids in meat, caused by a pro-antioxidant effect, and they are not recommended for feedlot animals [11]. Pelaes Vital et al. [54] indicated that the use of OEO in low doses (0.05%) can be considered a viable strategy to improve the shelf life and quality of lamb meat burgers. The antioxidant activity of OEO observed in this study may be due to carvacrol, which might be capable of reducing malondialdehyde (MDA) [56], considering that the main component of OEO used in this study was carvacrol (50.1%).

The pH, EE, and ash in the meat were not affected by the inclusion of OEO in the diets. The pH affects quality characteristics in the meat and decreases its useful life. The desirable pH ranges in sheep meat are between 5.4 and 5.8 [57]; the pH values obtained in this study were found within this range. Perez et al. [58] reported 1.68% EE and 1.2% ash in Merino lambs, similar in EE but lower in ash, compared to the present investigation. However, Ayele et al. [59] reported ash values between 4.08 and 6.15%, closer to those observed in the present study. The CP content at 24 h and 3 d was similar; however, on days 6, 12, and 24 of storage, the inclusion of OEO increased CP values. At 24 h, the addition of OEO did not affect the chemical composition of the meat, observing similar results to those reported in the study by Scarpa et al. [30] in the *Longissimus lumborum* muscle of lambs. Likewise, they are similar to those observed by Rivaroli et al. [60] when evaluating a mixture of essential oils (oregano, garlic, lemon, rosemary, thyme, eucalyptus, and orange) in diets for calves. Fat oxidation has an essential effect due to the formation of peroxides and aldehydes; these products react readily with meat proteins, affecting their quality during storage [61]. In this study, the EE content was not statistically different; however, the inclusion of 0.02 and 0.04% OEO in the diet decreased lipid oxidation, so CP was maintained longer when OEO was added. DM values were different over time, which may be a consequence of the fat content in the *Longissimus dorsi* muscle. Rotondi et al. [62] and Ayele et al. [59] reported that DM increased with increasing intramuscular fat content in lamb and kid meat. However, more research is necessary to identify the primary metabolic pathway of OEO and the active components that favor quality characteristics in lamb meat [11].

## 5. Conclusions

Under the conditions of the present study, as a phytogenic feed additive, the addition of 0.02% and 0.04% of essential oregano oil in the diet did not affect the productive performance, the production of ammoniacal nitrogen, volatile fatty acids, the nitrogen balance, or the backfat thickness of the loin eye area. The chemical composition of the meat was not affected by treatment, except for crude protein. Therefore, including 0.02% of OEO in the diet of growing lambs maintains the stability of the crude protein, and the antioxidant activity increases the shelf life of lamb meat.

**Author Contributions:** Conceptualization, A.M.-C., J.E.R.-B. and M.E.O.-C.; methodology, M.E.O.-C., C.N.-C. and C.G.-O.; software, J.G.H.-H. and P.Z.-C.; validation, C.N.-C. and C.G.-O.; formal analysis, J.G.H.-H. and P.Z.-C.; investigation, A.M.-C., M.E.O.-C. and P.Z.-C.; resources, M.E.O.-C. and J.E.R.-B.; data curation, J.G.H.-H. and P.Z.-C.; writing—original draft preparation, A.M.-C. and P.Z.-C.; writing—review and editing, A.M.-C., P.Z.-C., J.E.R.-B., C.N.-C., M.E.O.-C. and C.G.-O.; visualization, C.N.-C. and C.G.-O.; supervision, A.M.-C., J.E.R.-B. and C.G.-O.; project administration, M.E.O.-C. and C.N.-C.; funding acquisition, M.E.O.-C. and J.E.R.-B. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Animal care and handling procedures were conducted according to the technical specifications for the production, care and use of laboratory animals established in the Official Mexican Standard (NOM-062-ZOO-1999). The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the Colegio de Postgraduados (COBIAN/001/20), approved on 23 November 2020.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Conflicts of Interest:** The authors declare no conflict of interest.

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