



Article Greenhouse Gas Emission Reduction Potential of Lavender Meal and Essential Oil for Dairy Cows

Levend Coşkuntuna¹, Maximilian Lackner^{2,*}, Kadir Erten¹, Sevilay Gül³, Valiollah Palangi⁴, Fisun Koç¹, and Selim Esen^{5,*}

- ¹ Department of Animal Science, Faculty of Agriculture, Tekirdag Namik Kemal University, Tekirdag 59030, Türkiye
- ² Department of Industrial Engineering, University of Applied Sciences Technikum Wien, Hoechstaedtplatz 6, 1200 Vienna, Austria
- ³ Vocational School of Technical Sciences, Plant and Animal Production Department, Tekirdag Namik Kemal University, Tekirdag 59030, Türkiye
- ⁴ Department of Animal Science, Faculty of Agriculture, Ege University, Bornova, Izmir 35100, Türkiye
- ⁵ Balikesir Directorate of Provincial Agriculture and Forestry, Republic of Türkiye Ministry of Agriculture and Forestry, Balikesir 10470, Türkiye
- * Correspondence: maximilian.lackner@technikum-wien.at (M.L.); selim_esen01@hotmail.com (S.E.)

Abstract: This research aims to evaluate the potential of lavender meal (LM) and lavender essential oil (LEO) to mitigate methane emissions by dairy cows. Locally grown lavender was collected fresh for this purpose, and its oil was extracted using the cold-press method. The resultant LEO and LM and whole lavender (WL) were added to dairy cow concentrate feed at 0%, 0.05%, and 0.10%, and their effects on vitro gas production values and gas concentrations were subsequently assessed. Out of the 30 bioactive compounds isolated from LEO, linalool and linalyl acetate were the most common—accounting for 70.4% of the total. The lavender dose had a significant influence on gas production for up to 12 h. No significant variations were found across the lavender forms when gas kinetics, in vitro degradability, and predicted energy values were compared. The addition of WL to the concentrate feed of dairy cows produced the greatest quantities of methane, carbon dioxide, and hydrogen sulfide, whereas LEO resulted in the lowest values. In contrast, no significant difference in ammonia content was found across the various lavender forms added into dairy cow concentrate feed may decrease greenhouse gas emissions from dairy cows.

Keywords: lavender; byproduct; essential oil; methane; dairy cow; gas production

1. Introduction

A substantial amount of greenhouse gas (GHG) emissions, such as methane (CH₄) and nitrous oxide, come from livestock production—making it one of the main drivers of climatic change in the Anthropocene period [1,2]. Livestock production is responsible for around 13% of the world's GHG emissions [3]. CH₄ emissions, in particular, are significant contributors due to the fact that their potential to trigger global warming is 28 times more than that of carbon dioxide (CO₂) [3]. CH₄ production results in a loss of energy availability for the host ruminant animal, which typically accounts for between 2% and 12% of the overall energy availability [4]. As a result of ruminants' inability to convert this carbon into usable energy, the production of CH₄ has a negative impact on both the environment and the productivity and profitability of ruminant farming [5]. It is clear, therefore, that feasible methods of minimizing enteric CH₄ make livestock husbandry an important player in addressing climate change mitigation.

Selecting high-feed-efficiency animals, decreasing the forage-to-concentrate ratio in the diet, supplementing with nitrate and fat, and introducing feed additives are all effective



Citation: Coşkuntuna, L.; Lackner, M.; Erten, K.; Gül, S.; Palangi, V.; Koç, F.; Esen, S. Greenhouse Gas Emission Reduction Potential of Lavender Meal and Essential Oil for Dairy Cows. *Fermentation* **2023**, *9*, 253. https://doi.org/10.3390/ fermentation9030253

Academic Editor: Mengzhi Wang

Received: 23 February 2023 Revised: 28 February 2023 Accepted: 3 March 2023 Published: 4 March 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). ways to lower enteric CH₄ emissions. Essential oils (EOs) are one of the most common feed additives that are incorporated into the diet, along with ionophores [6], saponins [7], tannins [8], and flavonoids [9]. Fragrant plants, such as lavender, rosemary, rose, peppermint, and cypress, generate EOs to deter herbivorous insects and dangerous microorganisms and/or serve as signaling molecules [10]. EOs are known to have secondary metabolites that are both volatile and lipophilic, and their method of action in reducing CH₄ emissions has been the subject of several hypotheses. For example, it is believed that EOs may affect microbial activity by accumulating in the lipid bilayer and the cytoplasm [11]. Furthermore, it has been hypothesized that EOs raise propionate levels, hence reducing the amount of H₂ available to produce CH₄ [12].

Lavender, which belongs to the Lavandula genus (*Lamiaceae* family), is one of the most valued medicinal and fragrant plants of economic relevance, and it is produced for industrial uses [13]. Lavender EO (*Lavandula angustifolia* Mill., LEO) production is between 300 and 500 tons per year [13], whereas total EO production from all lavender species and hybrids is over 1500 tons per year [14]. Low efficiency, around 1% in mass, is produced by conventional and industrial ways of processing medicinal and fragrant plants, leading to massive quantities of solid and liquid waste [15]. Thus, natural waste valorization becomes inevitable if we are to lessen the impact on GHG emissions. Food-feed competitiveness issues, environmental ramifications, and the need for safer animal products may all be addressed by switching to alternatives that have been bioactively improved in place of traditional feed resources, as proven in previous research [16,17].

LEO contains 1,8-cineole, camphor, borneol, linalool, and linalyl acetate, which provide it with its potent antioxidant and antibacterial properties [18]. As mentioned earlier, LEO extracted from flowers is preferable for use in the formulation of cosmetics and other personal care products. This is due to the fact that linalyl acetate, which is present in the flower extract and contributes to the extract's signature aroma, is present in the flower extract [19]. However, the LEO extracted from the leaves and stems is superior for medicinal and insecticidal applications because of its greater 1,8-cineole and camphor content [19]. Lavandulol, eucalyptol, and geraniol are among the additional antibacterial and antifungal compounds isolated in LEO [20].

Although there have been limited evaluations of LEO's impact on CH₄ production [21,22], to the best of our knowledge, there has been no research investigating the effect of different amounts of lavender waste utilized during LEO extraction on CH₄ production in dairy cows in vitro. Therefore, the current study tested the hypothesis that different lavender forms would have different effects on CH₄ reduction due to the variation in bioactive components and the high antioxidant and antibacterial properties previously shown to be present in EOs obtained from the different parts of lavender (flower, leaf, stem). Thus, the objective of this study was to explore the effects of additional different lavender forms (whole lavender (WL), lavender meal (LM), and LEO) and doses (0, 0.05% and 0.010%) on the in vitro gas production and CH₄ ratio of dairy cows.

2. Materials and Methods

2.1. Chemical Composition of Diets

AOAC [23] methods were used to perform the proximate analysis of the dairy cow concentrate feed, whole lavender, and defatted lavender meal used during these in vitro studies. Briefly, the dry matter (DM) and ash content of the samples were determined by drying them at 102 °C overnight and ashing them in a muffle furnace for three hours at 550 °C. The crude protein (CP) ratio was calculated by multiplying the nitrogen content of samples by 6.25. Analyses of neutral detergent fibers (NDF) and acid detergent fibers (ADF) were conducted using a method described by Van Soest et al. [24]. Using the methodology of the ISO 10520 standard [25], the amount of starch in the samples was determined. The results of the chemical analyses of dairy cow concentrate feed are listed in Table 1.

Item	g/kg (Dry Matter Basis)
Dry matter (g/kg fresh matter basis)	898.7
Crude protein	203.8
Ash	74.0
Ether extract	31.3
Acid detergent fiber	105.3
Neutral detergent fiber	254.3
Starch	260.3

Table 1. Chemical composition of dairy cow concentrate feed.

2.2. Extraction and Characterization of Lavender Essential Oil

The current study utilized the cold-press method to extract oil from freshly harvested lavender, as 99.9% of all industrial and commercial essential oils are cold-pressed [26]. Locally cultivated lavender (Lavandula angustifolia Mill.) was freshly gathered from Tekirdag, Turkey (41.0° N, 27.5° E; located 5 m above sea level, with an annual mean temperature of 10.5 °C, and a total precipitation of 482 mm per year). Volatile compounds in the LEOs were characterized using a headspace solid-phase microextraction and gas chromatography/mass spectrometry (HS-SPME and GC/MS) approach, as detailed in earlier papers by Riu-Aumatell et al. [27]. Extraction was carried out using an SPME instrument (Supelco, Bellefonte, PA, USA) outfitted with a 10 mm fiber covered with 100 µm polydimethylsiloxane. A 5 mL sample of LEO was transferred to a 10 mL vial and extracted in headspace mode (a distance of 20 mm from the liquid surface) at 50 °C for 40 min with magnetic stirring. After extraction, the SPME apparatus was inserted into a gas chromatograph (Shimadzu GCMS-QP2010, Kyoto, Japan), maintained at 250 °C for 5 min, and equipped with a flame ionization detector. RTX-5 fused silica capillary columns were employed (Restek, Bellefonte, PA, USA; 30 m, id: 0.25 mm, ft: 0.25 µm). As the carrier gas, helium was used. The injector and detector had temperatures of 250 °C and 280 °C, respectively. In splitless injection mode, the temperature program ranged from 40 °C (held for 5 min) to 240 °C (held for 10 min) at a rate of 4 °C/min.

2.3. In Vitro Gas Production and Fermentation Characteristics

For each experimental group, about 1 kg of dairy cow concentrate feed was crushed and homogenized with 0, 0.05%, and 0.10% WL, LM, and LEO, respectively. The in vitro incubation of samples was performed with calibrated 100 mL glass syringes in triplicate in a 39 °C water bath for 48 h. The research did not use any animals directly; rather, 3 freshly slaughtered 2-year-old Holstein cattle were used in the extraction of rumen liquor. That liquor was pooled and subsequently filtered through four layers of cheesecloth. It was captured in a vessel pre-warmed to 39 °C and taken to the laboratory within 20 min. On each occasion, approximately 200 mg of dry samples were incubated in six replicates in glass syringes. Using pre-warmed glass syringes at 39 °C, 30 mL of rumen liquor-buffer mixture free of particles was injected under continuous injection of CO_2 as described by Menke and Steingass [28]. The total gas volume of each sample was determined at 3, 6, 12, 24, and 48 h, and the volume was corrected using blank bottles and alfalfa hay standard samples. The contents of CH₄, carbon dioxide (CO₂), ammonia (NH₃), and hydrogen sulfide (H₂S) were determined using a CH₄ analyzer (MX6 iBrid, Industrial Scientific Corporation, Pittsburgh, PA, USA) according to the protocol described by Goel et al. [29] after 24 h of incubation.

The cumulative gas production of samples was fitted to an exponential model proposed by Ørskov and McDonald (1979), by utilizing the Solver function in Excel (Equation (1)) [30].

$$Y = a + b(1 - e^{-ct})$$
(1)

It is indicated in Equation (1) that Y is the volume (mL) of gas produced at time t; *a* is the volume (mL) of gas produced from the instantly soluble fraction of samples; *b* is the

constant (mL/h); and *t* is the time of incubation (h). In order to calculate the metabolizable energy content (ME, (MJ/kg DM)), organic matter digestibility (OMD, (% DM)) and net energy lactation (NEL, (MI/kg DM)) of same

matter digestibility (OMD, (% DM)), and net energy lactation (NEL, (MJ/kg DM)) of samples, the following Equations (2) and (3) of Menke et al. [31] and Menke and Steingass [28] were used, respectively.

$$ME (MJ/kgDM) = 2.20 + 0.136 GP + 0.057 CP$$
(2)

$$OMD (\% DM) = 14.88 + 0.889 GP + 0.45 CP + 0.0651 Ash$$
(3)

$$NEL (MJ/kgDM) = 0.101 GP + 0.051 CP + 0.112 EE$$
(4)

In Equations (2)–(4), DM represents dry matter; GP is the net production of gas over a 24-h period (mL/200 mg); CP and EE are crude protein and ether extract, respectively, expressed in g/kg DM.

2.4. Statistical Analysis

This study utilized SAS-JMP software version 13.2 (SAS Institute Inc., Cary, NC, USA) to create a 3 × 3 factorial arrangement with the MIXED procedure, taking lavender form (WL, LM, LEO) and dosage (0%, 0.05%, and 0.10%) as the fixed effects, and experimental error as the random effect. The means were compared using Duncan's multiple range test, at the level of p < 0.05, which is considered to be a statistically significant difference.

3. Results

Table 2 provides a summary of the bioactive compounds identified by the HS-SPME and GC-MS approach in the LEO. Of the 30 compounds identified from LEO, the two most abundant were linalool (38.3%) and linalyl acetate (32.1%), together accounting for 70.4% of the total. The other most prevalent compounds in LEO were 4-terpinyl acetate (5.20%), neryl acetate (4.82%), β -farnesene (4.47%), caryophyllene (3.77%), and ocimene (2.21%), which together accounted for 20.47%.

Table 2. Percentage of volatile components isolated from lavender essential oils.

Number	R. Time	Area	Area%	Compounds
1	13.685	101,576	0.15	DL-Limonene
2	13.742	212,507	0.32	1,8-Cineole
3	13.900	57,827	0.09	Geranyl tiglate
4	14.342	1,085,072	1.61	α-Pinene
5	14.600	54,210	0.08	Farnesene
6	14.931	1,487,834	2.21	Ocimene
7	17.663	25,783,120	38.30	Linalool
8	18.338	525,190	0.78	Octenyl acetate
9	18.925	103,882	0.15	3-Octyl acetate
10	19.136	830,531	1.24	Alloocimene
11	19.619	236,533	0.35	Camphor
12	20.723	339,739	0.51	Borneol
13	20.920	591,330	0.88	Lavandulol
14	21.269	3,496,970	5.20	4-Terpinyl acetate
15	21.899	506,098	0.75	Linalyl propionate
16	24.722	21,628,616	32.10	Linalyl acetate
17	25.859	94,018	0.14	Fenchyl acetate
18	26.101	3,243,132	2 4.82 Neryl acetate	
19	29.534	359,315	0.53	Geranyl acetate
20	29.632	192,137	192,137 0.29 Hexyl hexanoa	
21	29.816	92,161	0.14 Zingiberene	
22	30.669	40,808	0.06	trans-α-Bergamotene

R. Time	Area	Area%	Compounds
30.852	2,533,028	3.77	Caryophyllene
31.211	87,501	0.13	Isocaryophyllene
31.400	87,541	0.13	α-Bergamotene
32.088	3,006,736	4.47	β-Farnesene
32.959	127,795	0.19	Germacrene D
33.075	33,468	0.05	β-Sesquiphellandrene
34.046	46,148	0.07	Eudesma-3,7(11)-diene
36.250	237,195	0.35	Caryophyllene oxide
-	67,222,018	100	-
	R. Time 30.852 31.211 31.400 32.088 32.959 33.075 34.046 36.250	R. Time Area 30.852 2,533,028 31.211 87,501 31.400 87,541 32.088 3,006,736 32.959 127,795 33.075 33,468 34.046 46,148 36.250 237,195 - 67,222,018	R. TimeAreaArea%30.8522,533,0283.7731.21187,5010.1331.40087,5410.1332.0883,006,7364.4732.959127,7950.1933.07533,4680.0534.04646,1480.0736.250237,1950.35-67,222,018100

Table 2. Cont.

In the current study, there was no evidence of an interaction between the lavender form and the dosage (p > 0.05; Table 3). On the other hand, the dosage of lavender had a significant effect on gas production for up to 12 h (p < 0.001 for 3 h; p < 0.01 for 6 h; p < 0.05 for 12 h), while the lavender forms had a significant effect on gas production at 3 h (p < 0.05). The gas production of the first 12-h period was reduced considerably when lavender (0.05% to 0.10%) was added to the diet of dairy cows. The decrease in gas production ranged between 24.24% and 25.13% in the third hour, 13.28% and 14.93% at the sixth hour, and 9.25% and 9.78% at the twelfth hour. The highest gas production was observed in LM (14.32 mL/200 mg DM) during the third hour, while the lowest production was observed in LEO (12.33 mL/200 mg DM).

Table 3. The effect of adding different forms of lavender on the cumulative gas production of concentrate feed of dairy cows.

Form	Dosage	3 h, mL	6 h, mL	12 h, mL	24 h, mL	48 h, mL		
	0%	16.00	29.00	36.00	58.23	62.23		
WL	0.05%	13.50	26.00	33.00	57.35	61.35		
	0.10%	12.00	26.50	33.50	56.78	60.78		
	0%	16.00	29.00	36.00	58.23	62.23		
LM	0.05%	14.00	26.00	35.00	59.47	62.97		
	0.10%	12.95	24.95	31.95	54.08	58.08		
	0%	16.00	29.00	36.00	58.23	62.23		
LEO	0.05%	10.00	22.00	30.00	50.36	53.86		
	0.10%	11.00	24.00	32.00	54.13	56.63		
SEM		0.83	1.34	1.26	1.76	2.19		
<i>p</i> -value		0.2142	0.5261	0.2930	0.1176	0.2856		
		Main e	ffect (Lavende	er form)				
WL		13.83 ^{ab}	27.17	34.17	57.45	61.45		
LM		14.32 ^a	26.65	34.32	57.26	61.09		
LEO		12.33 ^b	25.00	32.67	54.24	57.57		
SEM		0.48	0.78	0.73	1.01	1.27		
<i>p</i> -value		0.0417	0.1751	0.2587	0.0914	0.1089		
Main effect (Lavender dosage)								
	0%	16.00 ^a	29.00 ^a	36.00 ^a	58.23	62.23		
	0.05%	12.50 ^b	24.67 ^b	32.67 ^b	55.73	59.39		
	0.10%	11.98 ^b	25.15 ^b	32.48 ^b	55.00	58.49		
SEM		0.48	0.78	0.73	1.01	1.27		
<i>p</i> -value		0.0004	0.0063	0.0125	0.1140	0.1495		

WL: whole lavender; LM; lavender meal; LEO; lavender essential oil; SEM: standard error of mean. a: gas production from the immediately soluble fraction (mL); b: gas production from the insoluble fraction (mL); a + b: potential gas production (mL); ME: metabolizable energy (MJ/kg DM); NEL: net energy lactation (MJ/kg DM); OMD: organic matter digestibility (% DM).

A comparison of gas kinetics, in vitro degradability, and estimated energy values between lavender forms did not reveal significant differences (p > 0.05; Table 4). It was also established that there was no interaction between the form and dosage of lavender (p > 0.05). However, the immediately soluble fraction (a) of the diet was significantly reduced when lavender was added to the diets of dairy cows (p < 0.0001). However, the immediately soluble fraction (a) of the diet was significantly reduced when lavender was added to the diets of dairy cows (p < 0.0001). However, the immediately soluble fraction (a) of the diet was significantly reduced when lavender was added to the diets of dairy cows (p < 0.0001), whereas the gas production rate constant for the insoluble fraction (c), the gas production of the insoluble fraction (b), and the potential total gas production (a + b) were not affected. Additionally, the estimated energy values (ME and NEL) decreased linearly with increasing lavender dosage, and these differences were significant (p < 0.05). Moreover, adding lavender to dairy cow concentrate feed considerably decreased the OMD, which ranged from 5.17% to 6.14% in the groups with 0.05% and 0.10% lavender, respectively (p < 0.05).

Table 4. The effects of different doses and forms of lavender on gas kinetics, in vitro degradability, and estimated energy values.

Form	Dosage	С	а	b	a + b	ME	NEL	OMD
	0%	0.074	4.61	60.53	65.15	10.20	6.38	67.95
WL	0.05%	0.070	1.83	63.13	64.96	10.00	6.23	65.88
	0.10%	0.075	0.37	63.41	63.78	9.92	6.16	65.37
	0%	0.074	4.61	60.53	65.15	10.20	6.38	67.95
LM	0.05%	0.074	0.91	65.59	66.50	10.29	6.47	67.77
	0.10%	0.072	1.87	59.36	61.24	9.55	5.85	62.97
	0%	0.074	4.61	60.53	65.15	10.20	6.38	67.95
LEO	0.05%	0.073	0.00	56.89	56.89	9.05	5.43	59.66
	0.10%	0.076	0.00	59.85	59.85	9.56	5.86	63.01
SEM		0.003	0.51	1.95	2.28	0.23	0.18	1.49
<i>p</i> -value		0.7830	0.1561	0.1658	0.2741	0.0966	0.0806	0.0953
			Main e	ffect (Lavende	r form)			
WL		0.073	2.27	62.36	64.63	10.04	6.26	66.40
LM		0.073	2.46	61.83	64.29	10.02	6.23	66.23
LEO		0.074	1.54	59.09	60.63	9.60	5.89	63.54
SEM		0.002	0.29	1.12	1.32	0.13	0.11	0.86
<i>p</i> -value		0.8250	0.1133	0.1428	0.1101	0.0765	0.0648	0.0755
Main effect (Lavender dosage)								
	0%	0.074	4.61 ^a	60.53	65.15	10.20 ^a	6.38 ^a	67.95 ^a
	0.05%	0.072	0.91 ^b	61.87	62.78	9.78 ^{ab}	6.04 ^{ab}	64.44 ^b
	0.10%	0.074	0.75 ^b	60.87	61.62	9.68 ^b	5.96 ^b	63.78 ^b
SEM		0.002	0.292	1.12	1.32	0.13	0.11	0.86
<i>p</i> -value		0.7142	< 0.0001	0.6937	0.2100	0.0446	0.0486	0.0157

WL: whole lavender; LM; lavender meal; LEO; lavender essential oil; SEM: standard error of mean; *c*: the gas production rate constant (%) for the insoluble fraction (*b*); *a*: gas production from the immediately soluble fraction (mL); *b*: gas production from the insoluble fraction (mL); *a* + *b*: potential gas production (mL); ME: metabolizable energy (MJ/kg DM); NEL: net energy lactation (MJ/kg DM); OMD: organic matter digestibility (% DM).

In the current study, a significant effect of lavender forms was observed on CH₄ (p < 0.05), CO₂ (p < 0.01), and H₂S (p < 0.01) formation (Table 5). The addition of WL to the diet of dairy cows resulted in the highest concentrations of CH₄, CO₂, and H₂S, while LEO resulted in the lowest concentrations. On the contrary, there was no significant variation in NH₃ concentration between the different lavender forms added to the diet of dairy cows (p > 0.05). The addition of 0.05% lavender significantly reduced the concentration of CH₄ (p < 0.01); however, an increase in lavender dose did not yield a significant reduction in CH₄ concentration. With an increased dose of lavender, the concentration of NH₃ in dairy concentrate feed of dairy cows' increased significantly (p < 0.05). In addition, the presence

of lavender in the diet of dairy cows significantly reduced the generation of H_2S (p < 0.01). This study has also shown that there is a substantial interaction between lavender dosage and form that affects the production of CO₂, NH₃, and H₂S (p < 0.05).

Form	Dosage	CH ₄ , mL	CO ₂ , mL	NH ₃ , ppm	H ₂ S, ppm		
	0%	7.01	38.60 ^{abc}	150.6 ^{ab}	1373.8 ^a		
WL	0.05%	6.90	42.16 ^a	150.7 ^{ab}	1288.1 ^a		
	0.10%	7.23	41.60 ^{ab}	176.0 ^{ab}	1415.5 ^a		
-	0%	7.01	38.60 ^{abc}	150.6 ^{ab}	1373.8 ^a		
LM	0.05%	5.92	32.27 ^c	129.9 ^b	1069.2 ^{ab}		
	0.10%	6.83	37.86 ^{abc}	177.0 ^{ab}	1279.3 ^a		
-	0%	7.01	38.60 ^{abc}	150.6 ^{ab}	1373.8 ^a		
LEO	0.05%	5.51	32.74 ^{bc}	192.2 ^a	1024.7 ^{ab}		
	0.10%	6.41	31.93 ^c	176.6 ^{ab}	837.7 ^b		
SEM		0.27	1.63	9.4	69.9		
<i>p</i> -value		0.2058	0.0329	0.0448	0.0180		
		Main effect (L	avender form)				
WL		7.04 ^a	40.78 ^a	159.1	1359.1 ^a		
LM		6.58 ^{ab}	36.24 ^b	152.5	1240.8 ^a		
LEO		6.31 ^b	34.42 ^b	173.1	1078.8 ^b		
SEM		0.15	0.94	5.4	40.3		
<i>p</i> -value		0.0241	0.0028	0.0652	0.0028		
Main effect (Lavender dosage)							
	0%	7.01 ^a	38.60	150.6 ^b	1373.8 ^a		
	0.05%	6.11 ^b	35.72	157.6 ^{ab}	1127.3 ^b		
	0.10%	6.82 ^a	37.13	176.5 ^a	1177.5 ^b		
SEM		0.15	0.94	5.4	40.3		
<i>p</i> -value		0.0063	0.1522	0.0214	0.0045		

Table 5. The effects of different doses and forms of lavender on the formation of gases in vitro.

^{a-c}: Different superscripts on the same row differ by p < 0.05. WL: whole lavender; LM; lavender meal; LEO; lavender essential oil; SEM: standard error of mean; CH₄: methane; CO₂: carbon dioxide; NH₃: ammonia; H₂S: hydrogen sulfide.

4. Discussion

Two of the most urgent problems that humanity must solve as a result of the negative effects on the environment are a reduction in GHG emissions and the destruction of agricultural waste. Although studies on the influence of lavender on GHG emissions are scarce, these studies appear to focus on LEO [21,32]. Therefore, the purpose of this study was to examine the effect of different forms of lavender, which has increased in cultivation areas in recent years, on reducing GHG emissions.

In the current study, lavender had a dose-dependent influence on gas production, although the type of action altered as the incubation time increased. It is possible that, over a long period of incubation, the bacteria in the rumen may have developed tolerance to high doses of lavender. Similarly, during an in vitro investigation using LEO as an incubation medium at 0, 250, 750, and 1000 μ L/L, Yadeghari et al. [22] showed a dose–response effect of LEO on the rumen microbial ecosystem. Furthermore, previous studies have also indicated that LEO [22] and the dry extract of *L. officinalis* [33] have a stimulatory effect on ruminal fermentation. It was noted by Büyükkılıç Beyzi [21] that the stimulating action of LEO was dose-related, and that LEOs boost gas production even at low doses. In contrast to these studies, the current research found that adding lavender to dairy cow concentrate feed during the first 12 h of fermentation reduced gas production, although this effect disappeared as the incubation time increased.

It has been pointed out that the analysis of the various parameters of the gas production kinetics, including the fraction of easily degraded DM (*a*), the DM that is potentially degraded (a + b), as well as the rate kinetics of gas production (*c*), is reflective of the processes that occur during fermentation involving nutrition digestibility and ruminal microbial activity [34]. In the current study, adding 0.05% or 0.10% lavender to the concentrate feed of dairy cows had no significant influence on the potential for rumen degradation, indicating that the potential gas production curves remained the same as the control (0%), maintaining the rumen microbial community. The addition of LEO to dairy cow concentrate feed had a tendency to reduce OMD, ME, and NEL levels, and a statistically significant reduction was found as dosages increased. It is possible that this was due to the doses present in the concentrate (insoluble fraction, *b*) not reaching an inhibitory level for the enzymes required to break down lignocellulose. Furthermore, since the substrate was the same for all samples, the gas production capacity was determined by the extent to which the active compounds of each lavender form acted on the ruminal microorganisms.

Maintaining a healthy rumen microbiota, boosting feed digestibility for increased development and milk production, and minimizing ruminant CH₄ emissions are all key goals for nutritionists [33]. There is now a high need for feed additives that can modify rumen fermentation and are acceptable to consumers. Plant extracts and EOs appear to be the most suitable materials for this use. Previous research has indicated that these plant extracts and EOs can reduce CH₄ production in one of two ways: either by competing with substrates for propionate formation or by suppressing methanogenesis, therefore lowering CH₄ generation [34,35]. The analysis performed in this work was unable to ascertain which of the routes was responsible for the reduction in CH₄; nonetheless, LEO exhibited the highest rate of CH₄ reduction (-10.37%), followed by LM (-6.53%). After extraction, the active compounds in LEO are likely more concentrated, which explains why it is more effective in decreasing CH₄ generation. Cieslak et al. [35] attributed this occurrence to EOs' isoprenoid unit, a distinctive methanogen cell membrane structure that may result in cell death. In a similar vein, it is possible that this outcome occurred as a consequence of the fact that the isoprenoid units in LEO will be higher than those in WL and LM.

It is preferable to limit the conversion of dietary protein into NH₃ in the rumen, in addition to lowering the CH₄ emissions of the feed additives used. Previous research has shown that EO [11] and polyphenol-rich plant extract [36] increased the duodenal flow of undegraded protein in the rumen by reducing the conversion of ruminal protein into NH₃. Further research has shown that these feed additives did not reduce feed intake, dry matter intake, or total nutrient digestibility [10]. In the current study, a tendency of increased NH₃ levels was detected in dairy cow concentrate feed supplemented with LEO (p = 0.0652). The NH₃ ratio also increased significantly with higher lavender dosages (p < 0.05). This was an unexpected outcome, which may be related to lavender's limited ability to precipitate proteins. Similar to the current study's results, earlier research has shown that the concentrations, kinds, doses, and microbial species present in the rumen fluid all have a role in EOs' effectiveness in suppressing the growth of rumen microorganisms [11].

Animal feeding is connected to the production of H_2S , which is one of the toxic gases and has a severe impact on both the health of animals and the environment [37]. In the rumen and gut, sulfate-reducing bacteria create H_2S , which may be absorbed by the intestinal wall, leaving animals vulnerable to H_2S poisoning [38]. The present study found that adding lavender to dairy cows' diet lowered H_2S levels, with WL and LM being more effective than LEO in preventing the development of H_2S . With the addition of lavender to dairy cow concentrate feed, the H_2S ratio was reduced by 14.29–17.94%. Our results are comparable with those of Alvarado et al. [39], who reported that plant species, dose, and duration all contributed substantially to the decrease in H_2S emissions.

In the rumen, pyruvate decarboxylation results in the formation of acetyl-CoA, which is then converted into acetate. Changing the rumen's acetate-to-propionate ratio lowers gaseous emissions of CO_2 and H_2 [40]. Plant secondary metabolites have been shown to inhibit cellulolytic bacteria owing to their antibacterial and antiprotozoal capabilities [11].

As a consequence of decreasing the formation of short-chain fatty acids, levels of CO_2 and H_2 are brought down, leading to a corresponding reduction in CH_4 production [41]. In this study, LM and LEO were shown to be more successful in lowering CO_2 concentrations than WL. This finding is most likely associated with the tannin ratio in LM [42] and the 1,8-cineole and camphor levels in LEO [19].

5. Conclusions

A study was conducted to investigate the effect of waste released during the extraction of LEO on GHGs. The study's findings may have consequences for the livestock industry, as LM might be utilized as a feed addition to minimize GHG emissions. Similar to LEO, our results indicated that LM can be beneficial in lowering CH_4 , CO_2 , and H_2S ; however, there may be a drop in OMD and estimated energy values (ME and NEL) and an increase in NH_3 content with increasing dosage. Further research may be required to discover the optimum dosage of LM required to reduce GHG emissions while maintaining feed quality. The usage of LM could also aid the LEO industry economically, since waste material could be recycled as a feed supplement. On the other hand, this study was conducted in a controlled setting; thus, more research would be necessary to examine the influence of LM on GHGs in vivo using a cow model with an assessment of animal productive attributes.

Author Contributions: Conceptualization, L.C. and F.K.; methodology, L.C., M.L. and S.E.; software, S.E.; validation, L.C., S.G. and K.E.; formal analysis, L.C., S.G. and K.E.; investigation, M.L., V.P. and S.E.; resources, L.C., V.P. and S.E.; data curation, L.C., M.L., K.E., S.G., V.P. and S.E. writing—original draft preparation, M.L. and S.E.; writing—review and editing, M.L., S.E. and V.P.; supervision, F.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding. Open access funding was provided by the University of Applied Sciences Technikum Wien.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

References

- Singh, P.; Singh, J.; Kumar, A.; Wadhwa, M. Sustainable Utilization of Aloe Vera Waste in the Diet of Lactating Cows for Improvement of Milk Production Performance and Reduction of Carbon Footprint. J. Clean. Prod. 2021, 288, 125118. [CrossRef]
- Palangi, V.; Taghizadeh, A.; Abachi, S.; Lackner, M. Strategies to Mitigate Enteric Methane Emissions in Ruminants: A Review. Sustainability 2022, 14, 13229. [CrossRef]
- Kader Esen, V.; Palangi, V.; Esen, S. Genetic Improvement and Nutrigenomic Management of Ruminants to Achieve Enteric Methane Mitigation: A Review. *Methane* 2022, 1, 342–354. [CrossRef]
- 4. Bačėninaitė, D.; Džermeikaitė, K.; Antanaitis, R. Global Warming and Dairy Cattle: How to Control and Reduce Methane Emission. *Animals* 2022, 12, 2687. [CrossRef]
- 5. Pszczola, M.; Calus, M.P.L.; Strabel, T. Short Communication: Genetic Correlations between Methane and Milk Production, Conformation, and Functional Traits. *J. Dairy Sci.* 2019, 102, 5342–5346. [CrossRef]
- Mammi, L.M.E.; Guadagnini, M.; Mechor, G.; Cainzos, J.M.; Fusaro, I.; Palmonari, A.; Formigoni, A. The Use of Monensin for Ketosis Prevention in Dairy Cows during the Transition Period: A Systematic Review. *Animals* 2021, 11, 1988. [CrossRef]
- Beauchemin, K.A.; Ungerfeld, E.M.; Abdalla, A.L.; Alvarez, C.; Arndt, C.; Becquet, P.; Benchaar, C.; Berndt, A.; Mauricio, R.M.; McAllister, T.A.; et al. Invited Review: Current Enteric Methane Mitigation Options. J. Dairy Sci. 2022, 9297–9326. [CrossRef]
- 8. Honan, M.; Feng, X.; JM, T.; Kebreab, E. Feed Additives as a Strategic Approach to Reduce Enteric Methane Production in Cattle. *AFMA Matrix* 2022, *31*, 52–56. [CrossRef]
- Zeru, A.E.; Hassen, A.; Apostolides, Z.; Tjelele, J. Relationships between Agronomic Traits of Moringa Accessions and In Vitro Gas Production Characteristics of a Test Feed Incubated with or without Moringa Plant Leaf Extracts. *Plants* 2022, *11*, 2901. [CrossRef] [PubMed]
- 10. Wu, P.; Liu, Z.B.; He, W.F.; Yu, S.B.; Gao, G.; Wang, J.K. Intermittent feeding of citrus essential oils as a potential strategy to decrease methane production by reducing microbial adaptation. *J. Clean. Prod.* **2018**, *194*, 704–713. [CrossRef]

- Ugbogu, E.A.; Elghandour, M.M.M.Y.; Ikpeazu, V.O.; Buendía, G.R.; Molina, O.M.; Arunsi, U.O.; Emmanuel, O.; Salem, A.Z.M. The Potential Impacts of Dietary Plant Natural Products on the Sustainable Mitigation of Methane Emission from Livestock Farming. J. Clean. Prod. 2019, 213, 915–925. [CrossRef]
- 12. Fouts, J.Q.; Honan, M.C.; Roque, B.M.; Tricarico, J.M.; Kebreab, E. Enteric Methane Mitigation Interventions. *Transl. Anim. Sci.* **2022**, *6*, 1–16. [CrossRef] [PubMed]
- Ona, A.; Dan, V.; Muntean, L.; Rodica, V.; Stoie, A. Current Trends for Lavender (*Lavandula angustifolia* Mill.) Crops and Products with Emphasis on Essential Oil Quality. *Plants* 2023, 12, 357. [CrossRef]
- Wells, R.; Truong, F.; Adal, A.M.; Sarker, L.S.; Mahmoud, S.S. Lavandula Essential Oils: A Current Review of Applications in Medicinal, Food, and Cosmetic Industries of Lavender. *Nat. Prod. Commun.* 2018, *13*, 1403–1417. [CrossRef]
- Ratiarisoa, R.V.; Magniont, C.; Ginestet, S.; Oms, C.; Escadeillas, G. Assessment of Distilled Lavender Stalks as Bioaggregate for Building Materials: Hygrothermal Properties, Mechanical Performance and Chemical Interactions with Mineral Pozzolanic Binder. *Constr. Build. Mater.* 2016, 124, 801–815. [CrossRef]
- Bhatt, R.S.; Sarkar, S.; Sahoo, A.; Sharma, P.; Soni, L.; Saxena, V.K.; Soni, A. Dietary Inclusion of Mature Lemon Grass and Curry Leaves Affects Nutrient Utilization, Methane Reduction and Meat Quality in Finisher Lambs. *Anim. Feed Sci. Technol.* 2021, 278, 114979. [CrossRef]
- Bhatt, R.S.; Sahoo, A.; Sarkar, S.; Kumar, V.; Soni, L.; Sharma, P.; Gadekar, Y.P. Dietary Inclusion of Nonconventional Roughages for Lowering Enteric Methane Production and Augmenting Nutraceutical Value of Meat in Cull Sheep. *Anim. Feed Sci. Technol.* 2021, 273, 114832. [CrossRef]
- Mavandi, P.; Abbaszadeh, B.; Emami Bistgani, Z.; Barker, A.V.; Hashemi, M. Biomass, Nutrient Concentration and the Essential Oil Composition of Lavender (Lavandula Angustifolia Mill.) Grown with Organic Fertilizers. J. Plant Nutr. 2021, 44, 3061–3071. [CrossRef]
- González-rivera, J.; Duce, C.; Falconieri, D.; Ferrari, C.; Ghezzi, L.; Piras, A.; Rosaria, M. Coaxial Microwave Assisted Hydrodistillation of Essential Oils from Fi ve Different Herbs (Lavender, Rosemary, Sage, Fennel Seeds and Clove Buds): Chemical Composition and Thermal Analysis. *Innov. Food Sci. Emerg. Technol.* 2016, 33, 308–318. [CrossRef]
- Muscles, B.; Activity, A.; Responses, I.; Amer, S.A.; Abdel-Wareth, A.A.A.; Gouda, A.; Saleh, G.K.; Nassar, A.H.; Sherief, W.R.I.A.; Albogami, S.; et al. Impact of Dietary Lavender Essential Oil on the Growth and Fatty Acid Profile of Breast Muscles, Antioxidant Activity, and Inflammatory Responses in Broiler Chickens. *Antioxidants* 2022, 11, 1798. [CrossRef]
- 21. Büyükkılıç Beyzi, S. Effect of Lavender and Peppermint Essential Oil on In Vitro Methanogenesis and Fermentation of Feed with Buffalo Rumen Liquor. *Buffalo Bull.* **2020**, *39*, 311–321.
- Yadeghari, S.; Malecky, M.; Banadaky, M.D.; Navidshad, B. Evaluating in Vitro Dose-Response Effects of Lavandula Officinalis Essential Oil on Rumen Fermentation Characteristics, Methane Production and Ruminal Acidosis. *Vet. Res. Forum.* 2015, 6, 285–293. [PubMed]
- 23. AOAC. Official Methods of Analysis, 15th ed.; Association of Official Analytical Chemists: Arlington, VA, USA, 1990.
- 24. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef] [PubMed]
- 25. ISO 10520; Determination of Starch Content—Ewers Polarimetric Method. ISO: Geneva, Switzerland, 2000; pp. 1–8.
- 26. Manzur, M.; Luciardi, C.; Bl, M.A.; Alberto, R.; Cartagena, E. Citrus Sinensis Essential Oils an Innovative Antioxidant and Antipathogenic Dual Strategy in Food Preservation against Spoliage Bacteria. *Antioxidants* **2023**, *12*, 246. [CrossRef]
- Riu-Aumatell, M.; Castellari, M.; López-Tamames, E.; Galassi, S.; Buxaderas, S. Characterisation of Volatile Compounds of Fruit Juices and Nectars by HS/SPME and GC/MS. *Food Chem.* 2004, 87, 627–637. [CrossRef]
- Menke, K.H.; Steingass, H. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim. Res. Dev.* 1988, 28, 7–55.
- Goel, G.; Makkar, H.P.S.; Becker, K. Pycnocephalus Leaves and Fenugreek (*Trigonella foenum*-Graecum L.) Seeds and Their Extracts on Partitioning of Nutrients from Roughage- and Concentrate-Based Feeds to Methane. *Anim. Feed. Sci. Technol.* 2008, 147, 72–89. [CrossRef]
- Bağcık, C.; Koç, F.; Erten, K.; Esen, S.; Palangi, V.; Lackner, M. *Lentilactobacillus buchneri* Preactivation Affects the Mitigation of Methane Emission in Corn Silage Treated with or without Urea. *Fermentation* 2022, 8, 747. [CrossRef]
- 31. Menke, B.Y.K.H.; Raab, L.; Salewski, A.; Steingass, H.; Fritz, D.; Schneider, W. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor in vitro. *J. Agric. Sci.* **1979**, *93*, 217–222. [CrossRef]
- 32. Laleva, S.; Yordanova, D.; Kalaydzhiev, G.; Karabashev, V.; Ivanov, N.; Angelova, T.; Vasilev, V. Influence of Some Biologically Active Substances on Gas Production, Digesitibility, and Metabolic Energy in Different Feeds. *J. Hyg. Eng. Des.* **2022**, *39*, 3–8.
- Broudiscou, L.P.; Papon, Y.; Broudiscou, A.F. Effects of Dry Plant Extracts on Fermentation and Methanogenesis in Continuous Culture of Rumen Microbes. *Anim. Feed Sci. Technol.* 2000, 87, 263–277. [CrossRef]
- Nunes, H.P.B.; Dias, C.S.A.M.M.; Borba, A.E.S. Heliyon Bioprospecting Essential Oils of Exotic Species as Potential Mitigations of Ruminant Enteric Methanogenesis. *Heliyon* 2023, 9, e12786. [CrossRef]
- Cieslak, A.; Szumacher-Strabel, M.; Stochmal, A.; Oleszek, W. Plant Components with Specific Activities against Rumen Methanogens. *Animal* 2013, 7 (Suppl. 2), 253–265. [CrossRef] [PubMed]

- 36. Sinz, S.; Marquardt, S.; Soliva, C.R.; Braun, U.; Liesegang, A.; Kreuzer, M. Phenolic Plant Extracts Are Additive in Their Effects against in Vitro Ruminal Methane and Ammonia Formation. *Asian-Australas. J. Anim. Sci.* **2019**, *32*, 966–976. [CrossRef] [PubMed]
- Saksrithai, K.; King, A.J. Controlling Hydrogen Sulfide Emissions during Poultry Productions. J. Anim. Res. Nutr. 2018, 03, 1–14. [CrossRef]
- Santillán, M.K.G.; Khusro, A.; Salem, A.Z.M.; Pliego, A.B.; Elghandour, M.M.M.Y. Potential and Valorization of Salix Babylonica Waste Leaf Extract to Mitigate Equine Fecal Production of Methane, Carbon Monoxide, and Hydrogen Sulfide. Waste Biomass Valorization 2022. [CrossRef]
- Alvarado, T.D.; Elghandour, M.M.M.Y.; Ekanem, N.J.; Alcala-Canto, Y.; Velázquez, A.E.; Pacheco, E.B.F.; Purba, R.A.P.; Salem, A.Z.M. Influence of Azadirachta Indica and Cnidoscolus Angustidens Dietary Extracts on Equine Fecal Greenhouse Gas Emissions. J. Equine Vet. Sci. 2022, 116, 104049. [CrossRef] [PubMed]
- Elghandour, M.M.Y.; Vallejo, L.H.; Salem, A.Z.M.; Mellado, M.; Camacho, L.M.; Cipriano, M.; Olafadehan, O.A.; Olivares, J.; Rojas, S. Moringa Oleifera Leaf Meal as an Environmental Friendly Protein Source for Ruminants: Biomethane and Carbon Dioxide Production, and Fermentation Characteristics. J. Clean. Prod. 2017, 165, 1229–1238. [CrossRef]
- Zhou, W.; Pian, R.; Yang, F.; Chen, X.; Zhang, Q. The Sustainable Mitigation of Ruminal Methane and Carbon Dioxide Emissions by Co-Ensiling Corn Stalk with Neolamarckia Cadamba Leaves for Cleaner Livestock Production. J. Clean. Prod. 2021, 311, 127680. [CrossRef]
- 42. Nurzyńska-Wierdak, R.; Zawiślak, G. Chemical Composition and Antioxidant Activity of Lavender (*Lavandula angustifolia* Mill.) Aboveground Parts. *Acta Sci. Pol. Hortorum Cultus* **2016**, 15, 225–241.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.