Effect of oregano oil on milk yield, methane emissions and feed efficiency of dairy cows

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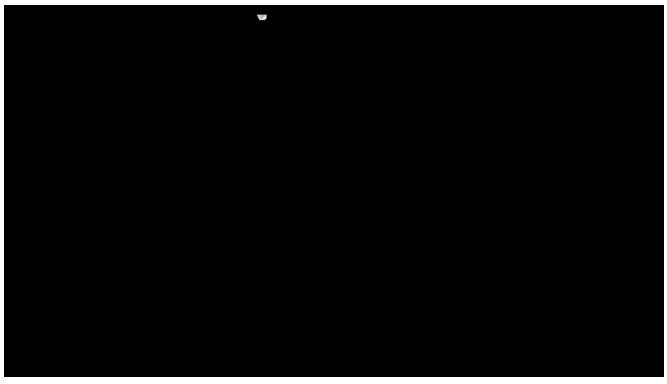
By



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Abstract

Agricultural systems contribute significant amounts of greenhouse gas emissions (**GHG**) emissions, being responsible for roughly 30% of emissions, which consist of carbon dioxide, nitrous oxide, and methane (**CH_4**), with the latter of which is released due to enteric fermentation by ruminant livestock and responsible for up to 14.5% of the global GHG emissions. Naturally occurring essential oils (EE) from oregano oil (**OO**) have the potential to reduce methane production, which is mediated through the rumen microbiome in ruminants, making EE an ideal mitigation strategy that would be useful to production systems including organic. The aim of this study is to assess the effect of **OO** in the pre and/or postpartum partial mixed ration (**PMR**) of dairy cows to assess the effect on postpartum milk yield, feed efficiency, and methane emissions of lactating dairy cows. Methane emissions were lower from cows offered OO (P 0.005), reducing daily ruminal methanogenesis from lactating cows offered OO by approximately 10% per cow and lowering **CH_4** emissions per kg of energy-corrected milk (P 0.001), milk protein and solids yield (P 0.002) compared to cows not offered OO. Moreover, cows offered OO had greater milk (P 0.005), energy-corrected milk (P 0.015), milk fat (P 0.015), and milk solids yield (P 0.006), and greater milk lactose concentrations (P 0.005) compared with cows not offered OO. Dry matter intake (**DMI**), live weight and body condition score did not differ between cows offered a PMR with and without OO. In conclusion, offering lactating cows OO in the PMR lowered daily CH_4 emissions per cow by 10%, and increased milk and milk fat, and solids yields, without increasing DMI, or affecting the live weight and body condition score of dairy cows. This lowered CH_4 emissions per kg of ECM, protein, and solids yield, which improves the sustainability of global food security.

Keywords: Oregano, Essential oil, Methane, Dairy, Yield

Literature Review

Global warming, greenhouse gas and methane emissions

Agricultural systems contribute significant amounts of greenhouse gas emissions (**GHG**) emissions, being responsible for roughly 30% of emissions, which consist of carbon dioxide (**CO_2**), nitrous oxide (**N_2O**) and methane (**CH_4**), with the latter of which is released due to enteric fermentation by ruminant livestock, which are responsible for up to 14.5% of the global GHG emissions (Kristiansen et al. 2020; Bačėninaitė et al. 2022). Carbon dioxide is known for having the greatest radiative forcing, by which the amount of energy that enters the atmosphere is not equal to the amount of energy that leaves the atmosphere, of all the anthropogenic GHG, followed by CH_4 (Anthropogenic and Natural Radiative Forcing, 2014). While carbon dioxide and CH_4 increase in the atmosphere, CH_4 has been identified as a short-lived climate pollutant (**SLCP**) due to the change in the radiative forcing and following long periods of constant emissions, there will be no further net accumulation in the atmosphere when the atmospheric SLCP and radiative forcing remains roughly constant (Anthropogenic and Natural Radiative Forcing, 2014). However, carbon dioxide warming will continue to increase while there are net positive carbon dioxide emissions (Pressman et al. 2023). While, methane has a short atmospheric lifetime of approx. 12.4 years (Myhre et al. 2013) and approx. 80 to 89 % of the total atmospheric CH_4 is removed by hydroxyl oxidation (Badr et al. 1992; Kirschke et al. 2013; He et al. 2019) and other sinks, which include reactions with stratospheric chlorine and oxygen atoms, uptake by soil, and reactions with marine chlorine atoms (Kirschke et al. 2013; Saunois et al. 2019). This means that the SLCP induced-warming related to CH_4 in the atmosphere should stabilize and not lead to warming in 20 years, due to an annual decline of 0.3% in the rate of CH_4 emissions (Cain et al. 2019). While ruminant livestock generates CH_4 from enteric fermentation by methanogenic microorganisms such as archaea that expel excess hydrogen (**H_2**) and produce CO_2 from rumen protozoa, bacteria, and anaerobic fungi (Morgavi et al. 2010; Martin et al. 2010; Tapio et al. 2017) that play an essential role in lowering CH_4 emissions to manage global warming, because the SLCP induced-warming from CH_4 represents one of the greatest opportunities to reduce the rate at which global temperatures are rising and as such reduce global warming over the next 20 years (Cain et al. 2019) and as such much research has assessed CH_4 emissions from ruminant livestock systems.

Measurement and estimation of methane emissions from ruminant livestock

Cattle produce CH_4 mainly by eructation of gases produced during rumen fermentation and only a very small amount of CH_4 from hind gut fermentation (EPA, 1995) and differing CH_4 collection and measurement methods hold various advantages and disadvantages that need

consideration when evaluating research studies (Bačėninaitė et al. 2022). Some studies have used respiration chambers for the measurement of CH_4 emissions, which produces accurate results, however, these are applied to individual animals that are placed in an enclosed and temperature controlled environment and while this facilitates the measurement of feed intake, rumen and hind gut enteric emissions, this type of measurement is relatively expensive due to equipment and labour costs (Tedeschi et al. 2022) and results in relatively short study periods of 2 to 7 d (Bačėninaitė et al. 2022) that involve relatively few (2 to 4) and even single animals that were separated from the herd and typical environment and this can be stressful and affect feed intake and CH_4 emissions (Tedeschi et al. 2022).

Studies that involved larger groups of animals have included the application of face masks, which are a relatively non-invasive method of collecting CH_4 emissions that requires animals to wear a mask to enable CH_4 levels to be recorded continuously in most environmental conditions either outdoors and/or indoors, while being relatively portable and inexpensive compared to respiration chambers and some other CH_4 measurement methods (Hill et al. 2016; Tedeschi et al. 2022). The main problem with the application of face masks is that this fails to measure hindgut emissions, which will underestimate the effect of diet on CH_4 emissions (Hill et al. 2016; Tedeschi et al. 2022) however, the large intestines of ruminants only produce a small portion of CH_4 that is expelled as flatulence (EPA, 1995). Some of the newer methods involve spot sampling of a larger group of animals, using a technique that uses gas-flux quantification systems, which are relatively low-cost and rely on sensors to capture a continuous measurement of emissions while cattle are feeding and/or being milked, using an automated data capture method that is non-invasive and is used to expresses the overall daily mean by calculating this from the intermittent measurements rather than the specific CH_4 emissions (Garnsworthy et al. 2019; Bačėninaitė et al. 2022). The application of face masks, spot sampling using a gas-flux quantification system and measurement of CO_2 using a sulphur hexafluoride (**SF6**) tracer technique has been found to be a relatively accurate method for measuring CH_4 emissions and have the advantage of allowing animals to remain in the herd within the typical environment reducing the potential effect of environmental changes that isolation and confinement may have, however SF_6 tracers are more invasive as this requires a greater amount of contact with the animal, which may affect natural animal behaviour, while variations due to the angle of the cow head and incorrect use can lead to over or under estimation of CH_4 levels (McGinn et al. 2006; Tedeschi et al. 2022).

Methane production by dairy cows

The amount of CH_4 emissions produced by dairy cows depends on the stage of production, age, live weight (Hardan et al. 2022; IPCC 2019; Shibata and Terada 2010) and the quantity

and composition of feed offered in the diet, due to its impact on the nutrients available to the rumen microorganisms and the microbiome profile (Shibata and Terada, 2010). The organisation of agriculture into production systems is essential to the efficient use of on-farm resources, which minimizes the adverse effects on food production costs, along with the environment and people by preserving the natural productivity and quality of the land and water, while increasing the production of food and fibre and subsequent agricultural production margins that helps to sustain vibrant rural communities (Sassenrath et al. 2009; Hofman-Bergholm 2022; dos Reis et al. 2023). Dairy production is no exception and this has long been described using a five-systems approach according to diet in the southern region of Australia, which comprises of the south-east seaboard regions of Victoria and New South Wales, and Tasmania (Soriano, 2022) and the North and South Island of New Zealand (Dairy NZ, 2023) where dairy cows are more likely to calve in Spring and largely graze perennial ryegrass for up to 365 d annually, utilizing more homegrown feeds and consuming less supplementary feeds (Margerison, 2022; Soriano, 2022; Dairy NZ, 2023).

In the UK dairy cows are typically not able to graze throughout the year due to greater annual rainfall and lower winter temperatures compared with dairy-producing areas of Australia and New Zealand and a declining number of grazing days increases supplementary forages and/or concentrated feed required (Margerison, 2022; Soriano, 2022; Dairy NZ, 2023), which increases feed imports onto the farm and into the country, which increases the financial cost and carbon footprint of feed provision unless substantial amounts of coproducts can be used and the carbon footprint can be attributed to the associated food and/or biofuel production (Wilkinson and Garnsworthy, 2016; Margerison 2022). Higher dietary fibre concentrations increase CH_4 production (Bačėninaitė et al. 2022; Wang et al. 2023), while greater starch and protein concentrations lower CH_4 production per cow (Wilkinson and Garnsworthy, 2009; Shibata and Terada, 2010). While greater feed intake increases CH_4 production, resulting in cows that consume more feed to produce milk producing more CH_4 due to more nutrients being available to the CH_4 producing microbes (Benchaar et al. 2001; Bačėninaitė et al. 2022; Pereira et al. 2023). Benchaar et al. (2001) found that increasing the proportion of concentrated feed and replacing more slowly degrading with rapidly degrading starch increased propionate and lowered organic matter fermentation in the rumen and decreased CH_4 production in relation to gross and digestible energy intake. While increasing feed efficiency, increasing the milk yield of dairy cows has been successful in increasing feed efficiency and lowering the carbon footprint associated with milk production by diluting the amount of nutrient related to the maintenance of the animal (Capper et al. 2009; Garnsworthy et al. 2020).

Reducing enteric methane emissions from ruminants

Methane emitted by ruminant species can be altered depending on the diet offered and a study that assessed nine differing diets to assess methane emissions in relation to the gross and digestible energy intake and found that CH_4 emissions were lowered due to changing fermentation towards propionate production and/or reducing the extent of ruminal fermentation by methanogens by increasing dietary starch and lowering dietary fibre concentrations using greater maize silage and supplementary feeds (Benchaar et al. 2001; Vellinga and Hoving. 2011). Other options include using diet supplements to lower methane production using synthetic (3-NOP) and naturally occurring products of which a well-known supplements being used includes seaweed (Asparagopsis), essential oils and oregano (which contain phytonutrients thymol and carvacrol) (Oh et al; 2017). Red seaweed in some cases has been shown to reduce enteric CH_4 production by up to 99% (Roque et al. 2021) due to bioactive bromoform that naturally occurs in red seaweed that reduces CH_4 production by ruminant livestock by acting as in inhibitor of CH_4 production (Bačėninaitė et al. 2022) from cereal and grass-based diets (Roque et al. 2019; Min et al. 2021a). Bromochloromethane and bromoform containing seaweed have been found to be the most effective inhibitors of enteric CH_4 production due to interference with methanogenesis (Wood et al, 1968; Goel et al. 2009). In a study performed by Bačėninaitė et al, bromoform was seen to increase the animal's weight and due to the mass growth of seaweed that would need to be harvested for this supplement to be commonly used, it is possible that the oceans pH could rise (ocean acidification). The long-term feeding effects and efficiency of seaweed is unknown but when red seaweed is added to cattle diets, it has effects on animal health, palatability, milk and meat quality as well as reproduction (Min et al. 2021b). The above study also looked at essential oils which could reduce methane production by up to 22% (Rossi et al. 2022). Some essential oils that are used to decrease methane formation include cinnamon bark, tea tree oil, cedarwood and cumin oils (Chao et al. 2000; Carrazco et al. 2020). These essential oils are seen to in some cases effect the gram-positive bacteria which may be beneficial as they can act as inhibitors for methane production (Patra. 2011; Carrazco et al. 2020). They also found that these essential oils could improve the milks fat and protein content and reduce the somatic cell count (SCC) (Belanche et al. 2020; Rossi et al. 2022). It is seen that these essential oils can alter the rumens microbial metabolism by reducing the plethora of archaea or by inhibiting fibrolytic bacteria that provide hydrogen for methanogenesis (Burt. 2004; Pauli and Schilcher. 2009; Patra. 2012; Cobellis et al. 2016). Essential oils from oregano are thought to have some antimicrobial properties which could be used in methane reduction strategies in organic production systems (Tekippe et al. 2011; Hristov et al. 2013; Olijhoek et al. 2019). Oregano oil can be seen to decreases methane production in a dose dependent way at high doses of >300mg/L in vitro (Benchaar and Greathead. 2011).

Methods and Materials

Aim, objectives and hypothesis

The aim of this study was to assess the effect of oregano oil (OO) in the pre and/or postpartum diets of dairy cows on the postpartum milk yield, feed efficiency, feed intake, and methane emissions of lactating dairy cows. The objective was to measure the effect on milk yield, milk component concentration, milk component yield, feed intake, rumen fluid pH, volatile fatty acid production and methane emissions and body condition, live weight, and feed efficiency of lactating dairy cows. The hypothesis was that the addition of Oregano oil to dairy cows diets prior to and following parturition may increase milk yield, feed efficiency, and lower methane emissions.

Materials and methods

Location

This study was carried out at the University of Nottingham Centre of Dairy Research and Innovation (CDSI) (Sutton Bonington, Loughborough, Leicestershire, UK) from December 2016 until August 2017, with the prior approval of the University of Nottingham Animal Ethics Committee. All procedures were completed in accordance with the requirements of the Animal Scientific Procedures Act (ASPA, 1986) and the Home Office.

Animals, treatment diet and animal feeding

The experiment was a randomized block design where 52 Holstein dairy cattle (22 primiparous; 30 multiparous) were selected at random according to the calving date from the CDSI dairy herd and allocated into equal blocks of similar calving date, parity, previous/genetic merit for live weight, milk yield, and body condition and offered one of three experimental diets consisting of offering a PMR that had no OO (None) or 10 g/hd/d of OO that was added to the diet postpartum (None:OO) and 21 d pre and postpartum (OO: OO) for 60 d pp. All animals were offered a partial mixed ration that was formulated to be iso-energetic and iso-nitrogenous on a CP and ME basis (Table 1).

The OO within the treatment diets was included as an active ingredient in combination with bulking agent's limestone flour and wheat feed to carry and substitute for OO in all PMRs. The nutrient requirements for maintenance, pregnancy, activity and in pp additional energy was provided for milk production of 32 L/d were met in each diet. The pp cows were offered a pelleted dairy concentrate (CP 18%; ME 12.4 MJ/Kg) during milking at 0.45 kg FM / L of milk

produced over and above 32 L/d rising from 2 kg/d to a maximum of 12 kg/d at 35 d pp. The maximum rate was offered for the following 60 d to a maximum of 95 d pp. All animals were offered ad-libitum access to fresh, clean water and PMR. The OO was included as an active ingredient in combination with bulking agent's wheat feed and limestone flour to carry and substitute for OO in all PMRs. The PMR was formulated to meet the requirements for maintenance, plus a milk production of 32 L/d, plus a pelleted dairy concentrate (CP 18%; ME 12.4 MJ/Kg) that was offered at milking at 0.45 kg FM / L of milk produced over and above 32 L/d rising from 2 kg/d to a maximum of 12 kg/d at 35 d pp. All animals were offered ad-libitum access to clean, fresh water and PMR.

Table 1: Diet formulation and nutrient composition of partial mixed rations

Ingredients (kg DM/d/cow)	Prepartum	Postpartum
Grass silage	12.69	28.64
Whole crop	12.55	22.40
Maize silage	16.52	21.94
Wheat straw	23.42	7.38
Soya and rape meal (50:50)	22.46	15.47
Limestone flour	-	0.33
Minerals [1]	0.14	0.15
Soyclor	10.35	-
Molasses	-	2.22
Treatment	0.58	0.58
Urea	-	0.34
Salt	-	0.26
Toxisorb	-	0.17
Binder	0.10	0.10
Composition		
Dry matter, %	37.0	46.6
CP, % DM	10.1	16.7
ME, MJ/Kg DM	10.2	11.7
NDF, % DM	37.2	39.4
Starch and sugar, %DM	21.1	26.1
Oil, %DM	4.7	4.0

[1] - Minerals and vitamins**;** calcium, 18%; phosphorus, 10%; magnesium, 5%; salt, 17%; copper, 2,000 mg/kg; manganese, 5,000 mg/kg; cobalt, 100 mg/kg; zinc, 6,000 mg/kg; iodine, 500 mg/kg; selenium, 25 mg/kg; vitamin A, 400,000 IU/kg; vitamin D_3, 80,000 IU/kg; and vitamin E, 1,000 mg/kg (ABN Ltd., Peterborough, UK).

Animal management

Prior to pp cows were maintained in deep litter straw beds and individually offered the experimental treatment. Following pp cows were transferred and maintained in a single pen fitted with free stall beds, which allowed 5% more stalls than animals. The stalls were fitted with cantilever divisions and rubber mattress, which was layered with sawdust and lime flour that was replenished daily.

Measurements

Milk yield, milk composition and live weight

Milk yield was recorded daily using a “Lely Astronaut A4” milking robot (Lely, Marknesse, the Netherlands) inline milk monitoring system up until 100 days in milk for individual animals. Milk composition was measured weekly from milk samples automatically collected in the milking robot (twice daily). The samples were analyzed for protein, milk fat, urea, and lactose whilst SCC were assessed via an infrared analyzer (Milko Scan FT 6000, Foss Electric, Hillerod, Denmark; AOAC International, 2002; method 972.16) and reported as a weekly mean. Animal weight was automatically recorded daily for each individual cows when milked in the robot (Lely Astronaut A3; Lely UK Ltd., St Neots, UK) using load cells (T4C 3.0; Lely UK Ltd., St Neots, UK).

Feed intake, feed sampling and composition

Individual pp feed intake was measured automatically using PMR feeding bins (Hokofarm Group B.V., Marknesse, the Netherlands) and pelleted concentrate feed intake was automatically recorded in the robotic milking system (Lely Astronaut A3; Lely UK Ltd., St Neots, UK). Forage and pelleted concentrate feed samples were taken weekly and analyzed for oven DM at 60°C/ 48 h to calculate DMI. The feed samples were ground (1mm) and analyzed for CP using the Kjeldahl method (method 984.13), ether extract (method 920.39), ADF, NDF (Van Soest *et al.,* 1991) and ash by heating at 600°C/ 8 h (method 942.05) according to AOAC (1990). To calculate total DMI, individual PMR and concentrate intake were used, using fresh matter intake and feed DM concentrations which were measured daily.

Body condition and animal health

Individual animal body condition score was assessed weekly using the Penn State University Method on a 1 to 5 scale. Pre and pp animal health was assessed and noted, including animals removed from treatment due to pre or pp ill health.

Feed conversion efficiency

Feed conversion efficiency of individual animals was calculated by dividing the daily mean DMI by the daily mean energy corrected (milk yield, kg x milk fat, g/kg / 35.0 g milk fat / kg) milk protein yield, milk yield and total milk solids yield (kg milk fat = kg milk protein). The feed efficiency was calculated by dividing the mean daily EC milk yield, milk protein yield and total milk solids yield by the daily total DMI.

Methane emissions

Methane from individual animals was measured via a methane analyzer equipped in the "Lely Astronaut A4" milking robot (Lely, Marknesse, the Netherlands) using an inline monitoring system developed at Nottingham University and the methane emissions were measured and recorded automatically during milking for each individual animal.

Statistical analysis

Data was collected and stored using Excel (Microsoft, USA) and statistical analysis was carried out using Minitab 17.0 (Minitab, 2015, USA). The data was evaluated for normality of distribution and using Log base^{10} the somatic cell count was transformed, showing the SCC, milk component yield, milk yield, methane production, milk composition, feed efficiency and DMI was normally distributed. All data was analysed using ANOVA general linear model (GLM) command to compare the treatment diets in a 'pair wise' basis, where the animal is deemed a random effect and the diet as a fixed effect. Data was reported as weekly least squares means with individual standard errors (± SE) in tables according to measurement and treatments pre partum: pp diets; None, OO and OO: OO. A Tukey' test was applied to identify the existence of significance difference between treatment diet means with a confidence interval of 0.95 and the relevant P values were reported in the Tables as P value <0.05 and tendencies were stated at $P < 0.10$ and were reported according to measurement and treatment.

Results

Milk yield and composition

Lactating dairy cows offered PMR with OO pre and postpartum had greater milk yield, EC milk yield, milk lactose concentrations, milk fat, and milk solids yield compared with cows not offered OO (None), and cows offered OO post-partum (Table 2). Milk protein yield was greater from cows offered OO pre and postpartum compared with cows offered no OO (None). The concentration of milk fat, protein, and urea did not differ between cows offered OO and not

offered OO, while cows offered OO following partition had lower milk SCC compared with cows not offered OO and offered OO pre and postpartum.

Table 2: Mean milk yield, milk component concentration and yield from dairy cattle offered no oregano oil (None); oregano oil postpartum (None: OO) and pre and postpartum (OO:OO)

	None	None: OO	OO: OO	P value
Milk yield, kg/d	39.1 (080) [b]	42.1 (0.93) [a]	42.6 (0.91) [a]	0.005
EC milk yield, kg/d [1]	39.1 (0.99) [b]	43.0 (0.99) [a]	43.1 (1.00) [a]	0.015
Milk fat, g/kg	41.1 (0.72)	41.5 (0.73)	41.1 (0.78)	0.951
Milk protein, g/kg	31.9 (0.31)	31.6 (0.29)	31.7 (0.32)	0.831
Milk lactose	47.1 (0.15) [b]	48.3 (0.15) [a]	48.1 (0.16) [a]	0.005
Milk fat, kg/d	1.55 (0.043) [b]	1.69 (0.023) [a]	1.70 (0.046) [a]	0.015
Milk protein, kg/d	1.22 (0.024) [b]	1.30 (0.026) [a, b]	1.33 (0.027) [a]	0.006
Milk solids, kg/d	2.76 (0.061) [b]	2.97 (0.065) [a]	3.01 (0.068) [a]	0.008
Milk urea,	23.1 (0.45)	24.1 (0.45)	24.0 (0.46)	0.149
SCC, cells/ml [2]	2.11 (0.039) [a]	1.91 (0.04) [b]	2.10 (0.26) [a]	0.019

[a, b] - Means in rows followed by differing superscript letters differ significantly [1] – EC to 4% butterfat and 3.3% protein. [2] - 00,000 in log base [10]

Live weight and body condition score

Table 3: Mean weekly live weight (±SE) of dairy cattle offered no oregano essential oil (None); oregano essential oil postpartum (None:OO) and pre and postpartum (OO: OO)

	None	None:OO	OO:OO	P value
7 d prepartum, kg	670 (17.0)	653 (18.3)	673 (18.9)	0.730
7 d postpartum, kg	665 (16.3)	651 (17.6)	665 (18.1)	0.807
14 d postpartum, kg	657 (16.1)	648 (17.4)	660 (17.9)	0.882
21 d postpartum, kg	653 (15.9)	646 (17.2)	659 (17.7)	0.875
28 d postpartum, kg	653 (15.5)	648 (16.7)	661 (17.2)	0.845
35 d postpartum, kg	653 (15.3)	648 (16.5)	661 (17.0)	0.897
42 d postpartum, kg	659 (15.5)	652 (16.7)	657 (17.2)	0.960
49 d postpartum, kg	660 (15.1)	655 (16.3)	661 (17.0)	0.960
56 d postpartum, kg	664 (15.0)	657 (16.2)	664 (16.6)	0.947
63 d postpartum, kg	667 (15.9)	560 (15.9)	668 (16.1)	0.962

There was no difference in the LW (table 3) and BCS (Table 4) of dairy cows offered and not offered OO.

Table 4: Mean weekly body condition (±SE) of dairy cattle offered no oregano essential oil (None); oregano essential oil postpartum (None:OO) and pre and postpartum (OO: OO)

21 d prepartum	2.8 (0.08)	2.8 (0.08)	2.9 (0.08)	0.314
14 d prepartum	2.8 (0.08)	2.8 (0.08)	2.9 (0.08)	0.315
7 d prepartum	2.8 (0.08)	2.8 (0.08)	2.9 (0.08)	0.315
7 d postpartum	3.0 (0.20)	2.8 (0.14)	3.0 (0.16)	0.516
14 d postpartum	2.7 (0.07)	2.7 (0.07)	2.7 (0.08)	0.745
21 d postpartum	2.7 (0.06)	2.6 (0.06)	2.7 (0.07)	0.795
28 d postpartum	2.5 (0.06)	2.6 (0.07)	2.7 (0.07)	0.373
35 d postpartum	2.5 (0.05)	2.5 (0.06)	2.6 (0.06)	0.358
42 d postpartum	2.5 (0.05)	2.6 (0.05)	2.6 (0.05)	0.395
49 d postpartum	2.5 (0.05)	2.6 (0.06)	2.6 (0.06)	0.691
56 d postpartum	2.5 (0.07)	2.5 (0.06)	2.6 (0.06)	0.277
63 d postpartum	2.5 (0.06)	2.6 (0.07)	2.6 (0.07)	0.460

Rumen fluid pH, fatty acid and ammonia concentrations, and methane emissions

There was no difference in the rumen concentrations of acetic, propionic, acetic:propionic ratio iso-butyric, n-butyric acids and ammonia between 7 and 28 d pp (Table 5), while mean propionic and iso-butyric acids tended to be greater in rumen fluid of cows offered OO and n-butyric acid concentration tended to be higher in rumen fluid of cows not offered OO.

Table 5: Postpartum mean acetic (A), propionic (P), A:P ratio, isobutyric, n-butyric acid concentrations (± SE) of rumen fluid of dairy cattle offered no oregano essential oil (None); oregano oil postpartum (None: OO) and pre and postpartum (OO: OO)

	None	None: OO	OO: OO	P value
Acetic acid				
At 7 d pp, umol/l	440 (25.1)	441 (24.9)	443 (22.8)	0.784
At 14 d pp, umol/l	439 (35.0)	485 (32.9)	469 (31.17)	0.590
At 28 d pp, umol/l	438 (33.2)	481 (32.1)	521 (31.1)	0.301
Mean acetic acid, umol/l	445 (18.5)	455 (18.2)	456 (17.7)	0.350
Propionic acid				
At 7 d pp, umol/l	343 (25.3)	332 (23.5)	349 (24.8)	0.801
At 14 d pp, umol/l	301 (45.1)	378 (43.2)	383 (40.1)	0.211
At 28 d pp, umol/l	323 (37.1)	361 (32.9)	398 (32.9)	0.201
Mean propionic acid, umol/l	312 (20.4)	360 (20.1)	376 (19.5)	0.091
Acetic: Propionic (A:P) ratio				
At 7 d pp, umol/l	1.4 (0.07)	1.5 (0.07)	1.4 (0.08)	0.512
At 14 d pp, umol/l	1.4 (0.09)	1.5 (0.08)	1.4 (0.08)	0.210
At 28 d pp, umol/l	1.4 (0.08)	1.3 (0.08)	1.2 (0.08)	0.461
Mean A:P acid ration, umol/l	1.5 (0.06)	1.5 (0.06)	1.4 (0.06)	0.211
Iso-butyric acid				
At 7 d pp, umol/l	11.1 (1.05)	14.0 (1.08)	13.1 (0.99)	0.312
At 14 d pp, umol/l	11.1 (1.46)	14.2 (1.40)	13.6 (1.30)	0.291
At 28 d pp, umol/l	11.5 (1.51)	12.1 (1.44)	14.4 (1.39)	0.309
Mean iso-butyric acid, umol/l	11.3 (0.75)	13.1 (0.73)	13.1 (0.59)	0.081
n-butyric acid				
At 7 d pp, umol/l	237 (16.5)	244 (18.4)	239 (17.4)	0.991
At 14 d pp, umol/l	271 (25.3)	292 (25.9)	241 (27.9)	0.381
At 28 d pp, umol/l	289.9 (24.8)	249.1 (27.1)	239 (26.1)	0.096
Mean n-butyric acid, umol/l	260.5 (12.5)	261.0 (13.1)	241.5 (12.9)	0.231
Ammonia				
At 7 d pp, umol/l	4601 (631.0)	5599 (678.0)	4811 (661.0)	0.501
At 14 d pp, umol/l	4501 (640.0)	4569 (675.0)	5006 (679.0)	0.492
At 28 d pp, umol/l	4554 (741.0)	5201 (759.0)	5671 (789.0)	0.681
Mean Ammonia, umol/l	4400 (381.0)	5301 (389.0)	5321 (389.0)	0.181

Dry matter intake, feed efficiency and feed conversion efficiency

Table 6: Postpartum mean rumen fluid pH, feed intake (DMI), feed efficiency and methane emissions (±SE) of dairy cattle offered no oregano essential oil (None); oregano essential oil postpartum (None: OO) and OO pre and postpartum (OO: OO)

	None	None: OO	OO: OO	P value
Rumen fluid pH, 7 d pp	6.4 (0.08)	6.5 (0.07)	6.4 (0.09)	0.461
Rumen fluid pH, 14 d pp	6.5 (0.07)	6.6 (0.08)	6.6 (0.08)	0.611
Rumen fluid pH, 28 d pp	6.6 (0.08)	6.5 (0.09)	6.6 (0.08)	0.401
Mean rumen fluid pH	6.6 (0.06)	6.6 (0.06)	6.6 (0.06)	0.598
Mean DMI, kg/d	19.9 (0.35)	19.9 (0.38)	19.4 (0.37)	0.441
DMI, kg/kg EC yield	0.52 (0.015) [a]	0.48 (0.015) [b]	0.47 (0.016) [b]	<0.001
DMI, kg/kg protein	16.6 (0.28) [a]	15.6 (0.30) [b]	14.8 (0.14) [b]	<0.001
DMI, kg/kg milk solids	7.24 (0.145) [a]	6.68 (0.158) [b]	6.44 (0.166) [b]	<0.001
CH_4 per cow, g/d	361 (1.4) [a]	330 (1.8) [b]	329 (1.9) [b]	0.005
CH_4 per kg EC yield, g	9.9 (0.28) [a]	8.1 (0.30) [b]	8.0 (0.32) [b]	0.001
CH_4 per kg milk protein, g	301 (6.6) [a]	275 (6.9) [b]	262 (7.4) [b]	0.002
CH_4 per kg milk solids, g	136 (3.5) [a]	122 (3.5) [b]	116 (3.7) [b]	0.002

[a, b] - Means in rows followed by differing superscript letters differ significantly

Rumen fluid pH and mean DMI did not differ between cows offered and not offered OO. Feed efficiency of cows offered OO was greater while CH_4 emissions were lower per cow, kg EC, milk protein and MS yield compared with cows not offered OO.

Discussion

This study assessed the effect of OO on the productivity, FE and CH4 emissions from lactating dairy cows. This is due to livestock farmers having to lower GHG emissions (Bellarby et al. 2013) and antimicrobial use by 2050 in accordance with regulations that ban the use of antimicrobials in livestock feed in Europe (European Union, 2003) and the UK necessitating the application of natural occurring substances, such as essential oils (**EO**), which will help enhance the public perception of the livestock industry by applying them as suitable alternatives to antibiotics (Benchaar et al. 2008; 2009) that have the potent to lower CH_4 emissions. These EO, including OO, that have high concentrations of phenolic compounds (e.g., carvacrol) and have mainly been evaluated using in-vitro and offered to cows using small scale studies to assess the effect on rumen fermentation (Calsamiglia et al. 2007; Cobellis et

al., 2016) due to their antimicrobial properties that may modulate rumen microbial activity (Benchaar and Greathead, 2011), which and lower methane emissions per kg of milk product production but required a larger scale study that is reported in this report, which used 52 lactating dairy cows to assess the effect of offering OO in a PMR both pre and postpartum during early lactation and it effect on milk yield, composition and feed efficiency.

Methane emissions and rumen fluid fatty acid concentrations

In relation to methane emissions, OO and carvacrol have been found to inhibit ruminal methanogenesis in in-vitro studies (Benchaar and Greathead, 2011; Cobellis et al. 2016) and lower methane emissions by up to 98% in-vitro batch cultures of ruminal fluid (Macheboeuf et al. 2008). In this study, the lactating cows offered OO in the PMR had methane emissions that were approx. 10% lower per cow compared with cows not offered OO (Table 4). These differences may be due to the limitations of in vitro techniques that apply short-term incubation using a buffered medium, which have a relatively limited ability to replicate the diversity and viability of the microbiome of the rumen (Benchaar, 2020) and some of the high concentrations of OO used in vitro would be impractical for feeding to dairy cows in-vivo and lead to such differences to the effects observed in-vitro, which are much less reliable than in-vivo application of EO in simulated rumen conditions (Beauchemin et al. 2009; Benchaar and Greathead, 2011; Benchaar, 2020).

In this study the CH_4 emitted by early lactation dairy cows was lower from offered OO in the PMR, despite there being no difference in acetic, propionic and acetic : propionic acid ratio (Table 3), which was in agreement with Tekippe et al. (2011), who found that animals offered a diet supplemented with oregano produced less methane and Kolling et al. (2018) who while they observed a tendency for DMI to increase, this was associated with a reduction in enteric CH_4 production (g/kg of DMI) when a small number of cows (n=4) that were observed in metabolic chambers. While Olijhoek et al. (2019) found that feeding low levels of OO (EO content of 0.12% of DMI) were not effective in lowering methane production per day or per kg-DMI, despite a linear increase in hydrogen production as the OO dose rate increased from 0-53g of oregano DM/kg of dietary DM across 4 diets (0g, 18g, 36g, and 53g). Feeding oregano did not affect CO_2 or O_2 consumption. Despite the lower CH_4 being emitted daily in this study by cows offered OO in the PMR, these cows showed no difference in acetic, propionic and acetic : propionic acid concentration ratio (Table 3) in the rumen fluid compared with cows not offered OO. This was in agreement with other studies that found that feeding cows increasing amounts of oregano leaves (250, 500, and 750 g/d) inhibited of CH_4 production, but that this was not associated with a change in rumen fermentation in VFA production towards propionogenesis (Tekippe et al. 2011; Hristov et al. 2013). Moreover, the studies showed that

there was no effect on protozoa or *Methanobrevibacter*, which are the predominant *Archaea* genus in the rumen (Tekippe et al. 2011; Hristov et al. 2013) and that increasing propionate production is known to influence ruminal methanogenesis (Beauchemin et al. 2009; Benchaar, 2020) and lower CH_4 production in the rumen. Finally, in this study there was no difference in rumen fluid ammonia concentrations (Table 3), which differed to shorter term in vitro studies, which showed that OO and its main component carvacrol affected N metabolism via the reduction of protein degradation and ammonia production (Benchaar et al. 2008). The rumen fluid pH did not differ between cows offered and not offered OO in the PMR this study (Table 4), which agreed with previous the study Hristov et al. (2012).

Milk yield and composition

In more recent years milk yield has been measured on the fat and protein corrected milk (FPCM) (PEFCR for Dairy Products, 2018) and while FAO (2016) has applied fat-corrected milk is the estimated quantity of milk which is calculated on a 4% milk fat energy basis as a means that evaluating milk production records of different dairy animals can be compared on a common energy basis. In this study the cows offered a PMR with OO had greater milk, EC milk, milk fat, and milk solids yields, and greater milk lactose concentrations compared with cows that were not offered OO in the PMR (Table 2), which was similar to the findings of a small study by Nowers (2016) who concluded that OO in *dairy* cow diets stimulated *milk* fat *production* and increased EC *milk yield and* Olijhoek et al. (2019) who found that milk yield responded quadratically to increasing dietary concentrations of OO, starting at relatively low inclusion rates of OO (OO content of 0.12% of DM). *W*hile milk fat, protein, and urea concentrations did not differ between cows offered OO and not offered OO, which was in agreement with Benchaar (2020) and Olijhoek et al. (2019), the c*ows in the study detailed in this report that were* offered OO pre and postpartum had *greater m*ilk protein yields compared with cows not offered but did not differ between cows offered OO pp and not offered OO. Some studies that have offered an *oregano extract* to dairy cows reported no change in *milk yield and* milk fat and protein concentration, however, these studies only used three (Lejonklev et al. 2016) to four cows (Benchaar, 2020) per treatment diet that were kept in metabolic chambers and were rumen fistulated respectively. While other studies have found that oregano supplemented diets increase the milk fat concentration but did not increase milk protein concentration and protein yield (Tekippe et al. 2011). In this study, methane emissions per kg EC milk, per kg milk protein and per kg milk solids yield in this study were lower from cows offered OO compared with cows not offered OO in the PMR (Table 4). There are no studies that reported the effect of OO on SCC and in this study that cows offered OO following partition had lower milk SCC compared with cows not offered OO and offered OO pre and postpartum.

Dry matter intake, feed efficiency, live weight and body condition

There was no difference in pp DMI between cows offered and not offered OO in this study (Table 5), which agreed with previous studies that equally found *oregano extract and oil at* 0.2 and 1.0 g/kg of DM, corresponding to intakes of 4.3 and 22.4 g/d, respectively for one day (Lejonklev et al. 2016*)* 10 g/d for longer periods of up to 58 days *(*Kolling et al. 2018; Benchaar, 2020) had no effect DMI of lactating dairy cows, which was in agreement with Olijhoek et al. (2019) who found that OO and oregano extracts (**OE**), that were grown in two different areas in Europe, had no effect on the DMI, while inclusion rate had no effect on DM digestibility. While, Tekippe et al. (2011) and Olijhoek et al. (2019) found there was no difference in the DMI and feed efficiency of cows offered OO, Kolling et al. (2018) observed a tendency for DMI to increase when a small number of cows (n=4) were observed in metabolic chambers, however, this variability may be due to the relatively small numbers of animals being used in these studies, over short periods of one to three weeks per diet treatment. As a result, there are no studies have reported the effect on live weight and body condition score and the results from this study showed that the LW and BCS and change in LW and BCS did not differ between the cows that were offered and not offered OO (Table 2) despite the increase in milk and protein yields during early lactation.

Conclusions

Overall, OO increased the milk yield and decreased methane production, potentially due to its antibacterial properties without affecting DMI, unlike other supplements such as red seaweed which can be unpalatable.

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