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PERFORMANCE OF HOLSTEIN DAIRY COWS FED A DIET SUPPLEMENTED WITH ROSEMARY AND GINGER ESSENTIAL OILS: MILK PRODUCTION, MILK FATTY ACID, AND RUMINAL FERMENTATION

Mahmoud A. Elazab[1], Soliman M. Zahran[2], Alaa E. Elkomy[1], Mohamed H. Ahmed[2], Ahmed E. Kholif[3,4♦], Einar Vargas-Bello-Pérez[5♦], Sobhy M.A. Sallam[6]

[1]Livestock Research Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Application, Borg El-Arab-Alexandria, Egypt
[2]Animal and Fish Production Department, Faculty of Agriculture (Saba-Basha), Alexandria University, Egypt
[3]Department of Animal Sciences, North Carolina Agricultural and Technical State University, Greensboro, NC 27411, USA
[4]Dairy Science Department, National Research Centre, Dokki, Giza, Egypt
[5]Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua, Periférico R. Aldama Km 1, 31031 Chihuahua, México
[6]Animal and Fish Production Department, Faculty of Agriculture (El-Shatby), Alexandria University, Egypt
♦Corresponding authors: ae_kholif@live.com, aekholif@ncat.edu (A.E.K.); evargasb@uach.mx (E.V.B.P.)

Abstract

The study determined the impact of *Rosmarinus officinalis* and/or *Zingiber officinale* essential oil (EO) on milk yield, composition, milk fatty acids profile, blood biochemicals, and rumen fermentation in dairy cows. Twenty-eight Holstein lactating cows were distributed into four groups using a completely randomized block design in a 70-d experiment. The control diet consisted of 13 kg of concentrate and 40 kg of fresh berseem clover per head per day, without supplementation. In the other treatments, the control diet was supplemented with 10 mL of EO per head per day, using either ginger EO (GEO treatment), rosemary EO (REO treatment) or a blend of both at a 1:1 v/v ratio (BEO treatment). Supplementation did not affect intake, milk production, or composition. Omega-3 and omega-5 were increased with REO (P<0.05) compared to the control. Both REO and BEO decreased (P = 0.003) serum globulin and increased (P<0.005) albumin to globulin ratio, high-density lipoprotein cholesterol, and total lipid. Serum total antioxidant capacity was increased (P<0.001) with the supplementation, without affecting glucose, total protein, albumin, serum cholesterol, low-density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, and urea concentrations. In conclusion, supplementing Holstein dairy cows with GEO and/or REO increased the level of omega-3 and omega-5 fatty acids while reduced saturated fatty acids in milk, without affecting feed intake, milk production or milk composition.

Key words: essential oil, *Rosmarinus officinalis* L., *Zingiber officinale* R., Holstein dairy cows, milk production, milk fatty acid, rumen fermentation

Over the past ten years, the application of essential oils (EOs), which are plant extracts with aromatic properties, has expanded in the fields of animal health and production (Kholif et al., 2018; Alabi et al., 2024; Ike et al., 2024). The physiologically active compounds in EOs, primarily polyphenolics, have been shown to modulate ruminal fermentation, hence improving nutrient use in ruminant models (Kholif et al., 2012, 2018).

Several studies reported mixed results ranging from –6.7 to + 5% differences from control with the use of EOs on milk production in dairy cattle (Patra, 2011). Increasing energy-corrected milk and feed efficiency (Kholif et al., 2018; Elcoso et al., 2019), milk yield and composition (Matloup et al., 2017; Kholif et al., 2018; 2021) or weak effects (Blanch et al., 2016) were observed. This shows that terpenes and other antioxidant compounds that are transferred to the milk might be able to increase the oxidative stability of the milk. Using EOs in feeding animals is a strategy to increase the amount of unsaturated fatty acids (UFA) and the total of conjugated linoleic acid isomers (Kholif and Olafadehan, 2021). As a result, when it is passed from animals to milk, it will have beneficial effects on both milk consumers and animals. Essential oils have been shown to positively affect starch and protein degradation, the production of ruminal ammonia, volatile fatty acids (VFAs), and methane (CH_4) due to their antimicrobial activities against some ruminal microorganisms, such as methanogenic archaea and hyper-ammonia-producing bacteria (Patra, 2011; Kholif and Olafadehan, 2021). However, these positive effects are often accompanied by adverse effects on fiber degradation (Patra and Yu, 2012).

Rosemary (*Rosmarinus officinalis* L.) is a species of Mediterranean origin, which is well known worldwide as a common spice for culinary purposes (Kholif et al., 2017; Huang et al., 2023). Rosemary EO (REO) contains several compounds 1,8-cineol, camphor, bornyl acetate, α- and β-pinene, limonene, camphene, terpineol, and ver-

benone (Huang et al., 2023) which can act to determine the biological properties in a synergic manner or regulate one another.

Ginger (*Zingiber officinale* R.) is another important aromatic medicine plant belonging to the family Zingiberaceae, which is well known in Asia. It has been listed in the Generally Recognized as Safe (GRAS) document of USFDA and has antimicrobial, anti-mycotoxigenic, and antioxidant properties (Tatsadjieu et al., 2009) because its aroma and taste have been used for culinary purposes for ages. The bioactive compounds of ginger are divided into three groups: diarylheptanoids, volatile oils, and gingerols (Zhang et al., 2021). There are two types of chemical components in ginger: volatiles (cineole, limonene, camphene, borneol, zingiberenol, linalool, and zingiberene. etc.) and non-volatiles the characteristic bioactive components of ginger (gingerols, shogaols, zingerone, and paradols) (Shaukat et al., 2023).

Some inconsistencies regarding the efficacy of EOs on animal performance still exist, mainly linked to the nature of the compounds and some intrinsic and extrinsic factors, such as infection, nutritional status, environment, and particularly diet composition (Omonijo et al., 2018). Only a few studies evaluated EO with a known chemical composition in modulating the rumen microbiome and function. Therefore, more research is recommended to determine the effects of ginger and rosemary EOs on the lactation performance of lactating animals. The current study was designed to evaluate the effect of GEO and/or REO on milk yield, composition, milk fatty acids profile, blood biochemicals, and rumen fermentation of Holstein dairy cows. We hypothesized that the active ingredients in either or both EOs (e.g., ginger and rosemary) would alter ruminal fermentation and modify feed digestion, which could then affect the quantity and quality of milk produced.

Material and methods

The study was carried out at the Milk Production Project and Animal Nutrition Laboratory, Faculty of Agriculture, Alexandria University (Egypt). The analyses of samples were carried out at the Animal Nutrition Laboratory, Department of Animal and Fish Production, Faculty of Agriculture, Alexandria University and Laboratory of Livestock Research Department, Arid Lands Cultivation Research Institute (ALCRI), City of Scientific Research and Technological Applications (SRTA-City), Borj El-Arab, Alexandria, Egypt. All procedures followed protocols approved and authorized by the Institutional Animal Care and Use Committee of the City of Scientific Research and Technological Applications (SRTA-City) (IACUC#F-M-4-23-10-24).

Animals, feeding, and experimental design

Twenty-eight Holstein cows with an average body weight of 498.5 ± 10.7 kg at early lactation (2–3 weeks after parturition), were divided randomly into four groups (7 animals/group) based on their weight, season of lactation, and previous milk production. The control group was fed a diet of 13 kg of concentrate and 40 kg of berseem clover per head per day without any supplementation. The second group received the same control diet supplemented with 10 mL of ginger EO (GEO) per head per day (GEO treatment). The third group was fed the control diet supplemented with 10 mL of rosemary EO (REO) per head per day (REO treatment). The fourth group received the control diet along with 10 mL of a blend of ginger and rosemary EOs at a 1:1 ratio per head per day (BEO treatment). The dosage of each oil or their blend was added and manually mixed to the feed before morning feeding at 06:00 h daily.

Cows were housed in open semi-shaded barns with free access to fresh water. Cows were fed three times daily at 06:00, 12:00 and 18:00 h. The concentrate mixture and berseem clover were used to formulate diets to provide cows with their requirements plus 10% margin (NRC, 2001). Feed intake was individually recorded daily as the difference between the offered concentrate mixture and forage and refusal. Concentrate mixture and forage samples were collected biweekly and dried for analysis. Ingredients of the concentrate mixture and chemical composition of concentrate mixture and clover are shown in Table 1 (DM basis).

Table 1. Ingredients of concentrate mixture and chemical composition of concentrate mixture and berseem clover fed to dairy cows (DM basis)

	Concentrate feed mixture	Berseem clover
Ingredient (%)		
corn with cobs	30	
wheat bran	25	
cottonseed meal	30	
soybean meal	12	
limestone	1.8	
NaCl	1.0	
minerals mixture	0.2	
Chemical composition (%)		
organic matter	89.9	88.7
crude protein	18.1	14.8
ether extract	3.7	1.7
neutral detergent fiber	44.1	52.8
acid detergent fiber	19.2	45.9
TDN[1]	727	61.3
ME (MJ/kg)[1]	10.92	8.58

[1]TDN and ME were calculated based on proximate analysis and digestibility of the ingredients published in NRC (2001). TDN: total digestible nutrients, ME: metabolizable energy.

Feed samples were dried in an oven at 70°C for 48 h then ground to pass a 1 mm screen for future analyses. The AOAC (2019) analytical procedures were used for dry matter (DM) by oven drying at 105°C for 24 h, and

organic matter (OM) by the difference after incinerating at 550°C for 4 h, crude protein (CP as 6.25×N) by Kjeldahl technique and ether extract using the extraction system. Neutral detergent fiber (NDF) organic matter, and acid detergent fiber (ADF) organic matter expressed exclusive of residual ash were determined in filter bags described by Van Soest et al. (1991). Sequential in the same sample, the lignin content of the feed was determined according to Van Soest et al. (1991) by solubilization of ADF with 72% sulfuric acid. Fiber analysis was performed using an Ankom 200 fiber analyzer unit (ANKOM Technology Corporation, Macedon, NY, USA). Total digestible nutrients (TDN) and metabolizable energy (ME) were calculated using NRC (2001) equations as follows: TDN (g/kg DM) = apparent digestible CP (g/kg DM) + digestible crude fiber (g/kg DM) + digestible nitrogen-free extract (g/kg DM) + digestible ether extract (g/kg DM) × 2.25); ME (Mcal/kg DM) = digestible energy (DE) × 0.82; where DE (Mcal/kg DM) = 0.04409 × TDN (%).

The investigated individual EOs of ginger (*Z. officinale* R.) and rosemary (*R. officinalis* L.) were produced by El-Hawag Factory for the extraction of Natural Oils and Cosmetics, Badr City, Egypt. The EOs blend was prepared at the laboratory by mixing equal amounts of ginger and rosemary.

Volatile components of the EO were determined using a gas chromatography-mass spectrometry (GC-MS) on a GC-2010 Shimadzu capillary gas chromatography directly coupled to the mass spectrometer system (GC-MS – model QP 2010; Shimadzu). A DB-5ms non-polar fused silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness) was used under following conditions: oven temperature program isotherm 2 min at 70ºC, 3ºC/min gradient to 200ºC and the final temperature kept for 35 min; injection temperature 200ºC; carrier gas is helium with flow rate 1.51 mL/min; linear velocity 45.1 cm/sec. The effluent of the GC column was introduced directly into the source of MS and spectra obtained in the EI mode with ionization energy 70 eV, in the electronic ionization mode and ion source temperature is 200ºC. The solvent cut time was 3 min. The sector mass analyzer was set to scan from 40 to 1000 m/z with an interface temperature of 240ºC.

Milk yield, composition, and milk fatty acid profile

Cows were machine milked three times daily at 05:00, 11:00, and 21:00 h. Milk yield for individual cows was recorded daily. Milk samples were collected biweekly after 2, 4, 6, 8, and 10 weeks, where milk fat, protein, lactose, solid not fat, and ash contents were analyzed immediately by the infrared method using Milk Analyzer (Milko Tester Instruments Inc., Bulgaria). Energy-corrected milk was calculated following the NRC (2001).

Milk samples from the 6th, 8th, and 10th weeks were collected from 5 animals of each treatment to quantify milk fatty acids profile using a GC-MS. Fatty acid methyl esters were prepared by transmethylation and were then quantified by using a gas chromatograph (Scion 456-GC/MS, Netherlands) with the following settings: scanning mass range of 40–500 amu, transfer line temperature of 240°C, source temperature of 280°C, quad temperature of 150°C and a solvent delay of 11.8 min. The separation of fatty acid methyl esters was achieved by a Rt-2560 column (100 m × 0.25 mm ID, 0.20 µm df, Restek) with a constant flow of 1.2 mL/min helium as carrier gas and the following oven temperature program: initial temperature of 100°C and held for 4 min, increased by 6°C min^{-1} to 170°C, and then increased by 3°C min^{-1} to 240°C and held for 11 min. The injection inlet temperature was 240°C and the injection volume was 1 µL with a split ratio of 20:1. A fatty acid methyl esters standard mix containing 37 fatty acid methyl esters was used to provide absolute quantification of each fatty acid.

Ruminal fermentation parameters

On the last day of the 6th to 10th weeks of the experimental periods, 5 animals were selected randomly from each treatment to collect rumen fluid samples (30 mL) using an esophageal probe 3 h after morning feeding. Ruminal pH was measured immediately using a portable digital pH meter and filtration from the feed particles by passing through four layers of gauze and then separating on two tubes: Tube 1: to classify and count protozoa using the method described by Onodera et al. (1977); Tube 2: stored at –20°C for measuring ammonia (NH_3-N) (Preston, 1995) and individual VFAs concentrations. Briefly, after thawing, an aliquot of 1.6 mL was prepared with 0.4 mL of 25% metaphosphoric acid (4:1 ratio) and centrifuged at 15,000 rpm for 20 min and 4ºC (K1015 Micro Prime; Centurion Scientific Ltd, Stoughton, Chichester, UK). The supernatant was used to determine VFAs concentrations using a gas chromatograph (Thermo Fisher Scientific, Inc., TRACE1300, Rodano, Milan, Italy) fitted with an AS3800 autosampler and equipped with a capillary column HP-FFAP (19091F-112; 0.320 mm o.d., 0.50 µm i.d., and 25 m length; J&W Agilent Technologies Inc., Palo Alto, CA). Hydrogen at 1.35 mL/min was used as carrier gas. Air, hydrogen, and nitrogen fluxes (make-up gas) were kept at 450, 40, and 35 mL/min, respectively. A 0.1 µL aliquot was injected in splitless mode for the entire run with 31.35 mL/min of H_2 flux (63.432 Pa). Injector and flame ionization detector temperatures were held isothermally at 250°C. The oven heating slope was 80°C (1 min), 120°C (20°C/min for 3 min), and 205°C (10°C/min for 2 min), with 9 min overall analytical time. Methane production was calculated by the equation of Wolin (1960): CH_4 = (acetic + (2 × propionic)) - fermentative CO_2; where fermentative CO_2 = (acetic/2) + (propionic/4) + (1.5 × butyric).

Blood biochemical parameters

Blood samples were collected biweekly without using an anticoagulant via vein tail puncture from cows for biochemical parameters assay. Serum was obtained by centrifugation of the blood at 3000 rpm for 20 minutes and was stored at –20ºC for later analysis.

Total protein, albumin, urea, total cholesterol, low-density lipoprotein cholesterol (LDL-cholesterol), glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total lipids, total antioxidant capacity were calorimetrically measured in blood serum using specific kits from Biodiagnostic Co., Giza, Egypt and following manufacturer instructions, with a visible light spectrophotometer. Serum globulin was determined by subtracting the serum albumin from the total protein. High-density lipoprotein cholesterol (HDL-cholesterol) was determined by subtracting the serum LDL-cholesterol from total cholesterol. The albumin/globulin ratio was determined by the division of serum albumin value on serum globulin value.

Statistical analysis

The data was statistically analyzed using statistical package for social sciences (SPSS®, version 2016). The following model for milk fatty acid profile was assumed:

$$Yij = \mu + Ti + e_{ij}$$

where: μ is the overall mean, *Ti* is the fixed effect of the treatment, e_{ij} is the random error term. Feed intake, milk yields, composition, blood parameters and ruminal fermentation were analysed using the statistical model:

$$Y_{ijk} = \mu + T_i + P_j + (T \times P)_{ij} + e_{ijk}$$

where Y_{ijk} is the observation; μ is the overall mean; T_i the fixed effect of the treatments; P_j the fixed effect of the period; $(T \times P)_{ij}$ the interaction between treatment and the period. and e_{ijk} the standard error. The significant differences among treatment means were compared using Duncan's new multiple-range test. The significant differences among treatment means were at a P-value ≤ 0.05 as a limit of significance.

Results

Chemical profiles of GEO and REO analyzed using the GC/MS are given in Table 2. Differences in the main components in the ginger and rosemary EOs were observed. The main constituent in the GEO is zingeberene (29.74%) followed by carveol (15.05%), *cyclohexene.*3-(1,5-dimethyl-4-hexenyl)-6-methylene (10.40%) and 9,12 octadecadienoic acid (9.20%) and other ingredients. The main three components in REO were limonene (23.03%), *cis*-vaccenic acid (12.91%), and *trans-4-decadienal (10.67%).*

Under the experimental conditions of the current study, the inclusion of GEO, REO, and BEO in lactating cows did not affect (P>0.05) feed consumption (Table 3). Supplementations did not affect milk production (Figure 1), or composition compared to the control treatment. The period of sampling affected milk production (P = 0.010) and the percentage or yield of milk protein, lactose, and SNF.

Table 2. List of active components of ginger essential oil (GEO) and rosemary essential oil (REO)

Component	Area (%)
GEO (*Zingiber officinale*)	
zingiberene	29.74
Alpha-Farnesene	2.88
Cis-alpha-Bisabolene	5.04
*Cyclohexene.*3-(1,5-dimethyl-4-hexenyl)-6-methylene	10.40
n-Hexadecanoic acid	3.93
9, 12 Octadecadienoic acid	9.20
carveol	15.05
REO (*Rosmarinus officinalis*)	
eucalyptol	3.98
Trans-3-Nonene	7.65
2-Decenal	2.96
Trans-4-Decadienal	10.67
limonene	23.03
octane, 2,4,6-trimethyl	9.14
2-Undecenal	2.49
4-Heptenal	6.90
linalool	3.50
9, 12 Octadecadienoic acid	8.77
Cis-Vaccenic acid	12.91
octadecanoic acid	1.61

The milk fatty acid profile (g/100 g of total milk fatty acids) showed that EOs did not significantly affect most of the measured fatty acids; however, GEO treatment decreased the proportions of C17:0 (P = 0.014) and C18:0 (P = 0.015), while both of GEO and REO treatments decreased the proportions of C20:0 (P = 0.001). Additionally, all treatments decreased the proportions of C24:0 (P = 0.009) compared to control (Table 4).

The UFA omega-3 (n3: C20:3-cis11, 14, 17) was found in the milk of cows supplemented with EO, without any presence in the milk of control cows (Figure 2). The highest omega-3 values were observed with REO and BEO (0.18 and 0.16 g/100 g, respectively), followed by GEO (0.09 g/100 g) with a significant difference (P<0.001) compared to the control group (0.01 g/100 g). Moreover, the REO and GEO increased (P = 0.032) omega-5 (n5: C14:1-*cis 9*) compared to the control.

As shown in Figure 3, poly-UFA (PUFA) to SFA was higher (P = 0.038) with cows fed the diet supplemented with GEO, REO and BEO (0.0035, 0.004 and 0.0039 g/100 g, respectively) compared to control (0.0032 g/100 g), GEO reduced (P = 0.042) the di-SFA to SFA as compared to the control and other treatments. REO and BEO did not affect unsaturated fatty acid (UFA), mono-UFA to SFA, and UFA to SFA compared to the control.

Table 3. Effects of ginger essential oil (GEO), rosemary essential oil (REO) and their blend at 1:1 (BEO) at 10 g daily on dry matter intake, milk production and composition of early lactating Holstein cows

	Treatments[1]				SEM	P value		
	control	GEO	REO	BEO		T	P	T × P
Intake (kg DM/d)								
concentrate	11.2	11.2	11.2	11.2	0.22	0.295	0.112	0.326
roughage	7.97	7.95	7.92	7.95	0.144	0.473	0.218	0.533
total intake	19.2	19.2	19.1	19.1	0.345	0.333	0.295	0.428
milk yield (kg/d)	23.8	23.2	22.8	23.9	0.24	0.281	0.010	0.964
ECM (kg/d)	24.6	24.3	24.2	24.4	0.40	0.979	<0.001	0.633
Milk fat								
(%)	3.93	3.83	4.00	3.75	0.056	0.464	0.108	0.133
(kg/d)	0.92	0.90	0.92	0.90	0.021	0.950	<0.001	0.314
Milk protein								
(%)	3.02	3.02	3.03	3.05	0.111	0.698	0.001	0.252
(kg/d)	0.72	0.71	0.70	0.73	0.010	0.793	<0.001	0.909
Solid not fat								
(%)	7.45	7.48	7.54	7.54	0.034	0.618	0.001	0.635
(kg/d)	1.77	1.76	1.73	1.79	0.032	0.857	0.001	0.900
Milk lactose								
(%)	4.53	4.55	4.58	4.59	0.020	0.574	0.001	0.499
(kg/d)	1.08	1.07	1.06	1.09	0.021	0.847	0.002	0.880

[1]Diet: Control diet contained 13 kg of concentrate feed mixture and 40 kg berseem clover without supplementation (Control), or supplemented with ginger essential oil (GEO), rosemary essential oil (REO) and their blend (BEO) at 10 g daily.

SEM: standard error of the means. T: significant differences between treatments. P: significant differences between periods. T × P: interaction between treatment and period. ECM: energy corrected milk.

Table 4. Effects of ginger essential oil (GEO), rosemary essential oil (REO) and their blend at 1: 1 (BEO) at 10 g daily on milk fatty acid profile (g/100 g of total milk fatty acids) of early lactating Holstein cows

	Treatments[1]				SEM	P value
	control	GEO	REO	BEO		
C4:0	1.01	1.12	1.02	1.05	0.022	0.230
C6:0	0.91	1.16	1.04	1.01	0.030	0.061
C8:0	0.87	1.22	1.13	1.12	0.056	0.154
C10:0	2.71	3.07	2.60	2.71	0.188	0.864
C11:0	0.16	0.20	0.19	0.11	0.010	0.077
C12:0	3.22	3.62	3.21	2.70	0.212	0.515
C13:0	0.14	0.21	0.21	0.17	0.014	0.197
C14:0	11.2	12.6	11.7	11.4	0.34	0.553
C15:0	0.99	1.32	1.28	1.21	0.045	0.071
C16:0	34.1	34.3	32.8	31.9	0.49	0.279
C17:0	0.93 a	0.81 b	0.86 ab	0.93 a	0.024	0.014
C18:0	12.8 a	9.2 b	10.7 ab	12.1 a	0.47	0.015
C20:0	0.14 a	0.11 b	0.08 c	0.12 ab	0.013	0.001
C22:0	0.06	0.06	0.06	0.06	0.014	0.987
C23:0	0.05	0.06	0.04	0.05	0.014	0.256
C24:0	0.15 a	0.02 b	0.01 b	0.03 b	0.022	0.009

[1]Diet: Control diet contained 13 kg of concentrate feed mixture and 40 kg berseem clover without supplementation (Control), or supplemented with ginger essential oil (GEO), rosemary essential oil (REO) and their blend (BEO) at 10 g daily.

a, b, c – means in the same row with different letters differ (P<0.05). SEM: standard error of the means.

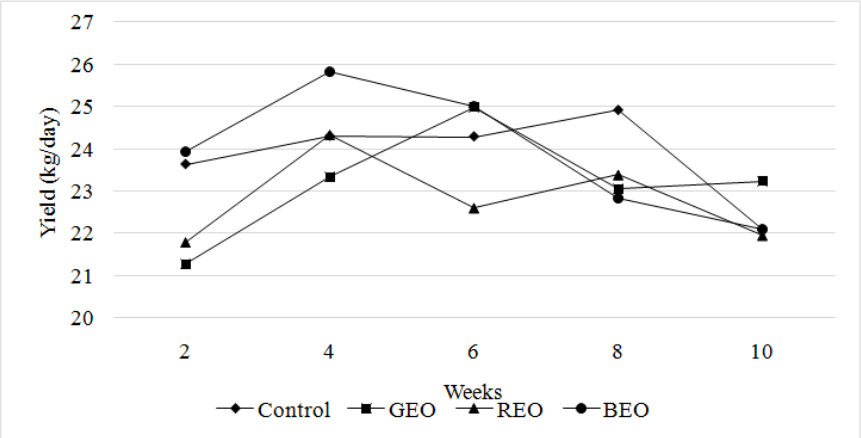

Figure 1. Effect of ginger essential oil (GEO), rosemary essential oil (REO) and their blend at 1: 1 (BEO) at 10 g daily per cow on milk yield through experimental period (10 weeks)

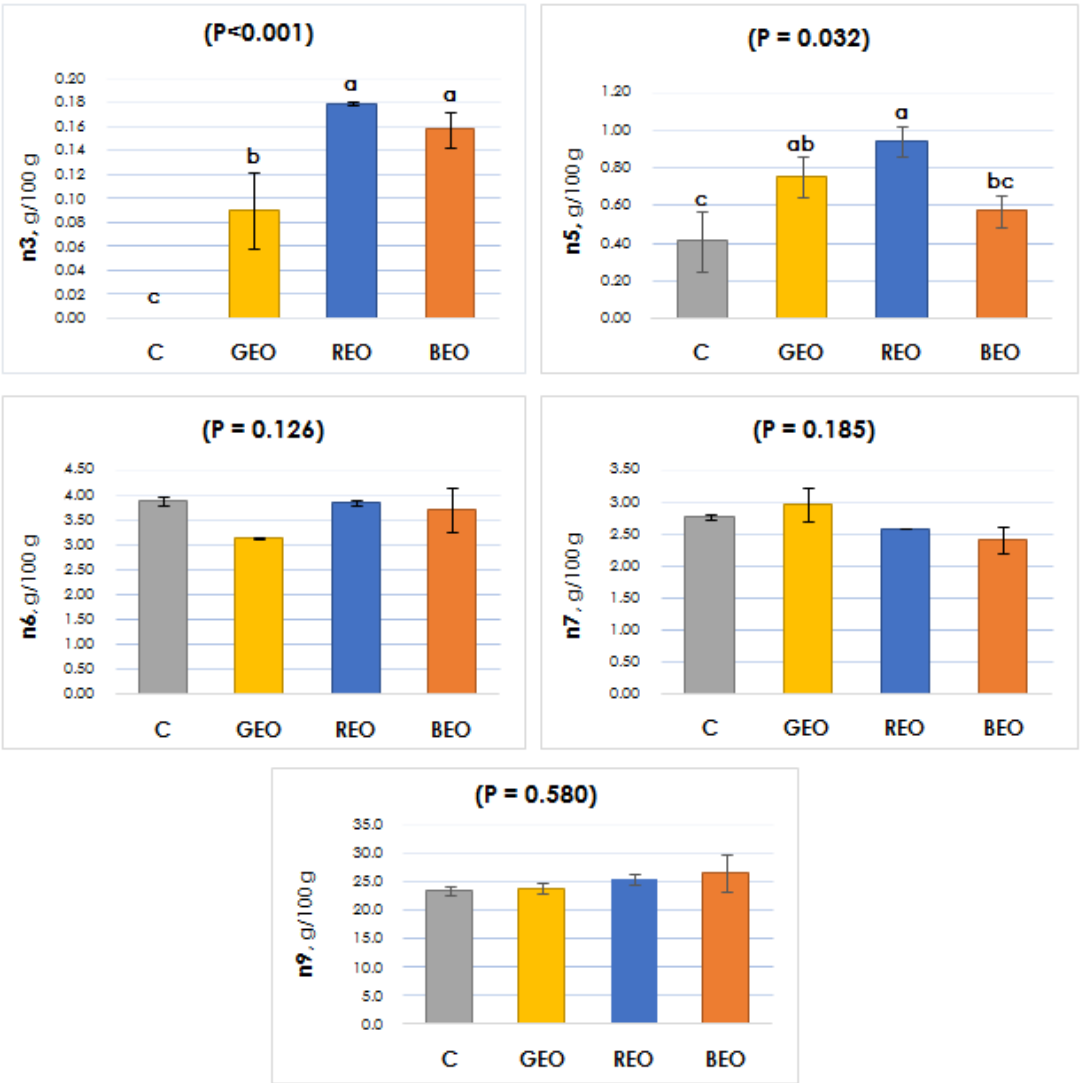

Figure 2. Effect of ginger essential oil (GEO), rosemary essential oil (REO) and their blend (BEO) at 1:1 at 10 g daily per cow on milk fatty acid profile. n3: C20:3-*Cis11, 14, 17*. n5: C14:1-*Cis 9*. n6 calculated as C18:2-*Trans9, 12*+ C18:1-Cis9, 12+ C20:3-*Cis8, 11, 14*+ C20:4. n7 calculated as C17:1-*Cis10* + C16:1-*Cis9*. n9: C18:1-*Trans9*+ C18:1-*Cis9*+ C20:1-*Cis11)*. Bars bearing different letters are significantly different (P≤0.05)

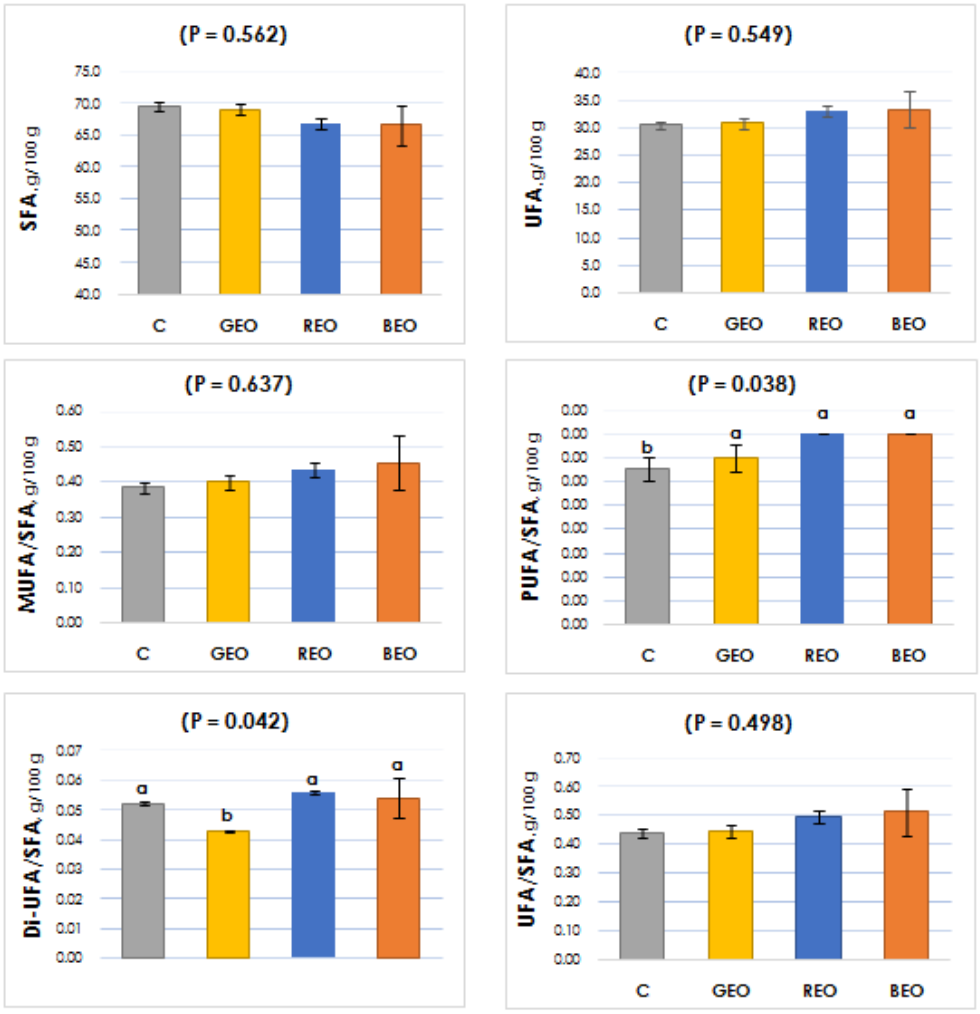

Figure 3. Effect of ginger essential oil (GEO), rosemary essential oil (REO) and their blend (BEO) at 1:1 at 10 g daily per cow on milk fatty acid profile of. SFA: saturated fatty acid. UFA: unsaturated fatty acid. MUFA/SFA: monounsaturated fatty acid/saturated fatty acid. PUFA/SFA: polyunsaturated fatty acid/saturated fatty acid. Di-UFA/SFA: di-unsaturated fatty acid/saturated fatty acid. Bars bearing different letters are significantly different (P≤0.05)

Table 5. Effects of ginger essential oil (GEO), rosemary essential oil (REO) and their blend at 1: 1 (BEO) at 10 g daily on some biochemical blood parameters of early lactating Holstein cows

	Treatments[1]				SEM	P value		
	control	GEO	REO	BEO		T	P	T × P
Total protein (g/dl)	7.74	7.64	7.44	7.61	0.05	0.124	0.302	0.022
Albumin (g/dl)	3.40	3.54	3.70	3.58	0.04	0.062	<0.001	0.015
Globulin (g/dl)	4.34 a	4.10 ab	3.37 c	4.03 bc	0.06	0.001	0.066	0.088
Albumin/Globulin ratio	0.78 b	0.86 b	1.10 a	0.89 ab	0.02	0.015	<0.001	0.025
Glucose (mg/dl)	58.01	55.36	57.66	55.82	0.51	0.174	0.007	0.141
Total cholesterol (mg/dl)	269.8	296.9	277.6	287.20	3.81	0.303	0.182	0.380
LDL (mg/dl)	249.1	279.9	260.8	270.80	3.83	0.169	0.024	0.185
HDL (mg/dl)	17.74 b	18.51 ab	20.06 a	19.78 a	0.31	0.035	0.119	0.596
Total lipid (mg/dl)	273.7 b	273.4 b	318.4 a	322.4 a	6.21	<0.001	<0.001	<0.001
ALT (U/L)	34.70	37.29	37.28	37.12	0.65	0.274	0.033	0.015
AST (U/L)	85.69	86.31	81.15	86.17	1.07	0.120	0.062	0.012
Urea (mg/dl)	16.22	15.90	17.09	15.97	0.28	0.414	0.001	<0.001
Total antioxidant (mM/l)	0.73 b	0.79 a	0.84 a	0.82 a	0.01	<0.001	<0.001	<0.001

[1]Diet: Control diet contained 13 kg of concentrate feed mixture and 40 kg berseem clover without supplementation (Control), or supplemented with ginger essential oil (GEO), rosemary essential oil (REO) and their blend (BEO) at 10 g per cow daily.

LDL: low-density lipoprotein cholesterol. HDL: high-density lipoprotein cholesterol. ALT: serum alanine aminotransferase. AST: serum aspartate aminotransferase.

a, b, c – means in the same row with different letters differ (P<0.05). SEM: standard error of the means. T: significant differences between treatments. P: significant differences between periods. T × P: interaction between treatment and period.

Table 6. Effect of ginger essential oil (GEO), rosemary essential oil (REO) and their blend at 1: 1 (BEO) at 10 g daily on ruminal pH, ammonia-N (NH_3-N), methane (CH_4), total and individual volatile fatty acids (VFAs) concentration in early lactating Holstein cows

Parameters	Treatments[1]				SEM	P value
	control	GEO	REO	BEO		
pH	6.28	6.30	6.17	6.20	0.052	0.800
NH_3-N (mg/100 mL)	25.2	28.2	23.3	23.45	0.94	0.231
CH_4 (mL/g digestible organic matter)	22.1	23.0	21.8	20.63	0.36	0.124
VFAs (mM)	90.0	90.0	90.0	90.04	0.01	0.415
Acetic (mM)	38.1	38.6	40.1	38.87	0.49	0.537
Propionic (mM)	26.4	24.3	22.8	26.11	0.70	0.228
Iso-butyric (mM)	2.49	2.46	2.23	2.47	0.081	0.691
Butyric (mM)	15.6	17.1	17.4	15.44	0.42	0.253
Iso-valeric (mM)	3.67	4.31	4.29	3.70	0.130	0.136
Valeric (mM)	3.75	3.27	3.24	3.45	0.131	0.474
Acetic/Propionic	1.49	1.61	1.76	1.50	0.052	0.213

[1]Diet: Control diet contained 13 kg of concentrate feed mixture and 40 kg berseem clover without supplementation (Control), or supplemented with ginger essential oil (GEO), rosemary essential oil (REO) and their blend (BEO) at 10 g daily.

SEM: standard error of the means.

The effect of GEO, REO, and BEO on serum blood biochemical parameters of early lactating Holstein cows is shown in Table 5. In comparison to the control group, the inclusion of REO and BEO resulted in a significant decrease ($P = 0.003$) in serum globulin, whereas increases ($P<0.05$) were observed for the albumin/globulin ratio, high-density lipoprotein cholesterol (HDL), and total lipid, without affecting glucose concentration. Total antioxidant capacity was increased ($P<0.001$) with the treatments (0.79, 0.84, and 0.82 mM/L; GEO, REO, and BEO, respectively) compared to the control (0.73 mM/I). Serum total protein, albumin, total cholesterol, low-density lipoprotein cholesterol (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and urea concentration were not affected by supplementations compared to the control.

Treatments did not affect ruminal pH, or the concentrations of NH_3-N, total or individual VFAs (Table 6). Additionally, the treatments did not affect the production of CH_4.

Discussion

The bioactivity of EOs depends on their complex mixture of volatile molecules produced by the secondary metabolism of aromatic and medical plants. As a result, the active ingredients might vary, particularly when EOs are mixed with other plant extracts, as observed in the current study (Stevanovic et al., 2019). The individual analysis of GEO and REO revealed that each of them has several compounds that determine its key bioactivities. This interaction among EO components may cause positive changes in rumen fermentation, but the combinations that will do this successfully are still unknown (Kholif and Olafadehan, 2021).

Enhancing animal performance is the main goal of ruminant production, and one of the ways to do this is by modifying feed intake and eating behavior. The current study revealed that concentrate or roughage feed intake was not affected by EOs supplementation. The effects of EO on feed intake might vary with EO source, type of diet, diet interaction, or adaptation of the rumen microbial population to EO (Kholif and Olafadehan, 2021). The main results approved by Smeti et al. (2015) illustrated that REO at 0.6 g/kg DM affected the silage DM intake without affecting hay intake. Additionally, Suksombat et al. (2017) reported that 2 mL/day of allicin, zingiberene, or citral did not affect feed intake in Holstein-Friesian cows. In contrast, other researchers demonstrated that feed intake was significantly decreased (Tassoul and Shaver, 2009) or increased because of the addition of a commercial EO complex (Kung et al., 2008).

The investigations on the effects of EOs on milk production varied in terms of the EO levels, the diet forage to concentrate ratio, lactation stage, and cows' output level (Tager and Krause, 2011). Therefore, information on the effects of EO on milk production and milk composition of dairy cows is less available with mixed results (Spanghero et al., 2009; Elcoso et al., 2019). Milk yield, compositions, and ECM did not differ between treatments; however, differences were noted during the experimental period. The last results agreed with Benchaar et al. (2007 a, 2006) who observed that 750 mg or 2 g of EOs daily did not affect milk production and composition. Moreover, Flores et al. (2013) showed that milk production and milk components did not differ with EOs supplementation. Other researchers found different results, either increased milk yield with garlic oil, cinnamon oil, and ginger oil supplementations or decreased milk production. Essential oils can impact milk production in cows and their impact depend on the type, dosage, and

composition of the oils used (Kholif et al., 2012; Kholif and Olafadehan, 2021). The potential for EOs to increase milk production can be attributed to their positive effects on feed utilization, improved nutrient availability, and energy utilization, as well as their ability to selectively inhibit harmful bacteria while promoting beneficial ones, thereby optimizing rumen fermentation (Cobellis et al., 2016; Kholif et al., 2018; Elcoso et al., 2019). However, some EOs may disrupt the balance of rumen microflora, leading to suboptimal fermentation, reduced production of VFAs, and, consequently, lower milk yields. Additionally, certain EOs have strong or unpalatable flavors that may decrease feed intake, resulting in reduced energy and nutrient intake and ultimately lowering milk production (Calsamiglia et al., 2007; Cobellis et al., 2016).

Milk fatty acids profile is an important variable that affects both the processing characteristics and the nutritional value of dairy products (Kholif and Olafadehan, 2022). Information on the effects of feeding REO and GEO on milk fatty acid profiles of dairy cows is scarce. Results of milk fatty acid showed that EOs caused some changes in fatty acid concentrations in milk. The mode of action of EO in the rumen microbial environment suggests that changes in bacterial growth rates result in changes in the proportion of rumen bacterial populations causing different proportions of rumen fatty acid and thus differences in milk fatty acid (Calsamiglia et al., 2007; Kholif and Olafadehan, 2022; Khattab and Elgandy, 2024). Modifying rumen populations with essential oils can impact certain rumen microorganisms involved in the biohydrogenation of polyunsaturated fatty acids (PUFAs), leading to changes in the fatty acid profile of rumen contents that are reflected in the milk of ruminants (Khattab and Elgandy, 2024). The milk fatty acid profile is mainly influenced by lipid metabolism in both the rumen and the mammary gland (Hanus et al., 2018; Bionaz et al., 2020; Kholif and Olafadehan, 2022). Supplementing with EOs can lower levels of some fatty acids, as EOs may produce strong inhibitors that impede the synthesis of *de novo* fatty acids in the mammary gland (Bauman and Griinari, 2003).

Reducing the intake of saturated fatty acids (SFAs) is associated with a significant reduction in cardiovascular disease risk, particularly when replaced with PUFAs in both randomized trials and cohort studies (Jakobsen et al., 2009; Farvid et al., 2014). The most effective EO on milk fatty acid was REO, followed by BEO, which also increases the PUFA/SFA, and omega-3 and 5. Our results agreed with Boutoial et al. (2013) and Kholif et al. (2017) who supplemented rosemary to lactating goats. Kholif et al. (2017) found that the supplementation of rosemary increased UFA. In contrast, profile of milk fatty acids in cows was not influenced by supplementation with a mixture of EOs (Benchaar et al., 2012).

Zingiberene EO has n-hexadecenoic (3.93%), which led to availability in cow milk and was represented in palmitate (GEO: 37.71 g/100 g) compared to control. The supplementation with GEO had minor effect on milk fatty acids, which decreased SFA; C17:0, C18:0, C20:0 and C24:0. Yang and He (2016) reported that EO supplementation may respond to milk fatty acid profiles in small ruminants compared to large ruminants such as dairy cattle, which could limit the ability of rumen bacteria to complete the biohydrogenation process in the rumen due to the high passage rate of feeds out of the rumen.

Based on the results of blood parameters, the addition of EOs had no influence on serum total protein, albumin, glucose, urea, AST, or ALT. These findings agreed with other experiments (Sahraei et al., 2014; Anassori et al., 2015; Malekkhahi et al., 2015; Khateri et al., 2017). The decreasing globulin concentration in our experiment was in agreement with that found by Kholif et al. (2012) who reported that GEO (2 mL/head/day ginger oil to goat) lowered serum globulin values, and this result may be due to the ruminal microbial protein synthesis. In contrast to our findings, other research observed that the concentration of blood total protein, cholesterol, and albumin: globulin ratio was decreased by the EO additives (Tassoul and Shaver, 2009; Yang et al., 2010). Serum cholesterol and LDL were not affected by the treatments; however, HDL concentration was increased with REO and BEO. These results may indicate the beneficial effect of EOs supplementation on dairy cattle because HDL-cholesterol has a vital role in preventing heart attack and stroke. Both REO and BEO significantly increased total lipid and total antioxidant capacity, which was confirmed by El-Essawy et al. (2019) who reported that clove herbal feeding at 5% of the diet (DM basis) or clove EO (2 mL/day in bark) significantly increased serum HDL-cholesterol, total antioxidant capacity and total lipid.

The variability of EO activity on ruminal fermentation and feed digestion may be affected by ruminal pH that can influence the dissociated or un-dissociated status of EO molecules as suggested by Cardozo et al. (2005). Rumen pH is a resultant of produced volatile fatty acids (acetate, propionate, and lactate), ammonia, rumen buffers and saliva (Van Soest, 1994) and is an index for fermentation. In this study, the ruminal pH ranged from 6.17 to 6.30 and was within the normal range for ruminal fiber degrading microbial activities and growth (Ryle and Ørskov, 1990). The ruminal pH was not affected with the EOs supplementation which may be reflected on the other ruminal fermentation parameters that were not altered with the supplementation. Similarly, Sahraei et al. (2014) observed that the high dose of REO (400 mg/d) affected ruminal pH, total and individual VFAs. Moreover, Chaves et al. (2008) illustrated that the supplementation with cinnamaldehyde, garlic, and juniper berry had no effect on *in vitro* ruminal fermentation parameters. Benchaar et al. (2007 b, 2006) reported no changes in total or molar proportions of individual VFAs, and ammonia concentration in ruminal fluid of lactating cows fed 0.75 or 2 g/day of a mixture of EO compounds. Moreover, Khateri et al. (2017) showed the addition of the specific mixture of EO at 0.8 and 1.6 mL/day did not affect total VFAs concentration, molar proportion of acetate, propi-

onate, butyrate, iso-valeric acid and acetate: propionate ratio in sheep.

A reduction in NH_3-N concentration is often considered a positive effect because it signifies a decrease in deamination and overall protein metabolism. Therefore, enhancing the escape of protein and dietary nitrogen from the rumen through the effects of EOs on amino acids deamination and protection from rumen degradation can lead to more efficient utilization and absorption (McIntosh et al., 2003; Kholif and Olafadehan, 2021). This improved nitrogen efficiency is necessary for efficient ruminant production. The main result indicated that NH_3-N concentration was not affected with REO and BEO supplementation. REO supplementation to sheep at 100, 200 and 400 mg/d had no effect on NH_3-N concentration (Sahraei et al., 2014). Khateri et al. (2017) reported that 0.8 or 1.6 BEO (50% thyme, 30% cinnamon and 20% clove) supplementation to sheep had not affected NH_3-N concentration. Also, Suksombat et al. (2017) reported that daily supplementation with 2 mL allicin, zingiberene or citral did not affect NH_3-N concentration in Holstein-Friesian cows. Others (Cobellis et al., 2015) observed that ammonia-N concentration was significantly reduced (59%–78%) with 1.0, 1.5 and 2 g/L REO (*Rosmarinus officinalis* L.) *in vitro*.

Results of calculated CH_4 indicated that REO and BEO tend to decrease CH_4 production (BEO: decreased CH_4 with 6.44% control), however the differences were not significant. The EOs exhibit different responses to methanogenesis depending upon the type of EO. Essential oils can directly inhibit methanogenic archaea and/or indirectly decrease CH_4 production by depressing some microbial metabolic processes contributing to methanogenesis (Hernandez et al., 2017; Ebeid et al., 2020; Kholif and Olafadehan, 2021). Moreover, EOs may cause changes in archaeal community structure and/or in activity of methanogenesis pathway, subsequently decreasing methanogen abundance and methane production (Ohene-Adjei et al., 2008). Wang et al. (2009) showed that feeding a mixture of EO derived from oregano (0.25 g/d) to sheep for 15 days lowered CH_4, while Beauchemin and McGinn (2006) and Tomkins et al. (2015) found no decrease in CH_4 production from beef cattle that were fed a commercial blend of EO (1 and 2 g/d; CRINA® ruminants; Akzo Surface Chemistry Ltd., Herfordshire, UK). Benchaar (2015) reported no effects of CH_4 production with dietary cinnamon oil (50 mg/kg DM intake), cinnamaldehyde (50 mg/kg DM intake), or monensin (24 mg/kg of DM intake) in dairy cows fed diet containing 60: 40 forage: concentrate ratio.

Conclusion

Supplementation of GEO, REO, or their blend at 1:1 (i.e., BEO) had a beneficial effect on improving dairy cows' productivity by enhancing milk fatty acids profile. REO and BEO supplementation improved conjugated omega 3, 5, 6, or 9 and polyunsaturated fatty acids, as well as decreased saturated fatty acids in milk. EOs were not deleterious to ruminal fermentation, or the health of cows improved the blood cholesterol components, and total antioxidant capacity, and tended to decrease the glucose in blood. Moreover, REO and BEO may be used in dairy cows to improve the health properties of milk by decreasing the proportions of C17:0, C18:0, C20:0, and C24:0 fatty acids. Finally, it is necessary to evaluate more EOs on dairy animals to define the optimum dose for producing healthy milk which reflects firstly on animals and then the consumer. Further studies using varying levels of the evaluated EOs should be conducted to explore their potential for enhancing milk production and composition. Additionally, it is recommended to analyze the rumen microbiome of dairy animals to understand how microbial communities and lactational performance are influenced by EOs.

Conflict of interest statement

The authors declare no conflict of interest.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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