Influence of diets supplemented with naturally protected or unprotected eucalyptus oil on methane production and lactating buffalo productivity

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Research Article

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Posted Date: August 10th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-721132/v2>

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Version of Record: A version of this preprint was published at Tropical Animal Health and Production on December 10th, 2021. See the published version at <https://doi.org/10.1007/s11250-021-03008-3>.

REGULAR ARTICLES

Influence of diets supplemented with naturally protected or unprotected eucalyptus oil on methane production and lactating buffalo productivity

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Abstract:

This study was designed to investigate the influence of naturally protected eucalyptus oil supplementation in a form of leaves (EUL) or mature seed capsules (EUS) compared to crude eucalyptus oil (EUO). The control group (G₁) received a diet containing concentrate feed mixture, fresh berseem, rice straw, and corn silage. Whereas the G₂, G₃, and G₄ animals have

a diet supplemented with 200 g/head/day of EUL or EUS or 4 mL/head/day EUO, respectively. Supplementation of EUL or EUS increased $\text{NH}_3\text{-N}$, SCFA's, and concentrations of acetic acid *in-vitro*. Bacterial total count, protozoa, and cellulolytic bacteria increased ($P < 0.05$) with EUL and EUS supplementation. Methane production dropped ($P < 0.05$) with EUS, EUL, and EUO supplementation. Milk fat decreased ($P < 0.05$) with EUO supplementation, while an adverse trend was shown for lactose. No differences in feed conversion were found among EUS, EUL, and EUO. Blood total protein, albumin, and urea increased ($P < 0.05$) with supplementation of EUL or EUS compared to EUO. EUO supplementation yielded increased ($P < 0.05$) AST, ALT, glucose, and creatinine. Supplementation with EUL, EUS, or EUO decreased ($P < 0.05$) DM, OM, and CP digestibility. While digestibility of EE with supplementation by EUL, EUS, or EUO was higher ($P < 0.05$). The digestion coefficient of NDF and ADF decreased ($P < 0.05$) with supplemental EUL, EUS, or EUO compared to the G1 diet. Feeding EUS increased the values of TDN and DCP compared to EUL, which increased than EUO. Our results confirm that the naturally protected form of leaves or seeds mitigates the undesirable effects of directly supplementing crude eucalyptus oil.

Keywords: Eucalyptus oil, eucalyptus leaves, eucalyptus seed capsules, methane, degradability, digestibility, milk production.

Introduction

The rumen is an intricate system in which nutrients used up by microorganisms at a suitable pH provide the main products of fermentation, short-chain fatty acids (SCFA's), and microbial biomass, which are exhausted by the host ruminants (Cieslak et al. 2013; Vakili et al. 2013). There has been increased interest recently to reduce the rate of rumen methane production. Methane (CH_4) production from enteric fermentation is of anxiety worldwide because of the increased accretion of greenhouse gases in the atmosphere as well as the waste of nutritious

energy (Sallam et al. 2010). There has been an interesting effort to reduce CH₄ release by inhibition of ruminal methanogens to increase the efficiency of feed energy utilization by ruminants; this would also rally economic efficiency and the environment (Benchaar and Greathead 2011). Many studies have been carried out to investigate the impact of supplementation with eucalyptus leaves and eucalyptus oil (EUO) on methane production (McIntosh et al. 2003; Castillejos et al. 2006) however, much is still unknown about using dried or ground mature seeds. Additionally, (Sallam et al. 2010) hypothesized that EUO might be used as a feed supplement to alter rumen biohydrogenation to reduce CH₄ release and increase the flow of SCFA's to the duodenum. Moreover, Abo-Donia and Nagpal (2015) reported that tannins have been shown to alter rumen biohydrogenation, while Sallam et al. (2010) stated that eucalyptus has an ionophore effect by affecting SCFA's formation in the rumen lead to inhibition of the final step in the biohydrogenation of SCFA's to stearic acid. Due to the volatile and reactive of essential oils (ESOs), it is possible that their effectiveness, when included in an animal's diet, possibly altered by conditions during the production season as well as storage of ESOs and conditions in the digestive system of the animals (Nguyen et al. 2009).

A topical study by Chouhan et al. (2017) found that the use of ESOs in a protected form has a high potential for antimicrobial resistance outstanding so that increasing chemical stability and solubility, reduced rapid evaporation, and reduced and reduced retardance of ESOs action. In addition, Lammari et al. (2020) established that the encapsulation of ESOs to make their release subject to continuous control enhances their bioavailability and effectiveness against microbes. In recent years, due to increasingly negative consumer perceptions, encouraged increase interest in adding ESOs to ruminant feeds to increase milk production and improve the animals' physiological performance (Thao et al. 2015). In the same line, Turek and Stintzing (2013) suggested that adding such oils in their natural form, whether in the form of leaves or grains, avoids the adverse effects of those crude oils and increases their

effectiveness in ruminant nutrition. However, knowledge is scarce about the role of naturally protected as opposed to crude oil on animal performance (Maes et al. 2019). Therefore, the current study was designed to investigate the impact of supplementation of eucalyptus oil vs naturally protected in form (leaves or seeds capsules) on methane production and productive performance.

Materials and Methods

Ingredients and the trial diets

Eucalyptus leaves (EUL) and green mature seed capsules (EUS) collected from trees on beach canals were dried under shade for a week, and then ground and stored at ambient room temperature until use. Eucalyptus oil (EUO) was obtained from El Hawag for Natural Oils, El Nasr City, Cairo, Egypt. Four experimental diets were formulated as total mixed ration (TMR) isonitrogenous and iso-caloric diets of lactating buffalo as recommended by Kearl (1982). Animals G₁ received the basal diet consisting of a concentrate feed mixture (CFM), fresh berseem (FB), rice straw (RS), and corn silage (CS) at a 40:60 concentrate: roughage ratio. The second (G₂), third (G₃), and fourth (G₄) groups have received a control diet with a supplement of 200 g/head/day of EUL, EUS, or 4 mL EUO, respectively. Supplemental EUL, EUS, or EUO were dissolved daily in 1 L tap water, and then blended and mixed directly with the concentrated feed to ensure consistency. Weekly homogeneous samples of tested diets were dried and ground, and then held in glass bottles for analysis and *in-vitro* studies. The chemical composition of ingredients and the tested diets are existing in Table 1.

Animals and management

Sixteen healthy lactating Egyptian buffalo (body weight: 457.4 ± 10.5 kg; in season: 2 to 4; after 14 days in lactation) were distributed into four similar groups and randomized according to their previous milk records using quadratic 4×4 Latin square experimental designs. Animals were individually fed the experimental diets twice daily (8 a.m. and 6 p.m.).

The diet was offered for 28 d (21 d as a preliminary period + 7 d as a collection period), and it was adjusted every week based on changes in body weight and milk production. Multimineral salt blocks were supplied for the animals to lick freely, along with access to drinking water.

***In-vitro* gas production and degradability**

In-vitro gas accumulation technique was conducted as confirmed by Theodorou et al. (1994) on obtained samples of the experimental diets. Rumen liquid was collected from two buffalo in each group before the morning meal using a stomach tube. About 600 mg of tested sample (1.0 mm) was incubated with 60 mL of previously prepared buffered rumen juice for each bottle (1:3 mL/mL) as proposed by Goering and Van Soest (1970) under continuous CO₂ reflux in a 100 mL calibrated glass bottle in a water bath at 39°C. Samples were incubated in quadratic groups together with four bottles containing only an incubation medium (blank). Headspace gas pressure was measured at 2, 4, 8, 16, 24, 36, and 48 h. The kinetic parameters of GP(t) (mL/g DM) were fitted using the NLIN option as a model of France et al. (2000) as:

$$Gv(t) = b \times (1 - e^{-c(t-L)}),$$

where $Gv(t)$ is the gas produced at time t , “ b ” is the asymptotic gas produced (mL/g DM) by the insoluble but slowly fermenting fraction, “ c ” is the constant gas production rate (mL/h), “ t ” is the time of fermentation, and “ L ” is lag time. *In-vitro* CH₄ production was determined as the procedure by Pellikaan et al. (2011).

After termination of the incubation, the bottle contents were used for the determination of *in-vitro* neutral detergent fiber degradability (IVNDFD). *In-vitro* liquor from each bottle was collected after filtration to determine the pH using a portable pH meter (HANNA-pH meter, (model HI8424), Woonsocket, RI, USA), NH₃-N concentration measured as the procedure AOAC (2016), and the total SCFA’s as mentioned by Eadie et al. (1967). Molar proportions of acetic, propionic, and butyric concentrations were analyzed by gas-liquid chromatography (GC 2010; PerkinElmer, Inc., Shelton, CN, USA) capillary column (HPINNOWAX, 30m_0.250

mm_0.25 mm). The counting of rumen ciliate protozoa was performed under a light microscope according to Dehority (2003). Bacteria and cellulolytic bacteria were counting according to Wanapat et al. (2000).

Digestibility and blood parameters

Composed feces were from the rectum directly of all animals in each group in the morning before feeding at the end of the collection period. Acid-insoluble ash (AIA) was used as an inner marker to estimate the digestion coefficient of nutrients (Van Keulen and Young 1977). For analysis of feeds and fecal samples were dried at 60°C and grinding to pass through a 1 mm screen. Estimated dry matter (DM), crude protein (CP), ash, and ether extract (BE) were as procedures of AOAC (2016). Neutral detergent fiber (NDF) was estimated according to Van Soest et al. (1991). Nutrient digestibility coefficients and the nutritive value were counting as equations of Schneider and Flatt (1975).

$$\text{DM digestibility (\%)} = 100 - \frac{100 \times \text{AIA \% in feed}}{\text{AIA \% in feces}}$$

$$\text{Digestibility of components} = 100 - \frac{100 \times \text{AIA \% in feed} \times \text{component \% in feed}}{\text{AIA \% in feces} \times \text{component \% in feces}}$$

Blood samples were obtained morning from the jugular vein of each experimental animal in each group before access to feed on the last day of the collection period. The samples were centrifuged at 4000 rpm/15 min to separate the serum and then stored at -18°C until analysis. Total proteins, albumin, urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and glucose concentration were determined using commercial kits (Bio Merieux 69280 Marcy-1, Etoile/France), as the manufacturer's instructions.

Milk production and composition

Lactating buffalo were milked twice daily (6:00 and 18:00), and milk production (MP) was recorded for each buffalo during the collection period. Daily milk samples were mixed in

the morning and afternoon for each animal and stored at -20°C for analysis of milk protein, fat, and lactose using infrared Milko-Scan (133BN Foss Electric, Denmark). Ash was estimated according to procedures of AOAC (2016), while total solids (TS) and solid not fat (SNF) were calculated as differences. Fat-corrected milk (FCM, 7%) was calculated according to Raafat and Saleh (1962) using the following equation:

$$\text{FCM} = [(0.265 \times \text{milk yield, kg}) + (10.5 \times \text{fat yield, kg})]$$

Energy-corrected milk (ECM) was calculated using fat and protein (adjusted to 3.5% fat and 3.2% protein) by Casasús et al. (2004) as the following formula:

$$\text{ECM (kg)} = \text{milk production (kg)} \times (383 \times \text{fat \%} + 242 \times \text{protein \%} + 783.2) / 3140.$$

Statistical analysis:

Kinetics of *in-vitro* gas production was analyzed using Statistical Analytical System (version 9.2; SAS Institute, Cary, NC) according to the General Linear Model as follows:

$$Y_{ij} = \mu + T_i + e_{ij}, (1)$$

where: Y_{ij} = observation; μ = overall mean; T_i = the fixed effect of the treatments; and e_{ij} = random error term common for all observations.

All obtained data from the feeding experiments were subjected to analysis of variance according to a 4×4 Latin square design using the general linear model procedures of the Statistical Analysis System Institute (version 9.2; SAS Institute, Cary, NC). The model was:

$$Y_{ijkl} = \mu + P_i + C_j + T_k + e_{ijkl} (2)$$

where Y_{ijkl} is the dependent variable under examination, μ is the overall mean, P_i is the fixed effect of the period ($i = 4$), C_j is the random effect of the cow ($j = 4$), T_k is the fixed effect of the dietary treatments ($k = 4$), and e_{ijkl} is the random error. The results are presented as mean values with the standard error of the means. Differences among means at $P < 0.05$ were

accepted as representing statistical differences. Treatment means were compared by orthogonal polynomials using Duncan's new multiple range test (MRT, 1955).

Results

In-vitro gas production kinetics and fermentation patterns

Data of leaves, seeds, and eucalyptus oil supplementation on *in-vitro* cumulative gas, methane production, and NDF degradability are exposed in Table (2). The pH value of the *in-vitro* incubated diet in G₄ was increased ($P < 0.05$) significantly compared to the other tested diets. The NH₃-N, SCFA's, and acetic acid concentrations were declined ($P < 0.05$) significantly in G₄ as compared to G₁, G₂, and G₃. The propionic acid concentrations of incubated rumen liquor in G₂ and G₃ were higher ($P < 0.05$) significantly compared to G₄ and G₁. The butyric acid concentrations of incubated rumen liquor in G₃ were increased ($P < 0.05$) significantly compared to G₁, G₂, and G₄. However, the C₂/C₃ ratio was reduced ($P < 0.05$) significantly in G₂ and G₃ compared to G₄ and G₁. The present study showed that EUO supplementation with buffalo diets led to a change in the end products of rumen fermentation with a drop in acetate. An *in-vitro* incubated diet G₄ reduced ($P < 0.05$) the total count of bacteria and cellulolytic bacteria compared to G₂, G₃, or G₁. No significantly different found of the bacterial total count in G₁, G₂, and G₃, but the cellulolytic bacteria count was lower ($P < 0.05$) in G₃ than in G₁. Conversely, the count of protozoa significantly increased ($P < 0.05$) with EUO supplementation (G₄) compared to supplementation with EUL (G₂), EUS (G₃), or the control (G₁).

As illustrated in Fig. (1), the cumulative gas volume (calculated as overall-means during incubation times) was lower ($P < 0.05$) significant for all treated diets (G₂, G₃, and G₄) than for the control (G₁). The lowest volume of gas accumulation was recorded with EUO (G₄), followed by *in-vitro* incubated diets with EUL (G₂) and EUS (G₃) (Table 2). The values of insoluble but slowly fermenting fraction (b) and constant gas released rate (c) were

significantly ($P < 0.05$) higher with EUO supplementation (G₄) compared to the other tested diets. Otherwise, the lag time was significantly ($P < 0.05$) lower with EUO supplementation (G₄) compared to the other tested diets. Supplemental EUL, EUS, or EUO diets cause a significant ($P < 0.05$) lower methane production and degradability of *IVNDFD* compared to the control diet. This study revealed that all supplemented forms of eucalyptus decreased ($P < 0.05$) methane and total gas production.

Feeding trials

As visible in Table 3, eucalyptus supplementation EUS, EUL, or EUO to buffalo diet caused a significant ($P < 0.05$) decrease in MP, 7% fat-corrected milk (FCM), and energy-corrected milk (ECM) compared to the control diet. Buffalo in G₄ had the lowest values of MP, 7% FCM, and ECM, followed by buffalo in G₂ and G₃. Milk fat was significantly ($P < 0.05$) decreased in G₄ than the other groups. An adverse trend was obtained for the milk content of lactose, while no significant effect was noticed on the content of proteins, ash, SNF, and TS by supplementing EUS or EUL.

Feed conversion values identified as DMI/FCM, TDNI/FCM, and NI/FCM were increased ($P < 0.05$) significantly by either supplemental form of eucalyptus in experimental diets compared to the control. The favorite values of feed conversion were noticed with either EUL or EUS supplementation in G₂ and G₃ compared to EUO supplementation in G₄.

The data in Table 4, appeared that EUL, EUS, or EUO supplementation into buffalo diet significantly ($P < 0.05$) decreased the digestion coefficient of DM, OM, CP, NDF, and ADF compared to the control diet. Additionally, the digestion coefficients of these parameters were significantly ($P < 0.05$) higher in G₂ and G₃ than in G₄. On contrary, the digestion coefficient of EE in the tested diets was increased ($P < 0.05$) significantly compared with the control diet. The nutritive values were significantly ($P < 0.05$) affected by EUS, EUL, or EUO supplementation. Values of TDN for G₃ and G₄ were decreased ($P < 0.05$) significantly

compared to G₁ and G₂, and G₄ had the lowest value of TDN. Data show that G₄ had the lowest value of DCP, followed by G₃ and G₂, respectively, and G₁ had the highest value of DCP.

Table 4 shows that serum total protein and albumin were significantly ($P < 0.05$) higher in G₂ and G₃ compared to G₄ or G₁, and G₄ had the lowest level of serum total protein. In contrast, AST and ALT concentrations in the serum of buffalo fed a diet containing EUO (G₄) were significantly ($P < 0.05$) increased compared to the other groups. The urea concentrations significantly ($P < 0.05$) decreased in G₄ serum compared to the other groups, while no significant differences were shown among G₂, G₃, and G₁. The concentration of serum glucose was significantly ($P < 0.05$) affected by supplementation form. Buffalo in G₄ had the highest serum glucose concentration, followed by buffalo in G₃ and G₂, respectively, and the lowest serum glucose concentration was estimated in buffalo in G₁. Creatinine in buffalo fed a diet containing EUS or EUO were significantly ($P < 0.05$) elevated compared to those fed a control diet (G₁) or a diet containing EUL (G₂).

Discussion

In-vitro gas production kinetics and fermentation patterns

There is a discrepancy in the results of studies conducted on ESOs supplements, especially eucalyptus oil, *in-vitro* rumen fermentation, as well as the extent of their various effects on gas accumulation and methane production (Sallam et al. 2010; Patra and Yu 2012; Thao et al. 2015; Giller et al. 2020; Al-Suwaiegh et al. 2020). Several results also showed a variation in the effect of these oils on the rate of degradability of nutrients components (Sallam et al. 2010; Castillejos et al. 2006). The reduction in pH values during *in-vitro* incubation of supplemental EUO in the diet in the present study was in line with the low values observed in several previous studies (Wang 2009; Sallam et al. 2010; Thao et al. 2014). Meanwhile, no observed effect of pH values with incubating of EUL or EUS supplementation, which was in line with the results obtained

via Manh et al. (2012); Thao et al. (2015). These results suggest that the use of natural protection for supplemental eucalyptus oil as the leaves or seed form reduces the undesirable effects of supplemental eucalyptus crude oil.

Regarding ammonia concentration, the obtained result agreed with those of Vakili et al. (2013); Thao et al. (2015). A study by Castillejos et al. (2006) showed that long-term EUO supplementation caused a reduction of rumen ammonia N than the control diet. Moreover, Patra (2012) suggested that ESOs may inhibit bacterial production of excess ammonia in the rumen, resulting in reduced deamination of amino acid consequently lowering rumen $\text{NH}_3\text{-N}$. Similarly, McIntosh et al. (2003) demonstrated that EUO inhibited the growth of some hyper-ammonia-producing bacterial species (i.e., *Clostridium sticklandii* and *Peptostreptococcus anaerobius*), but other bacterial species such as *Clostridium aminophilum* were less sensitive. Hyper-ammonia-producing bacteria are extant in few numbers in the rumen ($P < 0.01$) population, but they have very high deamination activity (Castillejos et al. 2006). In the same line, Vakili et al. (2013) reported that a higher level of EUO supplementation driving to a slight reduction of SCFA's concentrations in the rumen. Similar findings were observed by Wang (2009) when EUO supplementation was used in the sheep diet. Additionally, McIntosh et al. (2003) concluded that the effect of EUO supplementation in the rumen could be ascribed to chemical structures and bioactive components. In the same context, Castillejos et al. (2006); Giannenas et al. (2011) emphasized that EUO supplementation leading to modification in the end products of rumen fermentation with a drop in acetate. The studies by Cobellis et al. (2015) found similar results to those in this study, which indicated a decrease in feed degradability in the rumen. This could be attributed to the non-selective antimicrobial activities of supplemental ESOs, which affect a wide range of microbial subgroups such as cellulolytic bacteria. Furthermore, Patra and Yu (2012) found that adding all the tested ESOs of clove, eucalyptus,

garlic, oregano, and peppermint reduced the abundance of rumen archaea and protozoa; especially cellulolytic bacteria.

Along the same lines, Cieslak et al. (2013) stated that ESOs supplemented with ruminant diets could alter digestion and fermentation, and methanogenesis of diets in the rumen by microbial populations. Besides, Sallam et al. (2010) proposed that the potential effect of supplementation with fresh and residual eucalyptus leaves on mitigation of *in-vitro* CH₄ production be ascribed to a decrease in fermentable substrate rather than to a direct effect on methanogenesis. Analogous results were observed by Manh et al. (2012) in cows that received 100 g/day of eucalyptus leaf meal, which led to the mitigation of rumen CH₄ emissions. The study by Patra and Yu (2012) reported a drop in methane production by less than 15% when using eucalyptus extract.

Animal feeding and performance

Several studies have implied supplementation of eucalyptus leaves or eucalyptus oil on feed intake and palatability, but their results were variable and inconsistent (Ahmed et al. 2005). No differences were found in DMI with EUS, EUL, and EUO supplemented with buffalo diets. Similar findings were recorded by Benchaar et al. (2007); Vakili et al. (2013), while Giannenas et al. (2011) stated that the quantity of feed intake relies on the dose of ESOs supplemented. On the other hand, Cardozo et al. (2006) revealed that EUO supplementation decreases DMI. The effects of eucalyptus supplementation on DMI may fluctuate with the eucalyptus source, diet type, diet interactions, or adaptation of rumen microbial groups (Yang et al. 2010b). In another study, Sebei et al. (2015) mentioned that the major component of eucalyptus is 1,8-cineole, followed by α -pinene could be responsible for the degradation of the chemical constituent and also lead to the acceleration of oxidation. Increased feed conversion efficiency was observed when dairy cows were supplemented with eucalyptus leaf material (Thao et al. 2015) and also with eucalyptus oil (Giller et al. 2020; Al-Suwaiegh et al. 2020).

Despite MP and ECM decreased with supplanting eucalyptus, the reverse result was found by Giannenas et al. (2011), who confirmed that MP was increased with ESOs supplementation into the diets of dairy ewes. Milk contents of protein, fat, and lactose were very contradictory with ESOs supplementation. Some studies have reported an increase in protein content in milk (Wall et al. 2014), while others showed raising in milk fat (Santos et al. 2010). Nevertheless, other studies have found an increase in milk lactose (Benchaar et al. 2007) when dairy cows and ewes diets are supplemented with ESOs.

The digestibility of DM, OM, CP, NDF, and ADF differed ($P > 0.05$) among treatments in studies by Thao et al. (2014; 2015). Similarly, Sallam et al. (2010) concluded that supplementation with EUO influences the degradability of DM and OM *in-vitro*. Furthermore, Santos et al. (2010) found that feed digestibility was affected when using ESOs as a supplement to the diet of lactating dairy cows. The current results are supported by those found by Benchaar et al. (2007) who recognized that apparent total tract digestibilities of DM, CP, and NDF were affected in lactating cows supplemented with 2 g/ day of ESOs.

In the present study, eucalyptus supplementation in buffalo diets led to changes in blood components Table 4. Meanwhile, Morsy et al. (2012) found that dietary supplementation with different ESOs (anise, clove, and juniper) or their combination significantly elevated total protein, albumin, and globulin. In addition, Malekkhahi et al. (2015) demonstrated that sheep fed garlic ESOS or lambs fed a combined (thymol, carvacrol, eugenol, limonene, and cinnamaldehyde) supplemental diet did not affect plasma total protein and albumin. According to reviewed by Huang and Lee (2018) the improvement of serum protein of animals fed an ESOs blend could be because of the content of phytochemicals, which have immune stimulation and anti-inflammatory and antioxidative activities. Moreover, Yang et al. (2010b) reported that concentrations of some blood metabolites such as total protein and albumin could be influenced by the type of ESOs by changing the feed intake, and the lack of change in

glucose and creatinine concentration may be attributable to lack of DMI alternation by the ESOs.

Therefore, the synthesis of urea in the liver is performed from ammonia absorbed from the rumen; as a result, urea N concentration in the serum of buffalo is highly associated with the rumen $\text{NH}_3\text{-N}$ concentration (Davidson et al. 2003). This interpretation is consistent with the results obtained, as the concentrations of rumen $\text{NH}_3\text{-N}$ (Table 2) were not affected by EUS and EUL supplementation compared with the EUO supplement, which was reflected in BUN. These results disagree with those obtained from Yang et al. (2010a), who investigated different doses of ESOs in beef cattle but were consistent with some of those gotten by Davidson et al. (2003). Moreover, supplementation of EUO in the finishing diet of calves was expected to have pharmacological activity; however, these compounds did not affect the liver enzymes. Many previous studies have revealed that supplementation with ESOs did not affect significantly blood glucose concentration (Vakili et al. 2013; Yang et al. 2010b). These findings agreed with Malekhhahi et al. 2015) who reported that glucose levels showed an alteration in the blood of growing lambs when supplementation of different ESOs or blends of them. However, Yang et al. 2010b) found an increase in creatinine concentration in the blood with the addition of eucalyptus leaves, eucalyptus oil, or ESOs blend to the diet compared to G1 (Al-Suwaiegh et al. 2020). In contrast, Castillo et al. (2012) reported that the ESOs blend (carvacrol, cinnamaldehyde, and capsaicin) supplementation decreased the serum creatinine level in calves.

Conclusions

Despite the ability of crude eucalyptus oil to decline methane in the rumen of ruminants, it has negative effects on digestion and animal performance. The supplementation of camphor oil in the form of natural protection in the form of leaves or seed capsules mitigated the adverse

effects of adding the crude oil. The results showed that the leaves followed by the seed capsules were better in their results compared to the supplementation of crude oil on digestion, milk production, and its components, as well as blood parameters.

Fundings

Joint support between all of the following: Animal Production Research Institute; Faculty of Agriculture, Sohag University, and Faculty of Agriculture, Menoufia University, Egypt,

Authors' contributions:

Fawzy Abo-Donia: Conceptualization, Software, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision. **Usama Naye:** Data curation, Methodology, Writing draft preparation. **Mohamed Elaref:** Methodology, Writing-Original draft preparation, Investigation. **Abd El-Moniem Mahgoub:** Conceptualization, Resources, Formal analysis, Visualization. **Tarek Deraz:** Software, Validation Investigation, Formal analysis.

Data availability (data transparency) The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability (software application or custom code) Not applicable.

Declarations

Ethics approval All procedures and experimental protocols were carried out according to the guidelines for the care and use of animals in research and teaching per the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Consent to participate All authors agree on their participation in the work herein reported.

Conflict of interest The authors declare no competing interests.

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Table (1): Chemical composition of ingredients and the experimental diet (%) on a DM basis.

Item	Ingredients						Experimental diets			
	CFM	BF	RS	CS	EUL	EUS	G ₁	G ₂	G ₃	G ₄
OM	91.06	86.97	83.18	92.11	95.07	94.93	88.09	88.76	88.76	88.09
CP	16.35	15.07	2.69	8.62	8.63	12.94	12.28	12.37	12.34	12.28
NDF	55.65	34.78	73.41	60.00	60.82	62.01	53.78	54.21	54.20	53.78
ADF	38.26	23.91	48.64	45.64	50.01	51.82	36.86	37.22	37.21	36.86
EE	3.62	2.34	1.19	2.58	5.92	7.85	2.60	2.89	2.86	2.90
Ash	8.94	13.03	16.82	7.89	4.93	5.07	11.91	11.24	11.24	11.91

CFM concentrate feed mixture; BF fresh berseem; RS rice straw; CS corn silage; EUL eucalyptus leaves; EUS eucalyptus seeds; EUO eucalyptus oil; OM organic matter; CP crude protein; NDF neutral detergent fibre; ADF acid detergent fibre; SEM standard error of the mean; EE ether extract.

Table (2): Effect of leaves, seeds, and eucalyptus oil supplementation on *in vitro* cumulative gas, methane production, and NDF degradability.

Treatments	G ₁	G ₂	G ₃	G ₄	SEM	P-value
Basis pattern of <i>in vitro</i> fermentation						
pH	5.99 ^b	6.05 ^b	6.07 ^b	6.25 ^a	0.037	0.0018
NH ₃ -N (mg/L)	183.37 ^a	182.55 ^a	182.04 ^a	163.61 ^b	5.150	0.0522

SCFA's (mM/L)	89.02 ^a	88.98 ^a	88.86 ^a	83.55 ^b	0.695	0.0002
Acetic acid (mol/100 mol)	59.99 ^a	59.95 ^a	59.93 ^a	57.22 ^b	0.728	0.0484
Propionic acid (mol/100 mol)	20.83 ^b	21.53 ^a	21.54 ^a	19.99 ^c	0.252	0.0027
Butyric acid (mol/100 mol)	11.77 ^b	11.68 ^b	12.23 ^a	11.26 ^c	0.080	<.0001
C ₂ /C ₃ ratio	2.88 ^a	2.78 ^b	2.78 ^b	2.86 ^a	0.016	0.0013

Ruminal Microorganisms

Bacteria counts, ×10 ⁶ cfu/mL	6.48 ^a	6.42 ^a	6.37 ^a	6.07 ^b	0.035	<.0001
CB count, ×10 ⁵ cfu/mL	2.92 ^a	2.89 ^{ab}	2.87 ^b	2.73 ^c	0.016	<.0001
Protozoa counts, × 10 ³ cfu /mL	3.64 ^b	3.67 ^b	3.68 ^b	3.83 ^a	0.026	0.0016

Kinetic of gas production

A	103.03 ^a	98.00 ^b	97.93 ^b	77.70 ^c	0.500	<.0001
B	0.051 ^{ab}	0.050 ^b	0.050 ^b	0.053 ^a	0.001	0.0346
C	-0.155 ^b	-0.152 ^b	-0.156 ^b	-0.099 ^a	0.007	0.0002
L	2.332 ^a	2.286 ^a	2.481 ^a	0.978 ^b	0.186	0.0003
CH ₄ (mL/g DM)	14.10 ^a	8.41 ^b	8.35 ^b	8.35 ^b	0.142	<.0001
ME (MJ/kg DM)	11.664 ^a	10.513 ^b	10.449 ^b	9.685 ^c	0.098	<.0001
IVNDFD (%)	45.41 ^a	43.25 ^b	42.82 ^b	40.00 ^c	0.237	<.0001

^{abc}, Means within the same rows with differing superscripts are significantly different ($P \leq 0.05$).

SEM standard error of the mean; *CB* cellulolytic bacteria; *A* the exponential total gas mL; *B* the asymptotic gas produced (mL/g DM) by the insoluble but slowly fermenting fraction; *C* constant gas production rate (mL/h); *L* lag time; *IVNDFD* *In vitro* neutral detergent fiber degradability

Table (3): Effect of leaves, seeds, and eucalyptus oil supplementation on milk production, its constituents, and feed conversion.

Item	Experimental diets				SEM	P-value		
	G ₁	G ₂	G ₃	G ₄		T	P	T×P
TDMI (kg/h/d)	15.956	16.137	16.135	15.964	0.133	0.6265	0.2937	0.7791
MP (kg/h/d)	7.00 ^a	6.84 ^b	6.80 ^b	6.54 ^c	0.047	<.0001	0.8900	0.6526
FCM (kg/h/d)	6.38 ^a	6.19 ^b	6.10 ^b	5.82 ^c	0.059	<.0001	0.9926	0.9713
ECM (kg/h/d)	9.04 ^a	8.78 ^b	8.66 ^b	8.24 ^c	0.068	<.0001	0.9969	0.8982
<u>Milk composition (%)</u>								
Fat	6.16 ^a	6.08 ^{ab}	6.02 ^{ab}	5.95 ^b	0.060	0.1013	0.7209	0.6964
Protein	3.78	3.78	3.77	3.69	0.031	0.2065	0.6721	0.8121
Lactose	4.54 ^b	4.57 ^{ab}	4.66 ^{ab}	4.72 ^a	0.050	0.0612	0.3893	0.8416
Ash	1.39	1.38	1.38	1.38	0.018	0.9647	0.9634	0.1685
SNF	9.71	9.73	9.80	9.79	0.061	0.6439	0.2387	0.4375
TS	15.87	15.82	15.82	15.74	0.071	0.6798	0.5617	0.9866
<u>Feed conversion (kg intake/kg FCM 7% fat)</u>								
TDMI/FCM	2.503 ^c	2.616 ^b	2.649 ^b	2.746 ^a	0.032	<.0001	0.6758	0.5986
TDNI/FCM	1.563 ^b	1.642 ^a	1.633 ^a	1.609 ^{ab}	0.019	0.0333	0.6652	0.5867
NI/FCM	0.049 ^c	0.052 ^b	0.053 ^{ab}	0.054 ^a	0.001	<.0001	0.6015	0.6106

^{abc}, Means within the same rows with differing superscripts are significantly different ($P \leq 0.05$).

SEM standard error of the mean, *TDMI* total dry matter intake; *MP* milk production; *FCM* 7% fat-corrected milk; *ECM* energy-corrected milk; *SNF* solid not fat; *TS* total solids; *NI* nitrogen intake

Table (4): Effect of leaves, seeds, and eucalyptus oil supplementation on apparent digestibility coefficients and blood parameters of experimental diets.

Item	Experimental diets				$\pm$ SEM	<i>p</i> -Value		
	G ₁	G ₂	G ₃	G ₄		T	P	T×P
<u>Nutrient digestibility and Nutritive values (%)</u>								
DM	66.63 ^a	65.86 ^a	63.88 ^b	61.73 ^c	0.358	<.0001	0.3414	0.4809
OM	68.41 ^a	67.60 ^b	66.41 ^c	63.51 ^d	0.107	<.0001	0.0101	<.0001
CP	70.62 ^a	69.43 ^b	67.33 ^c	63.03 ^d	0.131	<.0001	0.1097	0.1292
EE	67.15 ^c	76.07 ^a	75.25 ^a	73.08 ^b	0.462	<.0001	0.7607	0.8997
NDF	68.11 ^a	67.28 ^b	64.24 ^c	60.01 ^d	0.209	<.0001	0.0409	<.0001
ADF	62.59 ^a	62.05 ^b	59.36 ^c	57.40 ^d	0.127	<.0001	<.0001	<.0001
TDN	62.45 ^a	62.75 ^a	61.63 ^b	58.60 ^c	0.093	<.0001	0.0109	<.0001
DCP	8.67 ^a	8.55 ^b	8.31 ^c	7.74 ^d	0.016	<.0001	0.1179	0.1125
<u>Blood parameters</u>								
Total protein (TP), g/dL	6.04 ^b	6.12 ^a	6.13 ^a	5.98 ^c	0.009	<.0001	0.6034	0.3950
Albumin (A), g/dL	3.04 ^b	3.15 ^a	3.15 ^a	3.00 ^b	0.027	0.0003	0.7482	0.0150
AST, u/L	36.38 ^b	36.47 ^b	36.56 ^b	37.56 ^a	0.314	0.0394	0.7197	0.9019
ALT, u/L	16.06 ^b	16.10 ^b	16.16 ^b	16.59 ^a	0.145	0.0508	0.6927	0.0003
Urea (BUN), mg/dL	15.39 ^a	15.22 ^a	15.23 ^a	14.10 ^b	0.073	<.0001	0.1348	0.1178
Glucose, mg/dL	58.50 ^d	61.80 ^c	63.73 ^b	65.23 ^a	0.120	<.0001	0.2217	0.1760
Creatinine, mg/dL	1.52 ^b	1.50 ^b	1.57 ^a	1.58 ^a	0.014	0.0003	0.0334	0.1999

^{abc}, Means within the same rows with differing superscripts are significantly different ($P \leq 0.05$).
SEM standard error of the mean; *DM* dry matter; *OM* organic matter; *CP* crude protein; *EE* ether extract;
NDF neutral detergent fibre; *ADF* acid detergent fibre; *TDN* total digestible nutrient; *DCP* digestible crude
protein; *AST* aspartate aminotransferase; *ALT* alanine aminotransferase

Fig. (1): Cumulative gas volume ($Gv^{(t)}$) for the experimental diets at different incubation times

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