THE EFFECT OF USING EUCALYPTUS OIL, LEAVES, AND SEED CAPSULES AS SUPPLEMENT IN DIETS ON LACTATING EGYPTIAN BUFFALO PRODUCTIVITY AND METHANE PRODUCTION

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SUMMARY

Sixteen lactating Egyptian buffaloes randomly assigned to a 4×4 Latin square design to investigate the effects of eucalyptus oil naturally protected in the form of leaves (EUL) or mature seed capsules (EUS) or unprotected crude oil (EUO). The control group (G1) got the basal diet consisting of concentrate feed mixture (CFM), fresh berseem (FB), rice straw (RS), and corn silage (CS) to be 40:60 concentrate: roughage ratio. In the G2, G3, and G4 animals fed the basal diet with a supplement of 200 g/head/day of EUL, EUS, or 4 ml EUO, respectively. Supplement of EUL or EUS increased NH3-N, SCFA’s, and acetate acid concentrations in-vitro than EUO. While C2/C3 ratio decreased (P<0.05) with supplement EUO or EUS compared to EUO or control diet. The total bacteria count, and cellulolytic bacteria increased (P<0.05) with supplement EUL or EUS, compared to EUO. While protozoa count increased with supplement EUO compared with EUL, EUS, or control. Methane production and degradability of NDF were lower (P<0.05) with the supplementation of EUS, EUL, or EUO compared to the control diet. Milk fat decreased (P<0.05) with EUO-supplement than the control diet, while an adverse trend was shown for lactose. No differences were found for feed conversion among EUS, EUL, or EUO. Total protein and albumin increased (P<0.05) with supplement EUO or EUS compared to EUO. Supplement EUO increased (P<0.05) AST; ALT, glucose, and creatinine. Blood urea increased (P<0.05) with feeding EUO or EUS compared to EUO, but no difference when compared to the control group. The supplementation of EUL, EUS, or EUO decreased (P<0.05) DM, OM, and CP digestibility compared to the control diet. Digestibility of EE with EUL, EUS, or EUO was higher (P<0.05) than the control diet, while it was higher (P<0.05) with supplementing EUO or EUS than supplementing EUO to the diet. Digestibility of NDF and ADF decreased (P<0.05) with supplement EUO, EUS, or EUO compared to the control diet. Feeding EUO increased (P<0.05) digestibility of NDF and ADF compared to EUL supplementation, which was increased (P<0.05) than feeding EUO. Feeding EUS increased values of TDN and DCP compared to EUL, which was higher than EUO. Finally, the results of the current study confirm that the effect of a supplement of EUO naturally protected in the form of leaves or seeds mitigates the negative effects of directly supplementing crude eucalyptus oil.

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

INTRODUCTION

The rumen is a complex ecosystem in which the nutrients consumed by microorganisms at a suitable pH to provide the main products of fermentation, basically, short-chain fatty acids (SCFA’a) and microbial biomass, which are used by the host ruminants (Cieslak et al. 2013 and Vakili, et al. 2013). Recently, there is increased interest in concerned with reducing the rate of rumen methane production.

Inasmuch methane (CH4) production from enteric fermentation is of concern worldwide because of the increased accumulation of greenhouse gases in the atmosphere, as well as being a waste of nutritious energy (Sallam et al., 2010). There is an interesting to reduce CH4 release by inhibition of ruminal methanogens to increasing the efficiency of feed energy utilization by ruminants, these would have also improved economic efficiency and environmental (Benchaar and Greathead, 2011). Many studies were conducted to investigate the effects of supplementation levels of eucalyptus leaves and eucalyptus oil.
(EO) on methane production (McIntosh et al., 2003; and Castillejos et al., 2006), furthermore, much is still unknown about using dried or ground mature seeds. Sallam et al. (2010) hypothesized that EO could be used as a feed supplement to alter rumen biohydrogenation to reduce CH4 release and increase the flow of volatile acids (VA) to the duodenum. 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 ionophores effect by affecting VA formation in the rumen through inhibiting the final step in the biohydrogenation of VA to stearic acid. Due to the volatile and reactive nature of EOs, it is possible that their effectiveness, when included in the animal’s diet, may be affected according to different conditions during the production season, as well as storage of EOs and conditions in the digestive system of animals (Nguyen et al., 2009).

A recent study by Chouhan et al. (2017) shown that using EOs in a protected form has great potential effect for antimicrobial resistance due to increased chemical stability and solubility, reduced rapid evaporation, and reduced degradation of the active EOs. Also, Lammani et al. (2020) supported that the application of the encapsulation of EOs to make their release subject to continuous control enhancing their bioavailability and effectiveness against microbes.

In recent years, due to increasingly negative consumer perceptions, there has been an increase in interest in adding EOs in ruminant feeds to increase milk production and improve the animal’s physiological performance (Thao et al., 2015). On the other hand, Turek and Stintzing (2013) suggested that adding such oils in their natural form, whether in the form of leaves or grains, avoids the negative effects of those crude oils and increases their effectiveness in ruminant nutrition.

However, there is a scarcity of knowledge of the effects of using naturally protected eucalyptus oil compared to using it as crude oil on animal performance (Maes et al., 2019). Therefore, this study aims to design new mixtures containing naturally protected eucalyptus oil compared to adding it in the form of crude oil and investigate the effect of adding these mixtures in dairy buffalo feed on methane production and production performance.

**MATERIALS AND METHODS**

This study was carried out according to the cooperation protocol between the Animal Production Research Institute (APRI), Agriculture By-product Utilization Research Department, and the Faculty of Agriculture, Menoufia University, the Animal Production Department, (Reference No. 2429.22.2019).

**Ingredients and the experimental diets:**

Eucalyptus leaves (EUL) and green mature seed capsules (EUS) collected from trees on beach canals were dried under shade for a week, 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 isonitrogenous and iso-caloric to cover the recommended requirements of lactating buffaloes according to Kearl (1982). Animals in the 1st group (G1) got the basal diet consisting of concentrate feed mixture (CFM), fresh berseem (FB), rice straw (RS), and eucalyptus leaves (EUL). The 2nd, 3rd, and 4th groups (G2, G3, and G4) were offered the basal diet formulated as concentrate feed mixture (CFM), fresh berseem (FB), rice straw (RS), and eucalyptus seeds (EUS), while the 4th group (G4) was offered the basal diet formulated as concentrate feed mixture (CFM), fresh berseem (FB), rice straw (RS), and eucalyptus oil (EUO). The table below shows the chemical composition of ingredients and the experimental diet (%). Table (1): Chemical composition of ingredients and the experimental diet (%) on a DM basis.

| Item | CFM | BF | RS | CS | EUL | EUS | G1 | G2 | G3 | G4 |
|------|-----|----|----|----|-----|-----|----|----|----|----|
| 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, and EUO: eucalyptus oil.
(RS), and corn silage (CS) to be 40:60 concentrate: roughage ratio. The 2\textsuperscript{nd} (G2), 3\textsuperscript{rd} (G3), and 4\textsuperscript{th} (G4) groups fed the basal diet with a supplement of 200 g/head/day of EUL, EUS, or 4 ml EUO, respectively. Supplemented EUL, EUS, or EUO were dissolved daily in 1 liter of tap water, then blended and mixed directly with the concentrated feed to ensure consistency. Weekly homogeneous samples of experimental diets were dried and ground, then held in glass bottles for analysis and in-vitro studies. The chemical composition of ingredients and the experimental diets are presented in Table (1).

**Animals and management:**

A total number of 16 healthy lactating Egyptian buffalo (body weight: 457.4 ± 10.5 kg; parity: 2 to 4; 14 day in lactation) were divided into four similar groups randomize according to their previous milk records using quadratic 4 x 4 Latin squares experimental designs. Animals were individually fed the experimental diets twice daily (8 a.m. and 6 p.m.). Diet was offered for 28 days (21 days as preliminary period + 7 days as collection period) and diet was adjusted every week according to changes in body weight and milk production. Mineral salt blocks left for the animals to lick freely, as well as access to drinking water.

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

In-vitro gas production technique was conducted according to Theodorou et al. (1994) on obtained samples of the experimental diets. Rumen fluid was collected from two buffalo cows of each group before the morning meal using a stomach tube. About 600 mg of tested sample (1.0 mm) were incubated with 60 mL of previously prepared buffered rumen fluid for each bottle (1.3 mL/mL) according to (Goering and Van Soest 1970) under continuous CO\textsubscript{2} reflux in 100 mL calibrated glass bottle in a water bath maintained at 39°C. Samples were incubated in quadratic together with four bottles containing only incubation medium (blank). Headspace gas pressure measured at 2, 4, 8, 16, 24, 36, and 48 h. Results of kinetic parameters of GP(t) (ml/g DM) were fitted using the NLIN option according to (France et al., 2000) as:

\[
G_{V(t)} = b \times (1 - e^{-k(L-t)})
\]

Where: \(G_{V(t)}\) 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 constant gas production rate (ml/h), ‘\(r\)’ is time of fermentation and ‘\(L\)’ is lag time. In-vitro CH\textsubscript{4} production was determine as described by Pellikaan et al. (2011).

After termination of the incubation, bottle content was used for determination of in-vitro neutral detergent fiber degradability (IVNDFD). In-vitro liquor from each bottle was collected after filtration to determine pH using a portable pH meter, the concentration of NH\textsubscript{3}-N according to AOAC (2016), and total short-chain fatty acids (SCFA’s) according to Eadie et al. (1967). Molar proportions of acetic, propionic, and butyric concentrations were analyzed by gas-liquid chromatography (GC 2010, PerkinElmer), 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 trial:**

The feces were collected directly from the rectum of all animals in each group once in the morning before feeding at the end of the collection period. Acid-insoluble ash (AIA) was used as an internal marker to estimate the digestibility of nutrients (Van Keulen and Young 1977). Feeds and fecal samples were dried at 60°C and ground to pass a 1-mm screen for analyze. Dry matter (DM), crude protein (CP), ash, and ether extract (EE) were determined according to the procedure of AOAC (2016). Neutral detergent fiber (NDF) was estimated according to Van Soest et al. (1991). Nutrient digestibility coefficients and the nutritive value were calculated from the equation stated by Schneider and Flatt (1975).

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

Digestibility of components = 100 –
Milk production and composition:

Lactating buffalo cows were milked twice daily (6:00 and 18:00) and milk production (MP) was recorded for individual buffalo during the collection period. Daily milk samples were mixed according to the ration of the morning and afternoon milk yield 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 determined according to AOAC (2016), while total solids and solid not fat (SNF) were calculated as differences. Fat correct milk (FCM, 7%) was calculated according to Raafat and Saleh (1962) using the following equation:

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

The yield of energy corrected milk (ECM) was calculated using fat and protein (adjusted to 3.5% fat and 3.2% protein) by the following formula (Casasús, et.al., 2004):

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

Blood samples:

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

Statistical analysis:

In-vitro gas production data were analyzed using Statistical Analytical System (SAS, 2009), according to the General Linear Model as following: \(Y_{ij} = \mu + T_i + e_{ij}\)

Where: \(Y_{ij}\) = the observation; \(\mu\) = Overall mean; \(T_i\) = the fixed effect of the treatments; \(e_{ij}\) = Random error term common for all observations. All obtained data of feeding experiments were subjected to analysis of variance according to a 4×4 Latin square design using the general linear model’s procedures of the Statistical Analysis System Institute (SAS, 2009). The results are presented as mean values with the standard error of the means. Differences among means with \(p<0.05\) were accepted as representing statistical differences. Treatment means were compared by orthogonal polynomials by Duncan’s (1955) New Multiple Range Test.

RESULTS AND DISCUSSION

In-vitro ruminal fermentation characteristics and gas production kinetic:

As shown in Table (2), the pH value of an in-vitro incubated diet in (G4) was increased (P<0.05) significantly compared to other experimental diets. Reduced the pH values with the supplement of eucalyptus oil to a diet during in-vitro incubation was in line with the value of low rumen pH observed by several studies (Sallam et al., 2010; Wang et al., 2009; Thao et al. 2014). While no effect was observed on rumen pH with supplemental EUL or EUS to buffalo diet which corresponds to results obtained by Manh et al. (2012); Thao et al. (2015). These results suggested that supplemental eucalyptus oil naturally protected in the leaves or seed reduces the negative effect of supplement eucalyptus crude oil.

The \(\text{NH}_3\text{-N}\), SCFA’s and acetic acid concentrations decreased (P<0.05) significantly in G4 than G1, G2, and G3. The propionic acid concentrations of incubated rumen liquor in G2 and G3 were increased (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, C2/C3 ratio decreased (P<0.05) significantly in G2 and G3 compared to G4 and G1. These findings agree with that mentioned by Vakili et al., (2013) and Thao et al., (2015), Castillejos et al. (2006) found that EUO supplementation for the long-term led to a reduction in rumen ammonia-N compared to those in the control diet. Moreover, Patra and Saxena (2009) suggested that essential crude oils may inhibit bacteria producing excess ammonia in the rumen, resulting in reduced consequently amino acid deamination, thus lowering rumen \(\text{NH}_3\text{-N}\). McIntosh et al. (2003) demonstrated that EUO inhibited the growth of some bacteria species (i.e., Clostridium sticklandii and Peptostreptococcus anaerobius) hyper-ammonia producing, but other bacteria species such as Clostridium aminophilium were less sensitive. Hyper-
ammonia-producing bacteria are present in low numbers in the rumen (P<0.01) of the rumen bacterial population, but they possess a very high deamination activity (Castillejos et al. (2006). Patra and Saxena (2010) and Vakili et al. (2013) reported that high levels of EUO supplementation led to a slight reduction in concentrations of total SCFA’s in the rumen. Similar findings were observed by Wang et al. (2009) when used EUO supplementation in the sheep diet. McIntosh et al. (2003) reported that the effect of EUO supplementation in the rumen was attributed to chemical structures and bioactive components.

Results of the present study noted that supplemented EUO to buffalo cows diets led to an alteration in the end products of rumen fermentation with a drop of acetate which was previously reported by Castillejos et al. (2006) and Giannenas et al. (2011).

Supplementation of EUO to the in-vitro incubated diet in G4 led to reduced (P<0.05) the total count of bacteria and cellulolytic bacteria than those in G2, G3, or G1. The total count of bacteria was not different significantly in G1, G2, and G3 but cellulolytic bacteria count was lower (P<0.05) in G3 than G1. Conversely, protozoa count was increased (P<0.05) significantly with EUO supplementation (G4), compared to the supplementation of EUL (G2), EUS (G3), or control (G1). Cobellis, et al., (2015) agreed on the result obtained in this study, which indicates a decrease in the feed degradability in the rumen, which attributed to the non-selective antimicrobial activities of supplemented EOs affecting a wide range of microbial subgroups such as, cellulolytic bacteria. Furthermore, Patra and Yu (2012) found that supplement of all the tested EOs of clove, eucalyptus, garlic, oregano, and peppermint reduce the abundance of rumen archaea and protozoa, especially in that of cellulolytic bacteria.

As illustrated in Fig. (1), the cumulative gas volume (calculated as a means for all incubation times) was significantly lower (P<0.05) for all treated diets (G2, G3, and G4) than for the control one (G1). The lowest volume of gas produced was recorded with EUO (G4) followed by those in-vitro incubated diets with EUL (G2) and EUS (G3) (Table 2).

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

The values of insoluble but slowly fermenting fraction (b) and constant gas production rate (c) significantly (P<0.05) increased with EUO supplementation (G4) than other experimental diets. Otherwise, Lag time significantly (P<0.05) reduced with EUO supplementation (G4) than other
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 experimental diets. All studied Supplementations (EUL, EUS, or EUO) led to a significant decrease (P<0.05) in methane production and Degradability of IVNDFD compared to the control diet. Findings of this study show that all supplemented forms of eucalyptus decrease (P<0.05) methane and total gas production. In the same line, Cieslak et al. (2013) stated that EOs supplemented to ruminant diets have altered digestion and fermentation, and methanogenesis of diets in the rumen by microbial populations. Sallam et al. (2010) suggest that the potential effect of supplementation fresh and residual eucalyptus leaves into the diets on mitigating the in-vitro CH$_4$ production, may be attributed 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 and led to mitigating of rumen CH$_4$ emission. Moreover, Patra and Yu (2012) reported a drop in methane production by less than 15% with using eucalyptus extract than the control group.

![Fig. (1): Accumulative gas volume (Gv(t)) for the experimental diets at different incubation times.](image)

**Feed intake, feed conversion and milk yield and composition:**

Table (3) shows that supplementary form of eucalyptus (EUS, EUL, or EUO) to buffalo diet led to a significant (P<0.05) decrease in milk production (MP), fat corrected milk (FCM) 7%, and energy corrected milk (ECM) than the control diet. Buffalo cows in G4 had the lowest values of MP, FCM 7%, and ECM followed by buffalo cows in G2 and G3. Several studies implied the effect of supplement eucalyptus leaves and others used eucalyptus oil on feed intake and palatability, but their results were variable and inconsistent (Ahmed et al., 2005; Hristov et al., 2013). The confirmed results show that supplementing EUS, EUL and EUO did not affect DMI, Similar findings were recorded by Benchaar et al. (2007); Vakili et al. (2013) and Hristov et al. (2013), while, Giannenas et al. (2011) stated that the amount of feed intake depends on the dose of EOs supplementation. On the other hand, Cardozo et al., (2006) reported that EUO supplementation decreases DMI. The effects of eucalyptus supplementation on DMI may differ with the eucalyptus source, diet type, diet interactions, or adaptation of rumen microbial groups (Yang et al., 2010b).

Feed conversion values as mentioned as DMI/FCM, TDNI/FCM, and NI/FCM increased (P<0.05) significantly by either supplementary form of eucalyptus to experimental diets than control one. Supplement of either EUL and EUS in G2 and G3 appeared preferred values of feed conversion compared to EUO supplementation in G4. Sebei et al., (2015) reported that the major component of the EOs in eucalyptus is 1,8-cineole followed by α-pinene. An 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).
Milk production as MP and ECM was significantly (P<0.05) decreased in experimental groups than the control group, also milk fat was significantly (P<0.05) decreased in G4 than the other experimental groups, while an adverse trend was obtained for the milk content of lactose, while contents of proteins, ash, SNF and TS were not affected (P<0.05) significantly by supplementing EUS or EUL. A reverse result was recorded by Giannenas et al. (2011) who refer to an increase in milk production with EOs supplementation into diets of dairy ewes. Effects of EOs supplementation on the contents of protein, fat, and lactose in the milk are very contradictory. Some studies reported an increase in milk protein content (Spanghero et al., 2009; Wall et al., 2014), but others show an increase in milk fat (Santos et al., 2010), while other studies found an increase in milk Lactose (Benchaaar et al., 2007) when dairy cows and ewes diets supplemented with EOs.

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

| Item                        | Experimental diets | SEM | p-Value |
|-----------------------------|--------------------|-----|---------|
| | G1 | G2 | G3 | G4 |
| TDMI (kg/h/d)               | 15.956            | 16.137 | 16.135 | 15.964 | 0.133 | 0.6265 | 0.2937 | 0.7791 |
| MP (kg/h/d)                 | 7.00a             | 6.84b | 6.80b | 6.54c | 0.047 | <.0001 | 0.8900 | 0.6526 |
| FCM (kg/h/d)                | 6.38a             | 6.19b | 6.10b | 5.82c | 0.059 | <.0001 | 0.9926 | 0.9713 |
| ECM (kg/h/d)                | 9.04a             | 8.78b | 8.66b | 8.24c | 0.068 | <.0001 | 0.9969 | 0.8982 |
| Milk composition (%)        |                   |      |       |      |       |       |       |       |
| Fat                        | 6.16a             | 6.08ab | 6.02ab | 5.95b | 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                    | 4.54b             | 4.57ab | 4.66ab | 4.72a  | 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 |
| Feed conversion (kg int/k FCM 7% fat) |       |      |       |      |       |       |       |       |
| DMI/FCM                    | 2.503a            | 2.616b | 2.649b | 2.746c | 0.032 | <.0001 | 0.6758 | 0.5986 |
| TDNI/FCM                   | 1.563b            | 1.642a | 1.633a | 1.609ab | 0.019 | 0.0333 | 0.6652 | 0.5867 |
| NI/FCM                     | 0.049a            | 0.052b  | 0.053ab | 0.054c | 0.001 | <.0001 | 0.6015 | 0.6106 |

*Means within the same rows with differing superscripts are significantly different (P<0.05).
SEM= standard error of the mean.
MP= Milk production, FCM= Fat corrected milk 7%, and ECM= Energy corrected milk.
SNF= solid not fat and, and TS: total solid.

Nutrient digestibility and nutritive values:

Data in Table (4) illustrated that the supplementation of EUL, EUS, or EUO to the buffalo diet significantly (P<0.05) decreased the digestion coefficient of DM, OM, CP, NDF, and ADF compared to the control diet. Also, the digestion coefficients of these parameters were significantly (P<0.05) higher in G2 and G3 than in G4. In contrast, the digestibility of EE in the experimental groups was increased (P<0.05) significantly compared with the control group. 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 than G1 and G2 and G4 had the lowest value of TDN. Also, G4 had the lowest value of DCP followed by G3 and G2, respectively and G1 had the highest value of DCP. For instance, the apparent digestibility of DM, OM, CP, NDF, and ADF were different (p>0.05) among treatments in the study by Thao, et al. (2014; 2015). Moreover, Sallam et al. (2010) concluded that supplementation of EUO influences the digestibility of DM and OM in-vitro. Furthermore, Santos et al. (2010) found that feed digestibility was affected when the EOs compound was added to the diet of lactating dairy cows. Current results are supported by the results obtained by Benchaaar et al. (2007) who shown that apparent total tract digestibilities of DM, CP, and NDF had been affecting lactating cows supplemented with 2 g/ day of EOs.
Table (4): Effect of leaves, seeds, and eucalyptus oil supplementation on apparent digestibility coefficients of experimental diets.

| Nutrient digestibility (%) | Experimental diets | \(\pm\ SEM\) | \(p\)-Value |
|---------------------------|--------------------|--------------|-------------|
|                           | G1                 | G2           | G3          | G4          |
| 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\(^a\)        | 76.07\(^a\)  | 75.25\(^a\) | 73.08\(^a\) | 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 |
| Nutritive values (%)      |                    |              |             |             |
| 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\(^a\)   | 8.31\(^a\)  | 7.74\(^a\)  | 0.016        | <.0001 | 0.1179 | 0.1125 |

Means within the same rows with differing superscripts are significantly different (\(P<0.05\)).

SEM= standard error of the mean.

Blood metabolites:

Table (5) shows that serum total protein and albumin significantly (\(P<0.05\)) increased in G2 and G3 compared to G4 or G1 and G4 had the lowest level of serum total protein. In the same context, (Morsy et al., 2012) found that the dietary supplementation of different EOs (anise, clove, and juniper) or their combination significantly increased total protein, albumin, and globulin. While Malekkhahi et al., (2015) stated that sheep fed garlic EO or lambs fed a combine (thymol, carvacrol, eugenol, limonene, and cinnamaldehyde) supplemented diet did not affect plasma total protein and albumin. Kirkpar et al. (2011) supposed that the improvement of serum protein of animals fed EOs blend could be due to the content of phytochemicals, which immune stimulation and anti-inflammatory and antioxidative activities. Moreover, Yang et al. (2010b) has been reported that concentrations of some blood metabolites such as total protein and albumin can be influenced by EO kind via changing of feed intake and no change in glucose and creatinine concentration may be contributed to lack of DMI alternation by the EO.

On contrary, the concentration of AST and ALT in serum of buffalo cows fed a diet containing EUO (G4) significantly (\(P<0.05\)) increased. In opposite, the concentration of AST and ALT in serum of buffalo cows fed a diet containing EUO (G4) significantly (\(P<0.05\)) increased than the other experimental groups. The urea concentrations were significantly different between the supplementary groups and the control group. Serum urea significantly (\(P<0.05\)) decreased in G4 compared to the other experimental groups. Ruminal ammonia-N over microbial requirement is absorbed across the rumen wall into portal blood, and most of it is converted to urea in the liver. Therefore, the synthesis of urea in the liver is performed from ammonia absorbed from the rumen; as a result, urea N concentration in blood is highly correlated with the rumen NH3-N concentration (Davidson et al., 2003). This interpretation is consistent with the results obtained, as the concentrations of rumen NH3-N, Table (2), were not affected by the supplement of EUS and EUL, compared with the EUO supplement, which was reflected on BUN. Although the results disagree with those obtained from Yang et al. (2010a) that investigated different doses of EO in beef cattle but were consistent with some of what is obtained by Tassoul and Shaver (2009). 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.

Serum glucose concentration was significantly (\(P<0.05\)) affected by supplementation type. Buffalo cows in G4 had the highest level of serum glucose concentration followed by buffalo cows in G3 and G2 respectively and the lowest level of serum glucose concentration was estimated in buffalo cows in G1. Many previous studies found that EOs supplementation did not affect significantly blood glucose concentration (Tassoul and Shaver, 2009; Yang et al., 2010b, and Vakili et al., 2013). While Malekkhahi et al. (2015) agree with the obtained result of glucose levels in this study that show alteration in glucose levels when goats and growing lambs fed different EO or EO blend.
Creatinine in buffalo cows fed a diet containing EUS or EUO significantly (P<0.05) increased compared to those fed a basal diet (G1) or fed a diet containing EUL (G2). The results of the study conducted by Yang et al. (2010b) indicated an increase in the concentration of creatinine in the blood when adding eucalyptus leaves, eucalyptus oil, or EOs blend to the diet compared to the control group (Al-Suwaiiegh et al., 2020). In contrast, Castillo et al. (2012) reported that the EOs blend (carvacrol, cinnamaldehyde, and capsaicin) supplementation decreased serum creatinine level in calves.

Table (5): Effect of leaves, seeds, and eucalyptus oil supplementation on blood parameters.

| Item                | Experimental diet | ± SEM | p-Value |
|---------------------|-------------------|-------|---------|
|                     | G1     | G2     | G3     | G4     | T      | P      | T×P    |
| Blood parameters    |        |        |        |        |        |        |        |
| Total protein (TP), g/dl | 6.04b  | 6.12a  | 6.13a  | 5.98c  | 0.009  | <.0001 | 0.6034 | 0.3950 |
| Albumin (A), g/dl   | 3.04b  | 3.15a  | 3.15a  | 3.00b  | 0.027  | 0.0003 | 0.7482 | 0.0150 |
| AST, u/l            | 36.38b | 36.47b | 36.56b | 37.56a | 0.314  | 0.0394 | 0.7197 | 0.9019 |
| ALT, u/l            | 16.06b | 16.10b | 16.16b | 16.59b | 0.145  | 0.0508 | 0.6927 | 0.0003 |
| Urea (BUN), mg/dl   | 15.39a | 15.22a | 15.23a | 14.10b | 0.073  | <.0001 | 0.1348 | 0.1178 |
| Glucose, mg/dl      | 58.50c | 61.80c | 63.73b | 65.23a | 0.120  | <.0001 | 0.2217 | 0.1760 |
| Creatinine, mg/dl   | 1.52b  | 1.50b  | 1.57b  | 1.58a  | 0.014  | 0.0003 | 0.0334 | 0.1999 |

Means within the same rows with differing superscripts are significantly different (P<0.05). SEM= standard error of the mean.

CONCLUSION

It could be inferred that EUS, EUL, and EUO supplementation in buffalo feed do not seem to have a protective effect on organ function associated with the blood measurements tested in this research, despite a slight decrease in milk production and fat content, so it is recommended to be careful about adding such substances to the animals diet. The results of the current study confirm that the effect of a supplement of EUO naturally protected in the form of leaves or seeds capsules mitigates the negative effects of directly adding EUO on nutrient digestibility, feeding value, milk yield and composition and blood parameters, where directly adding EUO reduces milk yield and the digestion coefficients of DM, OM, CP, EE, NDF, and ADF comparing with EUO naturally protected.

DECLARATION OF COMPETING INTEREST:

We declare that there is no conflict of interest in this project.

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تأثير إضافة أوراق وبذور زيت الكافور على إنتاج الجاموس المصري الحلال وإنتاج الميثان

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تم استخدام ستة عشر جاموس مصري محلل بشكل عشوائي في تصميم مربع لائني 4 × 4 لإعداد تأثير إضافة زيت الكافور (G1) على تحملات الكشر، ومعاملات الدهن وانتاج الميثان (G2، G3 و G4) في صفات الدعم ومعاملات الدهن في الجاموس الحلال. عند تغذية مجموعة T، وسراج (RS)، القياس المطر (CFM)، السراب البول (FB)، والدهن G1 (G1) على العلمة الأساسية والكوبر من لب لجسم المركز، نموذج البول G1 (G1) على المجموعة المعلمة في المجموعات المعاملة (G2، G3 و G4) مراة حيث كانت نسبة المواد المركزة في الخشنة 40٪، بينما تم تقسيم الحيوانات في المجموعات المعاملة (G2، G3 و G4) على المجموعة الأساسية باهظة البيها (200 جم/رس/يوم من EUL أو EUS) 4 أو 6 مل من EUS أو EUL.

أوراق الكافور إلى زيادة تركيزات كلاً من الأمونيا وأسماء الأحماض الدخيلة الطيارة وحمض الخليك معهيا مقارنة بزيت الكافور. بينما اختلفت نسبة حامض الخليك / حامض الريبوبونيك (C2/C3 مع فارق P<0.05) مع إضافة أوراق الكافور أو بذور الكافور مقارنة (C2/C3 مع فارق P<0.05) مع إضافة زيت الكافور أو زيت الكافور. زاد النتاج الإجمالي للكثيري والكثيري المتحول للسلولور (P<0.05) مع إضافة زيت الكافور أو زيت الكافور. زاد النتاج الزراعي بزيت الكافور أو بذور الكافور مقارنة بزيت الكافور أو بذور الكافور. بينما زاد عدد البروتوزويا مع إضافة زيت الكافور مقارنة بزيت الكافور أو بذور الكافور أو بذور الكافور. زاد النتاج الإجمالي للكثيري والكثيري المتحول للسلولور (P<0.05) مع إضافة زيت الكافور أو بذور الكافور أو بذور الكافور. زاد النتاج الزراعي بزيت الكافور أو بذور الكافور. زاد النتاج الزراعي بزيت الكافور أو بذور الكافور. زاد النتاج الزراعي بزيت الكافور أو بذور الكافور. زاد النتاج الزراعي بزيت الكافور أو بذور الكافور. زاد النتاج الزراعي بزيت الكافور أو بذور الكافور. زاد النتاج الزراعي بزيت الكافور أو بذور الكافور. زاد النتاج الزراعي بزيت الكافور أو بذور الكافور. زاد النتاج الزراعي بزيت الكافور أو بذور الكافور. زاد النتاج الزراعي بزيت الكافور أو بذور الكافور. زاد النتاج الزراعي بزيت الكافور أو بذور الكافور.