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

F.M. Abo-Donia*[1], A. Mahgoub[1], T. Deraz[1]. and U.A. Nayel[2]

[1]Agriculture By-product Utilization Research Department, Animal Production Research Institute (APRI), Giza, Egypt.

[2]Faculty of Agriculture, Menoufia University, Egypt.

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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 NH_3-N, SCFA's, and acetic acid concentrations i*n-vitro* than EUO. While C2/C3 ratio decreased (P<0.05) with supplement EUL 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 EUL or EUS compared to EUO. Supplement EUO increased (P<0.05) AST, ALT, glucose, and creatinine. Blood urea increased (P<0.05) with feeding EUL 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 EUL or EUS than supplementing EUO to the diet. Digestibility of NDF and ADF decreased (P<0.05) with supplement EUL, EUS, or EUO compared to the control diet. Feeding EUS 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's) 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, Lammari *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

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

Item	Ingredients						Experimental diets			
	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 2nd (G2), 3rd (G3), and 4th (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 × 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_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:

$$\mathbf{Gv_{(t)} = b\ x\ (1\text{-}e^{-c(t-L)})}$$

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 constant gas production rate (ml/h), '*t*' is time of fermentation and 'L' is lag time.*In-vitro* CH_4 produccion 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_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).

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

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

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; μ = 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 NH_3-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 NH_3-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 aminophilum* 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.

Treatment	G1	G2	G3	G4	SEM	*p-Value*
Basis pattern of *in-vitro* 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, $\times 10^6$ cfu/ml	6.48^{a}	6.42^{a}	6.37^{a}	6.07^{b}	0.035	<.0001
CB count, $\times 10^5$ cfu/Ml	2.92^{a}	2.89^{ab}	2.87^{b}	2.73^{c}	0.016	<.0001
Protozoa counts, $\times 10^3$ 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}	8.35^{b}	8.35^{b}	8.41^{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<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), and L: lag time.*
IVNDFD: In-vitro neutral detergent fiber degradability.

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

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.

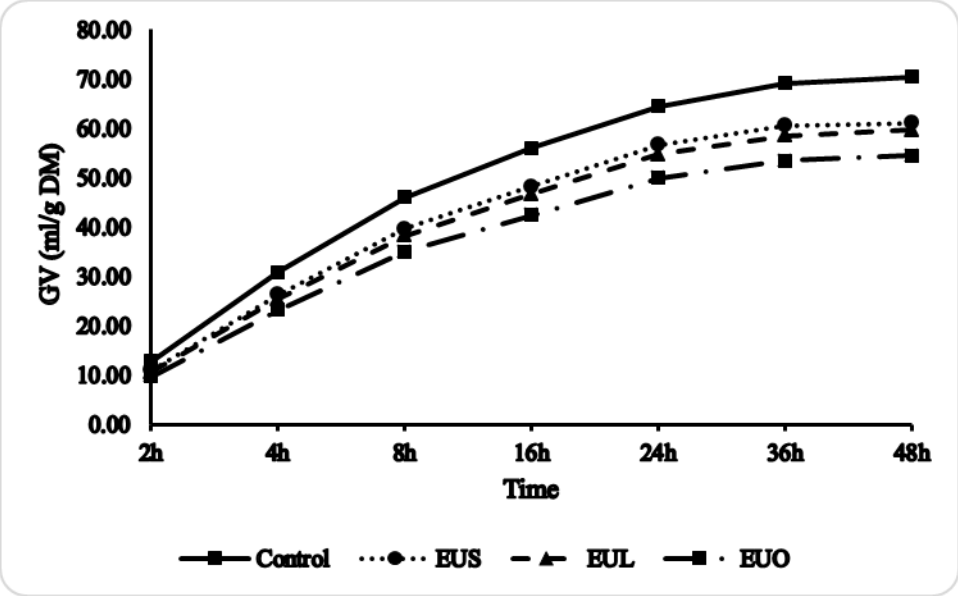

Fig. (1): Accumulative gas volume ($Gv_{(t)}$) for the experimental diets at different incubation times.

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 (Benchaar *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		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
Milk composition (%)								
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
Feed conversion (kg intake/kg FCM 7% fat)								
DMI/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<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 Benchaar *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.

Item	Experimental diets				± SEM	*p-Value*		
	G1	G2	G3	G4		T	P	T×P
Nutrient digestibility (%)								
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
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[b]	8.31[c]	7.74[d]	0.016	<.0001	0.1179	0.1125

[abc,] *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. Kırkpınar *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 NH_3-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-Suwaiegh *et al*., 2020). In contrast, Castillo et al. (2012) reported that the EOs bled (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	6.04^{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<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 وزيت الكافور على إنتاجية الجاموس المصري الحلاب وإنتاج الميثان

فوزى محمد أبودنيا[1] ، عبد المنعم محجوب[1] ، طارق دراز[1] و أسامه أبو العز نايل[2]

[1] قسم بحوث المخلفات الزراعية - معهد بحوث الانتاج الحيوانى - وزارة الزراعة - مصر.
[2] كلية الزراعة - جامعة المنوفية - مصر.

تم استخدام ستة عشر جاموسًه مصريًه حلاب بشكل عشوائي فى تصميم مربع لاتيني 4 × 4 لدراسة تأثير اضافة زيت الكافور المحمي طبيعيا في شكل أوراق (EUL) أو كبسولات بذور ناضجة (EUS) أو زيت خام غير محمي (EUO) على تخمرات الكرش , معاملات الهضم و انتاج الميثان معمليا وعلى انتاج اللبن , صفات الدم و معاملات الهضم فى الجاموس الحلاب . حيث تغذت المجموعة الكنترول (G1) على العليقة الأساسية والمكونة من خليط العلف المركز (CFM) ، البرسيم الطازج (FB) ، قش الأرز (RS) ، وسيلاج الذرة (CS) حيث كانت نسبة المواد المركزة الى الخشنة 60:40 , بينما تم تغذية الحيوانات فى المجموعات المعاملة (G2 و G3 و G4) على العليقة الأساسية مضاف اليها 200 جم / رأس / يوم من EUL أو EUS أو 4 مل من EUO على التوالي.أدت اضافة بذور الكافور أو أوراق الكافور إلى زيادة تركيزات كلا من الأمونيا و والاحماض الدهنية الطيارة وحمض الخليك معمليا مقارنة باضافة زيت الكافور. بينما انخفضت نسبة حامض الخليك / حامض البروبيونك (C2/C3) معنويا ($P<0.05$) مع اضافة أوراق الكافور أو بذور الكافور مقارنة باضافة زيت الكافور أو عليقة الكنترول . زاد العدد الإجمالي للبكتيريا والبكتيريا المحللة للسليولوز ($P <0.05$) مع اضافة أوراق الكافور أو بذور الكافور مقارنة بـ زيت الكافور. بينما زاد عدد البروتوزوا مع اضافة زيت الكافور مقارنةً بـ أوراق الكافور أو بذور الكافور أو الكنترول . انخفض معنويا إنتاج الميثان ومعامل الهضم المعملى ل NDF ($P <0.05$) مع اضافة بذور الكافور أو أوراق الكافور أو زيت الكافور مقارنة بالكنترول. انخفضت قيم دهن اللبن معنويا ($P <0.05$) باضافة زيت الكافور مقارنة بعليقة الكنترول ، بينما أخذ اللاكتوز مسار عكسي. لم يكن هناك فروق فى كفاءة تحويل الغذاء بين الثلاث معاملات (بذور الكافور أو أوراق الكافور أو زيت الكافور. زاد البروتين الكلى والألبيومين معنويا ($P <0.05$) باضافة أوراق الكافور أو بذور الكافور مقارنةً بـ زيت الكافور. أدت اضافة زيت الكافور الى زيادة كلا من AST و ALT والجلوكوز والكرياتينين ($P <0.05$). زادت اليوريا في الدم ($P <0.05$) مع اضافة أوراق الكافور أو بذور الكافور مقارنة مع زيت الكافور ، ولكن لم يكن هناك فرق عند مقارنتها بالكنترول . انخفضت معنويا معاملات هضم المادة الجافة , المادة العضوية و البروتين الخام ($P<0.05$) باضافة أوراق الكافور أو بذور الكافور أو زيت الكافور مقارنة بالكنترول , بينما زادت نفس قيم معاملات الهضم السابقة ($P<0.05$) باضافة أوراق الكافور أو بذور الكافور مقارنة باضافة زيت الكافور الى العليقة . كانت معاملات هضم الدهن الخام مع اضافة أوراق الكافور أو بذور الكافور أو زيت الكافور أعلى ($P <0.05$) منها فى الكنترول ، بينما زادت معاملات هضم الدهن الخام ($P<0.05$) باضافة أوراق الكافور أو بذور الكافور مقارنة باضافة زيت الكافور الى العليقة. انخفضت معامل هضم NDF وADF معنويا ($P <0.05$) باضافة بذور الكافور مقانة باضافة أوراق الكافور والتى ارتفعت هى الأخرى معنويا عند مقارنتها بزيت الكافور. أدت التغذية على بذور الكافور الى زيادة قيم مجموع المركبات الغذائية المهضومة (TDN) و البروتين المضوم (DCP) مقارنة بالتغذية على أوراق الكافور والتى ارتفعت معها نفس القيم مقارنة بالتغذية على زيت الكافور.

فى النهاية تؤكد نتائج الدراسة الحالية أن اضافة زيت الكافور المحمي بشكل طبيعي في شكل أوراق أو بذور يخفف من الآثار السلبية لاضافة زيت الكافور الخام بشكل مباشر.