

# Impact of Anise, Clove, and Thyme essential oils as feed supplements on the productive performance and digestion of Barki ewes

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## Abstract

The aim of this study was to evaluate the effects of anise, clove and thyme essential oils (EO) supply on ewes performance during late pregnancy and lactation. Forty Barki ewes ( $33.5 \pm 2.15$  kg BW and 2-3 years old) were allocated into four equal groups, 10 animals each and received the same basal diet, berseem hay and concentrate supplement. Control ewes were fed their basal diet without oil supplementation (control group) while the other groups were supplemented with one of the three EO as follow; control diet plus 2 ml/day of anise EO (Anise group), 2 ml/day clove EO (Clove group) or 2 ml/day thyme EO (Thyme group). The oils were daily introduced individually to dams from late pregnancy period till weaning time. The results revealed a similar body weight among treatments during the late pregnancy and lactation period. The clove EO supply significantly improved ( $P < 0.05$ ) milk yield at certain stages of lactation in comparison with other experimental groups. Thyme EO significantly ( $P < 0.05$ ) increased milk total solids, solid not fat, protein and lactose, while the three EO increased ( $P < 0.05$ ) the total anti-oxidant capacity ( $P < 0.01$ ) compared to control's milk and numerically increased the levels of unsaturated fatty acids profile, especially Linolenic C18:3n-3 (omega - 3) fatty acids that benefits consumer's health. The digestibility trial was performed at the end of lactation in which a similar feed intake was recorded among treatment. The digestibility was significantly ( $P < 0.05$  and  $P < 0.01$ ) affected by the EO supply, except for crude fiber and acid detergent fiber. The nitrogen excretion via feces was reduced ( $P < 0.01$ ) and nitrogen retention increased ( $P < 0.05$ ) by the clove EO supply. Ruminal pH and ammonia -nitrogen did not affected by sampling time ( $P > 0.05$ ) while EO supply decreased pH ( $P < 0.05$ ) and ammonia increased ( $P < 0.01$ ) with anise and clove EO supply. Volatile fatty acids were increased ( $P < 0.01$ ) at 4 h post feeding and affected ( $P < 0.05$ ) by treatment  $\times$  time interaction. Normal renal function and hepatic enzymes with EO supply, but decreased levels ( $P < 0.01$ ) of serum total protein with clove and thyme supply were obtained. Reduced levels of total cholesterol (TC) and triglycerides (TG) ( $P < 0.01$ ) were recorded with EO, whereas total lipids (TL) and lipase activity increased ( $P < 0.01$ ) with clove and thyme supply. Anise EO supplementation has variable effects on blood metabolites.

Clove and thyme EO supply to dams affecting positively ( $P < 0.01$ ) their lambs growth rate till weaning. Therefore, the presence of EO in Barki ewes nutrition could be considered as a promising and useful alternative feed supplement to enrich the nutritional properties of the dairy products and consequently adding value and benefits to the animal products for consumer health.

**Key words:** Essential oils, ewes, productive performance, lipids profile.

## INTRODUCTION

To improve nutritional efficiency in ruminants, dietary additives have a favorable effect. The use of antibiotics as feed additives in animal feeds was prohibited by the European Union after January 2006 (Fandino *et al.*, 2008) due to the health risk of transferring residues into meat and milk. For this reason, alternative additives such as those of plant origin are desired. Essential oils (EO) are generally recognized as safe for human and animal consumption. They could be used as alternatives for antibiotics and growth promoters in ruminant's nutrition (Vakili *et al.*, 2013). They are volatile aromatic compounds present in many plants. Chemically, they are a blend of secondary metabolites commonly composed of terpenoids and phenyl propanoids (Calsamiglia *et al.*, 2007). These compounds have been shown to favorably affect ruminal fermentation and improve nutrients utilization in ruminants (Hristov *et al.*, 1999).

Moreover, EO has been evaluated for their antimicrobial activity, and they investigated as rumen modifiers in ruminants (Wallace, 2005). Additionally, they exhibit cytotoxic effects on living cells, and these activities are mostly due to the presence of phenols, aldehydes, and alcohols (Sacchetti *et al.*, 2005). Recent research has focused on exploiting plant bio-actives as natural

feed additives that enhance protein metabolism (Patra and Saxena, 2010). The most important plant families from the point of the essential oils are *Asteraceae* or *Compositae*; *Lamiaceae* or *Labiatae* and *Apiaceae* or *Umbelliferae* (Bernath, 2009). So, three of EO with high potential for use in the ruminant diets such as Anise essential oil (AEO), Clove essential oil (CEO) and Thyme essential oil (TEO) are used to enhance digestibility, productive performance of ewes during pregnancy and post-lambing.

## MATERIALS AND METHODS

The present study was conducted at Mary out Research Station, Desert Research Center, Ministry of Agriculture, 35 Km south of Alexandria, Egypt, during 2015-2016.

### The experimental Animals, design, and diets:

Forty Barki ewes ( 2-3 years old and  $33.45 \pm 2.15$  kg body weight ) were ranked by weight to four homogenous groups (10 animals/ each) , they were housed in individual concrete pens (5m×5m) and randomly assigned to receive one of four dietary diets including: control group fed on basal diet (without essential oil supplement) that consisted of 60% concentrate feed mixture (CFM) and 40 % Berseem hay (BH) before mating and at early pregnancy and became (70 CFM: 30 BH) at late pregnancy and through lactation , till weaning. The CFM composed of 20% cotton seed, 10% soybean meal, 42% yellow corn, 15% wheat bran, 10% rice bran, 1% salt, 2% limestone. The other three groups were fed on the same basal diet and supplemented with one of three EO as follow: Control diet plus 2 ml/h/day of Anise (*Pimpinella anisum*) (AEO) or plus 2 ml/h/day of Clove (*Syzygium aromaticum*) (CEO) or plus 2 ml/h/day of Thyme (*Thymus vulgaris*) (TEO). The requirements during pregnancy and lactation were calculated according to the recommended feeding standards of Kearl (1982). The animals, adult and young, were weighed weekly before morning feeding till the end of lactation. The CFM was offered daily at 8:00 am and BH at 12:00 pm as group feeding. Free fresh, clean tap water was allowed free – choice drinking once daily after the morning feeding. The daily intake was recorded, and the orts were determined the next morning. The chemical composition of the basal diet is presented in Table, (1). Oils supplementation was started at the mid of pregnancy (75 days before birth date till weaning) once daily by syringe before morning feeding to ensure that ewes swallowed all the oil amount. Before starting the experiment, ewes were vaccinated against internal and external parasites and intro-toxemia.

During the lactation period samples of milk were collected biweekly for chemical analysis and fatty acids profile from the second week of lambing till 120 days. Diet apparent digestibility and animal N balance were measured *in vivo* at the end of the lactation period. The total collection of feces and urine was applied to four ewes from each group. They were fed the same diets as mentioned above for five days after ten days of adaptation to the metabolic cages. Drinking water was determined for each animal daily. Individual feed intakes were recorded to calculate average daily gain (ADG), average dry matter intake, and feed conversion ratio.

**Table (1): Nutrient composition of the basal diet (on DM basis %)**

Items	Concentrate feed mixture	Berseem hay
Dry matter	92.75	88.70
Organic matter	92.40	87.53
Crude protein	15.82	13.52
Crude fiber	13.16	30.65
Ether extract	2.47	1.65
Nitrogen-free extract	60.95	41.71
Ash	7.60	12.47
Neutral detergent fiber	34.12	48.12
Acid detergent fiber	17.05	33.90

### Measurements, Samples, and Analysis

#### Feed analysis

Proximate chemical analysis for feed ingredients, orts, nitrogen in fecal, and urine samples was determined according to the standard methods of A.O.A.C (2007). Neutral detergent fiber (NDF), acid detergent fiber (ADF) were determined by Goering and Van Soest (1970) using an Ankom fiber analyzer. During the whole trial (pregnancy and lactation), ewes consumed all the offered CFM and BH.

#### Feed additives (essential oils)

Essential oils were supplied from Natural oil extraction and Pressing unit, National Research Center, Dokki - Giza, Egypt). Essential oils analysis was performed using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. Instrument: A Trace GC Ultra Gas Chromatographs (Thermo Scientific Corp., USA), coupled with a Thermo mass spectrometer detector (IQS Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 µm film thickness). Analyses were carried out according to El - Gendy et al. (2017) using helium as carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 60°C for 1 min; rising at 3.0°C/min to 240 °C and held for 1 min. The injector and detector were held at 240°C. Diluted samples (1:10 hexane, v/v) of 0.2 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the

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compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection, and NSIT library). Compounds identified in the tested oils and their active components as a percentage (%) are listed in Table, (2).

<b>Table (2): The main active components of Anise, Clove and Thymol essential oils</b>			
<b>The plant EO</b>	<b>R T</b>	<b>Relative percentage (%)</b>	<b>Main components</b>
<b>Anise (<i>Pimpinella anisum</i>)</b>			
	7.45	0.17	$\beta$ -Terpinyl acetate
	14.22	1.81	p-Allylanisole
	16.21	3.24	l-Carvone
	16.58	3.35	p-Allylanisole
	17	1.64	Anisaldehyde
	18.32	81.86	Trans-anethole
	20.52	0.41	1,2-DIACETIN
	22.38	0.3	p-Anisylacetone
	24.28	0.26	$\alpha$ -Himachalene
	25.49	2.27	Humulen-(v1)
	25.7	0.88	$\alpha$ -Curcumen
	26.22	0.68	Zingiberene
	26.33	0.81	$\beta$ -Himachalene
	26.71	0.42	$\beta$ -Bisabolene
	38.9	1.9	Allocryptopine
<b>Clove (<i>Syzygium aromaticum</i>)</b>			
	16.75	0.12	Acetin, mono
	18.39	0.03	Thymol
	19	0.02	Dimethyl benzyl carbiny acetate
	21.17	99.07	Eugenol
	24.14	0.03	Diacetin monopropoanoate
	25.15	0.05	Isoeugenol
	27.56	0.02	Eugenol acetate
	47.16	0.19	1-Docosanol
	58.24	0.03	Squalene
<b>Thyme (<i>Thymus vulgaris</i>)</b>			
	4.63	7.52	$\alpha$ -Pinene
	5.83	11.21	$\beta$ -Pinene
	6.76	0.34	$\alpha$ -Phellandrene
	7.09	0.54	(+)-2-CARENE
	7.34	24.3	p-Cymene
	8.02	0.57	Benzyl Alcohol
	8.44	1.06	$\gamma$ -Terpinene
	9.46	0.64	Terpinolene
	10.05	2.84	L-linalool
	13.07	0.61	endo-Borneol
	13.36	1.24	4-Terpineol
	14.21	1.74	Anethole
	15.34	0.42	O-Methylthymol
	15.71	0.28	Carvacrol methyl ether
	18.49	40.59	Thymol
	18.68	1.9	Carvacrol
	20.54	3.49	Glycerol 1,2-diacetate
	23.57	0.48	$\alpha$ -Bergamotene
	26.9	0.24	$\gamma$ -CADINENE
Retention time (RT) is a measure of the time taken for a solute to pass through a chromatography column. It is calculated as the time from injection to detection. The RT for a compound is not fixed as many factors can influence it even if the same GC and column are used.			

### Ruminal liquor analysis

Rumen liquor was withdrawn by stomach tube from four ewes at 2, 4 and 6 h post feeding on the last day of the trial. The pH of rumen liquor was immediately recorded using digital pH meter, Gallen Kamp pH Stick pH K-120 – B. The entire contents were squeezed through 4 layers of cheesecloth and were kept frozen until analyzed for ammonia – nitrogen (NH<sub>3</sub>-N) (A. O. A. C, 1997) and total volatile fatty acids (TVFA's) Warner (1964).

### Biochemical blood analysis

Blood samples were collected from the same four ewes from the jugular vein at the end of the trial before morning feeding. All samples were centrifuged at 4,000 rpm for 15 minutes and the collected serum was frozen at -20°C for subsequent analysis of total protein, albumin, globulin was obtained by subtracting the total proteins values from the albumin values, urea-N, creatinine, ammonia, cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), total lipids (TL), lipase enzyme, Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and total antioxidant capacity (TAC) using Biodiagnostic laboratory kits.

### Milk production, composition, total antioxidant capacity and fatty acid analysis

Biweekly, the ewes were separated by their lambs and then milked by hand at 8.00 and 18.00 h from the second week of lambing till the end of lactation. Milk production was recorded. Milk samples were collected at each milking and mixed as a constant percentage of the morning and evening to obtain the sample of each animal and analyzed for fat, protein, lactose and total solids using a Milkotester (Master Pro Touch, Milkotester Ltd, Bulgaria). Total antioxidant capacity (TAC) of milk was estimated (Koracevic et al., 2001) as in blood. Fatty acids profile was determined by using High-Pressure Liquid Chromatography (HPLC) according to AOAC (1998).

### Statistical analysis

Analysis of variance (ANOVA) was used to test the obtained data using the general linear modeling procedure (SAS, 2000).

The used design was one-way analysis for data of feed intake, blood metabolites and milk composition. Results of ruminal parameters were subjected to a statistical analysis of variance using the general linear model (GLM) procedures by SAS (2004) that included the effect of treatment, sampling time and the interaction between treatment and sampling time followed this equation:  $Y_{ijk} = \mu + T_i + \text{Time}_j + \text{TTime}_{ij} + e_{ijk}$

Where:

$Y_{ijk}$  = observations value of the  $k^{\text{th}}$  animal,

$\mu$  = overall mean,  $T_i$  = effect of  $i^{\text{th}}$  treatment (i: 1-4),

$\text{Time}_j$  = effect of  $j^{\text{th}}$  time (j: 1-3),

$\text{TTime}_{ij}$  = the interaction

$e_{ijk}$  = experimental error.

Duncan's multiple tests (1955) were applied for comparison of means. Data of milk fatty acid profile were analyzed as a completely random design, where additives were the main source of variation.

## RESULTS

### Essential oils constituents

The main constituents of Anise, Clove and Thyme essential oils are presented in Table, (2). The data showed that the major bioactive constituents as % was anethole (81.86%) in anise oil, Eugenol (99.07 %) in clove oil and Thymol (40.59%) in thyme oil.

### Ewe's live body weight changes during pregnancy and lactation

The body weight (BW) evolution of ewes throughout the trial period was comparable among groups (Table, 3). Essential oils did not affect ewe's BW during late pregnancy and lactation compared with control ewes. All ewes had lost weight throughout the suckling period.

**Table (3) Effect of essential oils supply on body weight changes of ewes during pregnancy and lactation**

Items	control	Essential oils			SEM	Significant differences
		Anise	Clove	Thyme		
<b>Body weight, kg:</b>						
<b>Mid pregnancy (2 months pre-lambing)</b>	38.50	39.33	36.96	38.32	2.46	NS
<b>Late pregnancy (a week pre-lambing)</b>	45.89	45.95	43.22	44.12	2.68	NS
<b>Early lactation (a week post-lambing)</b>	41.13	39.84	37.95	39.21	2.53	NS
<b>Late lactation (3 months post-lambing)</b>	39.95	37.06	36.12	36.62	2.52	NS

a, b, c and d: values with different letters in the same row means statistically significant at (P<0.05)  
NS: non-significant, SEM: standard error for means

### Feed intake, digestibility and nutritive value

Across the digestibility trial, the EO supply did not affect total dry matter intake (DMI) and total crude protein (CP) intake when expressed as g/kg BW while EO supply especially clove EO increased DM, organic matter (OM), ether extract (EE), nitrogen-free extract (NFE), total digestible nutrients (TDN) (P<0.05), CP, NDF and digested crude protein (DCP) digestibilities (P<0.01) compared to other experimental ewes (Table, 4). However, the digested CF was not affected among all groups. Water intake showed that all amounts consumed by animal groups were comparable except anise supply resulted in a significant reduction (P<0.05).

**Table (4):** Effect of essential oils supply on dry matter and water intakes, digestibility, and nutritive value at the end of the lactation period

Items	control	Essential oils			SEM	Significant differences
		Anise	Clove	Thyme		
Body weight (kg)	35.77	32.22	32.22	35.35	2.53	NS
<b>Feed intake:</b>						
Total, DM intake g/kg BW:	19.56	20.31	21.23	20.49	0.88	NS
DMI (g/h/d)	696.02	651.67	684.79	719.31	31.90	NS
Total, CP intake g/kg BW	2.890	3.00	3.14	3.03	0.129	NS
Drink water ml/kg BW	74.0 <sup>a</sup>	51.7 <sup>b</sup>	74.2 <sup>a</sup>	78.7 <sup>a</sup>	1.76	***
Drink water ml/g DMintake	3.77 <sup>a</sup>	2.55 <sup>b</sup>	3.67 <sup>a</sup>	3.97 <sup>a</sup>	0.102	***
<b>Nutrients Digestibility%</b>						
Dry matter	52.13 <sup>b</sup>	54.82 <sup>ab</sup>	56.94 <sup>a</sup>	51.63 <sup>b</sup>	1.05	*
Organic matter	53.09 <sup>b</sup>	55.26 <sup>ab</sup>	57.95 <sup>a</sup>	53.36 <sup>b</sup>	0.98	*
Crude protein	66.80 <sup>c</sup>	70.41 <sup>ab</sup>	72.48 <sup>a</sup>	67.98 <sup>bc</sup>	0.89	**
Crude fiber	56.86	56.66	59.31	54.40	1.71	NS
Ether extract	58.89 <sup>a</sup>	53.44 <sup>b</sup>	59.40 <sup>a</sup>	57.43 <sup>ab</sup>	1.34	*
Nitrogen free extract	48.75 <sup>b</sup>	53.67 <sup>a</sup>	55.77 <sup>a</sup>	51.32 <sup>ab</sup>	1.41	*
Neutral detergent fiber	43.22 <sup>bc</sup>	46.97 <sup>b</sup>	51.82 <sup>a</sup>	41.77 <sup>c</sup>	1.24	***
Acid detergent fiber	35.43	36.96	42.55	38.27	1.97	NS
<b>Nutritive value (%)</b>						
Total digestible nutrients	50.12 <sup>b</sup>	52.93 <sup>ab</sup>	55.17 <sup>a</sup>	51.05 <sup>b</sup>	1.05	*
Digested crude protein	9.88 <sup>c</sup>	10.41 <sup>ab</sup>	10.72 <sup>a</sup>	10.05 <sup>bc</sup>	0.13	**

### Nitrogen retention

The nitrogen intake was not affected by EO supply ( $P>0.05$ ) (Table, 5) while anise and clove EO supply reduced excreted nitrogen via feces compared with thyme EO and control ewes ( $P<0.01$ ). Nitrogen retention and nitrogen balance as % of nitrogen intake were significantly ( $P<0.05$ ) increased by clove EO supply.

**Table (5):** Effect of essential oils supply on nitrogen (N) balance and utilization in lactating ewes

Items	control	Essential oils			SEM	Significant differences
		Anise	Clove	Thyme		
<b>Nitrogen utilization</b>						
N intake g/h/d	16.47	15.42	16.20	17.02	0.755	NS
N in feces g/h/d	5.44 <sup>a</sup>	4.56 <sup>b</sup>	4.45 <sup>b</sup>	5.43 <sup>a</sup>	0.168	***
N in urine g/h/d	6.97	7.27	6.96	8.47	0.76	NS
Total excretion g/h/d	12.42	11.84	11.42	13.90	0.90	NS
N retained, g	4.05 <sup>ab</sup>	3.59 <sup>b</sup>	4.78 <sup>a</sup>	3.12 <sup>b</sup>	0.34	*
N balance % of NI	25.02 <sup>ab</sup>	23.66 <sup>ab</sup>	29.56 <sup>a</sup>	18.35 <sup>b</sup>	2.53	*

a, b, c and d: values with different letters in the same row means statistically significant at ( $P<0.05$ )  
NS: non-significant, \*,  $P<0.05$ ; \*\*or \*\*\*,  $P<0.01$  SEM: standard error for means

### Ruminal fermentation characteristics

Data of table (6) indicated that ruminal pH did not affect by sampling time (Ti) or by treatment  $\times$  time (TrTi) ( $P>0.05$ ) while it decreased with EO supply ( $P<0.05$ ) especially with anise supply. The concentration of total volatile fatty acids (TVFA's) was significantly affected by Ti ( $P<0.01$ ) and by TrTi ( $P<0.05$ ). Ruminal ammonia- nitrogen ( $\text{NH}_3\text{-N}$ ) concentration was higher with clove supply ( $P<0.01$ ).

### Blood serum parameters

Supplementation with EO did not affect blood serum albumen, albumen /globulin (A/G) ratio, urea, creatinine, high density lipoprotein (HDL), low density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total antioxidant capacity (TAC) ( $P>0.05$ ) (Table, 7). However, a significant reduction ( $P<0.01$ ) in total protein (TP) was resulted with clove and thyme supply but not with anise EO supply. Ammonia-nitrogen ( $\text{NH}_3\text{-N}$ ) concentration showed controversial variations among the studied groups ( $P<0.05$ ). Clove and thyme EO supply were associated with hypocholesterolemic effect ( $P<0.01$ ) and high total lipids (TL) which reduced with anise EO supply ( $P<0.01$ ). EO supply reduced triglycerides (TG) ( $P<0.01$ ) and elevate lipase activity ( $P<0.01$ ) compared with control ewes.

**Table (6):** Effect of essential oils supply on some ruminal parameters

Item	Time	control	Essential oils			Overall mean	± SEM		
			Anise	Clove	Thyme		Tr	Ti	TrTi
pH	2h	6.42	5.92	6.37	6.45	6.29	0.08*	0.07 <sup>NS</sup>	0.14 <sup>NS</sup>
	4h	6.32	6.30	6.25	6.35	6.31			
	6h	6.50	6.23	6.20	6.0	6.23			
<b>Overall mean</b>		6.42 <sup>A</sup>	6.15 <sup>B</sup>	6.27 <sup>AB</sup>	6.27 <sup>AB</sup>				
TVFA's	2h	7.39 <sup>ab</sup>	7.31 <sup>ab</sup>	8.03 <sup>ab</sup>	8.05 <sup>ab</sup>	7.69 <sup>A</sup>	0.32 <sup>NS</sup>	0.28**	0.56*
	4h	9.01 <sup>a</sup>	8.09 <sup>ab</sup>	7.74 <sup>ab</sup>	7.52 <sup>ab</sup>	8.09 <sup>A</sup>			
	6h	5.25 <sup>c</sup>	7.01 <sup>b</sup>	7.84 <sup>ab</sup>	7.31 <sup>ab</sup>	6.85 <sup>B</sup>			
<b>Overall mean</b>		7.22	7.47	7.87	7.63				
NH <sub>3</sub> -N	2h	27.15	26.86	28.02	24.83	26.72	0.90**	0.78 <sup>NS</sup>	1.57 <sup>NS</sup>
	4h	25.73	24.11	26.17	22.21	24.56			
	6h	22.92	28.38	30.86	25.06	26.81			
<b>Overall mean</b>		25.26 <sup>B</sup>	26.45 <sup>AB</sup>	28.35 <sup>A</sup>	24.04 <sup>B</sup>				

a, b, c and A, B: values with different letters in the same row means statistically significant at (P<0.05)

NS: non-significant, \*, P<0.05; \*\*or \*\*\*, P<0.01 SEM: standard error for means

Ti: effect of sampling time, Tr: effect of treatment, TrTi: interaction of treatment and time

**Table (7):** Effect of essential oils supply on some blood serum metabolites

Items	control	Essential oils			SEM	Significant differences
		Anise	Clove	Thyme		
Total, protein g/ dl	7.32 <sup>ab</sup>	7.46 <sup>a</sup>	6.06 <sup>b</sup>	6.93 <sup>ab</sup>	0.257	***
Albumen g/ dl	4.17	3.63	3.92	4.15	0.203	NS
Globulin g/dl	3.15 <sup>ab</sup>	3.83 <sup>a</sup>	2.14 <sup>b</sup>	2.78 <sup>ab</sup>	0.228	**
Albumen / Globulin	0.76	1.06	0.55	0.67	0.234	NS
Urea mg/dl	54.52	50.66	52.23	52.02	2.07	NS
Creatinine mg/dl	1.58	1.59	1.67	1.85	0.06	NS
Ammonia μmol/ L	306.82 <sup>ab</sup>	268.7 <sup>ab</sup>	283.63 <sup>b</sup>	350.38 <sup>a</sup>	17.4	*
Cholesterol mg/dl	108.37 <sup>a</sup>	108.08 <sup>a</sup>	85.9 <sup>b</sup>	97.91 <sup>a</sup>	3.8	**
Triglyceride mg/dl	47.17 <sup>a</sup>	33.53 <sup>b</sup>	37.27 <sup>b</sup>	29.69 <sup>c</sup>	2.21	***
High density lipoprotein mg/dl	65.12	63.72	60.99	61.53	2.85	NS
Low density lipoprotein- mg/dl	270.5	257.5	269.7	255.4	7.93	NS
Total lipids	317.73 <sup>bc</sup>	285.95 <sup>c</sup>	525.92 <sup>a</sup>	352.84 <sup>b</sup>	17.2	***
Lipase U/ L	46.95 <sup>c</sup>	54.26 <sup>c</sup>	198.60 <sup>a</sup>	65.73 <sup>b</sup>	3.43	***
Alanine transferase U/ L	22	22.25	23	20.75	1.13	NS
Aspartate transferase U/ L	76.00	69.25	64.00	75.5	3.49	NS
Total antioxidant capacity M/ L	0.38	0.395	0.33	0.467	0.074	NS

a, b, c and d: values with different letters in the same row means statistically significant at (P<0.05)

NS: non-significant, \*, P<0.05; \*\*or \*\*\*, P<0.01 SEM: standard error for means

### Milk Production, composition and total antioxidant capacity

Data presented in Table, (8) showed that milk yield did not significantly affect by EO supply throughout all the lactation stages except at 15- and 105-days post-parturition, ewes receiving clove or thyme EO produced a greater amount of milk (P <0.05) in comparison to those receiving anise or not receiving any oil. However, clove and thyme EO supply tended to affect milk production at (90 days) (P=0.06). The highest yield reflected the improved feed efficiency (Milk yield/DMI) that uniformly associated with groups receiving clove, thyme, control and anise receiving ewes (P>0.05) (56.7 vs. 50.9, 48.2 and 46.6, respectively). Overall milk yield followed the same pattern with non-significant variations (p>0.05).

The composition of the milk was analyzed and shown in Table, (8). Essential oils supply tended to decrease milk fat content (P= 0.06). Increasingly, thyme EO supply affected milk protein (P< 0.01), lactose (P<0.05), total solids (TS) and solids nonfat (SNF) contents (P<0.01) compared with other milk of the studied animals. Regarding total antioxidant capacity (TAC), EO supply resulted in more healthy and nutritious milk with the highest levels of TAC compared with other values of control's milk.

### Milk fatty acids profile

Data relative to milk fatty acids profile are shown in Table, (9), and reflects a positive effect of EO supply. The unsaturation of milk fatty acids concentrations was increased with EO supply. Presumably, the studied EO affected on the composition of fatty acids decreasingly of the saturated fatty acids: Capric acid (10:0), Lauric acid (12:0) Myristic acid (14:0) and Pentadecanoic acid (15:0) and increasingly of the preformed Stearic acid (C18:0) and Oleic acid (C18:1 cis-9). Moreover, EO supply increased omega-3 (C18:3N3) ( $\alpha$ -linolenic acid), omega-6 (C18:3N6) ( $\gamma$ -linolenic acid) and the ratio omega 6/ omega 3

(N6/N3 ratio) in milk of all supplemented ewes except milk of thyme supplemented ewes, it shows a reduced ratio compared with control milk. Furthermore, elevated concentrations of arachidonic acid (C20:4) were recorded with EO supply.

**Table (8):** Effect of essential oils supply on milk production, chemical composition and total antioxidant capacity

Items	control	Essential oils			SEM	Significant differences
		Anise	Clove	Thyme		
<b>Zero- 15 days</b>	361.66 <sup>ab</sup>	327.71 <sup>b</sup>	432 <sup>a</sup>	392.8 <sup>ab</sup>	23.13	*
<b>30days</b>	468.24	413.78	494.99	488.14	40.15	NS
<b>45 days</b>	421.96	376.35	445.5	429.2	34.62	NS
<b>60 days</b>	379.8	334.9	400.9	396.26	28.03	NS
<b>75 days</b>	341.79	304.81	375.1	352.67	21.75	NS
<b>90 days</b>	290.52	259.08	332.19	314.27	19.32	NS
<b>105 days</b>	232.42 <sup>b</sup>	212.05 <sup>b</sup>	282.36 <sup>a</sup>	260.84 <sup>ab</sup>	16.07	*
<b>120 days</b>	185.93	176.33	217.42	198.23	12.56	NS
<b>Average daily milk yield, ml</b>	335.29	300.68	372.54	354.05	23.62	NS
<b>Milk yield/DMI (g/h/d)</b>	48.17	46.14	54.40	48.80	3.23	NS
<b>Overall milk yield, L. (16 weeks)</b>	37.55	33.67	41.72	39.56	2.46	NS
<b>Chemical composition of milk</b>						
<b>Fat %</b>	3.90	3.70	3.60	3.50	0.091	NS
<b>Total solids %</b>	13.43 <sup>a</sup>	12.23 <sup>c</sup>	12.73 <sup>b</sup>	13.44 <sup>a</sup>	0.88	***
<b>Solid not fat %</b>	9.53 <sup>b</sup>	8.53 <sup>d</sup>	9.13 <sup>c</sup>	9.94 <sup>a</sup>	0.016	***
<b>Protein %</b>	3.70 <sup>ab</sup>	3.30 <sup>c</sup>	3.60 <sup>b</sup>	3.90 <sup>a</sup>	0.071	**
<b>Lactose %</b>	5.60 <sup>ab</sup>	5.10 <sup>c</sup>	5.30 <sup>bc</sup>	5.80 <sup>a</sup>	0.1	**
<b>Salt %</b>	0.8	0.7	0.8	0.7	0.058	NS
<b>pH</b>	6.71 <sup>a</sup>	6.68 <sup>b</sup>	6.78 <sup>a</sup>	6.65 <sup>c</sup>	0.006	***
<b>Total antioxidant capacity mM/L</b>	9.57 <sup>d</sup>	13.02 <sup>b</sup>	14.14 <sup>a</sup>	12.90 <sup>c</sup>	0.032	***

a, b, c and d: values with different letters in the same row means statistically significant at (P<0.05)

NS: non-significant, \*, P<0.05; \*\*or \*\*\*, P<0.01 SEM: standard error for means

**Table (9)** Effect of essential oils supply on milk fatty acids profile of lactating ewes

Fatty acids %	control	Essential oils		
		Anise	Clove	Thyme
<b>C6, Caproic acid</b>	1.11	0.94	1.74	1.86
<b>C8, Caprylic acid</b>	3	2.64	3.6	2.79
<b>C10, Capric acid</b>	10.61	10	10.8	9.56
<b>C12, Lauric acid</b>	5.6	5.19	4.91	5.69
<b>C14, Myristic acid</b>	13.25	11.54	11.68	11.6
<b>C14:1, Myristoleic acid</b>	0.3	0.25	0	0.21
<b>C15:0, Pentadecanic acid</b>	1.21	1.1	0	1
<b>C15:1, Ginkgolic acid</b>	0	0.17	0	0.14
<b>C16:0, Palmitic acid</b>	28.71	30.14	29.75	29
<b>C16:1, Palmitoleic acid</b>	0.49	0.55	0.51	0.5
<b>C17:0, Margaric acid</b>	0	0.42	0.4	0.59
<b>C18:0, Stearic acid</b>	9.28	10.66	10.77	9
<b>C18:1n-9T, Elaidic acid</b>	21.17	17.89	16.46	20.3
<b>C18:1n-9C, Oleic acid</b>	0.9	2.45	2.79	1.96
<b>C18:3n-3, <math>\alpha</math>-linolenic acid</b>	0.1	0.13	0.12	0.15
<b>C18:3n-6, <math>\gamma</math>-linolenic acid</b>	1.3	1.5	1.84	1.31
<b>N6/N3 ratio</b>	13.00	11.54	15.33	8.73
<b>C20:0, Arachidic acid</b>	0.31	0.26	0.31	0.25
<b>C20:1, Gondoic acid</b>	0.15	0.2	0	0.19
<b>C20:4, Arachidonic acid</b>	2.51	3.97	4.32	3.9

### Lamb growth rates

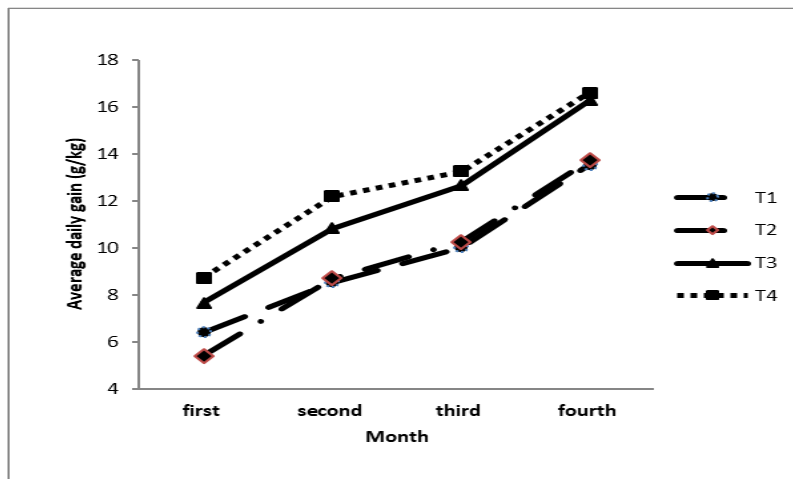
The indirect effect of suckling milk of EO supplemented ewes on growth rate of lambs is presented in Table, (10) and Figure (1). Lamb BW at birth was not affected by EO administration (P>0.05). Luckily, the EO supply in the current research has positive effect (P<0.001) on overall BW changes of lambs until weaning after (120 days) especially lambs fed milk of ewes receiving thyme followed by those of ewes receiving clove, lambs of control ewes then those of ewes receiving anise EO.

Consequently, average daily gain followed the same pattern ( $P < 0.001$ ) where it was (110.8 vs 109.7, 86.0 then 84.8 g respectively ones.

**Table (10):** Effect of essential oils supply on birth weight and daily gain of lambs until weaning

Items	control	Essential oils			SEM	Significant differences
		Anise	Clove	Thyme		
Birth weight, Kg	3.24	3.57	3.13	3.32	0.25	NS
Final B W, Kg	10.32 <sup>b</sup>	10.17 <sup>b</sup>	13.16 <sup>a</sup>	13.29 <sup>a</sup>	0.282	***
Average daily gain g/d	86.03 <sup>b</sup>	84.79 <sup>b</sup>	109.71 <sup>a</sup>	110.76 <sup>a</sup>	2.35	***

a, b, c and d: values with different letters in the same row means statistically significant at ( $P < 0.05$ )  
 NS: non-significant, \*,  $P < 0.05$ ; \*\* or \*\*\*,  $P < 0.01$  SEM: standard error for means



**Figure (1):** Lamb's body weight evolution from birth till weaning

## DISCUSSION

### Essential oils constituents

The current quantitative data of the EO active components (Table 2) were previously evaluated in different studies and inconsistent. Kilic et al. (2011) found 79.6% anethol in anise oil and Patra et al. (2009) reported a high eugenol content in stem part (74.5%) of the clove and its oil (78%). An elevated percentage of thymol (55.3% and 45.2%) were reported by Biricik et al. (2016) and Seirafy and Sobhanirad (2017), respectively. This variation in concentrations of the major components of EO may be mainly attributed to the plant part used in the extraction. Likewise, Gorgulu et al. (2010) found that cinnamon oil has eugenol as the major compound in the leaf part (69.6%) while the cinnamaldehyde was the major one in the inner bark part of the plant (60 %).

### Ewe's live weight changes during pregnancy and suckling period

The reduction in BW of ewes after parturition (Table 3) may be due to the negative energy balance during this physiological stage. In connection with Smeti et al. (2015) who attributed weight losses to the physiological situation of dairy ewes during this period. They are unable to ingest enough food and are forced to mobilize reserves to ensure good milk production (Atti et al., 1991 and Chilliard et al., 1998). The EO supply kept BW changes of supplemented ewes during pregnancy in the same range as control ewes. Although Zeid and Ahmed (2004) recorded that the BW tend to increase by feeding some medicinal herbs (Chamomile and thyme) in zaraibi doe rations and zaraibi goats (Abdelhamid et al., 2011).

### Feed intake, digestibility, and nutritive value

Lack of significant effect of EO on total DMI (Table 4) has been agreed with Benchaar et al. (2007) and (2008) in dairy cattle; Yang et al. (2007) in cow's diet; Fandino et al. (2008) in growing heifers; Morsy et al. (2012) in lactating goats and Vakili et al. (2013) in calves. Palatability aspect can explain the lack of EO effects on DMI.

Khamisabadi et al. (2016) found that using peppermint and *Thymus vulgaris* in the diets of Sanjabi male lambs increased dry matter intake. On the other hand, Calsamiglia et al. (2007) recorded depression of DMI in cattle because of palatability problems. Effects of EO on DMI are controversial where their effects vary with EO source, type of diet, diet interactions or adaptation of rumen microbial populations to EO (Geraci et al., 2012), and doses of EO because EO at low dose may stimulate intake whereas at higher doses may adversely affect intake in ruminants Patra, (2011).

Concerning differences in water intake among the experimental groups, it could be attributed to different chemical structures of the studied EO, their contents from active components which affecting their actions and activities. Improved feed digestibility in the current study, especially with clove supply, may be due to the phenolic nature of eugenol and its high potency



in stimulating bacteria involved in feed digestion. Besides, Williams and Losa (2001) reported stimulation of digestive secretions like saliva or endogenous digestive enzymes when using plant extracts. Moreover, Newbold et al. (2004) showed some EO protect the dietary protein from microbial degradation. Similarly, Rofiq (2016) reported increased OM digestibility with thyme. On the other hand, Khamisabadi et al. (2016) and Smeti et al. (2015) suggested that the addition of EO did not affect feed digestibility. However, Patra and Yu (2012) reported that clove, garlic, origanum, peppermint, or eucalyptus oil appeared to reduce feed digestibility differently.

Indeed, clove EO supply (Table 4) improved total digestible nutrients (TDN%) ( $P < 0.05$ ) and digested crude protein (DCP%) ( $P < 0.01$ ). These results agree with the findings of Castillejos et al. (2006) and Kilic et al. (2011) who found that eugenol improved N – utilization, the efficiency of energy and protein utilization in the rumen. Discrepancies among results of studies could be due to experimental conditions (in vitro vs. in vivo), ruminal evaluation vs. total tract evaluation, dose, type or chemical composition and amount of the basal diet. Besides, the source of anise, clove, and thyme and the procedures of EO extraction can be additional affecting factors (Vakili et al., 2013).

### **Nitrogen retention**

Across the digestibility trial, nitrogen intake was not affected by EO supply (Table 5) but nitrogen excretion via feces ( $P < 0.01$ ) and nitrogen retention were ( $P < 0.05$ ) affected by clove EO supply compared with other EO. This result is in harmony with improved CP – digestibility, DCP%, and lower total N excretion in the present study (Table 4). This may be the direct result of protein protection against degradation in the rumen (Newbold et al., 2004); consequently, nitrogen losses decreased (Terril et al., 1992). Tedeschi et al. (2003) agree with us.

### **Ruminal fermentation characteristics**

Varieties of results have been reported on the effect of EO supply on post feeding ruminal parameters. Matching with current results of pH, Patra (2010) who found that eugenol reduced rumen pH. However, Morsy et al. (2012 and Vakili et al. (2013) reported that EO supply did not affect pH.

The current results showed that EO supply resulted in higher levels of VFA's especially four h post feeding. The present findings for ruminal VFA's are in line with Newbold et al. (2004) and Castillejos et al. (2005) who found elevated levels of total VFA's on EO supply. Castillejos et al. (2006) used different doses of some EO and found a depression in total VFA's; they documented a reduction in diet fermentability through inhibition of rumen microbes that may be attributed to the antimicrobial activity of thymol. Als, McEwan et al. (2002) and Evans and Martin (2000) suggested that EO inhibits the rumen microbial fermentation. These inconsistencies among studies might be due to level, composition, and variety of the essential oils (Biricik et al., 2016). The EO supply resulted in increased ( $P < 0.01$ ) ruminal ammonia concentrations ( $\text{NH}_3 - \text{N}$ ), especially with clove and anise EO compared with control animals. Cardozo et al. (2004) consistent with the current results, and they concluded that anise extract stimulated peptidolysis and deamination resulting in an accumulation of  $\text{NH}_3 - \text{N}$ . Furthermore, Tekippe et al. (2011) and Biricik et al. (2016) agreed with the present findings suggesting an increase the amount of ammonia –N. However, Cardozo et al. (2006) and Morsy et al. (2012) inconsistent with the current results where they found that EO as anise, clove, and juniper inhibit deamination.

### **Blood serum parameters**

The current results (Table 7) showed reduced levels of total protein ( $P < 0.01$ ) in ewes supplemented with clove and thyme EO consequently decreased levels of globulins. Although DCP% and nitrogen retention was improved with EO supply (table 4 and 5) that resulted in increased milk's protein with these EO (table 9). However, Khateri et al. (2017) recorded that total protein and albumin were not influenced by the added mixture of EO. Serum urea and creatinine were unaffected by any of EO. Vakili et al. (2013) and Biricik et al. (2016) agree with us.

The increment of blood ammonia with thyme EO supply could be explained by its decreased levels in rumen. (table 6). Moreover, Chalupa, (1972) explained the relationship between rumen pH and absorption of ammonia that exists as free  $\text{NH}_3$  at high pH and as ammonium ions  $\text{NH}_4^+$  at lower pH. Reduction of blood total cholesterol (TC) and triglycerides (TG) in all studied supplemented ewes compared with control ones resulted in alterations of the lipid composition in animal products leading to human health benefit. These reduced levels of TC may be attributed to the reduction in cholesterol synthesis. Similar to Elson and Qureshi (1995) who found that isoprenoids and the end products of secondary plant metabolism suppress cholesterol synthesis by inhibiting the production of 3-hydroxy-3-methylglutaryl coenzyme A reductase which is the rate controlling enzyme in the synthetic cholesterol pathway. The present results are in agreement with those of Craig (1999) who recorded that aromatic plants and their oils were used extensively as hypocholesterolemic and as hypolipidemic. Besides, Priolo et al. (2007) and Morsy et al. (2012) consistent with the present findings. On the other hand, Vakili et al. (2013) and Alsaht et al. (2014) found TC and TG did not affect, but Chaves et al. (2008) reported elevated TG levels with EO.

The EO supply resulted in no change in high-density lipoprotein (HDL) and low-density lipoprotein (LDL) while total lipids (TL) and lipase activity ( $P < 0.01$ ) were elevated with clove and thyme EO supply compared with other groups. Unal and Kocabagli (2014) had reported that thyme EO added to lamb rations didn't affect TC, TG, HDL, and LDL ratios of the blood serum. These results indicate different responses to EO active component. Fascina et al. (2012) illustrated that the main active components in cinnamon and curcumin, stimulate pancreatic and intestinal enzyme secretion consequently production of bile salts, pancreatic and intestinal lipase increased leading to more effective nutrient absorption. Conflicting data were reported about the action of EO on hepatic enzymes, Morsy et al. (2012), Vakili et al. (2013) and Khateri et al. (2017) consistent with current results where they reported no change in liver function tests, while Seirafy and Sobhanirad (2017) reported elevated ALT and AST. These inconsistencies between studies may be due to the type and dosage rate of EO, components of EO, and experimental conditions (Benchaar et al. 2008).

Total antioxidant capacity (TAC) levels were insignificantly different among the supplemented ewes, and this result was explained by Seirafy and Sobhanirad (2017) who indicated non-specific stimulation of cellular immunity as a result of supplementation of thyme and oregano EO.

### **Milk Production, composition, and total antioxidant capacity**

Milk yield was improved with EO supply in few milking times (after 15 and 105 days) while the rest of milking times were not affected with EO supply (Table 8). Consistent with the current results, Morsy et al. (2012) and Tassoul and Shaver (2009) with dairy cattle. Also, milk's fat tended to decrease ( $P>0.05$ ) with EO supply.

Conversely, Kung et al. (2008) and Santos et al. (2010) recorded increases in milk and fat yield in dairy cows with EO. Total solids (TS), solid not fat (SNF) and lactose contents were increased ( $P<0.01$ ) with thyme EO. In agreement with the present work, Morsy et al. (2012) found elevated levels of milk lactose, TS, and SNF with thyme EO supply. This may be attributed to its phenolic structure. Also, increased milk protein ( $P<0.01$ ) resulted from improved feed protein utilization with thyme and clove EO (table 4).

Moreover, differences in chemical structures, active components contents, and properties of EO might be the main reason explaining the variations in milk pH. Essential oils are rich sources of natural antioxidants such as phenolic compounds (Zheng and Wang, 2001). In the present study, total antioxidants capacity (TAC) was positively affected ( $P<0.01$ ) by clove, anise and thyme EO, respectively resulted in more nutritious milk with higher levels of TAC than control milk.

Additionally, eugenol, was reported as an antibacterial agent against 24 different genera of bacteria, included animal and plant pathogens, also, thymol has an active antiseptic property, strong antimicrobial activities and these characteristics are due to their phenolic structure and their ability on cell wall membrane disruption (Dorman and Deans, 2000 and Trombetta et al., 2005). Moreover, the EO can exhibit anti-toxicogenic properties (Ultee and Smid, 2001). Also, Mastromatteo et al. (2010) cleared that antioxidants found in the structure of thyme EO, so it has transferred to their milk.

### **Milk fatty acids profile**

In harmony with other authors (Cannas et al., 2003) EO supply improved milk's fatty acids profile. All the previous studies on lactating goats and ewes observed an increase of the unsaturated of fatty acids, suggesting that EO can reduce the biohydrogenation process resulted in improving the nutritional value of their milk consequently, benefit human health (Nudda et al., 2013). Moreover, Tholstrup et al. (2003) found that saturated fatty acids play an essential positive role in the formation of blood cholesterol. Fortunately, the current results of blood cholesterol (table 7) support this explanation. So, all these figures produce a high quality and more healthy milk for the consumers.

### **Lamb growth rates**

It was clear that EO supply did not affect lamb's birth weight but increased ( $P<0.01$ ) lamb's growth rates throughout suckling period (table 10). This result agreed with Smeti et al. (2015) where they reported that rosemary EO supply did not affect lambs birth weight. Average daily gain (ADG) was positively affected by EO supply and this increment can be explained as a response to the amount of milk yield where thyme and clove EO supplemented ewes tend to produce higher amount of milk throughout lactation period as reported in (Table 8). Consequently, EO residues in milk may improve the lamb's appetite and weight where: anethol in anise oil was reported as digestion stimulant and galactagogue, eugenol in clove oil as appetite and digestion stimulant and antiseptic finally, thymol in thyme oil was reported as digestion stimulant, antiseptic and antioxidant (Richard, 1992 and Charalambous, 1994).

## **CONCLUSIONS**

Anise, clove or thyme EO supply to the pregnant ewes didn't affect DMI but improved nutrients digestibility, TDN, DCP and nitrogen retention, especially with clove and thyme EO. Rumen fermentation parameters were affected, especially with clove and thyme EO supply suggesting a different mode of actions on rumen microbial fermentation. EO affects lipid metabolism resulting in the low-fat content of the animal products. Also, they slightly increase milk production with considerable improvement in milk composition, and its fatty acids profile, especially with clove and thyme supply. Hence, they improved lamb's immunity; consequently, minimized lamb mortality and affecting lamb's growth rate positively. Therefore, using of EO in ruminant nutrition could be considered as a promising and useful alternative feed supplement in ruminant dairy nutrition to enrich the nutritional properties of the dairy products consequently, adding value and benefits to the animal products for consumer health.

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