

EFFECT OF ADDITION OF ANISE, CLOVE AND THYME ESSENTIAL OILS ON BARKI LAMBS PERFORMANCE, DIGESTIBILITY, RUMEN FERMENTATION, CARCASS CHARACTERISTICS AND INTRAMUSCULAR FATTY ACIDS.

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SUMMARY

Recently, some plants essential oils (EO) are used as natural feed additives for ruminants because their major bioactive compounds have antimicrobial properties that can be effective against undesirable rumen microbes. In addition, EO have not an adverse effect either on animal or human health and environment. This work was carried out to evaluate the effect of natural additives as essential oils addition on animal performance, feed intake, apparent digestibility, carcass characteristics, chemical composition and fatty acids profile of *longissimus lumborum* muscle of Barki lambs. Twenty weaned male Barki lambs (19.72 ± 2.09) kg BW, 3 months old were randomly assigned in one of four diet groups, 5 animals each, were received the same basal diet (50% berseem hay, BH, and 50% concentrate feed mixture). All experimental animals were kept in individual pens for 195 days for fattening trial and 15 days for digestibility trial. At the end of the experiment all lambs were slaughtered for carcass characteristics evaluation and meat fatty acids (FAs) profile determination. The control lambs were fed their basal diet without EO supply. The other three groups were orally given, by syringe, one of the three tested EO before morning feeding as follow: the basal diet plus 2 ml/day/lamb of anise EO (Anise group), 2 ml/day/lamb clove EO (Clove group) or 2 ml/day/lamb thyme EO (Thyme group). According to the results, the experimental EO had no significant effects on nutrients intake, lamb's performance (final body weight, average daily gain and feed conversion ratio) (P > 0.05). Clove EO improved dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), acid detergent fiber (ADF) digestibility (P < 0.05) and nitrogen balance% of intake (P < 0.05). Ruminal parameters showed higher ruminal ammonia with thyme EO supply (P < 0.05). Most of carcass characteristics were not affected by EO addition but lambs of clove group showed a significant increase in weight of feet (P < 0.05), full (P < 0.01) and empty (P < 0.05) digestive tract, tail fat (P < 0.01) and liver (P < 0.05) compared with control lambs. Some blood plasma metabolites were affected by EO where plasma creatinine and low-density lipoprotein (LDL) were increased (P < 0.05) in thyme group however, total lipids and cholesterol increased in clove group. Fatty acids content of *longissimus lumborum* muscle was slightly affected by addition of EO where palmitoleic (C16:1n-7) was increased and arachidonic acids (C20:4n-6) was decreased (P < 0.05) in meat of lambs fed clove. Also, clove inclusion in lambs diet increased monounsaturated fatty acids (MUFA) deposition in their meat (P < 0.05). Overall, addition of anise, clove or thyme EO to lambs' diets may not have the potential to improve animal growth performance, blood metabolites and meat chemical composition. Clove EO has a considerable positive effect on nutrients digestibility and improve carcass characteristics, meat FA profile. Further research are needed with higher concentrations of EO for longer duration of supply.

Keywords: essential oil, lambs, carcass, blood plasma, fatty acid profile, meat.

INTRODUCTION

The use of natural additives has reported as an essential principle of healthy nutrition. The ban on antibiotics use in animal nutrition as feed additives due to its residual effect found in milk and meat products and the increased awareness of the consumers about the health hazards occurs due to the use of antibiotics in animal nutrition triggered searching for natural and safe feed additives (Khamisabadi *et al.* 2016) because they have health benefits on animals when used as feed additives in animal nutrition.

The evaluation of EO for antimicrobial, antifungal and antioxidant properties has been conducted by humans in traditional medicine and there were much research on the use of plant extracts and EO in animal production all over the world (Khateri *et. al.*, 2017). Plants essential oils (EO) from clove, cinnamon and thyme were more favorable, due to their valuable effects on rumen fermentation such as decrease in methane emissions and ammonia nitrogen (N) concentration and an increase in propionate to acetate ratio (Jahani-Azizabadi *et. al.*, 2011). Thyme (*Thymus vulgaris* L) is a medicinal herb in the Lamiaceae family, has special functions such as antiseptic, antimicrobial and antioxidant (Sengul *et. al.*, 2008). Therefore, the objective of this study was to determine whether these essential oils can be used as feed additives in fattening lambs to improve animal performance, yield and quality of meat through measuring feed intake, growth rate, blood metabolites and carcass characteristics.

MATERIALS AND METHODS

The present study was conducted at Maryout Research Station, Desert Research Center, Ministry of Agriculture, 35 Km south of Alexandria, Egypt

Animals, diets and experimental procedures:

Fattening trial:

After weaning lambs at 3 months old on average, twenty male growing lambs with average live body weight of 19.72 ±2.09 kg body weight were used. The study lasted for 210 days including 195 days for fattening and 15 days for digestibility trials. Lambs were randomly divided into four homogenous groups (5 animals each). Lambs of each group were housed in a concrete pen (5m×5m) and randomly assigned to receive one of four tested diets. Animals of the control group were fed on the basal diet (without essential oil supplement) which consisted of 50% concentrate feed mixture (CFM) and 50 % Berseem hay (BH). The CFM composed of 17% soybean meal, 52.5% yellow corn, 28% wheat bran, 0.9% salt, and 1.1% limestone 0.5 vitamin mineral premix. The other three groups were fed on the same basal diet with supplementation of one of the three tested EO as follow: Control diet plus 2 ml/h/day of Anise (*Pimpinella anisum*, AEO, group) or plus 2 ml/h/day of Clove (*Syzygium aromaticum*, CEO, group) or plus 2 ml/h/day of Thyme (*Thymus vulgaris*, TEO, group).

Feeding requirements were calculated according to the recommended feeding standards of NRC (2007). The amount of ration was daily weighed and offered to the animals and changed biweekly depending on the live body weight changes till the end of the experimental period. The rations were offered twice a day for all groups. The CFM was offered daily at 8:00 am while BH was offered 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 feed intake was recorded, and the orts were determined next morning. The chemical composition of the basal diet is presented in Table, (1). The lambs were weighed at 15 day intervals throughout the study just before morning feeding to calculate the average daily gain (ADG) and feed conversion (FC: g of BW gain/kg of feed). Feed intake, utilization as well as daily live weight gains were recorded.

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

Item	Concentrate feed mixture	Berseem hay
Dry matter	92.18	90.66
Organic matter	96.23	85.57
Crude protein	14.23	13.12
Ether extract	3.88	3.47
Ash	3.77	14.43
Neutral detergent fiber	30.56	56.03
Acid detergent fiber	13.05	45.61

The CFM composed of 17 soybean meal, 52.5% yellow corn, 28% wheat bran, 0.9% salt, 1.1% limestone 0.5 Vitamin mineral premix.

Digestibility trial:

At the end of fattening period, 4 animals from each group were subjected to digestibility trial to determine nutrient digestibility coefficients. Animals were kept individually in metabolic cages for 15

days. The first 5 days were for adaptation period followed by 5 days for sample collection. Lambs were fed the same diets with the same additives as in fattening trial. Drinking water consumption was determined for each animal daily. The urine and fecal samples were collected, measured or weighed daily and 10% sample as a representative sample were collected and stored (urine) or dried (feces) for further analysis. Diet apparent digestibility and animal N balance were measured at the end of period.

Slaughter and carcass measurements

At the end of the study, on day 210 all of the lambs from the four fattening groups were starved for 12 h, weighed and slaughtered in the slaughter house of the station. After complete evisceration and dressing, the carcasses were weighed (the hot carcass weight). Non-carcass components, gastrointestinal tracts and viscera were separately weighed. Selected internal organs (liver, heart, and kidneys) were weighed. The contents of the digestive tract were removed, and their weight was subtracted from the slaughter live weight to obtain the empty body weight (EW). Dressing percentage was estimated as the percentage of carcass weight (CW) relative to the EW and as a percentage of the hot carcass relative to the EW. Carcasses were chilled at 4°C for 24 h and then weighed (the cold carcass). The cold carcass was split into two symmetrical parts along the backbone. Simple dissection of their right halves was carried out. The samples of *longissimus lumborum* muscle (L1) from the 10th to 13th rib were taken for chemical composition and FA profile. Muscle samples were minced, packed into polyethylene bags and stored at –30°C until analysis. To obtain the (L1) area, the exposed area of the (L1) muscle was drawn on tracing paper, and measured three times with a digital planimeter koizumi placom (kp-82) to the nearest cm². The values obtained were used to calculate the mean of the L1 area. Fatty acids profile was determined by using High Pressure Liquid Chromatography (HPLC) according to AOAC (2007).

Laboratory analytical methods:

Chemical analysis

The proximate chemical analysis was conducted on representative samples of feedstuffs, Orts, feces, muscles, and nitrogen in urine samples according to standard methods of AOAC (2007). Neutral detergent fiber (NDF), acid detergent fiber (ADF) were determined by using the automated ANKOM fiber analyzer as described by Goering and Van Soest (1970).

Analysis of feed additives (essential oils)

Essential oils were provided by 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 according to El-Gendy *et al.* (2017) as previously mentioned in El –Essawy *et al.* (2019). Compounds identified in the studied essential oils and their active components as percentage (%) are listed in Table, (2).

Ruminal liquor analysis

On the last day of the digestibility trial, rumen liquor was withdrawn by stomach tube from all lambs before morning feeding. 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 four layers of cheesecloth and kept frozen until analyzed for ammonia – nitrogen (NH₃-N) (AOAC, 2007) and total volatile fatty acids (TVFA's) Warner (1964).

Biochemical blood analysis

At the end of the digestibility trial blood samples were collected from all lambs in each group used in the digestibility trial via jugular vein in a heparinized tubes before morning feeding. All samples were centrifuged at 4.000 rpm for 15 minutes and the collected plasma was stored at -20°C for subsequent analysis. Plasma concentrations of total protein, albumin, (globulin was obtained by subtracting the total proteins values from the albumin values), blood urea–N, creatinine, total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), total lipids (TL), alanine amino transferase (ALT) , aspartate amino transferase (AST) and total antioxidant capacity (TAC) were determined using Bio diagnostic laboratory kits.

Statistical analysis

Analysis of variance (ANOVA) was used to test the obtained data using the general linear modeling procedure (SAS, 2000). Duncan (1955) Multiple Comparison Tests were applied with the P<0.05 value for significance.

Table (2): The main active components of Anise, Clove and Thymol essential oils

The plant EO	R T	Relative percentage (%)	Main components
<i>Anise (Pimpinella anisum)</i>			
	7.45	0.17	β -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	α -Himachalene
	25.49	2.27	Humulen-(v1)
	25.7	0.88	α -Curcumen
	26.22	0.68	Zingiberene
	26.33	0.81	β -Himachalene
	26.71	0.42	β -Bisabolene
	38.9	1.9	Allocryptopine
<i>Clove (Syzygium aromaticum)</i>			
	16.75	0.12	Acetin, mono
	18.39	0.03	Thymol
	19	0.02	Dimethylbenzyl carbinyl 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
<i>Thyme (Thymus vulgaris)</i>			
	4.63	7.52	α -Pinene
	5.83	11.21	β -Pinene
	6.76	0.34	α -Phellandrene
	7.09	0.54	(+)-2-CARENE
	7.34	24.3	p-Cymene
	8.02	0.57	Benzyl Alcohol
	8.44	1.06	γ -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	α -Bergamotene
	26.9	0.24	γ -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.

RESULTS AND DISCUSSION

Feed intake, digestibility and lamb performance:

Data of nutrient intake and growth performance are presented in Table (3) and Table (4), respectively.

Table (3): Effect of supplementation of Anise, Clove and Thyme oils to lamb diets on nutrient intake and digestibility.

Item	Treatment ¹				SEM ²
	Control	Anise	Clove	Thyme	
Nutrient intake, g/d					
DM	1433	1408	1406	1431	21.67
OM	1307	1285	1282	1305	18.18
CP	196.9	193.6	193.2	196.73	2.88
EE	52.79	51.93	51.68	52.73	0.761
NDF	611.2	598.7	602.25	612.1	11.58
ADF	408.7	398.99	403.94	410.08	9.22
Digestibility, %					
DM	70.77 ^{ab}	71.92 ^{ab}	73.18 ^a	68.5 ^b	1.35
OM	72.44 ^{ab}	73.44 ^{ab}	75.27 ^a	70.50 ^b	1.25
CP	69.42 ^{ab}	70.76 ^{ab}	73.17 ^a	67.43 ^b	1.51
EE	69.54 ^b	73.70 ^{ab}	76.61 ^a	72.71 ^{ab}	1.29
NDF	59.16	58.30	62.17	58.81	1.75
ADF	47.81 ^a	45.45 ^a	49.60 ^a	36.26 ^b	2.66

¹Control, basal diet without oil supplement; basal diet with Anise oil supplement; basal diet with clove oil supplement and basal diet with Thyme oil supplement, ² Standard error of the means.

^{a-b} Means within a row with different superscripts are significantly different ($P < 0.05$).

Table (4). Effect of supplementation of Anise, Clove and Thyme oils on lamb's performance

Item	Treatment ¹				SEM ²
	Control	Anise	Clove	Thyme	
Initial body weight, kg	20.05	19.51	19.53	19.80	2.09
Final body weight, kg	55.37	54.95	55.82	54.37	3.51
Average daily gain, g/ d	168.21	168.8	172.8	164.6	9.16
Average daily feed intake, kg/ d	1.26	1.24	1.25	1.24	0.905
Feed conversion ratio, kg/ kg	7.53	7.53	7.26	7.59	0.368

¹Control, basal diet without oil supplement; basal diet with Anise oil supplement; basal diet with clove oil supplement and basal diet with Thyme oil supplement; ² Standard error of the means.

Unfortunately, the desired object of adding EO as additives to increase feed intake by stimulating appetite was not confirmed. Dry matter (DM) and nutrient intake were not influenced ($P > 0.05$) by EO, which agree with Fandino *et al.* (2008) with anise EO, Chowdhury *et al.* (2018) with clove in Black Bengal goats, El-Essawy *et al.* (2019) in ewes with the same types of EO. Vakili *et al.* (2013) found that thyme and cinnamon EO had no effect on DMI and performance of the growing calves. The clove EO addition increased DM, OM, CP, EE and ADF ($P < 0.05$) digestibilities (table 3), which agree with El-Essawy *et al.* (2019) with ewes and they attributed this improvement in digestibility to the phenolic nature of eugenol and its potency in stimulating bacteria involved in feed digestion. Moreover, Williams *et al.* (2018) found that the effect of EO such as thymol, eugenol and others on the efficiency of the utilization of the ingested nitrogen is due to inhibition of the growth and activity of certain microorganisms. Indeed, EO rich in thymol affects proteolysis in the rumen due to its toxicity to certain species of microorganisms present in rumen, inhibiting the degradation of fibers (Cieslak *et al.*, 2013). However, Moura *et al.* (2017) observed no differences in nutrients intake between lambs fed copaiba EO and monensin. As shown in Table (4), the final weight of lambs, average daily gain (ADG), average daily feed intake or the feed conversion ratio were not affected during the entire study. So, addition of EO to the diet in fattening period had no adverse effects on growth performance when compared with lambs fed control diet. Similar results were recorded on incorporation of a mixture of EO (thymol, eugenol, vanillin and limonene) in beef cattle diet (Benchaar *et al.*, 2006), three encapsulated EO in sheep diet (Lin *et al.*, 2013), carvacrol and /or thymol in lambs diet (Biricik *et al.*, 2016). Other studies inconsistent with the present findings and found that nutrients digestibility were not influenced neither by addition of either essential oils active compounds in sheep diet (Lin *et al.*, 2013), or addition of EO complex to cows (Santos *et al.*, 2010).

Nitrogen retention:

The nitrogen intake was not affected by addition of EO (Table 5) while the nitrogen excretion, nitrogen balance and utilization were significantly affected by dietary additives (P<0.05). It is clear that clove EO fed lambs showed the minimum loss of N and consequently resulted in highest N balance and utilization. Dijkstra *et al.* (2013) explained that clove increased N – retention due to the higher level of active phenolic components. Also, Chowdhury *et al.* (2018) agreed with the present results and indicated that clove can reduce N wastage and increase N retention in small ruminants. On the other hand, thyme addition to lambs resulted in opposite trend of clove addition. El-Essawy *et al.* (2019) consistent with the current results with the same EO in Barki ewes. The higher nitrogen retention is resulted in response to lower nitrogen excretion (table 5) and higher nitrogen digestibility (table 4). Similarly, Smeti *et al.* (2015) reported higher nitrogen retention by rosemary supply in ewes and they attributed their findings to protein protection against ruminal degradation (Newbold *et al.*, 2004) that reducing the nitrogen losses (Terril *et al.*, 1992).

Table (5) Nitrogen utilization of the experimental rations fed to experimental lambs.

Item	Treatment ¹				SEM ²
	Control	Anise	Clove	Thyme	
Nitrogen intake g/head/day	31.50	30.98	30.91	31.47	0.461
Total excretion g/head/day	23.89 ^{ab}	23.13 ^{ab}	22.14 ^b	25.57 ^a	0.779
Nitrogen balance (NB) g/day	7.61 ^{ab}	7.85 ^{ab}	8.77 ^a	5.90 ^b	0.851
Nitrogen balance % of intake	24.10 ^{ab}	25.30 ^{ab}	28.34 ^a	18.72 ^b	2.56
N-utilization % of digested	34.72 ^{ab}	35.53 ^{ab}	38.73 ^a	27.41 ^b	3.25

¹Control, basal diet without oil supplement; basal diet with Anise oil supplement; basal diet with Clove oil supplement and basal diet with Thyme oil supplement; ² Standard error of the means.

^{a-b} Means within a row with different superscripts are significantly different (P<0.05).

Ruminal fermentation parameters:

The ruminal pH and total volatile fatty acids (TVFA) both were not influenced by adding EO (Table 6). Ammonia nitrogen (NH₃-N) concentration was increased significantly (P<0.05) in the three EO – added treatments. Kim *et al.* (2013) reported that EO affects the rumen microbial activity and consequently alters ruminal fermentation. The increased ammonia concentration in the present study agreed with those obtained by El-Essawy *et al.* (2019) who used the same EO in ewes and Cardozo *et al.* (2004) who emphasized that anise extract stimulated peptidolysis and deamination and as a consequence NH₃-N accumulated. EO had variable impacts on ruminal NH₃-N concentration in different studies. Previous researches suggesting that EO increased ruminal ammonia (Tekippe *et al.*, 2011), others reported that there were no effects (Vakili *et al.* 2013) with thyme and cinnamon EO and Chaves *et al.* (2008a) with carvacrol or cinnamaldehyde or there were a reduction in ammonia concentration (Lin *et al.* 2013).

Table (6): Effect of supplementation of Anise, Clove and Thyme and Clove oils to lambs on rumen fermentation

Item	Treatment ¹				SEM ²
	Control group	Anise group	Clove group	Thyme group	
pH value	6.18	6.37	6.41	6.46	0.096
Ammonia-N concentration, mg /dl	17.29 ^b	19.13 ^{ab}	18.89 ^{ab}	20.81 ^a	0.685
Volatile fatty acid, mg/ dl	9.606	10.02	8.91	9.14	0.369

¹Control, basal diet without oil supplement; basal diet with Anise oil supplement; basal diet with Clove oil supplement and basal diet with Thyme oil supplement; ² Standard error of the means.

^{a-b} Means within a row with different superscripts are significantly different (P<0.05).

Blood metabolites:

Table (7) represents the effect of the three EO on some blood metabolites. The studied EO have a noticeable effect on blood plasma creatinine, total lipids (TL), low density lipoprotein (LDL) and total cholesterol (TC) where they were significantly increased ($P<0.05$) in lambs given EO compared with control lambs. Garcia-Bojalil *et al.* (1998) suggested that oils supplementation is known to increase blood cholesterol. However, plasma levels of total protein (TP), albumin, globulin, urea, triglycerides (TG), high density lipoprotein (HDL), total antioxidant capacity, alanine aminotransferase (ALT) and aspartate

Table (7): Effect of supplementation of Anise, Thyme and Clove oils to lambs on some blood metabolites

Item	Treatment ¹				SEM ²
	Control	Anise	Clove	Thyme	
Total protein, mg/dl	7.78	6.98	6.75	6.66	0.22
Albumin, mg/dl	4.36	3.99	3.78	4.08	0.28
Globulin, mg/dl	3.42	2.99	2.97	2.58	0.22
A/G ratio	1.31	1.37	1.58	1.59	0.15
Urea, mg/dl	55.77	56.34	46.24	47.79	2.11
Creatinine, mg/dl	0.81 ^b	0.93 ^{ab}	1.21 ^{ab}	1.55 ^a	0.11
Triglycerides, mg/dl	11.58	11.55	11.38	11.41	0.10
Total lipid, mg/l	73.46 ^b	75.75 ^{ab}	76.40 ^a	74.80 ^{ab}	0.42
LDL, mg/l	31.59 ^c	33.84 ^c	37.37 ^b	40.03 ^a	1.01
HDL, mg/dl	70.63	71.84	71.05	70.88	0.41
Cholesterol, mg/dl	23.23 ^c	21.10 ^c	43.30 ^a	36.36 ^b	2.06
Total antioxidant capacity mM/l	0.55	0.44	0.46	0.43	0.05
Alanine, U/l	18.33	15.33	17.67	18.00	0.97
Aspartate amino transferase, U/l	13.67	14.33	17.67	17.67	0.81

¹Control, basal diet without oil supplement; basal diet with Anise oil supplement; basal diet with Clove oil supplement and basal diet with Thyme oil supplement; ² Standard error of the means.

^{a-b} Means within a row with different superscripts are significantly different ($P<0.05$).

aminotransferase (AST) were not influenced by addition of EO. The lack of influence of EO on some blood parameters consistent with Vakili *et al.* (2013) who used thyme oil in diets of feedlot calves, Unal and Kocabagli (2014) with thyme in lamb ration and they found no effect on TC, TG, HDL and LDL and in another study, Khateri *et al.* (2017) reported that serum TC, TP, albumin, BUN, ALT and AST concentration were not influenced by the addition of mixture of essential oils containing thyme, eugenol and cinnamon EO. Yang *et al.* (2010a) reported that levels of TG can be influenced by EO supply through changing of feed intake so, similar feed intake among groups in the current study contributed to similar blood levels of TG. Also, plasma creatinine concentration is reported to be a measure of muscle mass (Istasse *et al.*, 1990) consequently, increased levels of creatinine with EO addition especially thyme or clove EO reflect the increased muscle mass in lambs of the current study and this finding is supported by the results of rib -eye area and dressing % (Table 8). Therefore, addition of EO to lambs during the fattening period result in changes in blood metabolism that may improve production.

Carcass characteristics:

There was no effect of EO on carcass characteristics (Table 8). No statistically significant differences among groups regarding the main carcass indices such as slaughter weight, carcass weight and rib eye-area and also the chemical composition of the *longissimus lumborum* muscle. These results indicate the lack of influence of the anise, clove or thyme EO on the performance parameters such as feed intake and feed conversion ratio. Hence the results of performance and of the carcass are consistent with each other and with previous studies with different EO (Yang *et al.* (2010b), Unal and Kocabagli, (2014), Simitzis *et al.* (2014) and Biricik *et al.* (2016). Concerning the weight of carcass cuts, there was a significant increase with EO addition in weight of feet ($P<0.05$), full ($P<0.01$) and empty ($P<0.05$) digestive tract and tail fat ($P<0.01$) especially with clove and /or thyme addition. Besides, the weights of skin and neck tended to increase with clove or thyme addition ($P=0.081$ and $P=0.084$, respectively). Interestingly, lambs fed with clove, thyme or anise EO have heavier livers than those fed with control diet. Consistent with this result, Chaves *et al* (2008b) reported heavier livers in lambs supplemented with cinnamaldehyde

whereas Chaves *et al.* (2011) found no effect of dietary supplementation with cinnamaldehyde on lamb liver. In general, addition of clove or thyme EO improved carcass cuts and organs.

Table (8): Effect of Anise, Clove and Thyme essential oils addition on carcass characteristics of major cuts, internal organs and *longissimus lumborum* muscle area (cm²) and it's chemical composition of experimental lambs.

Item	Treatment ¹				SEM ²
	Control group	Anise group	Clove group	Thyme group	
Slaughter weight, kg	56.00	55.66	56.66	57.00	2.29
carcass weight, kg	25.33	24.73	25.20	26.66	1.34
Dressing %	45.23	44.39	44.52	46.68	1.12
Shoulder, kg	4.34	4.79	5.03	5.12	0.336
Loin, kg	1.971	2.120	2.688	2.438	0.224
Leg, kg	7.303	8.065	9.214	9.243	0.614
Skin, kg	7.03	8.32	9.83	8.33	0.636
Head, kg	3.23	3.37	4.03	3.80	0.258
Feet, gm	866.5 ^c	933.9 ^c	1065 ^{ab}	1117.7 ^a	53.78
Digestive tract full, kg	6.43 ^b	9.93 ^a	10.0 ^a	10.43 ^a	0.703
Digestive tract empty, kg	2.97 ^b	3.20 ^{ab}	3.90 ^a	3.90 ^a	0.233
Abdominal fat, gm	842.7	864.3	873.3	877.3	60.32
Kidneys fat, gm	662	639	612.3	671.7	22.93
Tail fat, gm	1005.3 ^c	1070 ^b	1163 ^a	1041 ^{cb}	14.79
9-10-11 ribs weight, Kg	1.462	1.483	1.485	1.543	57.41
Fat, gm	365	353.3	366.7	375	19.82
Fat %	24.96	24.08	24.91	24.33	1.97
Meat, gm	750	798.33	763.3	800	52.31
Meat %	51.27	53.53	51.68	51.84	2.63
Bone, gm	325	308.3	303.3	360	17.71
Bone %	22.22	21.47	23.14	23.39	1.05
Neck, kg	1.648	2.476	2.630	2.861	0.29
Ribs, kg	6.38	6.39	7.66	7.32	0.796
Flank, kg	0.675	0.780	0.995	0.753	0.102
Internal Organs , gm					
Lung	634.66	669.63	694.33	590.30	79.43
Heart	186.37	165.93	227.06	247.13	27.18
Liver	530.26 ^b	633.20 ^{ab}	782.60 ^a	678.03 ^{ab}	50.62
Spleen	59.47	68.17	57.93	67.50	6.09
Kidney	115.0	142.77	131.8	121.66	10.33
Testes	363.10	362.06	409.66	477.10	51.49
L.L muscle area (cm ²)	15.38	15.19	16.73	16.01	0.69
Chemical composition of <i>longissimus lumborum</i> muscle (%)					
Moisture	68.55	67.73	69.76	68.99	1.41
Crude protein	21.88	22.95	21.42	21.19	0.912
Ether extract	6.27	6.37	6.86	6.70	0.253
Ash	1.12	1.05	1.07	1.03	0.043

¹Control, basal diet without oil supplement; basal diet with Anise oil supplement; basal diet with Clove oil supplement and basal diet with Thyme oil supplement;

^{a-b} Means within a row with different superscripts are significantly different ($P < 0.05$).

² Standard error of the means.

Intramuscular fatty acid composition of *longissimus lumborum* muscle:

Intramuscular saturated fatty acids (SFA):

Most of intramuscular SFA were not affected by addition of EO (Table 9) except stearic acid which decreased significantly ($p < 0.05$) with EO especially with clove addition. Consequently, total SFA were numerically decreased in the same group, while its value was comparable among other studied groups. Tholstrup *et al.* (2003) found that saturated fatty acids play an important positive role in the formation of blood cholesterol.

Table (9): Effect of supplementation of Anise, Thyme and Clove oils to lambs on intramuscular fatty acid composition (% of total fatty acid) of the *longissimus lumborum* muscle of experimental lambs.

Item	Treatment ¹				SEM
	Control	Anise	Clove	Thyme	
SFA					
c14, myristic	2.03	2.22	2.12	2.30	0.16
c15, pentaenoic	0.55	0.66	0.49	0.57	0.07
c16, palmitic	26.27	26.99	26.78	27.09	0.60
c17, heptadecanoic	2.20	2.06	2.15	2.20	0.14
c18, stearic	17.44 ^a	16.97 ^{ab}	15.38 ^b	16.09 ^{ab}	0.56
∑SFA	48.49	48.90	46.93	48.24	0.74
MUFA					
c16:1ω7, palmitoleic	0.33	0.28	0.35	0.35	0.03
C16:1ω5	1.45 ^c	1.59 ^{bc}	1.99 ^a	1.82 ^{ab}	0.10
c18:1ω9, oleic	41.07	41.14	43.66	41.98	0.75
c18:1ω7, vaccinic	1.71 ^c	1.72 ^c	2.06 ^b	2.23 ^a	0.04
c18:1ω5, 6-octadecosaenoic	0.87	0.94	0.85	0.88	0.04
∑MUFA	45.42 ^b	45.66 ^b	48.91 ^a	47.26 ^{ab}	0.70
PUFA					
c18:2ω6, linoleic	3.95 ^a	3.54 ^{ab}	2.59 ^c	3.05 ^{bc}	0.16
c18:3ω3, linolenic	0.34 ^b	0.80 ^a	0.77 ^a	0.62 ^a	0.08
c18:4ω3, alphaoctadecatetraenoic	0.27 ^b	0.64 ^a	0.51 ^{ab}	0.37 ^{ab}	0.12
c20:4ω6, Arachidonic	1.57 ^a	0.47 ^b	0.29 ^b	0.47 ^b	0.10
∑PUFA	6.09 ^a	5.44 ^{Ab}	4.16 ^c	4.50 ^{bc}	0.31
∑UFA	51.51	51.10	53.07	51.76	0.74
∑ PUFA n-3	0.57 ^b	1.44 ^a	1.27 ^a	0.98 ^{ab}	0.14
∑ PUFA n-6	5.52 ^a	4.00 ^b	2.89 ^c	3.51 ^{bc}	0.24
Nutritional quality of meat					
n-6/n-3	9.77 ^a	2.85 ^b	2.27 ^b	3.89 ^b	0.50
PUFA/SFA	0.13 ^a	0.11 ^{Ab}	0.089 ^b	0.093 ^b	0.007

¹Control, basal diet without oil supplement; basal diet with Anise oil supplement; basal diet with Clove oil supplement and basal diet with Thyme oil supplement;

^{a-b} Means within a row with different superscripts are significantly different ($P < 0.05$).

² Standard error of the means.

Mono unsaturated fatty acids (MUFA):

Only vaccinic acid was increased in thyme EO provide group followed by that of clove group while the concentration of MUFA increased significantly ($p < 0.05$) in clove group followed by thyme added group. The presence of vaccinic acid which is an intermediate of bio-hydrogenation in rumen indicating incomplete bio-hydrogenation. Also El-Essawy *et al.* (2019) observed an increase of the unsaturated of fatty acids in milk with the same EO suggesting that EO can reduce the bio hydrogenation process resulted in improving the nutritional value of their milk consequently, benefit human health (Nudda *et al.*, 2013).

Polyunsaturated fatty acids (PUFA):

The proportion of all omega-3 fatty acids (PUFA n-3) were increased while, all omega-6 FA (PUFA n-6) were decreased significantly ($p < 0.05$) in all EO supplied groups compared to control one which is a good indicator for healthy meat for consumer. Although the total PUFA concentration was increased in control lambs, the unsaturated fatty acids (UFA) showed insignificant changes among studied animals but clove supply increased its level numerically compared to other animals groups. Lourenco *et al.* (2008) found that supplementation with cinnamaldehyde inhibited the apparent bio-hydrogenation of linoleic acid and linolenic acid. In lactating Damascus goats, Kholif *et al.* (2012) found that supplementation with garlic oil, cinnamon oil or ginger oil increased unsaturated FA and conjugated linoleic acid (CLA), and that cinnamon oil also increased n-3 linolenic acid.

Nutritional quality of meat:

The *n-6/n-3* and PUFA/SFA ratios are used to evaluate the nutritional value of fat for human consumption. The *n-6/n-3* ratio is strongly dependent on the dietary FA profile fed to ruminants. Lowering the *n-6/n-3* ratio in food production has been recommended to prevent or modulate certain human diseases, and it should range between (1-4) (WHO/FAO, 2003).

The current results indicate that *n-6/n:3* ratio Table (9) in muscle of lambs fed on EO were lower than 4 which is the maximum value recommended for the human diet by public health authorities, while muscle of control lambs were out of range (9.77). These results are consistent with findings of Jerónimo et al (2009), who reported that the *n-6/n-3* ratio in muscle of lambs was significantly decreased with sunflower oil.

Usually lamb meat has a high SFA content and low PUFA/SFA values. Increasing the PUFA concentration in the ration, by including a source rich in either *n-6* or *n-3* PUFA generally improves the PUFA/SFA ratio (Jerónimo *et. al.*, 2009). The PUFA/SFA (P:S) ratio was lower than 0.45, which is the minimum value recommended for the human diet by public health authorities. This is also observed and supported in the present experiment with EO addition.

CONCLUSION

In conclusion, this study attempt to evaluate the potential use of anise, clove or thyme essential oils in fattening period of Barki lambs. Among the three essential oils, clove showed greater DM, OM, CP, EE and ADF digestibility compared with control lambs. No weight change in all experimental lambs. Also clove fed group excreted the least nitrogen resulting in increased nitrogen retention and nitrogen balance. Ammonia increased with EO addition especially clove. Overall, the results of the present study showed that the studied EO have not negative effects on growth performance and blood metabolites, meat chemical composition but improve carcass cuts weights and organs, improve FA profile of the meat providing a safer and healthier animal products for consumer. Further researches are needed with higher concentrations of EO for longer duration of supply.

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تأثير إضافة الزيوت العطرية للينسون و القرنفل و الزعتر علي أداء الحملان البرقي و الهضم و تخمرات الكرش و صفات الذبيحة و الأحماض الدهنية العضلية

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استخدمت الزيوت العطرية لبعض النباتات كإضافات غذائية طبيعية للمجترات لإحتوائها علي مركبات نشطة بيولوجية لها خواص فعالة ضد الميكروبات و التي يمكن أن يكون لها تأثير مضاد للكائنات الدقيقة غير المرغوبة بكرش المجترات. كما أن هذه الزيوت العطرية ليس لها تأثيرات ضارة علي صحة الحيوان و الإنسان و البيئة . أجري هذا البحث لتقييم تأثير بعض الإضافات الطبيعية مثل الزيوت العطرية علي أداء الحيوان و المأكول و الهضم و صفات الذبيحة و التركيب الكيميائي و تركيب الأحماض الدهنية العضلية في الحملان البرقي. ولهذا تم تقسيم 20 من حملان البرقي المفطومة عمر 3 شهور ، و متوسط وزنها 19.72 ± 2.09 كجم) الي أربع مجموعات تتغذي علي نفس العليقة الأساسية المكونة من 50% دريس البرسيم + 50% مركز و استمرت التجربة لمدة 195 يوم لتجربة التسمين بالإضافة إلى 15 يوم لتجربة الهضم. في نهاية التجربة تم ذبح جميع الحملان لتقييم صفات الذبيحة و تقدير مكونات الأحماض الدهنية بلحم الحملان. حيوانات مجموعة الكنترول تم تغذيتها علي العليقة الأساسية فقط دون أي إضافات اما الثلاث مجموعات التجريبية الأخرى فقد تم التغذية على أحد الزيوت العطرية التجريبية باستخدام السرنجة قبل الوجبة الصباحية حيث اضيف للمجموعة الأولى 2 ملي لكل حيوان في هذه المجموعة من زيت الينسون (مجموعة الينسون) ، المجموعة الثانية اضيف لها 2 ملي زيت قرنفل (مجموعة القرنفل) ، المجموعة الثالثة اضيف لها 2 ملي زيت زعتر (مجموعة الزعتر). أظهرت النتائج عدم وجود أي تأثير للزيوت محل الدراسة علي مأكول الحيوانات ، و كفاءة الحيوان و المتمثلة في الزيادة اليومية في وزن الحيوان و الوزن النهائي للحيوان و مدي الإستفادة من الأكل. كان لزيت القرنفل تأثير ايجابي علي هضم المادة الجافة و المادة العضوية و البروتين الخام و الدهن واحد مكونات الألياف و كذلك ميزان النيتروجين كنسبة مئوية من المأكول. ارتفعت نسبة الأمونيا بكرش الحيوانات مع إضافة زيت الزعتر . لم تتأثر أغلب صفات الذبيحة بإضافة اي من الزيوت محل الدراسة إلا مجموعة القرنفل حيث زاد وزن الأرجل و وزن الجهاز الهضمي (مملوء و فارغ) و وزن دهن الذيل و كذلك وزن الكبد زيادة معنوية مقارنة بمجموعة الكنترول. كما ارتفع كرياتينين الدم و البروتينات الدهنية منخفضة الكثافة في مجموعة الزعتر بينما ارتفع الكوليستيرول و الدهون الكلية في مجموعة القرنفل. تأثرت مكونات الأحماض الدهنية في العضلة العينية بدرجة بسيطة بإضافة الزيوت حيث ارتفعت نسبة الأحماض الدهنية a غير المشبعة الأحادية في لحم حملان مجموعة القرنفل. الخلاصة فإن إضافة زيت الينسون و القرنفل و الزعتر في تغذية الحملان لم تحسن نموها أو أداؤها او يؤثر بإيجابية علي دلالات الدم أو علي التركيب الكيميائي للحم الحملان. ولكن زيت القرنفل كان له تأثيرا ايجابيا كبيرا علي هضم العناصر الغذائية و أيضا أدى إلى تحسن في أوزان بعض الأجزاء من الذبيحة و تركيب الأحماض الدهنية في اللحم . و مع هذا هناك حاجة لأبحاث أخرى مع تغيير تركيبات الزيوت وإعطائها للحيوان لفترة الزمنية أطول.