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Impact of Anise, Clove and Juniper Oils as Feed Additives on the Productive Performance of Lactating Goats

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ABSTRACT

This experiment was designed to study the effects of dietary addition of some essential oils on ruminal fermentation characteristics, blood parameters milk production and milk composition. Twenty lactating Damascus goats, seven days after parturition, were assigned randomly into four groups (five animals each) using complete randomized block design. Experimental period lasted for 90 days. Goats of each group were fed the same basal diet and received one of the following treatments; (C) control (without oil), (ANI) control diet+2 mL Anise oil/head/day (mL/h/d), (CLO) control diet+2 mL Clove oil/h/d, (JUN) control diet+2 mL Juniper oil/h/d. Ruminal Total Volatile Fatty Acids (TVFA) has achieved an increase while, ammonia nitrogen was decreased with Essential Oils (EO) additives. Values of serum total protein and globulin have recorded the highest concentrations, on the contrary, blood urea nitrogen and cholesterol concentrations were recorded the lowest values with EO additives. Milk yield and milk composition were not significantly affected by EO additives, while milk fat and milk non-protein nitrogen contents which decreased with EO additives and milk protein content increased with EO additives compared to control. Goats fed diet supplemented with Juniper oil produced milk fat have highest value of total and individual Conjugated Linoleic Acids (CLA) and C18.3N3 (omega 3). Results from this study suggested that feeding these EO (2 g/h/d) to lactating dairy goats had limited effects on milk production and milk composition but feeding 2 mL Juniper oil/h/d changed milk fatty acids profile for healthy effect on the consumers.

Key words: Lactating goats, essential oil, milk yield, rumen fermentation, milk fatty acids

INTRODUCTION

Recently, a number of studies have examined effects of Essential Oils (EO) and their active components, on rumen microbial fermentation and it is known that these EO contain active substances that may have a positive effect on the rumen microflora. One of the most important methods to improve nutritional efficiency in ruminants is to reduce the loss of energy, as methane, nitrogen (N) and as ammonia, from the rumen (Tamminga, 1996). Therefore, recent research has been greatly focused to exploit plant bioactives as natural feed additives to improve rumen fermentation such as enhancing protein metabolism, decreasing methane production (Patra and Saxena, 2009, 2010) and reducing nutritional stress such as bloat and improving animal health and productivity (Patra, 2007). Some plant extracts modify rumen fermentation as reducing methane and ammonia-N production in the rumen (Cardozo *et al*., 2006). It was observed

that EO and their constituents may inhibit deamination of amino acids and reduce methane production in the rumen (Benchaar *et al*., 2008). Feeding or supplementation with different plant materials appears to offer a means to alter the lipid composition in animal products, which may result in human health benefit (Rochfort *et al*., 2008). Additionally, essential oils have antimicrobial activities against gram-negative and gram-positive bacteria (Conner, 1993), which have been related to a number of small phenolic compounds (Helander *et al*., 1998). Anise, Clove and Juniper are considered essential oils which were potentially useful rumen fermentation modifiers. Very few studies were conducted on the effect of essential oils on the performance of lactating goats. The objective of this study was to determine the effect of plant EO Anise (*Pimpinella anisum*), Clove (*Syzygium aromaticum*), Juniper (*Juniperus communis*) supplementation to lactating goat's diets on dry matter intake, rumen fermentation, blood parameters, milk yield and milk fatty acids profile.

MATERIALS AND METHODS

This study was conducted at the Dairy Science Department, National Research Center, Dokki, Cairo, Egypt and Experimental Farm of Gemaza, Animal Production Research Institute, Dokki, Cairo, Egypt during January to July 2011.

Animals and rations: Twenty lactating Damascus goats, in the 2nd-3rd lactating seasons and weighting on average 44.0±3 kg after seven days of parturition were used in this experiment. Goats were grouped into four aged groups (five animals each) and were assigned at randomly to receive one of four dietary diets, using complete randomized block design. The four diets were (C) control (without oil), (ANI) control diet+2 mL Anise oil/h/d (*Pimpinella anisum*), (CLO) control diet+2 mL clove oil/h/d (*Syzygium aromaticum*), (JUN) control diet+2 mL juniper oil/h/d (*Juniperus communis)*. Control ration consisted of Concentrate Feed Mixture (CFM):berseem clover (1:1 dry matter bases). Chemical composition of the ingredients is shown in Table 1. The offered feeds were assessed to cover the maintenance and production requirements for each animal (NRC, 2001). The CFM for each animal was offered individually once daily at 8.00 am, while fresh berseem clover was offered at 10:00 am and 04:00 pm. Dry matter intake was measured at 30, 60 and 90 days by weighing the offered diets and refusals from the previous day. Clean water was available at all time.

Table 1: Chemical composition of Concentrate Feed Mixture (CFM) and Berseem clover (B)

	Diet ingredients	
Items	CFM*	B
Dry matter	91.29	13.30
Organic matter	89.89	88.20
Ash	10.11	11.80
Crude protein	14.15	14.20
Ether extract	4.05	2.60
Crude fiber	15.33	27.50
Nitrogen-free-extract	56.36	43.90

*The CFM consisted of 25% undecorticated cotton seed meal, 35% wheat bran, 30% corn, 3% rice bran, 3% molasses, 2% limestone, 1% urea and 1% salt (NaCl)

Feed analysis: Samples of feed ingredient were analyzed for dry matter, ash, crude protein, crud fiber and ether extract according to methods of AOAC (2007). Nitrogen-free extract was calculated by difference.

Sampling and analysis of rumen liquor: Rumen liquor samples were collected from three animals within each group by a stomach tube. Collection was performed four hours after morning feeding, at 30, 60 and 90 days. The rumen samples were filtered through two layers of cheese cloth and used as quickly as possible for the measurement of pH by using digital pH-meter. Strained rumen liquor was stored in glass bottles (25 mL) with few drops of toluene and paraffin oil just to cover the surface and stored at a deep freeze (-18°C) till chemical analysis. The concentration of ammonia-N in the rumen fluid was determined according to AOAC (2007). Ruminal Total Volatile Fatty Acids (TVFA's) and fractions of Volatile Fatty Acids (VFA) were determined by gas chromatography (Varian 3700; Varian Specialties Ltd, Brockville, Ontario, Canada).

Sampling and analysis of blood serum: Blood samples were collected from the jugular vein of each animal at 30, 60 and 90 days at four hours after morning feeding. The blood samples were directly collected into clean dried glass culture tubes and centrifuged at 4000 rpm for 20 min blood serum was then separated into a clean dried glass vial and then stored at -18°C till chemical analysis. Blood serum samples were analyzed for concentrations of total protein (Armstrong and Carr, 1964), albumin (Doumas *et al*., 1971), urea-N (Patton and Crouch, 1977), cholesterol (Raltiff and Hall, 1973), serum glutamic-oxaloacetate-transaminase (GOT) and glutamic-pyruvate-transaminase (GPT) (Reitman and Frankel, 1957). Globulin and albumin/globulin ratio were calculated by difference.

Sampling and analysis of milk: Individual milk samples were collected every two weeks during the experimental period (90 days). The goats were handily milked twice daily at 8.00 am and 6.00 pm. Milk yield was recorded daily. The sample of each animal represents a mixed sample of constant percentage of the evening and morning yield. Milk samples were analyzed for total solids, fat, protein, non-protein nitrogen and lactose using infrared spectrophotometry (Foss 120 Milko-Scan, Foss Electric, Hillerød, Denmark) according AOAC (2007) procedures. The ash content of milk was determined after heating in a muffle furnace at 550°C for 16 h. Solids, not fat content was calculated by difference. Fatty acids in milk were extracted and methylated according to method 996.06 of AOAC (1998). Using High Pressure Liquid Chromatography (HPLC) system.

Statistical analysis: All results were analyzed using the MIXED procedure of SAS (2004). Data of milk fatty acid profile were analyzed as a complete random design, where treatment was the main source of variation. Data of feed intake, ruminal parameters, blood parameters, milk production and milk composition were analyzed as a randomized block design. When a significant F-test was detected (i.e., $p<0.05$), treatment means were separated using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Rumen liquor parameters: Data of Table 2 shows the effect of treatments on goats' rumen parameters. Ruminal pH values were not affected by EO additives. In contrast, TVFA's concentration was significantly increased with EO additives which ANI recorded the highest value compared with control, which may indicate improved feed digestion. TVFA are the end

products of rumen microbial fermentation and represent the main supply of metabolizable energy for ruminants (Van Soest, 1982). So, the EO additives are possible that the improvement of energy production in ruminants. These results are in good agreement with those of Benchaar *et al.* (2007), who reported that total VFA concentration was tended to increase the rumen of lactating cows when the diet contained alfalfa silage supplemented with mixture of essential oil compounds. Molar proportion of acetate (C2) was significantly decreased for animals fed essential oils additives in which CLO recorded the lowest value compared with control animals. However, the propionate (C3) and butyrate (C4) proportions were significantly increased with EO additives, with the observation that CLO recorded the highest values. These data are in agreement with those of Cardozo *et al.* (2006) and Fandino *et al.* (2008), who reported that decreased acetate proportion and increased propionate proportion, with EO supplementation *in vivo* study. The increase in butyrate production with EO treatments may be due to activation of the gram positive bacteria *Butyrivibrio fibrisolvens*, a major butyrate producer in the rumen (Bryant and Small, 1956). Ammonia nitrogen was decreased with treated groups compared with control group. As, EO inhibit Hyper-Ammonia Producing (HAP) bacteria in the rumen, the concentrations of ammonia and deaminase activities sometimes decreases (Patra, 2011). The HAP bacteria comprise only around 1% of the rumen bacterial populations but they possess a very high deamination activity (Wallace, 2004). This could decrease the rate of ammonia production in the rumen, which may be beneficial nutritionally by increasing the efficiency of protein utilization in the rumen (Wallace *et al.*, 2002). EO reduces the number of ruminal protozoa and has a negative role on utilization of N by ruminants. Protozoa also possess proteolytic and deamination activities (Williams and Coleman, 1992). Thus the reduction in ammonia-N concentrations, supporting the hypothesis that ANI, CLO and JUN inhibit deamination. These results are consistent with changes observed under the study of Cardozo *et al.* (2006) and Castillejos *et al.* (2008). The main effects of EO in the rumen have been suggested to be due to reduction of protein and starch degradation and an inhibition of amino acid degradation due to selective action on certain rumen micro-organisms, specifically some bacteria (Hart *et al.*, 2008). One mode of action suggested for EO is an effect on the pattern of bacterial colonisation of particular starch rich substrates as they enter the rumen. A second possible mode of action is the inhibition of HAP bacteria involved in amino acid deamination.

Blood serum metabolites: Data in Table 3 showed the effect of treatments on some blood serum parameters. Groups of ANI, CLO or JUN supplemented have a higher value of serum total protein and globulin than animals fed control ration. In contrast serum urea concentration was decreased

Table 2: Rumen parameters of lactating goats fed rations added with essential oils

Items	Control	ANI	CLO	JUN	Pro. >F	SE
pH	6.09	6.00	5.83	5.80	0.235	0.126
TVFA (mM)	77.0^{b}	85.8^{a}	81.4^{a}	81.6^{a}	0.006	1.291
VFA (mol 100 mL)						
C2	69.3^{a}	63.8^{b}	57.2^{c}	60.8^{b}	0.036	6.185
C3	25.4^{b}	28.7^{a}	29.6^{a}	27.0^{ab}	0.029	1.992
C4	9.3^{b}	11.8^{a}	12.6^{a}	10.9^{ab}	0.005	1.921
C2:C3	2.72	2.22	1.93	2.25	0.009	0.655
NH_3-N (mg L^{-1})	281^{a}	263^{b}	258^{b}	260^{b}	0.036	0.261

Each value represents an average of 9 samples, Means at the same row with different superscript are significantly ($p<0.05$) different, ANI: Anise oil, CLO: Clove oil, JUN: Juniper oil, TVFA: Total volatille fatty acids, VFA: Voiatile fatty acids

Table 3: Blood serum parameters of goats fed on rations supplemented with essential oils

Item	Control	ANI	CLO	JUN	Pro. >F	SE
Total protein (g dL^{-1})	5.97^{b}	6.44^{a}	6.23^{a}	6.28^{a}	0.0001	0.047
Albumin (g dL^{-1})	2.93	3.08	2.99	2.96	0.4980	0.038
Globulin (g dL^{-1})	2.86^{b}	3.35^{a}	3.24^{a}	3.32^{a}	0.0020	0.053
A/G ratio	1.05	0.93	0.94	0.91	0.2380	0.027
Urea (mg dL^{-1})	37.11^{a}	30.11^{c}	31.14^{bc}	32.23^{b}	0.0001	0.437
Glucose (mg dL^{-1})	65.80	65.33	66.53	65.80	0.0220	0.514
Cholesterol (mg dL^{-1})	231.53^{a}	205.87^{b}	212.67^{b}	218.46^{b}	0.0010	2.457
GOT (Units mL^{-1})	33.33	34.73	32.67	32.47	0.1260	0.402
GPT (Units mL^{-1})	16.21	16.11	16.23	16.28	0.9210	0.085

Each value represents an average of 15 samples, Means at the same row with different superscript are significantly ($p<0.05$) different, ANI: Anise oil, CLO: Clove oil, JUN: Juniper oil

Table 4: Average daily milk yield and composition of lactating goats fed rations added with essential oils

Item	Control	ANI	CLO	JUN	Pro>F	SE
Live body weight (kg)	43.55	44.51	44.33	43.69	0.965	0.854
Dry matter intake (kg h^{-1} d^{-1})	1.39	1.40	1.41	1.45	0.569	0.074
Milk yield/DMI	0.808^{b}	0.898^{a}	0.900^{a}	0.855^{ab}	0.895	11.530
Milk yield (g d^{-1})	1122.90	1258.20	1268.60	1239.70	0.155	24.215
Fat (%)	4.37^{a}	4.15^{b}	4.11^{bc}	3.98^{c}	0.0001	0.030
Lactose (%)	4.82	4.88	4.90	4.85	0.883	0.035
Protein (%)	3.15^{b}	3.50^{a}	3.47^{a}	3.45^{a}	0.001	0.033
TS (%)	12.95	13.53	13.48	13.44	0.433	0.130
SNF (%)	8.70	9.25	9.15	8.96	0.076	0.089
Ash (%)	0.903	0.903	0.895	0.892	0.318	0.002
NPN (mg kg^{-1})	23.30^{a}	19.63^{b}	20.13^{b}	19.90^{b}	0.0001	0.247

Each value represents an average of 30 samples, Means at the same row with different superscript are significantly ($p<0.05$) different. ANI: Anise oil, CLO: Clove oil, JUN: Juniper oil, TS: Total solids, SNF: Solids-not-fat

with animals fed EO additives compared with control. These results reflects the decrease of ruminal ammonia N (Table 2) which support the hypophesis of improvement of ruminal microbial protein synthesis with treated groups. Serum cholesterol was decreased ($p<0.05$) with animals fed ANI, CLO or JUN compared with that fed control diet. Oils supplementation is known to increase blood cholesterol (Garcia-Bojalil *et al*., 1998), although the types of fatty acids in ANI, CLO or JUN would seem to differ resulting the decline of cholesterol concentration. Serum glucose contents and serum glutamic-oxaloacetate-transaminase (GOT) and glutamic-pyruvate-transaminase (GPT) contents were not affected by EO additives. These results indicated that feeding lactating goats on EO additives did not effect on liver function or general animals' health.

Dry matter intake: Dry Matter Intake (DMI) was not significantly affected with the experimental additives compared with the control (Table 4). When animals are in negative energy balance (early lactation) the additional energy available, due to the essential oil from medicinal supplementation is used to improve performance, reduce body reserve losses (Tedeschi *et al*., 2003). Tager and Krause (2011) and Benchaar *et al*. (2008) reported that dry matter intake was not affected by EO supplementation in the diet. Feeding of 2 g of juniper berry EO (containing 35% α-pinene) in cows kg^{-1} feed did not influence feed intake (Yang *et al*., 2007).

Milk yield and composition: Data presented in Table 4 showed the effect of treatments on milk yield and milk composition. Milk yield was not significantly affected by EO additives, the same trend was found in the studies of Benchaar *et al.* (2006, 2007) and Tassoul and Shaver (2009), who fed dairy cattle with a commercial blend of EO (thymol, eugenol, vanillina and limonene) at dietary doses of 0.75, 2 and 1.2 g day^{-1} and reported no differences in milk production in high producing cows. Feed efficiency (Milk yield/DMI) was improved with EO additives compared with control (Table 4). Similar results were obtained by Tassoul and Shaver (2009), who suggested that efficiency of milk production increased due to addition of EO in the diet of dairy cattle. Milk protein content was significantly higher while milk Non Protein Nitrogen (NPN) was lower with EO additives than control, this result probably due to the decrease of rumen ammonia nitrogen (Table 2) and increases the blood total protein (Table 3), which reflects the improvement in protein feed metabolism with the EO treatments. While, milk fat content was lower with animals fed EO additives than control. This decrease of milk fat may be due to the decline in rumen acetate proportion (Table 2) where they represent the precursor of the most milk short chain fatty acids synthesis. On contrast, Santos *et al.* (2010) observed that feeding of EO mixture containing eugenol, geranyl acetate and coriander oil as major components increased, the total yield of milk fat or fat percentage but has no effect on production of milk and other milk components.

Milk lactose content was not affected by ANI, CLO and JUN compared with control, these results take the same trend of milk yield (Table 4) and blood serum glucose (Table 3). Data of milk composition showed that milk total solids, solids not fat and ash contents were not affected by EO supplementation. Regarding the effect of treatments on milk component yield (Table 5), the values of milk components yields were higher with EO additives than control. Very little work has been published on the effects of EO on milk composition in dairy cows. Benchaar *et al.* (2007) and Tassoul and Shaver (2009) reported no effect of EO supplementation on milk components.

Milk fatty acids profile: Likely to change the C2, C3 and C4 proportion in the rumen and influenced by treatments led to a change in the form of fatty acids in the milk fat. The overall means of milk fatty acids Table 6 show that milk fat of EO additives had higher contents of C18:0, C18:1N9C and C18.3N3 omega 3 (n-3 fatty acid) than control. Also JUN treatment had higher contents of conjugated linoleic acid (C18:2 *trans*-10, *cis*-12 and C18:2 *cis*-9, *trans*-11), C18.1N9T and C18.3N3 than other treatments. On the other ward, C18.3N6 proportion was higher with CLO treatment than other treatments. N-6/N-3 ratio was decreased with EO additives compared with control which a positive healthy effect on the consumers. These results are in agreement with those of Benchaar *et al.* (2007), who reported that cows fed alfalfa silage supplemented with EO were higher in concentrations of 18:3, an n-3 FA and in *cis*-9, *trans*-11 18:2 and conjugated linoleic

Table 5: Average daily milk component yield (g day^{-1}) of lactating goats fed rations added with essential oils

Item (g day^{-1})	Control	ANI	CLO	JUN	Pro. >F	SE
Fat yield	490.7^{b}	528.66^{a}	515.67^{a}	507.19^{a}	0.0001	0.161
Lactose yield	541.2^{b}	638.21^{a}	606.10^{a}	600.94^{a}	0.058	0.152
Protein yield	353.7^{b}	443.31^{a}	424.03^{a}	413.44^{a}	0.0001	0.145
TS yield	1454.1^{b}	1701.91^{a}	1685.12^{a}	1633.35^{a}	0.043	0.265
SNF yield	976.9^{b}	1156.69^{a}	1153.55^{a}	1108.13^{a}	0.007	0.199
Ash yield	101.3	114.20	110.10	108.60	0.618	0.004

Each value represents an average of 30 samples, Means at the same row with different superscript are significantly ($p<0.05$) different.
ANI: Anise oil, CLO: Clove oil, JUN: Juniper oil

Table 6: Milk fatty acids composition of lactating goats fed rations added with essential oils

Fatty acids (g/100 g FA)	Control	ANI	CLO	JUN	SEM
C6	1.03	0.88	1.62	1.52	0.107
C8	2.95	2.44	3.21	2.66	0.285
C10	10.75	10.04	10.70	8.55	0.626
C12	5.59	5.06	4.87	3.90	0.398
C14.0	13.29	11.37	11.40	10.18	0.595
C14.1	0.40	0.25	0.00	0.21	0.048
C15.0	1.22	1.07	0.00	1.05	0.153
C15.1	0.00	0.14	0.00	0.33	0.044
C16.0	28.65	30.21	30.09	29.10	0.836
C16.1	0.44	0.54	0.53	0.17	0.051
C17.0	0.00	0.38	0.45	0.00	0.065
C18.0	9.35^{c}	11.15^{ab}	10.61^{b}	12.48^{a}	0.501
C18.1N9T	21.08^{b}	18.30c	16.84^{d}	23.44^{a}	0.983
C18.1N9C	0.70^{b}	2.87^{a}	2.83^{a}	3.00^{a}	0.304
C18:2 *trans*-10, *cis*-12	0.00	0.00	0.00	0.11	0.014
C18:2 *cis*-9, *trans*-11	0.00	0.00	0.00	0.23	0.032
Total CLA	0.00	0.00	0.00	0.34	0.027
C18.3N3	0.10^{c}	0.22^{b}	0.25^{b}	0.35^{a}	0.029
C18.3N6	1.45^{b}	0.45^{c}	1.69^{a}	0.52^{c}	0.173
N6/N3 ratio	14.50^{a}	2.05^{c}	6.76^{b}	1.49^{c}	0.978
C20.0	0.42	0.36	0.51	0.25	0.037
C20.1	0.19	0.23	0.00	0.19	0.033
C20.4	2.39^{b}	4.04^{a}	4.40^{a}	1.76^{c}	0.327

Each value represents an average of three samples, Means with different superscripts are significantly ($p<0.05$) different, ANI: Anise oil, CLO: Clove oil, JUN: Juniper oil

acid. In general, supplementation of juniper oil changed the fatty acids profile of the milk fat so that the proportions of CLA and omega 3 fatty acids were increased, proportions of unsaturated fatty acids were increased and saturated fatty acids were decreased which a good indicator for healthy milk for consumers. (Lourenco *et al.*, 2008) found that supplementation with cinnamaldehyde in that study inhibited the apparent bio-hydrogenation of C18:2 (linoleic acid) and C18:3 (linolenic acid) as reflected by the accumulation of intermediates such as *trans*-10 C18:1, *trans*-10, *cis*-12 C18:2 and *trans*-11, *cis*-15 C18:2. Similarly, fatty acid profile of milk of cows supplemented daily with 750 mg of a mixture of EO compounds was not changed (Benchaar *et al.*, 2007). However, supplementing the same mixture at a higher concentration (i.e., 2 g day^{-1}) increased the concentrations of conjugated linoleic acid (*cis*-9, *trans*-11 18:2.) in milk fat (Benchaar *et al.*, 2006).

CONCLUSIONS

It could be concluded that supplementing ANI, CLO or JUN for lactating goat's improved rumen fermentation as propionate production and reduce acetate proportion and improved milk protein of lactating goats. Juniper oil supplementation improved conjugated linoleic acid and omega 3 fatty acids in milk fat. Under the conditions of the present study the recommended juniper oil supplementation to achieve the highest concentration of CLA and linolenic acid is 2 mL/goat/day. In addition, juniper oil supplementation to dairy animals can contribute to improve the health

properties of milk and suggesting that its consumption benefits human health. Further work is necessary to determine if the essential oils additive would be effective on the products from this milk and their effect on the human health.

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