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Effect of Supplementing Lactating Goats Rations with Garlic, Cinnamon or Ginger Oils on Milk Yield, Milk Composition and Milk Fatty Acids Profile

S.M. Kholif [*1], T.A. Morsy [1], M.M. Abdo[***1], O.H. Matloup[****1] and A.A. Abu El-Ella[2]**

[1]Dairy Science Department, National Research Center, [2]Animal Production Research Institute, Agriculture Research Center, Dokki, Cairo, Egypt 12622
E-mail: [*]<sobhykholif@yahoo.com>; [**]<tareknrc@yahoo.com>,
[***]<abdo_soft1988@hotmail.com>; [****]<osmatloup@yahoo.com>,

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ABSTRACT Plant essential oils are volatile aromatic compounds with antimicrobial activity that can alter ruminal fermentation when used as dietary supplements. Consequently, both milk quantity and quality can be modified. This work aims at verifying the effect of different plant essential oils (garlic, cinnamon and ginger oils) on the milk production when used as dietary additives. Twenty- eight lactating Damascus goats, seven days after parturition, were divided into four groups using complete randomized block design, with 90-days period to study the effect of adding Garlic oil (*Alilum sativum*) (GAR), Cinnamon oil (*Cinnamomum cassia*) (CIN) or Ginger oil (*Zingiber officinale*) (GIN) to their rations on milk yield and composition. These treatments included: (1) control ration consisted of concentrate feed mixture: bersem clover (40:60 dry matter bases); (2) control + 2 ml/head/day garlic oil; (3) control + 2 ml/head/day cinnamon oil; (4) control + 2 ml/head/day ginger oil. Ruminal volatile fatty acids and propionate proportions were increased and ruminal acetate proportion and ammonia nitrogen concentration were decreased with experimental additives. Blood serum proteins and glucose concentrations were increased and urea nitrogen and cholesterol concentrations were decreased with CIN and GAR additives. Results indicated that experimental additives, significantly increased ($p<0.05$) milk yield, protein and solids not fat contents compared with the control, however fat percent and milk non protein nitrogen were decreased ($p<0.05$) by treatments compared to the control. Total solids and ash were not affected by the experimental additives. The experimental additives were increased ($p<0.05$) unsaturated fatty acids in milk specially C18:1n9c and conjugated linoleic acids (CLA). CIN treatments increased C18:3N3 and C18:3N6 (omega 3 and omega6) compared with other treatments. In conclusion, plant essential oils especially CIN oil supplementation to ration of lactating goats had beneficial effects on milk yield and milk protein and so enhance healthy fatty acids (CLA and omega 3) contents in milk.

INTRODUCTION

Essential oils (EOs) are concentrated extracts of aromatic oily liquids from various plant materials obtained by steam distillation. Many EOs have strong antimicrobial activities against a wide range of bacteria, yeasts and moulds (Baratta et al. 1998; Giordani et al. 2004), and can be used to substitute antimicrobial growth promoters by the animal feed industry. Chemically, EOs are complex mixtures of mono- and sesquiterpenes and biogenically related phenolics or monophenols (Hummelbrunner and Isman 2001). These compounds have been shown to modulate ruminal fermentation to improve nutrient utilization in ruminants (Wang et al. 1996; Hristov et al. 1999). Furthermore, EOs are classified and generally recognized as safe food additives and have been proposed as a safe alternative to antibiotics (Calsamiglia et al. 2006). Manipulation of rumen microbial ecosystem for enhancing fibrous feed digestibility, reducing methane emission and nitrogen excretions by ruminants to improve their performance are some of the most important goals for animal nutrition (Patra et al. 2006). Plant extracts with high concentration of secondary metabolites are good candidates for achieving one or more of these objectives (Teferedegne 2000; Wanapat et al. 2008). Very few studies have been conducted on the effect of essential oils on the performance of lactating goats. Therefore, the objective of this study was to evaluate the effect of adding garlic oil (*Alilum sativum),* cinnamon oil (*Cinnamomum cassia)* or ginger oil (*Zingiber officinale)* to the rations on ruminal fermentation, milk yield, milk composition and milk fatty acids profile in lactating Damascus goats.

Address for correspondence:
Sobhy Mahmoud Kholif,
Dairy Science Department,
National Research Center,
Dokki, Cairo, Egypt
Mobile: +201143070321
Fax: +2027601877
E-mail: sobhykholif@yahoo.com

MATERIAL AND METHODS

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

Animals and Rations

Twenty- eight lactating Damascus goats, in the 3rd or 4th lactating seasons and weighting an average 46.6 ± 2 kg were used in this experiment starting by the first week of lactation and extended to 90 days. Goats were divided into four groups (seven animals each) and were assigned at random to receive one of four dietary treatments using complete randomized block design. The treatments included: (1) the control ration, consisted of concentrate feed mixture (CFM): bersem clover (40:60 dry matter bases); (2) control ration + 2 ml/head/day garlic oil (GAR); (3) control ration + 2 ml/head/day cinnamon oil (CIN) and (4) control ration + 2 ml/head/day ginger oil (GIN). The tested EOs were mixed with CFM for the morning meal. The 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 was offered for each animal individually once daily at 8.00 am, while fresh berseem clover was offered at 10.00 and 16.00 hrs. Dry matter intake was measured at 30, 60 and 90 days by weighing the offered diets and refusals from the previous day. Drinking water was available at all time.

Table 1: Ingredient and chemical composition of total mixed rations of lactating goats

Ingredient (g/kg)	*Control ration*
Berseem clover	600
Yellow corn	61
Soybean meal	88
Wheat bran	200
Sunflower meal	20
Urea	5
Calcium carbonate	3
Minerals and vitamins[a]	23
Chemical composition (g/kg DM)	
Dry matter	901.1
Organic matter	896.6
Crude protein	167
Ether extract	37.1
Crude fiber	234.2
Neutral detergent fiber (NDF)	387
Acid detergent fiber (ADF)	231
NE_L (Mj/kgDM)[b]	6.2

[a] Contained 141 g/kg of Ca, 27 g/kg of P, 65 g/kg of Mg, 14 g/kg of S, 120 g/kg of Na, 6 g/kg of K, 944 mg/kg of Fe, 1613 mg/kg of Zn, 484 mg/kg of Cu,1748mg of Mn, 58mg/kg of I, 51 mg/kg of Co, 13 mg/kg of Se, 248,000 U/kg of vitamin A, 74,000 UI/kg of vitamin D3 and 1656 IU/kg of vitamin E.
[b] Calculated using published values of feed ingredients (NRC, 2001).

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). NDF and ADF were determined according to Van Soest et al. (1991). Nitrogen-free extract and organic matter were 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 feeding of morning concentrate, at 30, 60 and 90 days. The rumen samples were filtered through two layers of cloth and used 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 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 from three animals within each group 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 r.p.m. for 20 minutes; blood serum was then separated into a clean dried glass vial and stored at -18°C till chemical analysis.

Blood serum samples were analyzed for concentrations of total protein as described by Armstrong and Carr (1964), albumin (Doumas et al. 1971), glucose (Siest et al. 1981), cholesterol (Raltiff and Hall 1973) and serum glutamic-oxaloacetate-transaminase (GOT) and glutamic-pyruvate-transaminase (GPT) (Reitman and Frankel 1957). Globulin and albumin/globulin ratio were calculated.

Sampling and Analysis of Milk

Individual milk samples were collected from all animals every two weeks during the experimental period (90 days). The goats were hand milked twice daily at 8.00 and 16.00 hrs. and the milk yield was recorded. 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 to AOAC (2007) procedures. The ash content of milk was determined after heating in a muffle furnace at 550 ^{O}C for 16 hour and the 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 HPLC system.

Statistical Analysis

All results were analyzed using the procedure of SAS (2004). Data on milk fatty acid profile were analyzed as a complete random design where treatment was the main source of variation. Data on feed intake, ruminal parameters, blood parameters, milk production and milk composition were analyzed as a randomized block design. Data were expressed as mean values when there was no interaction between week and treatment (that is, $P>0.10$). When a significant F-test was detected (that is, $P<0.05$), treatment means were separated using Duncan's multiple range test was used to test (Duncan 1955).

RESULTS AND DISCUSSION

Rumen Liquor Parameters

Effects of the experimental additives on some rumen parameters are shown in Table 2. Ruminal pH values were not significantly affected by the experimental additives. The observed decreases in pH in most oils treatments are reflect the higher concentration of VFA in the rumen fluid. These results agree with the observations of Busquet et al. (2005), who reported that low doses of different essential oils, tested on *in vitro* study. The pH value was related with normal range, where microbial digestion of fiber and protein has been found to be optimal (Firkins 1996). Ammonia nitrogen was decreased in groups fed the experimental additives compared with the control group. These results are in agreement with those of Cardozo et al. (2004) who reported that garlic oil in continuous culture reduced ammonia N and increased peptide and amino acids nitrogen concentrations. Moreover, Cardozo et al. (2005) found that ammonia nitrogen concentration was reduced in rumen fluid of cow fed rations supplemented with cinnamon oil compared with the control group. In *in vitro* studies, Busquet et al. (2006) reported that oregano EO reduced the concentration of ammonia N when supplied at the same concentration (3000 mg/l). In the same concern, Castillejos et al. (2008) observed a decrease in ruminal ammonia N concentration with some essential oils supplementation. Lower ammonia nitrogen concentration might be attributed to the action of essential oils additives as regulators in absorbing and releasing ammonia nitrogen in the rumen. These advantages may provide favorable conditions in the rumen for microorganisms activity for best utilization of ruminal ammonia and beneficial conversion into microbial protein. These results may be due to the higher uptake of ammonia to microbial protein syntheses with animals fed essential oils additives than with the control animals. Reduction in ammonia nitrogen concentration may be due to the essential oils modified the microbial population profile in a continuous culture experiment, reducing the contribution of *Prevotella* spp, which is mainly responsible for protein degradation and amino acids de-amination, suggesting a mechanism of action of essential oils on protein metabolism (Ferme et al. 2004). Supplementation of essential oils were increased TVFA's concentrations, which might indicate essential oils action in a stimulating rumen micro flora activity and enhancing digestibility. These results are in accordance with those noted by Busquet et al. (2005), Castillejos et al. (2008),

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

Items	*Control*	*CIN*	*GAR*	*GIN*	*Pro>F*	*SE*
pH value	6.03	5.83	5.90	5.92	0.195	0.126
TVFA(mMol/L)	77.0^{c}	93.7^{a}	95.1^{a}	91.5^{b}	0.001	2.353
VFA, mol/dl.C2	69.3^{a}	59.9^{b}	61.7^{b}	52.4^{c}	0.051	5.785
C3	25.4^{b}	30.3^{a}	30.9^{a}	32.2^{a}	0.031	2.337
C4	9.3^{c}	14.0^{a}	12.5^{b}	15.7^{a}	0.001	2.014
C2:C3	2.72^{a}	1.97^{b}	1.99^{b}	1.62^{b}	0.004	0.893
NH_3-N(mg/L)	281^{a}	267^{b}	278^{b}	271^{b}	0.051	0.274

Each value represents an average of twenty one samples.
$^{a, b, c}$ means at the same row with different superscript are significantly (P<0.05) different.
CIN= cinnamon oil - GAR=garlic oil - GIN=ginger oil

Kongmun et al. (2011). Volatile fatty acids are the end products of rumen microbial fermentation and represent the main supply of metabolizable energy for ruminants (Van Soest 1982). Therefore, ascension in their production would be nutritionally favorable for the animal. Molar proportions of individual TVFA's and the acetate to propionate ratio were significantly (P<0.05) affected by essential oils additives which increased the propionate and butyrate proportions and decreased the acetate proportion. Consequently, there was a decrease in the acetate to propionate ratio compared with the control.

The results of this study were consistent with Busquet et al. (2006), who reported that cinnamaldehyde (main component of cinnamon) tended to decrease the acetate proportion, and increased propionate proportion. Moreover, Cardozo et al. (2004) and Busquet et al. (2005) reported that garlic oil resulted in lower acetate proportion; whereas, propionate proportion increased due to the decreased of acetate to propionate ratio as compared with the control. In general terms, the reduction in the acetate-to-propionate ratio and the increase in butyrate concentration observed as a result of supplementation with some essential oils or their active components suggested that the main mechanism of action is the inhibition of methanogenesis (Calsamiglia et al. 2007).

Blood Serum Metabolites

Serum albumin values (Table 3) were increased while serum urea nitrogen was decreased with the EOs supplementation. Also, serum globulin values were increased with CIN and GAR and decreased with GIN supplementation compared to control. These results may be due to the improvements of ruminal microbial protein synthesis. Also serum glucose was improved with CIN and GAR supplementation and was parallel with ruminal propionate (Table 2) which the precursor of glucose synthesis. On the other hand, serum cholesterol was decreased in animals fed Eos compared with those fed control diet. These results indicated the healthy effect of EOs supplementation to goat's diets to decrease cholesterol concentrations. Oils supplementation is known to increase blood cholesterol (Garcia-Bojalil et al. 1998), although the types of fatty acids in CIN, GAR and GIN would seem to differ resulting the decline of cholesterol concentration. Blood serum glutamic-oxaloacetate-transaminase (GOT) and glutamic-pyruvate-transaminase (GPT) values were not affected by treatments. The results of cholesterol and GOT and GPT enzymes indicated the healthy effect of CIN, GAR and GIN supplementation to goat's diets as a decrease of cholesterol.

Dry Matter Intake, Live Body Weight

Dry matter intake (DMI) was increased with the experimental additives compared with the control (Table 4). The increase of feed intake may be due to the improvement of nutrients palatability. 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 and reduce body reserve losses (Tedeschi et al. 2003). However, Tager and Krause (2011) reported that dry matter intake was not affected by EOs supplementation in the diet. Yet, the live body weight of lactating goats was not significantly affected by experimental additives.

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

Items	*Control*	*CIN*	*GAR*	*GIN*	*Pro>F*	*SE*
Total protein (g/dl)	6.28	6.65	6.62	6.24	0.0001	0.056
Albumin (g/dl)	2.93[b]	3.21[a]	3.21[a]	3.20[a]	0.002	0.035
Globulin (g/dl)	3.35[b]	3.44[a]	3.41[a]	3.04[c]	0.0001	0.055
A/G ratio	0.87	0.94	0.95	1.07	0.153	0.026
Urea (mg/dl)	37.11[a]	29.11[bc]	28.06[c]	30.45[b]	0.0001	0.564
Glucose (mg/dl)	65.80[c]	69.67[a]	68.87[ab]	66.67b[c]	0.006	0.441
Cholesterol (mg/dl)	231.53[a]	201.93[b]	200.07[b]	206.0[b]	0.0003	2.957
GOT (Units/ml)	33.33	33.93	33.60	33.80	0.954	0.404
GPT (Units/ml)	16.21	15.83	16.13	15.75	0.212	0.093

Each value represents an average of twenty one samples.
[a, b, c] means at the same row with different superscript are significantly (P<0.05) different.

Milk Yield and Composition

Milk yield were significantly higher (P<0.05) in goats fed experimental additives compared to the control (Table 4). In other words, CIN, GIN and GAR treatments increased milk yield by 23.7, 18.9 and 16.5%, respectively compared to the control. It is interesting to note that serum glucose (Table 3) had the same trend as milk yield which was also in agreement with the results of Clark et al. (1977) who claimed a positive correlation between blood glucose and milk yield. The increase in milk yield reported herein may be due to the higher ruminal TVFA and propionate proportion and the reduction of methane production (Patra and Saxena 2010). On contrast, Benchaar et al. (2006; 2007) and Tassoul and Shaver (2009) tested a commercial blend of EOs (that is, thymol, eugenol, vanillina and limonene) at dietary doses of 0.75, 2 and 1.2 g/d and reported no differences in milk yield in high producing cows.

The data of milk composition of the experimental goats are also summarized in Table 4. These date revealed that milk fat was decreased for animals fed essential oils additives compared with the control animals. The reduction in milk fat can be also attributed to the negative relationship with a higher milk yield. The lower milk fat may be due to the lower ruminal acetate proportion and acetate to propionate ratio in goats received essential oils compared to the control (Table 2). While, milk protein was increased with essential oils additives as a result of improvement of ruminal microbial protein synthesis. Similar results were obtained by Spanghero et al. (2008) who reported that EOs supplementation to rations had increased the milk protein content. Although milk lactose was not affected by EOs supplementation, Santos et al. (2010) reported that milk lactose percent was increased by EOs supplementation.

Milk total solids and ash percentages were not significantly affected by the treatments. However, all EOs supplementation showed an increase in milk solids not fat and decreased milk non-protein nitrogen percentages compared with the control group. The decrease in

Table 4: Average dry matter intake, live body weight and daily milk yield and composition of lactating goats fed rations supplemented with essential oils

Items	*Control*	*CIN*	*GAR*	*GIN*	*Pro>F*	*SE*
Number of animals	7	7	7	7		
Live body weight (kg)	45.75	47.40	46.20	47.40	0.868	0.808
Dry matter intake (kg/h/d)	1.37[b]	1.40[a]	1.38[ab]	1.40[a]	0.009	0.004
Milk yield/DMI	0.819	0.992	0.947	0.953	0.458	0.578
Milk yield(gm/d)	1122.92[b]	1389.45[a]	1307.96[a]	1335.09[a]	0.0006	23.227
Fat %	4.37[a]	4.17[b]	4.15[b]	4.11[b]	0.0002	0.023
Lactose %	4.82	5.12	5.03	5.02	0.156	0.044
Protein %	3.15[b]	3.52[a]	3.57[a]	3.48[a]	0.0001	0.034
Total solids %	12.95	13.67	13.83	13.48	0.169	0.138
Solids not fat %	8.7[b]	9.5[a]	9.43[a]	9.28[a]	0.074	0.099
Ash %	0.903	0.902	0.897	0.898	0.742	0.003
NPN %	23.3[a]	19.53[b]	19.18[b]	19.48[b]	0.0001	0.267

Each value represents an average of forty two samples.
[a, b] means at the same row with different superscript are significantly (P<0.05) different.

Table 5: Milk fatty acids profile of lactating goats fed rations supplemented with essential oils

Fatty acids (g/100g FA)	*Control*	*CIN*	*GAR*	*GIN*	*SEM*
C6	1.03	0.29	1.61	0.21	0.179
C8	2.95^{a}	2.39^{a}	2.94^{a}	0.0^{b}	0.435
C10	10.75^{b}	12.0^{a}	10.26^{b}	5.42^{c}	0.943
C12	5.59^{b}	6.14^{a}	4.79^{c}	3.62^{d}	0.410
C14.0	13.2^{9a}	12.02^{b}	10.7^{d}	11.14^{c}	0.576
C14.1	0.4^{a}	0.0^{b}	0.0^{b}	0.55^{a}	0.075
C15.0	1.22^{b}	0^{d}	1.02^{c}	2.91^{a}	0.318
C15.1	0.0^{c}	0.0^{c}	0.34^{b}	7.17^{a}	0.922
C16.0	28.65	29.51	27.23	30.05	0.874
C16.1	0.44	0.65	0.54	0.56	0.033
C17.0	0.0^{b}	0.0^{b}	0.24^{a}	0.0^{b}	0.034
C18.0	9.35^{c}	10.45^{b}	12.02^{a}	12.55^{a}	0.531
C18.1N9T	21.08^{b}	16.79^{d}	23.01^{a}	20.75^{c}	0.918
C18.1N9C	0.70^{d}	3.14^{b}	4.19^{a}	3.01^{c}	0.397
C18:2 *trans*-10, *cis*-12	0.0^{b}	0.08^{a}	0.09^{a}	0.0^{b}	0.013
C18:2 *cis*-9, *trans*-11	0.0^{b}	0.29^{a}	0.26^{a}	0.29^{a}	0.043
Total CLA	0.0^{b}	0.37^{a}	0.35^{a}	0.29^{a}	0.035
C18.3N3	0.1^{0c}	0.5^{0a}	0.09^{d}	0.25^{b}	0.051
C18.3N6	1.45^{ab}	1.62^{a}	0.46^{b}	0.34^{b}	0.179
N6/N3 ratio	14.5^{a}	3.98^{c}	5.11^{b}	1.36^{d}	0.935
C20.0	0.42^{b}	0.39^{b}	0.21^{c}	1.0^{a}	0.069
C20.1	0.19^{b}	0.66^{a}	0.0^{c}	0.18^{b}	0.081
C20.4	2.39^{b}	3.08^{a}	0.0^{c}	0.0^{c}	0.426

Each value represents an average of three samples.
$^{a, b, c}$ means with different superscripts are significant ($P<0.05$) difference.
CIN= cinnamon oil - GAR=garlic oil - GIN=ginger oil

milk non protein nitrogen reflects the increase in milk protein and blood albumin may be due to the improvements of ruminal microbial protein synthesis. On the contrary, Benchaar et al. (2006, 2007) and Tassoul and Shaver (2009) reported no differences in milk composition in high producing cows when fed EO supplementation.

Milk Fatty Acid Profile

The overall means of milk fatty acids (Table 5) show that CIN treatment induced a higher content of C10, C12, C20: 1 and C20:4 than other treatment. However, GIN treatment induced a higher content of C15, C15:1 and C20 than other treatment. On other wards, conjugated linoleic Acid (CLA) proportion was increased with EOs treatments and CIN treatment had the highest value. It is well documented that conjugated linoleic acid (CLA) is important for human health and it is being sold as a panacea that has the capability of reducing or eliminating cancer, preventing heart disease, improving immune function and altering body composition to treat obesity or build lean body mass (Whigham et al. 2000). In the current study, CIN supplementation significantly increased the proportion of n-3 (C18:3 N-3) and n-6 FAs (C18:3 N-6) but significantly decreased n-6: n-3 ratio in milk fat. In general, supplementation of CIN 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.

CONCLUSION

Supplementation of CIN, GAR and GIN oils could be used to improve rumen fermentation as propionate production and reduce methane gas emission and enhancing milk production and milk protein of lactating goats. CIN oil supplementation improved conjugated linoleic acid and omega3 fatty acids in milk fat. Under the conditions of the present study the recommended cinnamon oil supplementation to achieve the highest concentration of CLA and omega 3 fatty acids is 2 ml/goat/day. In addition, cinnamon 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 for high producing cows and the products from this milk and their effect on the human health.

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