Effects of dietary essential oil and live yeast supplementation on dairy performance, milk quality and fatty acid composition of dairy cows

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SUMMARY

This study aimed to determine the effects of live yeast (LY) and essential oil (EO) on dairy cattle diets on performance and milk composition traits. A total of 120 multiparous (in 2nd and 3rd lactations) Holstein dairy cows were used and 30 animals were allocated to each treatment group. Treatment groups were as follows: 1) control, (C, without any supplementation), 2) essential oil mixture addition (EO, 10 g/day/cattle) 3) live yeast, (LY, Saccharomyces cerevisiae, 10 g/day/cattle, 4×10^9 CFU/g) 4) EO+LY (10 g/day/cattle +10 g/day/cattle). Experiments were performed for 16 weeks. Body weight, milk yield, and feed conversion ratio were not influenced by the treatments. Milk fat increased with EO supplementation to the diet. Milk protein decreased in the LY+EO group. Somatic cell counts (SCC) decreased significantly with EO supplementation. Milk lactose, casein, and density were not significantly influenced by the treatments. Milk urea concentration increased in the LY group. Milk-free fatty acids significantly increased in the EO group. Milk citric acid increased in the LY group. The control (C) group had greater pentadecanoic acid (C15: 0) content than the other groups. The myristic acid ratio (C14: 0) of the C and EO groups was greater than the myristic acid ratio of the LY and LY+EO groups. Based on present findings, EO mixture supplementation to dairy cattle diets had positive effects on milk fat content and SCC.

KEY WORDS

Milk fatty acids, somatic cell count, milk urea, natural feed additives.

INTRODUCTION

Optimal ruminal fermentation and microbial digestion is a key part of the feeds to milk process in dairy animals. In this sense, feed additives are frequently used in animal feeding to ensure microbial growth, to control pathogenic microorganisms and to prevent negative effects of microorganisms on rumen fermentation [1,2]. Use of some feed additives like antibiotics, hormones and hormone-like substances in ruminant feeding is restricted by laws since such substances have some negative effects on human health through cross-immunity against the pathogenic microorganisms. Therefore, various other additives with positive effects on animal metabolism and performance are widely used. These additives play a great role also in regulation of digestive system and protection of physiological balance and the other characteristics. These substances may also improve feed consumption, feed conversion ratio, meat and milk yields [3]. Yeasts and plant essential oils are considered among such substances to be used in ruminant diets. Probiotics supplemented into ruminant diets may improve rumen conditions, increase milk yield and quality. They do not allow harmful microorganisms to survive through producing substances in gastrointestinal system [4]. Many plant extracts have been reported to have antibacterial, antiparasitic and antiviral properties[5]. Essential oils are known to have antimicrobial effects against a broad spectrum of microorganisms, including bacteria, protozoa and fungi [6,7]. Essential oils may also increase digestion and absorption of nutrients through promotion of beneficial microbial population [6], anti-methanogenic affects[8], thus improve feed conversion ratios and increase milk yield and quality [9,10].

In a study, the leaf powder supplementation to diet were significantly reduced somatic cell count in the milk and were showed immunomodulatory effects with subclinical mastitis in dairy cows [11]. Another study the effect of *Origanum vulgare* was given at 0.9 mL by intramammary infusion: S. aureus and E. coli were not detected in milk[12]. Also, the sage essential oil as intramammary infusion to ewes resulted in a significant decrease in somatic cell count. The infusion of aqueous extracts by intramammary (Fumariaindica, Nepatacataria and Adiantumcapillus, 750 mg/tube for 5 days) significantly improved sub-clinical mastitis ratio with all extracts in cows[14]. Those results summarize the essential oils in some plants which could be used as antimicrobials or as adjuvants, especially the

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control of mastitis. In a review study, essential oils related to animal nutrition, hygiene and protection; it is part of a sustainable, natural option to improve animal health and food derived from animal products and to reduce the use of antimicrobials in livestock has been reported [15].

Essential oils and live yeast, as mentioned, with their strong antioxidant and antimicrobial properties, to support and improve animal performance and health; it is also a potential feed additive that can be used as a natural antioxidant in animal nutrition to improve the quality of products. In this context, the aim of this study was to evaluate the effects of in vivo dietary essential oils and live yeast (Saccharomycess cerevisia) supplementation on milk yield, composition and fatty acid profile of lactating dairy cows. In addition, it was aimed to evaluate whether essential oils and live yeast can be used as a natural feed additive that can improve product quality in milk.

MATERIAL AND METHODS

Animal and feed material

This study was carried out in Saray Agriculture and Livestock Inc. ranches, operating in Kayseri (38° 22 24.8376» and 35° 27 49.8384») province of Turkey.

A total of 120 lactating and clinically healthy (parasitic drugs and other requirements for health were applied before the experiments) cows were used to determine the effects of essential oil mixture and live yeast supplementation to diet on their dairy performance and milk quality. All animals used in the study were cared for according to Erciyes University Ethics committee reports. The cows were selected among 1900 Holstein cows and allocated at age, lactation number, milk yield and live weight into 4 treatment groups of 30 cows each group in a paddock. The shelter barns were 120 m long and 28 m wide. They were free-stall barns with group-feeding and had free access to fresh water with automatic waterers. The barns have sufficient ventilation and suitable for animal breeding in all seasons and animal welfare. The treatment groups were as follows: 1: Control (no additives), 2: Essential oil mixture (EO, Bionat SB, Origanum (oregano), Cuminum (cumin), Cinnamomum (cinnamon), Allium (garlic) extract and Lignosulfonic acid as organic acid, similar doses to those reported by [16-18], 3: Live yeast (LY, contains 4x109 cfu/g yeast (*Saccharomyces cerevisia* NCYC R618) per gram, similar doses to those reported by [10] (Global Nutritech Co. Ltd., VA, USA, Branch of Turkey, Kocaeli-Turkey) and 4: EO + LY.

Experimental design

Following two weeks adaptation period, the experimental period was performed in 16 weeks. The experiment was performed in summer-autumn season, the average temperature was 20.1 ºC and the average humidity was 48.4% in the barn. During the experimental period, the health status and behavior of all cows was monitored on daily basis (data not shown). Alfalfa hay, corn silage and vetch-wheat grass were used as roughage. The feed additives used in the research were homogeneously mixed with milled grains and oilseed meal in 500 kg bunkers and transferred to the feed distribution wagons.

The cows were fed at 4 times at day (08:00, 13:00, 17:00 and 21:00) and feed intake measured daily for each group. The amount of feed consumed to cows was monitoring for 7 days, and feed intake was calculated by collecting the remaining feeds in the feeders every 7-day periods before feeding in the morning. The diets were formulated to meet nutrient requirements of dairy cows for lactation NRC [19] based on animal live weights and milk yields. The total mixture ration (TMR) composition is given in Table 1.

Determination of body weight

Body weight (BW) of cattle was determined each month. However, the data are shown in only initial and final body weights in tables, because of the cows were not any significant differences in body weight of the treatment groups.

Determination of chemical composition of feed material

Dry matter (DM), crude protein (CP), crude ash (CA), crude oil (CO) and crude cellulose (CS) analyses of silages were performed according to the methods specified in [20]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were performed according to [21] method.

Determination of chemical composition of milk samples

The cows were machine milked twice daily with a commercial herd tracking system (Afimilk, version 4.5, Israel) with milk-

Table 1 - Ingredients and chemical composition of diets.

Ingredients	Kg/d per cow
Corn	4.0
Barley	3.24
Soybean seed meal	2.75
Cotton seed meal	2.0
Sunflower seed meal	1.0
Wheat bran	1.0
Maize silage	21.0
Alfalfa hay	4.0
Vetch-wheat hay	0.5
Malt pulp	3.5
Salt	0.05
Marble powder	0.12
Vitamin-mineral premix[1]	0.10
By-pass oil	0.25
Chemical composition (g/kg DM)	
Dry matter	524.4
Crude protein	187.5
Ether extract	46.4
Organic matter	921.6
Acid detergent fiber	212.1
Neutral detergent fiber	394.2
Crude fiber	164.3
Non-fiber carbohydrates	293.5
Total digestibility	660.5
Metabolizable Energy[2] Mcal/Kg	2.59
Net Energy Lactation[2] Mcal/Kg	1.64

[1]Each kg of premix provides; Vitamin A 15.000.000 IU, Vitamin D3 3.000.000 IU, Vitamin E 30.000 mg, Manganese 50.000 mg, Iron 50.000 mg, Zinc 50.000 mg, Copper 10.000 mg, Iodine 800 mg, Cobalt 150 mg and Selenium 150 mg.
[2] Calculated according to NRC ([19]).

ing machine. Milk yield, dry matter, fat, protein, and somatic cell counts was recorded at 14 days intervals from consecutive milkings. Milk samples were obtained from each cow from the animals every 14 days during 3 consecutive milking (all milking in a day), and pooled and kept refrigerated until chemical (4 °C) and fatty acid (FA) analysis (-20 °C). The milk density, acidity, lactose, casein, urea, free fatty acid, citric acid, and freezing point of the milk samples were analyzed in milk analyzer (Milko Scan FT 120, Foss, Padova, Italy).

Determination of fatty acid composition in milk

The milk fatty acid composition were determined by gas chromatography (Shimadzu GC 2010 Plus) according to Fritsche and Steinhart[22]. The milk samples of each cattle were taken into numbered tubes and milk fat was separated and the fatty acid profile of those fat samples was analyzed).

Statistical analyses

The data analyzed with SPSS statistical software [23]. One-Way ANOVA procedure was used to determine whether the differences between the groups were significant. Duncan multiple comparison test was used to determine the differences between significant means. Significance level was considered as $P<0.05$.

RESULTS

Performance

Effects of EO, LY and EO+LY supplementations into dairy cattle diets on body weight (BW), feed intake, milk yield and FCR are provided in Table 2. Present treatments did not have significant effects on body weights ($P>0.05$). Dairy cattle were housed in a single compartment as a group during the experiment; therefore, the total feed intake was obtained as a group average (based on dry matter) for each dietary treatment. Therefore, the statistical analysis could not be done since there was no replicate for feed intake. EO, LY and EO+LY supplementation did not have significant effects on milk yield and FCR ($P>0.05$).

Milk composition

Effects of EO, LY and EO+LY supplementations into dairy cattle diets on milk composition are given in Table 3. It was observed that present additives did not have any significant effects on milk dry matter ($P>0.05$). Milk fat varied between 3.62 - 3.77 and milk fat increased significantly with EO supplementations. The greatest milk protein was observed in EO group, and this value was significantly greater than those of C and EO + LY groups. Present treatments did not have significant effects on milk density, freezing point, lactose, and casein. However, EO and LY treatments increased milk urea and the greatest value was observed in LY-supplemented group ($P<0.01$). The lowest acidity was observed in the control group, while the greatest value was observed in EO-supplemented group ($P<0.05$). The greatest free fatty acids were observed in LY group and the lowest value was observed in the control group ($P<0.05$). The greatest citric acid was observed in LY-supplemented group ($P<0.05$). EO supplementations into dairy cattle diets significantly reduced somatic cell counts.

Milk fatty acid composition

Effects of EO, LY and EO+LY supplementation into cattle diets on milk fatty acid composition are provided in Table 4. Accordingly, milk fat C6:0, C16:0, C16:1, C18:0, C18:1 n9*t*, C18:2 n6*c*, C20:2, C22:0, C22:1 n9, C24:0, C22:6 n3 fatty acids were not influenced by the treatments ($P>0.05$). However, C14:0 fatty acid was found to be the greatest in control and EO-supplemented groups and the lowest in LY and EO+LY- supplemented groups ($P<0.05$). The control group also had the greatest C15:0 fatty acid content ($P<0.05$).

DISCUSSION

Performance

Dietary EO, LY and EO+LY supplementations did not have significant effects on BW and feed conversion ratio. Such findings were supported by previous studies [16,24-31] indicating insignificant effects of live yeast additions to dairy cow diets on BW of cattle. However, in another studies, essential oil [32] and yeast additives increased body weight gain [33]. On the other hand, some other researchers reported that such additives did not influence BWG of dairy cattle [34,35]. Dairy cattle start to increase body condition after a negative energy balance (especially after the 2nd month of lactation) and weight gain may occur from the middle of lactation. In this study, there was not significant live weight losses or gains. At the same time, there were not any contributions of additives to that constant structure of cattle body.

Table 2 - Effects of essential oil, live yeast, and their combinations on dairy cow performance.

	Treatments					
	C	EO	LY	EO+LY	SEM	P
Initial body weight, kg	617.97	620.07	620.67	619.03	7.98	0.636
Final body weight, kg	652.60	662.90	671.57	666.60	8.28	0.435
BW change, kg	34.63	42.83	50.9	47.57	1.28	0.454
Feed intake, DM, kg/day	22.73	22.80	22.80	23.07	-	-
Milk yield, L/day	24.65	25.26	25.16	25.80	0.74	0.752
Feed conversion ratio[1]	1.73	1.72	1.73	1.70	0.06	0.978

C: control; EO: essential oil addition 10 g/d per cow; LY: live yeast addition 40x10^9 cfu g/d per cow; EO+LY: essential oil+ live yeast; SEM: Standard error of means; P: probability;
[1] The data calculated as: average daily dry matter intake / average daily milk yield.

Table 3 - Effects of essential oil, live yeast, and their combinations on milk quality parameters.

	Treatments					
	C	EO	LY	EO+LY	SEM	P
Total solid, g/100 g	12.11	12.16	12.18	12.10	0.06	0.659
Fat, g/100 g	3.70[b]	3.77[a]	3.62[b]	3.67[b]	0.03	0.002
Protein, g/100 g	3.22[b]	3.28[a]	3.24[ab]	3.20[b]	0.02	0.013
Lactose, g/100 g	4.76	4.79	4.74	4.72	0.05	0.780
Casein, g/100 g	2.93	2.95	3.00	3.05	0.04	0.277
Urea, g/100 g	0.049[c]	0.054[b]	0.057[a]	0.054[b]	0.002	0.007
Acidity, SH	8.25[b]	9.67[a]	9.02[ab]	8.75[b]	0.19	0.001
Free fatty acid, Mol/L	7.86[c]	12.08[ab]	13.71[a]	11.27[b]	0.87	0.001
Freezing point (-°C)	0.57	0.59	0.60	0.59	0.01	0.094
Citric Acid, g/kg	0.13[c]	0.15[ab]	0.16[a]	0.14[bc]	0.00	0.003
Density	1032.5	1032.4	1032.6	1032.6	0.36	0.946
Somatic cell count	381627[a]	335831[b]	366631[a]	372419[a]	8319	0.001

C: control; EO: essential oil addition 10 g/d per cow; LY: live yeast addition 40x10^9 cfu g/d per cow; EO+LY: essential oil+ live yeast; SEM: Standard error of means; P: probability; [a,b,c]: Values with different superscript in a same line differ significantly between treatment groups.

The essential oil, live yeast and essential oil + live yeast supplementation to dairy cattle diets did not have significant effects on milk yields. [16-18,36] reported that addition of essential oil to dairy cattle rations did not affect milk yield. Contrarily, [16] reported that essential oil mixture containing thymol, eugenol vanillin, guaiacol and limonene increased milk yield as compared to the control group. Similarly, [37] reported that the addition of eucalyptus oil, menthol and peppermint oil to drinking water of lactating dairy cattle at a level of 16 mg/l improved milk yield however higher levels (32 and 48 mg/l) decreased milk yield. In addition, the addition of essential oil mix obtained from oregano, cinnamon and orange peels did not affect the milk yield of the cows [18]. In a study, the addition of cinnamaldehyde and eugenol mixture with vitamins and minerals did not affect milk yield of dairy cattle[36]. Similarly, some studies reported that addition of live yeast [38-40] did not affect milk yield. Another study indicated that 10 g/day yeast addition to the diet increased milk yield[10]. Although different results have been reported by the researchers, the effects of feed additives were more pronounced in animals that were housed and fed under unfavorable conditions [4,41].

Milk composition

The essential oil, live yeast, and essential oil + live yeast supplementation to dairy cattle diets did not have any significant effects on milk dry matter. Milk protein, fat and mineral may affect the DM of milk. In this study, although milk fat and protein were affected, milk DM rate was not affected by the treatments. It was reported in previous study that essential oil addition to dairy cattle diets did not affect milk dry

Table 4 - Effects of essential oil, live yeast, and essential oil + live yeast addition to dairy cattle diets on milk fatty acid composition.

	Treatments					
	C	EO	LY	EO+LY	SEM	P
C6:0	3.77	7.50	4.52	4.86	1.22	0.303
C14:0	4.41[a]	3.84[a]	2.67[b]	2.84[b]	0.49	0.011
C15:0	1.39[a]	0.25[b]	0.46[b]	0.15[b]	0.27	0.028
C16:0	14.36	10.47	11.02	12.07	1.27	0.274
C16:1	6.44	6.94	5.84	5.57	0.43	0.241
C18:0	5.65	4.63	5.81	5.24	0.59	0.678
C18:1	7.31	7.47	10.52	8.99	0.76	0.092
C18:2	28.02	27.51	27.59	26.87	1.51	0.962
C20:2	9.47	11.19	10.94	10.87	0.70	0.375
C22:0	1.77	1.76	1.55	1.44	1.33	0.086
C22:1	2.21	3.52	2.71	2.52	0.59	0.976
C24:0	0.25	0.07	0.00	0.00	0.06	0.105
C22:6	0.08	0.59	0.00	0.00	0.16	0.303

C: control; EO: essential oil addition 10 g/d per cow; LY: live yeast addition 40x10^9 cfu g/d per cow; EO+LY: essential oil+ live yeast; SEM: Standard error of means; P: probability; [a,b,c]: Values with different superscript in a same line differ significantly between treatment groups.

matter [17]. Similarly, live yeast addition to the dairy cattle diet [31,40] did not affect dry matter of milk. However, in another study reported that live yeast addition to the diet increased the dry matter content of milk[42].

EO supplementations increased milk fat. Similarly, increasing milk fat and protein with essential oil mix including eugenol, geranyl acetate and coriander oil[43]. In another study determined that mixture of eucalyptus, menthol and peppermint oil with drinking water reduced milk fat yield[37]. However, some other studies reported that such supplementations did not have any significant effects on milk fat yield [16-18,36,44]. Similarly, addition of live yeast did not have any significant effects on milk fat [38-40,45,46]. However, there are some other studies reporting increased milk fat contents with live yeast addition to dairy cattle rations [38,42,47,48]. In another study reported that addition of 10 g/day live yeast per dairy cattle reduced milk fat[10].

Addition of herbal essential oil to dairy cattle diets increased milk protein. Milk protein can be influenced by a few factors and is generally more stable. In contrast, a study indicated that eugenol, geranyl acetate and coriander essential oil mixture increased milk protein content[49]. Contrarily, addition of live yeast to dairy cattle diets did not have any significant effects on milk protein [38,40,45,46]. Previously reported that addition of live yeast to dairy cattle diets increased milk protein[10,42].

The supplementation of EO to dairy cow diets caused a significant decrease in milk somatic cell count (SCC). Somatic cell count in milk is an indicator of healthy udder and animal structure. It is well known that essential oils have a strong antimicrobial and antioxidant activity. The reduction in SCC obtained in this study can be attributed to the antimicrobial effect of the EO mixture. In another study reported that the addition of an EO mixture reduced the number of somatic cells in milk[50]. Similarly, it was found that yeast culture addition to dairy cow diets did not have significant effects on somatic cell counts [10,31,51,52].

The addition of EO, LY and EO+LY to the diet did not have significant effects lactose, casein, and density of milk. There are not enough studies about the effects of live yeast and EO on chemical composition of milk. It was indicated in a previous study that addition of yeast culture to dairy cattle diet did not affect lactose of milk [35,46,51]. However, a study stated that the addition of 10 g/animal of live yeast increased the lactose rate in milk, while the lactose rate in milk did not change when 14 g per day was used[10]. In another study reported that LY addition did not cause any changes in milk lactose[52]. Also reported that EO supplementation did not affect lactose of milk[17].

The addition of EO, LY and EO+LY to the diet influenced urea, acidity, free fatty acid, citric acid and freezing point of milk. It was determined that the amount of free fatty acid and citric acid in milk increased with yeast addition. Also, it was observed that the EO addition increased the acidity and freezing point of milk. However, there is not enough published experimental results on potential effects of such additives on milk quality traits. The greatest milk urea was observed in LY group and urea content of EO+LY mixtures was greater the control group. It was found that the addition of EO reduced the amount of urea in milk [50]. It was reported in another study that the amount of urea in milk did not change by LY supplementation [51].

Milk fatty acid composition

There was no significant effect of EO and LY and combinations on the fatty acid composition of milk. It was observed that only myristic acid (C14:0) ratio decreased in LY and EO+LY groups, C15:0 fatty acid also decreased in treatment groups. It was reported in a previous study that the essential oil additive did not have significant effects on fatty acid composition of milk [53]. It was reported in another study that flax oil increased n-3 fatty acid in milk, but the addition of yeast did not affect the fatty acid composition of milk [46]. Also showed that live yeast additive had no effect on fatty acid composition of milk[39].

CONCLUSION

It was concluded based on present findings that essential oil mixture increased milk fat, protein, acidity, citric acid, and freezing point and decreased somatic cell counts. Despite some differences in milk urea and free fatty acids, live yeast and EO+LY groups generally had similar with the control group. Based on present findings, EO mixture can be recommended for dairy cattle diets just because of positive impacts on some milk quality parameters, but live yeast was not recommended for ruminant diets due to the addition of an extra feed cost. In addition, the income and outgoings obtained by adding EO mixture to the diet should be compared and it should be decided to use it as a feed additive.

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