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Effect of whole cottonseed, monensin and vitamin E on milk compositions and CLA content of milk fat of lactating dairy cows

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Abstract

The present study was aimed at determining the effects of adding vitamin E and monensin on milk production, milk composition, and the milk fatty acid profile of lactating dairy cows when whole cottonseed, as the main source of FA, was included in the diet. In a balanced 4×4 repeated Latin square design, eight lactating Holstein cows were randomly assigned to one of the four dietary treatments for 28-d treatment period: 1) control diet (without whole cottonseed, monensin and vitamin E; C), 2) diet with 20 percent whole cottonseed of DM (WCS), 3) diet with 20 percent whole cottonseed of DM plus 12000 IU of vitamin E/cow per d (WCS+E), and 4) diet with 20 percent whole cottonseed of DM plus 24 ppm of monensin/kg DMI per cow per d (WCS+M). Protein and lactose percentages were not significantly affected by experimental treatments, but 4% FCM, milk fat percentage and milk fat yield (kg/day) were significantly different among treatments ($p<0.05$). Feeding vitamin E supplement increased SCFA and decreased LCFA ($p<0.05$). The concentration of cis-9, trans-11 CLA was affected by monensin and vitamin E supplementations ($p<0.05$) and treatments WCS and WCS+M had the highest amount within the experimental treatments. The supplementation of diet by monensin had significant effect on trans-10, sic-12 CLA concentration and increased it ($p<0.05$). The results of this study showed that supplementation of vitamin E or monensin did not have any effect on milk fat concentration when whole cottonseed is included in the diet whereas affected milk fat compositions.

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Introduction

Consumers are increasingly aware of the link between diet and health, and recently FA have gained special attention for their potential health benefits. Fat is the major energy containing component in milk and accounts for many of the physical properties, manufacturing characteristics, and organoleptic qualities of milk and milk products (Bauman and Griinari, 2003). Conjugated linoleic acid (CLA) is one such bioactive FA that may function to improve health maintenance and prevent chronic diseases. When consumed as a natural component of the diet, cis-9,trans-11 18:2 CLA (rumenic acid, RA) has been consistently shown to offer anticarcinogenic and antiatherogenic effects in biomedical studies using animal models of human disease (Parodi, 2004; Bauman *et al.,* 2006). Dietary modification shows the most promise as a management strategy to change milk composition in the short term. The fatty acid content of the lactating cow diet affects the type and the proportion of the fatty acids in the milk fat (Grummer, 1991). Conjugated linoleic acid is an intermediate product of biohydrogenation of linoleic acid by the rumen bacterium, *Butyrivibrio fibrosolvens* (Harfoot and Hazelwood, 1988). Whole cottonseed is a unique feedstuff because of its high content of energy, mainly in the form of oil, moderately high level of CP, and high quality fiber (Harvatine *et al*., 2002). It has been shown that feeding unsaturated oils to ruminants impaired ruminal BH of unsaturated FA (Bauman and Griinari, 2001, 2003) and increased ruminal outflow of BH intermediates such as trans-10, cis-12 CLA that were considered to be potent inhibitors of milk fat synthesis (Baumgard *et al.,* 2000). Whole cottonseed (WCS) has become an important ingredient in diets for high-producing dairy cows. Cottonseed oil is also a good source of unsaturated fatty acids and contains approximately 500 g/kg linoleic acid (NRC, 2001). Biohydrogenation of unsaturated fatty acids in the rumen is affected by the type and amount of fatty acid substrate, forage to grain ratio, and nitrogen content of the diet (Harfoot and Hazelwood, 1988).

Monensin is a carboxylic polyether ionophore antibiotic produced by the fermentation of Streptomyces cinnamonensis (Russell, 2002). It is extensively used in the diet of dairy cows, and its effects on milk production and composition are well documented (Da Silva *et al.,* 2007; Odongo *et al.,* 2007; Alzahal *et al.,* 2008; Fatahnia *et al.,* 2010). The benefits of feeding monensin to dairy cows include the increased milk production and improved energy balance associated with reduced incidence of subclinical ketosis, clinical acidosis and displaced abomasums (Duffied and Bagg, 2000). Monensin is also known to decrease rumen biohydrogenation (BH) of polyunsaturated fatty acids (PUFAS; Van Nevel and Demeyer, 1995) in vitro, and to increase total conjugated linoleic acid in vitro (CLA; Fellner *et al.,* 1997). Thus, dietary supplementation of monensin might increase concentration of PUFAs and CLA in milk. Increasing specific USFA such as CLA, linoleic acid and linolenic acid (C18:3n-3) in milk would increase consumer interest and acceptance of milk due to health benefits associated with these FA (Ramaswamy *et al.,* 2001).

Pottier *et al.* (2006) studied the effect of vitamin E supplementation (12 000 mg/d) in the diets containing linseed oil on milk fat composition of lactating dairy cows. They observed that dietary fat from linseed oil decreased milk fat concentration and addition of vitamin E to the diet eliminated the fat-depressing effect of linseed oil. Kay *et al.* (2005) observed that addition of 10 000 mg vitamin E/d to a TMR increased milk fat content by 6%. Bell *et al.* (2006) studied the effect of diets containing 60 g of safflower oil/kg DM, 60 g of safflower oil plus 150 mg of vitamin E/kg of DM, 60 g of safflower oil plus 24 mg of monensin/kg DM, or 60 g of safflower oil plus 24 mg of monensin and 150 mg of vitamin E/kg DM on milk fat percentage and milk fatty acid profile in lactating dairy cows. They observed that cows fed with safflower oil plus vitamin E or safflower oil plus vitamin E and monensin containing diets had higher milk fat percentage in relation to those fed with safflower oil or safflower oil plus monensin ones. The alleviating effect of vitamin E on milk fat depression suggested that it could minimise the formation of

trans-10 isomers in the rumen.

The present study was aimed at determining the effects of adding vitamin E and monensin on milk production, milk composition, and the milk fatty acid profile of lactating dairy cows when whole cottonseed, as the main source of FA, was included in the diet.

Materials and methods

Cows and experimental diets

Eight lactating Holstein cows (4 primiparous; 550± 15 kg of live weight, 4 third parity; 600 ± 20 kg of live weight; 550 ± 5 days in milk) were separated from the rest of the herd and housed in the individual stalls. In a balanced 4×4 repeated Latin square design, cows were randomly assigned to one of the four dietary treatments for 28-d treatment period: 1) control diet (without whole cottonseed, monensin and vitamin E), 2) diet with 20 percent whole cottonseed of DM, 3) diet with 20 percent whole cottonseed of DM plus 12000 IU of vitamin E/cow per d and 4) diet with 20 percent whole cottonseed of DM plus 24 ppm of monensin/kg DMI per cow per d (Table 1). The diets contained 460 g/kg DM forage and 540 g/kg DM of a concentrate mixture. Each experimental period lasted 28 days with 21 days of treatment adaptation and 7 days of data collection. All the diets were formulated based on NRC (2001) recommendations. Cows were fed the total mixed rations (TMR) ad libitum with a 10% of daily refusal. All cows were individually fed twice daily at 08:30 and 16:30 hours. Cows had free access to drinking water and were milked three times daily at 06:00, 14:00 and 22:00 hours. Milk samples were obtained from six consecutive milkings, days 27 to 28 of each experimental period and pooled within cow and period relative to production to obtain one composite milk sample per cow per period for chemical analysis. Milk samples were used for determination of milk composition (Milk-O-Scan 133B Foss Electric, Denmark). Fat corrected milk (4% FCM) is defined as milk with 4% of fat (NRC, 2001). Milk protein, fat and lactose yields were calculated by multiplying milk yield from the respective day by protein, fat and lactose contents of the milk for each cow. Milk nitrogen was calculated as (milk protein/6.38).

Milk samples without adding any preservative were stored at 20° C until analysed for FA profile. For GC–MS analysis, an Agilent 6890 gas chromatography with a 30m to 0.25mm HP-5MS capillary column coupled with an Agilent 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA) operating in EI mode at 70 eV was used. The temperatures of injector and detector ports were set at 250 and 150 o C respectively. Initially, the column temperature was held at 60 oC for 3 min and then was increased at a rate of 5 oC/min to 220 oC. The temperature of column was held at 220 oC for 10 min.

Statistical analysis

All statistical analyses were performed using PROC MIXED of SAS (1999). Data on milk production, milk composition, and blood parameters were analyzed using the following model:

$$Y_{ijk} = \mu + P_i + C_j + T_k + e_{ijk}$$

Where,

Y_{ijk} = the dependent variable

μ = overall mean

P_i = effect of period

C_j = random effect of cow

T_k = effect of treatment

e_{ijk} = residual error

Statistical significance was declared at $p<0.05$.

Results and discussion

Ingredients and chemical composition of the experimental diets were shown in Table 1. Whole cottonseed had a higher concentration of C18:2 in relation to the other FA (Table 2).

Effects of whole cottonseed, monensin and vitamin E on milk production and milk composition of dairy were shown in Table 3. Protein and lactose percentages were not significantly affected by experimental treatments, but 4% FCM, milk fat percentage and milk fat yield (kg/day) were significantly different among treatments ($P<0.05$). Adding whole cottonseed to diet (20%) decreased milk fat percentage ($P<0.05$). According to Coppock *et al.* (1987), including 100 to 300 g/kg WCS in the diet increased milk fat percentage in 8 out of 13 trials,

although only four trials showed significantly higher values than the control diet. Moreover, in some later studies (Kajikawa *et al.,* 1991; Wu *et al.*, 1994; Adams *et al.,* 1995; Dhiman and Satter, 1995; Bitman *et al.,* 1996) no response in milk fat percentage or yield was found when WCS was supplemented to dairy cow diets. In other studies supplementing WCS to dairy cattle rations, milk-fat percentage and yield increased (Sklan *et al.,* 1992; Belibasakis and Tsirgogianni, 1995; Harrison *et al.,* 1995) or decreased (Wilks *et al.,* 1991; Smith *et al.,* 1993). According to Smith *et al.* (1993), the reducing effect of WCS on milk fat yield is larger in corn silage than in alfalfa diets. The effect of WCS on fat metabolism may be attributed to both ruminal and post-ruminal effects (Kajikawa *et al.,* 1991; Wu *et al.,* 1994; McNamara *et al.,* 1995).

Table 1. Ingredients and chemical composition of the experimental diets.

	Dietary treatment[1]			
	C	WCS	WCS+E	WCS+M
Ingredient (g/kg DM)				
Alfalfa hay	190	190	190	190
Corn silage	270	270	270	270
Ground barley	190	100	100	100
Ground corn	150	90	90	90
Cottonseed meal	90	40	40	40
Soybean meal	80	80	80	80
Wheat bran	20	20	20	20
Salt	2	2	2	2
Ca carbonate	2	2	2	2
Mineral premix[2]	4	4	4	4
Vitamin premix[3]	4	4	4	4
Whole cottonseed	-	200	200	200
Monensin (ppm/cow day)	-	-	-	24
Vitamin E (IU/cow day)	-	-	12000	-

[1]Diets, Control (C) diet without whole cottonseed or vitamin E or monensin supplementation; WCS, control diet plus whole cottonseed 200 g/kg DM; WCS+E, WCS diet plus 12 000 IU of vitamin E/cow day; WCS+M, WCS diet plus 24 ppm of monensin/kg DMI per cow day.

[2] Each kg (DM basis) of mineral permix contained 200 g of Ca; 90 g of Mg; 13.5 g of Mn; 17.5 g of Fe; 14.3 g of Zn; 3.5 g of Cu; 210 mg of I; 35 mg of Co; 90 mg of Se.

[3] Each kg (DM basis) of vitamin permix contained 1 500 000 IU of vitamin A; 400 000 IU of vitamin D3; 6000 mg of vitamin E; and 400 mg of antioxidant.

Supplementing WCS in diets increased milk yield, but it was not significantly diference (P>0.05). The high fiber and energy content of WCS has the potential to alter milk composition and yield when fed to lactating dairy cows. Milk yield increased in lactating cows fed WCS compared with those fed none (Mooney and Allen, 1997). Anderson *et al.* (1979) noted that milk yield increased from 24.1 to 26.9 kg/d when cows were fed WCS compared with cows fed no WCS. Others (Clark and Armentano, 1993; DePeters *et al.,* 1985) have not observed any change in milk yield when WCS were included in the diet.

In the present study, milk protein content and yield was not decreased when WCS was added. Anderson *et al.* (1979), Smith *et al.* (1981) and DePeters *et al.* (1985) reported decreased milk protein percentage and yield with cottonseed feeding. The mechanism

whereby supplementary fat, especially protected fat, sometimes depresses protein in milk has yet to be understood (Palmquist & Jenkins, 1980). Smith *et al.* (1993) found a decrease in milk-protein yield by WCS feeding. Pires *et al.* (1997) found an increase in milk-protein yield and content when WCS was supplemented to dairy cow diets.

Table 2. Fatty acid profile of the experimental diets and wholecottonseed fed to Holstein dairy cows.

	Dietary treatment*				Whole cottonseed
	C	WCS	WCS+E	WCS+M	
C14:0	3.41	4.18	4.18	4.18	1.85
C16:0	49.85	44.00	44.00	44.00	28.41
C16:1	-	-	-	-	1.12
C18:0	1.40	1.41	1.41	1.41	3.07
C18:1 cis-9	24.87	23.54	23.54	23.54	15.30
C18:2 cis-9 cis-12	17.25	25.28	25.28	25.28	48.82
C18:3 n-3	-	-	-	-	0.14
C20:4	-	-	-	-	0.32
Others†	3.22	1.59	1.59	1.59	0.97
Saturated‡	54.66	49.59	49.59	49.59	33.33
Unsaturated§	42.12	48.82	48.82	48.82	65.38

FA, fatty acids are expressed as g/100 g FA methyl esters.

*Abbreviations are explained in Table 1.

†Unidentifiable peaks.

‡Sum of C14:0, 16:0 and C18:0 FA.

§Sum of C16:1, C18:1, C18:2, C18:3 and C20:4 FA.

Reduced milk fat is another important side effect of high-fat diets. This phenomenon, referred to as milk fat depression (MFD), is typically observed with low-fiber diets including high levels of concentrates (either readily digestible carbohydrates or unsaturated fat; Bauman and Griinari, 2003). Several theories have been proposed to explain MFD (Bauman and Griinari, 2003). However, currently, the most supported theory involves trans-10, cis-12 CLA as a direct inhibitor of milk fat synthesis in the mammary gland. In this study adding vitamin E to diet containing WCS (WCS+E) increased milk fat percentage compared to diets containing WCS (WCS or WCS+M diets), but it was not significantly difference (P>0.05). Pottier *et al.* (2006) studied the effect of vitamin E supplementation (12 000 mg/d) in the diets containing linseed oil on milk fat composition of lactating dairy cows. They observed that dietary fat from linseed oil decreased milk fat concentration and addition of vitamin E to the diet eliminated the fat-depressing effect of linseed oil. Kay *et al.* (2005) observed that addition of 10 000 mg vitamin E/d to a TMR increased milk fat content by 6%. Bell *et al.* (2006) studied the effect of diets containing 60 g of safflower oil/kg DM, 60 g of safflower oil plus 150 mg of vitamin E/kg of DM, 60 g of safflower oil plus 24 mg of monensin/kg DM, or 60 g of safflower oil plus 24 mg of monensin and 150 mg of vitamin E/kg DM on milk fat percentage and milk fatty acid profile in lactating dairy cows. They observed that cows fed with safflower oil plus vitamin E or safflower oil plus vitamin E and monensin containing diets had higher milk fat percentage in relation to those fed with safflower oil or safflower oil plus monensin ones. The alleviating effect of vitamin E on milk fat depression suggested that it could minimize the formation of trans-10 isomers in the rumen. Charmley and Nicholson (1994) studied the effect of fat source on milk fat composition of cows receiving different levels of dietary vitamin E. They

observed that dietary fat from micronized soybeans decreased milk fat concentration. However, a high concentration of vitamin E supplement in the diet (8 000 IU/d) eliminated the fat-depressing effect. Similarly, Focant *et al.* (1998) showed that a huge dietary vitamin E supplement (9600 IU/d) prevented the drop in milk fat yield, as well as resistance to oxidation, which was induced by the incorporation of unsaturated fat-rich extruded linseeds and rapeseeds. Kay *et al.* (2005) observed that addition of 10000 IU of α-tocopherol/d to a TMR increased milk fat content by 6%.

Table 3. Effects of whole cottonseed, monensin and vitamin E on milk production and milk composition of dairy cows.

Item	Dietary treatment				SE	Mixed effects		
	C	WCS	WCS+E	WCS+M		square	period(square)	treatment
Milk yield (kg/d)								
Milk	42.6	43.5	43.9	42.0	1.278	0.0158	0.9079	0.7197
4% FCM	40.6[a]	35.8[b]	38.4[ab]	35.1[b]	1.336	0.0015	0.7198	0.0209
Fat	1.534[a]	1.266[b]	1.353[b]	1.296[b]	0.075	0.0024	0.5240	0.0063
Protein	1.312	1.390	1.372	1.380	0.042	0.0031	0.9586	0.5540
Lactose	1.889	1.944	2.027	1.859	0.060	0.0079	0.4405	0.2541
Nitrogen	0.206	0.218	0.215	0.216	0.007	0.0031	0.9586	0.5540
Milk composition (%)								
Fat	3.41[a]	2.99[b]	3.18[b]	2.78[b]	0.178	0.0218	0.3612	0.0072
Protein	3.08	3.20	3.13	3.28	0.068	0.0788	0.5140	0.1576
Lactose	4.43	4.48	4.63	4.42	0.071	0.2936	0.0648	0.1301

[a, b, c] Least square means within a row without common superscript differ (P<0.05). 1Standard error of the mean. 2P-values for mixed effects.

Diets, Control (C) diet without whole cottonseed or vitamin E or monensin supplementation; WCS, control diet plus whole cottonseed 200 g/kg DM; WCS+E, WCS diet plus 12 000 IU of vitamin E/cow day; WCS+M, WCS diet plus 24 ppm of monensin/kg DMI per cow day.

Monensin supplementation had no effect on milk production. This disagrees with earlier trials establishing that the inclusion of 300 mg of monensin/d in dairy cow diets for the first 25 wk of lactation would increase milk yield (Van der Werf *et al.,* 1998; Phipps *et al.,* 2000). However, feeding monensin at 24 and 22 mg/kg of DM, respectively, for 15-d (Bell *et al.,* 2006) and 35-d (Osborne *et al.,* 2004) periods had no effect on DMI and milk yield of dairy cows. Discrepancies between studies could be related to factors such as stage of lactation, diet composition, and length of the trial. Moreover, Sauer *et al.* (1998) suggested that some adaptive changes occur in the rumen microflora following monensin supplementation and cows that had previously received monensin no longer respond. The response to monensin regarding milk production and composition also differs with genetic line, cows with the highest capacity of milk production responding best to monensin supplementation (Van der Werf *et al.,* 1998). A supply of glucogenic precursors, resulting from changes in the pattern of rumen fermentation, can also be a likely mechanism of supporting additional milk yield by monensin supplementation. Adding monensin decreased milk fat concentration (WCS diet vs. WCS+M diet), but does not significantly difference (P>0.05). Supplementation of monensin has been shown to decrease milk fat percentage (Ramanzin *et al.,* 1997; Phipps *et al.,* 2000) but not total milk fat yield (McGuffey *et al.,* 2001). The reduced ruminal production of acetate and butyrate is frequently attributed as the main factor reducing milk fat percentage when cows are fed diets supplemented with monensin (Van Der Werf *et al.,* 1998). Phipps *et al.* (2000), Da Silva *et al.* (2007), Odongo *et al.*

(2007), and Alzahal *et al.* (2008) reported a reduction in milk fat percentage with monensin supplementation. The milk fat depressing effect of monensin has been attributed to the reduced ruminal production of acetate and butyrate, which might result in a shortage of lipogenic precursors for the synthesis of fatty acids in the lactating mammary gland (Dye *et al.,* 1988; Van der Werf *et al.,* 1998). Fellner *et al.* (1997) reported that monensin inhibited the rate of biohydrogenation of long chain fatty acids, which can inhibit the de novo synthesis of fatty acids in the mammary gland (Bauman and Griinari, 2003), and therefore reduced milk fat output when cows were fed diets supplemented with monensin (Ipharraguerre and Clark, 2003).

Table 4. Milk FA profile of Holstein dairy cows fed the experimental diets.

FA	Dietary treatment*			
	C	WCS	WCS+E	WCS+M
C6	2.84^{b}±0.26	3.23^{b}±0.26	5.09^{a}±0.40	3.05^{b}±0.26
C8	3.10±0.16	2.91±0.16	3.70±0.25	3.12±0.16
C10	7.15^{b}±0.55	6.32^{b}±0.55	10.04^{a}±0.84	7.63^{b}±0.55
C12	8.23±0.63	6.48±0.63	8.99±0.96	6.93±0.63
C14	18.02±0.94	16.26±0.94	20.26±1.43	19.02±0.94
C14:1	2.29^{a}±0.17	1.48^{b}±0.17	1.48^{b}±0.26	1.31^{b}±0.17
C15	2.15±0.11	1.88±0.11	1.59±0.17	1.99±0.11
C16	24.63±1.23	25.04±1.23	25.21±1.76	25.03±1.23
C16:1	2.35^{ab}±0.15	2.00^{bc}±0.15	2.67^{a}±0.23	1.81^{c}±0.15
C17	0.92±0.16	1.51±0.16	0.77±0.24	1.05±0.16
C18	5.37^{a}±0.85	6.60^{a}±0.85	2.99^{b}±0.96	6.67^{a}±0.85
C18:1 trans-11	5.57^{a}±0.59	2.67^{b}±0.59	2.44^{b}±0.66	4.47^{a}±0.59
C18:1 cis-9	11.51^{ab} ±1.27	14.65^{a}±1.27	8.93^{b}±1.94	8.50^{b}±1.27
C18:2	2.65±0.27	2.11±0.27	1.36±0.42	2.79±0.27
Cis-9, trans-11 CLA	0.08^{c}±0.12	0.95^{a}±0.12	0.62^{b}±0.18	1.32^{a}±0.12
Trans-10, cis-12 CLA	0.01^{b}±0.067	0.03^{b}±0.067	0.04^{b}±0.101	1.41^{a}±0.067
Others	3.15±0.78	5.91±0.78	2.97±1.19	5.15±0.78
SFA	72.40^{b}±1.55	70.23^{b}±1.55	78.99^{a}±2.33	74.49^{ab}±1.55
USFA	24.45^{a}±1.09	23.86^{a}±1.09	17.66^{b}±1.67	21.50^{ab}±1.09
MUFA	21.71^{a}±1.22	20.80^{a}±1.22	15.52^{b}±1.82	16.09^{b}±1.22
PUFA	2.74^{b}±0.41	3.06^{b}±0.41	2.10^{b}±0.62	5.53^{a}±0.41
SCFA	21.32^{b}±1.38	18.94^{b}±1.38	27.82^{a}±2.10	20.73^{b}±1.38
MCFA	50.35±1.67	48.17±1.67	52.34±2.39	50.21±1.67
LCFA	25.18^{a}±1.85	26.98^{a}±1.85	16.79^{b}±2.83	24.15^{a}±1.86

FA, fatty acids expressed as g/100 g fatty acid methyl esters. CLA, conjugated linoleic acid; Others, unidentifiable peaks; SFA, saturated fatty acids; USFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCFA, short-chain fatty acids (C4:0–C12:0); MCFA, medium-chain fatty acids (C14:0–C17:0); LCFA, long-chain fatty acids (C18:0–C18:3n-3).

[a,b]Mean values with different superscripts in the same row are different ($p < 0.05$). Multi-treatment comparison method: Fisher's protected LSD.

*Abbreviations are explained in Table 1.

Cows fed monensin had lower 4% FCM yield as a result of the decrease in milk fat concentration (P>0.05). This agrees with the well-documented decrease in milk fat concentration reported when adding monensin to a dairy cow diet (Sauer *et al.,* 1998; Dhiman *et al.,* 1999; Phipps *et al.,* 2000; Bell *et al.,* 2006) due to the reduction of molar proportions of acetate and butyrate and the increase of propionate (Sauer *et al.,* 1998).

Monensin supplementation increased protein concentration compared to treatments (P>0.05). Yang and Russell (1993) found that monensin feeding reduced in vitro and in vivo ruminal NH_3 formation from protein hydrolysates by suppressing certain bacteria with high deamination activity. Thus, monensin supplementation may improve N efficiency by increasing gut absorption of α-amino N. Because of the high levels of free AA, monensin might be particularly effective in lactating cows fed alfalfa silage. Ruiz *et al.* (2001) observed that adding monensin to the diet reduced ruminal NH_3, suggesting a N-sparing effect.

Feeding monensin supplement had no effect on milk concentrations of short-chain fatty acids (SCFA) (4:0–12:0) and long-chain fatty acids (LCFA) (18:0 to 18:3n-3) (p > 0.05; Table 4), but feeding vitamin E supplement increased SCFA and decreased LCFA (p <0.05). Khodamoradi *et al.* (2012) found that Feeding monensin and vitamin E supplements had no effect on the concentration of SCFA in milk fat that was in agreement with the findings of others (Pottier *et al.,* 2006; Da Silva *et al.,* 2007). However, Fatahnia *et al.* (2010) reported that feeding monensin to dairy cows decreased SCFA concentration of milk, whereas Odongo *et al.* (2007) found that the concentration of SCFA in milk fat was increased by monensin supplementation.

Dietary treatments had no effect on milk concentrations of medium chain fatty acids (MCFA) (p>0.05; Table 4). However, the lowest and the highest concentrations of milk MCFA (14:0–17:0) were observed in treatments WCS and WCS+E, respectively (Table 4) that was in agreement with previously published studies (Da Silva *et al.,* 2007; Odongo *et al.,* 2007; Fatahnia *et al.,* 2010). In a previous study, vitamin E supplementation at 12 000 mg/day had no effect on the concentrations of SCFA and MCFA in milk fat (Pottier Adding WCS, vitamin E and monensin decreased the concentration of 14:1 (p <0.05). In agreement with our results, Da Silva *et al.* (2007) and Fatahnia *et al.* (2010) found that monensin had no effect on concentration of LCFA in milk fat, whereas Odongo *et al.* (2007) showed that milk fat concentration of LCFA was increased by dietary supplementation with monensin. Fatty acids in milk originate from two sources; uptake from circulation and the de novo synthesis within the mammary epithelial cells (Neville and Picciano, 1997). Short chain FA and MCFA arise almost exclusively from the *de novo* synthesis using circulating acetate and butyrate that are originated from the rumen, whereas LCFA are derived from the uptake of circulating lipids (Mansbridge and Blake, 1997).

Feeding monensin and vitamin E supplements had no effect on milk concentration of 16:0 (p >0.05; Table 4). Feeding cows with the M diet (WCS+M treatment) decreased the concentration of 16:1, whereas the E diet (WCS+E) tended to increase it (p = 0.05; Table 4). The concentration of 18:0 was affected by vitamin E supplementation and decreased with adding vitamin E (p <0.05). Fatahnia *et al.* (2010) and Alzahal *et al.* (2008) reported that the concentration of 18:0 in milk fat was decreased by dietary supplementation of monensin, Odongo *et al.* (2007) observed that feeding monensin at 24 mg/kg DM increased the concentration of 18:0 in milk fat.

The concentration of trans-11 18:1 was not affected by monensin but its concentration reduced in treatments WCS and WCS+E (p >0.05). Pottier *et al.* (2006) found no differences in milk fat concentrations of trans-11 18:1 and cis-9, trans-11 CLA by dietary supplementation of vitamin E at 12 000 mg/day. Trans-11 18:1 can be converted to cis-9, trans-11 CLA in the mammary gland and other tissues catalyzed by D9-desaturase (Bauman and Griinari, 2003). Therefore, an increased flow of trans-11 18:1 from the rumen to small intestine is desirable because it would elevate the concentration of cis-9, trans-11 CLA in milk fat. Turpeinen *et al.* (2002) reported that on average 19% of dietary trans-11 18:1 can be converted to cis-9, trans-11 CLA in humans.

Adding vitamin E or monensin to diets decreased the concentration of cis-9 18:1 (p<0.05). The

concentration of 18:2 n-6 was not affected by monensin and vitamin E supplementations (p>0.05; Table 4), whereas the WCS+E diet tended to decrease it. The concentration of cis-9, trans-11 CLA was affected by monensin and vitamin E supplementations (p <0.05) and treatments WCS and WCS+M had the highest amount within the experimental treatments. The supplementation of diet by monensin had significant effect on trans-10, sic-12 CLA concentration and increased it (p <0.05). Focant *et al.* (1998) observed that dietary supplementation with vitamin E (9600 IU/d) resulted in the reduction of trans-10 C18:1 fatty acid in milk fat, suggesting that the effect of vitamin E on milk fat concentration was mediated by the changes in rumen BH. This suggestion is also supported by findings of Kay *et al.* (2005) who reported that plasma trans-11 C18:1 tended to increase and plasma trans-10 C18:1 numerically decreased in dairy cows when the TMR was supplemented with 10 000 IU of α-tocopherol/d. The milk fat-depressing effect of monensin has been attributed to the reduced ruminal production of acetate and butyrate, the inhibition of ruminal BH of LCFA and the enhanced supply of trans-10, cis-12 CLA to the mammary gland (Fellner *et al.,* 1997; Van der Werf *et al.,* 1998; Bauman and Griinari, 2001). Trans-10, cis-12 CLA is thought to be responsible for milk fat depression (Baumgard *et al.,* 2000). The increased concentration of trans-10, cis-12 CLA with the WCS+M diet can be kept responsible for decreasing the synthesis of MCFA (Baumgard *et al.,* 2000). Literatures indicate that cis-9, trans-11 CLA have numerous health benefits including reduction of body fat mass, anticarcinogenic, antiatherogenic, antidiabetogenic and immune modulating effects. Therefore, nutritional strategies that increase its content in milk fat of dairy cows are of interest. Supplementing dairy cows' ration with vitamin E and monensin is one such a strategy (Pottier *et al.,* 2006; Fatahnia *et al.,* 2010).

The concentrations of saturated fatty acids (SFA), USFA, MUFA and PUFA were affected by the dietary treatments (p<0.05; Table 4). The concentration of SFA was numerically higher, whereas USFA and LCFA were numerically lower in milk fat of the cows fed the vitamin E diet than those fed the other diets (Table 4).

Conclusion

In this study, monensin and vitamin E supplementations had no effects on milk yield, but decreased milk fat concentration. Concentrations of trans-11 18:1, cis-9, trans-11 CLA and trans-10, cis-12 CLA were affected by the dietary treatments. The results of this study showed that supplementation of vitamin E (at 12 000 IU/cow day) or monensin (at 24 mg of monensin/kg of DM) did not have any effect on milk fat concentration when whole cottonseed is included in the diet whereas affected milk fat compositions.

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