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Supplementation with Selenium and Vitamin E Improves Milk Fat Depression and Fatty Acid Composition in Dairy Cows Fed Fat Diet*

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ABSTRACT : This experiment was conducted to evaluate the effect of supplementing a fat diet with selenium (Se) and vitamin E on performance of cows, blood antioxidant status and milk fatty acid composition. Sixty-three lactating Holstein cows were randomly divided into seven groups of nine cows each and each group was fed one of the following diets: i) a basal diet (control); ii) a basal diet with 0.15 mg Se/kg DM (LSe); iii) a basal diet with 0.3 mg Se/kg DM (HSe); iv) a basal diet with 5,000 IU/cow d vitamin E (LVE); v) a basal diet with 10,000 IU/cow d vitamin E (HVE); vi) a basal diet with 0.15 mg Se/kg DM and 5,000 IU/cow d vitamin E (LSeVE); vii) a basal diet with 0.3 mg Se/kg DM and 10,000 IU/cow d vitamin E (HSeVE). Milk fat percentage and conjugated linoleic acid (CLA) yield in HVE and HSeVE diets increased ($p<0.05$) compared with the control diet. In milk fat, dietary supplementation of Se tended to increase the proportion of the sum of unsaturated fatty acids (UFA) and significantly decreased ($p<0.05$) the proportion of the sum of saturated fatty acids (SFA). In addition, compared with the control, thiobarbituric acid reactive substances (TBARS) content was lower and glutathione peroxidase (GSH-Px) was higher when fat diets were supplemented with Se. Our data showed that supplementation with Se and/or VE improved these nutrients in blood and milk. The results indicated that fat diets supplemented with Se improved both antioxidant status in blood and fatty acids in milk fat, and fat diets supplemented with vitamin E alleviated milk fat depression. Therefore, the combination of Se and vitamin E caused synergistic effects on the nutritional quality of milk fat and performance of cows fed a fat diet. (**Key Words :** Cows, Selenium, Vitamin E, Unsaturated Fatty Acid, Milk Fat)

INTRODUCTION

Since the deleterious effects of saturated fat on LDL cholesterol are beyond any doubt (Riccardi and Rivellese, 1993; Kris-Etherton, 1997), human dietary guidelines have recommended reductions in the intake of saturated fatty acids (SFA) and total fat as a means of reducing the prevalence of coronary heart disease. Moreover, SFA should provide no more than 10% of total calories (FAO/WHO, 1998). It is well known that unsaturated fatty acids (UFA) play key roles in human nutrition and health. Certain highly UFA, such as conjugated linoleic acid (CLA), are already known to be required for normal human health. Furthermore, developments have brought new insights into the function of highly UFA and special attention has been paid to dietary polyunsaturated fatty acids (PUFA) that could play a positive role for human health (Sacks and Katan, 2002; Tapiero et al., 2002).

Numerous factors could influence fatty acid profiles of milk, and dietary manipulation may be the most important one. Considerable studies (Sarrazin et al., 2004; Chichlowski et al., 2005; Chen et al., 2008; Purushothaman et al., 2008) have been carried out to increase milk UFA content when high plant oils or oilseeds as a source of UFA were included in the diet of dairy cows. Unfortunately, the addition of plentiful fat to the bovine diet may lead to adverse effects on the rumen environment and influence the normal growth and function of rumen microbes. In addition, a high proportion of fat in diets was observed to affect the milk production capacity of cows and reduced milk fat content (Bell et al., 2006; Fatahnia et al., 2008; Liu et al., 2008), referred to as milk fat depression.

Studies on the effects of Se and/or vitamin E on fatty acid composition in bovine milk are rare. In humans, Dodge et al. (1999) reported that a selenium (Se) supplement significantly increased the concentration of PUFA and decreased the concentration of SFA in human milk. In other

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Table 1. Nutrient composition of experimental diet

Composition	Basal diet
Ingredients (% of DM)	
Alfalfa hay	17.0
Corn silage	33.0
Crack corn	32.2
Soybean meal	13.5
Soybean oil	2.0
Dicalcium phosphate	0.8
Limestone	0.5
Mineral and vitamin mix[1]	1.0
Chemical analysis (% of DM)	
CP	17.9
NDF	35.1
ADF	18.9
Ca	0.83
P	0.39
DM (% of diet)	55.7
NE_L (Mcal/kg)	1.71

[1] Content (per kilogram): NaCl, 400 g; $NaHCO_3$, 200 g; I, 0.1 g; Fe, 3 g; Mg, 20 g; Cu, 2 g; Mn, 3 g; Zn, 6 g; Se, 0.06 g; Co, 0.02 g; vitamin A, 800,000 IU; vitamin D, 100,000 IU; and vitamin E, 4,000 IU.

species, dietary Se has also been indicated to influence the fatty acid composition of mouse muscle (Czauderna et al., 2004) and chicken eggs (Pappas et al., 2005). Several studies (Bell et al., 2006; Pottier et al., 2006) showed that adding vitamin E to the diet prevented the drop in milk fat yield which was induced by fat-rich diets, as well as improving resistance to oxidation. Se and vitamin E as antioxidants could inhibit the oxidation of the oxidative-labile UFA more effectively than that of the oxidative-stable SFA. Therefore, we hypothesized that increased dietary Se and vitamin E proportionally should protect UFA from oxidation, and hence increase UFA content in milk. The purpose of the present study was to evaluate the response of supplementing with Se and/or vitamin E on milk fat depression and milk fatty acid concentration in cows fed high-UFA diets, and the effects of treatments on antioxidant status in blood were also assessed.

MATERIALS AND METHODS

Animals and treatments

Twenty-eight primiparous and thirty-five multiparous healthy Holstein cows were used in this study. Cows were on average 94 days in milk at the start of the study. They were randomly divided into seven groups of nine cows each and each group was fed one of the following diets: i) a basal diet (control); ii) a basal diet with 0.15 mg Se/kg DM (LSe); iii) a basal diet with 0.3 mg Se/kg DM (HSe); iv) a basal diet with 5000 IU/cow d vitamin E (LVE); v) a basal diet with 10,000 IU/cow d vitamin E (HVE); vi) a basal diet with 0.15 mg Se/kg DM and 5,000 IU/cow d vitamin E (LSeVE); vii) a basal diet with 0.3 mg Se/kg DM and 10,000 IU/cow d vitamin E (HSeVE). The composition of the basal diet is presented in Table 1. Diets were formulated to meet energy and nutrition requirements (NRC, 2001). The vitamin E and Se were added to the concentrates prepared to give the correct concentration for each treatment. The vitamin E product contained 50% DL-α-tocopheryl acetate. Se was in the form of Nano-Se (Bosar Biotec Ltd., China). The composition of the main UFA in soybean oil were: C18:1 (14.7%), C18:2 (48.2%), and C18:3 (5.4%). The study lasted 6 weeks and all cows were housed in tie stalls during the experimental period. Cows were fed a total mixed ration (TMR) twice daily at 07:30 and 15:30 h. Daily dry matter intake (DMI) was recorded and water was provided *ad libitum*.

Sampling and analysis

Samples of diets were taken once weekly. Contents of crude protein (CP), Ca and P were determined according to the AOAC (1990). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined by the methods of Van Soest et al. (1991). Cows were milked twice daily starting at 06:30 and 18:30 h. Milk yield was recorded weekly and milk samples were taken from each cow on two consecutive milkings on 13, 14, 27, 28, 41 and 42 d during the experimental period. The samples taken on consecutive two days from each cow were then blended, and sub-samples of milk from each cow were kept at 4°C until analysis of milk fat, protein and lactose content by infrared spectrophotometry (Bentley, 2000; Bentley Instruments, US). The rest of the sample was stored at -20°C for later analysis.

The frozen milk samples were rapidly thawed and then centrifuged at 12,000×g at 8°C for 1 h to harvest the fat layer for fatty acid analysis. Lipid extraction of milk fat was performed according to Hara and Radin (1978) in a mixture of hexane and isopropanol (3:2; v/v). Methyl esters of the fatty acids in milk fat were prepared by transesterification with sodium methoxide according to the method of Christie (1982).

Fatty acid methyl esters in hexane were then injected into a Finnigan Trace Gas Chromatograph (Thermo Electron Co., US) equipped with an electron ionization detector. Separation of fatty acid methyl esters was performed with a fused silica capillary column (HP-88, 100 m×0.25 mm i.d.×0.25 μm film thickness; Agilent, US). Analysis of fatty acids in total samples of lipid fractions required injection of 1 μl of methyl-esters in hexane. Helium was the carrier gas and was set at a split ratio of 1:40. The injector and detector were maintained at 230°C. For fatty acids in milk, the initial column temperature was 70°C for 2 min, increased by 8°C/min to 120°C and then increased at 15°C/min to 160°C, held for 40 min, then increased at 5°C/min to 215°C and held for 10 min. All fatty acids were eluted at a flow of 1 ml/min. The

Table 2. Milk yield and composition from cows fed different diets

Item	Diets							SEM
	Control	LSe	HSe	LVE	HVE	LSeVE	HSeVE	
DMI (kg/d)	19.09	18.89	19.07	19.24	19.11	19.36	19.01	0.26
Milk (kg/d)	21.10	21.00	21.10	21.40	21.30	21.40	21.80	0.36
Fat (%)	3.31^{c}	3.25^{c}	3.25^{c}	3.54^{bc}	3.81^{ab}	3.51^{c}	3.86^{a}	0.17
Protein (%)	3.53	3.46	3.52	3.43	3.44	3.48	3.54	0.14
Lactose (%)	4.46	4.69	4.65	4.62	4.61	4.63	4.51	0.11
Fat (kg/d)	0.70^{bc}	0.68^{c}	0.68^{c}	0.76^{ab}	0.81^{a}	0.75^{ab}	0.80^{a}	0.04
Protein (kg/d)	0.75	0.73	0.74	0.73	0.73	0.74	0.74	0.03
Lactose (kg/d)	0.98	0.98	0.98	0.99	0.98	0.99	0.94	0.03

[a, b, c] Means within row with different superscrips differ ($p<0.05$).

methylated fatty acid standards (Matreya, Inc., US) including CLA were used to identify the retention times and to determine correction factors for individual fatty acids.

Blood samples from each cow were collected from the jugular vein into heparinized tubes, on the last day of this experiment. One aliquot of each sample was stored at -20°C for analysis of glutathione peroxidase (GSH-Px) and thiobarbituric acid reactive substances (TBARS); another aliquot was centrifuged at 3,000×g for 10 min at 4°C to obtain plasma which was stored at -20°C for Se and vitamin E analysis.

α-Tocopherol was extracted from the milk and blood samples and quantified using HPLC (Hogan et al., 1990). Se concentration of the samples was measured with hydride generation atomic absorption spectrophotometry as described by Sturman (1985). The TBARS concentration was determined by the method of Trotta et al. (1982). The GSH-Px activity was measured by a modification of the method of Paglia and Valentine (1967) and expressed as U/g of protein. The protein concentration was determined by the method of Lowry et al. (1951).

Statistical analysis

The data were analyzed by the analysis of variance (ANOVA) procedure of Statistical Analysis Systems (SAS Version 8.2, 2001). Duncan's multiple range method was used to compare the difference in treatment means.

RESULTS

Production measures

Different diets showed no distinct effect on DMI, milk yield, concentrations and production of milk protein and lactose (Table 2). However, vitamin E supplementation increased the concentration and production of milk fat which were significantly higher ($p<0.05$) in HVE and HSeVE groups. Compared with the control diet, the milk fat percentages were increased 15.1 and 16.6% by the HVE and HSeVE diet, respectively.

Fatty acid composition in milk

The composition of fatty acids in milk fat are summarized in Table 3. There was no overall effect of Se and/or vitamin E supplementation on individual fatty acids in milk. The only differences observed were in the C10:0 and C16:0 levels, which were lower ($p<0.05$) in the HSeVE group compared to the control group. Dietary supplementation of Se tended to increase the summed concentration of UFA, compared with the control group. Meanwhile, dietary supplementation of Se decreased the summed content of SFA, which was significantly lower ($p<0.05$) in HSe and HSeVE groups compared to the control group. Especially when cows were fed HSeVE, the summed concentration of UFA was increased ($p<0.05$) by 4.6% and the summed concentration of SFA was decreased ($p<0.05$) by 7.1%, compared with the control diet.

The production of fatty acids was also influenced by the Se and/or vitamin E supplementation. The production of *cis*-9, *trans*-11 CLA was significantly higher ($p<0.05$) in HVE and HSeVE groups compared with the control group. Nevertheless, the production of *cis*-12, *trans*-10 CLA was not significantly different among all treatments.

Antioxidant status

Se supplementation (including LSe, HSe, LSeVE and HSeVE groups) significantly increased ($p<0.05$) the concentration of Se in plasma and milk (Table 4). Cows fed diets which contained 0.3 mg Se/kg DM (HSe and HSeVE groups) had higher ($p<0.05$) Se concentration in plasma and milk than those cows fed diets which contained 0.15 mg Se/kg DM (LSe and LSeVE groups). However, the concentration of Se in plasma or milk was not affected by vitamin E supplementation.

Diets that contained 5,000 IU/cow d vitamin E (LVE and LSeVE groups) did not alter vitamin E concentration in plasma or milk. Cows fed diets which contained 10,000 IU cow d vitamin E (HVE and HSeVE groups) had higher ($p< 0.05$) concentration of vitamin E in plasma and milk than those cows fed diets that contained no (control group) or 5,000 IU/cow d vitamin E (LVE and LSeVE groups). However, the concentration of vitamin E in plasma or milk

Table 3. Composition of fatty acid in milk fat from cows fed different diets

	Diets							SEM
	Control	LSe	HSe	LVE	HVE	LSeVE	HSeVE	
Fatty acid (g/100 g of fatty acids)								
C4:0	3.49	3.49	3.42	3.48	3.51	3.52	3.24	0.23
C6:0	1.86	1.61	1.66	1.82	2.02	1.99	1.62	0.16
C8:0	1.14	1.08	1.00	1.16	1.14	1.10	1.05	0.07
C10:0	2.85^{a}	2.72^{ab}	2.77^{ab}	2.76^{ab}	2.81^{ab}	2.75^{ab}	2.67^{b}	0.09
C12:0	1.61	1.46	1.23	1.69	1.50	1.44	1.32	0.27
C14:0	8.39	8.32	8.28	8.23	8.11	7.96	7.42	0.57
C16:0	21.57^{a}	21.33^{a}	21.09^{a}	21.65^{a}	21.36^{a}	21.11^{a}	19.87^{b}	0.51
C16:1	2.02	2.00	2.03	1.96	2.00	1.87	1.98	0.08
C18:0	14.29	14.01	13.92	13.71	13.88	14.57	14.08	0.55
C18:1c9	26.59	27.22	27.72	27.04	26.70	26.95	27.95	0.81
C18:1t6-8	0.39	0.38	0.38	0.39	0.46	0.43	0.43	0.06
C18:1t9	0.43	0.42	0.39	0.39	0.47	0.41	0.40	0.06
C18:1t10	1.16	1.19	1.14	1.12	1.19	1.19	1.15	0.07
C18:1t11	1.31	1.37	1.43	1.31	1.40	1.42	1.47	0.11
C18:2	3.63	3.54	3.57	3.49	3.51	3.47	3.67	0.14
CLA c9, t11	0.91	0.87	0.93	0.88	0.95	0.85	0.96	0.06
CLA c10, t12	0.03	0.02	0.02	0.02	0.03	0.03	0.02	0.01
C18:3	0.34	0.34	0.38	0.32	0.37	0.44	0.43	0.07
C20:0	0.22	0.19	0.21	0.21	0.20	0.23	0.20	0.03
ΣSFA	55.41^{a}	54.17^{ab}	53.27^{b}	54.71^{ab}	54.75^{ab}	54.81^{ab}	51.47^{c}	1.03
ΣUFA	36.79^{b}	37.35^{ab}	37.98^{ab}	36.94^{ab}	37.08^{ab}	37.06^{ab}	38.49^{a}	0.90
Fatty acid yield (g/d)								
CLA c9, t11	6.38^{b}	5.96^{b}	6.34^{b}	6.69^{ab}	7.69^{a}	6.43^{b}	7.67^{a}	0.58
CLA c10, t12	0.17	0.15	0.13	0.18	0.19	0.19	0.15	0.04

$^{a, b, c}$ Means within row with different lowercase superscripts differ ($p<0.05$).

Table 4. Contents of Se and vitamin E in milk and blood, and antioxidant parameters in blood of cows fed different diets

	Diets							SEM
	Control	LSe	HSe	LVE	HVE	LSeVE	HSeVE	
Selenium								
Plasma (μg/L)	41.6^{d}	52.3^{b}	61.5^{a}	43.1^{cd}	46.2^{bcd}	50.7^{bc}	62.2^{a}	2.88
Milk (μg/L)	13.0^{c}	18.4^{b}	29.7^{a}	12.9^{c}	15.2^{c}	19.9^{b}	31.5^{a}	0.86
α-Tocopherol								
Plasma (mg/L)	2.66^{b}	2.71^{b}	2.90^{b}	3.31^{ab}	4.11^{a}	3.61^{ab}	4.34^{a}	0.40
Milk (mg/L)	0.82^{b}	0.83^{b}	0.87^{b}	0.90^{b}	1.17^{a}	0.98^{b}	1.19^{a}	0.06
GSH-Px activity								
Blood (U/g protein)	96.5^{b}	94.3^{b}	108.7^{a}	98.9^{b}	103.2^{ab}	110.4^{a}	109.6^{a}	3.15
TBARS content								
Blood (nmol/L)	14.06^{a}	12.8^{ab}	11.47^{bc}	13.69^{a}	12.2^{ab}	9.79^{cd}	9.19^{d}	0.66

$^{a, b, c}$ Means within row with different superscrips differ ($p<0.05$).

was not affected by Se supplementation.

The activity of GSH-Px in blood was significantly increased ($p<0.05$) and the content of TBARS in blood was significantly reduced ($p<0.05$) when HSe, LSeVE, and HSeVE were fed. The activity of GSH-Px and content of TBARS in blood was not affected by LVE, HVE and LSe diets when compared with the control diet.

DISCUSSION

Production measures

The lack of any effect of dietary Se and/or vitamin E supplementation on DMI, milk yield, milk protein and milk lactose agrees with other findings (Baldi et al., 2000; Givens et al., 2004). Both percentage and yield of milk fat were significantly increased when diets were supplemented with vitamin E, and milk fat percentage was increased ($p<0.05$) by, respectively, 15 and 17% when HVE and HSeVE were fed compared with the control diet. Previous studies showed that vitamin E was capable of reducing the extent of diet-induced milk fat depression. Pottier et al. (2006) observed that addition of 12,000 IU of vitamin E to fat diets increased milk fat content by 18%. Kay et al. (2005) reported that milk fat percentage was 6% higher

when cows were fed a TMR containing 10,000 IU of α-tocopherol.

Fatty acid composition in milk

Milk fat consists of approximately 70% SFA, 27% monounsaturated fatty acids (MUFA), and 3% PUFA (Deeth, 1997). UFAs, especially PUFA of membranes, are particularly susceptible to oxidation due to the double bonds in the fatty acids. Se and vitamin E are essential micronutrients that share a common biological role as antioxidants (Bendich, 1990, Hogan et al., 1996). Vitamin E is the most effective chain-breaking antioxidant (Huang et al., 2002), and Se, an integral component of GSH-Px (Erskine, 1993), acts synergistically with vitamin E to protect the body against free radicals (Kim et al., 1997). As antioxidants, it is reported that Se and vitamin E could inhibit the oxidation of the oxidative-labile UFA more than that of the oxidative-stable SFA. Therefore, it is possible that increased dietary Se and vitamin E should increase tissue and milk UFA and decrease SFA contents. Barja et al. (1996) ascribed the increased PUFA levels observed in the liver of guinea pigs treated with vitamin E to a protective effect of vitamin E against PUFA peroxidation. Clement and Bourre (1993) suggested that vitamin E deficiency may alter the enzymatic activities of chain elongation and desaturation. Dodge et al. (1999) indicated that Se plays a unique role in influencing the lipid content of human breast milk, which increased the concentration of PUFA (41%) and decreased the concentration of SFA (11%) in human milk. In the present study, although vitamin E had a limited effect on the composition of fatty acids in milk, HSe numerically increased the proportion of the sum of UFA and significantly decreased the proportion of the sum of SFA. The proportion of the sum of UFA was highest and the proportion of the sum of SFA was lowest in milk fat for cows fed HSeVE, which confirms their synergistic relationship. This implies that Se exerted potent effects on UFA, protecting them from oxidation and leading to increased concentrations in milk. The protective effect was found to be more obvious when Se was combined with vitamin E in the bovine diet. Therefore, our results showed that Se, to a certain extent, modified the composition of fatty acids in bovine milk when fat diets were fed. Furthermore, fat diets supplemented with Se and vitamin E are more beneficial in improving the composition of fatty acids in milk.

CLA, as one of the UFA, has received much attention because several positive responses have been discovered, including reduced tumorigenesis, decreased atherogenesis, enhanced immune response, increased feed efficiency and reduced body fat (Pariza et al., 2001). In our research, the yield of *cis*-9, *trans*-11 CLA significantly increased when 10,000 IU/cow d vitamin E (HVE and HSeVE groups) were fed. This increase was related to the increased milk fat percentage. In the bovine mammary gland, *trans*-11 C18:1 could be a substrate for endogenous synthesis of *cis*-9, *trans*-11 CLA via Δ 9-desaturase (Bauman et al., 2001). The proportion of *cis*-9, *trans*-11 CLA in milk fat did not alter due to the similar level of *trans*-11 C18:1 in milk fat for all treatments.

Antioxidant status

Se and vitamin E are important components of the antioxidant defense system of tissues and cells. Se is an integral component of GSH-Px, which functions to convert hydrogen peroxide to water and lipid hydroperoxides to the corresponding alcohol (Lyons et al., 2007). Vitamin E, as the primary lipid-soluble antioxidant, can quench peroxidation reactions in membranes. They work together to maintain low amounts of free radicals that are extremely unstable and react with proteins, lipids, carbohydrates and nucleic acids (Solomons and Fryhle, 2002). In the present study, Se supplementation increased the activity of GSH-Px in blood. In addition, peroxidation status was also assessed by investigating TBARS in plasma. TBARS content represents the compromised outcome of whole blood resistance to free radical aggression and was measured to estimate the amount of lipid peroxidation products in blood. Interestingly, antioxidant status in plasma of cows fed LSe and LVE showed no change, but GSH-Px activity increased and TBARS content was reduced in plasma of cows fed LSeVE. This result implied that there was a synergistic relationship between Se and vitamin E which improved antioxidant status in blood.

Higher concentration of Se in plasma and milk was obtained with Se supplementation in the current study, which is consistent with results of other studies (Ortman and Pehrson, 1999; Shinde et al., 2007). Se supplementation increased the activity of GSH-Px in blood, which suggested that Se increased the antioxidant status. Tissue concentrations of Se are highly correlated with GSH-Px activity and directly related to Se intake. Ortman and Pehrson (1999) showed that cows supplemented with 3 mg of Se daily had significantly higher activity of GSH-Px in erythrocytes than non-supplemented cows.

Vitamin E concentration in plasma and milk was significantly higher in HVE and HSeVE groups than in other groups. As confirmed by other studies (Panda et al., 2006; Samanta et al., 2007; Shinde et al., 2007), vitamin E supplement could effectively increase vitamin E status in plasma and milk. However, vitamin E concentration in plasma and milk was not affected by supplementation with vitamin E at 5,000 IU/cow d in LVE and LSeVE groups. The data showed that increasing vitamin E concentration in plasma and milk might need more dietary vitamin E when

cows were fed a high-oil diet.

CONCLUSION

Overall, this study demonstrated that supplementation with Se and vitamin E alleviated milk fat depression and improved antioxidant status in blood when cows were fed a fat diet. Moreover, Se combined with vitamin E increased the proportion of the sum of UFA and decreased the proportion of the sum of SFA and lead to improved nutritional quality of milk fat.

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