Effects of Silage Species and Supplemental Vitamin E on the Oxidative Stability of Milk

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ABSTRACT

Two experiments were conducted to study the effects of feeding legume silages and providing supplemental vitamin E in concentrates on the oxidative stability of milk. In experiment 1, six multiparous Holstein-Friesian cows were offered 1 of 6 silage treatments in a cyclical changeover-design experiment, with four 4-wk periods. The silages were grass, red clover, white clover, alfalfa, grass and red clover mixture (50:50 on a DM basis), and grass and white clover mixture (50:50 on a DM basis). In experiment 2, 8 cows were used in a changeover-design experiment with three 4-wk periods. The 4 treatments were a factorial combination of forages (grass silage or red clover silage) and supplemental vitamin E in the form of all-rac- α -tocopheryl acetate (29 or 290 IU/kg of DM in the concentrate). All forages were offered *ad libitum* and a flat rate of concentrates (8 kg/d) was fed in both experiments. Red clover silage led to significantly higher forage intakes, milk yields, and milk protein percentage in experiment 2, which was in agreement with results from experiment 1. There was no effect of vitamin E on feed intake, milk production, or milk fat and protein percentage. Red clover silage also led to significant changes in milk fatty acid profiles, particularly increased levels of polyunsaturated fatty acids. Milk samples were stored at 4°C and 20°C and analyzed for α -tocopherol and thiobarbituric acid reactive substances at intervals to determine oxidative stability. Diets based on red clover and alfalfa silages were associated with more rapid loss of α -tocopherol and increased production of thiobarbituric acid reactive substances during the storage of milk in comparison with diets based on grass silage. The increased oxidative deterioration of milk produced from cows fed red clover silage was avoided by vitamin E supplementation.

(**Key words:** legume silage, milk oxidation, red clover, vitamin E)

Abbreviation key: AS = alfalfa silage, GRCS = mixture of grass silage and red clover silage (50:50 on a DM basis), GWCS = mixture of grass silage and white clover silage (50:50 on a DM basis), GS = grass silage, GS+ = grass silage + supplemental vitamin E, PUFA = polyunsaturated fatty acids, RCS = red clover silage, RCS+ = red clover silage + supplemental vitamin E, TBARS = thiobarbituric acid reactive substances, WCS = white clover silage.

INTRODUCTION

Legumes are important components of ruminant diets in many parts of the world. They are a vital part of organic (ecological) dairy systems, where they provide the major source of N for the system. Earlier studies have established the high intake characteristics and milk production potential of red clover silage (RCS) in comparison with grass silage (GS; Thomas et al., 1985), alfalfa silage (AS; Broderick et al., 2000, 2001), and corn silage (Hazard et al., 2001).

This article considers the effect of RCS on aspects of milk quality. There have been substantial improvements in the hygienic and keeping qualities of milk over the last century, and the market now expects milk with a long shelf life. The growing importance of extended shelf-life milk products means that this requirement will increase. Recent results from our group (Dewhurst et al., 2003a) showed that feeding RCS led to increased levels of polyunsaturated fatty acids (PUFA), particularly α -linolenic acid, in milk. This is a beneficial effect because milk is criticized for its low PUFA and high saturated fatty acid contents in relation to targets for the human diet (Department of Health, 1994). In addition to fish products, ruminant products are one of the few sources of beneficial omega-3 PUFA, which are increasingly regarded as being deficient in the human diet (Simopoulos, 2001). However, this change in fatty acid composition could also have a negative effect on

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milk, with PUFA making milk more susceptible to oxidation (e.g., Charmley and Nicholson, 1994).

The objective of this work was to confirm the effect of RCS on milk fatty acid profiles and investigate effects on the oxidative stability of milk produced from RCS. The use of supplemental vitamin E (in the concentrates) to overcome problems with RCS feeding was also investigated.

MATERIALS AND METHODS

Two experiments were conducted to investigate the effects of legume silages on the oxidative stability of milk. Milk was taken from 2 feeding experiments, 1 of which was described previously (Dewhurst et al., 2003b) and used for evaluations of oxidative stability. Oxidative stability was assessed according to the loss of α -tocopherol and appearance of thiobarbituric acid reactive substances (TBARS) during storage at 4°C or 20°C, for 0, 48, and 96 h, respectively.

Animal Feeding Experiments

Experiment 1. The feeding experiment has been described previously (Dewhurst et al., 2003b). Six multiparous Holstein-Friesian cows (mean initial DIM = 76, SD = 36.0) were allocated to 6 forage treatments according to a 4-period cyclical changeover design with 4-wk periods (Davis and Hall, 1969). The forage treatments were GS, RCS, white clover silage (WCS), AS, and 50:50 (DM basis) mixtures of GS and RCS (GRCS) and GS and WC (GWCS) prepared as big bales. All cows received 8 kg/d of a standard concentrate, which contained (as mixed) 30.0% wheat, 15.0% palm kernel expeller, 14.0% corn gluten feed, 11.0% solvent-extracted rapeseed meal (low glucosinolate, low erucic acid), 9.0% solvent-extracted sunflower meal, 5.0% molasses, 5.0% expeller linseed meal, 5.0% groundnut meal, 2.0% solvent-extracted soybean meal, 1.5% vegetable fat, and 2.5% minerals/vitamins. The mineral/vitamin premix supplied (on a concentrate DM basis): 11,600 IU of vitamin A/kg, 2300 IU of vitamin D₃/kg, 35 mg of Cu/kg, 140 mg of Mn/kg, 0.46 mg of Se/kg, and 14 mg/kg of Zn. In addition to the recordings of milk yield and composition already reported (Dewhurst et al., 2003a), samples of evening and morning milk were also taken from each cow during the final week of each period and used immediately for the storage experiments described below. Blood samples were collected from the tail vessels at the end of wk 4 in each period.

Experiment 2. Eight multiparous Holstein-Friesian cows (initial DIM = 77, SD = 3.2) were randomly assigned to 4 dietary treatments according to a 3-period

changeover design (2 incomplete Latin squares) with 4-wk periods. The 4 treatments were a factorial arrangement of forage types (GS or RCS) and concentration of vitamin E in the concentrates (29 or 290 IU of vitamin E/kg of DM in the form of all-*rac*- α -tocopheryl acetate). Thus there were 4 treatments, designated: GS, RCS, grass silage + supplemental vitamin E (GS+), red clover silage + supplemental vitamin E (RCS+).

All cows received 8 kg/d of the standard concentrate (as experiment 1 apart from the different concentrations of vitamin E). Concentrates were offered through out-of-parlor feeders with 4 meals spaced at least 4.5 h apart. The silages were prepared from single cuts of ryegrass and red clover during 1999. The cows were housed in a free-stall barn and had ad libitum access to the forages through roughage intake control feeders (Insentec B.V., Marknesse, The Netherlands). Feed intake and milk yields were recorded continuously, with results from the final week of each period used in the statistical analysis. Consecutive evening and morning milk samples were taken from each cow for analysis of fat, protein, and lactose concentrations by infrared milk analyzer (Milkoscan 605; Foss Electric, Hillerød, Denmark). Bulked (by volume) samples were also collected and stored frozen for subsequent fatty acid analysis. Samples of evening and morning milk were also used immediately in the storage experiments described below. Blood samples were collected from the tail vessels at the end of wk 4 in each period.

Milk Stability Experiments

Milk stability experiments were conducted using bulked samples of evening and morning milk taken from each cow. Twenty-milliliter aliquots of each milk sample were stored in glass tubes with teflon-lined screw caps at either 4°C or 20°C and held in the dark for periods of 0, 48, and 96 h (1 tube for each incubation duration). At the end of these incubations, samples were analyzed for content of α -tocopherol and TBARS.

Chemical Analysis

Methods of feed processing and chemical analysis in our laboratory have been described previously (Dewhurst et al., 2000). Concentrations of fatty acids in samples of feeds and milk were analyzed according to the methods described by Sukhija and Palmquist (1988). The concentration of α -tocopherol in silages was determined according to McMurray et al. (1980). α -Tocopherol was extracted and analyzed according to Hidirgölu (1989) for milk and a modification of Hoelher et al. (1998) for plasma (with plasma, deionized water, internal standard in ethanol, ethanol, hexane, and

Table 1. Chemical composition of the silages used in Exp. 2 (% of oven DM, unless stated otherwise).

Item	Grass silage	Red clover silage
Oven DM (%)	20.7	21.1
pH	4.2	4.5
OM	90.5	88.5
CP	19.9	20.2
Ammonia N (% of total N)	12.6	15.3
Ether extract	4.62	4.32
Water-soluble carbohydrates	0.86	0.71
NDF	56.3	50.5
ADF	34.5	37.0
Lactic acid	7.17	7.04
Acetic acid	1.37	2.54
n-Butyric acid	0.71	1.24
α -Tocopherol (mg/kg of DM)	17.1	20.7
Fatty acids		
Total	1.39	1.40
C _{16:0}	0.36	0.37
C _{18:2}	0.20	0.26
C _{18:3}	0.67	0.58

methanol used in the ratios 1:1:1:4:3:3). Thiobarbituric acid-reactive substances in milk were determined according to Vyncke (1975) and are expressed as malondialdehyde equivalents ($\mu\text{g/mL}$) using 1,1,3,3-tetraethoxypropane as a standard.

Statistical Analysis

Data were analyzed using REML (Genstat 5; Lawes Agricultural Trust, 1997). Preliminary overall REML analysis (storage time $\times$ storage temperature $\times$ diet) showed that there were highly significant effects ($P < 0.001$) of storage time and storage temperature, as well as interactions with the effects of diets. Further analysis was conducted using values for each time and temperature combination separately. A fixed effect of diet and a random effect of period + cow were used for experiment 1. A Student-Newman-Keuls test was used to compare treatment means when the overall analysis showed a significant effect of diet. For experiment 2, the model used forage, vitamin E, and their interaction as fixed effects and period + cow as random effects.

RESULTS

Feed Composition

Experiment 1. Details of feed composition were presented earlier (Dewhurst et al., 2003a). The concentration of α -tocopherol in GS, RCS, WCS, and AS was 10.7, 16.5, 18.6, and 11.3 mg/kg of DM, respectively.

Experiment 2. The results of the chemical analysis of the silages used in experiment 2 are shown in Table 1. The silages were typical of moderate fermentation

quality silages, although the N content of the grass silage was at the high end of the normal range. The fatty acid composition of the 2 silages was very similar, with slightly higher concentrations of C_{18:3} in the grass silage. The concentrate used in experiment 2 contained 4.48% of DM as fatty acids, with 0.83% as C_{16:0}, 1.57% as *cis*-9 C_{18:1}, 1.18% as C_{18:2}, and 0.15% as C_{18:3}. The concentrations of α -tocopherol in GS and RCS are given in Table 1.

Milk Production

Feed intake and milk production results from experiment 2 are shown in Table 2, with detailed fatty acid composition of the milk given in Table 3. Milk fatty acids are presented as proportions of reported fatty acids. Corresponding results for experiment 1 were presented previously (Dewhurst et al., 2003a, 2003b); it is important to note that there was no significant effect of diet on milk fat percentage.

Plasma α -Tocopherol

The effects of forage and supplemental vitamin E treatments on concentrations of α -tocopherol in plasma are given in Tables 4 and 5 for experiments 1 and 2, respectively.

Milk Storage Experiments

Concentrations of α -tocopherol and TBARS (expressed as malondialdehyde equivalents [$\mu\text{g/mL}$] using a 1,1,3,3-tetraethoxypropane standard) in fresh and stored milk are shown in Tables 4 and 6, respectively, for experiment 1, and Tables 5 and 7, respectively, for experiment 2.

DISCUSSION

Feed Intake and Milk Production

Increased voluntary forage (and total) intake and milk production for cows offered RCS rather than GS in experiment 2 are consistent with earlier observations (Thomas et al., 1985; Dewhurst et al., 2003a). Earlier studies showed no effect (e.g., Thomas et al., 1985) or a slight reduction (Hoffman et al., 1998) in milk protein percentage when feeding RCS in place of GS. By contrast, in the current experiment, RCS led to a significant increase in milk protein percentage. It seems likely that the increase in milk protein percentage reflects the improved energy status of cows resulting from the 35% increase in forage voluntary intake. Milk protein

Table 2. Effects of legume silages and supplementary vitamin E on feed intake, milk production, and milk composition in Exp. 2.

Item	Dietary treatment ¹				SED (crop)	Significance ²		
	GS	GS+	RCS	RCS+		Crop	Vitamin E	Interaction
Silage DM intake (kg/d)	9.2	9.5	12.7	12.8	0.33	***	NS	NS
Total DM intake (kg/d)	15.9	16.4	19.4	19.7	0.33	***	NS	NS
α -Tocopherol intake (mg/d)	153	1080	262	1182	10.5	***	***	NS
Milk yield (kg/d)	23.1	23.7	24.9	25.1	0.783	*	NS	NS
Milk fat, %	3.68	3.73	3.78	3.47	0.179	NS	NS	NS
Milk protein, %	2.90	2.87	2.94	2.99	0.012	***	NS	***
Milk lactose, %	4.56	4.58	4.55	4.57	0.029	NS	NS	NS
Milk fat yield, g/d	844	867	922	857	32.6	NS	NS	NS
Milk protein yield, g/d	671	676	732	747	22.4	**	NS	NS
Milk lactose yield, g/d	1058	1082	1145	1151	37.6	*	NS	NS
Milk α -tocopherol yield (mg/d)	7.93	15.95	6.93	15.55	0.781	NS	***	NS

* $P < 0.05$.** $P < 0.01$.*** $P < 0.001$.¹GS = grass silage, RCS = red clover silage, GS+ = grass silage + vitamin E, RCS+ = red clover silage + vitamin E.²NS = not significant. SED = standard error of the difference.

percentage was particularly low for cows offered GS, suggesting that low energy status was a dominant issue in determining production responses in this study (Thomson et al., 1985).

Milk Fatty Acids

The 30% increase in the concentration of C_{18:2} in milk fat and the doubling in concentrations of C_{18:3} when

Table 3. Effects of legume silages and supplementary vitamin E on the fatty acid composition of milk (% total milk fatty acids; Exp. 2).¹

Fatty acid	Dietary treatment ²				SED (crop)	Significance ³		
	GS	GS+	RCS	RCS+		Crop	Vitamin E	Interaction
C _{4:0}	4.89	5.00	5.26	5.29	0.096	***	NS	NS
C _{6:0}	2.60	2.50	2.63	2.58	0.054	NS	NS	NS
C _{8:0}	1.22	1.11	1.19	1.15	0.031	NS	*	NS
C _{10:0}	2.48	2.24	2.34	2.24	0.073	NS	*	NS
C _{12:0}	3.06	2.81	2.89	2.79	0.069	NS	*	NS
C _{14:0}	10.60	10.22	10.53	10.15	0.153	NS	*	NS
C _{14:1}	0.73	0.80	0.73	0.74	0.053	NS	NS	NS
C _{15:0}	1.07	1.11	1.19	1.19	0.025	***	NS	NS
<i>anteiso</i> C _{15:0}	0.40	0.39	0.42	0.41	0.009	*	NS	NS
<i>iso</i> C _{15:0}	0.21	0.21	0.19	0.18	0.004	***	NS	NS
C _{16:0}	31.0	30.7	30.5	30.7	0.54	NS	NS	NS
C _{16:1}	1.16	1.25	1.20	1.25	0.084	NS	NS	NS
C _{17:0}	0.50	0.50	0.58	0.57	0.015	***	NS	NS
<i>anteiso</i> C _{17:0}	0.34	0.33	0.37	0.35	0.010	**	NS	NS
<i>iso</i> C _{17:0}	0.29	0.28	0.27	0.25	0.006	***	NS	NS
C _{18:0}	12.59	12.18	11.88	11.98	0.485	NS	NS	NS
Total C _{18:1} (excluding C _{18:1 trans-11})	23.2	24.6	23.5	23.8	0.35	NS	*	NS
C _{18:1 trans-11}	1.31	1.28	1.16	1.16	0.044	**	NS	NS
CLA (<i>cis</i> -9, <i>trans</i> -11)	0.45	0.47	0.40	0.38	0.014	***	NS	NS
CLA (<i>trans</i> -10, <i>cis</i> -12)	0.026	0.031	0.027	0.031	0.0029	NS	NS	NS
C _{18:2}	1.24	1.12	1.54	1.51	0.034	***	NS	NS
C _{18:3}	0.48	0.46	0.92	0.94	0.019	***	NS	NS
C _{20:0}	0.21	0.22	0.20	0.23	0.007	NS	*	NS
C _{20:1}	0.08	0.09	0.08	0.10	0.002	***	***	NS

* $P < 0.05$.** $P < 0.01$.*** $P < 0.001$.¹Total fatty acids are all those listed in this table.²GS = grass silage, RCS = red clover silage, GS+ = grass silage + vitamin E, RCS+ = red clover silage + vitamin E.³NS = not significant. SED = standard error of the difference.

Table 4. Effects of legume silages on the concentration of α -tocopherol ($\mu\text{g/mL}$) in plasma and in milk after storage at 4°C or 20°C for 48 or 96 h (Exp. 1).¹

Item	GS	GRCS	RCS	GWCS	WCS	AS	SED	Significance ²
Plasma	2.05 ^{ab}	1.51 ^{ab}	2.24 ^{ab}	2.58 ^b	3.00 ^b	0.98 ^a	0.412	***
Milk								
0 h	0.368	0.380	0.268	0.384	0.413	0.299	0.0790	NS
48 h at 4°C	0.290 ^b	0.302 ^b	0.224 ^{ab}	0.300 ^b	0.365 ^b	0.120 ^a	0.0708	*
96 h at 4°C	0.210	0.176	0.132	0.134	0.231	0.105	0.0489	NS
48 h at 20°C	0.181 ^b	0.196 ^b	0.092 ^a	0.142 ^{ab}	0.167 ^{ab}	0.134 ^{ab}	0.0308	**
96 h at 20°C	0.142 ^b	0.110 ^{a,b}	0.041 ^a	0.087 ^{ab}	0.122 ^b	0.067 ^{ab}	0.0309	**

* $P < 0.05$.** $P < 0.01$.¹GS = grass silage, RCS = red clover silage, WCS = white clover silage, AS = alfalfa silage, GRCS = mixture of GS and RCS (50:50 DM basis), GWCS = mixture of GS and WCS (50:50 DM basis).²NS = not significant. SED = standard error of the difference.^{a,b}Values in the same row with different superscripts are significantly different at the 5% confidence level (Student-Newman-Keuls test).

replacing GS with RCS are consistent with earlier results from this laboratory (Dewhurst et al., 2003a). Similarly, feeding RCS led to lower concentrations of biohydrogenation intermediates ($\text{C}_{18:1}$ *trans*-11 and conjugated linoleic acid [*cis*-9, *trans*-11]) in milk.

The increased concentrations of $\text{C}_{18:3}$ in milk from cows offered RCS occurred despite the slightly lower concentrations of $\text{C}_{18:3}$ in RCS in comparison with GS. In fact, the apparent recovery of $\text{C}_{18:3}$ from feed into milk increased from 5.4% for GS to 9.6% for RCS. The increased apparent recovery of $\text{C}_{18:3}$ into milk and lower levels of biohydrogenation intermediates with RCS confirm the reduction in biohydrogenation noted previously in this laboratory (Dewhurst et al., 2003b; Lee et al., 2003).

α -Tocopherol in Forage, Plasma, and Milk

Concentrations of α -tocopherol were higher in both of the clover silages (RCS and WCS) in comparison with

GS and AS in experiment 1. This pattern was not fully reflected in plasma concentrations of α -tocopherol: feeding white clover silage led to the highest concentrations, while concentrations were significantly lower when feeding AS. Concentrations of α -tocopherol in plasma were similar for cows fed RCS or GS, despite the higher intakes and higher concentrations of α -tocopherol in RCS in experiment 2. The doubling in plasma α -tocopherol in response to supplemental vitamin E was highly significant, but proportionately much less than the increase in vitamin E intake (approximately 5-fold). Other workers have shown that vitamin E supplementation increased plasma and milk α -tocopherol, but only to a limited extent (e.g., St-Laurent et al., 1990; Jackson et al., 1997).

The concentration of α -tocopherol in fresh milk samples generally increased in line with concentrations in plasma (Tables 4 and 5). Cows fed RCS were an exception to this relationship in both experiments, with lower concentrations of α -tocopherol in milk relative to

Table 5. Effects of silage crop (grass vs. red clover) and supplemental vitamin E on the concentration of α -tocopherol ($\mu\text{g/mL}$) in plasma and in milk after storage at 4°C or 20°C for 48 or 96 h (Exp. 2).

Item	Dietary treatment ¹				SED (crop)	Significance ²		
	GS	GS+	RCS	RCS+		Crop	Vitamin E	Interaction
Plasma	2.46	4.94	2.45	4.75	0.164	NS	***	NS
Milk								
0 h	0.336	0.668	0.281	0.611	0.0346	*	***	NS
48 h at 4°C	0.252	0.429	0.217	0.443	0.0389	NS	***	NS
96 h at 4°C	0.206	0.293	0.126	0.283	0.0184	*	***	NS
48 h at 20°C	0.131	0.193	0.087	0.183	0.0110	**	***	NS
96 h at 20°C	0.085	0.126	0.037	0.110	0.0066	***	***	***

* $P < 0.05$.** $P < 0.01$.*** $P < 0.001$.¹GS = grass silage, RCS = red clover silage, GS+ = grass silage + vitamin E, RCS+ = red clover silage + vitamin E.²NS = not significant. SED = standard error of the difference.

Table 6. Effects of legume silages on the concentration of thiobarbituric acid-reactive substances (expressed as malondialdehyde equivalents in $\mu\text{g/mL}$) in milk after storage at 4°C or 20°C for 48 or 96 h (Exp. 1).¹

Item	GS	GRCS	RCS	GWCS	WCS	AS	SED	Significance ²
0 h	0.3	0.0	0.8	0.8	0.5	0.3	0.38	NS
48 h at 4°C	4.5	4.8	4.5	3.8	3.0	4.8	1.16	NS
96 h at 4°C	7.6	7.6	8.4	6.2	7.7	7.5	1.17	NS
48 h at 20°C	7.8 ^{ab}	7.5 ^{ab}	10.2 ^b	5.7 ^a	6.3 ^{ab}	8.9 ^b	1.19	**
96 h at 20°C	13.0	13.3	15.0	11.8	14.5	13.0	1.76	NS

** $P < 0.01$.

¹GS = grass silage, RCS = red clover silage, WCS = white clover silage, AS = alfalfa silage, GRCS = mixture of GS and RCS (50:50 DM basis), GWCS = mixture of GS and WCS (50:50 DM basis). ²NS = not significant. SED = standard error of the difference.

^{a,b}Values in the same row with different superscripts are significantly different at the 5% confidence level (Student-Newman-Keuls test).

plasma concentrations. This suggests that the increased oxidation in these milks started—either in the udder, the collection vessels, or both—before milk samples were analyzed. Similarly, a smaller proportion of α -tocopherol intake was yielded in milk when feeding RCS (2.7%) than when feeding GS (5.2%). This effect may also reflect greater oxidation of α -tocopherol within the animal and its tissues.

Oxidative Stability of Milk

Diets based on either RCS or AS led to increased levels of TBARS and reduced concentrations of α -tocopherol in stored milks. Concentrations of α -tocopherol were numerically lower in fresh milk from cows fed RCS or AS, although this difference was not statistically significant owing to large variability. Charmley and Nicholson (1994) also found that significant differences in concentrations of α -tocopherol developed during milk storage. Conversely, there were no significant differences in TBARS for milk stored for 96 h at 20°C: this is in agreement with the observations of Lundin and Palmquist (1983) and reflects the fact that all of the milks had deteriorated extensively by this stage.

The consistent and highly significant increase in concentrations of PUFA in milk from cows fed RCS provides an explanation for the reduced oxidative stability of milk. Many earlier studies have shown that increasing concentrations of unsaturated fatty acids in milk, particularly of PUFA C_{18:2} and C_{18:3}, will increase the susceptibility of milk to oxidation (e.g., Sidhu et al., 1975; Granelli et al., 1998; Timmons et al., 2001).

Supplemental vitamin E led to production of milk from RCS-based diets with the same oxidative stability as was observed for milk from GS-based diets. The effects of supplemental vitamin E on TBARS in stored milk are in agreement with earlier studies (e.g., Lundin and Palmquist, 1983; Atwal et al., 1991).

Legumes are important components of dairy systems where farmers prefer not to use N fertilizer, such as organic (ecological) systems or in regions where N fertilizer is not available at an economic price. Consequently, there may be a need to look for alternative solutions to the problem of milk oxidation for use with organic dairy systems, which do not permit the use of supplemental vitamin E for this purpose. However, results with the mixed forage diet (GRCS) suggest that reduced oxidative stability of milk may not be a major problem with

Table 7. Effects of silage crop (grass vs. red clover) and supplemental vitamin E on the concentration of thiobarbituric acid-reactive substances (expressed as malondialdehyde equivalents in $\mu\text{g/mL}$) in milk after storage at 4°C or 20°C for 48 or 96 h (Exp. 2).

Item	Dietary treatment ¹				SED (crop)	Significance ²		
	GS	GS+	RCS	RCS+		Crop	Vitamin E	Interaction
0 h	0.33	0.20	0.65	0.28	0.020	***	***	***
48 h at 4°C	4.52	3.25	4.23	3.25	0.106	NS	***	NS
96 h at 4°C	7.39	5.98	7.90	6.43	0.137	***	***	NS
48 h at 20°C	7.33	6.00	10.04	6.30	0.299	***	***	***
96 h at 20°C	13.02	10.77	15.54	12.39	0.729	***	***	NS

¹GS = grass silage; RCS = red clover silage; GS+ = grass silage + vitamin E; RCS+ = red clover silage + vitamin E.

²NS = not significant. SED = standard error of the difference.

*** $P < 0.001$.

these mixed forage diets that are more typical of organic farms.

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