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Effects of Feeding a Dietary Antioxidant in Diets with Oxidized Fat on Lactation Performance and Antioxidant Status of the Cow

M. Vázquez-Añón,*[1] J. Nocek,† G. Bowman,* T. Hampton,* C. Atwell,* P. Vázquez,‡ and T. Jenkins§
*Novus International Inc., St. Louis, MO 63147
†Spruce Haven Research Center, Auburn, NY 13021
‡Universidad de Santiago, Lugo, Spain 27002
§Clemson University, Clemson, SC 29634

ABSTRACT

The objective of the study was to evaluate the effect of feeding the dietary antioxidant Agrado Plus (AOX; Novus International, St. Louis, MO) in diets that contained 2% fresh fat (FF) or oxidized fat (OF) on milk production and composition and antioxidant status of cows during mid to late lactation. Forty-four mid to late lactating primiparous cows housed in a tie-stall barn were fed a diet that contained 2% FF for 15 d as adaptation period and then randomly allocated to 1 of the 4 dietary treatments (FF, FF+AOX, OF, OF +AOX) for 6 wk. Feeding AOX increased dry matter intake, 3.5% fat-corrected milk, and milk fat yield, and decreased milk protein content but not yield. Feeding OF increased milk fat yield, but decreased dry matter intake and body weight gain. Milk fat composition changed with treatments: AOX increased *cis* 18:1 and decreased *trans*-11 18:1, whereas OF decreased *trans*-9 and *trans*-11 18:1 and *cis*-9, *trans*-11 18:2 in milk. Plasma antioxidant enzymes and status were affected by treatments. Feeding OF increased superoxidase dismutase activity but decreased plasma antioxidant status, whereas AOX supplementation increased glutathione peroxidase activity across fat types and increased the antioxidant status and superoxidase dismutase activity when feeding OF diets. It can be concluded that feeding AOX improved lactation performance and the antioxidant status of the cow across fat types, and feeding OF increased milk fat yield but decreased dry matter intake, body weight gain, and antioxidant status. The negative effects of feeding OF were partially alleviated by AOX.

Key words: oxidized fat, dietary antioxidant, Agrado Plus

INTRODUCTION

In the body there is a natural balance between the formation of free radicals during the normal metabolism of the cells and the endogenous antioxidant capacity of the animal that would prevent free radicals from accumulating and harming the cells. However, the level of free radicals can exceed the antioxidant capacity of the animal leading to oxidative stress (Miller and Brezeinska-Slebodizinska, 1993; Weiss, 1998). High-producing dairy cows are prone to oxidative stress, and the situation can be exacerbated under certain environmental, physiological, and dietary conditions (Bernabucci et al., 2002, 2005; Castillo et al., 2005; Lohrke et al., 2005). Generation of free radicals during peroxidation of essential fatty acids in the lipid membranes can damage cells and impair the production and health status of the animal (Miller and Brezeinska-Slebodizinska, 1993). Dietary lipids such as supplemental fat, oil seeds, and distiller grains, if not stabilized, can be significant contributors to the load of free radicals in the animal (Andrews et al., 2006). Decreased performance, increased gut turnover, and compromised immune response have been reported in production animals fed oxidized fat (Cabel et al., 1988; Dibner et al., 1996). Inclusion of dietary antioxidants ameliorates these negative effects by scavenging peroxides and reducing peroxidation of fatty acids (Frankel, 2005). Feeding oxidized fat not only increases the load of peroxides to the animal, but can also negatively affect rumen fermentation by reducing microbial protein yield and efficiency (Vázquez-Añón and Jenkins, 2007). The negative ruminal effect of feeding OF was partially alleviated when adding an antioxidant in the diet. Feeding an antioxidant also increased fiber digestibility in fresh and oxidized fat, suggesting an overall positive effect of antioxidants on rumen microflora. Feeding 50 ppm of Agrado (Agrado is a trademark of Novus International Inc. and is registered in the United States and other countries) increased milk yield and efficiency as well as OM digestibility in the rumen (Smith et al., 2002). The objective of the study

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[1]Corresponding author: Mercedes.vazquez@novusint.com

Table 1. Ingredient and nutrient composition (% DM basis) of treatment diets

	Treatment[1]			
Item	FF	FF + AOX	OF	OF + AOX
Corn silage	47.07	47.07	47.07	47.07
Haylage	10.51	10.51	10.51	10.51
Fine ground corn	10.51	10.51	10.51	10.51
Soybean meal	12.61	12.61	12.61	12.61
Citrus pulp	8.40	8.40	8.40	8.40
Corn distillers	2.18	2.18	2.18	2.18
SoyPlus[2]	1.47	1.47	1.47	1.47
Corn gluten meal	0.43	0.43	0.43	0.43
Fishmeal	0.44	0.44	0.44	0.44
Bloodmeal	0.44	0.44	0.44	0.44
Urea	0.24	0.24	0.24	0.24
Celmanax[3]	0.19	0.19	0.19	0.19
Alimet[4]	0.08	0.08	0.08	0.08
Mineral vitamin mix[5]	3.44	3.44	3.44	3.44
Agrado Plus liquid[6]	—	0.02	—	0.02
Fresh soybean oil	2.0	2.0	—	—
Oxidized soybean oil	—	—	2.0	2.0
CP	17.8	18.1	18.0	18.2
Soluble protein (% CP)	38.6	36.3	43.8	37.6
NDF	31.1	31.9	32.1	31.5
ADF	18.8	19.7	18.7	18.8
NSC[7]	29.9	28.8	30.9	30.4
Starch	24.5	23.3	25.5	24.9
Sugar	5.4	5.5	5.4	5.5
Ash	7.6	7.5	7.6	7.5
NFC[8]	38.9	37.7	37.0	38.0
Total fatty acids (FA), % of total FA	4.3	3.8	4.0	4.4
14:0	0.568	0.605	0.623	0.563
16:0	13.04	13.22	13.39	13.31
18:0	3.42	3.41	3.46	3.53
18:1	21.16	20.61	21.61	21.51
18:2	47.89	47.99	46.93	46.56
18:3	8.02	8.42	7.40	7.63
22:0	0.376	0.370	0.371	0.384
24:0	0.073	0.196	0.186	0.201
Unsaturated FA[9]	77.07	77.02	75.95	75.70
Saturated FA[10]	17.48	17.80	18.03	18.02

[1]Treatments: FF = fresh fat; +AOX = with antioxidant; OF = oxidized fat.

[2]West Central (Ralston, IA).

[3]Celmanax (VI-COR, Varied Industries Corporation, Mason City, IA) consists of a preparation of yeast components, yeast culture, yeast extract, and hydrolyzed yeast.

[4]Alimet (Novus International, St Louis, MO) feed supplement contains 88% 2 hydroxy-4-methylthio butanoic acid.

[5]Contained 11.5% Ca, 1.5% P, 5.1% Mg, 10.7% Na, 4.8% Cl, 938 mg/kg Zn, 221 mg/kg Cu, 938 mg/kg Mn, 16 mg/kg Se, 12.8% I, 117,000 IU/kg vitamin A, 23,000 IU/kg vitamin D, and 1250 IU/kg vitamin E.

[6]Agrado Plus Liquid (Novus International). Dietary antioxidant was added to FF+AOX and OF+AOX diets in the form of Agrado Plus Liquid.

[7]Includes starch and sugar.

[8]Calculated NFC.

[9]Unsaturated FA include C18:1, C18:2, and C18:3 (% of total FA).

[10]Saturated FA include: C14:0, C16:0, C18:0, C21:0, C22:0, and C24:0 (% of total FA).

was to evaluate the benefits of feeding a dietary antioxidant in diets that contain fresh or oxidized soybean oil on milk production and antioxidant status of cows during mid to late lactation.

MATERIALS AND METHODS

Treatments and Experimental Design

Forty-four primiparous mid- to late-lactation Holstein cows housed in a tie-stall barn at Spruce Haven Research facility (NY) were randomly assigned to treatments at 171 ± 10 DIM. For the first 15 d of the study, all cows were fed the fresh fat (FF) diet for ad libitum intake (Table 1) that contained 2% fresh nonstabilized soybean oil. During this time, individual daily milk and feed intake were measured and BCS, BW, and blood samples were taken at the end of the adaptation period and served as covariate. Following this period, cows were assigned to the 4 treatments and

Table 2. Fatty acid (FA) composition of the oxidized and fresh soybean oil in the presence and absence of the antioxidant (AOX)

Item, g/100 g of total FA	Treatment[1]			
	FF	FF + AOX	OF	OF + AOX
14:0	0.08	0.08	0.09	0.08
16:0	10.81	10.83	11.36	11.51
18:0	4.22	4.22	4.45	4.49
18:1	21.69	21.75	22.40	22.84
18:2	53.44	53.41	50.10	51.23
18:3	6.80	6.78	5.84	5.90
21:0	0.04	0.04	0.05	0.05
22:0	0.34	0.34	0.36	0.36
24:0	0.13	0.12	0.16	0.16
Other FA	2.47	2.43	5.18	3.37
Total FA, % of DM	90.11	89.61	84.09	84.12

[1]Treatments: FF = fresh fat; +AOX = with antioxidant; OF = oxidized fat.

fed the treatment diets (Table 1) for 6 wk. Treatment assignments were balanced for DIM, milk yield during covariate period, and BCS. The experimental diet consisted of 58% forage and 42% concentrate mixture that contained 2% experimental fat on a DM basis. The diets were formulated using Cornell-Penn-Miner model (Fox et al., 1990) following NRC (2001) recommendations and current industry practices. The experimental fat consisted of nonstabilized soybean oil. Half of the experimental fat was oxidized by bubbling air through the fat at 92°C for 24 h to achieve a peroxide value of 240 mEq/kg (method Cd 12-57; AOCS, 1997). Peroxide values were used to assess the quality and stability of the experimental fats before adding the antioxidant.

The study consisted of 4 treatments: 1) fresh nonoxidized soybean oil (fresh fat, **FF**) added to the diet at 2%; 2) fresh nonoxidized soybean oil added to the diet at 2% plus 200 mg/kg of dietary antioxidant (**FF + AOX**); 3) oxidized soybean oil (oxidized fat, **OF**) added to the diet at 2%; 4) oxidized soybean oil added to the diet at 2% plus 200 mg/kg of dietary antioxidant (**OF**

Table 3. Effect of feeding fresh and oxidized fat in the presence or absence of an antioxidant (AOX) to dairy cows on milk production and constituents

Item	Treatment[1]				SE	*P*-value						
	FF	FF + AOX	OF	OF + AOX		AOX	Fat	AOX × Fat	Week	Week × Fat	Week × AOX	Week × AOX × Fat
Milk, kg/d	27.35	28.12	27.30	28.00	0.36	0.08	0.84	0.92	<0.01	0.99	0.60	0.07
3.5% FCM, kg/d	27.23	27.82	27.30	28.78	0.36	0.01	0.20	0.26	0.04	0.97	0.89	0.32
DMI, kg/d	20.58	20.99	19.90	20.73	0.22	0.01	0.04	0.35	<0.01	0.88	0.91	0.97
Unsaturated FA intake,[2] g/d	635	647	605	628	6.9	0.01	<0.01	0.43	<0.01	0.88	0.92	0.97
Saturated FA intake,[3] g/d	144	150	144	150	1.6	<0.01	0.99	0.87	<0.01	0.89	0.93	0.97
Milk constituent												
Fat, %	3.49	3.47	3.49	3.64	0.06	0.25	0.14	0.14	0.27	0.92	0.87	0.81
Fat yield, kg/d	0.94	0.97	0.96	1.03	0.02	0.01	0.02	0.24	0.22	0.95	0.98	0.25
Protein, %	3.01	2.99	3.04	2.94	0.03	0.03	0.68	0.15	0.15	0.98	0.61	0.59
Protein yield, kg/d	0.81	0.84	0.83	0.82	0.01	0.57	0.92	0.12	<0.01	0.99	0.65	0.92
BCS change[4]	0.062	0.075	0.059	0.003	0.04	0.57	0.34	0.37				
BW change,[5] kg	26.4	28.2	15.2	18.4	4.1	0.54	0.01	0.87				
Energy output,[6] Mcal/d	33.03	33.28	31.82	31.89	0.44	0.71	0.01	0.83	0.46	0.98	0.97	0.73
Energy input, Mcal/d	35.18	35.89	33.85	35.29	0.39	0.01	0.02	0.35	<0.01	0.94	0.90	0.95
Energy balance,[7] Mcal/d	2.13	2.67	2.04	3.35	0.49	0.07	0.55	0.43	0.21	0.89	1.00	0.83

[1]Treatments: FF = fresh fat; +AOX = with antioxidant; OF = oxidized fat.

[2]Unsaturated fatty acids (FA) include isomers C18:1, C18:2, and C18:3.

[3]Saturated FA include: C14:0, C16:0, C18:0, C21:0, C22:0, and C24:0.

[4]BCS (scored on a 5-point scale, where 1 = thin to 5 = obese) at wk 6 minus wk 1; average at wk 1 was 3.60, 3.64, 3.52, and 3.57 for FF, FF+AOX, OF, and OF+AOX, respectively.

[5]BW at wk 6 minus wk 1; average at wk 1 was 615, 633, 620, and 607 for FF, FF+AOX, OF, and OF+AOX, respectively.

[6]Energy output = maintenance energy + gain/loss energy + milk energy.

[7]Energy balance = energy input – energy output.

Table 4. Effect of feeding fresh or oxidized fat in the presence or absence of antioxidants (AOX) to dairy cows on milk fatty acid (FA) composition at the end of the 6-wk trial

	Treatment[1]					*P*-value		
Milk FA, g/100 g of FA	FF	FF + AOX	OF	OF + AOX	SE	AOX	Fat	AOX × Fat
4:0 to 12:0	15.16	14.18	15.17	14.28	0.35	0.02	0.86	0.89
14:0	11.00	10.69	11.06	10.91	0.16	0.16	0.40	0.60
16:0	24.71	24.78	25.70	25.17	0.48	0.64	0.16	0.51
18:0	10.64	10.54	10.09	10.60	0.32	0.52	0.46	0.35
Trans-9 18:1	0.353	0.351	0.323	0.332	0.01	0.76	0.05	0.66
Trans-10 18:1	0.758	0.892	0.661	0.801	0.11	0.22	0.38	0.98
Trans-11 18:1	1.661	1.370	1.263	1.180	0.08	0.04	0.00	0.22
Trans-12 18:1	0.71	0.71	0.70	0.69	0.02	0.95	0.68	0.86
Cis-9 18:1	19.47	20.47	19.31	20.11	0.44	0.05	0.54	0.81
18:2	2.870	2.963	2.967	2.951	0.08	0.64	0.60	0.49
18:3	0.497	0.496	0.510	0.477	0.01	0.83	0.29	0.33
Cis-9, *trans*-11 18:2	0.721	0.642	0.575	0.561	0.03	0.13	<0.01	0.48
Trans-10, *cis*-12 18:2	0.0027	0.0035	0.0026	0.0018	0.002	0.36	0.55	0.79
Trans-9, *trans*-11 18:2	0.058	0.062	0.061	0.057	0.006	0.94	0.91	0.57

[1]Treatments: FF = fresh fat; +AOX = with antioxidant; OF = oxidized fat.

+ AOX). The dietary antioxidant consisted of a liquid blend of ethoxyquin and tertiary-butyl-hydroquinone (Agrado Plus, Novus International, St. Louis, MO). The dietary antioxidant was added to the experimental fat to achieve 200 mg/kg of final diet on a DM basis. Fresh and oxidized soybean oils were stored at –20°C for up to 2 d before feeding.

The amount of feed offered, orts, and a.m. and p.m. milk weights were recorded daily for each cow. Individual milk samples were taken weekly during one 24-h period, composited based upon the amount of milk produced at each milking, and analyzed for milk protein, fat, lactose, and urea by infrared spectrophotometry (DHIA, Ithaca, NY; AOAC, 2000; method 972.16). Milk samples during one 24-h period were taken at the beginning (covariate period) and end (wk 6) of the trial for fatty acids. Fifty-milliliter samples were collected, placed in a tightly sealed container, and frozen for later analysis. Whole milk was centrifuged at 21,000 × *g* for 30 min at 4°C, and the top fat layer was removed for fatty acid analysis. A sample of the fat layer was methylated in 0.5 *M* sodium methoxide in methanol followed by a second methylation in acetyl chloride:methanol (1:10, vol/vol) as described by Kramer et al. (1997). Analysis of milk fatty acids was done on a gas chromatograph (HP5890A GC, Agilent Technologies Inc., Santa Clara, CA) equipped with a flame-ionization detector and a 100-m × 0.25-mm Supelco SP-2560 column. The injector and detector temperatures were held at 250 and 260°C, respectively. The carrier gas was He (20 cm/s) with an inlet pressure of 104 kPa. The column temperature was programmed for starting at initial temperature of 50°C for 4 min, then increased 13°C/min to 165°C for 42 min, and then increased at 4°C/min to 220°C, with the final temperature held for 11 min. Identity of the peaks was determined by comparison of retention times to known standards. Unidentified milk fatty acid peaks were identified based on comparison of retention times to a commercial mixed standard (catalog no. GLC-90, Nu-Chek Prep, Elysian, MN). The individual *trans* 18:1 isomers were identified by comparing to purchased or donated standards. Body weight and BCS were measured at start of the trial and at the end. Dry matter content of the TMR was measured weekly. Concentrate and silage samples were taken every 2 wk, composited monthly, and frozen for later analysis. The source of forage was maintained constant during the entire study. Feed samples were analyzed for CP, NDF, ADF, NSC, ether extract, and fatty acids as described by Vázquez-Añón and Jenkins (2007). Energy balance was calculated as the total energy input minus the total energy output, following the NRC (2001). Total energy input was calculated as DMI × NE_L of the diet; total energy output was calculated as [(BW gain/BW loss) energy + maintenance energy + milk energy]. The energy for BW gain was calculated as 5.12 Mcal/kg of BW, and energy for BW loss was calculated at 4.92 Mcal/kg of BW. Energy for maintenance was calculated as $BW^{0.75} \times 0.08$ (NRC, 2001). Milk energy per day was calculated as milk yield × [(0.0929 × fat) + (0.0547 × protein/0.93) + (0.0395 × lactose)].

Blood samples were taken every 2 wk for evaluation of antioxidant status. Blood samples were taken from each cow via tail vein using heparin plasma tubes at 2 h after feeding, immediately placed on ice, and centrifuged at 1,000 × *g* for 10 min. The supernatant plasma was stored in a freezer for later analysis of

superoxide dismutase (**SOD**) using the assay kit supplied by Cayman Chemical Company (catalog no. 706002, Ann Arbor, MI), total antioxidant status (**TAS**) using the kit supplied by Calbiochem (catalog no. 615700, Darmstadt, Germany), and glutathione peroxidase (**GPX**) using the assay kit supplied by Cayman Chemical Company (catalog no. 703102) and as described by Castillo et al. (2005). Enzyme activity was expressed as units per milligram of plasma protein, which was measured following Bradford (1976) procedure. Daily health status of the cows was monitored during the entire study.

Statistical Analyses

The study was designed as a completely randomized design with repeated measurements with a 2×2 factorial treatment arrangement, where the main effects were type of fat (FF vs. OF) and the presence or absence of AOX. Data were analyzed using the MIXED procedure (SAS Institute, 2003) with cow as the error term to test for main effects and interactions. When measurements were taken over time, repeated measure data were analyzed using the mixed procedure (SAS Institute, 2003) with cow within treatment as the subject and the error term to test for main effects and interaction, and the residual error was used to test for week and week by treatment interaction. Pretreatment measurements were used during analysis of covariate. Significance differences were declared at $P < 0.05$ and trends at P-values ≤ 0.1 and > 0.05. Mean comparisons across treatments were evaluated when the interaction terms of the model were significant ($P \leq 0.05$).

The statistical model used to analyze the lactation performance and plasma parameters was

$$Y = \text{covariate week} + \text{treatment} + \text{cow(treatment)} + \text{week} + \text{week} \times \text{treatment} + \text{residual}.$$

The statistical model used to analyze milk fatty acids and BW gain at the end of the trial was

$$Y = \text{covariate week} + \text{treatment} + \text{residual}.$$

RESULTS

Oxidation of the experimental fat by bubbling air during heating increased the levels of peroxides from 0.5 to 240 mEq/kg of fat and decreased the total fatty acids content of the oil. Specifically, the content of polyunsaturated fatty acids such as 18:2 and 18:3 in the OF vs. FF soybean oil (Table 2) were reduced with a concomitant increase in 18:1, which is one of the end products of oxidation of 18:2 and 18:3. Similar changes were observed in the fatty acids (Table 1) of the OF diets. Loss of polyunsaturated fatty acids during oxidation lead to lesser unsaturated ($P < 0.01$; Table 3) fatty acid intake in cows fed OF diets.

Performance Parameters

Cows responded to feeding AOX by increasing DMI ($P = 0.007$), intake of saturated and unsaturated fatty acids ($P < 0.01$), 3.5% fat-corrected milk ($P = 0.01$), and increasing milk fat yield ($P = 0.01$) as described in Table 3. A trend was observed for milk yield to increase ($P = 0.08$) in the presence of AOX. Feeding AOX decreased milk protein content ($P = 0.03$) but not yield. Although the reduction in milk protein content was significant, the magnitude of the reduction was minor with limited biological meaning. Feeding OF decreased DMI ($P = 0.04$), intake of unsaturated fatty acids ($P < 0.01$), and BW gain ($P = 0.01$) and increased milk fat yield ($P = 0.02$). No significant AOX by type of fat interaction was observed for any of the production parameters; however, the magnitude of the response to AOX for FCM, DMI, and fat yield was numerically greater in cows fed OF (5.4, 4.2, and 7.3%, respectively) compared with FF (2.2, 2.0, and 2.7%, respectively).

Milk ($P = 0.0003$), FCM ($P = 0.04$), DMI ($P < 0.01$), protein yield ($P < 0.01$), and total energy input ($P < 0.01$) were gradually decreased during the 6-wk study (data not shown) as a reflection of the mid to late stage of lactation of the cows used in the trial (171 DIM at start). Feeding AOX increased total energy inputs ($P = 0.01$), which resulted in a trend to improve overall energy balance ($P = 0.07$). Total energy inputs ($P = 0.02$) and outputs ($P = 0.01$) decreased when OF was fed. Milk fat content and yield and protein content did not change over time. No dietary treatment by week interaction was observed for any of the performance parameters.

Fatty Acid Profile in Milk

Changes were observed in the fatty acid profile of milk as described in Table 4. Feeding AOX decreased the concentration of short-chain fatty acids (4:0 to 12:0; $P = 0.02$) and *trans*-11 18:1 ($P = 0.04$) and increased the concentration of *cis* 18:1 ($P = 0.05$) in milk. Feeding OF decreased *trans*-9 ($P = 0.05$) and *trans*-11 18:1 ($P < 0.01$) and *cis*-9 *trans*-11 18:2 ($P < 0.01$). No significant AOX by fat type interaction was observed for any of the fatty acids. When the milk fatty acid profile was expressed in grams per day, smaller differences were observed (data not shown) between treatments, mostly because of the overall response of AOX and OF on milk

fat yield. The effect of AOX in reducing short-chain fatty acids and *trans*-11 18:1 or in increasing *cis* 18:1 yield disappeared, mostly due to its large total milk fat yield response. For OF, its effects at reducing *trans*-9 18:1 disappeared but the reduction of *trans*-11 18:1 and *cis*-9 *trans*-11 18:2 yield was maintained.

Plasma Antioxidant Parameters

Glutathione peroxidase, SOD, and TAS were evaluated every 2 wk to determine the antioxidant status of the cows as described in Table 5. For TAS there was a significant week × AOX × type of fat interaction ($P < 0.01$). Cows fed OF + AOX showed the greatest improvement in TAS over the trial. These animals started the trial with the lowest TAS values but showed the greatest values by the end of the trial, whereas cows fed OF showed a sharp drop in the TAS values during the last week of the study. Plasma GPX activity increased when AOX was added to the diet ($P < 0.01$) but no changes were observed with type of fat or week of trial, whereas plasma SOD activity increased when feeding OF ($P < 0.01$) and was increased further when AOX was added to the OF diet ($P = 0.05$).

DISCUSSION

Dairy cows responded to dietary antioxidants by increasing FCM, milk fat yield, and DMI at the expense of milk protein content regardless of the degree of oxidation of the fat. However, the magnitude of the AOX response was numerically greater in cows fed oxidized compared with fresh fat. The improvements in FCM and milk fat yield might be associated with the ruminal effects of AOX reported recently (Vázquez-Añón and Jenkins, 2007). Feeding the antioxidant Agrado Plus to continuous culture fermenters resulted in increased fiber digestibility and microbial N efficiency independent of the degree of oxidation of the dietary fat. It appears that the ruminal and lactation responses to AOX are independent of the degree of oxidation of the dietary fat. Smith et al. (2003) reported improvements in milk yield and OM digestibility when feeding antioxidants in the form of ethoxyquin but did not report the degree of oxidation or quality of the dietary fat. Other antioxidants such as vitamin E have been reported to improve milk fat content but not milk yield or FCM when fed at high levels. Bell et al. (2006) and Pottier et al. (2006) linked supplementation of tocopherols with lower concentrations of *trans*-10 18:1 in milk and decreased milk fat depression. In the current study, AOX supplementation did not reduce the fatty acids associated with milk fat depression such as *trans*-10, *cis*-12 18:2 (Lock et al., 2007) or *trans*-10

Table 5. Effect of feeding fresh or oxidized fat in the presence or absence of antioxidants (AOX) to dairy cows on total antioxidant status (TAS), glutathione peroxidase (GPX), and superoxide dismutase (SOD) in plasma

		Treatment[1]					*P*-value					
Item	Week	FF	FF + AOX	OF	OF + AOX	SE	AOX	Fat	AOX × Fat	Week	Week × Fat	Week × AOX	Week × AOX × Fat
TAS, m*M*	Overall mean	0.19	0.24	0.15	0.25	0.02	<0.01	0.53	0.27	0.04	0.23	<0.01	<0.01
	Wk 2	0.16^{abc}	0.24^{c}	0.18^{bc}	0.10^{ab}	0.04							
	Wk 4	0.19^{bc}	0.25^{c}	0.20^{bc}	0.22^{c}	0.04							
	Wk 6	0.21^{bc}	0.23^{c}	0.07^{a}	0.42^{d}	0.04							
GPX,[2] units/mg of protein	Overall mean	0.34	0.51	0.43	0.59	0.05	<0.01	0.07	0.96	0.06	0.07	0.34	0.34
SOD,[2] units/g of protein	Overall mean	22.02^{c}	19.34^{bc}	23.74^{ab}	26.35^{a}	8.4	0.55	<0.01	0.05	0.12	0.20	0.53	0.76

$^{a-d}$Means in rows with different superscripts differ, $P < 0.05$.

[1]Treatments: FF = fresh fat; +AOX = with antioxidant; OF = oxidized fat.

[2]Only overall means are reported because of lack of significant week or week by treatment interaction effect.

18:1. Other conjugated linoleic acid isomers associated with milk fat depression such as *cis*-10, *trans*-12 18:2 or *trans*-9, *cis*-11 18:2 were not analyzed in this study. However, in the current study, AOX supplementation increased *cis* 18:1 and decreased *trans*-11 18:1 and short-chain fatty acids but not yield in milk. The greater concentration of *cis*-18:1 in milk might be the result of AOX preserving unsaturated fatty acids from oxidation before its absorption (Andrews et al., 2006) or systemically (Focant et al., 1998) by improving the antioxidant capacity of the animal (see discussion below). Lower concentration of *trans*-11 18:1 coupled with a greater concentration of *cis*-18:1 would also reflect lower ruminal hydrogenation and *trans*-isomerization of 18:1. A 1 percentage unit reduction in the concentration of short-chain fatty acids in milk could reflect lower de novo fatty acid synthesis with AOX; however, it most likely is the result of the 1 percentage unit increase in *cis* 18:1 content without changes in the total milk fat percentage. The mechanism by which AOX improves milk fat yield is not yet clear. Improvements in digestibility of fiber (Vázquez-Añón and Jenkins, 2007) and OM (Smith et al., 2002) together with greater *cis* 18:1 and lower *trans*-11 18:1 in milk would suggest a dual AOX effect: 1) preserving *cis* 18:1 from oxidation in the feed and systemically, and 2) modulating rumen microorganisms toward greater cellulolytic and lower hydrogenation and *trans*-isomerization activity.

Feeding OF to cows decreased DMI in the absence of AOX. The negative effect of feeding OF on feed consumption has been previously reported in other species and has been associated with the rancid smell of the oxidized fat (Cabel et al., 1988; Dibner et al., 1996). In spite of the feed intake reduction, cows fed OF continued to produce the same amount of milk and FCM as cows fed FF but at the expense of BW gain. When energy balance was calculated, cows fed OF were able to maintain positive energy balance by reducing the energy output associated with BW gain and maintaining the energy output due to milk production.

Interestingly, milk fat yield was increased and *trans*-9 and *trans*-11 18:1 and *cis*-9, *trans*-11 18:2 in milk were decreased with OF in the presence or absence of AOX. The surprising effects of OF on milk fat yield and fatty acid composition might be related to the lower content of polyunsaturated fatty acids in the OF oil (Jenkins, 1993) due to loss during oxidation, but also to the direct effects of feeding OF on rumen fermentation. Feeding oxidized fat to continuous culture fermenters has been shown to change the microbial population toward butyrate-producing bacteria that are capable of taking the biohydrogenation process to completion (Vázquez-Añón and Jenkins, 2007). In the current study, the lower content of *trans*-9 and *trans*-11 18:1 and *cis*-9, *trans*-11 18:2 in milk from cows fed OF would suggest greater rates of complete biohydrogenation of unsaturated fatty acids and, therefore, less metabolites that would cause milk fat depression.

Plasma antioxidant enzymes and status of the cows were affected by dietary treatments. By the end of the trial, feeding OF without AOX decreased the total antioxidant status of the cow at the same time as it increased the SOD enzyme activity. Superoxidase dismutase is the first enzyme involved in the conversion of oxygen radicals to peroxides (Yu, 1994). It is possible that its activity was increased by OF as a defense mechanism to reduce the load of oxygen radicals coming from the diet; however, the magnitude of the increase was not enough to maintain the antioxidant status of the cow. Superoxidase dismutase activity was also increased by AOX supplementation, but the effect was dependent on the type of fat fed. Dietary antioxidants increased plasma SOD activity when feeding OF but not with FF. Glutathione peroxidase is the enzyme involved in the second step of removing the peroxides produced by SOD enzyme and converting them into water (Yu, 1994), and its activity was increased by AOX across types of fat fed. The effect of AOX on antioxidant enzymes and status of the cow was most noticeable when feeding OF. Greater SOD and GPX activity with AOX resulted in increased TAS in cows fed OF. It might be required for both enzymes (SOD and GPX) to be active for adequate removal of end products of oxidation and increased antioxidant status of the animal (Collins, 2005). The mechanism by which AOX increases the activity of antioxidant enzymes in plasma is not clear. It is possible that AOX, by removing reactive oxygen molecules and end products of oxidation from the diet and digesta, would reduce the load of peroxides coming into the animal and, consequently, spare the endogenous antioxidant defense system. Previous work with broiler chickens fed oxidized fat (Sui-Ying et al., 1997) showed greater concentrations of the reduced over the oxidized form of glutathione in the duodenum and ileum tissue when the dietary antioxidant ethoxyquin was supplemented in the diet. The authors speculated that ethoxyquin, by removing peroxides from the diet and digesta, would spare the required oxidation of glutathione by GPX in the duodenum and ileum. The mechanism by which dietary antioxidants increased antioxidant enzyme activity and status requires further research.

CONCLUSIONS

Feeding AOX improved lactation performance by increasing DMI, FCM, and milk fat yield independent

of the level of oxidation of the fat, and by increasing the plasma antioxidant enzymes and status of the cow. Changes in milk fat composition toward greater *cis* 18:1 and lower *trans*-11 18:1 indicated decreased oxidation and ruminal biohydrogenation of 18:1. Feeding OF decreased DMI without negatively affecting milk yield but at the expenses of BW gain and antioxidant status of the cow. Greater milk fat yield coupled with lower content of several *trans* 18:1 isomers and *trans*-11, *cis*-9 18:2 would suggest changes in microbial population toward complete biohydrogenation, reducing the likelihood of milk fat depression. The negative effect of feeding OF was partially ameliorated with AOX supplementation.

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REFERENCES

Andrews, J., M. Vázquez-Añón, and G. Bowman. 2006. Fat stability and preservation of fatty acids with AGRADO antioxidant in feed ingredients used in ruminant rations. J. Dairy Sci. 89(Suppl. 1):60. (Abstr.)

AOAC. 2000. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Washington, DC.

AOCS. 1997. Official Methods of Analysis. AOCS Press, Champaign, IL.

Bell, J. A., J. M. Griinari, and J. J. Kennelly. 2006. Effect of safflower oil, flaxseed oil, monensin, and vitamin E on concentration of conjugated linoleic acid in bovine milk fat. J. Dairy Sci. 89:733–748.

Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. 2002. Markers of oxidative status in plasma and erythrocytes of transition cows during hot season. J. Dairy Sci. 85:2173–2179.

Bernabucci, U., B. Ronchi, and A. Nardone. 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy Sci. 88:2017–2026.

Bradford, M. M. 1976. A reapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254.

Cabel, M. C., P. W. Waldroup, W. D. Shermer, and D. F. Calabotta. 1988. Effects of ethoxyquin feed preservative and peroxide level on broiler performance. Poult. Sci. 67:1725–1730.

Castillo, C., J. Hernandez, A. Bravo, M. Lopez-Alonso, V. Pereira, and J. L. Benedito. 2005. Oxidative status during late pregnancy and early lactation in dairy cows. Vet. J. 169:286–292.

Collins, A. R. 2005. Assays for oxidative stress and antioxidant status: Applications to reach into the biological effectiveness of polyphenols. Am. J. Clin. Nutr. 81(Suppl.):261S–267S.

Dibner, J. J., M. L. Kitchell, C. A. Atwell, and F. J. Ivey. 1996. The effect of dietary ingredients and age on the microscopic structure of the gastrointestinal tract in poultry. J. Appl. Poult. Res. 5:70–77.

Focant, M., E. Mignolet, M. Marique, F. Clabots, T. Breyne, D. Dalemans, and Y. Larondelle. 1998. The effect of vitamin E supplementation of cow diets containing rapeseed and linseed on the prevention of milk fat oxidation. J. Dairy Sci. 81:1095–1101.

Fox, D. G., L. D. Tedeschi, T. P. Tylutki, J. B. Russell, M. E. Van Amburgh, L. E. Chase, A. N. Pell, and T. R. Overton. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim. Feed Sci. Technol. 112:29–78.

Frankel, E. N. 2005. Antioxidants. Pages 209–258 in Lipid Oxidation. 2nd ed. E. N. Frankel, ed. The Oily Press, Bridgwater, UK.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76:3851–3863.

Kramer, J. K. G., V. Fellner, M. E. R. Dugan, F. D. Sauer, M. M. Mossoba, and M. P. Yurawecz. 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total *trans* fatty acids. Lipids 32:1219–1228.

Lock, A. L., C. Tyburczy, D. A. Dwyer, K. J. Harvatine, F. Destaillats, Z. Mouloungui, L. Candy, and D. Bauman. 2007. *Trans*-1-octadecenoic acid does not reduce milk fat synthesis in dairy cows. J. Nutr. 137:71–76.

Lohrke, B., T. Viergutz, W. Kanitz, B. Losand, D. G. Weiss, and M. Simko. 2005. Short communication: Hydroperoxides in circulating lipids from dairy cows: Implications for bioactivity of endogenous-oxidized lipids. J. Dairy Sci. 88:1708–1710.

Miller, J. K., and E. Brezeinska-Slebodizinska. 1993. Oxidative stress, antioxidants and animal function. J. Dairy Sci. 76:2812–2823.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Pottier, J., M. Focant, C. Debier, G. De Buysser, C. Goffe, E. Mignolet, E. Froidmont, and Y. Larondelle. 2006. Effect of dietary vitamin E on rumen biohydrogenation pathways and milk fat depression in dairy cows fed high-fat diets. J. Dairy Sci. 89:685–692.

SAS Institute. 2003. SAS User's Guide: Statistics. SAS Inst. Inc., Cary, NC.

Smith, J. L., L. G. Sheffield, and D. Saylor. 2002. Impact of ethoxyquin on productivity of dairy cattle. J. Dairy Sci. 85(Suppl. 1):358. (Abstr.)

Sui-Ying, W., W. Bottje, P. Maynard, J. Dibner, and W. Shermer. 1997. Effect of Santoquin and oxidized fat on liver and intestinal glutathione in broilers. Poult. Sci. 76:961–967.

Vázquez-Añón, M., and T. Jenkins. 2007. Effects of feeding oxidized fat with or without dietary antioxidants on nutrient digestibility, microbial nitrogen, and fatty acid metabolism. J. Dairy Sci. 90:4361–4367.

Weiss, W. P. 1998. Requirements of fat-soluble vitamins for dairy cows:A review. J. Dairy Sci. 81:2493–2501.

Yu, B. P. 1994. Cellular defenses against damage from reactive oxygen species. Physiol. Rev. 74:139–162.