Effect of different rumen-inert fatty acids supplemented with a dietary antioxidant on performance and antioxidative status of early-lactation cows

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ABSTRACT

The objective of the study was to evaluate the effect of diets supplemented with fatty acids of different degrees of saturation, in the absence or presence of an antioxidant (AOX; Agrado Plus, Novus International Inc., St. Charles, MO), on dairy cow lactation performance. Calcium salts of long-chain fatty acids were supplemented as a source of lower saturation fatty acid, and a palm acid product was supplemented as the higher saturation fatty acid source. Sixty early-lactation Chinese Holstein cows (100 ± 23 d in milk) were randomly allocated to 4 dietary treatments in a 2×2 factorial design: (1) lower saturation fatty acid (LS), (2) LS and AOX, (3) higher saturation fatty acid (HS), and (4) HS and AOX. The Ca salts of long-chain fatty acids and palm acid product were supplied at 1.8 and 1.5% on a dry matter basis, respectively, to form isoenergetic diets. The AOX was added at 0.025% in the ration. The experiment lasted 9 wk, including 1 wk for adaptation. Lactation performance was recorded and milk was sampled and analyzed weekly. Blood samples were taken from the coccygeal vein to determine metabolism parameters on d 16, 36, and 56 during the experiment. Neither fatty acid type nor AOX supplementation showed a significant effect on dry matter intake during the study. Milk yield was lower in the LS-fed cows compared with the cows fed HS. Milk fat and milk protein concentrations were not affected by fatty acid type or AOX supplementation. Adding AOX increased the yield of milk in the LS-fed cows, but did not affect those fed HS. Activity of plasma superoxide dismutase was significantly lower, plasma glucose tended to be lower, and plasma malondialdehyde was higher in the LS-fed animals compared with those fed HS. Addition of AOX decreased both plasma nonesterified fatty acids and hydrogen peroxide contents and increased total antioxidant capacity across the fatty acid types. Plasma β -hydroxybutyrate was not affected by fatty acid type

or AOX treatment. Cows fed LS had higher *cis*-9 $C_{18:1}$ and *trans*-10, *cis*-12 $C_{18:2}$ in milk at the expense of $C_{18:0}$, whereas AOX addition increased milk *cis*-9 $C_{18:1}$ at the expense of milk $C_{12:0}$, $C_{16:0}$, and *trans*-10, *cis*-12 $C_{18:2}$. It is inferred that feeding LS resulted in inferior lactation performance, whereas addition of antioxidant partially alleviated these negative effects.

Key words: antioxidant, antioxidative status, dairy cow, fatty acid

INTRODUCTION

High-yielding dairy cows are often in severe negative energy balance during early lactation because of insufficient feed intake to meet energy requirements for maintenance of body tissues and milk production (Block et al., 2001; Grummer et al., 2004). Increasing the energy density in diets by increasing grain or fatty acid supply is an alternative to alleviate this negative energy balance. Excessive grain feeding increases energy density but often causes undesirable rumen fermentation and milk fat depression. The addition of fatty acids, if not from ruminally inert fatty acids, could be detrimental to fiber digestion and DMI. Unsaturated fatty acids (UFA) are usually biohydrogenated by the microorganisms in the rumen. Some fatty acids, especially long-chain UFA released into the ruminal environment, could inhibit the ruminal microbes and then decrease fiber digestion (Coppock and Wilks, 1991). Supplementation of UFA in the form of Ca salts or saturated fatty acids (SFA) may minimize the likelihood of adverse effects associated with fat supplementation (Chalupa et al., 1986; Bernard and Kertz, 2009). Dietary fatty acids, especially polyunsaturated fatty acids (PUFA), may play an important role in oxidative stress. Diets with a high content of PUFA may be easily oxidized (Shiota et al., 1999). Dietary lipids such as supplemental fat rich in PUFA, oilseeds, and distiller grains, if not stabilized, can be significant contributors to the free radical load in animals (Andrews et al., 2006). Consumption of diets rich in PUFA renders tissues susceptible to free radical-mediated lipid peroxidation. Sies et al. (2005) suggested that a mixture of antioxidant compounds is

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required to provide protection from the oxidative effect of diets rich in fat.

An antioxidant (**AOX**), Agrado Plus (Novus International Inc., St. Charles, MO), was reported to be effective at improving oxidative balance and performance in lactating cows by improving rumen metabolism (Vázquez-Añón and Jenkins, 2007) and plasma oxidative status (Vázquez-Añón et al., 2008). Addition of AOX may alleviate the negative effect of feeding UFA on rumen microbes, especially when oxidized fat is included in the diet (Vázquez-Añón and Jenkins, 2007). The essential difference between fresh and oxidized fat lies in the production of free radicals and changes in the degree of saturation. We hypothesized that different rumen-inert fatty acids induce different effects on dairy cows, thereby influencing the response of the antioxidant utilization. Because the effects of adding UFA and SFA to the diets of lactating dairy cows have been documented (Mandebvu et al., 2003), a control diet without supplemental fatty acids was not included in this study. The objective of this study was to determine the effects of supplementing a dietary antioxidant with different inert fatty acids on performance and antioxidant status in early-lactation cows.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

Sixty Chinese Holstein cows ($\text{DIM} = 100 \pm 23$) were allocated to 15 blocks of 4 cows and were fed 4 diets varying in fatty acid type, with or without a dietary antioxidant, in a 2×2 factorial design. Calcium salts of long-chain fatty acids (Megalac, Jiali, China) was supplemented as a source of lower saturation fatty acid, and a palm acid product (BergaFat rumen-protected fat, Berg+Schmidt, Selangor Darul Ehsan, Malaysia) was supplemented as the higher saturation fatty acid source. The palm acid product contained a high percentage of palmitic acid in the form of free fatty acids. The AOX was formulated to consist of a dry granular blend of ethoxyquin and tertiary butylhydroquinone. The treatments were: (1) lower saturation fatty acid (**LS**), (2) LS and AOX, (3) higher saturation fatty acid (**HS**), (4) HS and AOX. The Ca salts of long-chain fatty acids and the palm acid product were supplied at 1.8 and 1.5%, respectively, to form isoenergetic diets, and AOX was added at 0.025% in the ration. Additional limestone was added in the HS-fed cows to make diets equal in calcium content. The ingredients and composition of the experimental diets are presented in Table 1. Fatty acid compositions of the Ca salts of long-chain fatty acids, palm acid product, and experimental diets are presented in Table 2.

The experiment lasted 9 wk, with the first week for adaptation. Cows were housed in tie-stall barns and milked 3 times daily at 0600, 1330, and 2030 h when receiving diets. All cows had free access to water throughout the experiment.

Sampling, Measurement, and Analysis

Feed was offered to ensure approximately 10%orts. To determine DMI, diets offered and refused were weighed for 2 consecutive days on a weekly basis. The dietary samples were collected weekly and composited to analyze the chemical compositions of DM, CP, ash, and ether extracts (AOAC, 1990). The ADF and NDF were determined according to Van Soest et al. (1991). The health status of cows was recorded by an experienced veterinarian. Milk production was recorded on 1 d (3 milkings) a week using milk-sampling devices (Waikato Milking Systems NZ Ltd., Waikato, Hamilton, New Zealand), and 50 mL of milk was collected weekly for analysis of fat, protein, lactose, and total solids by infrared analysis with a spectrophotometer (Foss-4000, Foss, Hillerød, Denmark).

Blood samples were taken from the coccygeal vein on d 16, 36, and 56 during the experiment and were immediately transferred into heparinized tubes. Plasma was obtained by centrifuging at $3,000 \times g$ for 10 min and was frozen at -20°C for later analysis of glucose, NEFA, BHBA, superoxide dismutase (**SOD**), hydrogen peroxide (H_2O_2), total antioxidant capacity (**TAOC**), malondialdehyde (**MDA**), and glutathione peroxidase (**GSH-Px**) activity. Plasma glucose and NEFA levels were determined by enzymatic analysis, as described by McCutcheon and Bauman (1986). Plasma concentrations of BHBA were quantified by enzyme immunoassay with a commercial kit (RT110371, ADL, Fremont, CA). Activities of SOD and GSH-Px and MDA content were measured as described by Zhang et al. (2006). The TAOC was measured by ferric-reducing/antioxidant power assay (Benzie and Stain, 1996). Contents of H_2O_2 were measured by reaction with molybdate and tested with a spectrophotometric method (Chai et al., 2004).

Milk samples during a 1-d period were taken at the end of the experiment for analysis of fatty acid profiles. The fatty acids in milk were methylated by *in situ* transesterification with 0.5 *N* methanolic NaOH followed by 14% boron trifluoride in methanol. Samples were injected by autosampler into a Hewlett-Packard 6890A GC equipped with a flame-ionization detector (Hewlett-Packard, Sunnyvale, CA). The fatty acid composition of Ca salts of long-chain fatty acids, palm acid product, and final rations were also determined. Results for each fatty acid were expressed as a percentage of the sum of all identified fatty acids.

Table 1. Ingredients and composition of the experimental diets

Item	Treatment ¹			
	LS	LS + AOX	HS	HS + AOX
Ingredient, % DM basis				
Corn silage	18.9	18.9	18.9	18.9
Grass hay	11.2	11.2	11.2	11.2
Beer brewing wastes	11.5	11.5	11.5	11.5
Alfalfa meal	7.1	7.1	7.0	7.0
Concentrate ²	46.0	46.0	46.0	46.0
Ca salts of long-chain fatty acids	1.8	1.8	0	0
Palm acid product	0	0	1.5	1.5
Antioxidant	0	0.025	0	0.025
Limestone	0.5	0.5	0.9	0.9
Salt	0.5	0.5	0.5	0.5
Premix ³	0.5	0.5	0.5	0.5
Dicalcium phosphate	1.3	1.3	1.3	1.3
Sodium bicarbonate	0.7	0.7	0.7	0.7
Composition				
CP, % of DM	15.2	15.2	15.3	15.2
Ether extract, % of DM	5.0	5.1	5.2	5.1
NDF, % of DM	41.2	41.3	41.3	41.4
ADF, % of DM	24.0	24.1	24.0	24.2
Ash, % of DM	7.7	7.6	7.8	7.8
Ca, ⁴ % of DM	1.05	1.05	1.04	1.04
NE _L , ⁴ Mcal/kg of DM	1.63	1.63	1.62	1.62

¹LS = lower saturation fatty acid; HS = higher saturation fatty acid; AOX = antioxidant.

²The ingredients (% TMR) of concentrate: 6.0 distillers dried grains with solubles, 20.3 ground corn, 4.9 wheat, 4.0 wheat bran, 4.3 soybean meal, 4.3 cottonseed meal, 2.2 cottonseed.

³Formulated to provide (per kg of premix) 1,000,000 IU of vitamin A, 200,000 IU of vitamin D, 1,250 IU of vitamin E, 14,000 mg of Zn, 100 mg of Se, 180 mg of I, 3,000 mg of Fe, 40 mg of Co, 3,000 mg of Mn, and 3,000 mg of Cu.

⁴Calculated based on individual feedstuffs in CNSAPH (2000).

Statistical Analysis

Data were subjected to covariate analysis using the SAS MIXED procedure (SAS Institute, 2000). Pre-treatment measurements were included in the model as a covariate. When measurements were taken over time, repeated measurement data were analyzed using the

MIXED procedure of SAS. Fatty acid type, AOX, week, and their interactions were included in the model. Cows within treatment were subjected as random to test for main effects and interactions using the covariance type auto-regressive order 1 [AR (1)], and the residual error was used to test for week and week × treatment interaction. Mean comparisons across treatments were

Table 2. Fatty acid composition of the Ca salts of long-chain fatty acids, palm acid product, and experimental diets¹

Fatty acid, %	Ca salts of long-chain fatty acids	Palm acid product	LS	HS
C _{8:0}	1.29	1.25	2.65	2.64
C _{12:0}	4.61	1.65	5.99	5.00
C _{14:0}	1.65	0.65	1.95	1.62
C _{16:0}	41.32	81.13	38.68	51.95
C _{18:0}	5.01	3.94	7.65	7.29
C _{18:1}	36.75	3.65	34.42	23.38
C _{18:2}	7.01	5.61	7.15	6.68
C _{20:3}	0.06	0.09	0.04	0.05
C _{20:5}	0.11	0.09	0.11	0.10
C _{22:4}	0.07	0.05	0.05	0.04
C _{22:6}	2.12	1.89	1.31	1.25
Saturated	53.88	88.62	56.92	68.50
Unsaturated	46.12	11.38	43.08	31.50

¹LS = lower saturation fatty acid; HS = higher saturation fatty acid.

Table 3. Performance of cows fed different fatty acid (FA) types in the presence or absence of antioxidant

Item	Treatment ¹				SEM	P-value		
	LS	LS + AOX	HS	HS + AOX		FA	AOX	FA × AOX
DMI, kg/d	22.7	22.8	22.9	23.0	0.39	0.66	0.55	0.67
BW increase, kg	24.4	24.5	22.5	29.2	5.74	0.81	0.57	0.57
Milk yield, kg/d	31.2 ^b	32.5 ^a	33.4 ^a	33.2 ^a	0.35	<0.01	0.14	0.02
Composition, %								
Protein	3.02	2.99	3.00	2.95	0.025	0.24	0.18	0.71
Fat	3.56	3.57	3.56	3.51	0.042	0.48	0.68	0.50
Lactose	4.93	4.92	5.00	4.97	0.016	<0.01	0.39	0.59
TS	12.09	12.06	12.18	11.97	0.06	0.97	0.11	0.20

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

¹LS = lower saturation fatty acid; HS = higher saturation fatty acid; AOX = antioxidant.

made when the interaction terms of the model were significant ($P \leq 0.05$) using LSMEANS and PDIF separation of all the treatments.

For all analyses, least squares means were calculated and differences between treatments were detected with Tukey's adjustment. Significant differences were declared at $P < 0.05$ and trends at $P \leq 0.10$ and $P > 0.05$.

RESULTS

Feed Intake and Lactation Performance

There was no interaction between week and treatments for DMI, lactation performance, and plasma parameters except for MDA; corresponding data are not shown. The BW changes were not significantly affected by fatty type and AOX. Neither fatty acid type nor AOX significantly affected DMI (Table 3). Compared with that in cows fed HS, milk yield was lower in the LS-fed cows ($P < 0.05$). Milk fat and milk protein contents were not affected by fatty acid or AOX supplementation. Cows fed LS had lower milk lactose compared with HS-fed cows. There were interactions between fatty acid type and AOX for milk yield ($P < 0.05$). Feeding AOX increased the milk yield in LS-fed cows, but did not show significant effects in the HS-fed cows.

Plasma Parameters

Plasma parameters of the subject cows are presented in Table 4. Plasma SOD activity was significantly lower ($P < 0.01$), glucose tended to be lower ($P = 0.10$), and plasma MDA was higher in the LS-fed animals compared with those fed HS ($P < 0.01$). The addition of AOX significantly decreased H_2O_2 content ($P = 0.02$), tended to decrease NEFA concentration ($P = 0.06$), and tended to increase TAOC in plasma ($P = 0.09$). Plasma BHBA was not affected by fatty acid type or AOX treatments. Plasma GSH-Px activity was

not influenced by fatty acid type or AOX addition, but a trend for an interaction between fatty acids and AOX ($P = 0.06$) was observed. Addition of AOX decreased GSH-Px activity in HS, but not in LS. For overall means, neither fatty acid type nor AOX showed a significant effect on plasma MDA. However, there were week × treatment interactions for MDA. Both fatty acid type and AOX showed significant effects on plasma MDA on d 16 of the experiment, but not on d 36 or 56.

Fatty Acid Composition in Milk

Profiles of milk fatty acid from the cows fed different diets are shown in Table 5. There was a significant difference in *cis*-9 $C_{18:1}$ because of its increased concentration in LS + AOX compared with in HS + AOX. The concentration of *trans*-10, *cis*-12 $C_{18:2}$ was higher and that of $C_{18:0}$ lower for LS than for HS cows. Addition of AOX increased milk *cis*-9 $C_{18:1}$ at the expense of $C_{12:0}$, $C_{16:0}$, and *trans*-10, *cis*-12 $C_{18:2}$. Although $C_{12:0}$ was higher in HS + AOX than in HS, addition of AOX appeared to decrease $C_{12:0}$. Neither fatty acid type nor AOX showed any significant effect on $C_{14:0}$, $C_{18:2}$, or $C_{18:3}$ contents. There were significant fatty acid type × AOX interactions for $C_{12:0}$, *cis*-9 $C_{18:1}$, and *trans*-10, *cis*-12 $C_{18:2}$ compositions. Fatty acid type and AOX tended to interact for $C_{14:0}$, $C_{18:2}$, SFA, and PUFA ($P \leq 0.10$). The effect of AOX was more pronounced in LS versus HS diets for the above fatty acids. Feeding LS increased the concentrations of monounsaturated fatty acid (MUFA) and PUFA, but decreased the concentrations of SFA. Addition of AOX increased the concentrations of MUFA at the expense of SFA and PUFA.

DISCUSSION

Feed Intake and Lactation Performance

The milk yield was recorded on 1 d (3 milkings) a week and DMI was recorded on 2 consecutive days weekly in this study. Results and discussion of lactation

Table 4. Plasma parameters of cows fed different fatty acid (FA) types in the presence or absence of antioxidant

Item ¹	Treatment ²					P-value		
	LS	LS + AOX	HS	HS + AOX	SEM	FA	AOX	FA × AOX
SOD, U/mL	158.3	159.4	163.9	169.3	2.52	<0.01	0.20	0.38
MDA, nmol/mL	2.79	2.73	2.25	2.40	0.157	<0.01	0.79	0.49
GSH-Px, U/mL	175.0	178.8	198.4	173.9	7.64	0.24	0.17	0.06
H ₂ O ₂ , mmol/L	39.3	31.2	43.0	34.4	3.64	0.36	0.02	0.95
TAOC, U/mL	3.47	3.73	3.65	4.23	0.241	0.17	0.09	0.50
Glucose, mmol/L	3.82	3.70	3.84	3.90	0.063	0.10	0.63	0.17
NEFA, mmol/L	0.24	0.23	0.27	0.19	0.226	0.91	0.06	0.14
BHBA, mmol/L	0.29	0.33	0.34	0.35	0.025	0.17	0.24	0.58

¹SOD = superoxide dismutase; MDA = malondialdehyde; GSH-Px = glutathione peroxidase; TAOC = total antioxidant capacity.

²LS = lower saturation fatty acid; HS = higher saturation fatty acid; AOX = antioxidant.

performance were based on this protocol. For overall means, the milk yield curves in different treatment were parallel in this study, as shown in Figure 1. The data over 8 wk showed similar treatment effects, and there was no week by treatment effect on milk yield or milk composition, indicating that other factors play little role in the study.

Responses of DMI to supplemental fatty acids have been inconsistent. Compared with diets rich in SFA, diets with UFA are more likely to depress DMI because of the toxicity of UFA to rumen microbes and its inhibition of fiber digestion (Bremmer et al., 1998). However, in the current study, no treatment effect on DMI was observed, possibly because of the small amount of fatty acid inclusion. In contrast to other

studies (Mandebvu et al., 2003; Relling and Reynolds, 2007), which reported that inclusion of fatty acids with different degrees of saturation resulted in similar milk yield, lower milk yield was observed in the LS-fed cows compared with those fed HS. Although UFA was fed in the form of Ca salts, the incomplete protection of Ca salts from rumen hydrogenation has been described previously (Chouinard et al., 1998; Lundy et al., 2004), where postruminal flow of UFA was less than expected. Plasma glucose, which is the main source of milk lactose, may contribute to the decrease of milk lactose in LS-fed cows. In agreement with us, Relling and Reynolds (2007) reported that cows supplemented with UFA showed lower milk lactose than those fed SFA and that plasma insulin and glucose-dependent insulintropic

Table 5. Fatty acid composition (% of total fatty acids measured) in milk for cows fed different fatty acid types in the presence or absence of antioxidant

Fatty acid, %	Treatment ¹					P-value		
	LS	LS + AOX	HS	HS + AOX	SEM	FA	AOX	FA × AOX
C _{12:0}	3.55 ^a	3.05 ^b	3.13 ^b	3.24 ^b	0.095	0.21	0.05	<0.01
C _{14:0}	11.71 ^a	11.03 ^b	11.42 ^{ab}	11.62 ^{ab}	0.213	0.49	0.27	0.05
C _{16:0}	35.63	34.59	36.24	34.69	0.304	0.26	<0.01	0.42
C _{18:0}	14.77	14.68	15.60	16.23	0.425	<0.01	0.53	0.40
<i>cis</i> -9 C _{18:1}	24.71 ^b	27.80 ^a	24.92 ^b	25.74 ^b	0.410	0.03	<0.01	<0.01
<i>cis</i> -9 C _{18:1} /(C _{18:0} + <i>cis</i> -9 C _{18:1})	0.63	0.65	0.61	0.61	0.009	<0.01	0.17	0.12
<i>trans</i> -11 C _{18:1}	3.25	3.06	3.04	2.98	0.097	0.16	0.20	0.52
C _{18:2} (n = 6)	4.17	3.64	3.73	3.72	0.150	0.24	0.08	0.09
<i>cis</i> -9, <i>trans</i> -11 C _{18:2}	0.38	0.46	0.36	0.36	0.064	0.39	0.54	0.54
<i>cis</i> -9, <i>trans</i> -11 C _{18:2} /(<i>cis</i> -9, <i>trans</i> -11 C _{18:2} + <i>trans</i> -11 C _{18:1})	0.10	0.12	0.11	0.11	0.011	0.67	0.40	0.61
<i>trans</i> -10, <i>cis</i> -12 C _{18:2}	0.088 ^a	0.049 ^b	0.050 ^b	0.035 ^c	0.0031	<0.01	<0.01	<0.01
C _{18:3}	0.22	0.24	0.21	0.21	0.032	0.51	0.83	0.73
Others ²	1.49	1.40	1.30	1.19	0.039	<0.01	0.01	0.68
Saturated fatty acids	65.66	63.35	66.38	65.77	0.465	<0.01	<0.01	0.07
MUFA ³	29.17 ^b	32.06 ^a	29.09 ^b	29.76 ^b	0.421	<0.01	<0.01	0.01
PUFA ⁴	5.14	4.59	4.53	4.47	0.124	<0.01	0.02	0.06

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹LS = lower saturation fatty acid; HS = higher saturation fatty acid; AOX = antioxidant; FA = fatty acid.

²Including C_{20:3}, C_{20:5}, C_{22:4}, and C_{22:6}.

³MUFA = monounsaturated fatty acids.

⁴PUFA = polyunsaturated fatty acids.

Figure 1. Milk yield curve of cows fed different fatty acid types in the presence or absence of antioxidant (AOX). LS = lower saturation fatty acid; HS = higher saturation fatty acid. Bars indicate the SEM.

polypeptide were greater for MUFA- and PUFA-fed cows than for those on SFA, whereas glucagon-like peptide-1 and cholecystokinin showed the opposite results. These hormones may modulate the distribution of plasma glucose to synthesize milk lactose. The response of milk yield and composition to addition of AOX appears to be dependent on fatty acid type. The magnitude of the AOX response was significantly greater in cows fed LS compared with those fed HS. The increase in milk yield in cows fed LS + AOX compared with those fed LS only was not the result of increased DMI, but may be associated with the improved antioxidative status. Consumption of diets rich in UFA renders tissues more susceptible to free radical damage (Sies et al., 2005). The addition of AOX was observed to increase DM, OM, and fiber digestibility in vitro (Han et al., 2002; Vázquez-Añón and Jenkins, 2007), which might also be associated with improved milk production (Vázquez-Añón et al., 2008). Neither fatty acid type nor AOX significantly affected milk protein and fat contents, but cows fed HS had higher yields of milk protein and fat because of higher milk yield.

Plasma Parameters

Lack of significant effects on plasma glucose, NEFA, and BHBA indicated that the 2 types of fatty acids were utilized similarly postabsorption. Plasma concentrations of glucose, NEFA, and BHBA were within the ranges of normally fed cows, showing that these cows did not suffer from ketosis. A similar treatment effect was also found when fatty acids were supplemented at 1.7% of expected DMI (Mandevu et al., 2003; Ye et al., 2009). However, cows supplemented with UFA at 1

kg/d per cow had higher concentrations of cholesterol, high-density lipoprotein, low-density lipoprotein, and NEFA in blood (Bernard and Kertz, 2009) compared with those fed SFA. Another study has shown that UFA partly exerted a positive effect on lipid metabolism in the liver, but adverse effects on the antioxidative status of the cows remained a concern (Večeřa et al., 2003). Of all parameters tested, SOD is the first enzyme involved in the conversion of oxygen radicals to peroxides (Yu, 1994), and MDA is one of the lipid peroxidation products that has been used as an indicator of oxidative stress (Armstrong and Browne, 1994). Lower plasma SOD and higher MDA in LS-fed cows indicated that these cows were more prone to oxidative stress. Extensive literature has documented the relationship between dietary-induced UFA and lipid peroxidation (Shiota et al., 1999; Young et al., 2005). Compared with SFA, UFA are susceptible to free radical-mediated lipid peroxidation, whereas AOX terminates peroxidative chains by reacting directly with a variety of free radicals. Although free radicals such as superoxide (O_2^-) were not determined in this study, H_2O_2 , the product of oxygen radicals converted by SOD, decreased when AOX was added to the diet. Supplementation of AOX reduced plasma peroxides without decreasing SOD activity, indicating that AOX could remove free radicals directly, reduce the load of peroxides coming into the body, and consequently spare the endogenous antioxidant system. Cows fed AOX tended to have higher antioxidative capacity, which was consistent with an experiment carried out by Vázquez-Añón et al. (2008).

Fatty Acid Composition in Milk

Compared with palm acid product, Ca salts of long-chain fatty acids contained more *cis*-9 $C_{18:1}$ at the expense of $C_{16:0}$. Interestingly, milk $C_{16:0}$ was not affected by fatty acid type. Because $C_{16:0}$ is incorporated into milk from both de novo synthesis and preformed fatty acids, dietary $C_{16:0}$ was probably not the sole factor that affected milk content of $C_{16:0}$. Bauman and Griinari (2003) reported that high concentrations of milk *trans*-11 $C_{18:1}$ and *trans*-10, *cis*-12 $C_{18:2}$ may be responsible for milk fat depression. However, milk fat was similar between the treatments in the current study. In the study carried out by Pottier et al. (2006), high-fat diets could induce milk fat depression and vitamin E could counteract the depression. The degree of saturation in fat and type of AOX may result in the different responses. In the present study, the supplemented fatty acids contained less PUFA than the linseed oil used by Pottier et al. (2006), which may not be enough to induce milk fat depression. However, both AOX used in the above 2 studies increased milk yield and decreased

trans-10, *cis*-12 C_{18:2}, and the response of milk fat to AOX depended on the saturation degree of fatty acids and types of AOX. In the current study, addition of AOX to LS increased milk *cis*-9 C_{18:1} and MUFA at the expense of C_{12:0} and C_{14:0}, but no significant difference was observed between HS and HS + AOX for these fatty acids. The response of AOX on LS may relate to its particular effect on UFA, which coincides with the result of milk performance.

The fatty acid composition in the rumen and plasma was not determined in the present study. Therefore, the biohydrogenation of fatty acid should be interpreted with caution. The major difference in the fatty acids in milk was *cis*-9 C_{18:1} (Table 5), which was significantly higher in the LS + AOX than in others. The *cis*-9 C_{18:1} may be produced through desaturation of C_{18:0} by stearoyl-CoA desaturase (SCD). The activity of SCD in the rumen is low, and ratios of *cis*-9 C_{18:1} to (*cis*-9 C_{18:1} + C_{18:0}) and *cis*-9, *trans*-11 C_{18:2} to (*cis*-9, *trans*-11 C_{18:2} + *trans*-11 C_{18:1}) in milk could be used to evaluate the activity of SCD (Sol Morales et al., 2000). Indeed, the ratio of *cis*-9 C_{18:1} to (C_{18:0} + *cis*-9 C_{18:1}) was significantly higher in LS-fed cows compared with HS-fed cows (Table 5). The higher milk *cis*-9 C_{18:1} in LS-fed cows suggested the specific effect of dietary C_{18:1} on C_{18:0} desaturation. Compared with soybean, tallow that had similar fatty acid composition to the Ca salts of long-chain fatty acids used in the present study could increase the ratio of C_{18:1} to C_{18:0} (Sol Morales et al., 2000). Expression of SCD mRNA is highly correlated with the amount of oleic acid in ovine subcutaneous adipose tissue (Barber et al., 2000). The ratio of *cis*-9, *trans*-11 C_{18:2} to (*cis*-9, *trans*-11 C_{18:2} + *trans*-11 C_{18:1}) was reported to vary little among diets, but to a greater extent within tissues (Palmquist et al., 2004). The effect of diet on this ratio was not observed in milk in the present study.

Although diets and fatty acids were not oxidized in the study, the response of milk profiles to AOX and fatty acid type was greater than that observed in previous trials where the effect of AOX on fresh and oxidized fat was evaluated (Vázquez-Añón et al., 2008). The large difference in degree of saturation between treatments in the current study could explain the response. However, outflow and transfer efficiency of fatty acids were not evaluated in the study. Further study is needed to understand the process of biohydrogenation and the role of dietary antioxidant supplementation.

CONCLUSIONS

Compared with cows fed the HS diet, cows fed the LS diet had decreased milk yields with no changes to milk composition except for milk lactose. Feeding LS resulted

in inferior plasma antioxidative status, as indicated by lower plasma SOD and higher MDA. The addition of AOX restored the negative effect of feeding LS on milk yield and improved antioxidative status regardless of fatty acid type. The response to AOX addition was more pronounced in cows fed LS compared with those fed HS.

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