Effects of Feeding Oxidized Fat With or Without Dietary Antioxidants on Nutrient Digestibility, Microbial Nitrogen, and Fatty Acid Metabolism

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ABSTRACT

A dual-effluent continuous culture system was used to investigate, in a 2 × 2 factorial design, the effect of feeding a fresh (FF) or oxidized (OF) blend of unsaturated fats (33% fish oil, 33% corn oil, 26% soybean oil, and 7% inedible tallow) when supplemented with a blend of antioxidants (AO; Agrado Plus, Novus International Inc.; Agrado Plus is a trademark of Novus International Inc. and is registered in the United States and other countries) on nutrient digestibility, bacterial protein synthesis, and fatty acid metabolism. Twice a day for 10 d, 12 fermenters were fed a diet that consisted of 52% forage and 48% grain mixture that contained 3% (dry matter basis) FF or OF, with or without AO. The OF contained a higher concentration of peroxides (215 vs. 3.5 mEq/kg), and a lower concentration of unsaturated fatty acids than the FF. Feeding OF reduced nitrogen digestibility, microbial nitrogen yield, and efficiency (expressed as kilograms of dry matter digested) and increased the outflow of saturated fatty acids in the effluent when compared with feeding FF. Adding AO improved total carbohydrate, neutral, and acid detergent fiber digestibilities and the amount of digested feed nitrogen converted to microbial nitrogen across the types of fats. From this study, we concluded that feeding OF reduced microbial nitrogen and increased the outflow of saturated fatty acids. Feeding AO improved fiber digestibility by rumen microorganisms, regardless of the type of fat.

Key words: oxidized fat, antioxidant, Agrado Plus

INTRODUCTION

In the body of an animal 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 prevents free radicals

from accumulating and harming the cells. However, the levels 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 can impair the production and health status of the animal (Miller and Brezeinska-Slebodizinska, 1993). The impact of free radicals and oxidative stress is unknown in ruminal microorganisms. Dietary lipids such as supplemental fat, oilseeds, and distillers 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 a compromised immune response have been reported in production animals fed oxidized fat (OF; Cabel et al., 1988; Dibner et al., 1996). Inclusion of dietary antioxidants (AO) ameliorates these negative effects by scavenging peroxides and reducing the peroxidation of fatty acids (Frankel, 2005).

In a compilation of feedlot trials, feeding 150 mg/kg of ethoxyquin in the form of Agrado Plus (Novus International Inc., St. Louis, MO; Agrado Plus is registered in the United States and other countries) was found to reduce the incidence of liver abscess and improve BW gain across studies (Vázquez-Añón et al., 2005a,b). In dairy cattle, feeding 50 mg/kg of ethoxyquin (Smith et al., 2002) improved milk yield and efficiency as well as OM digestibility, suggesting an antioxidant effect on rumen fermentation. The objective of this study was to evaluate the effect of feeding fresh fat (FF) or OF with or without dietary AO on nutrient digestibility, fatty acid metabolism, and bacterial protein synthesis by using continuous culture fermenters.

MATERIALS AND METHODS

A lactating dairy ration was formulated to support 40 kg/d of milk production with a predicted DMI of 24

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Table 1. Ingredient and nutrient composition of the basal diet

Item	Amount, % (DM basis)
Alfalfa balage	4.56
Corn silage	28.12
Mixed grass hay	19
Soybean meal (44%)	15.58
Corn gluten meal	0.66
Soyhulls	3.8
Flaked barley	5.22
Steam-flaked corn	17.48
Fresh fat ¹	0 or 3
Oxidized fat ²	0 or 3
Dietary antioxidant ³	0 or 0.02
Urea	0.66
Magnesium oxide	0.01
Dicalcium phosphate	0.28
Sodium bicarbonate	0.97
Limestone	0.28
Trace minerals and salts	0.19
Vitamin A, D, E mix	0.11
Vitamin E	0.06
CP	18.6
Soluble protein, % of CP	34.4
NDF ⁴	28.3
ADF	18.1
NSC ⁴	31.6
Starch	25.7
Sugar	5.9
Ether extract	5.5
Ash	6.1
NFC ⁵	41.7

¹Fresh fat was added to the fresh fat (FF) and fresh fat plus antioxidant (FF + AO) treatment diets.

²Oxidized fat was added to the oxidized fat (OF) and oxidized fat plus antioxidant (OF + AO) treatment diets.

³Dietary antioxidant was added to FF + AO and OF + AO treatment diets in the form of Agrado Plus (Novus International, St. Louis, MO).

⁴Includes starch + sugar.

⁵Calculated NFC.

kg/d. Dietary ingredients and nutrient composition are shown in Table 1 and 2. The diet consisted of 52% forage and 48% concentrate mixture that contained 3% experimental fat on a DM basis. The experimental fat consisted of a blend of nonstabilized unsaturated fats that contained 33% fish oil, 33% corn oil, 26% soybean oil, and 7% inedible tallow. Half of the experimental fat was oxidized (OF) by bubbling air through the fat at 92°C for 24 h to achieve a peroxide value of 215 mEq/kg (method Cd 12-57; AOCS, 1997). The study consisted of 4 treatments: 1) fresh nonoxidized fat (FF) added to the diet at 3%; 2) FF added to the diet at 3% plus 200 mg/kg of dietary AO (FF + AO); 3) OF added to the diet at 3%; 4) OF added to the diet at 3% plus 200 mg/kg of dietary AO (OF + AO). The dietary AO consisted of a liquid blend of ethoxyquin and tertiary-butyl-hydroquinone and was added to the experimental fat just prior to mixing of the diets at a rate of 200 mg/kg of DM of the final diet. The diets were stored at

Table 2. Effect of feeding fresh (FF) or oxidized fat (OF) in the presence and absence of antioxidants (AO) on the daily amounts of fatty acids fed to continuous cultures

Item, mg/d	Treatment ¹			
	FF	OF	FF + AO	OF + AO
12:0	4	4	4	4
14:0	55	89	93	52
15:0	8	8	8	8
16:0	675	632	660	690
16:1	106	104	111	114
18:0	152	141	146	155
18:1	851	793	836	865
18:2	1,739	1,591	1,725	1,703
18:3	229	209	227	224
20:0	24	22	24	25
20:4	9	7	9	8
21:0	20	15	20	17
22:0	18	16	16	19
20:5	124	88	125	99
24:0	12	7	20	7
22:6	70	44	69	51
Other fatty acids	405	342	340	440
Unsaturated fatty acids ²	3,136	2,841	3,107	3,072
Saturated fatty acids ³	1,013	970	1,034	1,020

¹Treatments: FF = fresh fat; FF + AO = fresh fat with antioxidant; OF = oxidized fat; OF + AO = oxidized fat with antioxidant.

²Unsaturated fatty acids included 14:1, 16:1, 18:1-isomers, 18:2, 18:3, 22:1, 20:5, and 22:6.

³Saturated fatty acids included 12:0, 14:0, 15:0, 16:0, 18:0, 20:0, 21:0, 22:0, and 24:0.

0°C between feedings and allowed to come to room temperature prior to feeding. Peroxide values and changes in fatty acid profile were used to assess the quality and stability of the experimental fats prior to adding the AO.

Continuous Culture System

A 12-unit dual-effluent continuous culture system, as described by Hoover et al. (1976), was used in this study. Ruminal inoculum was obtained from 2 rumen-cannulated lactating Holstein cows. Ruminal inoculum was pooled before inoculating the 1,164-mL fermenters. Fermenters were fed the experimental diets (ground to pass a 4-mm sieve) for 10 d in 2 equal feedings at 12-h intervals (Table 1). All treatments were fermented in triplicate for 10 d in continuous cultures. Continuous culture conditions were defined to represent average in vivo flow rates as follows: liquid dilution rate, 12%/h; solids retention time, 24 h; feed intake, 100 g of DM/d; fermentation temperature, 39°C. The pH was recorded at 0.5-h intervals. The dual-flow continuous culture technique, under the conditions defined herein, was established by Hannah et al. (1986) as a valid method of simulating in vivo rumen fermentation. It represents a practical and appropriate alternative to in vivo methods.

The artificial saliva of Weller and Pilgrim (1974) was continuously infused at a rate to provide the 12%/h liquid flow for fermentation periods of 10 d. The first 7 d were for equilibration. During the last 3 d, the effluents were collected and a 1-L sample was composited and saved for analysis. After the effluent was collected on d 10, the contents of the fermenters were stirred vigorously prior to being allowed to settle to dislodge some of the solids associated with microbes. The upper fluid layer was used for collection of microbes as described by Lean et al. (2005).

Chemical Analysis

Feed DM was determined by oven-drying at 100°C for 24 h. Effluent DM was determined by centrifuging a 34- to 40-g sample of effluent at $30,000 \times g$ for 45 min as described by Lean et al. (2005). For digestibility determination, DM digested and OM digested were corrected for microbial DM and OM. Determination of NDF and ADF contents in the feed was as described by Goering and Van Soest (1970) and Van Soest et al. (1991), and in continuous culture effluents as described by Crawford et al. (1983). Total nitrogen in feed and effluents, and bacterial, ammonia, and ether extraction were determined according to AOAC (1990). Analysis of VFA was performed in accordance with the gas chromatographic separation procedure described by Lean et al. (2005). Effluent and bacterial concentrations of purines were determined by the procedures of Zinn and Owens (1986) to partition effluent nitrogen flow into microbial and dietary fractions and to calculate DM and OM. The sugars and starches of the feeds and effluents were determined by the procedure of Smith (1969), except that ferricyanide was used to detect reducing sugars.

Fermenter outflow samples were freeze-dried and converted to methyl esters in sodium methoxide-methanolic HCl as described by Kramer et al. (1997). Analysis of fermenter outflow fatty acids was done on a high-performance gas chromatograph (HP5890A GC, Agilent Technologies, Inc., Santa Clara, CA) equipped with a flame-ionization detector and a 30 m $\times$ 0.25 mm (0.2 μ m film) Supelco 2380 fused-silica capillary 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 140°C for 3 min, then increased to 220°C at 2°C/min, and held at 220°C for 2 min. Peaks were quantified by comparison with an internal standard (17:0), which was added prior to methylation.

Statistical Analysis

Data were analyzed as a completely randomized design by ANOVA with the GLM procedure of SAS (SAS Institute, 2003). Main effects of type of fat and presence of dietary AO were tested as a 2×2 factorial arrangement. Significance differences were declared at $P < 0.05$ and $P > 0.05$, but $P \leq 0.10$ were considered trends.

RESULTS

Nutrient Digestibility

Digestion coefficients are shown in Table 3. Digestion of CP was reduced ($P = 0.01$) by OF compared with FF. The rates of CP digestibility observed in this study were high but within the range reported for other continuous culture fermenters fed a variety of diets (Bargo et al., 2003; Lean et al., 2005). One possible explanation for the high digestibility may lie in the method used to arrive at microbial nitrogen flow. Altering feed conditions increased the variability of RNA concentrations within the mixed rumen microbial population (Smith and McAllan, 1974). Such variability in the RNA nitrogen content of the bacterial cells could have resulted in an overestimation of bacterial nitrogen flow and in inflated protein digestion. Digestion of NDF was not affected by fat source, whereas AO significantly increased NDF digestion ($P = 0.02$) when added to either the FF or the OF diet. Digestion of ADF was numerically improved in the OF diet ($P = 0.08$) compared with that of the FF diet and, as with NDF digestion, addition of AO increased ADF digestion ($P = 0.04$) when added to either fat source. Digestion of DM, OM, and NSC was not affected by the treatments. However, digestion of total carbohydrates (in g/d) was increased ($P = 0.05$) by AO regardless of fat source, mostly by improving NDF digestibility. Production rates (in mmol/d) and molar ratios of VFA, along with average fermentation pH, were not affected by fat source in the presence or absence of AO, with the exception of butyrate (Table 4). Fermenters fed OF diets had higher butyrate production (53 vs. 59 ± 2.01 ; $P = 0.02$) and molar ratios (13.8 vs. 14.8 ± 0.46 ; $P = 0.02$) than those fed FF diets.

Nitrogen Metabolism

Table 5 shows the effects of the treatments on nitrogen partitioning and on microbial efficiency and composition. Microbial nitrogen yield was less for the OF than the FF diet ($P = 0.03$), but bypass nitrogen was greater ($P = 0.01$) for the OF diet, due in part to the lower protein digestibility. This resulted in higher NAN for the OF treatment ($P = 0.01$). The addition of AO caused a numerical ($P = 0.09$) improvement in

Table 3. Effect on digestion coefficients for DM, OM, CP, fiber, and NSC of feeding fresh (FF) or oxidized fat (OF) in the presence and absence of antioxidant (AO) to continuous culture fermenters

Digestion, %	Treatment ¹				SE	P-value		
	FF	OF	FF + AO	OF + AO		Fat	AO	Fat × AO
DM	67.4	69.8	69.5	70.9	2.00	0.49	0.56	0.86
OM	61.0	61.8	65.0	63.1	1.77	0.76	0.17	0.47
CP	97.9	87.5	98.5	93.0	2.35	0.01	0.22	0.33
NDF	35.7	40.1	46	45.2	2.64	0.51	0.02	0.35
ADF	44.0	50.0	50.9	54.0	2.23	0.08	0.04	0.53
NSC ²	69.5	70.7	69.4	69.0	1.02	0.72	0.43	0.45
Total carbohydrates, ³ g/d	32.2	33.5	34.8	34.9	0.89	0.47	0.05	0.534

¹Treatments: FF = fresh fat; FF + AO = fresh fat with antioxidant; OF = oxidized fat; OF + AO = oxidized fat with antioxidant.

²Includes sugar and starch.

³Grams of NDF + grams of NSC digested per day.

microbial nitrogen in both fat treatments. As a result, the FF + AO treatment produced more microbial nitrogen than any other treatment. Addition of AO numerically reduced ammonia levels ($P = 0.06$) for both fat sources.

Because of the low carbohydrate digested (in g/d) and the overall high yield of microbial nitrogen, efficiencies were high across all treatments. Compared with OF, however, FF resulted in higher microbial nitrogen per unit of digested DM ($P = 0.03$), with numerical improvements in OM ($P = 0.1$) and total carbohydrates ($P = 0.08$). The significant improvement in microbial nitrogen per unit of digested DM with FF diets was the result of a significant improvement in microbial nitrogen yield, with a concomitant slight numerical reduction in DM digested. Incorporation of feed nitrogen into microbial nitrogen was greater ($P = 0.01$) in the presence of AO, regardless of fat source. The nitrogen content of the microbes was reduced with the AO ($P =$

0.02) and OF ($P = 0.02$) diets, suggesting changes in the microbial population when feeding AO and OF (Table 5).

Fatty Acid Metabolism

Oxidation of the experimental fat by bubbling air during heating oxidized the fat, as reflected by the higher levels of peroxides in the OF compared with the FF (215 vs. 3.5 mEq of peroxides/kg of fat) and the lower supply of unsaturated fatty acids in the OF and OF + AO final diets (Table 2).

The outflow of fatty acids in the effluent varied with AO and type of fat as described in Table 6. Feeding FF diets resulted in lower outflows of 16:0 ($P = 0.01$), 18:0 ($P = 0.01$), and 24:0 ($P = 0.01$), with a trend for 22:0 ($P = 0.06$), and resulted in higher outflows of 20:5 ($P = 0.04$) and other fatty acids ($P = 0.01$), with a trend for *trans*-18:1 ($P = 0.07$), compared with when OF diets

Table 4. Effects of treatments on volatile fatty acid (VFA) production, molar ratios, and average daily pH in the fermenter

Item	Treatment ¹				SE	P-value		
	FF	OF	FF + AO	OF + AO		Fat	AO	Fat × AO
Total VFA, mmol/d	385	397	396	386	7.92	0.87	0.99	0.22
	molar %							
Acetic acid	53.3	52	52.4	54	0.93	0.85	0.57	0.17
Propionic acid	28.4	28.8	29.1	26.9	1.17	0.46	0.62	0.3
Isobutyric acid	0.79	0.74	0.69	0.74	0.03	0.99	0.14	0.16
Butyric acid	13.8	14.8	13.2	15	0.46	0.02	0.61	0.39
	mmol/d							
Acetic acid	205	206	207	209	4.1	0.76	0.61	0.99
Propionic acid	109	114	115	104	6.1	0.62	0.7	0.22
Isobutyric acid	3.1	3	2.7	2.9	0.08	0.93	0.04	0.19
Butyric acid	53	59	52	58	2.01	0.02	0.66	0.97
Average pH	6.29	6.14	6.15	6.18	0.05	0.27	0.32	0.1

¹Treatments: FF = fresh fat; FF + AO = fresh fat with antioxidant; OF = oxidized fat; OF + AO = oxidized fat with antioxidant.

Table 5. Effect of feeding fresh (FF) or oxidized fat (OF) in the presence or absence of antioxidants (AO) to continuous culture fermenters on nitrogen partitioning, microbial growth, efficiency, and composition

Item	Treatment ¹				SE	P-value		
	FF	OF	FF + AO	OF + AO		Fat	AO	Fat × AO
CP digested, %	97.9	87.5	98.5	93	2.35	0.01	0.22	0.34
NAN, g/d	2.59	2.69	2.68	2.69	0.01	0.01	0.01	0.10
Ammonia N, mg/dL	18.93	17.98	17.58	16.62	0.61	0.16	0.06	0.99
Bypass N, g/d	0.07	0.41	0.05	0.23	0.08	0.01	0.21	0.31
Microbial N, g/d	2.52	2.28	2.63	2.47	0.08	0.03	0.09	0.66
Efficiencies								
Microbial N, g/kg of DM digested	37.4	32.7	38.1	34.8	1.53	0.03	0.39	0.63
Microbial N, g/kg of OM digested	43.8	39.2	43.1	41.8	1.57	0.10	0.54	0.32
Microbial N, g/kg of total carbohydrate digested	78.4	68.2	75.7	71	3.66	0.08	0.99	0.48
Feed N, ² %	79.7	78.4	81.3	81	0.67	0.26	0.01	0.49
Total VFA, ³ mol/kg of carbohydrate digested	12.01	11.88	11.38	11.08	0.33	0.53	0.06	0.8
Total VFA, mol/kg of microbial N	153	175	150	158	6.38	0.05	0.16	0.3
Bacterial composition								
Nitrogen, %	9.89	9.70	9.68	9.48	0.08	0.05	0.02	0.81
Ash, %	8.76	10.00	9.03	13.71	1.81	0.13	0.33	0.40
RNA-N, % of total N	10.3	11.00	10.09	10.47	0.36	0.24	0.49	0.81

¹Treatments: FF = fresh fat; FF + AO = fresh fat with antioxidant; OF = oxidized fat; OF + AO = oxidized fat with antioxidant.

²Digested feed N converted to microbial N (%).

³VFA = volatile fatty acids.

were fed. In general, feeding FF diets reduced the outflow of saturated fatty acids ($P = 0.01$), with a trend for improvement in the outflow of unsaturated fatty acids ($P = 0.09$), when compared with OF diets. The presence of AO in the diets tended to reduce the outflow of 18:3 ($P = 0.08$) and increase that of 24:0 ($P = 0.02$) across the types of fats as described in Table 6. A significant AO × type of fat interaction was observed only for 22:6. The mechanism by which AO reduced 22:6 in the OF diets, but not in the FF diets, is unclear and might require further research.

DISCUSSION

The microorganisms present in the continuous culture fermenters responded differently to the 2 types of fat. Feeding OF reduced protein digestibility and microbial nitrogen yield, content, and efficiency (expressed as DM digested) when compared with feeding FF. In prokaryotic and eukaryotic organisms, oxidative stress occurs when there is an undesirable accumulation of free radicals in the cells, resulting in disrupted cellular membrane integrity, DNA replication, and life

Table 6. Effect of feeding fresh (FF) or oxidized fat (OF) in the presence or absence of antioxidant (AO) to continuous cultures of mixed ruminal microbes on the daily outflow of fatty acids in the effluent

Item, mg/d	Treatment ¹				SE	P-value		
	FF	OF	FF + AO	OF + AO		Fat	AO	Fat × AO
16:0	677	705	665	726	13	0.01	0.77	0.25
16:1	60	53	61	58	6	0.38	0.62	0.81
18:0	193	299	199	273	26	0.01	0.71	0.56
<i>Trans</i> -18:1	1,408	1,235	1,448	1,367	61	0.07	0.19	0.47
<i>Cis</i> -18:1	380	395	394	427	17	0.21	0.21	0.61
18:2	340	340	329	354	20	0.54	0.94	0.56
20:0	20	20	19	21	1	0.24	0.34	0.18
18:3	63	64	51	52	6	0.79	0.08	0.97
<i>Cis</i> -9, <i>trans</i> -11 18:2 conjugated linoleic acid	18	14	14	10	4	0.36	0.26	0.98
22:0	19	22	21	23	1	0.06	0.14	0.63
20:5	40	22	37	25	4	0.01	0.90	0.48
24:0	16	17	17	20	0.5	0.01	0.02	0.08
22:6	26	34	32	15	4	0.26	0.10	0.01
Other fatty acids	846	769	802	811	14	0.05	0.93	0.02
Unsaturated fatty acids ²	1,964	1,774	1,982	1,890	73	0.09	0.38	0.52
Saturated fatty acids ³	988	1,185	983	1,130	27	0.01	0.31	0.38

¹Treatments: FF = fresh fat; FF + AO = fresh fat with antioxidant; OF = oxidized fat; OF + AO = oxidized fat with antioxidant.

²Unsaturated fatty acids included 14:1, 16:1, isomers-18:1, 18:2, 18:3, CLA, 22:1, 20:5, and 22:6.

³Saturated fatty acids included 12:0, 14:0, 15:0, 16:0, 18:0, 20:0, 21:0, 22:0, and 24:0.

span of the cells (Scandalios, 2005). Ruminal microorganisms are predominantly anaerobes, with a less-developed antioxidant capacity than aerobic organisms (Brioukhanov and Netrusov, 2004). It is possible that the amount of peroxides in the OF diet would be enough to create oxidative stress in the ruminal microorganism, compromising their proteolytic activity and optimal growth.

Changes in the output of fatty acids in the effluent with type of fat might reflect changes in microorganism fatty acid metabolism. Feeding OF increased the outflow of saturated fatty acids such as 16:0 and 18:0 and reduced the outflow of unsaturated FA such as 20:5, with a trend for *trans*-18:1, compared with feeding FF. A lower outflow of *trans*-18:1 and a higher outflow of 18:0 would imply more complete conversion of these fatty acids to 18:0 when feeding OF diets. The higher biohydrogenation with OF diets was further substantiated by the higher outflow of saturated fatty acids. Changes in bacterial composition, lower CP digestibility, lower microbial nitrogen yield, higher concentration of butyrate, lower *trans* 18:1 content, and higher 16:0 and 18:0 contents in the outflow from fermenters fed OF would reflect changes in the microbial population toward butyrate-producing microorganisms with biohydrogenation activity and low proteolytic activity. It is possible that those rumen microorganisms capable of taking the biohydrogenation process to completion were less susceptible to OF.

The presence of AO in the diet was effective in both the FF and OF diets, causing several positive responses. Antioxidants mostly improved fiber and carbohydrate digestibilities, with modest improvements in microbial nitrogen yield or changes in VFA and pH, regardless of the degree of oxidation of the fat. Improvements in fiber digestibility with no alteration in VFA or pH with AO supplementation would suggest microbial metabolic changes favoring cellulolytic activity. According to Hino et al. (1993), feeding AO such as α -tocopherol and β -carotene alleviates the negative effect of a high level of unsaturated fat supplementation on microbial growth, cellulose digestion, and fatty acid utilization by the rumen microflora, similarly to the present study. The mechanism by which AO compounds ameliorate the toxic effect of excessive unsaturated fatty acids has not been well depicted and might vary with the AO compound and type of fat. The AO fed in the current study are highly lipophylic quinoline compounds. Under an aqueous solution such as rumen fluid, these compounds stay within the lipid moieties, protecting the fatty acids from further peroxidation (Frankel, 2005) and perhaps reducing the toxic effect of unsaturated fatty acids.

The potential role of AO in the biohydrogenation process in the rumen cannot be ruled out. In the current study, AO supplementation tended to reduce the outflow of 18:3, suggesting increased 18:3 biohydrogenation. Recent studies (Bell et al., 2006; Pottier et al., 2006) have linked supplementation of AO such as tocopherol with a lower concentration of *trans*-10 18:1 in milk and reduced milk fat depression. In our current study, no changes were observed in conjugated linoleic acid or total *trans* 18:1 with AO supplementation; however, the concentrations of the different *trans* isomers were not evaluated. The understanding of the role of AO in the biohydrogenation process requires further research.

CONCLUSIONS

Oxidized fat reduced microbial protein metabolism, as measured by lower digestion of CP, lower microbial nitrogen yield, and increased biohydrogenation, by increasing the outflow of saturated fatty acids and reducing *trans*-18:1 when compared with FF. Feeding AO improved the utilization of both OF and FF diets by increasing the fiber and carbohydrate digestibilities and efficiency of use of feed nitrogen for microbial nitrogen. Feeding AO might alleviate the negative effect of feeding unsaturated fatty acids to rumen microbes, regardless of the level of oxidation.

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