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Influence of Linolenic Acid Content on the Oxidation of Milk Fat

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Increasing the content of α-linolenic acid in milk fat might be desirable to meet consumer concerns about dietary healthfulness. However, the rich content of polyunsaturated fatty acids (PUFAs) will influence the oxidative stability of milk fat. This experiment was carried out to determine the effects of infusion with different amounts of high-linolenic perilla fatty acid (HLPFA) emulsion into the duodenum of dairy cows on milk fatty acid profile and the susceptibility of milk fat to oxidation. In a crossover design, 4 multiparous Holstein cows were infused duodenally with increasing amounts (0, 40, 80, 120, or 160 g/day) of free fatty acids from HLPFA emulsion or with carrier alone. Continuous infusions (20 to 22 h/day) were for 7 days at each amount. Infusions were homogenates of HLPFA with 15 g/day of xanthan gum, 5 g/day sodium alginate, and 25 g/day Tween 80; controls received carrier only. The concentration of n-3 PUFAs, especially α-linolenic acid, in milk fat increased linearly as HLPFA infusion increased, but the saturated fatty acids decreased linearly. The milk production and the activity of superoxide dismutase, glutathione peroxidase, and catalase in milk tended to decrease quadratically. The milk fat percentage, however, tended to increase. The concentration of malondialdehyde increased quadratically in milk fat. Results suggest that infusion with HLPFA emulsion at varying amounts enhanced the content of n-3 PUFAs in milk fat over the length of experiment but decreased the oxidative stability of milk fat.

KEYWORDS: Oxidative stability; high-linolenic perilla fatty acid; milk fat

INTRODUCTION

Milk fat is an important dietary source of nutrients and energy. It contains 66% saturated fatty acids, 30% monounsaturated fatty acids, and 4% polyunsaturated fatty acids (*1*). However, an increase in the ratio of saturated to unsaturated fatty acids is associated with an increased risk for cardiovascular disease (*2*). To optimize the balance of fatty acids in milk fat with regard to human health, many measures have been applied. Researchers have fed fats that are rich in unsaturated fatty acids to dairy cows to increase the content of PUFAs in milk fat (*3*, *4*). But, any increase in PUFAs content would have negative effects on milk, with increasing concentration of PUFAs making milk more susceptible to oxidation (*5*).

Recently, it was observed that oxidative reactions in milk are detrimental, because these reactions not only decrease the nutritional value of milk but also increase the amount of off-flavor. Lipid oxidation of milk is highly influenced by content of long-chain unsaturated fatty acids, which are particularly susceptible to oxidation and can give rise to development of off-flavor (*6*, *7*). The rate at which off-flavors develop especially depends on the extent of unsaturation of milk fatty acids.

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Our hypothesis was that HLPFA reaching the small intestine would increase the content of α-linolenic acid in milk fat and decrease the oxidative stability of milk fat in a dose-dependent manner. The objective of this study was to confirm the effect of infusion with increasing amounts of HLPFA into the duodenum of dairy cows on milk fatty acid profile and to investigate effects on the oxidative stability of milk fat.

MATERIALS AND METHODS

Animal Selection. Animal care and procedures were approved and conducted under established standards of the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China. Four multiparous Holstein cows (BW = 556 ± 19 kg, DIM = 93 ± 9 days) that had been fitted with ruminal cannulas and the T-type duodenal cannula were housed in individual tie stalls, and fresh water was provided for ad libitum consumption. Cows were milked at 0700 and 1900 h, the ration was fed in amounts to ensure ad libitum intake at the same time, and milk weights were recorded at each milking. Cows were allowed to exercise daily in an outside lot except during the infusion time. The lactation diet (**Table 1**) contained 50% forage and 50% concentrate (DM basis) to meet China NY/t 34 (*8*) guidelines of nutrient specification for a 550 kg cow producing 20 kg of milk per day. Treatments consisted of homogenized aqueous mixtures of free fatty acids (FFA) from HLPFA emulsion or a control containing only the emulsifying ingredients.

The Preparation of Infusates. Treatments were administered by duodenum infusion to avoid ruminal biohydrogenation of unsaturated FFA. The control infusate consisted of 15 g/day of xanthan gum (Fufeng

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Table 1. Ingredient and Composition of the Basal Diet on a DM Basis (%)

items	% of DM
ingredient	
alfalfa hay	7.0
Chinese wildrye	22.0
corn silage	21.0
ground corn	23.0
wheat bran	3.5
soybean meal	10.5
cotton seed meal	5.5
rapeseed meal	4.0
calcium carbonate	1.2
calcium phosphate, dibasic	1.3
sodium chloride	0.5
mineral−vitamin premix[a]	0.5
total	100.0
component	
NE_L,[b] Mcal/kg DM	1.55
DM, %	51.9
CP, % of DM	16.9
NDF, % of DM	37.42
ADF, % of DM	22.43
EE, % of DM	3.09
Ca, % of DM	0.95
P, % of DM	0.64

[a]Contained 5500 of mg Fe/kg, 4080 mg of Cu/kg, 17500 mg of Zn/kg, 4980 mg of Mn/kg, 110 mg of Se/kg, 180 mg of I/kg, 88.5 mg of Co/kg, VA > 2000 IU/g, 600 IU of vitamin D3/g, and 10.8 mg of vitamin E/g. [b] Calculated according to China NY/t 34 (2004) guidelines.

Table 2. Schematic of Experimental Design and Application of Treatments

	amount of HLPFA[a] infused (g/day)											
		week of experiment										
	preliminary	period 1					washout		period 2			
cow	−1	1	2	3	4	5	6	7	8	9	10	11	12
3860	W[b]	0[c]	0	0	0	0	W	W	0	40	80	120	160
356	W	0	0	0	0	0	W	W	0	40	80	120	160
2863	W	0	40	80	120	160	W	W	0	0	0	0	0
746	W	0	40	80	120	160	W	W	0	0	0	0	0

[a]HLPFA, high-linolenic perilla fatty acid. [b]W, cows were infused with water only. [c]0, For 0 g/day of HLPFA, cows received carrier in water.

Fermentation Co., Ltd., ShanDong, China), 5 g/day sodium alginate (Qingdao Bright Moon Seaweed Group Co., Qingdao ShanDong, China), and 25 g/day Tween 80 (Sigma Aldrich, Germany) in 10 L of purified water (no excess iron or copper). The HLPFA emulsion contained the same ingredients as the control plus HLPFA (Li Nuo Long Biocemistry Inc., HeNan, China) at 40, 80, 120, or 160 g/day. As determined by gas chromatography of methyl esters (*9*), the HLPFA contained (weight basis) 2.8% *cis*-9 18:1, 14.7% *cis*-9 *cis*-12 18:2, 82.4% *cis*-9 *cis*-12 *cis*-15 18:3 and 0.1% other FA. The reason for choosing these particular doses was because free linolenic acid is easily hydrogenated in the rumen of the dairy cows, and generally 18:3 *cis*-9 *cis*-12 *cis*-15 duodenal flow is much lower compared to other C18 fatty acid, such as 18:0 and 18:1 *cis*-9, even 18:2 *cis*-9 *cis*-12. The average duodenal flow of 18:3 *cis*-9 *cis*-12 *cis*-15 is less than 30 g/day. So we adopted such amount with an increment up to approximately 160 g/day HLPFA to evaluate the possible effect on lactating dairy cows.

Experimental Design. The design of our experiment (**Table 2**) was essentially as previously described (*10*). The 4 cows were administered the 2 treatments in a crossover design. After a 1 week preliminary period in which cows were infused only with water, two cows received the control infusate and 2 cows received the HLPFA infusate. During this 5 week experimental period (period 1), the 2 cows receiving the HLPFA infusate received each amount (0, 40, 80, 120, and 160 g/day) sequentially, with each amount continuously being infused for 7 days (20 to 22 h/day) before increasing to the next amount. During period 1, the 2 control cows received only the carrier infusate for the entire 5 week. Measurements were made during the last 3 day of each infusion amount. At the end of week 5, all cows were returned to water infusion for a 2 week washout period before being changed to the opposite treatment for period 2. In period 2, the procedures were repeated, so that the other 2 cows received the HLPFA doses in sequentially increasing amounts and the cows that previously received HLPFA received the control infusate.

The Processing of Infusates. Infusates were prepared daily. The method of operation was according to the previous study (*10*, *11*), but we modified a few details noted below. The ingredients were mixed and heated to 72 °C in steam-jacketed stainless steel vats and then were homogenized into stable emulsions with a homogenizer (model APV 1000, GRUNDFOS Homogenizers, Denmark) at 17.24 MPa during the first stage and 3.45 MPa during the second stage to form stable emulsions. Homogenized mixtures were cooled and stored at 4 °C until use. Each day, the appropriate amount of infusate for each cow was weighed into tared containers while being infused for 20 to 22 h daily. The amount of infusate left in the container was weighed daily to determine the dose of infusion, and the actual infusion amounts were 0, 38.7, 75.4, 117.1, and 155.7 g/day for each expected amounts (0, 40, 80, 120, and 160 g/day) respectively. Placement of the infusion apparatus was confirmed daily to ensure postruminal delivery of infusion treatments. Solutions were pumped into the duodenum by using peristaltic pumps (Watson-Marllow, London, U.K.) via the Norprene food tubing (06402-14; Cole-Parmer Instrument Company, IL).

Milk Collection. Milk was sampled from the last 6 milkings (3 days) of each infusion period. The samples then were composited cold after gentle inversion and pouring between containers. An aliquot of the composite sample was tested in duplicate for concentration of fat and FFA. The concentration of milk fat and FFA were determined by near mid infrared procedures using a MilkoScan Minor machine (MilkoScan Type FT 120, Foss Electric, Hillerød, Denmark). A second set of milk samples from individual cows was composited from the last 2 milkings (1 day) according to the milk yield and stored at −70 °C for further analysis of fatty acid profile, the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-px), and catalase (CAT), the concentration of malondialdehyde (MDA) and cholesterol (CHO) in milk fat.

Milk Fatty Acid Analysis. According to the previous assay method (*4*) for milk fatty acid analysis, frozen milk samples from individual cows were thawed in a refrigerator at 4 °C and centrifuged at 17800*g* for 30 min at 8 °C to separate fat. Fat cake (1 g) was transferred to a 5 mL tube and centrifuged again at 20000*g* for 20 min at 20 °C. Fat (20 mg) was esterified using a method described by Kramer et al (*12*). Separation of FA was achieved by gas chromatography (model 6890 Series II; Hewlett-Packard Co., Avondale, PA) fitted with a flame-ionization detector. Samples containing FA methyl esters in hexane (1 μL) were injected through the split injection port (100:1) onto an SP-2560 fused silica 100 m × 0.25 mm column with a 0.20 μm film (Supelco Inc., Bellefonte, PA). The oven temperature was initially 170 °C for 30 min, and then increased to 200 at 1.5 °C/min and held for 20 min. The temperature then was increased again at 5 °C/min to 220 °C and held for 20 min. Injector and detector temperatures were maintained at 240 °C; total run time was 94 min. Heptadecadienoic acid was used as a qualitative internal standard. Each peak was identified using FA and FA methyl esters (Nu-Chek Prep, Elysian, MN; Matreya, Pleasant Gap, PA; and Supelco 37 Component FAME mix, Supelco, Bellefonte, PA). The percentage of each FA was calculated by dividing the area under the FA peak by the sum of the areas under the total reported FA peaks. Fatty acids were reported as grams per hundred grams of FA methyl esters.

Cholesterol and Antioxidant Enzymes Analysis. For milk CHO, SOD, GSH-px, CAT and MDA analysis, milk samples were thawed and centrifuged for 30 min at 13700*g* at 4 °C. The supernatant was collected, used to precipitate the casein in milk with 4% acetic acid, and then centrifuged at 13700*g* at 4 °C for 30 min. At last, the supernatant was collected for the level of CHO and enzyme activity determinations. All samples and reagents were maintained at 4 °C throughout the preparatory steps until analysis for the above-mentioned indexes. The concentration of cholesterol in milk was assayed by using a kit (Zhongsheng Beikong Bio-Technology and Science Inc., Beijing, China) and performed in series on a HITACHI 7060 autoanalyzer (HITACHI Co., Japan). Commercial assay kits for determining SOD, GSH-Px, CAT, and MDA in the whey were

Table 3. Effects of Increasing Amounts of High-Linolenic Perilla Fatty Acid (HLPFA) Infused into the Duodenum of Dairy Cows on the Yield and Composition of Milk

		HLPFA (g/day)						treatment by amount, *P*		
treatment	variable	0	40	80	120	160	SEM[a]	linear	quadratic	cubic
HLPFA[b]	milk, kg/day	18.5	17.2	16.9	15.9	16.3	1.7	0.40	0.05	0.30
CK[c]		18.2	15.9	16.1	16.7	16.0	1.7			
HLPFA	fat, %	4.01	4.12	3.96	4.32	4.41	0.25	0.09	0.23	0.16
CK		3.69	4.36	4.05	3.95	4.0	0.26			
HLPFA	fat, kg/day	0.74	0.70	0.67	0.68	0.72	0.03	0.40	1.00	0.68
CK		0.68	0.68	0.63	0.66	0.64	0.04			
HLPFA	FFA, %	3.42	2.87	2.43	2.25	2.51	0.53	0.054	0.002	0.40
CK		4.09	5.84	6.63	4.19	5.19	0.56			
HLPFA	cholesterol, mmol/L	0.079	0.073	0.064	0.059	0.092	0.015	0.02	0.80	0.59
CK		0.051	0.10	0.11	0.073	0.10	0.016			

[a]SEM, standard error of the mean. [b]HLPFA, infusion with high-linolenic perilla fatty acid. [c]CK, infusion with carrier only.

bought from Nanjing Jiancheng Bioengineer Institute, China. The principles of these assay kits are briefly described as follows.

SOD activity was determined using the xanthine oxidase method, as based on its ability to inhibit the oxidation of hydroxylamine by the xanthine−xanthine oxidase system. CAT activity was measured according to the ammonium molybdate spectrophotometric method, as based on the fact that ammonium molybdate could rapidly terminate the H_2O_2 degradation reaction catalyzed by CAT and react with the residual H_2O_2 to generate a yellow complex which could be monitored by the absorbance at 405 nm. GSH-Px activity was measured by quantifying the rate of H_2O_2-induced oxidation of GSH to oxidized glutathione (GSSG), catalyzed by GPH-Px. MDA was determined by the thiobarbituric acid (TBA) method, based on its reaction with TBA to form thiobarbituric acid-reactive substances (*13*).

Statistical Analysis. The statistical analysis in our experiment was according to Drackley et al (*10*). Data were analyzed statistically by using PROC MIXED of SAS (version 9.1, SAS Institute Inc., Cary, NC). The model contained the random effect of cow and fixed effects of period (the 5 week sets of infusion amounts within each treatment type; 1 df), treatment (control or HLPFA; 1 df), amount infused (as a subplot; 4 df), and the interaction of treatment and amount (4 df). In this analysis, the statistical parameter of interest is the interaction of treatment by amount, which determined whether cows receving the HLPFA treatment responded differently with advancing amount (weeks) compared with control cows. Polynomial contrasts were constructed to partition the treatment by amount interaction into single degree of freedom interactions of the linear, quadratic, and cubic effects of amount by treatment, and the *P*-values associated with these contrasts are tabulated. Degrees of freedom were determined by using the Kenward−Roger method (*14*). Model residuals were examined and, for all variables, were normally distributed. Least squares means were calculated and are presented with their standard error of the mean throughout. Significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Milk Yield and Composition. Milk yield tended to decrease quadratically as HLPFA infusion increased ($P = 0.05$), which was likely attributable at least in part to the tendency of decreased DMI (data not tabulated) and nutrient intake (**Table 3**). Previously, researchers demonstrated that abomasumally infused unsaturated FA also decreased milk yield (*15*, *16*), but the kind and amount of fatty acids infused were different from those in this study. Increasing the amount of HLPFA infused into the duodenum tended ($P = 0.09$) to linearly increase the milk fat concentration, but milk fat yield was unaffected (**Table 3**). Our result was in accordance with another similar study (*17*), in which infusion with rapeseed oil into duodenum of the dairy cows slightly increased the milk fat content.

The present study showed that the cholesterol content decreased linearly as HLPFA infusion increased (**Table 3**), which may be because the linolenic acid decreases plasma cholesterol concentration (*18*), but the effect is dependent on the dosage of linolenic acid. In our study, the figure of cholesterol from 160 g/day increased in milk fat, which maybe due to the 160 g/day is a high dosage to dairy cows.

With regard to decreasing concentration of FFA in milk fat with increasing dose of HLPFA, the increasing amounts of HLPFA infused into duodenum of the dairy cows might have decreased the susceptibility of milk fat to spontaneous lipolysis. The concentration of FFA was determined primarily to exclude the possibility that the variation of oxidative stability in milk fat was the result of lipolysis of the milk lipids. The extent of lipolysis could increase off-flavor in milk fat, because FFA are more liable to oxidation than are esterified fatty acids, and the content of FFA in our study was in the normal concentration of level (*19*), which did not influence the oxidative stability of milk fat.

Fatty Acid Composition. To meet human dietary requirements, the consumption of α-linolenic acid might be promoted. Concerns about excess saturated fatty acids (SFA) and a deficiency of n-3 PUFA in the human diet have led to recommendations that PUFA/SFA ratio should be close to 0.45 and the n-6/n-3 PUFA ratio should be less than 4 (*20*). In the present experiment, increasing HLPFA infused into the duodenum of dairy cows resulted in a linearly increase in the proportion of most n-3 PUFA, especially $C_{18:3n-3}$ in the milk fat and a concomitant decrease in C_4 to C_{17} saturated fatty acids as well as in oleic acid. Our results showed that changes were qualitatively similar to those seen previously, showing that $C_{18:3n-3}$ and $C_{20:5n-3}$ are increased in swine muscle and adipose tissue, when the concentration of linseed increased in the diet (*21*, *22*) and also infusion with rapeseed oil into the duodenal of dairy cows increased the content of $C_{18:3}$, but the oleic acid was not decreased in milk fat (*23*). Moreover, it was reported that $C_{16:0}$ and $C_{14:0}$ have been implicated as being hypercholesterolemic (*24*). In our study, the majority of saturated fatty acids, including $C_{16:0}$ and $C_{14:0}$ decreased linearly, which demonstrates an improvement for human health.

The atherosclerotic index has been proposed as a measure of the propensity of the human diet to influence the incidence of coronary heart disease. The lower the atherosclerotic index, the lower the likelihood of the milk to cause atherosclerosis in humans. Also the ratio of PUFA to SFA has been used as a measure of atherogenicity (*2*). The additional α-linolenic acid in the milk fat is expected to have a preventive effect against cardiovascular disease when consumed by human (*25*), and the changes in fatty acid profile resulted in a linear decrease in the atherogenic index ($P = 0.05$). The ratio of PUFA to SFA increased linearly, and the ratio of n-6 to n-3 PUFA ($P = 0.13$) with increasing amounts of HLPFA infusion tended to decrease linearly with increasing amounts of HLPFA infusion (**Table 4**), as previously reported by Petit (*26*) for cows fed whole flaxseed. Although the content of fatty acids often is stable in milk, dietary

Table 4. Effects of Increasing Amounts of High-Linolenic Perilla Fatty Acid (HLPFA) Infused into the Duodenum of Dairy Cows on the Composition of Fatty Acids in Milk Fat

		HLPFA (g/day) (g/100 g of fatty acid methyl esters)						treatment by amount, *P*		
treatment	fatty acid	0	40	80	120	160	SEM[a]	linear	quadratic	cubic
HLPFA[b]	12:0	3.98	3.57	3.82	3.27	3.19	0.26	0.45	0.82	0.25
CK[c]		4.05	3.39	3.43	3.17	3.55	0.27			
HLPFA	14:0	12.42	11.64	10.91	9.72	8.85	0.33	0.0001	0.038	0.068
CK		12.35	11.43	11.23	11.11	11.42	0.35			
HLPFA	16:0	35.32	34.65	31.13	29.39	24.94	1.43	0.005	0.017	0.16
CK		36.45	37.29	36.06	38.86	36.17	1.51			
HLPFA	18:1 c9	17.43	16.63	13.68	11.73	11.50	0.81	0.0070	0.053	0.067
CK		16.39	16.79	17.17	16.92	17.08	0.92			
HLPFA	18:2 n-6	2.38	2.94	3.19	3.77	4.16	0.14	0.0001	0.011	0.13
CK		2.25	2.54	2.60	2.45	2.43	0.15			
HLPFA	18:3 n-3	0.61	6.49	12.42	18.75	25.38	1.54	0.0001	0.0001	0.004
CK		0.78	0.96	1.29	1.81	1.47	1.73			
HLPFA	atherogenic index[d]	4.21	3.18	2.72	2.11	1.65	0.20	0.05	0.18	0.005
CK		4.45	4.09	3.92	3.65	3.66	0.20			
HLPFA	PUFA	3.85	10.45	16.53	23.67	30.44	1.53	0.0001	0.0001	0.022
CK		3.88	4.44	4.84	5.13	4.57	1.72			
HLPFA	SFA	74.85	70.13	66.93	62.20	55.98	1.21	0.0001	0.005	0.032
CK		76.01	75.40	74.79	75.04	75.05	1.31			
HLPFA	PUFA/SFA	0.05	0.15	0.25	0.38	0.57	0.04	0.04	0.22	0.0001
CK		0.05	0.06	0.07	0.07	0.06	0.04			
HLPFA	n-6/n-3	4.81	0.55	0.28	0.44	0.21	0.61	0.15	0.13	0.10
CK		3.89	4.21	2.65	5.42	4.44	0.69			

[a]SEM, standard error of the mean. [b]HLPFA, infusion with high-linolenic perilla fatty acid. [c]CK, infusion with carrier only. [d]Calculated as [12:0 + (4 × 14:0) + 16:0]/(16:1 *cis*-7 + 18:1 *cis*-9 + 18:2n-6 + 18:3n-3), based on Ulbright and Southgate (*2*).

Table 5. Effects of Increasing Amounts of High-Linolenic Perilla Fatty Acid (HLPFA) Infused into the Duodenum of Dairy Cows on the Enzymatic Radical Scavenging Systems in Milk Fat

		HLPFA (g/day)						treatment by amount, *P*		
treatment	variable	0	40	80	120	160	SEM[a]	linear	quadratic	cubic
HLPFA[b]	SOD, U/mL	19.91	14.23	17.85	19.50	15.48	1.40	0.56	0.05	0.049
CK[c]		19.82	16.83	14.16	18.27	13.14	1.44			
HLPFA	GSH-px, U/mL	23.58	23.52	22.93	23.92	23.47	0.43	0.70	0.09	0.88
CK		23.70	23.26	23.39	23.63	22.48	0.44			
HLPFA	CAT, U/mL	3.28	3.89	3.08	2.08	2.98	0.59	0.50	0.10	0.41
CK		2.36	3.57	3.34	2.29	4.98	0.57			
HLPFA	MDA,nmol/mL	3.60	3.63	3.53	3.87	3.79	0.14	0.16	0.005	0.02
CK		3.82	3.82	3.78	3.67	3.70	0.15			

[a]SEM, standard error of the mean. [b]HLPFA, infusion with high-linolenic perilla fatty acid. [c]CK, infusion with carrier only.

fatty acids and fatty acid infusions could alter the fatty acid profile in milk (*3*, *4*, *10*).

Oxidative Stability of Milk Fat. Increasing amounts of HLPFA infused into the duodenum did not significantly affect the enzymatic radical scavenging systems (SOD, GSH-px, and CAT) in milk (**Table 5**). The HLPFA infusion, however, tended to quadratically decrease the actvity of SOD ($P = 0.05$), GSH-px ($P = 0.09$), and CAT ($P = 0.10$). In this experiment, the enzymatic radical scavenging systems obviously are not high enough to prevent oxidation of PUFAs in the milk fat, because the concentration of MDA increased quadratically. Also, the consistent and highly significant increase in concentrations of PUFAs in milk fat, as a result of cows receiving increasing amounts of infused HLPFA provides an explanation for the decreased oxidative stability of milk fat. Many earlier studies have shown that increasing concentrations of unsaturated fatty acids in milk fat, particularly of PUFA, $C_{18:2}$ and $C_{18:3}$, will increase the susceptibility of milk to oxidation (*6*, *27*). Moreover, in our study, the content of oleic acid in milk fat decreased significantly as HLPFA infusion increased (**Table 4**), which maybe influence the oxidative stability of the milk fat. However, the previous experiment showed that the higher concentration of oleic acid in milk from cows fed hay did not result in a higher accumulation of lipid hydroperoxides (*28*). So in our study, the variation content of oleic acid in milk fat maybe not the main factor that affected the oxidative stability of the milk fat.

Previous study has shown that linolenic acid is less stable than linoleic acid or more saturated fatty acids, because two pentadienyl groups are more susceptible to extraction of an electron by free radicals, resulting in formation of a lipid free radical (*29*). Evidently, linolenic acid provides more substrates for free radical attack as HLPFA infusion increased. As the content of PUFA in milk increased linearly, the activity of SOD tended to decrease quadratically ($P = 0.05$). This response was consistent with the previous study on the breast milk from human mothers of term (FT) and preterm infants (PT). PT milk contains more long-chain PUFAs than does FT milk, and it also contains higher SOD concentration (in nanograms per milliliter) in milk for FT infants than in milk for PT infants (*30*). One possible cause for the SOD response in cows is that the decreasing DMI (data not shown) would result in a tendency of decreasing absorption of copper and zinc by the dairy cows, which could decrease the activity of Cu−Zn SOD and total SOD activities in milk. Because of SOD catalyzes dismutation of the superoxide anion into hydrogen

peroxide, GSH-Px and CAT catalyze lipid derived hydrogen peroxide into nontoxic alcohols (*31*). The activity of GSH-Px had the same tendency as activity of SOD. Our result was in accordance with previous study with fish oils rich in n-3 PUFA feed to rats that demonstrated a decrease of GSH-px activity in animal tissues (*32*) in response to elevated lipid peroxidation. As in our study, the activity of SOD and GSH-px was decreased and the oxidative stability of milk fat was decreased as the HLPFA infusion increased. The activity of CAT also tended to decrease quadratically (**Table 5**) in milk fat.

In this experiment, the activity of SOD, GSH-px, and CAT tended to decrease quadratically, but the content of PUFAs increased linearly in milk fat as HLPFA infusion increased. Just as the oxidation mechanism of polyunsaturated lipids in milk fat, the PUFAs reacted with harmful free radicals, for example, superoxide anion, and then continued the lipid peroxidation, eventually forming a complex series of compounds that include reactive carbonyl compounds such as MDA (*33*). MDA is a measure of the oxidation of macromolecules by oxygen radicals, which is produced from the oxidation of protein, DNA and lipids by oxygen radicals (*34*). So, MDA formation is the most widely used index of lipid peroxidation is MDA formation (*13*). In that process of mechanism, the SOD would compete with PUFA for reacting with superoxide anion to alleviate the lipid peroxidation. However, as reported by previous study, the increase in PUFA was correlated positively with the concentration of MDA (*35*). In our study, the content of PUFA in milk increased linearly, but the antioxidative effect of SOD, GSH-px, and CAT did not prevent the increase in content of MDA in milk.

Milk fat composition, especially the PUFAs, can be altered by the linolenic acid supplementation into the intestine of dairy cows, but the variation of PUFA profile in milk fat would influence the oxidative stability of milk fat. Our results indicated that abomasum infusion of HLPFA increased proportion of healthy fatty acid (α-linolenic acid) in the milk fat and decreased the oxidative stability of milk fat. For future research, we should consider the nutritive value of milk to human health and the oxidative stability of milk fat as we study dietary regulation of fatty acid composition of milk.

ABBREVIATIONS USED

PUFAs, polyunsaturated fatty acids; HLPFA, high-linolenic perilla fatty acid; FFA, free fatty acid; SFA, saturated fatty acid; SOD, superoxide dismutase; GSH-px, glutathione peroxidase; CAT, catalase; MDA, malondialdehyde; SEM, standard error of the mean; FT, human mothers of term infants; PT, human mothers of preterm infants; CK, infusion with carrier only.

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