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Effects of Different Ratios of Short-Medium Chain Fatty Acids to Long Chain Fatty Acids on Rumen Fermentation and Metabolism of Blood Lipids in Lactating Dairy Cows

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Abstract: The objectives of this study were to investigate the effects of different ratios of Short-Medium Chain Fatty Acids (SMCFA) to Long Chain Fatty Acids (LCFA) on rumen fermentation and metabolism of blood lipids in lactating dairy cows. Thirty six Holstein cows (183±46 DIM) were allotted to three treatments according to randomized block design with 12 cows in each treatment. Cows in treatments were supplemented with 80 g day^{-1} SMCFA mixture and 320 g day^{-1} LCFA mixture, 400 g day^{-1} of butterfat, 240 g day^{-1} SMCFA mixture and 160 g day^{-1} LCFA mixture. Rumen liquid and blood samples were collected from all the cows. The results showed that supplementing different ratios of SMCFA to LCFA did not affect rumen fermentation. As the ratios of SMCFA to LCFA increased, concentrations of trans-11 18:1 linearly increased and the cis-9, cis-12 18:2 quadratically increased and peaked at 40SM60L. The contents of LCFA (>16:0) and PUFA were quadratically increased but that of SFA decreased quadratically. Simultaneously, concentrations of triglycerides in the serum quadratically increased whereas that of insulin decreased quadratically. This study demonstrated that dietary supplementation of different ratios of SMCFA to LCFA did not influence rumen fermentation but altered the plasma fatty acid composition in dairy cows.

Key words: Short-medium chain fatty acid, long chain fatty acid, rumen fermentation, blood lipid, dairy cow

INTRODUCTION

Milk fat is the component that can be significantly influenced by diets (Sutton, 1989; Shingfield *et al*., 2013). Evidence shows that supplementation of fatty acids or some oil containing certain kinds of fatty acids in diet may alter the milk fat percentage yield and composition (Harvatine and Allen, 2006; Khas-Erdene *et al*., 2010; Shingfield *et al*., 2013). It is known that the ratio of Short-Medium Chain Fatty Acids (SMCFA) to Long Chain Fatty Acids (LCFA) is approximately 40:60 in milk fat (Chilliard *et al*., 2000). The previous study has revealed that increasing the ratios of Short-Medium Chain Fatty Acids (SMCFA) to Long Chain Fatty Acids (LCFA) increased milk fat percentage and total SMCFA concentration in milk fat as well as milk total solids proportion (Sun *et al*., 2013). Therefore, it can be deduced that supplemented different ratios of SMCFA to LCFA might influence the metabolism of blood lipids.

It has been well postulated that supplementation of lipids in the diets tends to pose adverse effects on rumen fermentation in dairy cows because most of the dietary lipids may be altered by ruminal biohydrogenation due to the existence of rumen microorganisms. Previous publications have reviewed the metabolic mechanisms of LCFA in the rumen (Doreau and Chilliard, 1997; Chilliard *et al*., 2000). Investigations regarding the metabolism of individual SMCFA in the rumen have also been reported (Liu *et al*., 2009; Fievez *et al*., 2012). However, to the knowledge, the researches on different ratios of SMCFA to LCFA on rumen fermentation are seldom.

As mentioned above with the alteration of milk fat, researchers assumed that metabolism of blood lipids might be altered which has been proved in the previous study (Sun *et al*., 2012). Therefore, the objectives of the present study were to evaluate the effects of dietary supplementation of different ratios of SMCFA to LCFA on rumen fermentation and metabolism of blood lipids in lactating dairy cows which might help explain the possible mechanism and alteration of milk fat caused by supplementation of SMCFA and LCFA.

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MATERIALS AND METHODS

Animals, diets and experimental design: This study was conducted in Beijing Cangdafu Dairy Farm (Beijing, China). All animals used in the present study were maintained according to standards of the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China. Thirty six lactating Holstein cows (DIM = 183±46, average milk yield = 21±3.37 kg day^{-1}, parity = 1.83±1.25) were used in the present study. All the animals were housed in a tie stall barn and had free access to fresh water. The Total Mixed Ration (TMR) was fed individually to the cows at 0700, 1400 and 2000 h, respectively. Ingredients and chemical composition of the basal diet which contained 50% forage and 50% concentrate are shown in Table 1. Cows were daily milked three times at 0730, 1430 and 2030 h when the milk yield was recorded. Thereafter, the cows were allowed to exercise outside every day.

All the cows were randomly assigned to 1 of 3 treatments with 12 cows in each treatment. Treatment mixes were added to diets as follows: 80 g day^{-1} SMCFA mixture and 320 g day^{-1} LCFA mixture (20SM80L, ratio of SMCFA to LCFA was 20:80); 400 g day^{-1} of butterfat (40SM60L, ratio of SMCFA to LCFA was 40:60); 240 g day^{-1} SMCFA mixture and 160 g day^{-1} LCFA mixture (60SM40L, ratio of SMCFA to LCFA was 60:40). The FA composition of the three treatments supplemented is shown in Table 2.

Table 1: Ingredient and chemical composition of the experimental diet

Diet ingredients (DM basis %)	Ratio
Alfalfa hay	10.79
Chinese wildrye	10.79
Corn silage	21.77
Dry distillers grains	5.52
Soybean hull	1.20
Ground corn	23.34
Wheat bran	5.29
Soybean meal	7.34
Cottonseed meal	1.68
Rapeseed meal	1.68
Sunflower seed meal	1.20
Yeast	5.29
Sodium bicarbonate	1.08
Calcium phosphate dibasic	0.48
Limestone	0.84
Sodium chloride	0.60
Calcium carbonate precipitated light	0.48
Magnesium chloride	0.12
Mineral-vitamin premix[a]	0.48
Nutrient composition[b]	
Dry matter (%)	50.00
Crude protein (%)	16.07
Neutral detergent fiber (%)	39.78
Acid detergent fiber (%)	22.39
NE_L (Mcal kg^{-1} DM)[c]	1.48
Ca	0.90
P	0.42

[a]Contained (per kilogram of DM) a minimum 250,000 IU of vitamin A; 65,000 IU of vitamin D; 2,100 IU of vitamin E; Zn 2,100 mg; Cu 540 mg; Fe 400 mg; Mn 560 mg; Se 15 mg; I 35 mg; Co 68 mg; [b]Analysed value; [c]Calculated value (China NY/t34, 2004)

The butterfat supplemented in 40SM60L treatment was purchased from Bright Dairy Co. (Shanghai, China) and was melt at 37°C and separated the oil layer after cooling. SMCFA mixture and LCFA mixture were prepared according to the fatty acid composition in the butterfat. The SMCFA mixture composed of 6% caproic acid, 4% octanoic acid, 9% capric acid, 10% lauric acid, 32% myristic acid and 39% palmitic acid. The LCFA mixture consisted of 59% cocoa butter, 16% olive oil and 25% palm oil. The SMCFA and LCFA provided by the butterfat were approximately 40 and 60% of the total FA.

The experiment lasted for 9 weeks with a pretrial period of 1 week. The FA supplements and butterfat were mixed thoroughly with 800 g soy hulls (Jinhai Foods Inc., Qinhuangdao, China) and consumed by cows of each treatment every morning before feeding and then more TMR was fed subsequently.

Sampling and analyses: Rumen liquid samples were collected using a stomach vacuum pump 2 h post-feeding on 56 day after feeding the experimental diets. After discarding the initially obtained 100-150 mL liquid samples which might be contaminated by saliva, 200 mL of rumen liquid samples were collected from each cow. The pH of the liquid was measured with a pH meter (370 model pH meter; Jenway, London, UK) and the NH_3 concentrations were assayed using the micro-diffusion method of Zitnan *et al.* (1998, 2005). The rumen liquid samples were filtered through gauze before storing at -20°C for later SCFA analysis. The SCFA levels in rumen fluid samples were determined by gas chromatography (Zitnan *et al.*, 1998, 2005).

Table 2: Fatty acids compositions of the fatty acids supplements

	Treatments (g/100 g of FAME[b])[c]		
Fatty acids[a]	20SM80L	40SM60L	60SM40L
4:0	0.00	4.28	0.00
6:0	1.31	2.41	3.92
8:0	0.84	1.39	2.43
10:0	1.85	3.33	5.46
12:0	2.73	3.78	6.66
14:0	6.55	11.09	18.63
14:1	0.00	1.53	0.00
15:0	0.00	1.18	0.00
16:0	30.11	28.67	33.19
16:1	0.25	1.28	0.32
17:0	0.14	0.57	0.07
18:0	18.12	10.74	8.99
cris-9 18:1	32.35	20.77	16.65
trans-11 18:1	0.03	3.32	0.02
CLA	0.11	1.70	0.06
18:2	5.13	2.51	3.26
18:3	0.26	0.87	0.13
20:0	0.36	0.25	0.18
cis-8,11,14 20:3	0.00	0.18	0.00

[a]Expressed as No. of carbons: No. of double bonds; [b]Fatty acid methyl esters; [c]20SM80L supplemented 400 g day^{-1} FA supplement, containing 20% SMCFA mixture and 80% of LCFA mixture; 40SM60L supplemented 400 g day^{-1} of butterfat providing 40% SMCFA and 60% LCFA (approximately); 60SM40L supplemented 400 g day^{-1} FA supplement containing 60% SMCFA mixture and 40% LCFA mixture

About 4 h after morning feeding on the last day of the experiment, blood samples were collected from the tail root vein. Plasma samples were obtained by collecting 7.0 mL of blood into tubes containing lithium heparin for determination of plasma fatty acid composition, Non-Esterified Fatty Acids (NEFA) and Beta-Hydroxybutyric Acid (BHBA). Another 7.0 mL of blood was taken in 9 mL vacutainers (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) for serum collection for analysis of blood parameters.

The fatty acid compositions in the plasma were determined as described by Palmquist and Jenkins (2003). Concentrations of NEFA and BHBA in the plasma were measured by a Synchron Clinical System chemistry analyzer CX5PRO autoanalyzer (Beckman Company, Topeka, Kansas, USA) with bovine kits commercially obtained from Randox (Crumlin, UK). While serum glucose, phospholipid, Total Cholesterol (TC) and Triglycerides (TG) were analyzed using a Synchron Clinical System chemistry analyzer CX5PRO autoanalyzer (Beckman Company, Topeka, Kansas, USA) with bovine kits purchased from Beckman. Plasma samples were also measured for growth hormone (Reynaert and Franchimont, 1974; hGH kit, Beijing Furui Biotech Ltd. Beijing, China) and insulin (Starr *et al*., 1979; INS kit, Beijing Furui Biotech Ltd.).

Statistical analysis: All data were analyzed using the GLM procedures of SAS (Version 9.0, SAS Institute Inc., Cary, NC). The data were tested for linear and quadratic effects of the three fatty acid supplements. All data were presented as least squares means with their standard error of the mean throughout. The significance level was declared at $p<0.05$ and the trends were declared at $p<0.10$.

RESULTS AND DISCUSSION

Rumen fermentation: The results of different ratios of SMCFA to LCFA on rumen fermentation are shown in Table 3. No difference was observed in rumen pH and concentrations of NH_3-N as the ratios of SMCFA to LCFA increased. Concentrations of isobutyrate butyrate and valerate as well as the acetate to propionate ratio did not differ with the supplementation of different ratios of SMCFA to LCFA except that isovalerate concentration tended to be elevated linearly ($p = 0.08$). Although, the acetate, propionate and TVFA concentrations slightly increased as the ratios of SMCFA to LCFA elevated, still no differences were observed in the three treatments. Additionally, acetate to propionate ratio tended to decrease quadratically lowered at 40SM60L ($p = 0.07$).

Plasma fatty acids compositions: As the ratios of SMCFA to LCFA increased, concentrations of trans-11 18:1 linearly increased ($p<0.05$) and the cis-9, cis-12 18:2 quadratically increased and peaked at 40SM60L ($p<0.05$). The contents of LCFA (>16:0) and PUFA were quadratically increased ($p<0.05$) but that of SFA and SCMFA (<16:0) decreased quadratically ($p<0.05$). There was no difference in other fatty acids in the three treatments (Table 4).

Blood parameters: As presented in Table 5 there were no significant effects in blood glucose, cholesterol, phospholipids, NEFA, BHBA and growth hormone among different treatments. As the ratios of SMCFA to LCFA increased in the diets, concentrations of triglycerides quadratically increased ($p<0.05$). Different ratios of SMCFA to LCFA supplements did not influence the concentration of growth hormone but quadratically decreased that of insulin ($p<0.01$) in the sera of dairy cows as the percentage of SMCFA increased.

The present study investigated the effects of dietary supplementation of different ratios of SMCFA to LCFA on rumen fermentation and metabolism of blood lipids in lactating dairy cows. Available literatures have demonstrated that different from non-ruminants, absorbed fatty acids in the intestine of ruminants are different from

Table 3: Ruminal fermentation parameters from cows fed 20SM80L, 40SM60L and 60SM40L

	Treatments[a]				p-value		
Items	20SM80L	40SM60L	60SM40L	SEM[b]	Treatment	Linear	Quadratic
pH	6.67	6.65	6.59	0.10	0.81	0.54	0.89
NH_3-N (μmol mL^{-1})	32.92	35.22	34.01	2.26	0.78	0.73	0.55
Fatty acids (μmol mL^{-1})							
Acetate	70.09	71.52	77.36	4.42	0.49	0.26	0.70
Propionate	18.21	20.53	20.57	1.42	0.41	0.25	0.53
Isobutyrate	0.84	0.89	0.98	0.06	0.25	0.10	0.78
Butyrate	9.01	10.39	9.61	0.72	0.43	0.55	0.26
Isovalerate	1.54	1.82	1.81	0.11	0.13	0.08	0.29
Valerate	1.39	1.53	1.62	0.11	0.32	0.14	0.86
TVFA	101.07	106.68	111.33	6.58	0.55	0.28	0.95
Acetate/Propionate ratio	3.86	3.54	3.76	0.11	0.15	0.55	0.07

[a]20SM80L supplemented 400 g day^{-1} FA supplement, containing 20% SMCFA mixture and 80% of LCFA mixture; 40SM60L supplemented 400 g day^{-1} of butterfat providing 40% SMCFA and 60% LCFA (approximately); 60SM40L supplemented 400 g day^{-1} FA supplement containing 60% SMCFA mixture and 40% LCFA mixture; [b]SEM = Standard Error of least squares Means

Table 4: Plasma fatty acids compositions from cows fed 20SM80L, 40SM60L and 60SM40L (g/100 g of FAME[z])

	Treatments[y]				p-values		
Items	20SM80L	40SM60L	60SM40L	SEM[x]	Treatment	Linear	Quadratic
Fatty acid[w]							
14:0	1.52	1.30	1.47	0.09	0.20	0.77	0.08
16:0	10.65	10.18	10.73	0.21	0.10	0.82	0.04
16:1	0.88	0.86	0.83	0.07	0.92	0.70	0.97
18:0	10.72	9.77	11.17	0.54	0.12	0.61	0.07
trans-9 18:1	0.12	0.11	0.08	0.03	0.72	0.44	0.96
trans-11 18:1	0.29[b]	0.33[ab]	0.38[a]	0.02	0.03	0.01	0.80
cis-9 18:1	7.27	6.52	7.21	0.49	0.43	0.93	0.21
cis-9, cis-12 18:2	53.05[b]	56.95[a]	52.97[b]	1.17	0.03	0.97	0.01
18:3	3.87	4.00	3.92	0.17	0.86	0.85	0.59
cis-9, trans-11 CLA[v]	0.13	0.26	0.15	0.14	0.76	0.93	0.47
Summations							
<16:0	2.81	2.21	2.69	0.20	0.08	0.70	0.03
>16:0	85.66[b]	86.75[a]	85.75[b]	0.33	0.04	0.86	0.01
≥20:0	10.21	8.83	9.86	0.60	0.24	0.72	0.10
SFA[u]	27.68[a]	25.55[b]	27.85[a]	0.63	0.02	0.87	0.01
MUFA[t]	10.00	8.40	9.66	0.72	0.24	0.77	0.10
PUFA[s]	62.31[b]	66.05[a]	62.49[b]	1.05	0.02	0.92	0.01

[a, b]Means in the same row with different superscripts differ significantly for treatment effect; [z]Fatty acid methyl esters; [y]20SM80L supplemented 400 g day^{-1} FA supplement containing 20% SMCFA mixture and 80% of LCFA mixture; 40SM60L supplemented 400 g day^{-1} of butterfat providing 40% SMCFA and 60% LCFA (approximately); 60SM40L supplemented 400 g day^{-1} FA supplement, containing 60% SMCFA mixture and 40% LCFA mixture; [x]SEM = Standard Error of least squares Means; [w]Expressed as numbers of carbons: numbers of double bonds; [v]Conjugated linoleic acid; [u]Saturated fatty acids; [t]Monounsaturated fatty acids; [s]Polyunsaturated fatty acids

Table 5: Blood parameters from cows fed 20SM80L, 40SM60L and 60SM40L

	Treatments[z]				p-value		
Items	20SM80L	40SM60L	60SM40L	SEM[y]	Treatment	Linear	Quadratic
Glucose (mmol L^{-1})	2.84	2.74	2.89	0.20	0.88	0.88	0.62
Triglycerides (mmol L^{-1})	0.39	0.45	0.39	0.02	0.12	0.89	0.04
Cholesterol (mmol L^{-1})	6.84	5.59	6.29	0.46	0.18	0.40	0.10
Phospholipids (mmol L^{-1})	3.15	3.24	3.11	0.05	0.22	0.56	0.10
NEFA (mmol L^{-1})	0.49	0.53	0.46	0.02	0.19	0.39	0.10
BHBA (mmol L^{-1})	0.49	0.49	0.54	0.03	0.50	0.31	0.53
Growth hormone (ng mL^{-1})	3.07	3.88	3.74	0.29	0.13	0.12	0.20
Insulin (ng mL^{-1})	18.26[b]	14.93[c]	21.41[a]	1.01	<0.01	0.03	<0.01

[a-c]Means in the same row with different superscripts differ significantly for treatment effect; [z]20SM80L supplemented 400 g day^{-1} FA supplement containing 20% SMCFA mixture and 80% of LCFA mixture; 40SM60L supplemented 400 g day^{-1} of butterfat providing 40% SMCFA and 60% LCFA (approximately); 60SM40L supplemented 400 g day^{-1} FA supplement, containing 60% SMCFA mixture and 40% LCFA mixture; [y]SEM = Standard Error of least squares Means

consumed dietary fatty acids due to the ruminal hydrogenation (Doreau and Chilliard, 1997). Some literatures reported that dietary addition of LCFA have potentially negative effects on rumimal activity including alteration of ruminal pH, Volatile Fatty Acids (VFA) and micro-organisms, etc. (Doreau and Chilliard, 1997; Dohme *et al*., 2001; Hristov *et al*., 2005; Liu *et al*., 2012) found that >20 carbon LCFA influenced rumen fermentation by promoting the alteration in the bacterial population of fiber digestion and hydrogenation. But others indicated that ruminal pH, ammonia concentration and major VFA concentrations were not affected when the beef cattle were fed with the diets containing linoleic acid- or oleic acid-rich oils (Hristov *et al*., 2005). Dohme *et al*. (2001) revealed that supplementation of pure medium-chain fatty acids such as C8:0 and C14:0 had no effects on pH, ammonium and VFA concentrations using the rumen simulation technique. Consistent with those reports in the present study, no differences were observed in rumen pH, NH_3-N and TVFA of the dairy cows from different treatments, although, the acetate: propionate ratio in 40SM60L tended to be lower than the other two treatments which suggested that under the conditions of the present study, different ratios of SCMFA to LCFA in the diets did not affect rumen fermentation in dairy cows.

Many studies have presented the effects of dietary application of fatty acids or certain oils on plasma fatty acid composition and relative parameters. Moore *et al*. (1969) reported that addition of stearic acid and palmitic acid increased the concentrations of C18:0 and C16:0 in the plasma lipids. Subsequently, Rindsig and Schultz (1974) found that feeding lauric acid to lactating cows enhanced plasma cholesterol but did not alter blood glucose, ketones and acetate, free fatty acids and triglycerides concentrations. Gonthier *et al*. (2005)

demonstrated that feeding dairy cows with flaxseed increased plasma cholesterol, NEFA, LCFA and MSFA but decreased plasma medium-chain and saturated fatty acids.

It is known that LCFA in milk fat originated from feed and body adipose tissues (Mansbridge and Blake, 1997; Kalac and Samkova, 2010). In the present study, feeding SMCFA and LCFA mixture did not affect plasma concentrations of C16:1, C18:1trans and C18:1cis, thus, MUFA concentration did not differ in different treatments. However, different ratios of SMCFA to LCFA in diets altered the sum of C18:2 and C18:3 which contributed to the quadratically increased PUFA concentration peaked in 40SM60L. Furthermore, when the ratio of SMCFA to LCFA at 40:60 in the diet, the plasma SFA concentration was the lowest as a result of the relatively lower C14:0, C16:0 and C18:0 in the plasma of dairy cows. In addition, the variation of LCFA in the plasma in the current study agreed with the alteration of that in the milk in the previous study (Sun *et al*., 2013). These results indicated that supplementation of SMCFA and LCFA at the ratio of 40:60 helped elevate the concentrations of LCFA, especially PUFA but lowered that of SFA.

NEFA, cholesterol, triglycerides and phospholipids are the important components in blood lipids. Fatty acids uptake by mammary gland mainly originated from blood NEFA (Chilliard *et al*., 2000). Plasma NEFA concentration is closely related to body lipolysis (Chilliard *et al*., 1984). In the present study, no difference was observed in plasma NEFA of the dairy cow from different treatments, implying that different ratios of SMCFA to LCFA in diets did not influence metabolism of body lipids.

In this study, serum triglycerides quadratically increased as the ratios of SMCFA to LCFA increased in the diets. Simultaneously, different ratios of SMCFA to LCFA in the diets had no effects on serum concentrations of glucose, cholesterol and BHBA. The reasons might be that in the three treatments, the amounts of SCMFA and LCFA supplements are the same except for the supplemental ratios which might be responsible for the obtained results in the current study.

A finding of the present study is that blood growth hormone was not changed but insulin was significantly different in the three treatments. Previous publications have proposed that supplemental oil or fat had no effects on blood growth hormone (Gagliostro and Chilliard, 1991; Grummer and Carroll, 1991; Bu *et al*., 2007). Although, insulin could stimulate the lipogenesis rate but inhibit lipolysis rate in adipose tissue (Griinari *et al*., 1997), it had no effects on milk fat yield even if the concentrations increased at four or five folds in the circulation system (Mcguire *et al*., 1995). The increased insulin would reduce fatty acid mobilization thus reduce the circulating NEFA in the plasma (Griinari *et al*., 1997). In agreement with those documents in the present study, the variation of insulin concentrations in the 60SM40L treatment was higher but that of NEFA slightly lower than the other two treatments. Furthermore, different plasma insulin concentrations as a consequence of the increased supplemental ratios of SMCFA to LCFA did not influence the milk fat yield which had been presented in the previous study (Sun *et al*., 2013).

CONCLUSION

Supplementation of different ratios of SMCFA to LCFA in diets did not influence rumen fermentation but altered the plasma fatty acid composition in dairy cows which resulted in the increased fat content and total SMCFA concentration in milk fat in the previous study. Combined with the results obtained in the previous study, ratio of SMCFA to LCFA at 60:40 was a relatively better combination increasing the concentrations of LCFA, especially PUFA but lowering that of SFA in plasma of dairy cows which had also been regarded as the better ratio for milk fat synthesis.

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