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Effects of supplemental energy sources and *trans*-10, *cis*-12 conjugated linoleic acids (CLA) on milk yield and composition in lactating Holstein cows

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Abstract The effects of supplemental energy sources (corn or saturated fatty acids) and *trans*-10, *cis*-12CLA on milk yield and composition were investigated in the present study. Four multiparous Holstein cows (560 ± 31 kg BW, 98 ± 10.5 DIM) were designated to a 2×2 factorial 4×4 Latin Square experiment. Treatments were isoenergetic supplementation of corn or calcium salt of saturated fatty acids (CaFA) with or without calcium salt of CLA. Both milk yield and composition were affected by sources of supplemental energy. Compared to CaFA supplementation, corn supplementation increased the milk yield, the lactose yield, and the content and yield of milk protein but decreased the yield and content of milk fat. Plasma insulin was higher, plasma glucose tended to be higher, and plasma non-esterified fatty acid (NEFA) was lower when corn rather than CaFA was supplemented. CaCLA supplementation did not affect milk yield and the yield of measured milk components, but increased the content of milk protein and decreased the content of total solids. Plasma glucose was decreased by CaCLA supplementation. Content and yield of short and medium chain fatty acids (≤ 16 carbon atoms) in milk fat increased or remained unchanged while those of long chain fatty acids (> 16 carbon atoms) decreased or remained unchanged by corn supplementation. CaCLA supplementation failed to increase the content and yield of *trans*-10, *cis*-12CLA in milk fat, which explained the unobserved depression of milk fat synthesis by CaCLA supplementation. Yields of *de novo* synthesized fatty acids in the mammary gland were increased rather than decreased by corn supplementation. The decreased milk fat yield by corn supplementation could be exclusively attributed to

reductions in preformed fatty acids, which might be a result of depressed lipolysis by stimulated insulin secretion.

Keywords glucose, insulin, CLA, milk composition

1 Introduction

Glucogenic-insulin and *trans*-fatty acid are the two major theories to explain diet-induced milk fat depression (MFD) that still receive support. Davis and Brown (1970) noticed that diet-induced MFD was usually accompanied by elevated *trans*-C18:1 in milk fat and suggested that the diet-induced MFD might be related to shifted biohydrogenation of polyunsaturated fatty acids in the rumen. Later research work revealed that ruminal polyunsaturated fatty acid biohydrogenation shifted towards producing more *trans*-10, *cis*-12 conjugated linoleic acid (CLA) when high concentrate/low roughage (HC/LR) diets were fed, and *trans*-10, *cis*-12 CLA was proven to be a potent inhibitor of milk fat synthesis (Chouinard et al., 1999; Baumgard et al., 2002; Bauman and Griinari, 2003).

More propionate is usually produced in the rumen with HC/LR diets, which may in turn promote hepatic gluconeogenesis and insulin secretion. Increased circulating insulin depresses lipolysis in adipose tissue and directs lipogenic precursors away from the mammary gland. The glucogenic-insulin theory believes it is the increased circulating insulin that depresses milk fat synthesis. In experiments on the theory variable results were observed. Though milk fat was consistently depressed by ruminal propionate infusion (Hurtaud et al., 1993; Miettinen and Huhtanen, 1996; Maurizio et al., 1997; Li et al., 2007) or postruminal glucose infusion (Rigout et al., 2002; Zhang, 2006), the extents varied from about 3% to 23%. McGuire et al. (1995) and Griinari et al. (1997) used hyperinsulinemic-euglycemic clamp techniques to investigate the effect of insulin on milk fat synthesis in lactating cows. Milk fat

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content and yield decreased only slightly and the differences were insignificant. Corl et al. (2006) observed a 27% decrease in milk fat yield in clamped Holstein cows in the immediate post partum period, and the analysis of milk fatty acid composition revealed that the decrease could exclusively be attributed to reduced supply of preformed fatty acids to the mammary gland. Furthermore, there were evidences that yields or contents of *de novo* synthesized fatty acids in milk fat were increased by postruminal glucose infusion (Rigout et al., 2002; Zhang, 2006) or hyperinsulinemic-euglycemic clamp (Gariinari et al., 1997), which implied that insulin may direct lipogenic precursors towards rather than from the mammary gland. Insulin seems to affect milk fat synthesis in a reversed dual direction manner and depresses milk fat synthesis by reducing the supply of preformed fatty acids on one hand, and promotes it by facilitating the synthesis of *de novo* synthesized fatty acid on the other.

More research work is clearly needed to elucidate the effect of elevated circulating insulin on milk fat synthesis, and the effect of insulin may be cumulative with that of *trans*-fatty acids if both of them contribute to MFD. Corn or CaFA (Megalac) was supplemented in the diet of mid-lactating Holstein cows with or without co-supplementation of a CaCLA product containing *trans*-10, *cis*-12 CLA on isoenergetic basis in our study. The objectives were to investigate if corn supplementation could depress milk fat synthesis through stimulating insulin secretion, and if the milk fat depressing effect of *trans*-10, *cis*-12 CLA could be superimposed on that of corn supplementation.

2 Materials and methods

2.1 Animals, basal diet, and treatments

Four multiparous Holstein cows (560 ± 31 kg BW, 98 ± 10.5 DIM) were designated to a 2×2 factorial 4×4 Latin Square experiment. Basal diet consisted of 2 kg alfalfa hay, 1 kg Chinese wildrye, 25 kg whole corn silage, and a mixed concentrate. The daily offered roughage was calculated to contain 48.36 MJ of NE_L and 406.49 g of MP, and the mixed concentrate was calculated to contain $7.43 \text{ MJ} \cdot \text{kg}^{-1}$ of NE_L and 114.55 g of $MP \cdot \text{kg}^{-1}$ (Table 1) based on a set of data measured by the China Agricultural University. Daily amount of mixed concentrate fed to each cow was calculated on the basis that concentrate plus roughage could meet the nutrient requirements of the animals according to the recommendations of NRC (2001).

900 g calcium salt of saturated fatty acids (Megalac, VOLAC International Ltd., UK, Table 2) or 2.5 kg corn was supplemented to the basal diet on isoenergetic basis ($19 \text{ MJ } NE_L$). 70 g of a product (CaCLA, Qingdao Auhai Biotech Co. Ltd., Qingdao, China, Table 2) containing $33.7 \text{ g} \cdot 100 \text{ g}^{-1}$ *trans*-10, *cis*-12 conjugated linoleic fatty

acid was supplemented on the basis of Megalac or corn supplementation. Seventy g of Megalac instead of CaCLA was supplemented when CaCLA was not supplemented to keep the energy balanced among treatments. So the ingredients supplemented in the four treatments were 970 g Megalac (CaFA), 900 g Megalac plus 70 g CaCLA (CaFA + CLA), 2.5 kg corn plus 70 g CaCLA (corn +

Table 1 Composition and nutrient level of the mixed concentrate

ingredient	composition ($\text{g} \cdot \text{kg}^{-1}$)	nutrients ²⁾	contents
corn	509.9	DM, $\text{g} \cdot \text{kg}^{-1}$	897.0
wheat bran	51.1	NE_L , $\text{MJ} \cdot \text{kg}^{-1}$ _DM	7.43
soybean meal	234.2	MP, $\text{g} \cdot \text{kg}^{-1}$ _DM	114.55
cottonseed meal	113.5	NDF, $\text{g} \cdot \text{kg}^{-1}$ _DM	76.6
CaHPO ₄	22.0	ADF, $\text{g} \cdot \text{kg}^{-1}$ _DM	32.2
limestone	12.0	Ca, $\text{g} \cdot \text{kg}^{-1}$ _DM	4.0
salt	12.0	P, $\text{g} \cdot \text{kg}^{-1}$ _DM	3.6
premix ¹⁾	45.3	—	—

Note: 1) representing the premix, is composed of $500 \text{ g} \cdot \text{kg}^{-1}$ limestone, $180 \text{ g} \cdot \text{kg}^{-1}$ CaHPO₄, $10 \text{ g} \cdot \text{kg}^{-1}$ Fe, $2 \text{ g} \cdot \text{kg}^{-1}$ Cu, $9 \text{ g} \cdot \text{kg}^{-1}$ Zn, $40 \text{ mg} \cdot \text{kg}^{-1}$ CoSO₄, $32 \text{ mg} \cdot \text{kg}^{-1}$ Na₂SeO₃, $290 \text{ g} \cdot \text{kg}^{-1}$ MgSO₂, $750 \text{ mg} \cdot \text{kg}^{-1}$ Vitamin A, $200 \text{ mg} \cdot \text{kg}^{-1}$ Vitamin D and $750 \text{ mg} \cdot \text{kg}^{-1}$ Vitamin E3; 2) representing the data were calculated using a set of data measured by the China Agricultural University.

Table 2 Ingredients and composition of Megalac and CaCLA

Megalac		CaCLA	
ingredient	content($\text{g} \cdot \text{kg}^{-1}$)	ingredient	content($\text{g} \cdot \text{kg}^{-1}$)
calcium	90.0	calcium	72
moisture	50.0	moisture	19
others	20.0	C16:0	50
C14:0	12.6	C18:0	20
C16:0	369.6	<i>cis</i> -9- C18:1	99
C18:0	42.0	<i>cis</i> -9, <i>cis</i> -12- C18:2	20
C18:1	336.0	<i>cis</i> -9, <i>trans</i> -10- CLA	336
C18:2	79.8	<i>trans</i> -10, <i>cis</i> -12 CLA	337
—	—	other CLAs	46

Note: Megalac is a product of calcium salts of saturated fatty acids produced by VOLAC International Ltd., UK; CaCLA is calcium salt of conjugated linoleic acid.

CLA), and 2.5 kg corn plus 70 g CaFA (corn), respectively.

Animals were kept in individual free stalls and allowed free access to water during the experiment. Diets were divided into three equal portions and fed to the animals at 07:00, 14:00, and 20:00 each day. No significant feed refusal was observed during the experiment. Milking was performed at the time of each feeding. All the handling procedures were approved by the Academic Committee of the College of Animal Science, Shandong Agricultural University.

2.2 Experimental procedure, sampling, and sample determination

The experiment consisted of four periods of 18 d each. Cows were differently treated in a cross-over manner at different periods. After 14 days of pre-feeding, the average of milk yields recorded in the successive three days was used for comparison among treatments. Milk and blood samples were taken at the last day (18th day) of each period. Weighed samples of milk taken at morning, noon, and evening milking were pooled together according to the milk yield in each milking and stored at -20°C for further analysis. A 10 mL blood sample was drained from the jugular vein into a heparin tube 2 hours after morning feeding, immersed into ice slush immediately, and centrifuged at $3000\text{ r}\cdot\text{m}^{-1}$ for 10 min as soon as possible. Plasma was divided into four equal portions and stored at -70°C for further analysis of glucose, insulin, and non-esterified fatty acids (NEFA).

Extracted milk fat was used to prepare fatty acid methyl esters (FAME) by a mild transesterification with $0.4\text{ mol}\cdot\text{L}^{-1}\text{ H}_2\text{SO}_4$ in methanol, and the composition of FAME was determined by GLC analysis according to the method of Piperova et al. (2004). Plasma NEFA and glucose were determined enzymatically, and plasma insulin was determined by immunoassay using commercially available kits (NEFA, Nanjing Jiancheng Bio-engineering Institute; glucose, Lideman Biochemical Co. Ltd, Beijing; insulin, Northern Biotech Institute, Beijing). Milk fat, protein, and lactose were measured using the Bentley 150 Infrared Milk Analyzer.

2.3 Statistics

Data were subjected to ANOVA using the general linear models procedure of SAS. The statistical model used was as follows:

$$Y_{ijkl} = \mu + N_i + I_j + NI_{ij} + P_k + C_L + e_{ijklm},$$

where Y_{ijklm} = observation for the L th cow in the k th period; μ = overall mean; N_i = effect of supplemental energy, $i =$

900 g Megalac or 2.5 kg corn; I_j = effect of CLA; $j = 70\text{ g CaCLA}$ or 70 g Megalac ; NI_{ij} = effect of the interaction of supplemental energy and CLA; P_k = effect of period, $k = 1, 2, 3, 4$; C_L = effect of cow, $L = 1, 2, 3, 4$; e_{ijklm} = random error. Significance was declared at $P < 0.05$, and a tendency was declared at $P < 0.1$.

3 Results

3.1 Milk yield and composition

Cows tended to produce more milk when receiving corn instead of CaFA supplementation, and an average of $1.43\text{ kg}\cdot\text{d}^{-1}$ or more milk was produced in the two corn supplementation treatments ($P < 0.1$) (Table 3). Milk composition was affected by sources of supplemental energy but not by CaCLA in the present study. Compared to CaFA supplementation, corn supplementation decreased milk fat content ($P < 0.05$) and increased milk protein yield ($P < 0.05$) as well as the content of non-fatty solids ($P < 0.05$), tending to decrease milk fat yield and the content of milk total solids ($P < 0.1$). On average, the corn supplementation decreased milk fat content by 12.27% and milk fat yield by 7.96%. Though the effects of sources of supplemental energy on milk protein content ($P = 0.1511$), and lactose yield ($P = 0.1443$) were statistically insignificant, the P values were quite near the tendency level. Numerically, corn supplementation increased milk protein content and lactose yield. Unexpectedly, *cis*-10, *trans*-12 CLA supplementation did not change milk fat content and yield in the present study ($P > 0.1$), although the daily amount of *cis*-10, *trans*-12 CLA offered to each cow reached 23.59 g in the two CaCLA supplementation treatments.

3.2 Plasma NEFA, insulin, and glucose

The corn supplementation tended to increase circulating insulin but decreased plasma NEFA as compared to CaFA supplementation ($P < 0.1$) (Table 4). The plasma glucose

Table 3 Effects of sources of supplemental energy (corn or CaFA) and CaCLA on milk yield and composition in Holstein cows

item	treatment				SEM	P value		
	CaFA	CaFA + CLA	corn + CLA	corn		energy	CLA	energy \times CLA
milk yield/($\text{kg}\cdot\text{d}^{-1}$)	17.9	18.47	19.83	19.4	1.43	0.0728	0.8756	0.6194
milk fat/%	4.78	4.51	4.07	4.08	0.27	0.0182	0.4019	0.5737
milk fat/($\text{g}\cdot\text{d}^{-1}$)	854.23	848.16	802.22	764.74	83.09	0.0842	0.99	0.2496
milk protein/%	3.14	3.26	3.38	3.33	0.14	0.1511	0.3365	0.4337
milk protein/($\text{g}\cdot\text{d}^{-1}$)	560.83	580.73	666.03	654.05	42.86	0.0156	0.3323	0.3091
Lactose/($\text{g}\cdot\text{d}^{-1}$)	887.42	851.47	966.89	929.6	83.6	0.1443	0.8711	0.3026
non-fat solids (NFS)/%	8.57	8.5	8.73	8.78	0.12	0.0402	0.5092	0.4844
total solids (TS)/%	13.36	12.93	12.82	12.88	0.23	0.0757	0.1489	0.6193

Note: CaCLA, CaFA and CLA represent calcium salts of conjugated linoleic acid, calcium salts of saturated fatty acids and conjugated linoleic acid respectively.

Table 4 Effects of sources of supplemental energy (corn or CaFA) and CaCLA on concentrations of NEFA, insulin, and glucose ($\text{mmol} \cdot \text{L}^{-1}$) in the plasma of the jugular vein of Holstein cows

item	treatment				SEM	P value		
	CaFA	CaFA + CLA	corn + CLA	corn		energy	CLA	energy \times CLA
NEFA	1.49	1.35	0.36	0.31	0.1	0.0704	0.6431	0.3996
insulin	10.25	10.17	11.33	11.84	1.33	0.0819	0.6608	0.7736
glucose	3.44	3.3	3.39	3.52	0.12	0.1516	0.0777	0.7265

Note: NEFA means non-esterified fatty acids.

concentration in the two treatments of corn supplementation was numerically higher than that of the two CaFA supplementations, but the difference was statistically insignificant though the P value was quite near the tendency level ($P = 0.1516$). However, the CaCLA supplementation tended to decrease plasma glucose ($P < 0.1$), without effects on plasma NEFA and insulin ($P > 0.1$). There was no interaction between these two factors on the measured plasma concentrations ($P > 0.1$).

3.3 Fatty acid profile and yields of milk fatty acids

The content of short and medium chain fatty acids (≤ 16 carbon atoms) in milk fat was generally increased or remained unchanged, while that of long chain fatty acids (≥ 16 carbon atoms) was generally decreased or remained unchanged by corn supplementation (Tables 5, 6). The corn supplementation increased the content of C6, C8, C10, C12, and C14 ($P < 0.05$), however, it decreased the content of *cis*-9C18:1 ($P < 0.05$), and tended to decrease the contents of *trans*-12C18:1 ($P < 0.1$). As for the CaCLA supplementation, it decreased the content of *trans*-11C18:1 and *cis*-9, *trans*-11CLA ($P < 0.5$), and tended to decrease the content of *trans*-12C18:1 ($P < 0.1$). Sources of supplemental energy modified the depressing effect of CaCLA on these three fatty acids. The depressing effects were more prominent when corn was supplemented ($P < 0.05$), and the content of *trans*-12C18:1 was actually slightly increased by CaCLA when CaFA was supplemented ($P < 0.05$).

The corn supplementation increased the yield of C10 and simultaneously decreased the yield of *cis*-9C18:1 and C18:2 ($P < 0.05$). The yields of C8 and C12 tended to be increased while the yields of *trans*-12C18:1 and C18:3 tended to be decreased by corn supplementation ($P < 0.1$). The CaCLA supplementation only decreased the yield of *trans*-11C18:1 and *cis*-9, *trans*-11CLA ($P < 0.05$). There was no interaction between CaCLA and sources of supplemental energy on the yield of individual milk fatty acids ($P > 0.1$). What should be noticed is that both the content and yield of *trans*-10, *cis*-12CLA were not increased by CaCLA supplementation ($P > 0.1$), which may explain the unobserved depressing effect of *trans*-10, *cis*-12CLA on milk fat.

4 Discussion

4.1 High insulin/glucose condition

The corn supplementation tended to increase circulating insulin ($P < 0.1$) without significant influence on circulating glucose in the present study, though the level of circulating glucose of the two corn supplementation treatments was numerically higher than that of the two CaFA supplementation treatments and the difference was quite near the statistical tendency standard ($P = 0.1516$). Therefore, while the corn supplementation stimulated insulin secretion, there was no clear clue on whether the corn supplementation could also promote glucose production or not. Previous researches revealed that increased circulating propionate itself could stimulate insulin secretion (Cole and Hallford, 1994; Sano et al., 1995), and the increased gastrointestinal supply of propionate might decrease rather than increase the endogenous glucose production in lactating cows (Knowlton et al., 1998; Lemosquet et al., 2004). Therefore, the elevated circulating insulin could be exclusively due to the increased ruminal propionate production by corn supplementation. Unfortunately, ruminal propionate production was not monitored in our study.

4.2 CaCLA and plasma glucose

CaCLA supplementation tended to decrease the plasma glucose concentration in our study ($P < 0.1$). The phenomenon was not observed in several other studies using transition on the early lactating (Bernal-Santos et al., 2003; Selberg et al., 2004; Castañeda-Gutiérrez et al., 2005), mid-lactating (de Veth et al., 2006), and late lactating (Baumgard et al., 2002) cows. There was evidence in isolated rat hepatocytes that gluconeogenesis was decreased after being incubated in Ham's nutrient medium containing $20 \text{ mg} \cdot \text{L}^{-1}$ of a CLA mixture for 3 h (Cantwell et al., 1999). As we found no further relevant reports and the phenomenon remained unexplained at present, more supporting evidences are needed to deduce the argumentation that the decreased circulating glucose by CaCLA supplementation in our study was due to an inhibition effect of CLA on gluconeogenesis.

Table 5 Effects of sources of supplemental energy (corn or CaFA) and CaCLA on the fatty acid composition ($\text{mg} \cdot \text{g}^{-1}$) of milk fat of Holstein cows

fatty acid	treatment				SEM	P value		
	CaFA	CaFA + CLA	corn + CLA	corn		energy	CLA	energy \times CLA
C4	35.08	33.97	37.71	26.94	2.38	0.1270	0.6708	0.2187
C6	18.75	16.93	22.45	15.99	1.89	0.0063	0.8446	0.1366
C8	11.8	10.17	14.87	10.61	1.43	0.0031	0.6610	0.1172
C10	24.25	20.28	32.61	22.86	3.53	0.0022	0.7347	0.1079
C12	27.11	23.38	35.43	24.47	4.37	0.0082	0.9519	0.1541
C14	92.07	86.45	116.57	77.89	11.32	0.0119	0.4712	0.1286
C14:1	7.31	9.21	11.1	6.91	4.29	0.4365	0.3870	0.9670
C16	339.19	339.59	359.33	256.44	24.09	0.4642	0.4072	0.4242
C16:1	4.83	11.52	5.59	6.82	6.66	0.8107	0.6492	0.1764
C18	73.44	67.48	74.67	54.92	13.63	0.6678	0.7958	0.5980
<i>t11C18:1</i>	11.08	9.65	7.94	9.42	1.02	0.6934	0.0012	0.0263
<i>t12C18:1</i>	2.75	2.94	1.5	2.25	0.54	0.0634	0.0588	0.0232
<i>c9C18:1</i>	317.41	333.38	252.45	216.89	32.76	0.0144	0.5962	0.1758
<i>c12C18:1</i>	5.09	5.6	5.16	4.90	2.22	0.6661	0.7098	0.4288
C18:2	19.8	19.93	14.78	14.06	3.39	0.1054	0.3299	0.3001
C18:3	2.26	2.34	1.85	1.60	0.42	0.1781	0.6771	0.4396
<i>c9t11CLA</i>	3.28	3.01	2.14	2.61	0.34	0.0796	0.0036	0.0225
<i>t10c12CLA</i>	0.55	0.47	0.31	0.25	0.24	0.1649	0.7008	0.8047
<i>c9c11CLA</i>	0.18	0.06	0.07	0.00	0.19	0.4041	0.7561	0.3553
<i>t9t11CLA</i>	1.93	1.78	1.52	1.36	0.24	0.1500	0.1299	0.6292
C20:3	1.17	1.18	1.21	0.85	0.24	0.9117	0.5571	0.7256
C20:5	0.21	0.19	0.27	0.16	0.05	0.1499	0.5396	0.1971
C22:4	0.49	0.51	0.49	0.44	0.17	0.7161	0.6959	0.5079
total CLA	5.94	5.31	4.04	5.56	0.76	0.0724	0.0296	0.2838
<i>Trans-C18:1</i>	13.82	12.59	9.45	15.31	1.25	0.2351	0.0013	0.0100
<i>Cis-C18:1</i>	322.50	338.98	257.61	193.27	32.81	0.0150	0.5801	0.1632
C4-8	65.62	61.07	75.03	70.47	5.12	0.0105	0.9974	0.1255
C10-16 ¹⁾	322.74	314.86	378.17	346.80	30.83	0.0298	0.4750	0.2501
C4-16 ¹	388.36	375.93	453.20	417.26	33.10	0.0184	0.5042	0.1943
C16-22 ¹⁾	605.70	618.76	542.76	577.18	33.00	0.0199	0.5414	0.2003

Note: 1) means half of the C16 fatty acids were included.

4.3 Milk yield, composition, and milk fatty acid profile

Lactose is a major osmoregulator for mammary gland uptake of water, which enhances the lactose synthesis and transport into the alveolar lumen, followed by enhancing water transport and thus milk yield (Rigout et al., 2002). The corn supplementation tended to increase milk yield ($P < 0.1$) and also numerically increased lactose yield ($P = 0.1443$). In literature, the effects of carbohydrate supplementation on milk yield are inconsistent. Both positive (Hurtaud et al., 2000; Rigout et al., 2002; Lemosquet et al., 2004; Janicek et al., 2007) and null results (Arieli et al., 2001) have been reported. Responses of milk yield to the increased carbohydrate supply may be limited by genetic potential of the cows and positive responses can only be observed when the glucose balance is negative.

The effect of *cis*-10, *trans*-12CLA on depressing milk fat was quite consistent in literature, which was not observed in our study. The CaCLA product we used contained $33.7 \text{ g} \cdot 100 \text{ g}^{-1}$ *cis*-10, *trans*-12CLA. A supplemental dosage of $70 \text{ g} \cdot \text{d}^{-1}$ CaCLA was equivalent to $23.59 \text{ g} \cdot \text{d}^{-1}$ *cis*-10, *trans*-12CLA, which was higher than the lower limit dosage ($17.3 \text{ g} \cdot \text{d}^{-1}$) used by Giesy et al. (2002). The lower dosage treatments depressed milk fat yield by 29% in the Giesy experiment. There were evidences that *trans*-10, *cis*-12 CLA acted on the mammary tissue to depress milk fat synthesis. The results of Baumgard et al. (2002) demonstrated that *trans*-10, *cis*-12 CLA decreased the expression of genes encoding enzymes involved in circulating fatty acid uptake and transport, *de novo* fatty acid synthesis, desaturation of fatty acids and triglyceride synthesis in mammary tissues.

Table 6 Effects of sources of supplemental energy (corn or CaFA) and CaCLA on the fatty acid yields ($\text{g} \cdot \text{d}^{-1}$) of milk fat of Holstein cows

fatty acid	treatment				SEM	P value		
	CaFA	CaFA + CLA	corn + CLA	corn		energy	CLA	energy \times CLA
C4	29.72	28.19	30.10	26.94	4.29	0.8477	0.7170	0.3161
C6	15.91	14.23	17.96	15.99	2.74	0.2123	0.9205	0.2302
C8	10.01	8.58	11.93	10.61	1.84	0.0756	0.9554	0.1852
C10	20.58	17.15	26.21	22.86	4.08	0.0320	0.9836	0.1475
C12	22.96	19.59	28.49	24.47	4.78	0.0721	0.8962	0.1723
C14	78.07	71.78	93.40	77.89	14.79	0.1973	0.5560	0.1907
C14:1	6.11	7.27	8.92	6.91	3.90	0.5529	0.4473	0.8362
C16	288.10	275.59	286.59	256.44	42.93	0.6474	0.6954	0.3589
C16:1	4.05	7.48	4.43	6.82	3.21	0.9351	0.7586	0.1201
C18	62.24	56.29	59.60	54.92	12.81	0.7646	0.9246	0.4382
<i>t11</i> C18:1	9.40	7.87	6.34	9.42	1.62	0.3862	0.0298	0.3775
<i>t12</i> C18:1	2.35	2.35	1.19	2.25	0.611	0.0835	0.1359	0.1359
<i>c9</i> C18:1	268.66	265.91	201.27	216.89	32.55	0.0117	0.5929	0.7065
<i>c12</i> C18:1	4.30	4.49	4.13	4.90	1.55	0.8786	0.7182	0.5601
C18:2	16.74	15.96	11.86	14.06	2.22	0.0226	0.2281	0.5457
C18:3	1.89	1.90	1.49	1.60	0.29	0.0513	0.7405	0.6679
<i>c9t11</i> CLA	2.75	2.37	1.71	2.61	0.41	0.1021	0.0215	0.2567
<i>t10c12</i> CLA	0.45	0.35	0.24	0.25	0.17	0.1194	0.5610	0.5989
<i>c9c11</i> CLA	0.14	0.03	0.05	0.00	0.14	0.4283	0.6799	0.3030
<i>t9t11</i> CLA	1.61	1.40	1.21	1.36	0.27	0.1604	0.2225	0.8385
C20:3	0.99	0.98	0.97	0.85	0.22	0.5288	0.6136	0.5563
C20:5	0.18	0.16	0.22	0.16	0.05	0.4689	0.5207	0.2655
C22:4	0.42	0.42	0.53	0.44	0.12	0.3541	0.5353	0.5045
Total CLA	4.95	4.14	3.21	4.22	0.70	0.0547	0.0397	0.7757
<i>Trans</i> -C18:1	11.75	10.22	7.53	11.66	2.06	0.2274	0.0339	0.2554
<i>Cis</i> -C18:1	272.96	270.39	205.40	221.79	32.41	0.0116	0.5798	0.6847
C4-8	55.63	50.99	59.99	53.54	8.59	0.4512	0.8398	0.2439
C10-16 ¹	273.80	257.31	302.54	263.76	45.30	0.4668	0.6403	0.2683
C4-16 ¹	329.43	308.30	362.53	317.30	52.81	0.4557	0.6643	0.2557
C16-22 ¹	513.23	497.85	432.97	437.11	54.38	0.0411	0.7320	0.8431

Note: ¹ means half of the C16 fatty acids were included.

Unlike what was observed by Giesy et al. (2002), the CaCLA supplementation did not increase the content of *trans*-10, *cis*-12 CLA in milk fat in our study, which explains the unobserved milk fat depressing effect of *trans*-10, *cis*-12 CLA.

The corn supplementation decreased milk fat content ($P < 0.05$), tending to decrease milk fat yield ($P < 0.1$) in our study compared to the CaFA supplementation. Total yield of the measured fatty acids accounted for 99.2%, 95.5%, 99.6%, and 99.2% of milk fat yield in CaFA, CaFA + CaCLA, corn + CaCLA, and corn treatments, respectively (Table 6). On average, the CaFA treatment produced $67.7 \text{ g} \cdot \text{d}^{-1}$ more milk fat (Table 3), $70.5 \text{ g} \cdot \text{d}^{-1}$ more C16-C22 fatty acids and $21.1 \text{ g} \cdot \text{d}^{-1}$ less C4-C16 fatty acids (Table 6) compared to the corn treatment. These apparent calculations suggest that the decreased milk fat yield by

corn supplementation was exclusively attributed to a reduction in preformed fatty acids. The increased *de novo* synthesized fatty acids ($21.1 \text{ g} \cdot \text{d}^{-1}$) by corn supplementation compensated for 23.8% of the potential decrease in milk fat yield ($67.7 + 21.1 = 88.8 \text{ g} \cdot \text{d}^{-1}$). Statistically, the corn supplementation increased or tended to increase the yield of C10, C8, and C12; and decreased or tended to decrease the yield of *cis*-9C18:1, C18:2, *trans*-12C18:1, and C18:3. Therefore, data from our study are in agreement with the hypothesis that insulin might increase milk fat production by promoting *de novo* synthesis of fatty acids in the mammary gland and decrease it by reducing the supply of preformed fatty acids to the mammary gland.

The positive effects of corn supplementation on milk proteins observed in the present study are in agreement with those of previous researches. Rigout et al. (2002)

analyzed the data from 10 ruminal propionate or post-ruminal glucose infusion experiments. Milk protein content increased linearly ($R^2 = 0.96$; $SEM = 0.05$; $P < 0.001$) with an increase in glucogenic precursors energy (GPE). Evidences from the experiments of Mackle et al. (2000) and Rulquin et al. (2004) indicated that the positive effects of corn supplementation on milk protein could be related to the increased mammary arterial amino acid flux, or/and the increased mammary amino acid efficiency. The negative effects of CaFA supplementation on milk protein were also reported by other authors (Erickson et al., 1992; Karunanandaa et al., 1994; Cervantes et al., 1996; Avila et al., 2000; Schroeder et al., 2002), but the effects of FA supplementation on milk protein reported in literature are inconsistent and are usually negative or null (Wu et al., 1993; Pantoja et al., 1996; Ruppert et al., 2003). A positive exception was observed by Harvatine and Allen (2005). The saturated FA supplementation in the diet of lactating cows increased milk protein content, and simultaneously the circulating insulin was elevated. It is well documented that insulin could promote milk protein synthesis (Bequette et al., 2000; Mackle et al., 2000; Molento et al., 2002); therefore, the positive effects observed by Harvatine and Allen (2005) might be caused by increased circulating insulin rather than FA supplementation itself.

5 Conclusion

Our results indicated that both milk yield and composition of mid-lactation Holstein cows were influenced by sources of supplemental energy. Compared to isoenergetic CaFA supplementation, the corn supplementation increased the yields of milk protein and non-fatty solids, but decreased the content of milk fat, while the CaCLA supplementation did not increase the content of *cis*-10, *trans*-12CLA in milk fat effectively, which explained the unobserved effect of CaCLA supplementation in depressing milk fat synthesis in the present study. Analysis of milk fatty acid profiles indicated that the depressed milk fat yield could be exclusively attributed to decreasing the incorporation of preformed fatty acids into milk fat. The corn supplementation increased the yield of *de novo* synthesized fatty acids in the mammary gland, which partially offset the potential reduction in milk fat yield. However, the CaCLA supplementation decreased plasma glucose. In general, the underlying mechanisms still remain unexplained at present.

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References

- Avila C D, DePeters E J, Perez-Monti H, Taylor S J, Zinn R A (2000). Influences of saturation ratio of supplemental dietary fat on digestion and milk yield in dairy cows. *Journal of Dairy Science*, 83: 1505–1519
- Bauman D E, Griinari J M (2003). Nutritional regulation of milk fat synthesis. *Annual Review Nutrition*, 23: 203–227
- Baumgard L H, Matitashvili E, Corl B A, Dwyer D A, Bauman D E (2002). *trans*-10, *cis*-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. *Journal of Dairy Science*, 85: 2155–2163
- Bequette B J, Hanigan M D, Calder A G, Reynolds C K, Lobley G E, MacRae J C (2000). Amino acid exchange by the mammary gland of lactating goats when histidine limits milk production. *Journal of Dairy Science*, 83: 765–775
- Bernal-Santos G, Perfield J W, Barbano D M, Bauman D E, Overton T R (2003). Production responses of dairy cows to dietary supplementation with conjugated linoleic acid (CLA) during the transition period and early lactation. *Journal of Dairy Science*, 86: 3218–3228
- Cantwell H, Devery R, O'Shea M, Stanton C (1999). The effect of conjugated linoleic acid on the antioxidant enzyme defense system in rat hepatocytes. *Lipids*, 34: 833–839
- Castañeda-Gutiérrez E, Overton T R, Butler W R, Bauman D E (2005). Dietary supplements of two doses of calcium salts of conjugated linoleic acid during the transition period and early lactation. *Journal of Dairy Science*, 88: 1078–1089
- Cervantes A, Smith T R, Young J W (1996). Effects of nicotinamide on milk composition and production in dairy cows fed supplemental fat. *Journal of Dairy Science*, 79: 105–113
- Chouinard P Y, Corneau L, Barbano D M, Metzger L E, Bauman D E (1999). Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. *Journal of Nutrition*, 129 (8): 1579–1584
- Cole N A, Hallford D M (1994). Influence of a propionate load in fed or unfed lambs on blood metabolites and hormone patterns. *Journal of Animal Science*, 72: 2141–2148
- Corl B A, Butler S T, Butler W R, Bauman D E (2006). Regulation of milk fat yield and fatty acid composition by insulin. *Journal of Dairy Science*, 89: 4172–4175
- Davis C L, Brown R E (1970). Low-fat milk syndrome. In: Phillipson A T, ed. *Physiology of Digestion and Metabolism in the Ruminant*. Newcastle upon Tyne: Oriel Press, 545–565
- de Veth M J, Castañeda-Gutiérrez E, Dwyer D A, Pfeiffer A M, Putnam D E, Bauman D E (2006). Response to conjugated linoleic acid in dairy cows differing in energy and protein status. *Journal of Dairy Science*, 89: 4620–4631
- Erickson P S, Murphy M R, Clark J H (1992). Supplementation of dairy cow diets with calcium salts of long-chain fatty acids and nicotinic acid in early lactation. *Journal of Dairy Science*, 75: 1078–1089
- Griinari J M, McGuire M A, Dwyer A, Bauman D E, Palmquist D L (1997). Role of insulin in the regulation of milk fat synthesis in dairy cows. *Journal of Dairy Science*, 80: 1076–1084
- Giesy J G, McGuire M A, Shafii B, Hanson T W (2002). Effect of dose

- of calcium salts of conjugated linoleic acid (CLA) on percentage and fatty acid content of milk fat in midlactation Holstein cows. *Journal of Dairy Science*, 85: 2023–2029
- Harvatine K J, Allen M S (2005). The effect of production level on feed intake, milk yield, and endocrine responses to two fatty acid supplements in lactating cows. *Journal of Dairy Science*, 88: 4018–402
- Hurtaud C, Lemosquet S, Rulquin H (2000). Effect of graded duodenal infusions of glucose on yield and composition of milk from dairy cows. 2. Diets based on grass silage. *Journal of Dairy Science*, 83: 2952–2962
- Hurtaud C, Rulquin H, Verite R (1993). Effect of infused volatile fatty acids and caseinate on milk composition and coagulation in dairy cows. *Journal of Dairy Science*, 76: 3011–3020
- Janicek B N, Kononoff P J, Gehman A M, Karges K, Gibson M L (2007). Effect of increasing levels of corn bran on milk yield and composition. *Journal of Dairy Science*, 90: 4313–4316
- Karunanandaa K, Goodling L E, Varga G A, Muller L D, McNeill W W, Cassidy T W, Lykos T (1994). Supplemental dietary fat and ruminally protected amino acids for lactating Jersey cows. *Journal of Dairy Science*, 77: 3417–3425
- Knowlton K F, Dawson T E, Glenn B P, Huntington G B, Erdman R A (1998). Glucose metabolism and milk yield of cows infused abomasally or ruminally with starch. *Journal of Dairy Science*, 81: 3248–3258
- Lemosquet S, Rigout S, Bach A, Rulquin H, Blum J W (2004). Glucose metabolism in lactating cows in response to isoenergetic infusions of propionic acid or duodenal glucose. *Journal of Dairy Science*, 87: 1767–1777
- Li B, Wang Z H, Li F C, Lin X Y (2007). Milk fat content was changed by ruminal infusion of mixed VFAs solutions with different acetate/propionate ratios in lactating goats. *Small Ruminant Research*, 72: 11–17
- Mackie T R, Dwyer D A, Ingvarsen K L, Chouinard P Y, Ross D A, Bauman D E (2000). Effects of insulin and postruminal supply of protein on use of amino acids by the mammary gland for milk protein synthesis. *Journal of Dairy Science*, 83: 93–105
- Maurizio R, Bailoni L, Schiavon S, Bittante G (1997). Effect of monensin on milk production and efficiency of dairy cows fed two diets differing in forage to concentrate rations. *Journal of Dairy Science*, 80: 1136–1142
- McGuire M A, Griinari J M, Dwyer D A, Bauman D E (1995). Role of insulin in the regulation of mammary synthesis of fat and protein. *Journal of Dairy Science*, 81: 1353–1375
- Miettinen H, Huhtanen P (1996). Effects of the ratio of ruminal propionate to butyrate on milk yield and blood metabolites in dairy cows. *Journal of Dairy Science*, 79: 851–861
- Molento C F M, Block E, Cue R I, Petitclerc D (2002). Effects of insulin, recombinant bovine somatotropin, and their interaction on insulin-like growth factor-I secretion and milk protein production in dairy cows. *Journal of Dairy Science*, 85: 738–747
- Pantoja J, Firkins J L, Eastridge M L (1996). Fatty acid digestibility and lactation performance by dairy cows fed fats varying in degree of saturation. *Journal of Dairy Science*, 79: 429–437
- Piperova L S, Moallem U, Teter B B, Sampugna J, Yurawecz M P, Morehouse K M, Luchini D, Erdman R A (2004). Changes in milk fat in response to dietary supplementation with calcium salts of *trans*-18:1 or conjugated linoleic fatty acids in lactating dairy cows. *Journal of Dairy Science*, 87: 3836–3844.
- Rigout S, Lemosquet S, van Eys J E, Blum J W, Rulquin H (2002). Duodenal glucose increases glucose fluxes and lactose synthesis in grass silage-fed dairy cows. *Journal of Dairy Science*, 85: 595–606
- Rulquin H, Rigout S, Lemosquet S, Bach A (2004). Infusion of glucose directs circulating amino acids to the mammary gland in well-fed dairy cows. *Journal of Dairy Science*, 87: 340–349
- Ruppert L D, Drackley J K, Bremmer D R, Clark J H (2003). Effects of tallow in diets based on corn silage or alfalfa silage on digestion and nutrient use by lactating dairy cows. *Journal of Dairy Science*, 86: 593–609
- Sano H, Hayakawa S, Takahashi H, Terashima Y (1995). Plasma insulin and glucagon responses to propionate infusion into femoral and mesenteric veins in sheep. *Journal of Animal Science*, 73: 191–197
- Schroeder G F, Gagliostro G A, Becu-Villalobos D, Lacau-Mengido I (2002). Supplementation with partially hydrogenated oil in grazing dairy cows in early lactation. *Journal of Dairy Science*, 85: 580–594
- Selberg K T, Lowe A C, Staples C R, Luchini N D, Badinga L (2004). Production and metabolic responses of periparturient holstein cows to dietary conjugated linoleic acid and *trans*-octadecenoic acids. *Journal of Dairy Science*, 87: 158–168
- Wu Z, Huber J T, Sleiman F T, Simas J M, Chen K H, Chan S C, Fontes C (1993). Effect of three supplemental fat sources on lactation and digestion in dairy cows. *Journal of Dairy Science*, 76: 3562–3570
- Zhang N (2006). A study on the mechanism of milk fat depression in ruminants. Dissertation of the Master's Degree. Taian: Shandong Agricultural University (in Chinese)