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Original Research Article

Milk fatty acids profiles and milk production from dairy cows fed different forage quality diets

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ABSTRACT

Thirty lactating Holstein cows were used to investigate the effects of different forages quality on milk fatty acids (FA) profiles and production. The cows were assigned to 3 dietary treatments (n = 10 per treatment) in a randomized block design with 3 repeated measures. They were fed the experimental diets for 90 d with 3 days of collection of samples for analysis at about 27 d intervals (samples were collected on days 28, 29, 30, 58, 59, 60, 88, 89 and 90). The treatments were (DM basis): 1) mixed forages diet (MF) consisting of 3.7% Chinese wild rye, 26.7% corn silage and 23.4% alfalfa hay; 2) corn stalk diet 1 (CS1) where corn stalk was used to formulate a similar chemical nutrient level to MF; 3) corn stalk diet 2 (CS2) which used corn stalk to formulate a similar forage level to MF for the diet. Dry matter intake and BW were similar between treatments, but daily milk yield, milk fat and protein yield decreased ($P < 0.05$) in CS1 and CS2 compared with MF, with CS2 being the lowest ($P < 0.05$). In total FA of milk, the compositions of C18:1c9, C18:3 and unsaturated FA increased ($P < 0.05$) in CS1 and CS2 compared with MF, and C18:0 and trans-C18:1 were trended to increase ($P < 0.10$), but C4:0-C16:0 were decreased ($P < 0.05$). Compared with cows fed CS2, cows receiving CS1 increased the compositions of C4:0 to C12:0 and C18:2 ($P < 0.05$). The results suggests feeding corn stalk could produce a greater proportion of unsaturated fatty acid (UFA) in milk fat without resulting in milk fat depression (MFD) in mid lactation cows, but simply increasing the ratio of concentrate in low forages diets is not an effective way to increase milk fat synthesis and milk production.

1. Introduction

The secretion of milk fat and the composition of milk fatty acids (FA) are of great interest with regard to human nutrition, and altering them in dairy cows via dietary manipulation has gained considerable attention because of the implications for human health (Parodi, 1999). For FA in milk, short chain FA (4 to 8 carbons) and medium chain FA (10 to 14 carbons) arise almost exclusively from *de novo* synthesis, and long chain FA (LCFA; >16 carbons) are derived from the uptake of circulating lipids, while FA with 16 carbons originate from both sources (Neville and Picciano, 1997). In contrast to short and medium chain FA, there is very little LCFA synthesized (>18 carbons) *de novo* by ruminants and therefore most LCFA must be ingested in the feed if these moieties are to be present in the milk (Elgersma et al., 2006; Chilliard et al., 2007).

Milk FA composition is often affected by rumen biohydrogenation and Δ9-desaturase enzyme conversion (C18:0 into C18:1, Bauman and Griinari, 2003), but the large changes of milk fat composition can be achieved by changing the nature of forages in the diets. For example, Chilliard et al. (2007) reviewed data relating to the FA composition of milk from animals fed hay, fresh grass and maize silage and reported changes to the content of C18:0, C18:1 and C18:3, unsaturated FA, (especially C18:3), and saturated FA, (especially C16:0). Milk fat content (MFC) was largely affected by the fiber content of feed source and its ability to maintain rumen function, and generally increased with increasing fiber content of different forages

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(Loor et al., 2005; Bauman and Griinari, 2003). Furthermore, those diets which were formulated with higher quality forages increased MFC and milk yield in dairy cows, whereas low quality forages containing fewer nutrients would result in the reduction of milk yield and a decrease in milk fat synthesis (Sutton, 1989; Onetti et al., 2002; Zhu et al., 2013). High quality forages have been widely used in diets for ruminant production, however Chinese producers still prefer to feed poor quality agricultural residues such as corn stalks to reduce feeding costs (Pang et al., 2008; Zhu et al., 2013). There are 3 types of dairy production in China, which are small dairy production by small farmers, medium size dairy production in the dairy production zones, and large scale dairy production in modern dairy farms. Small farmers feed more corn stalks as major roughage of animals and feed less concentrate; farmers in the dairy production zone use less corn stalks and relatively more concentrate to feed animals; large dairy farms use total mixed ration (TMR) consisting of high quality roughage such as hay, silage and alfalfa and commercial concentrate to feed animals. Ideally, one would be able to modify low quality forages to formulate a diet with similar chemical nutrient levels to that of high quality forages diets simply by adjusting the forage to concentrate ratio, yet to still produce similar levels of milk production. Unfortunately diets with a high proportion of concentrate may provide large amounts of readily digestible carbohydrates and reduced amounts of fibrous components, so that it would cause milk fat depression (MFD) and change milk FA profiles (Bauman and Griinari, 2003); and if the neutral detergent fiber (NDF) is adequate, the higher level of concentrate may not result in MFD, but if the fiber is low quality, we have no definite conclusion at present. Therefore, in this study we designed 3 diets which represent the diets in those 3 models of dairy production, to investigate the influence of different forage quality on milk production and milk FA profiles.

2. Materials and methods

2.1. Animals and experimental design

The experiment was conducted in a dairy farm belonging to a dairy cooperative and located about 20 km west of Hohhot in Inner Mongolia of China. The company provided a milk composition analyzer and a somatic cell counter. Animals were cared by following the guidelines approved by the Animal Care Advisory Committee of the Inner Mongolia Agriculture University. Thirty lactating Holstein cows (days in milk [DIM] = 120 ± 24 d; parity = 2 to 3; BW = 554 ± 21 kg; milk yield = 24.30 ± 1.47 kg/d) were divided into 3 groups based on averages of these indexes, and then randomly assigned to 3 dietary treatments (n = 10 cows per treatment) in a randomized block design. They were fed the experimental diets for 90 d with 3 days of collection of samples for analysis at about 27 d intervals (samples were collected on days 28, 29, 30, 58, 59, 60, 88, 89 and 90). Cows were housed in individual stalls and fed TMR diets and milked twice daily at the 06:00 and 18:00. Corn stalks, which were relatively fresh and harvested by farmers, were dried and chopped (3 to 4 cm) in a half of month and ensure adequate to our entire experiment. The treatments were (DM basis): 1) mixed forages diet (MF) consisting of 3.7% Chinese wild rye, 26.7% corn silage, and 23.4% alfalfa hay; 2) corn stalk diet 1 (CS1) using corn stalks formulated to provide a similar chemical nutrient level to MF; and 3) corn stalk diet 2 (CS2) using corn stalks to formulate a similar forage level to MF diet (see Table 1).

2.2. Sample collection and measurements

Feed intake and milk yield were recorded daily, and BW of each cow were measured before morning feeding and after milking at the first day of each sampling period. Representative TMR samples and orts were collected for 3 consecutive days and stored at −20°C for later chemical analysis. Dried feed samples were analyzed for DM, CP, crude fat (ether extract), and ash according to the Association of Official Analytical Chemists (AOAC) (1999). Acid detergent fiber and NDF were analyzed by the method of Van Soest et al. (1991). Net energy for lactation and starch were calculated by near-infrared spectroscopy (FOSS NIRS DS 250). Diets compositions that were calculated according to the chemical analysis and inclusion rates of ingredients are presented in Table 2.

Milk samples were collected at the same TMR sampling days, and were pooled according to the proportion of morning and afternoon milk yield and transported to the laboratory for fat, protein, lactose and total solid (TS) content analyses using MilkoScanTMMinor-Type78110 (FOSS Analytical A/S 69, DK-3400, Denmark). Somatic cell counts in milk were using ADAM-SCC-II (Nanoentek Inc, Korea) each day during 3 d sampling period. These samples were kept at −20°C for later FA analysis. Fatty acids of TMR and milk analysis were performed according to the internal method containing NaOCH3/methanol followed by HCl/methanol, as described by Khas-Erdene et al. (2010), and was analyzed using a GC-2014 gas chromatograph (Shimadzu, Shimadzu Technologies) fitted with a flame-ionization detector. These samples containing methyl esters in hexane (2 μL) were injected through the split injection port (50:1) onto an HP-88 fused silica 100 mm × 0.25 mm column, 0.20 μm film (Agilent, Agilent Technologies). The oven temperature was initially set at 120°C for 10 min and was then increased to 230°C at a rate of 3.2°C per min and held at that temperature for 35 min. The injector and detector temperatures were maintained at 250 and 300°C, respectively, and the total run time was 79.38 min. The qualitative external standard method was used in this study. Each peak was identified using known standards of FA and FA methyl esters (FAME, Nu-Chek Prep, Elysian, MN, USA; Matreya, Pleasant Gap, PA, USA; and Supelco 37 Component FAME mix, Supelco Inc.). The percentage of each FA was calculated by dividing the area under the FA peak (minus the peak area for heptadecanoic acid) by the sum of the areas for all the reported FA peaks. Fatty acids were reported as grams per hundred grams of FAME (Han et al., 2014).

2.3. Calculation of results and statistical analysis

These data of 3 repeated measures were collected together, and were analyzed as a randomized block design using the MIXED procedures of (Han et al., 2014). The variance for cows nested

Table 1
Ingredients of the experimental diets (% of DM).

Item	Diets[1]		
	MF	CS1	CS2
Hay	3.7	0	0
Corn silage	26.7	0	0
Alfalfa hay	23.4	0	0
Corn stalk	0	35	53.8
Ground corn grain	24.6	34.61	24.6
Soybean meal (49.0% CP)	14.8	20.82	14.8
Whole cottonseed	5.1	7.18	5.1
Calcium bicarbonate	0.6	0.84	0.6
Sodium chloride	0.5	0.7	0.5
Mineral-vitamin mix[2]	0.6	0.84	0.6
Total	100	100	100

MF = mixed forages; CS1 = corn stalk diet 1; CS2 = corn stalk diet 2.
[1] The MF diet consisted of 3.7% Chinese wild rye, 26.7% corn silage and 23.4% alfalfa hay; CS1 used corn stalk to formulate similar chemical nutrients levels with MF; and CS2 used corn stalk to formulate similar forages level as MF diet.
[2] The mineral-vitamin mix per kilogram of DM provided: 500,000 to 700,000 IU vitamin A; 110,000 to 120,000 IU vitamin D_3; 8,000 to 10,000 IU vitamin E; 7,000 to 10,000 mg Zn; 40 to 80 mg Se; 84 mg I; 1,400 to 1,750 mg Fe; 30 to 40 mg Co; 1,400 to 3,500 mg Mn; and 1,400 to 1,600 mg Cu.

Table 2
Chemical composition (% of DM).

Item	Diets[1]		
	MF	CS1	CS2
Chemical composition			
CP	18.14	18.38	13.61
EE	3.97	4.10	2.84
NDF	32.30	33.10	44.30
ADF	21.30	20.20	29.10
Ash	7.47	7.39	7.93
Starch[2]	21.50	25.39	15.32
NEL[2], Mcal/kg	1.57	1.58	1.04
Fatty acid composition in diets (g per 100 g of total fatty acid)			
C16:0	24.35	21.22	19.98
C16:1	0.62	0.42	0.51
C18:0	3.01	3.38	3.38
C18:1c9	21.73	23.1	24.58
C18:2c6	44.16	44.8	42.69
C18:3n3	2.55	4.75	3.92
others	3.58	4.16	3.11
UFA	67.23	71.38	69.74
LCFA	86.71	85.62	83.34

MF = mixed forages diet; CS1 = corn stalk diet 1; CS2 = corn stalk diet 2; EE = crude fat (ether extract); NDF = neutral detergent fiber; ADF = acid detergent fiber; NEL = Net energy for lactation; UFA = unsaturated fatty acid; LCFA = total long chain fatty acid.

[1] The MF diet consisted of 3.7% Chinese wild rye, 26.7% corn silage and 23.4% alfalfa hay; CS1 used corn stalk to formulate chemical nutrients levels similar to that of MF; and CS2 used corn stalk to formulate forages level similar to that of MF.

[2] Starch and NEL were calculated by near-infrared spectroscopy (FOSS NIRS DS 250).

Table 3
The effects of dietary treatments on milk performance.

Item	Diets[1]			SEM	P-value
	MF	CS1	CS2		
Milk yield, kg/d	26.43^{a}	22.62^{b}	17.41^{c}	1.36	<0.01
DMI, kg/d	16.75	16.71	16.19	0.41	0.58
Fat, %	4.26^{a}	3.71^{b}	4.03^{ab}	0.14	0.04
Fat yield, kg/d	1.11^{a}	0.83^{b}	0.70^{c}	0.05	<0.01
Protein, %	3.20^{a}	3.10^{ab}	3.00^{b}	0.05	0.02
Protein yield, kg/d	0.84^{a}	0.70^{b}	0.53^{c}	0.041	<0.01
SCC, × 1000/mL	24.67	25.24	29.87	6.59	0.35
Weight, kg/cow	568	534	554	21	0.98

MF = mixed forages; CS1 = corn stalk diet 1; CS2 = corn stalk diet 2; SEM = pooled standard error of the means; DMI = dry matter intake; SCC = somatic cell count.
$^{a-c}$ Means within a row with different superscripts differ ($P < 0.05$).

[1] The MF consisted of 3.7% Chinese wild rye, 26.7% corn silage and 23.4% alfalfa hay; CS1 was using corn stalk to formulate a chemical nutrient level similar to that of MF; CS2 was using corn stalk to formulate a forages level similar to that of MF.

within treatment was used as random error term to test the main effect of treatment. The cow variance was considered random and data of DIM throughout the whole experiment were used as covariates. Data were presented as covariate adjusted least squares means. The SEM was pooled standard error of the means. The significance level was declared at $P < 0.05$ and a trend was declared at $P < 0.10$.

3. Results and discussion

3.1. Dry matter intake, milk production and composition

There were no significant changes observed with dry matter intake (DMI, $P = 0.58$), BW (BW, $P = 0.98$) and somatic cell count ($P = 0.35$) in milk among treatments for the entire experimental period (Table 3). Multiple mechanisms regulate DMI of ruminants, but DMI generally declines with increasing NDF content when animals are fed energetic diets (Allen, 2000). In our study, MF and CS1 had the same NDF content and net energy for lactating cows (NEL) and no effect on DMI of dairy cows (Table 2), and the result was consistent with that reported by Zhu et al. (2013) who fed different forage source diets containing the same NEL to dairy cows. For different energy level diets CS1 and CS2, our result was in agreement with the findings of Hoogendoorn and Grieve (1970), who found that there was no effect on DMI of increasing the energy and using hay as the only forage.

Milk, milk protein and fat yield were decreased in CS1 and CS2 compared with MF, and were lower ($P < 0.05$) in CS2 than in CS1. The nutrient levels such as CP, crude fat, NDF and NEL were kept approximately the same by altering the forage quality between CS1 and MF (Table 2), and this result implied that the intake of nutrients had the same effect relative to DMI for both treatments. Higher quality diets could improve rumen function and the efficiency of milk production synthesis, thereby further increasing the yields of milk, milk protein, fat and milk protein content (MPC) (Onetti et al., 2002). The lower nutrient levels of CS2 diet resulted in a strong negative calculated energy balance (NEL < 1.37 Mcal kg/L; NRC, 2001) and might help to explain the lowered milk production when compared with CS1 (Hoogendoorn and Grieve, 1970). The MPC of the MF group was significantly higher than that of the CS2 group ($P < 0.05$), and that of CS1 was between the two, but with no statistically significant differences ($P > 0.05$).

The milk fat content was decreased in CS1 compared with MF (3.71%, 4.26% and 4.03%, respectively, $P < 0.05$), but it was not significantly different among CS1, CS2 and MF. Diets of CS1 and MF contained the same level of fiber, but Ruppert et al. (2003) reported that diet of CS1 containing lower quality forages could decrease the fiber digestibility of cows. In ruminants, low fiber and high grain diets were considered as a group of diets that cause MFD encompassing concomitant low MFC and milk yield (Bauman and Griinari, 2003), but the fiber of CS1 diet was not low and this may be the reason why MFD did not occur in the cows fed CS1 diet (the concentration of milk fat was over 37 g/L). Cows fed CS2 produced higher MFC than cows fed CS1, and a similar finding had been reported for dairy cows fed diets with increasing hay proportion and NDF content (Loor et al., 2005). However, there is no significant difference between these two treatments, and the lower NEL and crude fat intake may cause this result. From another point of view, when rapidly fermentable carbohydrate or starch are fed, there is a greater production of glucose, propionate and microbial protein leading to signals in the cow's body to produce more milk and milk protein, to improve MPC and milk fat yield, and to reduce MFC (Jenkins and Mcguiret, 2006).

3.2. Milk fatty acid composition

In total FA of milk, the compositions of C18:1 and C18:3 were increased ($P < 0.05$) in CS1 and CS2 compared with MF, and C18:0 ($P = 0.08$) and trans-C18:1 ($P = 0.09$) tended to increase (Table 4). Diets of CS1 and CS2 containing higher C18:1 and C18:3 compositions (Table 2) contributed to a higher intake of these two FA proportions (relative to total FA) by cows, and resulted in higher proportions of C18:1 and C18:3 in milk fat. Those FA, which are partly bio-hydrogenated into C18:0 and trans-C18:1, are partly absorbed intact in the gut and secreted into milk (Chilliard et al., 2007; Bauman and Griinari, 2003) causing the C18:0 and trans-C18:1 to increase. The milk fat content of trans-C18:1 is generally correlated with the depression of MFC and milk fat synthesis (MFD, Bauman and Griinari, 2003). From the results point of view, lower MFC and milk fat production of CS1 group also have a close relationship with its highest trans-C18:1. In reality, if lower quality roughage and higher concentration diets (comparison CS1, even

Table 4
Effects of dietary treatments on milk profiles of fatty acids (FA) (g/100 g of total FA).

Item	Diets[2]			SEM	*P*-value
	MF	CS1	CS2		
C4:0	0.71[a]	0.68[b]	0.60[c]	0.03	0.01
C6:0	0.62[a]	0.55[b]	0.47[c]	0.03	0.02
C8:0	0.86[a]	0.70[b]	0.61[c]	0.04	<0.01
C10:0	2.83[a]	2.45[b]	2.07[c]	0.11	<0.01
C12:0	3.77[a]	3.22[b]	2.79[c]	0.14	<0.01
C14:0	12.49[a]	11.47[b]	11.10[b]	0.32	0.02
C14:1	0.98	0.91	0.95	0.94	0.86
C15:0	1.21[a]	1.04[b]	1.03[b]	0.38	<0.01
C16:0	36.14[a]	33.09[b]	34.08[b]	0.68	0.01
C16:1	1.25	1.11	1.36	0.98	0.20
C17:0	0.60	0.62	0.66	0.07	0.87
C18:0	11.84	13.34	13.12	0.58	0.08
trans-18:1	1.08	1.28	1.13	0.08	0.09
C18:1c9	21.44[b]	24.71[a]	26.09[a]	0.78	<0.01
C18:2c6	2.72[b]	3.08[a]	2.76[b]	0.11	0.03
C18:3n3	0.47[b]	0.58[a]	0.63[a]	0.03	<0.01
Others	0.99	1.17	0.55	0.86	0.46
SFA	72.98[a]	69.23[b]	68.24[b]	0.76	<0.01
UFA	27.02[b]	30.77[a]	31.76[a]	0.76	<0.01
DNFA[1]	39.44[a]	36.33[b]	35.64[b]	0.71	<0.01
LCFA[1]	77.67[b]	79.36[a]	80.15[a]	0.68	0.04

FA = fatty acids; MF = mixed forages; CS = corn stalk; SEM = pooled standard error of the means; SFA = saturated fatty acid; UFA = unsaturated fatty acid; DNFA = *de novo* FA; LCFA = total long chain FA.
[a–c] Means within a row with different superscripts differ ($P < 0.05$).
[1] The DNFA included C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, and half of C16 content; LCFA included 16C and more than 16C content.
[2] The MF consisted of 3.7% Chinese wild rye, 26.7% corn silage and 23.4% alfalfa hay; CS1 was using corn stalk to formulate a chemical nutrient level similar to that of MF; CS2 was using corn stalk to formulate a similar forages level similar to that of MF.

with the same NDF) to be fed to cows, a MFD may be result in, the trend of trans-C18:1 might predict this result. The C18:2 in milk fat of cows fed CS1 increased ($P < 0.05$) and that of CS2 group had not reduced ($P > 0.05$, Table 2) when compared with MF, this was consistent with corn stalk test results of Shingfield et al. (2005). The short and medium chain FA (C4:0-C16:0) were reduced (P < 0.05), whereas C14:1, C16:1 and C17:0 were not different (P > 0.05) between treatments, these results were similar to those of Chilliard et al. (2001) who fed cows using hay and silage.

Compared with cows fed CS2 diet, cows receiving CS1 diet increased ($P < 0.05$) the compositions of C4:0 to C12:0 and C18:2 in milk fat. It is well accepted that short- and medium-chain fatty acids are synthesized *de novo* in the mammary gland from short-chain fatty acids derived from microbial fermentation of carbohydrates in the rumen (Chilliard et al., 2000). Loor et al. (2005) reported similar changes in these FA in the milk of cows fed hay with concentrations increasing, but the values for C18:0 were reduced significantly in that study. The difference between Loor's diet and ours was that our CS1 diet contained more C18:3 and C18:2, and for effects of FA hydrogenation, the composition of C18:0 in milk fat of CS1 was not reduced, but it did not exclude a possibility that a high proportion of whole cottonseed in CS1 diet would depress mammary Δ9-desaturation.

The compositions of unsaturated fatty acid (UFA) and LCFA in milk fat were increased ($P < 0.05$) in CS1 and CS2 compared with MF, but no difference ($P > 0.05$) were observed between cows fed CS1 and CS2, indicating that the source of forage and concentrate ratio did not affect their profiles. These results are consistent with those of Han et al. (2014) who replaced corn stover by mixed forages, and those of Bargo et al. (2006) who fed cows using increased amount of hay. Our results showed that feeding cows diets with only corn stalk as forages increased the composition of UFA which might help promote consumers' health (Parodi, 1999; Khas-Erdene et al., 2010) and LCFA proportion in milk compared with feeding cows mixed forages diets which contained silage.

4. Conclusions

Diets with different forage quality affect the milk fatty acids profiles and milk production. Dairy cows fed corn stalk could produce a greater proportion of UFA in milk fat without resulting in MFD in mid lactation cows. But simply increasing the ratio of concentrate in low forages diets is not an effective way to increase milk fat synthesis. For improving milk fatty acid profiles and milk production, the combined effects of corn stalk and other roughage still need to be studied.

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