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Full Length Research Paper

Effects of different corn silage: Alfalfa silage ratios and full fat extruded soybeans on milk composition, conjugated linoleic acids content in milk fat and performance of dairy cows

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The objective of this study was to investigate the effects of different corn silage : alfalfa silage ratios and full fat extruded soybeans on milk composition, especially that of conjugated linoleic acids content in milk fat and performance of dairy cows. Sixty Holstein cows were divided into 6 groups for a 12 week study. Treatments were arranged in a 2 × 3 factorial with 0 or 5% full fat extruded soybeans meal (dry matter basis) and three forage treatments (dry matter basis): (1) 13.5% corn silage, (2) 10.1% corn silage and 4.1% alfalfa silage, (3) 6.8% corn silage and 7.8% alfalfa silage. Full fat extruded soybeans addition increased the ether extract in diets and content of conjugated linoleic acids in milk, but it had no effect on fat, protein and lactose content in milk. Milk fat and lactose were not affected by replacing corn silage with alfalfa silage in diets. Increasing alfalfa silage content in the diets increased milk yield and milk content of conjugated linoleic acids and protein. Results from this study suggested that more alfalfa silage can be used in the diet of cow as a good source of forages.

Key words: Conjugated linoleic acid, alfalfa silage, corn silage, full fat extruded soybeans.

INTRODUCTION

Alfalfa is the predominant forage legume fed to lactating dairy cows in China. Cherney et al., (2004) reported that cows fed alfalfa silage (40.5 kg/day) had higher milk yield than second-cutting orchardgrass silage (34.4 kg/day) and second-cutting fescue silage (36.9 kg/day). (Broderick et al., 2002) showed that feeding an alfalfa silage-based diet led to greater feed intake (25.2 kg/day of DMI) and higher milk yield (41.1 kg/day) than feeding ryegrass silage-based diet (16.8 kg/day of DMI, 35.6 kg/day of milk yield). Kammes et al., (2008) reported that cows that consumed alfalfa silage had higher milk and 4% fat corrected milk (FCM) yield (32.8 kg/day) than cows fed with kura clover-reed canary grass silage (30.9 kg/day of 4% FCM). When cows were fed alfalfa silage, yields of milk, 3.5% FCM milk yield, fat and content of protein in milk were higher than those fed with earlier or later maturity red clover silage (Broderick et al., 2007).

In recent years, many researchers (Onetti et al., 2002; Benchaar et al., 2007) investigated the effects of replacing corn silage with alfalfa silage for dairy cows adding plant extracts, animal fats or their blends in diets. High quality AS was found essentially equal in energy DM basis to CS for

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Abbreviation: ADF, Acid detergent fiber; **AS,** alfalfa silage; **BW,** body weight; **CLA,** conjugated linoleic acid; **CP,** crude protein; **CS**, corn silage; **DIM,** days in milk; **DM,** dry matter; **DMI,** dry matter intake; **EE,** ether extract; **ESB,** full fat extruded soybeans; **FA,** fatty acids; **FCM,** fat collected milk; **IVDNDF,** *in vitro* digestible dry matter; **NDF,** neutral detergent fiber; **SARA**, subacute ruminal acidosis; **SCC,** somatic cell counts; **SNF,** solid not fat; **TMR,** total mixed ration; **TVA,** *trans*11-vaccenic acid.

milk production (Broderick 1985). Some researchers found no significant effect of forage on milk production as part of CS replaced by AS (Onetti et al., 2002; Ruppert et al., 2003; Onetti et al., 2004; Benchaar et al., 2007). In previous studies, milk yield responded differently when fats were added to the diets. Some research showed milk yield decreased with addition of fats (Onetti et al., 2001) while other studies found milk yield increased after addition of fats (Shaver, 1990; Drackley et al., 1994; Onetti et al., 2002). Others indicated there was no change of milk yield when fats were added to the diets (Elliott et al., 1993; Grummer et al., 1993; Ruppertet et al., 2003; Onetti et al., 2003; Benchaar et al., 2007).

When AS replaced 25% CS in the diets with 2% supplemental tallow, *cis*-9, *trans*-11 CLA concentration in milk tended to increase, however, *tran*-10, *cis*-12 CLA content decreased (Onetti et al, 2004). Benchaar et al. (2007) reported that CLA in milk increased (P< 0.01) by replacing CS with AS in the diets. But the content of CLA in milk was not affected by the specific mixture of essential oil compounds. Zhiqiang et al. (2003) indicated that increasing proportion of alfalfa hay in diet of cow can increase milk yield. Shengli (2001) also showed that feeding cows with alfalfa hay can improve quality of milk. The object of this study was to investigate the effects of AS replacing part of CS when adding full fat ESB on milk yield, milk composition and content of CLA in milk.

MATERIALS AND METORDS

Animals

Sixty multiparous (2.57 ± 0.62 parity) Holstein cows that averaged 46.28 ± 14.04 DIM and 600 ± 54 kg of BW were divided into six groups of 10 cows. Cows within each group were randomly assigned to one of six treatments. This feeding trial lasted 12 weeks. The first 4 weeks were considered to be the adaptation period. Measurements were made during the last 8 weeks. Cows were housed individually in a tie-stall barn and had free choice access to water. Cows were fed with TMR three times daily. Animals care and procedures were approved by the Department of Grassland Science, College of Animal Science and Technology, China Agricultural University, Beijing, China.

Diets

Experiment treatments were 2 × 3 factorial with 0 or 5% ESB (DM basis). As a percentage of total DM, the three forage treatments are: (1) 13.5% Corn silage, (2) 10.1% corn silage and 4.1% baled alfalfa silage, (3) 6.8% corn silage and 7.8% baled alfalfa silage. ESB was incorporated into concentrates and then added to TMR. Table 1 is ingredient composition of the experimental diets. Diets were formulated to meet NRC (2001) nutrient allowances of lactating cow (600 kg of BW) producing 35kg milk with 3.0% fat and 3.0% protein and taking 22.7 kg DM per day. When corn silage was replaced by alfalfa silage in the diets with ESB, corn, cottonseed meal and soybeans meal was decreased to equalize energy and protein of diets. Alfalfa silage and corn silage were made in Beijing Sanyuanlvhe Company. Alfalfa silage was baled with 74% moisture. Corn silage was whole crop corn silage. ESB was produced by Beijing Hongfutianlv high-tech development center. Average content of DM of alfalfa silage, corn silage and ESB were 26.19, 25.45 and 94.53%, respectively. CP, NDF, ADF and EE (DM basis) of alfalfa silage were 19.52, 46.10, 36.64 and 2.54% of corn silage were 9.73, 54.29, 30.35 and 2.34% of ESB were 31.21, 17.41, 11.61 and 17.04%, respectively.

Chemical composition of experimental diets is shown in Table 2. The six diets had similar calculated NE_L (1.66 Mcal/kg of DM). EE was increased when supplementing ESB in diets, while there were no significant change of content of CP, NDF and ADF. When replacing corn silage with alfalfa silage in diets, CP was increased, but NDF, ADF content were unchanged. Table 3 showed FA content and composition of experimental diets. As corn silage was replaced by alfalfa silage, FA contents in diets increased. Adding ESB enhanced the FA contents of diets.

Sampling and analysis

Samples of alfalfa silage, corn silage, alfalfa hay, *Chinese leymus* hay, concentrates and TMR were collected weekly. The TMR amount offered and refused was recorded daily. Orts were collected on d 14 to 21 and d 42 to 49 and dried 48 h in a 60℃ oven for DMI determination. Dry matter content of forages and concentrates was determined by oven-drying at 60℃ for 48 h. Results were used to adjust as fed ratios in the TMR. Amounts of TMR fed and refused were recorded everyday for individual cow.

Weekly dried feed samples were ground through a 2 mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA). Samples were further dried at 105℃ for 24 h to collect to 100% DM. Samples were analyzed for CP (AOAC, 2000), EE (AOAC, 1990), FA (Sukhija and Palmquist, 1988), NDF and ADF with amylase and sulfite (Van Soest et al, 1991).

Cows were milked three times daily at 0700, 1430 and 2030. Milk yield was recorded twice a week. Duplicate milk samples (50 ml) were collected each week. One set of milk samples were analyzed for fat, protein, lactose and SNF by near mid infrared procedures (MilkoScan 4000, Foss Electric, Denmark). SCC was detected by FOSS Somatic 5000 (Foss Electric, Denmark). The other set of milk samples was stored at -20℃ for further analysis of FA profile. Milk fat was extracted using the method of Hara and Radin (1978) and fatty acid methyl esters were prepared according to Kramer et al. (1997). Fatty acid methyl esters were analyzed using a gas chromatograph (GC-2010, Shimadzu Co., Japan) fitted with a flame-ionization detector. Fatty acid methyl esters were separated using a fused silica 100m × 0.25 mm column with a 0.20 μm film (Supelco Inc., Bellefonte, PA). Helium was utilized as the carrier gas. Samples including methyl esters in hexane (1 μL) were injected through the split injection port (40:1) with a column flow of 1.5 ml/min of helium. The oven temperature was initially 180℃ for 45 min then increased ramped to 215℃ at 10 min and held for 17 min. Injector and detector temperatures were maintained at 250℃. Hepta-decadienoic acid was used as a qualitative internal standard. Fatty acid peaks were identified using pure methyl ester standards (Supelco 37 Component FAME mix, Supelco, Bellfonte, PA; Matreya, Pleasant Gap, PA).

Statistical analysis

Performance data of dairy cows and fatty acid composition of milk fat were analyzed as a randomized block design using the PROC MIXED models of SAS (SAS Institute, 2000). The model used was:

$$Y_{ijk} = \mu + C_i + W_j + B_k + F_l + B_k F_l + E_{ijkl}$$

Where Y_{ijk} = dependent variable for cow i consuming diet of treatment k during week j, μ= population mean, C_i = the random effect of

Table 1. Ingredient composition of the experimental diets.

Corn silage[1]	0% ESB[2]			5% ESB		
	13.5%	10.1%	6.8%	13.5%	10.0%	6.8%
	(% of the DM)					
Corn silage	13.5	10.1	6.8	13.5	10.1	6.8
Alfalfa silage	0.0	4.1	7.8	0.0	4.1	7.8
Alfalfa hay	12.1	12.0	11.9	12.0	11.8	11.7
Chinese leymus	6.8	6.8	6.7	6.8	6.5	6.5
Corn	28.7	28.5	28.5	27.9	27.9	27.9
Peanut meal	2.9	2.9	2.9	2.8	2.8	2.8
Corn germ cake	7.2	7.2	7.2	7.0	7.0	6.9
Cottonseed meal	3.6	3.6	3.6	1.8	1.8	1.8
Soybeans meal	4.5	4.5	4.4	2.4	2.4	2.4
Full fat extruded soybeans	0.0	0.0	0.0	5.4	5.4	5.4
Wheat gluten	3.4	3.3	3.3	3.2	3.2	3.2
Whole cottonseed	5.0	4.9	4.9	5.0	4.9	4.8
Calcium soaps of fatty acid	0.8	0.8	0.8	0.8	0.8	0.8
Dried brewers grain	1.1	1.1	1.1	1.1	1.1	1.1
Wet brewers grain	4.4	4.3	4.3	4.3	4.3	4.2
Brewers dried yeast	2.8	2.8	2.7	2.8	2.7	2.7
Premix feed[3]	0.5	0.5	0.5	0.5	0.5	0.5
Calcium hydrogen phosphate[4]	1.2	1.2	1.2	1.2	1.2	1.2
NaCl	0.5	0.5	0.5	0.5	0.5	0.5
$NaHCO_3$	1.0	1.0	1.0	1.0	1.0	1.0

[1]Forage treatment were: (1) 13.5% corn silage (DM basis), (2) 10.1% corn silage and 4.1% alfalfa silage (DM basis), (3) 6.8% corn silage and 7.8% alfalfa silage (DM basis).
[2]ESB is full fat extruded soybeans.
[3]Containing 280,000 IU of vitamin A, 70,000 IU of vitamin D, 1400 IU of vitamin E, Fe 900 mg; Cu 500 mg; Zn 1500 mg; Mn 1200 mg; Se 12 mg; I 15 mg; and Co 50 mg (per kilogram of DM).
[4]Containing 21.85% Ca and 18.64% P.

cow i, W_j= the effect of week j, B_k=the effect of extruded soybeans, F_i= the effect of forages, B_kF_i= the interaction of extruded soybeans and forages, and E_{ijkl} = residual error. Least squares means ± standard errors of the mean were reported in the results. Significance was declared at $P < 0.05$ unless otherwise noted.

RESULTS AND DISCUSSION

Dry matter intake, milk yield and milk composition

Effects of dietary treatments on dry matter intake, milk yield and milk composition are shown in Table 4.

Extruded soybeans effects

Adding ESB did not significantly affect DMI in the diets regardless of forage type. These results are similar to previous studies where DMI did not differ whether cows were fed ESB or not (Abu-Ghazaleh et al., 2002a, Whitlock et al., 2002; Chen et al., 2008), but differed from Solomon et al. (2000) and Dhiman et al., (1999) who reported higher DMI for cows fed ESB diets compared with cows fed the control diet because increased energy and protein intake would lead to higher DMI for cows fed ESB. Milk yield (P > 0.05) and yield of 3.5% FCM (P > 0.05) was not affected when ESB was added to the diets. Chen et al. (2008) and Abu-Ghazaleh et al. (2002) observed that cows fed ESB tended Milk yield (P > 0.05) and yield of 3.5% FCM (P > 0.05) was not affected when ESB was added to the diets. Chen et al. (2008) observed and Abu-Ghazaleh et al. (2002) observed that cows fed ESB tended to have higher milk yield than those fed control diets (P = 0.09) but yield of 4% FCM was similar for all diets (P > 0.05). In other researches supplementing ESB increased milk yield and yield of 3.5% FCM (Dhiman et al., 1999; Whitlock et al. 2002; Gramenzi et al., 2003). Abu-Ghazaleh et al. (2002a) thought the increase of milk yield addition of ESB could not be assigned to DMI change since it was not significantly different among treatments (P > 0.05).

In this study content and yield of milk fat were noIn this study content and yield of milk fat were not affected by the addition of ESB (P > 0.05). Solomon et al. (2000) showed that adding ESB had no influence on milk fat content (P > 0.2) but decreased the milk fat yield (P ≤ 0.01).

Table 2. Chemical composition of experimental diets.

Corn silage[1]	0% ESB[2]			5%ESB		
	13.5%	10.1%	6.8%	13.5%	10.0%	6.8%
DM %	58.9	58.9	58.8	59.4	59.2	59.5
	(% DM)					
NE_L[3], Mcal/kg of DM	1.66	1.66	1.66	1.66	1.66	1.66
CP	15.99	16.49	16.74	16.26	16.68	16.99
NDF	44.04	43.74	43.65	44.09	43.66	43.60
ADF	21.69	21.03	22.03	21.63	21.37	21.81
EE[4]	4.02	4.07	4.02	4.93	4.90	4.91
Ca	0.84	0.81	0.84	0.87	0.87	0.80
P	0.41	0.42	0.45	0.38	0.43	0.39

[1]Forage treatment were: (1) 13.5% corn silage (DM basis), (2) 10.1% corn silage and 4.1% alfalfa silage (DM basis), (3) 6.8% corn silage and 7.8% alfalfa silage (DM basis).
[2]ESB is full fat extruded soybeans.
[3]Estimated from the NRC (2001).
[4]EE=Ether extract.

Table 3. Fatty acids content and composition of experimental diets.

Corn silage[1]	0% ESB[2]			5% ESB		
	13.5%	10.1%	6.8%	13.5%	10.0%	6.8%
	Fatty acid, DM% (g/100 g of total fatty acid)					
C14:0	0.52	0.64	0.65	1.1	1.15	1.17
C16:0	16.4	16.7	16.6	17.6	17.7	17.5
C16:1	0.23	0.26	0.30	1.01	1.10	1.15
C18:0	3.25	2.98	2.64	4.82	4.51	4.20
C18:1	17.66	16.42	14.87	14.33	13.04	11.45
C18:2	34.24	33.41	32.94	37.56	36.82	36.23
C18:3	5.31	8.84	11.29	3.5	6.9	9.2
Others	22.39	20.75	20.71	20.08	18.78	19.12

[1]Forage treatment were: (1) 13.5% corn silage (DM basis), (2) 10.1% corn silage and 4.1% alfalfa silage (DM basis), (3) 6.8% corn silage and 7.8% alfalfa silage (DM basis).
[2]ESB is full fat extruded soybeans.

These results were different from some previous reports. Abu-Ghazaleh et al. (2002a) and Chen et al. (2008) found milk fat percentage and milk fat yield were decreased in cows fed ESB. Dhiman et al., (1999) and Whitlock et al. (2002) reported that milk fat yield tended to increase (P > 0.05) and milk fat proportion decreased when ESB was supplemented to the diets. Dhiman et al., (1995, 1997 and 1999) explained that ESB supplying more $C_{18:2}$ fatty acids resulted to the lower milk fat content. ESB with rich $C_{18:2}$ fatty acids had been shown to increase the $C_{18:1}$ fatty acid content of milk by rumen biohydrogenation. Milk fat content was decreased by a *trans*-isomer of $C_{18:1}$ fatty acid (Romo et al., 1996).

Content and yield of milk protein did not change when ESB was added in the diets (P > 0.05). These results were consistent with the previous studies from Whitlock et al. (2002) and Chen et al. (2008). On the other hand, Solomon et al. (2000), and Abu-Ghazaleh et al. (2002) reported milk protein content decreased when fat was added to the diet. Reduced milk protein could be due to the negative effects on rumen microbial growth if cows were fed high fats diets (Jenkins, 1993; Satter et al., 1994). A response could be attributed to a better balance of amino acids for milk protein synthesis or more rumen undegradable protein by addition of ESB (Block et al., 1981; Chen et al., 2008). Milk protein yield increased because of the increasing milk yield (Dhiman et al., 1999). There was no significant difference in milk lactose content, milk lactose yield, milk SNF content, milk SNF production and milk SCC (P > 0.05) when ESB was supplemented to the diets.

Forage effects

No significant effect on DMI was observed (P = 0.07), as the proportion of AS was increased in the diets. These

Table 4. DMI, milk yield, SCC and milk compositon for cows fed dietary treatments.

Corn silage[1]	0% ESB[2]			5% ESB				Significant effects(P=)[3]		
	13.5%	10.1%	6.8%	13.5%	10.0%	6.8%	SEM	ESB	silage	ESB× Silage
DMI, Kg/d	22.1	22.1	22.1	22.2	22.1	22.0	0.04	0.19	0.07	0.36
Milk, Kg/d	34.93	35.64	36.84	35.05	36.28	36.95	1.1	0.47	< 0.001	0.32
3.5% FCM, Kg/d[4]	36.37	36.96	38.03	36.46	37.25	38.21	0.9	0.33	< 0.001	0.29
Fat, %	3.76	3.77	3.76	3.78	3.78	3.77	0.04	0.11	0.64	0.26
Fat, Kg/d	1.31	1.32	1.37	1.32	1.34	1.38	0.15	0.69	< 0.0001	0.64
Protein, %	3.00	3.08	3.16	3.00	3.11	3.19	0.05	0.61	< 0.0001	0.59
Protein, Kg/d	1.06	1.08	1.15	1.05	1.11	1.16	1.1	0.67	< 0.0001	0.77
Lactose, %	4.47	4.50	4.55	4.48	4.50	4.53	0.32	0.15	0.19	0.22
Lactose, Kg/d	1.56	1.61	1.68	1.57	1.62	1.67	1.22	0.10	< 0.001	0.13
SNF, %	8.17	8.28	8.41	8.18	8.31	8.42	0.45	0.11	< 0.001	0.10
SNF yield, g/d	2.86	2.97	3.10	2.86	3.00	3.11	1.77	0.20	< 0.001	0.31
SCC[5], $\times 10^3$/ml	234	232	231	236	237	236	0.32	0.65	0.56	0.38

[1]Forage treatment were: (1) 13.5% corn silage (DM basis), (2) 10.1% corn silage and 4.1% alfalfa silage (DM basis), (3) 6.8% corn silage and 7.8% alfalfa silage (DM basis).
[2]ESB is full fat extruded soybeans.
[3]*P*-value for the effect of ESB (0 or 5% DM), silage source (AS vs. CS) and the interaction between ESB and silage source (ESB × silage)
[4]3.5% FCM = (0.432×kg milk) + (16.216×kg fat)
[5]SCC = somatic cell counts.

results are similar to previous reports of Smith and Harris (1993) and Wattiaux and Karg (2004a) reporting that DMI was not affected whether cows were fed 14% AS and 41% CS or 41% AS and 14% CS. Similarly, Benchaar et al. (2007) observed no change in DMI when cows were fed 50% of the DM as either AS or CS. In contrast, Onetti et al., (2002) observed that DMI was increased by 1.6 kg/d as the proportion of AS was increased by 25% (DM) in the diets. When forage accounted for 40% of the DM, cows fed AS-based diets had a higher DMI, compared with cows consumed CS-based diets (Ruppert et al. 2003). Cows fed with higher proportion of AS increased DMI significantly, milk yield was not enhanced (0netti et al., 2002; Ruppert et al., 2003).

Despite no effect of forage on DMI when the proportion of AS in the diets was increased, milk yield was increased significantly (P < 0.0001). Brito and Broderick (2006) also observed that cows fed with diets with 40% corn silage and 10% alfalfa silage (DM basis) had less milk yield (39.5 kg/day) than those fed with diets with 51% alfalfa silage (41.5 kg/day). In contrast, Wattiaux and Karg (2004a) showed that cows fed CS-based diets had higher milk yield than those fed with AS-based diets. Results of present study are different from many previous studies that milk yield was not changed when cows were fed with AS-based diets compared with cows fed with CS-based diets (Broderick, 1985; Ruppert et al., 2003; Wattiaux and Karg, 2004b; Benchaar et al., 2007). Yield of 3.5% FCM also increased linearly (P < 0.0001). Similarly, Ruppert et al. (2003) and Brito and Broderick (2006) observed that yield of 3.5% FCM increased when the proportion of AS was raised in the diets. On the contrast, Groff and Wu (2005) showed that yield of 3.5% FCM decreased as AS was replaced by CS gradually when CP of dietary treatment was 16.25%. The increased milk yield and milk protein content may have occurred because when CS was replaced by part of AS, the supply of fermentable carbohydrate in rumen increased, which possibly lead to increasing microbial protein supply to the small intestine. It was easier to reach a balance between RUP and RDP when AS and CS were fed together (Dhiman et al. 1997). It would result in the increase of milk yield and milk protein content. Dairy cows are at high risk of (SARA) if fed diet with high concentrate/low fiber diets (Alzahal et al., 2009). SARA could decrease milk yield (McLaughlin et al., 2009). Replacing some CS with AS, increased *in vitro* digestible dry matter (IVDNDF) significantly (P < 0.05), which increased milk yield and milk protein content (Xu. 2005).

Feeding cows either CS or AS had no influence on the milk fat content. Some researchers indicated similar results (Broderick, 1985; Charmley et al., 1993). However, other studies reported that milk fat content decreased when cattle were fed diets based on CS rather than with AS (Onetti et al., 2002; Ruppert et al., 2003; Wattiaux and karg, 2004a,b; Brito and Broderick 2006; Benchaar et al., 2007). As the milk yield increased, a higher milk fat yield was observed when cows were fed with AS based diets than fed with AS based diets. Some other researchers observed that milk fat yield increased when AS replaced CS in the diets (Onetti et al., 2002; Krause and Combs, 2003).

The milk protein content and milk protein yield was higher for cows fed with AS based diets than for those fed with CS based diets. Similarly, Wattiaux and Karg (2004b) reported that milk protein content increased for cows fed

Table 5. Mean fatty acid content of milk from cows fed diets.

Corn silage[1]	0% ESB[2]			5%ESB			Significant effects(P=)[3]			
	13.5%	10.1%	6.8%	13.5%	10.0%	6.8%	SEM	ESB	silage	ESB× silage
C4:0	2.6	2.65	2.68	2.08	2.15	2.16	0.05	< 0.0001	0.22	0.23
C6:0	1.97	2	2.02	1.51	1.56	1.57	0.03	< 0.0001	0.27	0.18
C8:0	1.03	1.04	1	0.87	0.81	0.77	0.01	< 0.0001	0.13	0.11
C10:0	2.34	2.22	2.32	2.51	2.44	2.48	0.01	< 0.0001	0.21	0.20
C12:0	3.02	2.89	2.82	3.05	2.91	2.9	0.01	0.55	< 0.0001	0.62
C14:0	10.32	9.98	10.13	11.2	10.46	10.95	0.06	< 0.0001	< 0.0001	< 0.0001
C15:0	1.01	1.03	1.02	0.98	1	1.01	0.005	0.63	0.49	0.59
C16:0	27.9	28.1	28	28.9	28.9	29	0.07	< 0.0001	0.26	0.56
C16:1	0.13	0.12	0.14	0.3	0.31	0.29	0.01	< 0.0001	0.42	0.52
C17:0	0.49	0.51	0.51	0.5	0.51	0.5	0.004	0.65	0.51	0.64
C18:0	7.8	8.1	8.2	8.1	8.4	8.7	0.05	< 0.0001	< 0.0001	< 0.0001
C18:1	27.1	27.3	27.2	25.7	25.8	25.75	0.10	< 0.0001	0.42	0.38
C18:2	4.4	4.3	4.5	4.2	4.2	4.3	0.02	0.35	0.23	0.34
CLA[4]	0.83	0.91	0.93	1	1.02	1.08	0.09	< 0.0001	< 0.0001	< 0.0001
C18:3	0.33	0.56	0.77	0.51	0.74	0.89	0.02	< 0.0001	< 0.0001	< 0.0001
C20:0	0.23	0.23	0.24	0.23	0.24	0.23	0.01	0.80	0.53	0.60
C20:4	0.11	0.12	0.11	0.12	0.12	0.12	0.01	0.73	0.78	0.67
Short[5]	10.96	10.8	10.84	10.02	9.87	9.88	0.13	< 0.0001	0.17	0.16
Medium[6]	39.85	39.74	39.8	41.88	41.18	41.75	0.22	< 0.0001	0.22	0.31
Long[7]	40.8	41.52	41.95	39.86	40.52	41.07	0.25	< 0.0001	< 0.0001	< 0.0001
Unsaturated	58.82	58.87	59.05	60.05	59.5	60.39	0.73	< 0.0001	0.13	0.21
Saturated	32.79	33.19	33.54	31.71	32.07	32.31	0.53	< 0.0001	< 0.0001	< 0.0001
Others	8.39	7.94	7.41	8.24	8.43	7.3	0.31	< 0.0001	< 0.0001	< 0.0001

[1]Forage treatment were: (1) 13.5% corn silage (DM basis), (2) 10.1% corn silage and 4.1% alfalfa silage (DM basis), (3) 6.8% corn silage and 7.8% alfalfa silage (DM basis)
[2]ESB is full fat extruded soybeans
[3]*P*-value for the effect of ESB (0 or 5% DM), silage source (AS vs CS) and the interaction between ESB and silage source (ESB×silage)
[4]CLA= *cis*-9, *trans*-11 conjugated linoleic acid
[5]Short= C4:0 to C12:0 fatty acids
[6]Medium= C14:0 to C17:0 fatty acids
[7]Long= C18:0 to C20:4 fatty acids.

with recommended diets containing 41% AS and 14% CS compared with those fed with recommended diets containing 14% AS and 41% CS. Other researchers showed that there was no change in content and yield of milk protein when cows were fed with diets based on AS or CS (Dhiman and Satter, 1997; 0netti et al., 2002; Ruppert et al., 2003). On the contrary, Benchaar et al. (2007) and Brito and Broderick (2006) showed that the protein content of milk was higher ($P < 0.01$) and the protein yield of milk tended to be higher ($P = 0.05$) when cows fed CS based diets than those fed AS based diets. Milk lactose content, milk lactose yield and SCC did not differ among diets based on AS or CS. However, milk lactose yield was raised with increasing the proportion of AS.

Milk fatty acid composition

Extruded soybeans effects

Table 5 shows the changes of FA composition in milk fat when diets supplemented with ESB. With the addition of ESB, the proportion of short chain FA (C4:0 to C12:0) in milk fat was decreased ($P < 0.0001$) from 10.87 to 9.92 g/100 g of FA. Table 5 shows the changes of FA composition in milk fat when diets were supplemented with ESB. With the addition of ESB, the proportion of short chain FA (C4:0 to C12:0) in milk fat was decreased ($P < 0.0001$) from 10.87 to 9.92 g/100 g of FA. Similarly, content of long chain FA (41.40 vs. 40.43 g/100 g of FA) was lowered ($P<0.0001$) by the ESB-added diets, while medium chain FA concentration (39.80 to 41.53 g/100g of FA) was raised significantly ($P < 0.0001$). This is contrary to the results that were observed in previous studies where concentration of short and medium chain fatty acids in milk decreased and long chain fatty acids of milk increased when adding ESB to the diets (Schingoethe et al., 1996, Abu-Ghazaleh et al., 2002b and Chen et al., 2008). Grummer, (1991) and Dhiman et al., (1995) observed that milk fat concentration could be increased by the increasing supply of long chain fatty acids (FA) in the diets. However, de novo synthesis of short and medium chain FA would be inhibited by the increasing diet-added long chain FA in the mammary gland. Total unsaturated fatty acids concentration in milk was higher ($P < 0.0001$) and proportion of saturated fatty acids was lower ($P < 0.0001$) when cows fed additional ESB. Possibly the lower proportion of C12:0, C14:0 and C16:0 and higher concentration of TVA, *cis*-9 C18:1, *cis*-9, *cis*-12 C18:2 and CLA in milk would lead to the variation in the proportion of saturated fatty acids with ESB-added diets (Abu-Ghazaleh et al., 2002a). Ney (1991 reported that medium chain fatty acids compose the hypercholes-terolemic part of milk fat and therefore, the reduction of medium chain FA in milk should improve the profile of fatty acids in milk fat. The proportion of C4:0, C6:0, C8:0 and C18:1 in milk fat was decreased with supplemental fat ($P < 0.0001$). The concentration of C10:0, C14:0, C16:0, C16:1, C18:0, CLA and C18:3 fatty acids in milk fat were increased by feeding ESB ($P < 0.0001$). No effect of dietary fat was observed for C12:0, C15:0, C17:0, C18:2, C20:0 and C20:4 fatty acids**.**

The major object of this research was to determine the effects of forages resources, addition of ESB and their interactions on CLA content in milk fat. The *cis-9*, *trans*-11 CLA is produced from linoleic and linolenic acids through biohydrogenations in rumen (Griinari et al., 1999). Linoleic acid was the main fatty acid in the diets. The CLA was produced by cow in two ways. The first way is that *cis-9*, *cis-12* linoleic acid was converted to *cis*-9, *trans*-11 CLA by 12-*cis,* 11-*trans* isomarase through ruminal biohydrogenations (Griinari and Bauman, 1999). The other way to produce *cis*-9, *trans*-11 CLA by cow was that *cis*-9, *trans*-11 CLA can be synthesized from TVA through Δ^9-desaturase activity in mammary gland (Ip et al., 1999; Salminen et al., 1998). At least 65% production of *cis*-9, *trans*-11 CLA in milk fat should be due to the Δ^9-desaturase reaction (Corl et al., 2000). In present study concentration of CLA increased from 0.89 to 1.03 g/100 g of FA ($P < 0.0001$) when feeding cow with ESB. Abu-Ghazaleh et al. (2002b) and Whiltlock et al. (2002) also indicated that total CLA proportion in milk was higher after addition of ESB in the diets due to the increasing supple-mentation of linoleic and linolenic acids from adding ESB. When fat was supplemented to the diet of cows, *trans*-C18:1 fatty acids-products of incomplete ruminal biohy-drogenation into the duodenum increased. Research has shown that adding ESB in diets could improve the incomplete biohydrogenation (Kalscheur et al., 1997). The increased milk CLA concentration mainly was due to the increasing production of TVA in rumen when adding fat (Abu-Ghazaleh et al., 2002b).

Yield changes of fat acids in milk were similar to variation of fat acids content in milk (Table 6) because of lack of change in milk yield and milk fat content when adding ESB in diets. Yield of C4:0, C6:0, C8:0 and C18:1 fatty acids decreased when ESB was added to the diets of cows. However, yield of C10:0, C14:0, C16:0, C16:1, C18:0, CLA and C18:3 fatty acids increased. No differences of yield of C12:0, C15:0, C17:0, C18:2, C20:0 and C20:4 fatty acids were observed.

Forage effects

Replacing CS with AS increased proportions of C18:0, CLA and C18:3 fatty acids in milk (Table 5). Milk from cows fed more, AS had decreased concentrations of C12:0, C14:0 and C18:1fatty acids compared with those fed with high CS diet. There was no effect on contents of C4:0, C6:0, C8:0, C10:0, C15:0, C16:0, C16:1, C17:0, C18:2, C20:0, C20:4 by silage source ($P < 0.10$). Increasing AS:CS ratio resulted in increase of CLA content of milk fat as reported by Benchaar et al. (2007), Onetti et al. (2002) also showed that contents of C18:0 and C18:3 fatty acids in milk were increased by increasing the AS:CS ratio. But no change of CLA concentration in milk fat was observed when AS in the diets was increased. Onetti et al. (2004) reported that *cis*-9, *trans*-11 CLA isomer content was not changed, but proportion of *trans*-10, *cis*-12 CLA isomer and trans019 C18:1 was decreased ($P < 0.04$) when corn silage was replaced by alfalfa. Compared with cows fed only one forage-corn silage, cows fed half forage as alfalfa in the diet had higher milk fat content and yield, which is agreement with the decrease content of *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA in milk. Smith et al. (1993) reported that dietary forage source did not change the composition of FA in milk. Short and medium chain fatty acids were not affected by dietary forage source. Long chain FA increased when CS was substituted for AS. Meanwhile, saturated fatty acids were increased and no difference was observed in unsaturated FA.

Alteration of milk fat concentration and milk fat yield led to the changes of FA yields in milk (Table 6). Yield of C4:0, C6:0, C16:0, C18:0, C18:1, C18:2, C18:3, C20:0 and C20:4 fatty acids were increased by replacing AS with CS in the diets. Silage source did not affect content of C10:0, C14:0, C15:0, C16:1 and C17:0 fatty acids. CLA yield was also higher when cows fed alfalfa silage, compared to those fed with corn silage. Although, C18:1 isomers and CLA isomers were not separated in present research, adding alfalfa in the diets would keep a more stable rumen environment, which enhanced the process of rumen biohydrogenation to produce CLA.

Conclusions

This study focused on effect of forage source and adding ESB on milk CLA content and milk production perfor-mance of dairy cows. While this study found changes in milk yield and content similar to previous reports, the significant findings of this study were that, content of milk CLA was increased when CS was replaced by AS. Even

Table 6. Mean fatty acid yield of milk from cows fed diets (g/day).

Corn silage[1]	0% ESB[2]			5%ESB			Significant effects (P=)[3]			
	13.5%	10.1%	6.8%	13.5%	10.0%	6.8%	SE	ESB	silage	ESB× silage
C4:0	36.56	38.27	38.51	29.81	30.04	31.29	0.14	< 0.0001	< 0.0001	< 0.0001
C6:0	27.74	28.92	28.99	21.61	22.48	22.78	0.42	< 0.0001	< 0.0001	< 0.0001
C8:0	14.5	15.04	14.35	12.45	11.67	11.17	0.20	< 0.0001	< 0.0001	< 0.0001
C10:0	32.95	32.1	33.29	35.92	35.16	35.98	0.21	< 0.0001	0.20	0.17
C12:0	42.52	41.79	40.47	43.65	41.93	42.08	0.13	0.13	< 0.0001	0.17
C14:0	145.31	144.31	145.37	160.27	150.73	158.88	0.85	< 0.0001	0.11	0.15
C15:0	14.22	14.89	14.64	14.02	14.41	14.66	0.07	0.35	0.43	0.34
C16:0	392.83	406.33	401.8	413.56	416.45	420.79	1.28	< 0.0001	< 0.0001	< 0.0001
C16:1	1.83	1.74	2.01	4.29	4.53	4.21	0.17	< 0.0001	0.13	0.11
C17:0	6.9	7.37	7.32	7.16	7.34	7.26	0.07	0.13	0.11	0.15
C18:0	109.82	117.13	117.67	115.91	121.04	136.24	0.76	< 0.0001	< 0.0001	< 0.0001
C18:1	381.57	394.76	390.32	367.77	371.78	373.63	1.33	< 0.0001	< 0.0001	< 0.0001
C18:2	61.95	62.18	64.58	60.1	60.52	62.39	0.34	0.16	0.23	0.21
CLA[4]	11.68	13.42	14.59	13.05	14.4	15.7	0.43	< 0.0001	< 0.0001	< 0.0001
C18:3	4.65	8.1	11.05	7.3	10.66	12.91	0.36	< 0.0001	< 0.0001	< 0.0001
C20:0	3.21	3.37	3.56	3	3.39	3.33	0.32	0.13	< 0.0001	0.15
C20:4	1.56	1.76	1.71	1.58	1.68	1.73	0.10	0.54	< 0.0001	0.60
Short[5]	154.27	156.12	155.61	143.44	141.28	143.3	0.85	< 0.0001	0.45	0.20
Medium[6]	561.09	574.64	571.14	599.3	593.46	605.8	0.79	< 0.0001	< 0.0001	< 0.0001
Long[7]	574.44	600.72	603.48	568.71	583.47	595.93	0.44	< 0.0001	< 0.0001	< 0.0001
Unsaturated	463.24	481.96	484.26	454.09	463.57	470.57	0.51	< 0.0001	< 0.0001	< 0.0001
Saturated	826.56	849.52	845.97	857.36	854.64	874.46	0.87	< 0.0001	< 0.0001	< 0.0001
Others	118.57	114.13	110.29	116.85	121.3	109.38	0.82	< 0.0001	< 0.0001	< 0.0001

[1]Forage DM basis), (2) 10.1% corn silage and 4.1% alfalfa silage (DM basis), (3) 6.8% corn silage and 7.8% alfalfa silage (DM basis)
[2]ESB is full fat extruded soybeans
[3]*P*-value for the effect of ESB (0 or 5% DM), silage source (AS vs CS) and the interaction between ESB and silage source (ESB×silage)
[4]CLA= *cis*-9, *trans*-11 conjugated linoleic acid
[5]Short= C4:0 to C12:0 fatty acids
[6]Medium= C14:0 to C17:0 fatty acids
[7]Long= C18:0 to C20:4 fatty acid.

higher CLA content in milk was obtained by using both ESB and AS in the diets. This research has resulted in increased recognition of the value of the use of alfalfa in diets of dairy cows in China and hence, increased its use.

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