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Changes in Milk Production and Metabolic Parameters by Feeding Lactating Cows Based on Different Ratios of Corn Silage: Alfalfa Hay with Addition of Extruded Soybeans

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ABSTRACT: The objective of this study was to investigate the effects of different ratios of corn silage (CS): alfalfa hay (AH), and extruded soybeans (ESB) on milk yield, milk composition, blood metabolites, and fatty acids in milk fat and plasma. Ninety multiparous Holstein cows were arranged in a randomized block design experiment which lasted 14 weeks. Treatments were arranged as a 3×3 factorial with 0%, 5% or 10% ESB (dry matter basis) and three forage treatments: i) 30% CS, 10% AH and 10% *Leymus chinense* hay (LC); ii) 20% corn silage, 20% alfalfa hay and 10% LC; iii) 10% CS, 30% AH and 10% LC. Cows were allowed to consume a total mixed ration *ad libitum*. There was no change of dry matter intake when cows were fed the experimental diets. As more AH was added to the diets, milk yield, milk protein content and yield, and *trans*9, *cis*11-conjugated linoleic acids (CLA) concentrations in milk fat and plasma increased. When ESB were supplemented to the diets, milk yield, and *trans*9, *cis*11-CLA concentration in milk fat and plasma increased. When 10% ESB was added to the diet containing 30% AH the *trans*9, *cis*11-CLA content (1.46 g/100 g of total fatty acids) in milk was the highest among all treatments. These results suggests that AH could replace part of a CS diet and be a good forage source of diet for dairy cows to improve milk yield and milk composition. Meanwhile, ESB could be included in the diet with high AH to improve production performance of dairy cows. (**Key Words :** Alfalfa hay, Extruded Soybean, Milk Yield, Fatty Acid)

INTRODUCTION

In recent years, many studies have focused on investigating the effects of plants and plant extracts as the source of exogenous fat on dairy cows performance. Commonly, adding fat would be expected to increase milk yield and milk fat, however, different types and sources of fat have dissimilar effects on these parameters (Chouinard et al., 1997). Extruded soybeans (ESB) are commonly used as a source of energy and protein in diets for lactating dairy cows. The high oil content of ESB makes it an attractive energy-dense feed for animals with high energy requirements (Ye et al., 2010). Inclusion of ESB in diets of cows could alter the fatty acids (FA) profile in milk fat, especially the conjugated linoleic acids (CLA) concentration. Chen et al. (2008) showed that feeding ESB to cows increased *trans*9, *cis*11-CLA by 72%. Ye et al. (2010) also indicated that supplementation of ESB was effective in increasing *trans*9, *cis*11- CLA in milk.

Corn silage (CS) and alfalfa hay (AH) are the predominant forages fed to dairy cows in China. Previous studies have compared the effects of feeding cows different ratios of CS and AH on digestion, milk production and milk compositions (Broderick, 1985; Onetti et al., 2004; Oelker et al., 2009). Data from Kleinschmit et al. (2007) demonstrated that increasing the proportion of AH could increase DMI, and it tended to increase the milk yield. Onetti et al. (2004) reported that cows fed CS+tallow diet had equivalent DMI and milk yield with those cows fed with AH+tallow diet. West et al. (1997) indicated that cows fed diets including AH had greater milk fat percentage than those fed a CS diet. Previous studies indicated different CS and AH ratios in diets could change the FA profile. Ruppert et al. (2003) showed that corn silage and legume or grass forages may interact differently with various fat sources. Oelker et al. (2009) showed that AH diets had a similar *cis*9,

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*trans*11-CLA content to CS diets, and had a higher C18:3 content than cows fed CS diets. Onetti et al. (2004) also demonstrated that C18:2 and C18:3 contents increased when cows were fed AH diets with tallow compared with those fed CS diets with tallow.

However, little attention has been given to the influence of ESB combined with different ratios of CS and AH on production performance of dairy cows and fatty acids concentration in milk contents. The objective of this study was to investigate the effects of different ratios of CS and AH combined with the addition of ESB on milk yield, milk compositions, and fatty acids in milk fat and plasma, and blood metabolites.

MATERIALS AND METHODS

Animals and diets

Ninety multiparous Holstein cows (73±27 DIM, 627±52 kg of BW) were used in a randomized block design experiment over a 14-week period. The first 2 weeks were for adjustment and data collected during this time weren't used in the eventual analysis. Milk samples were collected in the last 12 weeks. Ten cows were randomly assigned to each treatment and housed in tie stalls and had free-choice access to water. Cows were fed *ad libitum* three times daily at 06:00 h, 14:00 h and 20:00 h, allowing for 5% to 10% orts. All procedures were approved by the Department of Grassland Science, College of Animal Science and Technology, China Agricultural University (Beijing, China).

Table 1 shows ingredient compositions of the experimental diets. Treatments were arranged with 0, 5% of 10% supplemental ESB (Produced by Sanyuanlvhe Co. Ltd, Beijing) and three forage treatments: i) 30% CS, 10% AH and 10% LC; ii) 20% CS, 20% AH and 10% LC; iii) 10% CS, 30% AH and 10% LC. Concentrates accounted for about 50% DM of the diets. When adding ESB, corn and soybean meal were reduced to make the diets isonitrogenous and isoenergetic. All feeds were made or purchased by Sanyuanlvhe Company in Beijing. All diets were formulated to meet or exceed the recommendations for nutrition requirements of NRC (2001) of lactating cows (600 kg of BW) producing 35 kg milk per day with 3.0% fat and 3.0% protein.

Sample collection and analysis

Samples of diet ingredients and TMR were collected weekly. Dry matter content was determined on feed samples dried in a 60°C forced-air oven for 48 h. Results were used to adjust as-fed ratios of forages and concentrate in the TMR. Feeds samples were ground to pass through a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA). The amounts of TMR offered and refused were measured daily. DMI for individual cows was determined by subtracting weekly mean of orts from weekly mean of feed offered. Organic matter was determined by combustion of feed at 550°C in a muffle furnace for 720 min. NDF and ADF content of feed samples was analyzed by method A of Van Soest et al. (1991) with a Ankom 2000 Fiber Analyzer (Ankom Technology, Fairport, NY), and were corrected for residual ash. Measurement of NDF used sodium sulfite and α-amylase. CP content was determined with macro-Kjeldahl nitrogen test (AOAC, 2000) by Kjeltec 2300 (Foss Electric, Denmark). Crude fat content was measured by ether extract (EE) content (AOAC, 2000). The NFC component was

Table 1. Ingredients compositions of the experimental diets

Corn silage (% DM)	Treatments[1]								
	0% ESB[2]			5% ESB			10% ESB		
	30%	20%	10%	30%	20%	10%	30%	20%	10%
	% of the DM								
Corn silage	30.1	20.2	10.1	30.1	20.2	10.2	30.1	20.1	10.2
Alfalfa hay	10.1	20.0	30.2	10.2	20.1	30.1	10.2	20.1	30.1
Leymus Chinese hay	9.8	9.8	9.7	9.7	9.7	9.7	9.7	9.8	9.7
Corn	20.8	20.9	20.9	19.1	19.0	19.0	17.1	17.0	17.0
Extruded soybeans	0	0	0	5.0	5.0	5.1	10.0	10.1	10.1
Wheat gluten	8.2	8.2	8.0	8.0	8.1	8.1	8.0	8.1	8.1
Soybean meal	13.8	13.8	13.8	10.8	10.8	10.7	7.8	7.7	7.7
Cottonseed meal	4.2	4.1	4.3	4.1	4.1	4.1	4.1	4.1	4.1
Premix feed[3]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
NaCl	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

[1] Treatments: i) 30% corn silage and 10% alfalfa hay; ii) 20% corn silage and 20% alfalfa hay; iii) 10% corn silage and 30% alfalfa hay; iv) 30% corn silage, 10% alfalfa hay and 5% ESB; v) 20% corn silage, 20% alfalfa hay and 5% ESB; vi) 10% corn silage, 30% alfalfa hay and 5% ESB; vii) 30% corn silage, 10% alfalfa hay and 10% ESB; viii) 20% corn silage, 20% alfalfa hay and 10% ESB; ix) 10% corn silage, 30% alfalfa hay and 10% ESB.

[2] ESB is extruded soybeans.

[3] Containing 260,000 IU of vitamin A; 150,000 IU of vitamin D; 1,500 IU of vitamin E; Fe 1,000 mg; Cu 800 mg; Zn 2,100 mg; Mn 700 mg; Se 18 mg; I 25 mg; and Co 20 mg (per kilogram of DM).

calculated as 100-(NDF+EE+CP+ash).

Cows were milked 3 times daily at 0730, 1500 and 2100 h. Milk yield from individual cows was measured on Tuesday of every week. Duplicated milk samples (100 ml) were mixed from three milkings in the ratio of 4:3:3. Milk samples were collected on Tuesday every week. One set of milk samples was preserved with bronopol-B2 for analysis of milk fat, protein, lactose and TS by near mid infrared procedures (MilkoScan 4000, Foss Elcetric, Denmark). SCC was measured by FOSS Somatic 5000 (Foss Elcetric, Denmark). The other set of milk samples was stored at -20°C for further analysis of FA profile. The *in vitro* dry matter digestibility (IVDMD), *in vitro* crude protein digestibility (IVCPD), *in vitro* ether extract digestibility (IVEED) and *in vitro* NDF digestibility (IVNDFD) of samples were determined by incubating feeds in rumen fluid for 48 h. Digestibility of chemical components was calculated with the equation: digestibility = (weight before incubation-weight after incubation)/weight before incubation×100%. Three cows fitted with a ruminal cannula were used for collecting rumen fluid and were fed 50% forages and 50% concentrates (DM basis).

Milk fat was extracted according to Hara and Radin (1978) and esterified by the method of Kramer and Zhou (2001). Separation of FA methyl esters of all samples was achieved using a gas chromatograph (GC-2010, Shimadzu Co., Japan) with a flame-ionization detector. Samples including methyl esters in hexane (1 μl) were injected through the split injection port (with ratio of 60:1) into a fused silica 100 m×0.25 mm×0.20 μm column (Supelco Inc., Bellefonte, PA). Carrier gas was helium, and the column flow of helium was 1.5 ml/min. The oven temperature was initially maintained at 120°C for 10 min, and then raised to 230°C at rate of 3.2°C/min and held for 35 min. Temperature of the injector was 250°C and of the detector was 300°C. Pure methyl ester standards were used to identify the fatty acids peaks (Supelco 37 Component FAME mix, Supelco, Bellfonte, PA; Matreya, Pleasant Gap, PA). Fatty acids were reported using the unit of grams per hundred grams of total FA methyl esters.

Duplicated blood samples from individual cows (20 ml) were collected at 3 week intervals from the coccygeal vein 4 hours before feeding. According to methods of Bu et al. (2007), one set of blood samples was collected for separating plasma in Vacutainer tubes containing anticoagulant (Lithium heparin, Greiner bioore GmbH). Plasma samples were collected by centrifuging at 3,000×*g* for 15 min at 4°C from blood samples. The other set of blood samples were collected in serum separator tubes (Serum Clot Activator, Greiner Bioone GmbH, A-4550 Kremsumunster, Austria). Blood samples were allowed to clot for 60 min at room temperature and centrifuged at 3,000×*g* for 15 min at 4°C to separate the serum. Blood plasma and serum samples were stored at -20°C for further analysis. One set of plasma samples were analyzed by clinical analyzer 7600 (Hitachi High-Tec, Co., Japan) for total protein (TP), triglyceride (TG), cholesterol (CHOL), BUN, BHBA, NEFA, lactic acid (LA), and glucose (GLU). Plasma samples were used for analysis of FA profile. Lipids from plasma were extracted with the procedure of Christie (1982). The residues (free FA, phospholipids and cholesterol) were dissolved in hexane under nitrogen. The solvent was analyzed for FA profile as described above for milk samples at 20:1 split ratio.

Statistical analysis

The data of chemical compositions of experimental diets and concentration of FA in experimental diets were analyzed as one factor design using the PROC GLM models of SAS (SAS Institute, 2000).

The data of milk yield, milk compositions, blood metabolites, FA in milk fat and plasma were analyzed as a 3×3 factorial randomized block design using the PROC MIXED models of SAS (SAS Institute, 2000). The model contained forage, ESB, and forage×ESB interaction. Cow and time were random effects. Forage (different CS and AH ratios), ESB, and forage×ESB interaction were the fixed effects. Significance was declared at $p<0.05$ unless otherwise noted. Tendencies for treatment effects were considered to exist if $0.05<p<0.10$. Least squares means± standard errors of the mean were reported in the results.

RESULTS AND DISCUSSION

The chemical compositions of the experimental diets are shown in Table 2. There was no difference in NE_L between the experimental diets. When CS was partially replaced by AH, DM percentage and CP content increased, while NDF and NFC contents decreased. After the addition of ESB in diets, CP and EE content increased, but NDF contents declined.

DMI, milk yield and milk composition

Table 3 shows DMI, milk yield, milk compositions, body weight and SCC of cows fed experimental diets.

Forage effects : DMI, which was averaged 23.9 kg/d, was not affected ($p = 0.20$) when CS was replaced by AH. This result was consistent with previous studies. Oelker et al. (2009) reported that no difference of DMI was found whether cows were fed AH or CS. Broderick (1985) also showed that DMI was not affected by feeding cows with CS-based or alfalfa-based diets. But some authors reported that DMI increased as more alfalfa was included in the diets (Ruppert et al., 2003; Gehman and Kononoff (2010).

In this research, there was a linear ($p<0.0001$) increase

Table 2. Chemical compositions and digestibilities of experimental diets

CS (% DM)	Treatments									SEM	Feeds		
	0% ESB			5% ESB			10%ESB						
	30%	20%	10%	30%	20%	10%	30%	20%	10%		CS	AH	ESB
DM (%)	51.8^{c}	57.3^{b}	64.1^{a}	51.9^{c}	57.2^{b}	64.2^{a}	52.0^{c}	57.2^{b}	65.2^{a}	0.18	25.3	91.2	93.5
OM	94.8	94.9	94.9	94.9	94.8	94.9	94.8	94.9	94.9	0.03	94.0	91.6	92.7
NE_L[3] (Mcal/kg)	1.61	1.62	1.62	1.62	1.62	1.63	1.62	1.63	1.63	0.01	1.50	1.42	2.2
CP	16.0^{h}	17.1^{e}	18.2^{c}	16.7^{g}	17.6^{d}	18.7^{b}	17.0^{f}	18.1^{c}	19.1^{a}	0.03	8.7	20.3	37.7
EE	3.3^{c}	3.2^{c}	3.2^{c}	4.1^{b}	4.0^{b}	4.1^{b}	4.6^{a}	4.6^{a}	4.7^{a}	0.02	3.0	2.3	18.3
NDF	37.9^{a}	37.3^{b}	36.8^{d}	36.4^{d}	35.9^{e}	35.5^{f}	35.4^{f}	34.9^{g}	34.6^{h}	0.08	44.7	36.5	13.9
ADF	19.5	19.5	19.6	19.5	19.5	19.6	19.4	19.5	19.5	0.07	26.5	28.9	8.0
Ash	5.2	5.1	5.1	5.1	5.2	5.1	5.2	5.1	5.1	0.03	6.0	7.4	7.3
NFC[4]	37.6^{a}	37.3^{b}	36.7^{c}	37.7^{a}	37.2^{b}	36.7^{c}	37.7^{a}	37.3^{b}	36.6^{c}	0.09	37.6	33.5	22.8
Ca	0.83	0.78	0.81	0.80	0.82	0.81	0.82	0.82	0.81	0.01	0.52	1.80	0.33
P	0.42	0.41	0.42	0.41	0.42	0.43	0.42	0.43	0.42	0.01	0.22	0.32	0.67
IVDMD[5] (% DM)	62.17^{c}	65.03^{b}	67.09^{a}	62.16^{c}	65.03^{b}	67.20^{a}	62.09^{c}	65.27^{b}	67.35^{a}	0.09	45.7	65.3	82.3
IVOMD[6] (% DM)	62.11^{c}	64.17^{b}	66.43^{a}	61.86^{c}	64.09^{b}	66.41^{a}	61.91^{c}	64.46^{b}	66.55^{a}	0.12	45.5	63.5	82.8
IVCPD[7] (% CP)	65.52^{h}	67.06^{g}	69.52^{d}	67.37^{f}	69.88^{c}	70.79^{b}	68.19^{e}	70.07^{c}	72.21^{a}	0.07	49.0	68.8	85.6
IVNDFD[8] (% NDF)	52.34^{c}	53.45^{b}	54.96^{c}	52.57^{c}	53.33^{b}	54.98^{c}	52.33^{c}	53.37^{b}	55.00^{c}	0.08	46.0	57.7	75.9
IVEED[9] (% EE)	57.47^{c}	57.51^{c}	57.32^{c}	60.54^{b}	60.65^{b}	60.4^{b}	64.52^{a}	64.67^{a}	64.63^{a}	0.09	53.8	57.9	71.1

[1] CS is corn silage. [2] SEM is standard error of the mean. [3] NE_L was calculated from NRC (2001).
[4] NFC = 100-(CP+EE+NDF+Ash). [5] IVDMD is *in vitro* dry matter digestibility.
[6] IVOMD is *in vitro* organic matter digestibility. [7] IVCPD is *in vitro* crude protein digestibility.
[8] IVNDFD is *in vitro* neutral detergent digestibility. [9] IVEED is *in vitro* ether extract digestibility.

in milk yield and 4% FCM yield when CS was replaced by AH. Kleinschmit et al. (2007) also indicated that there was a linear increase in milk yield when alfalfa was added to the diet. Broderick (1985) found that milk yield was higher when cows were fed 60% AH diet compared with 76% CS diet. Brito and Broderick (2006) concluded that it was unnecessary to feed high levels of CS for maximal milk production. However, Onetti et al. (2004) showed no change of milk yield when cows were fed AH diet compared with CS diet. Ruppert et al. (2003) explained that chemical composition and digestibility of CS and alfalfa was different, but milk yield might be expected to be similar if diets with different forage contain a similar supply of energy, metabolizable protein, minerals, and vitamins. In the present study, it seems that more nutrients were available for milk synthesis because both OM digestibility and intake of digestible OM increased when cows were fed high AH diets.

Table 3. DMI, milk yield, milk compositions, SCC and body weights of cows fed experimental diets

CS (% DM)	Treatments									SEM	p value[1]		
	0% ESB			5% ESB			10% ESB						
	30%	20%	10%	30%	20%	10%	30%	20%	10%		F	ESB	F×ESB
DMI (kg/d)	24.0	23.9	23.9	24.0	23.8	23.9	23.9	23.7	23.9	0.20	0.51	0.67	0.92
Milk yield (kg/d)	30.5^{e}	31.3^{d}	32.6^{c}	31.2^{d}	32.3^{b}	34.0^{b}	34.1^{b}	34.7^{a}	35.1^{a}	0.93	0.005	<0.0001	0.81
4% FCM[2] (kg/d)	29.4^{d}	30.5c^{d}	31.7^{bc}	30.1^{cd}	31.5^{bcd}	32.9^{ab}	32.6^{ab}	33.9^{ab}	34.3^{a}	0.96	0.002	<0.0001	0.95
Fat (%)	3.7	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.80	0.07	0.29	0.95	0.96
Fat yield (kg/d)	1.15^{d}	1.20^{cd}	1.25^{bcd}	1.18^{cd}	1.24^{bcd}	1.29^{ab}	1.27^{abc}	1.33^{ab}	1.35^{a}	0.04	0.003	0.0003	0.98
Protein (%)	3.0^{e}	3.1^{cd}	3.2^{ab}	3.1^{de}	3.2^{bc}	3.3^{a}	3.1^{e}	3.2^{bc}	3.3^{a}	0.04	<0.0001	0.16	0.90
Protein yield (kg/d)	0.93^{e}	0.98^{cde}	1.05^{bc}	0.95^{de}	1.02^{cd}	1.11^{ab}	1.04^{bc}	1.10^{ab}	1.15^{a}	0.03	<0.0001	<0.0001	0.88
Lactose (%)	5.2	5.1	5.1	5.2	5.1	5.2	5.1	5.2	5.2	0.03	0.97	0.43	0.46
Lactose (kg/d)	1.56^{c}	1.59^{c}	1.65^{bc}	1.57^{c}	1.65^{bc}	1.74^{b}	1.74^{ab}	1.73^{ab}	1.76^{ab}	1.78^{a}	0.004	<0.0001	0.65
TS[3] (%)	12.6	12.6	12.5	12.6	12.4	12.4	12.5	12.6	12.7	0.10	0.97	0.09	0.23
TS yield (kg/d)	3.84^{d}	3.96^{d}	4.09^{bcd}	3.92^{d}	4.01^{cd}	4.21^{bc}	4.26^{abc}	4.37^{ab}	4.47^{a}	0.12	0.008	<0.0001	0.98
SCC ($\times 10^3$/ML)	144.5^{a}	101.9^{b}	96.6^{b}	140.2^{a}	103.7^{b}	99.7^{b}	165.8^{a}	90.8^{b}	88.7^{b}	11.68	<0.0001	0.98	0.18
Body weight (kg)	634	611	632	603	625	619	602	629	617	4.34	0.76	0.83	0.91

[a-f] Means in the same row with different superscripts differ ($p<0.05$).
[1] p-value for the effect of forage, ESB, and interaction between forage and ESB.
[2] 4% FCM = 0.4×kg milk+15.0×kg fat. [3] TS is total solids.

Milk protein content increased from 3.1% to 3.3% after replacing CS with AH in this study. Kleinschmit et al. (2007) and Oelker et al. (2009) showed that there was no effect on milk protein content due to forage source. There were some reasons for the change of milk CP content. In our study, partially replacing CS with AH, CP content and digestibility of diets were higher. Cows could consume more digestible protein and had high RDP when fed more AH, which could result in the increased CP content in milk. Otherwise, CP content of 30% AH diets was higher than in 10% and 20% AH diets, and 30% AH diets probably supplied more metabolizable protein than 10% and 20% AH diets. When cows consumed 30% AH diets, milk protein and yield were higher but the blood BUN was lower than 10% and 20% AH diets. Milk protein yield increased because of the increased milk yield and milk protein content when CS was replaced by AH in the diets.

No change was observed on milk fat content after replacing CS with AH in this research, which was in accordance with another study (Kleinschmit et al., 2007). Milk fat yield increased with AH due to an increase in milk yield. However, in some other studies (Krause and Combs, 2003; Ruppert et al., 2003) milk fat and content increased when CS was replaced by alfalfa. Like the results from previous studies (Kleinschmit et al., 2007; Kowsar et al., 2008) forage source did not affect the lactose content in milk. No difference of TS content was detected among treatments. The increasing inclusion of AH in diets linearly increased milk lactose and TS yield ($p<0.0001$). SCC content decreased when AH replaced part of CS in the diet. The increasing proportion of AH in diets could lead to a more stable environment in rumen, which could balance the rumen pH and may partly explain the decrease of SCC when more AH was included in the diets.

ESB effects : There was no effect of ESB on DMI ($p = 0.99$), which was consistent with other previous work (Abu-Ghazaleh et al., 2002a, b; Ye et al., 2009). But Solomon et al. (2000) indicated that DMI was improved significantly by the addition of ESB, which was related to the temperature of the extrusion treatment (Chouinard et al., 1997).

In contrast to previous studies (AbuGhazaleh et al., 2002a; Whitlock et al., 2002) in which no effect on milk yield was found with the inclusion of ESB, milk yield was increased in this study. This increase of milk yield with cows fed ESB diets could not be attributed to DMI because treatments had no effects on DMI. The increased milk yield could be due to the increased RUP, elevating protein in the small intestine and increasing energy intake (Block et al., 1981). After adding ESB to the diets, CP content and digestibility of CP in diets increased. Higher CP fed to cows may explain the increased milk yield by increasing protein delivery to the small intestine (Abu-Ghazaleh et al., 2002b). Adequate supply of protein to the small intestine of lactating dairy cows is essential for maximum milk yield (Calsamiglia et al., 1995).

Milk protein percentage (Table 4) was not affected by ESB supplements compared with diets without ESB and was similar in other researches (Whitlock et al., 2002; Ye et al., 2009). Solomon et al. (2000) showed that milk protein content was decreased with the addition of ESB, which would be associated with negative effects on rumen microbial growth observed for cows fed high fat diets (Jenkins, 1993; Satter et al., 1994). The decrease in milk protein content is likely to be consistent with the increased milk urea nitrogen concentrations observed with diets containing ESB and would also be consistent with a dilution

Table 4. Composition and concentration of fatty acids in experimental diets

CS (% DM)	Treatments									SEM
	0% ESB			5% ESB			10% ESB			
	30%	20%	10%	30%	20%	10%	30%	20%	10%	
Fatty acids (g/100 g of total fatty acids)										
C14:0	0.60	0.62	0.60	0.63	0.61	0.61	0.61	0.61	0.60	0.01
C16:0	25.12^{c}	27.58^{b}	30.09^{a}	23.24^{e}	25.15^{c}	27.63^{b}	22.01^{f}	23.64^{d}	25.44^{c}	0.13
C18:0	4.75^{c}	3.92^{e}	3.18^{g}	5.45^{b}	4.40^{d}	3.42^{f}	5.96^{a}	4.47^{d}	3.84^{e}	0.05
C18:1	21.38^{d}	22.91^{c}	24.16^{a}	20.55^{e}	22.76^{c}	23.47^{b}	19.82^{f}	20.88^{e}	23.40^{b}	0.11
C18:2	35.88^{d}	31.37^{g}	27.08^{i}	38.18^{b}	33.68^{e}	30.40^{h}	39.74^{a}	37.05^{c}	32.28^{f}	0.11
C18:3	9.26^{i}	10.56^{f}	11.87^{c}	9.67^{h}	11.05^{e}	12.11^{b}	10.11^{g}	11.58^{d}	12.60^{a}	0.08
c9 t11CLA[1]	0.09^{a}	0.09^{a}	0.10^{a}	0.06^{b}	0.07^{b}	0.07^{b}	0.04^{c}	0.05^{c}	0.05^{c}	0.01
C20:0	0.61^{a}	0.57^{ab}	0.54^{b}	0.49^{c}	0.46^{c}	0.41^{d}	0.41^{d}	0.36^{e}	0.32^{f}	0.01
C20:1	0.30	0.32	0.33	0.26	0.29	0.31	0.24	0.26	0.30	0.01
C20:2	0.28^{a}	0.27^{a}	0.25^{a}	0.19^{b}	0.14^{c}	0.13^{cd}	0.11^{d}	0.07^{e}	0.05^{e}	0.01
C22:0	0.61^{d}	0.74^{b}	0.83^{a}	0.45^{f}	0.62^{d}	0.69^{c}	0.29^{g}	0.43^{f}	0.55^{e}	0.01
C22:1	0.45^{a}	0.33^{b}	0.22^{c}	0.31^{b}	0.22^{c}	0.14^{d}	0.23^{c}	0.13^{d}	0.07^{e}	0.01
C24:0	0.67^{b}	0.72^{ab}	0.75^{a}	0.52^{de}	0.55^{d}	0.61^{c}	0.43^{f}	0.47^{ef}	0.50^{e}	0.01

[1] CLA-conjugated linoleic acids.

of milk protein as milk yield increased (Solomon et al., 2000). In this study, the high CP content and digestibility of diets containing ESB appeared to avoid a decrease in milk CP concentration. Milk protein yield increased due to the increase of milk protein content and milk yield.

Like other studies (AbuGhazaleh et al., 2002a; Solomon et al, 2000), there was no difference ($p>0.05$) in milk fat content when cows were fed ESB. However, Ye et al. (2009) and Chen et al. (2008) found lower milk fat content with addition of ESB in the diet. Griinari et al. (1998) suggested that depression of milk fat was closely related to increases in *trans*-C18:1 FA. But our results do not support this theory, since milk *trans*-C18:1 FA content increased in this study, but no depression of milk fat concentration was observed. Baumgard et al. (2000) observed that *trans*10, *cis*12-CLA are negatively correlated with milk fat percentage and would decrease milk fat synthesis, which could cause milk fat depression. In this study, no change was found in milk *trans*10, *cis*12-CLA concentration and could explain the no difference result in milk fat percentage. Milk fat yield improved because of the increased milk yield.

Milk lactose percentage, TS content and SCC were not influenced ($p>0.05$) by the supplementation of ESB, while milk lactose and SNF yield increased due to the increase of milk yield.

No forage×ESB interaction was found in DMI, milk yield, milk compositions and SCC when cows were fed experimental diets.

No change of body weights of cows was found among all treatments.

Milk fatty acids profile

Forage effects : Composition and concentration of fatty acids in experimental diets are shown in Table 4. Partially replacing CS with AH, C16:0, C18:1, C18:3, C22:0 and C24:0 concentrations in diets increased, while C18:0, C18:2 and C20:0 contents declined.

Table 5 shows FA compositions in milk fat. Feeding cows with more AH reduced the proportion of short chain fatty acids (SCFA, from C4:0 to C13:1), but increased both medium chain fatty acids (MCFA, from C14:0 to C17:1) and long chain fatty acids (LCFA, from C18:0 to C24:0) content in milk fat. When CS was replaced by AH, percentage of saturated fatty acids (SFA) decreased and unsaturated fatty acids (USFA) content increased.

One of the main objectives of this study was to examine the effects of forages on CLA content in milk fat. In this study, *cis*9, *trans*11-CLA and *trans*10, *cis*12-CLA isomers were identified. Content of *cis*9, *trans*11-CLA increased by

Table 5. Fatty acids compositions in milk fat

CS (% DM)	Treatments									SEM	p value		
	0% ESB			5% ESB			10% ESB						
	30%	20%	10%	30%	20%	10%	30%	20%	10%		F	ESB	F×ESB
Fatty acids (g/100 g of total fatty acids)													
C14:0	12.64^{a}	9.74^{d}	6.86^{g}	11.76^{b}	8.68^{e}	6.11^{h}	10.72^{c}	7.64^{f}	5.14^{i}	0.07	<0.0001	<0.0001	0.06
C16:0	28.89^{ab}	29.29^{ab}	30.56^{a}	25.46^{cd}	28.25^{abc}	27.33^{bc}	22.85^{d}	23.33^{d}	24.30^{d}	1.07	0.15	<0.0001	0.75
C16:1	1.49^{f}	1.67^{e}	1.72^{d}	1.64^{e}	1.84^{c}	1.92^{b}	1.74^{d}	1.94^{b}	2.05^{a}	0.02	<0.0001	<0.0001	0.24
C17:0	0.70^{a}	0.71^{a}	0.70^{a}	0.53^{b}	0.55^{b}	0.54^{b}	0.45^{c}	0.46^{c}	0.46^{c}	0.01	0.28	<0.0001	0.84
C17:1	0.28	0.28	0.28	0.28	0.28	0.28	0.29	0.27	0.28	0.004	0.30	0.67	0.29
C18:0	10.46^{e}	10.84^{d}	11.60^{c}	11.09^{d}	11.67^{c}	12.01^{b}	11.75^{bc}	12.54^{a}	12.79^{a}	0.10	<0.0001	<0.0001	0.07
t9 C18:1	0.16^{i}	0.38^{h}	0.55^{f}	0.48^{g}	0.68^{e}	0.90^{c}	0.76^{d}	0.98^{b}	1.23^{a}	0.02	<0.0001	<0.0001	0.11
t-10 C18:1	0.88^{d}	0.77^{e}	0.48^{f}	1.03^{c}	0.91^{d}	0.66^{e}	1.39^{a}	1.25^{b}	1.04^{c}	0.02	<0.0001	<0.0001	0.26
t-11C18:1	1.80^{c}	1.77^{c}	1.78^{c}	2.41^{b}	2.40^{b}	2.41^{b}	3.47^{a}	3.48^{a}	3.46^{a}	0.03	0.92	<0.0001	0.98
c-9C18:1	18.87^{c}	20.45^{abc}	20.97^{abc}	20.34^{abc}	19.62^{bc}	22.39^{a}	20.57^{abc}	21.69^{ab}	22.70^{a}	0.92	0.02	0.14	0.70
c-11C18:1	0.68	0.68	0.71	0.68	0.71	0.67	0.66	0.68	0.68	0.01	0.46	0.29	0.22
C18:2	4.42^{e}	4.44^{e}	4.38^{e}	5.17^{b}	5.15^{b}	5.16^{b}	6.04^{a}	6.01^{a}	6.04^{a}	0.05	0.86	<0.0001	0.88
C18:3 n-3	0.43^{e}	0.52^{d}	0.65^{c}	0.52^{d}	0.65^{c}	0.76^{b}	0.62^{c}	0.72^{b}	0.82^{a}	0.02	<0.0001	<0.0001	0.65
C18:3 n-6	0.07	0.07	0.07	0.08	0.08	0.08	0.07	0.07	0.07	0.002	0.78	0.09	0.34
c9 t11 CLA	0.67^{i}	0.84^{h}	1.04^{f}	0.96^{g}	1.11^{e}	1.26^{c}	1.18^{d}	1.34^{b}	1.46^{a}	0.02	<0.0001	<0.0001	0.17
t10 c12 CLA	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.001	0.30	0.10	0.12
C20:4	0.24^{a}	0.25^{a}	0.24^{a}	0.2^{bcd}	0.21^{bc}	0.21^{b}	0.18^{cd}	0.18^{d}	0.18^{d}	0.01	0.92	<0.0001	0.76
SCFA[1]	26.98^{a}	24.11^{d}	21.35^{g}	26.19^{b}	22.96^{e}	20.50^{h}	25.12^{c}	22.12^{f}	19.55^{i}	0.14	<0.0001	<0.0001	0.691
MCFA[2]	33.26^{ab}	33.81^{ab}	35.13^{a}	29.81^{cde}	32.79^{abc}	31.93^{bcd}	27.16^{e}	27.88^{e}	28.94^{de}	1.07	0.08	<0.0001	0.76
LCFA[3]	39.76^{d}	42.08^{cd}	43.52^{c}	44.00^{c}	44.25^{c}	47.57^{b}	47.72^{b}	50.00^{ab}	51.51^{a}	0.94	<0.0001	<0.0001	0.78
USFA[4]	31.90^{d}	33.99^{cd}	34.70^{c}	35.67^{c}	35.48^{c}	38.56^{b}	38.84^{b}	40.50^{ab}	41.89^{a}	0.93	0.001	<0.0001	0.72
SFA[5]	68.10^{a}	66.01^{ab}	65.30^{ab}	64.33^{bc}	64.52^{b}	61.44^{cd}	61.16^{de}	59.50^{de}	58.11^{e}	1.08	0.005	<0.0001	0.80

[1] SCFA = Short chain fatty acids, from C4:0 to C13:1. [2] MCFA = Medium chain fatty acids, from C14:0 to C17:1.
[3] LCFA = Long chain fatty acids, from C18:0 to C24:0. [4] USFA = Unsaturated fatty acids.
[5] SFA = Saturated fatty acids.

about 33% (from 0.94 to 1.25 g/100 g FA) when 30% AH was included in the diet. It is reported that the consumption of *cis*9, *trans*11-CLA is beneficial to human health (Ip et al., 1999). C18:2 and C18:3 are precursors of CLA produced through ruminal biohydrogenations (Griinari and Bauman, 1999). Harfoot and Hazlewood (1988) indicated that the biohydrogenation of C18:1 by mixed ruminal microbes involved not only direct biohydrogenation to produce C18:0 but also the formation of several positional isomers of *trans*monoenes, including transvaccenic acid (TVA), an intermediate of biohydrogenation. Therefore, CLA was also produced by the incomplete biohydrogenation of linoleic acid in rumen and by endogenous synthesis from TVA through activities of Δ^9-desaturase in the mammary gland (Griinari and Bauman, 1999; Ip et al., 1999). Griinari et al. (2000) observed that most milk CLA was derived from TVA in the mammary gland and a small amount of milk CLA was generated in the rumen. In this study, milk from cows fed high AH diets contained higher *cis*9, *trans*11-CLA content than from those fed high CS diets. Benchaar et al. (2007) also indicated that replacing alfalfa with CS in the diets increased the milk fat content of *cis*9, *trans*11-CLA. Although content of C18:2 in diets decreased, C18:3 concentrations increased when CS was replaced by AH, which could improve biohydrogenaration in rumen to increase *cis*9, *trans*11-CLA concentration in milk fat. Otherwise, increased C18:1 content in high AH diets could raise the TVA content in the mammary gland, which also may increase milk *cis*9, *trans*11-CLA content by endogenous synthesis. While in other studies (Onetti et al., 2004; Oelker et al., 2009), there was no difference on milk *cis*9, *trans*11-CLA content in CS based or AH based diets. Like the results of Onetti et al. (2004), no change was found in milk *trans*10, *cis*12-CLA content in this study when part of corn CS was replaced by AH.

When cows were fed high AH diets, TVA content in milk did not change, while *trans*-9 18:1, *cis*-9 C18:1 and *cis*-11 18:1 contents increased and *trans*-10 C18:1 concentration decreased. There was no difference on C18:2 content after replacing CS with AH. Because of increased C18:3 in high AH diet, milk C18:3 content increased, as well as concentrations of C16:0, C16:1 and C18:0, while C14:0 content declined.

ESB effects : After adding ESB, contents of C18:0, C18:2 and C18:3 in diets were increased, but C16:0, C18:1, *cis*9, *trans*11-CLA, C20:0, C20:2, C22:1 and C24:0 contents were decreased (Table 4). The milk FA profile was changed with ESB addition (Table 5). Feeding ESB reduced the proportion of both SCFA and MCFA, and increased LCFA content in milk fat. Ramaswamy et al. (2001) also reported a reduction in SFA and MCFA, and an increase in LCFA contents when ESB was supplemented to the diets. Like previous studies (Donovan et al., 2000; Chen et al., 2008), feeding ESB increased percentage of USFA and decreased SFA content. Regarding human health, these changes could allow an improvement in milk FA profile since medium-chain FA and SFA have been reported to constitute the hypercholesterolemic portion of milk fat (Ney, 1991; AbuGhazaleh et al., 2002a).

No change of *trans*10, *cis*12-CLA (average of 0.05 g/100 g of total FA) was detected in ESB diets, which was similar to the result of Chen et al. (2008) and agreeded with assertions of Baumgard et al. (2000) that *tran*-10 isomers of 18-carbon FA are partially responsible for the content of milk fat and yield when fat was added to the diets.

Griinari and Bauman (1999) observed that adding plant oils to diets can substantially increase milk CLA concentration. However, Solomon et al. (2000) indicated that plant oils are not commonly used in ruminant diets because they tend to restrain rumen microbial growth. An alternative is to use full fat seeds, but these need to be processed so that a portion of their unsaturated FA becomes available in the rumen for bacterial biohydrogention (Solomon et al., 2000). The present study found that supplementation of ESB had positive effect with *cis*9, *trans*11-CLA concentration in milk fat increasing from 0.81 to 1.08 g/100 g of total FA when 10% ESB was added to the diets. The increase in *cis*9, *trans*11-CLA observed with addition of ESB was similar to the results of Ramaswamy et al. (2001) who observed that *cis*9, *trans*11-CLA content increased to 114% with ESB diet. After adding ESB in the diets, conversion from C18:2 and C18:3 to *cis*9, *trans*11-CLA seemed to be improved due to the increased of C18:2 and C18:3 with supplemented ESB. TVA concentration in milk also increased after adding ESB. Both ways to produce *cis*9, *trans*11-CLA were enhanced, which resulted in the increase of *cis*9, *trans*11-CLA proportion in milk fat.

TVA can be converted into CLA in human body (Salminen et al., 1998). Similarly to the increase of *cis*9, *trans*11-CLA content, it is also beneficial to increase TVA concentration in milk fat. Milk from cows fed 10% ESB diets contained higher TVA (3.47 g/100 g of total FA at 10% ESB) content than from those fed without ESB (1.78 g/100 g of total FA). Besides TVA, percentages of other C18:1isomers also were altered with addition of ESB. Concentration of total C18:1 increased ($p<0.0001$) after adding ESB to diets. These results were generally consistent with the report of Whitlock et al. (2002) that all C18:1 concentration increased in ESB-containing diets.

Adding ESB to diets increased percentages of C16:1, C18:0, C18:2 and C18:3 in milk fat, while content of C14:0, C16:0, C17:0 and C20:4 decreased (Table 5).

There was no forage×ESB interaction effect on FA profile of milk.

Table 6. Fatty acids compositions in plasma

CS (% DM)	Treatments									SEM	p value		
	0% ESB			5% ESB			10% ESB						
	30%	20%	10%	30%	20%	10%	30%	20%	10%		F	ESB	F×ESB
Fatty acids, (g/100 g of total fatty acids)													
C14:0	2.14^{c}	2.17^{c}	2.15^{c}	2.40^{b}	2.40^{b}	2.39^{b}	2.65^{a}	2.65^{a}	2.67^{a}	0.01	0.12	<0.0001	0.12
C14:1	0.72	0.73	0.73	0.74	0.73	0.73	0.74	0.74	0.73	0.01	0.93	0.15	0.53
C16:0	22.86^{a}	20.79^{c}	18.51^{e}	21.61^{b}	18.48^{e}	15.31^{g}	19.62^{d}	16.71^{f}	14.34^{h}	0.14	<0.0001	<0.0001	<0.0001
C16:1	0.65	0.65	0.64	0.65	0.64	0.64	0.63	0.64	0.64	0.01	0.99	0.54	0.89
C18:0	24.17^{c}	24.85^{b}	25.33^{a}	21.21^{f}	22.13^{e}	23.06^{d}	19.87^{g}	20.89^{f}	21.09^{f}	0.14	<0.0001	<0.0001	0.03
t-9C18:1	0.65	0.66	0.64	0.65	0.66	0.65	0.66	0.66	0.64	0.01	0.08	0.85	0.92
t-11C18:1	0.48^{c}	0.47^{c}	0.48^{c}	0.60^{b}	0.60^{b}	0.59^{b}	0.75^{a}	0.74^{a}	0.74^{a}	0.01	0.84	<0.0001	0.95
c-9C18:1	5.76^{h}	6.34^{g}	7.11^{e}	6.98^{f}	7.86^{d}	9.21^{b}	8.22^{c}	9.14^{b}	10.43^{a}	0.05	<0.0001	<0.0001	<0.0001
c-11C18:1	0.82	0.81	0.82	0.83	0.83	0.83	0.82	0.82	0.82	0.02	0.96	0.81	0.98
Total C18:1	7.71^{g}	8.28^{f}	9.05^{e}	9.06^{e}	9.95^{d}	11.28^{b}	10.45^{c}	11.36^{b}	12.63^{a}	0.05	<0.0001	<0.0001	<0.0001
C18:2	26.41^{c}	26.35^{c}	26.36^{c}	32.04^{b}	32.10^{b}	32.06^{b}	35.76^{a}	35.82^{a}	35.76^{a}	0.20	0.98	<0.0001	0.99
C18:3	4.70^{c}	5.44^{b}	6.34^{a}	3.50^{e}	4.71^{c}	5.52^{b}	2.20^{g}	3.04^{f}	3.89^{d}	0.08	<0.0001	<0.0001	0.06
c9 t11 CLA	0.70^{h}	0.76^{g}	0.83^{f}	0.86^{f}	0.96^{e}	1.05^{d}	1.15^{c}	1.21^{b}	1.29^{a}	0.02	<0.0001	<0.0001	0.33
C20:0	1.22	1.22	1.23	1.23	1.22	1.23	1.23	1.23	1.22	0.01	0.70	0.94	0.15
C21:0	1.82^{a}	1.80^{a}	1.83^{a}	1.22^{b}	1.21^{b}	1.22^{b}	1.02^{c}	1.02^{c}	1.03^{c}	0.04	0.84	<0.0001	0.98
C22:0	2.23^{a}	2.24^{a}	2.24^{a}	2.09^{b}	2.09^{b}	2.09^{b}	1.68^{c}	1.69^{c}	1.69^{c}	0.03	0.88	<0.0001	0.98
C23:0	3.52^{a}	3.53^{a}	3.60^{a}	2.38^{b}	2.42^{b}	2.38^{b}	2.06^{c}	2.05^{c}	2.10^{c}	0.21	0.97	<0.0001	0.98
C24:0	1.16^{a}	1.16^{a}	1.17^{a}	1.05^{b}	1.03^{b}	1.04^{b}	0.97^{c}	0.97^{c}	0.96^{c}	0.01	0.24	<0.0001	0.06

Fatty acids profile in plasma

Forage effect0s : Fatty acids compositions in plasma are shown in Table 6. When part of CS was replaced by AH the FA profile in plasma changed. The content of USFA increased and SFA concentration declined. Like the trend in milk fat, *cis*9, *trans*11-CLA in plasma increased as more AH was fed to cows. No *trans*10, *cis*12-CLA was detected in plasma. No change of TVA, *trans*-9 C18:1 and *cis*-11 C18:1 content in plasma was found, but *cis*-9 C18:1 concentration increased after CS was partially replaced by AH. Otherwise, C18:0 and C18:3 percentages in plasma increased but C16:0 content decreased as AH content increased in the diets.

ESB effects : When ESB was added to diets USFA concentration in plasma increased, while SFA content decreased**.** C14:0, *cis*9, *trans*11-CLA, TVA, *cis*-9 C18:1 and C18:2 contents in plasma increased with supplementation of ESB, whereas, C16:0, C18:0, C18:3, C21:0, C22:0, C23:0 and C24:0 concentrations in plasma decreased.

Blood metabolites

Forage effects : Concentration of metabolites in blood from cows fed different treatments is shown in Table 7. Concentrations of GLU in blood were not affected by dietary forage source, which was in accordance with previous studies (Alenander and Broderick, 1996; Dhiman and Satter, 1997). Concentration of BUN was lower when cows were fed high AH diets. No difference was observed in TG content in plasma. NEFA can be used as an energy source by many tissues, including skeletal muscle and hepatocytes. NEFA is considered a biomarker of negative energy balance (Ye et al., 2009). Blood NEFA, TP and CHOL concentration were higher when part of CS was replaced by AH. Ruppert et al. (2003) explained that increased concentrations of NEFA and cholesterol in plasma may be attributed to greater FA intakes when cows were fed

Table 7. Concentration of metabolites in blood from cows fed different treatments

CS (% DM)	Treatments									SEM	p value		
	0% ESB			5% ESB			10% ESB						
	30%	20%	10%	30%	20%	10%	30%	20%	10%		F	ESB	F×ESB
GLU[1] (mmol/L)	3.62	3.61	3.62	3.66	3.63	3.60	3.64	3.65	3.62	0.01	0.20	0.22	0.21
TP[2] (g/L)	68.03^{f}	70.28^{de}	73.37^{c}	69.51^{e}	71.67^{d}	77.98^{a}	70.12^{e}	75.55^{b}	78.65^{a}	0.50	<0.0001	<0.0001	<0.0001
BUN (mmol/L)	137.4^{a}	126.8^{c}	121.6^{d}	135.2^{ab}	125.0^{c}	113.7^{e}	134.2^{b}	118.8^{d}	112.4^{e}	1.7	<0.0001	<0.0001	0.001
NEFA (μEq/L)	172.7^{g}	245.9^{f}	345.3^{cd}	294.3^{e}	365.3^{e}	423.8^{b}	334.3^{d}	445.6^{b}	557.2^{a}	8.25	<0.0001	<0.0001	<0.0001
CHOL[3] (mmol/L)	4.37^{cd}	5.72^{b}	7.17^{a}	4.21^{d}	4.42^{c}	7.16^{a}	3.35^{e}	5.85^{b}	7.13^{a}	0.06	<0.0001	<0.0001	0.001
BHBA (mmol/L)	0.76^{a}	0.61^{c}	0.53^{d}	0.69^{b}	0.55^{d}	0.39^{f}	0.61^{c}	0.44^{e}	0.39^{f}	0.02	<0.0001	<0.0001	0.002
TG[4] (mmol/L)	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.13	0.13	0.004	0.72	0.08	0.93
LACT[5] (mmol/L)	3.11^{a}	2.89^{b}	2.85^{b}	3.01^{ab}	2.67^{c}	2.30^{d}	2.97^{ab}	2.24^{d}	1.79^{e}	0.06	<0.0001	<0.0001	<0.0001

[1] GLU = Glucose. [2] TP = Total protein. [3] CHOL = Cholesterol. [4] TG = Triglyceride. [5] LA = Lactic acid.

high alfalfa diet. Grummer and Carroll (1991) also indicated that increases in plasma NEFA could be attributed to mobilization of adipose tissue or to a decrease in NEFA clearance by the tissues or both. Lactic acid and BHBA contents declined after replacing CS by AH.

ESB effects : Adding ESB could alter fat mobilization and deposition in animal tissues. Concentrations of GLU and TG in blood were unchanged when AH was included in the diets. Grummer and Carroll (1991) indicated that supplemented fat could spare glucose concentration; however, spared glucose could be utilized for lactose synthesis and milk production (Abdelqader et al., 2009). BUN, CHOL and BHBA contents decreased with supplementation of ESB, while concentration of NEFA and TP increased linearly with increased ESB addition. Abdelqader et al. (2009) indicated that the plasma NEFA concentrations were linearly increased as the concentration of fat increased in the diets and cows were in a neutral or positive energy balance. Therefore, this increase in plasma NEFA is more likely to be a result of inefficient uptake of fatty acids by peripheral tissue as has been proposed by Grummer (1993). Ye et al. (2009) showed that the higher NEFA in cows supplemented with fat may be associated with their greater nutrient demands for milking.

CONCLUSIONS

Replacing CS with AH and adding ESB improved production performance of cows. Increasing AH percentages in diets, milk yield, milk protein content and yield, and *cis*9, *trans*11-CLA concentration in milk fat and plasma increased. Supplementation of ESB to diet resulted in higher milk yield and *cis*9, *trans*11-CLA concentration in milk fat and plasma. Milk yield and *cis*9, *trans*11-CLA concentration were highest among the treatments when 30% AH and 10% ESB was concluded in diets. It is suggested from the results that partially replacing CS with AH and adding ESB to diets of cows could improve production performance of cows and milk compositions.

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