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Effects of Feeding Extruded Soybean, Ground Canola Seed and Whole Cottonseed on Ruminal Fermentation, Performance and Milk Fatty Acid Profile in Early Lactation Dairy Cows

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ABSTRACT : Four ruminally cannulated Holstein cows averaging 43 days in milk (DIM) were used in a 4×4 Latin square to determine the effect of feeding extruded soybean, ground canola seed and whole cottonseed on ruminal fermentation and milk fatty acid profile. One hundred and twenty lactating Holstein cows, 58 (±31) DIM, were assigned to four treatments in a completely randomized block design to study the effects of the three types of oilseeds on production parameters and milk fatty acid profile. The four diets were a control diet (CON) and three diets in which 10% extruded soybean (ESB), 5% ground canola seed (GCS) and 10% whole cottonseed (WCS) were included, respectively. Diets consisted of concentrate mix, corn silage and Chinese wild rye and were balanced to similar concentrations of CP, NDF and ADF. Ruminal fermentation results showed that ruminal fermentation parameters, dry matter intake and milk yield were not significantly affected by treatments. However, compared with the control, feeding cows with the three oilseed diets reduced C14:0 and C16:0 and elevated C18:0 and C18:1 concentrations in milk, and feeding ESB increased C18:2 and cis9, trans11 conjugated linoleic acid (CLA). Production results showed that feeding ESB tended to increase actual milk yield (30.85 kg/d vs. 29.29 kg/d) and significantly decreased milk fat percentage (3.53% vs. 4.06%) compared with CON. Milk protein (3.41%) and solid non-fat (13.27%) from cows fed WCS were significantly higher than from cows fed CON (3.24% and 12.63%, respectively). Milk urea N concentrations from cows fed the ESB (164.12 mg/L) and GCS (169.91 mg/L) were higher than cows fed CON (132.31 mg/L). However, intake of DM, 4% fat corrected milk, energy corrected milk, milk fat and protein yields, milk lactose percentage and yield, somatic cell count and body condition score were not affected by different treatments. The proportion of medium-chain fatty acid with 14 to 16 C units in milk was greatly decreased in cows fed ESB, GCS and WCS. Feeding ESB increased the concentration in milk of C18:1, C18:2, C18:3 and cis9, trans11-CLA content by 16.67%, 37.36%, 95.24%, 72.22%, respectively, feeding GCS improved C18:0 and C18:1 by 17.41% and 33.28%, respectively, and feeding WCS increased C18:0 by 31.01% compared with feeding CON. Both ruminal fermentation and production trial results indicated that supplementation of extruded soybean, ground canola seed and whole cottonseed could elevate the desirable poly- and monounsaturated fatty acid and decrease the medium chain fatty acid and saturated fatty acid content of milk fat without negative effects on ruminal fermentation and lactation performance. (**Key Words :** Extruded Soybean, Ground Canola Seed, Whole Cottonseed, Ruminal Fermentation, Milk Fatty Acid, Dairy Cows)

INTRODUCTION

High producing cows, especially in early lactation (the first 100 days after calving), are typically in negative energy balance. Oilseeds, such as extruded soybean, ground canola seed and whole cottonseed, are commonly used as feed ingredients for dairy cows with additional non-starch energy (Chilliard, 1993). These oilseeds are readily available in most areas of China, but they are less used in Chinese dairy production. The main feeds for most dairy farms in China are grain, crop by-products and limited amounts of natural forage. The efficiency of feed energy utilization is low, resulting in the production of low milk volumes with low fat and protein content and a high morbidity from rumen acidosis and other nutritional metabolic diseases (Xu, 2006).

The advantages of inclusion of these oilseeds in dairy rations have been well documented (Hermansen, 1995; Dhiman et al., 1999). The oil in the seed is believed to be slowly released when the seed is masticated, which may help decrease detrimental effects on rumen fermentation

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Table 1. Ingredient and nutrient composition of experimental diets[1]

Component	Experimental treatments			
	CON	ESB	GCS	WCS
	---------- % DM basis ----------			
Corn silage	22.79	22.81	22.78	22.74
Chinese wild rye	21.81	21.83	21.78	17.09
Corn, ground shelled	28.44	23.20	22.60	24.80
Soybean meal	10.72	6.71	11.50	11.10
Bran	1.99	1.77	1.70	2.42
Cottonseed meal	2.85	4.39	6.50	2.13
Soybean, extruded	-	10.53	-	-
Canola seed, ground	-	-	5.00	-
Whole cottonseed	-	-	-	10.16
DDGS	5.19	4.62	4.00	5.42
Maize protein powder	2.07	-	-	-
Premix[2]	0.82	0.82	0.82	0.82
$NaHCO_3$	0.58	0.58	0.58	0.58
Calcium hydrogen phosphate	1.20	1.20	1.20	1.20
Limestone	0.58	0.58	0.58	0.58
Salt	0.58	0.58	0.58	0.58
"XP" Yeast culture[3]	0.38	0.38	0.38	0.38
Composition				
NE_L[4] (Mcal/kg of DM)	1.64	1.70	1.70	1.71
OM (% of DM)	95.59	95.36	95.06	95.49
CP (% of DM)	16.21	16.47	16.21	16.13
NDF (% of DM)	36.49	36.52	36.77	37.81
ADF (% of DM)	21.26	21.54	21.34	23.52
Ether extract (% of DM)	3.43	5.86	5.95	6.07

[1] CON = Control, ESB = Extruded soybean, GCS = Ground canola seed, WCS = Whole cottonseed.
[2] Contained 100 mg/kg of I_2; 3,000 mg/kg of Fe; 2,000 mg/kg of Cu; 2,500 mg/kg of Mn; 8,000 mg/kg of Zn; 60 mg/kg of Se. 20 mg/kg of Co; 950,000 IU/kg of vitamin A; 400,000 IU/kg of vitamin D; 7,500 IU/kg of vitamin E.
[3] Bought from Diamond V Mills, Inc.
[4] Estimated using NE_L values for feedstuffs from NRC (2001).

(Harfoot and Hazelwood, 1988) and increase the efficiency of milk fat synthesis (Schauff and Clark, 1992; Palmquist et al., 1993). Milk fatty acid (FA) profile can be modified to increase the proportion of monounsaturated and polyunsaturated fatty acids (PUFA) and conjugated linoleic acids (CLA) and decrease the proportion of medium-chain FA by supplying oilseed to dairy cows (Whitlock et al., 2002; Chichlowski et al., 2005; Reveneau et al., 2005). A wide range of positive health effects has been demonstrated for PUFA and CLA by lowering insulin resistance associated with cardiovascular disease (Kennelly, 1996; Aminot-Gilchrist and Anderson, 2004). Thus, increasing the concentration of PUFA and CLA in milk may be beneficial to public health and enhance the consumption of dairy products.

However, data on the relationship between addition of these oilseeds and performance of lactating dairy cows are inconsistent because the impact is influenced by the source of oilseed, feeding condition, and the stage of lactation (Palmquist and Jenkins, 1980). For these reasons, the objective of the present study was to evaluate and compare the influence of extruded soybean, ground canola seed and whole cottonseed on rumen fermentation characteristics, feed intake, milk yield, milk composition and milk fatty acid profile in early lactation dairy cows which were fed dry Chinese wild rye and corn silage as the forage resource in China, and to investigate the relationships among these indices.

MATERIALS AND METHODS

Preparation of oilseeds

Extrusion of full fat soybean was performed at 150°C at a rate of 1.0 tonne/h using a multi-purpose twin-screw extrusion system (MUYANG TPH135 Series High Efficient Extruder) at the feed manufacturing workshop in the Ministry of Agriculture Feed Industry Center and contained 36% CP and 19.5% ether extract (EE). Canola seed was obtained from Hubei canola production base and contained 19.5% CP and 40.5% EE, and levels of glucosinolate and erucic acid were acceptably low. Linted whole cottonseed contained 25% CP and 19% EE and was purchased locally

from Beijing.

Experimental diets

Dietary treatments consisted of a control diet without any oilseed added (CON), an extruded soybean diet (ESB), a ground canola seed diet (GCS) and a whole cottonseed diet (WCS). The ESB and WCS diets contained approximately 10% extruded soybean and whole cottonseed respectively, and the GCS diet contained 5% ground canola seed (dry matter, DM basis) to maintain a similar EE level. The four experimental diets consisted of a 55:45 concentrate:forage ratio (DM basis). All diets were formulated to meet nutrient requirements of early lactating dairy cows (NRC, 2001). The CON diet was balanced with maize protein powder to contain approximately 16% CP in the dietary DM. The forage part of the diets consisted of 51% corn silage and 49% dry Chinese wild rye (DM basis), except that WCS contained less dry Chinese wild rye to produce a similar NDF and ADF level to the other diets. The CON diet was estimated at 1.64 Mcal NE_L/kg using NRC (2001) equations, whereas the other three diets containing supplemental oilseed had a higher estimated NE_L concentration (1.70, 1.70, 1.71 vs. 1.64 Mcal/kg). The formulation and proximate analyses of the four diets are shown in Table 1.

Ruminal fermentation trial

Four ruminally fistulated cows averaging 43 (±23) days in milk (DIM) were used for one of the four treatment groups in a 4×4 Latin square design with four 3-wk periods. The first 18 d of each period were for adaptation to diets and the last 3 d were for sample collection. Cows were housed in open stalls and had free access to water. Animal care and use of all animals in the ruminal fermentation trial and the production trial were conducted under the approval of the China Agricultural University Animal Science and Technology College Animal Care and Use Committee.

Different experimental diets were supplied as a total mixed ration (TMR) for *ad libitum* intake three times daily and amounts fed and refused were recorded. Cows were milked three times daily, with individual milk weights recorded. Milk was sampled from each cow daily during the collection period and stored at -20°C for analysis of fatty acids.

Samples of ruminal digesta were collected from the rumen of each cow on sampling days for each period prior to morning feeding (0 h) and at 2 h intervals for 24 h daily. Following immediate determination of pH using a portable pH meter (pH/temp meter 199 Model No 3D; Fisher Scientific, Pittsburgh, PA), a portion of the ruminal fluid was collected to measure ammonia N (NH_3-N) concentration as described by Broderick and Kang (1980) using a spectrophotometer (UV-VIS 8500, Shanghai Tianmei Scientific Instrument Co., China). The remaining portion of the supernatant was acidified with 25% metaphosphoric acid (wt/vol) and then frozen at -20°C for analysis of volatile fatty acids (VFA, Erwin et al., 1961). Concentrations of VFA were determined using gas chromatography (6890 N, Agilent technologies) equipped with a 30 m HP-INNOWax 19091N-213 (Agilent) capillary column (0.32 mm i.d. and 0.50 mm film thickness). The chromatograph oven was programmed as follows: 120°C for 3 min, 10°C/min increment to 180°C, and then held for 1 min. The injector and detector were maintained at 220°C and 250°C respectively. Nitrogen was used as carrier gas (flow rate 2.0 ml/min).

Production trial

One hundred and twenty lactating Holstein cows (34 primiparous cows and 86 multiparous cows), 58 (±31) DIM, were used in the production trial. Cows were housed in four free-stall barns at Beijing Qin Feng Xiong Te dairy farm. Normal herd management and surgical practices were followed during the experiment. The cows were fed the control diet for a 2 wk adaptation period, which was used as a covariate period. Then cows were assigned, based on their parity, DIM, and milk yield during the covariate period, to 4 groups in a completely randomized block design prior to random allocation to the experimental diets. The cows were fed the experimental diets for 12 wk after the adaptation period.

Diets were fed as a total mixed ration (TMR) for *ad libitum* intake in a feeding trough three times daily at 07:00, 14:00 and 21:00 h to allow for between 5 and 10% refusal, and all the cows were tied when feeding. Fifteen cows of each group were fed individually, and the amount of TMR offered and refused was recorded daily to measure individual intake. All refused TMR was moved after feeding for all treatments, after that the cows were free to move in the barns.

The DM content of corn silage was measured weekly, and the amount of forage fed was adjusted to maintain a 55:45 concentrate to forage ratio. Samples of TMR, feed refusals, corn silage, dry Chinese wild rye and concentrate were collected weekly and combined into monthly samples for testing. Weekly feed samples were dried at 60°C in a forced air oven for 48 h, and then were ground through a 1 mm screen of a standard Wiley mill (model 3; Arthur H. Thomas Co., Philadelphia, PA). All feeds were analyzed for OM, CP and EE according to the methods of AOAC (1997). NDF and ADF were determined using the modified filter bag method of Van Soest et al. (1991). Subsamples of feed composites were dried in a forced air oven at 105°C for 24 h to determine the absolute DM, and chemical analyses

Table 2. Fatty acid composition of experimental diets, forage, extruded soybean, ground canola seed and whole cottonseed

Composition	Experimental treatments				Corn silage	Chinese wild rye	Extruded soybean	Canola seed	Cotton seed
	CON	ESB	GCS	WCS					
Fatty acid (g/100 g of fatty acids)									
C14:0	0.56	0.40	0.36	0.59	0.98	4.56	0.15	0.09	0.68
C14:1	0.01	0.01	0.01	0.01	0.00	0.25	0.00	0.00	0.00
C16:0	17.69	15.20	11.16	20.30	23.30	25.98	11.97	3.66	23.70
C16:1	0.30	0.22	0.35	0.44	0.62	1.50	0.09	0.38	0.61
C18:0	3.40	4.27	2.89	2.93	4.29	5.99	5.18	2.15	2.38
C18:1	21.93	20.30	38.55	19.01	9.49	11.98	18.91	57.76	15.59
C18:2	44.30	47.26	31.84	49.69	27.24	21.48	51.52	18.82	55.77
C18:3	7.95	9.66	11.38	4.50	24.40	11.83	10.96	14.36	0.21
c9,t11-CLA	0.07	0.06	0.07	0.06	0.19	0.39	0.03	0.03	0.04
t10,c12-CLA	0.00	0.01	0.00	0.02	0.00	0.00	0.01	0.00	0.05
Others[1]	3.79	2.61	3.39	2.45	9.49	16.04	1.18	2.75	0.97
Medium[2]	18.56	15.83	11.88	21.34	24.90	32.29	12.21	4.13	24.99
Long[3]	81.44	84.17	88.12	78.66	75.10	67.71	87.79	95.87	75.01
Unsaturated	76.40	78.58	84.47	74.87	68.14	53.6	81.72	93.94	72.63
Saturated	23.60	21.42	15.53	25.13	31.86	46.4	18.28	6.06	27.37

[1] Other fatty acids: (C20:0 to C22:6). [2] Medium-chain fatty acid: (C14:0 to C16:1). [3] Long-chain fatty acid: (≥C18:0).

were expressed on this final DM determination.

Cows were milked three times daily at 06:30, 13:30 and 20:30 h, with individual milk weights recorded at each milking. Composite milk samples were obtained from 2 consecutive days milking before the start of the trial and every 2 wk for the duration of the experiment. The 24 h composite of each cow's milk was split into two portions for analysis. One portion was refrigerated at 4°C and sent to Beijing Dairy Cattle Center laboratory for analyses of fat, protein, lactose and solid non-fat (SNF) by near-infrared spectroscopy (Foss MikoScan 4000; Foss Technology, Eden Prairie, MN) and somatic cell count (SCC) by fluorescence (Fossomatic 5000; Foss Technology). Fat corrected milk (FCM) and energy corrected milk (ECM) were determined using the NRC (2001) equation: 4% FCM = 0.4×kg milk+15.0×kg fat and ECM = (0.3246×kg milk)+(12.86×kg fat)+(7.04×kg protein), respectively. Milk urea N (MUN) was determined on defatted milk samples using a colorimetric diagnostic kit (Sigma Diagnostics, St. Louis, MO, procedure 535) by Crocker (1967). The remaining portion was stored at -20°C until analysis of fatty acids. Body condition score (BCS, Wildman et al., 1982) was recorded at the beginning of the trial and every 4 wk for the experiment.

Fatty acids of extruded soybean, canola seed, whole cottonseed, forage and experimental diets were extracted and methylated by the one-step procedure (Sukhija and Palmquist, 1988) using hexane instead of benzene. Lipids from the milk were extracted with chloroform/methanol (2:1, v/v) according to the method described by Lin et al. (2003). Fatty acids were quantified by incorporating internal standard, heptadecanoic (C17:0) acid methyl ester (Sigma, St Louis, MO), into each sample. The fatty acids were methylated with $NaOCH_3$/methanol at 50°C for 30 min and HCl/methanol at 60°C for 1 h according to the method of Magdi (2001). The methylated sample was then thoroughly mixed with 1 ml distilled water and 2 ml hexane, and the hexane extract was used for fatty acid analysis.

The fatty acid methyl esters (FAME) of feed and milk samples were separated by gas-liquid chromatography (GLC, 6890 N, Agilent technologies) on a 60 m Supelco SP 2560 (Supelco, Bellefonte, PA) capillary column (0.25 mm i.d. and 0.20 mm film thickness) with a split ratio of 20:1 using helium as carrier gas. Column oven initial temperature was 180°C (held for 10 min), increased by 4°C per min and then was held at 220°C for 15 min. The injector and detector temperature were maintained at 250°C. Peaks of CLA isomers and other PUFA were identified by comparison of retention times with standards (Sigma, St Louis, MO).

Statistical analysis

Data of dry matter intake (DMI), milk yield and milk fatty acid profile in the ruminal fermentation trial were analyzed as a 4×4 Latin square using PROC MIXED of SAS (1999) according to the following model: $Y_{ijkl} = \mu+T_i+P_j+C_k+e_{ijkl}$, where: Y_{ijkl} = observation; μ = population mean; T_i = treatment (i = 1, 2, 3, or 4); P_j = period (j = 1, 2, 3, or 4), C_k = random effect of cow (k = 1, 2, 3, or 4), $C_{k\sim N}$ (0, σ^2_{cow}), and e_{ijkl} = residual error. Rumen fermentation parameters were analyzed over time using the model described previously but with the addition of repeated measurements within cows by periods.

Data were analyzed as a randomized complete block

Table 3. Ruminal fermentation characteristics in dairy cows fed control (CON), extruded soybeans (ESB), ground canola seed (GCS) and whole cottonseed (WCS) diets[1]

Item	Experimental treatments				SEM	p value
	CON	ESB	GCS	WCS		
pH	6.45	6.46	6.38	6.47	0.07	0.23
NH_3-N (mg/dl)	9.93	10.52	10.47	9.56	0.19	0.10
Total VFA (mM)	154.19	136.60	128.59	131.74	5.71	0.09
VFA (g/100 g VFA)						
Acetic acid (A)	67.25	65.83	66.75	66.82	1.04	0.31
Propionic acid (P)	19.59	21.08	20.55	20.86	0.72	0.59
Butyric acid	1.56[a]	0.98[b]	0.91[b]	0.83[b]	0.12	0.04
Isobutyric acid	10.23[a]	9.76[ab]	9.51[b]	9.43[b]	0.17	0.04
Valeric acid	1.13	1.35	1.39	1.24	0.06	0.31
Isovaleric acid	0.88	1.00	0.89	0.82	0.06	0.15
A:P	3.42	3.13	3.24	3.21	0.22	0.19

[a, b] Means within a row with different superscripts differ ($p<0.05$).

design using the PROC MIXED of SAS (1999) in which cows were blocked by week of entry to the production trial. Corresponding data from the preliminary week were included as a covariate for each variable. The model used was: $Y_{ijk} = \mu+D+T_i+P_j+TP_{ij}+W_k+PW_{jk}+TW_{ik}+TPW_{ijk}+b_l+C_{ijm}+B_j(X_{ijm}-X_j)+E_{ijk}$, where Y_{ijkl} = observation; μ = population mean; D = day in milk; T_i = treatment; P_j = parity; W_k = week and its respective interactions TP_{ij}, PW_{jk}, TW_{ik} and TPW_{ijk}; b_l = random effect of block l ~ N (0, σ^2_b); C_{ijm} = random effect of cow m within treatment i and parity j ~ N (0, σ^2_c); X_{ijm} = covariate measurement for cow m within treatment i and parity j; X_j = mean covariate for parity j; and E_{ijk} = residual error.

Significance was declared at $p<0.05$ and trend was ascribed at $0.05<p\leq0.10$.

RESULTS AND DISCUSSION

Fatty acid composition of experimental diets, forage, extruded soybean, ground canola seed and whole cottonseed

Fatty acid composition of experimental diets is presented in Table 2. C18:2 fatty acid was numerically higher in both ESB and WCS than in CON and GCS. The GCS diet contained more C18:1 and less C18:2 fatty acid than the other three diets.

Ruminal fermentation trial

Rumen fermentation parameters : There was no interaction between sampling time and treatment for any measurement of ruminal fermentation characteristics ($p<0.05$). Therefore, only dietary effects are presented (Table 3). The ruminal pH values observed for the four treatments were similar and within an acceptable range to maintain a healthy rumen environment (NRC, 2001). ESB and GCS tended to increase the ruminal NH_3-N level ($p = 0.10$). However, Chen et al. (2002) reported that extruded soybean meal increased dietary undegradable protein, decreased the NH_3-N in rumen, and Chichlowski et al. (2005) found a GCS diet tended to decrease the ruminal NH_3-N. In this study, it is likely that the oil in extruded soybean and canola seed was associated with negative effects on rumen microbial growth or the extensive degradation of GCS in the rumen.

There was a tendency for total VFA to be decreased by feeding the three oilseeds diets ($p<0.10$). This was consistent with other studies where oilseed supplementation in the form of extruded soybean (Madison-Anderson et al., 1997), canola seed (Chichlowski et al., 2005) or whole cottonseed (Reveneau et al., 2005), resulted in a decrease in total VFA concentration. Ruminal fluid from cows fed GCS, WCS and ESB had lower proportions of butyric acid and isobutyric acid compared with cows fed CON ($p<0.05$). The addition of dietary fat partly replaced the nonstructural carbohydrate in the diet. As a result, there was less fermentable carbohydrate available for VFA production, which probably caused a decrease in total VFA, butyric acid and isobutyric acid (Schauff and Clark, 1992) or acetic:propionic acid ratio (Oldick and Firkins, 2000).

Reveneau et al. (2005) reported that the amount of free oil in ESB, GCS or WCS was not large enough for the lipolysis rate to build up sufficient concentrations of free FA to alter microbial processes within the rumen.

DMI, milk yield and milk fatty acids profile : DMI and milk yield were not significantly different among treatments ($p>0.05$, Table 4). Compared with CON, feeding different oilseed diets reduced C14 to C16 fatty acids in milk (Table 3, $p<0.05$). This result agreed with other reports (Dhiman et al., 1999; Delbecchi et al., 2001; Whitlock et al., 2002; Chichlowski et al., 2005). Medium-chain fatty acids are mainly synthesized *de novo* in the epithelial cells of the mammary gland of the dairy cow, and their synthesis is susceptible to inhibition when dietary levels of certain long-chain fatty acids are increased (Palmquist et al., 1993).

Table 4. DMI, milk yield and milk fatty acid profile in ruminal fermentation trial

Item	Experimental treatments				SEM	p value
	CON	ESB	GCS	WCS		
DMI (kg/d)	18.29	17.94	17.79	18.35	0.47	0.35
Milk yield (kg/d)	23.45	24.51	22.85	23.10	0.35	0.26
Fatty acid (g/100 g of fatty acids)						
C14:0	14.14[a]	11.05[c]	12.65[b]	10.73[c]	0.23	0.01
C14:1	0.96	0.70	0.96	0.79	0.08	0.39
C16:0	36.43[a]	28.56[b]	26.84[b]	29.53[b]	0.58	0.01
C16:1	1.53	1.19	1.57	1.41	0.17	0.91
C18:0	13.85[b]	17.00[a]	18.97[a]	18.85[a]	0.61	0.04
C18:1	28.07[b]	35.44[a]	32.72[a]	33.72[a]	0.93	0.04
C18:2	2.67[b]	3.20[a]	2.82[b]	2.73[b]	0.17	0.01
C18:3	0.52	0.56	0.58	0.53	0.04	0.09
c9,t11-CLA	0.79[b]	1.36[a]	0.95[b]	0.81[b]	0.04	0.01
t10,c12-CLA	0.01	0.02	0.01	0.01	0.002	0.20
Others[1]	1.03	0.92	1.93	0.89	0.02	0.22
Medium[2]	53.06[a]	41.52[b]	42.02[b]	42.46[b]	1.03	<0.001
Long[3]	46.94[b]	58.48[a]	57.98[a]	57.54[a]	1.03	<0.001
Unsaturated	34.96[b]	42.86[a]	40.64[a]	40.35[a]	0.74	0.01
Saturated	65.04[a]	57.14[b]	59.36[b]	59.65[b]	0.74	0.01

[a, b] Means within a row with different superscripts differ ($p<0.05$).

[1] Other fatty acids: (C20:0 to C22:6). [2] Medium-chain fatty acid: (C14:0 to C16:1). [3] Long-chain fatty acid (≥C18:0).

The concentrations of C18:0 and C18:1 in milk fat from cows fed ESB, GCS and WCS were higher than that from cows fed CON ($p<0.05$). Both ESB and WCS diets that are rich in C18:2 fatty acids have been shown to increase the C18:0 and C18:1 fatty acid content of milk through rumen biohydrogenation (Dhiman et al., 1997). On the other hand, the average increase in the proportion of C18:1 in the GCS treatment can be attributed to higher concentration of C18:1 in canola seed.

The proportions of C18:2 and cis9, trans11-CLA in the milk of cows fed ESB were higher than those in the milk of cows from all other treatments ($p<0.05$). Previous work, such as Dhiman et al. (1999), Solomon et al. (2000) and Abu-Ghazaleh et al. (2002), observed a similar result. This could have been due to the slightly higher fatty acid content in the ESB or differences in the availability of fatty acids in the rumen. Both canola seed and whole cottonseed were rich in C18:2 and C18:3 fatty acids which may serve as precursors for cis9,trans11-CLA by partial ruminal biohydrogenation (Griinari et al., 1999). However, in this experiment, neither GCS nor WCS treatment influenced the C18:2, C18:3 or cis9,trans11-CLA percentage in milk relative to CON ($p>0.05$). Previous studies, such as Chichlowski et al. (2005) which used GCS and Sullivan et al. (2004) which used WCS, also found similar results. This probably occured because dietary oil in the form of intact seeds does not change milk CLA content and the hydrolysis and hydrogenation of C18:2 or C18:3 in GCS and WCS by ruminal microorganisms (Murphy et al., 1990; Dhiman et al., 1997). Alternatively, it is more likely that oil released from ground raw canola seed and whole cottonseed is not as readily available as oil from the process of extrusion, thereby affecting subsequent availability of the oil to ruminal microbes (Chichlowski et al., 2005). Dhiman et al. (1999) found that cows fed with the extruded cottonseed diet had a higher C18:2 and CLA content in milk fat than the control. The increase in milk PUFA suggests a portion of the oil escaped ruminal metabolism and saturation. Compared with cows feeding on the CON diet, ESB, GCS and WCS treatments decreased medium-chain and saturated fatty acids in milk fat and increased long-chain and unsaturated fatty acids in milk fat significantly ($p<0.05$).

Production trial

DMI, milk yield and milk composition : Daily individual DMI of cows in the production trial were not affected by the supplementation of ESB, GCS and WCS ($p>0.05$, Table 5). These results were consistent with previous reports from Abu-Ghazaleh et al. (2002) and Lee et al. (2006) who used extruded soybean, Chichlowski et al. (2005) who used ground canola seed and Reveneau et al. (2005) who used whole cottonseed. Milk yield and composition was affected by treatment diets ($p<0.05$). Cows fed ESB had a trend to increased milk yield compared with those fed CON, GCS and WCS ($p<0.10$). This result was similar to other studies (Dhiman et al., 1999; Solomon et al., 2000; Whitlock et al., 2002). Considering that DMI was not affected by the experimental diets, the increased milk yield observed as a consequence of ESB treatment could be attributed to increasing the ruminally undegraded protein (RUP), elevating protein in the small intestine and more energy intake (Block et al., 1981). Adequate supplement of available protein and essential amino acids to the small intestine of lactating dairy cows is essential for maximum

Table 5. DMI, milk yield, BCS and milk composition from cows fed different diets in production trial

Item	Experimental treatments				SEM	p value
	CON	ESB	GCS	WCS		
DMI (kg/d)	20.96	21.48	20.50	20.43	0.67	0.86
Milk yield						
Actual milk yield (kg/d)	29.29	30.85	29.97	29.84	0.55	0.09
4% FCM[1] (kg/d)	30.81	29.12	29.48	30.79	1.31	0.30
ECM[2] (kg/d)	32.51	31.73	31.59	32.48	0.79	0.44
Milk efficiency (4% FCM/kg of DMI)	1.49	1.42	1.46	1.50	0.05	0.15
BCS[3]	2.57	2.70	2.65	2.67	0.06	0.16
Milk composition						
Fat (%)	4.06[a]	3.53[b]	4.11[a]	4.26[a]	0.18	0.04
Fat yield (kg/d)	1.21	1.13	1.24	1.27	0.06	0.13
Protein (%)	3.24[b]	3.23[b]	3.28[b]	3.41[a]	0.03	0.04
Protein yield (kg/d)	0.96	1.01	0.99	1.02	0.05	0.23
Fat:protein	1.26	1.11	1.27	1.25	0.06	0.13
Lactose (%)	4.71	4.78	4.79	4.73	0.03	0.42
Lactose yield (kg/d)	1.40	1.49	1.45	1.43	0.06	0.56
SNF (%)	12.63[b]	12.34[b]	12.81[ab]	13.27[a]	0.13	0.04
SNF yield (kg/d)	3.74	3.82	3.84	3.97	0.18	0.28
SCC ($\times 10^3$ cfu/ml)	389.49	205.32	226.85	265.63	58.71	0.49
MUN (mg/L)	132.31[b]	164.12[a]	169.91[a]	134.56[b]	6.72	0.04

[a, b] Means within a row with different superscripts differ ($p<0.05$).

[1] 4% FCM (Fat corrected milk) = 0.4×kg milk+15.0×kg fat.

[2] ECM (Energy corrected milk) = (0.3246×kg milk)+(12.86×kg fat)+(7.04×kg protein).

[3] Scored on a five-point scale where 1 = emaciated to 5 = overly fat.

milk yield (NRC, 2001). However, GCS and WCS showed no significant effect on milk yield in the present study ($p>0.05$).

Milk fat percentage was decreased in cows fed the ESB diet than those fed the GCS and WCS diets ($p<0.05$). The same effect was observed in previous studies (Abu-Ghazaleh et al., 2002; Whitlock et al., 2002). Both GCS and WCS had no negative effect on the milk fat percentage. Aldrich et al. (1997) reported that feeding lactating dairy cow diets supplemented with canola (11.2% of DM) maintained or increased milk fat percentage. Reveneau et al. (2005) observed there was no difference in milk fat percentage between cows fed WCS or control diets. Milk fat contains fatty acids derived from mammary uptake of preformed fatty acids (Palmquist et al., 1993). Ruminally protected fats tend to increase milk fat percentage while fats that are susceptible to ruminal biohydrogenation may reduce milk fat percentage (Kennelly, 1996). One possible explanation of the reduced milk fat percentage in this study for cows fed ESB may be that extruded soybean was not well protected from microbial biohydrogenation in the rumen, or it could be the result of dilution as milk yield increased in cows fed ESB. Furthermore, Block et al. (1981) reported that heating oil produced reducing agents that were capable of capturing hydrogen ions and possibly inhibited methanogenesis in the rumen. Reduced methanogenesis spares other hydrogen ions for the production of propionic acid, which leads to milk fat depression.

Milk protein percentage often decreases when supplemental fat is fed to lactating dairy cows (Wu and Huber, 1994; Delbecchi et al., 2001), but in this study we observed that increased milk production was not accompanied by a lower milk protein percentage in cows fed ESB. It was possible that ESB provided either more rumen undegradable protein, or a better balance of amino acids for milk protein synthesis (Block et al., 1981). The WCS diet significantly increased milk protein and SNF percentage compared with ESB, GCS and CON ($p<0.05$). Dhiman et al. (1999) and Mabjeesh et al. (2000) also reported that milk protein percentage increased when WCS was fed to lactating cows. Harvatine and Firkins (1997) found that microbial protein synthesis was improved when cows were fed with WCS, resulting in an increased protein flow to the small intestine. Consequently, there was an improved flow of amino acids to the mammary gland and a high milk protein content. This indicates that the seed coat of WCS offers better protection from ruminal degradation than with ESB and GCS. However, when corrected for total fat yield and total protein yield of the milk, all the differences were negligible ($p>0.05$).

Because of the drop in milk fat percentages or milk protein percentage and increased actual milk yields with ESB, yields of 4% FCM and ECM were similar for all treatments ($p>0.05$). There was no significant effect on milk efficiency, milk lactose percentage, milk yield and BCS of

Table 6. Fatty acid composition of milk fat for dairy cows fed different diets

Item	Experimental treatments				SEM	p value
	CON	ESB	GCS	WCS		
Fatty acid (g/100 g of fatty acids)						
C14:0	15.68[a]	13.76[b]	12.91[b]	13.21[b]	0.49	0.01
C14:1	1.42	1.29	1.52	1.16	0.06	0.13
C16:0	38.17[a]	32.13[b]	27.20[b]	33.46[b]	1.65	0.01
C16:1	1.39	1.34	1.47	1.32	0.18	0.67
C18:0	13.06[b]	15.14[ab]	17.41[a]	17.11[a]	0.46	0.03
C18:1	26.15[c]	30.51[ab]	33.28[a]	28.79[bc]	1.23	0.02
C18:2	2.65[b]	3.64[a]	3.01[b]	3.15[b]	0.14	0.04
C18:3	0.21[b]	0.41[a]	0.29[b]	0.24[b]	0.01	0.01
c9,t11-CLA	0.54[b]	0.93[a]	0.53[b]	0.51[b]	0.04	<0.001
t10,c12-CLA	0.01	0.01	0.01	0.01	0.001	0.12
Others[1]	0.72[b]	0.84[b]	2.37[b]	1.04[b]	0.04	<0.001
Medium[2]	56.66[a]	48.52[b]	43.10[b]	49.15[b]	1.49	<0.001
Long[3]	43.34[b]	51.48[a]	56.90[a]	50.85[a]	1.49	<0.001
Unsaturated	32.81[b]	38.62[a]	41.23[a]	35.79[ab]	1.28	0.02
Saturated	67.19[a]	61.38[b]	58.77[b]	64.21[ab]	1.28	0.02

[a, b, c] Means within a row with different superscripts differ ($p<0.05$).

[1] Other fatty acids: (C20:0 to C22:6). [2] Medium-chain fatty acid: (C14:0 to C16:1). [3] Long-chain fatty acid: (≥C18:0).

cows fed different diets ($p>0.05$).

For cows fed diets containing ESB and GCS the levels of MUN increased significantly ($p<0.05$). This response agreed with the observation of Johnson et al. (2002), who reported an increase in the level of MUN when cows were fed with oilseed. This was probably the result of high ruminal NH_3-N concentration and increased nitrogen absorption across the rumen wall when cows fed with ESB and GCS.

Fatty acid composition of milk : Changes in fatty acid composition of milk are presented in Table 6. Diets with the three types of oilseeds decreased the proportion of C14:0 and C16:0 fatty acids in milk ($p<0.05$). This result was similar to the previous ruminal fermentation trial. In terms of human health, these alterations may represent an improvement in the FA profile of milk because medium-chain FA have been reported to constitute the hypercholesterolemic portion of milk fat (Ney, 1991).

The concentration of C18:0 in milk fat from cows fed GCS and WCS was significantly higher (33.31% and 31.01%, respectively) than that from cows fed CON ($p<0.05$). The proportion of C18:1 in milk fat was increased significantly by 16.67% and 27.27% ($p<0.05$) with ESB and GCS treatments, respectively. The proportions of C18:2, C18:3 and cis9,trans11-CLA were higher in the milk of cows fed ESB than those in the milk of cows fed CON, GCS and WCS ($p<0.05$). The effects of different diets on fatty acid profile in milk fat were consistent with the results from the ruminal fermentation trial results and other research (DePeters et al., 1985; Sullivan et al., 2004; Chichlowski et al., 2005).

Feeding cows with ESB, GCS and WCS significantly increased the long-chain and unsaturated fatty acids and decreased the medium-chain and saturated fatty acid concentrations in milk fat ($p<0.05$) compared with cows on the CON diet. Fatty acids such as C18:0 are considered to be neutral in their effects upon human cholesterol levels while C18:1 and other PUFA are considered to be beneficial in lowering blood cholesterol in humans (Kennelly, 1996). The change of milk fatty acid profile would improve the quality of milk and be beneficial to human health (Ney, 1991).

CONCLUSION

Results showed that feeding lactating dairy cows with ESB, GCS and WCS had no adverse effect on ruminal fermentation and DMI. However, feeding cows with ESB tended to increase milk yield and decreased milk fat percentage in dairy cows. Supplementation of the three oilseeds to the diet resulted in decreased proportions of medium-chain FA in milk and increased proportions of long-chain and unsaturated FA. Supplementing extruded soybean to cows could increase the concentration of cis9,trans11-11CLA in milk. The observed increase in PUFA is considered beneficial for human health and indicated that extruded soybean, ground canola seed and whole cottonseed supplementation could potentially modify the fatty acid composition of milk.

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