Journal of Livestock Science and Technologies, **2018, 6 (1): 19-29** ***http://lst.uk.ac.ir***

DOI: 10.22103/jlst.2018.10824.1207

Effects of replacing soybean meal with canola meal and decreasing crude protein on milk production and nutrient utilization of dairy cows in early lactation

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Abstract This study investigated the effects of replacing soybean meal (SM) with canola meal (CM) and decreasing crude protein (CP) levels in CM based diets on performance of dairy cows when different sources of processed grains and CM were used. Canola meal was untreated or roasted at 130°C for 30 minutes and grains sources (corn and barley) were either ground or steamed-flaked. Eight Holstein cows (2nd lactations; 42 ± 2 d in milk; 600±20 kg body weight) were used in a 4×4 Latin square design with 4 treatments. Treatments were a control diet based on SM containing 16.5% CP (SM), or 1 of 3 diets based on CM as (1) untreated CM + ground grain sources containing 17.7% dietary CP (CM-17.7), 2) treated CM + ground grain sources containing 16.5 % dietary CP (CM-16.5), and 3) treated CM + steam flaked grain sources containing 15.3 % dietary CP (CM-15.3). Diets were iso-energetic, however, the estimated metabolizable protein (MP) and Lys: Met ratio were the highest in the SM diet but both values were gradually decreased with reducing dietary CP in the CM diets. Feeding CM diets led to similar intake, milk yield and efficiency, as well as nitrogen (N) efficiency (milk N/N intake) as compared to SM diet. However, milk fat content tended to be lower in SM diet than in CM diets. Digestibility of dry matter (DM) and neutral detergent fiber (NDF) was lower in cows fed CM-17.7 than those fed either SM, or CM-16.5, CM-15.3 diets. Decreasing dietary CP in the CM diets had no significant effects on DM intake, milk production or milk composition, whereas N efficiency was linearly increased. Different diets or dietary CP level had no significant effect on plasma parameters. In conclusion, the present study indicated that replacing SM with CM resulted in similar milk yield and efficiency. Decreasing dietary CP from 17.7 to 15.3 % by feeding a mixture of treated CM and steam flaked grain sources did not affect milk production but improved N efficiency of dairy cows in early lactation.

Keywords: canola meal, crude protein, dairy cows, soybean meal

Received: 07 Sep. 2017, *accepted:* 14 May. 2018, *published online:* 23 Apr. 2018

Introduction

Dietary CP levels have major influences on dry matter intake (DMI), milk yield, and milk composition, feed costs, and reproductive efficiency in dairy cows. In some experiments, positive relationship between the dietary levels of CP (14 to 22%) and DMI (Sinclair et al., 2014) as well as between CP concentration (up to 23%) and milk yields (NRC, 2001) were reported. However, feeding high CP diets, especially highly soluble or degradable CP, was reported to increase production costs and contribute to environmental N pollution (Broderick, 2006). Reducing dietary CP (RDP and/or RUP) while improving amino acid (AA) profile of metabolizable protein (MP) is the most significant means by which to avoid such problems and to enhance efficiency of dietary N utilization without adversely affecting milk yield (Lee et al., 2012; Bahrami-Yekdangi et al., 2014; Sinclair et al., 2014). Met, Lys, and His have been identified as the most often limiting AAs in diets of lactating dairy cows (Schwab et al., 2007; Lee et al., 2012). Supplementation of low-protein diets (~14%) with those AA has been shown to be a promising strategy to counteract the potential negative effect of overall MP deficiency on dairy cow productivity (Lee et al., 2012; Giallongo et al., 2016). Another way to increase N efficiency in low protein diets is to improve efficiency of microbial protein synthesis and/or feeding RUP supplements with balanced AA profile. Cereal grains are the major sources of starch in the diets of lactating dairy cows. Shifting the site of starch digestion from the intestine to the rumen increased recycled N and RDP utilization and resulted in greater microbial N supply to the duodenum (Davies et al., 2013).

Cereal grain processing that increases ruminal starch fermentation (e.g. steam-flaking), increases cycling of urea to the gut, and microbial protein flow to the small intestine and, consequently enhances milk production and milk protein yield (Theurer et al., 1999). Therefore, more degradable starch in the rumen can be a promising method to increase recycled urea-N and the efficiency of microbial N production as well as lowering dietary CP concentration (Davies et al., 2012).

Soybean meal (SM) is the most commonly used protein supplement in dairy cow diets because of its high concentration of net energy and CP compared with other oilseed meals (NRC, 2001). However, with respect to essential AA (especially Lys, Met and His), only the AA profile of fishmeal and canola meal (CM) closely resembles that of milk protein; being complementary to microbial protein (Piepenbrink and Schingoethe 1998). Inclusion of CM in dairy cow diets had positive effects on DMI and yield of milk and milk protein compared with SM or other protein sources (Huhtanen et al., 2011; Martineau et al., 2013). The positive effects of CM have been attributed to increased absorption of essential AA such as Met and His (Martineau et al., 2013; Mirzaie goudarzi et al., 2017). Heat treatment increased RUP fraction in CM (65 % of CP), and cows fed treated CM had greater milk production and N efficiency across a wide range of dietary CP concentrations as compared with SM (Gidlund et al., 2015). Based on the proper profile of essential AA in CM, we hypothesized that increasing RUP fraction of CM as well as ruminally starch availability of grain sources would improve N efficiency, thereby reducing dietary CP requirement. Therefore, the objective of the current study was to examine the potential responses to processed CM and grain sources along with reducing thedietary CP level on nutrient utilization and performance of high-producing dairy cows in early lactation.

Materials and methods

Treatment of canola meal and grain sources

Commercially available CM was either left untreated, or sprayed with molasses (0.2 L water/kg DM containing 150 g molasses/L) and heated for 30 min at 130°C in a nut huge cook pot and consequently, spread out on the ground for cooling. Beet molasses, as a source of sugar, was included to induce non-enzymatic browning reaction and increase ruminal escape of CM protein (Cleale et al., 1987). The method had been previously optimized in terms of temperature, time, and molasses concentration in our laboratory. To prepare grain sources, whole barley and corn grains were either ground by a hammer mill (with a screen size of 3 mm) or barley and corn of the same sources were steamed for 45 min (psi=100) in a vertical steam chamber in a commercial feed processing and equipment complex (Chavdaneh Co., Shahreza, Isfahan, Iran) before flaking between preheated large rollers (100×30.5 cm) to a desired flake density (380 g/L).

Animals and experimental diets

The *in vivo* study was conducted at the farm facilities of Fodeh milk and meat complex (2000 milking cows, Isfahan, Iran). Eight high-producing Holstein cows in early lactation (2 lactations; 42 ± 2 DIM; 600 ± 20 kg BW) were used for an 84-d experimental period. Cows were housed in individual pens (3 × 3 m) within an opened barn with a concrete feed bunk and water trough. Clean sands were used for bedding and refreshed once daily. The experimental design was a 4 × 4 Latin square with 21-d periods. The first 14 d of each period were used to adapt the cows to treatments, and the remaining 7 d were used for sample collection. Dietary treatments were, 1) a diet containing 16.5 % CP based on SM which was considered as the control group (SM), 2) a diet containing 17.7% CP based on untreated CM + ground grains sources (CM-17.7), 3) a diet containing 16.5% CP based on treated CM + ground grains sources (CM-16.5), and 4) a diet containing 15.3% CP based on treated CM + steam flaked grains (CM-15.3). Diets were isoenergetic (1.6 Mcal of NEL/kg DM) at a forage-to-concentrate ratio of 35:65 (Table 1). The diets were formulated to meet or exceed the nutrients requirements, except for MP in diets 3 and 4, for Holstein dairy cows yielding 46 kg of milk/d, 3.3% milk fat and 3.0% milk protein at 25 kg of DMI/d, and 600 kg of BW, according to the NRC (2001) model. Concentrations of CP were reduced by 1.2 and 2.4 % units in diets 3 and 4, respectively, compared to the diet 2. The diets were prepared once daily as a TMR and fed in equal portions at 0830 and 1530 h. Cows had free access to diets and fresh water. Cows were milked 4 times daily at 0400, 1000, 1600 and 2200 h.

Sampling and data collection

Dry matter intake and orts (5 to 10 % of TMR) were

Table 1. Feed ingredients of the experimental diets (DM basis)

Item, %	Diets[1]			
	SM	CM-17.7	CM-16.5	CM-15.3
Alfalfa hay	15.5	15.5	15.5	15.5
Corn silage	19.3	19.3	19.3	19.3
Dried beet pulp, shreds	5.8	1.9	3.2	6.0
Barley grain, ground	14.9	14.9	14.9	-
Corn grain, ground	22.7	21.9	22.7	-
Barley grain, flaked	-	-	-	14.9
Corn grain, flaked	-	-	-	22.7
Soybean meal	13.1	1.33	0.95	0.12
Untreated canola meal	0.92	14.4	-	-
Treated canola meal	-	-	14.4	14.4
Soybean whole extruded, coarse	2.50	3.07	3.07	1.99
Fish meal	0.19	1.54	0.50	0.08
Feather meal	0.15	0.77	0.38	0.08
Meat and bone meal, rendered	0.15	0.77	0.38	0.08
Fat, powder	1.92	2.27	2.27	2.27
Sodium bicarbonate	0.80	0.80	0.80	0.80
Calcium carbonate	0.42	0.38	0.42	0.46
Dicalcium phosphate	0.50	-	0.08	0.19
Magnesium oxide	0.20	0.20	0.20	0.20
Mineral premix[2]	0.39	0.39	0.39	0.39
Vitamin premix[3]	0.39	0.39	0.39	0.39
Common salt	0.20	0.20	0.20	0.20

[1] SM= diet containing 16.6 % CP based on soybean meal; CM-17.7= diet containing 17.7 % CP based on untreated canola meal and ground grains (barley and corn); CM-16.5= diet containing 16.5 % CP based on heated canola meal and ground grains (barley and corn), CM-15.3= diet containing 15.3 % CP based on heated canola meal and steam flaked grains (barley and corn).

[2] Mineral premix contained (per kilogram DM) 10 g Mn, 16 g Zn, 4 g Cu, 0.15 g I, 0.12 g Co, 0.8 g Fe, and 0.08 g Se.

[3] Vitamin premix contained (per kilogram DM) 1,00,000 IU vitamin A, 360,000 IU vitamin D3, 15,000 IU vitamin E.

measured and recorded daily throughout of the experiment. Samples of individual feed ingredients, TMR and orts were collected during the last 5 d of each period. A composite orts sample was prepared for each cow and period combination by mixing the orts from each day in a day proportion by wet weight to the orts from that week. Fecal samples were collected from the rectum every 9 h over a 72-h period of each experimental period and were composited by cow and period. Milk production was recorded during the 5-d sampling period. Samples for milk composition determination were collected during the sampling period. The cow body weight was recorded at 0800 h at the beginning of each treatment period and at the end of the experiment. Chewing activities were monitored for 24 h on d 20 of each period. Eating and ruminating activities were recorded every 5 min and each activity was assumed to persist for the entire 5-min interval between observations. Total time spent chewing was calculated as the total time spent eating and ruminating. Blood samples were collected 4 h after the morning feeding (12:30) from the coccygeal vein using a vacutainer tube on d 21 of each experimental period. Blood samples (EDTA tube) were centrifuged (4°C; 3,000 × g; 20 min) and plasma stored at −20°C until analysis.

Chemical analyses

Frozen TMR and fecal samples were thawed overnight at room temperature, pooled per collection period for each cow, and subsequently dried in an oven at 60°C for 48 h. Dried TMR and fecal samples were then ground through a 1-mm screen. Samples were analyzed for DM (AOAC, 1990), CP using the macro-Kjeldahl procedure (AOAC, 1990), and aNDF (Van Soest et al., 1991) using the Ankom200/220 system (Ankom Technology Corp. Fairport, NY) and heat stable alpha-amylase (Sigma-A3306) without sodium sulfite. The apparent digestibility of DM, CP and NDF was determined using the acid-insoluble ash ratio technique (Van Keulen and Young, 1977). Nitrogenous fractions of SM and CM were partitioned into 5 fractions, A, B1, B2, B3 and C according to CNCPS using standardization of procedures for nitrogen fractionation (Licitra et al., 1996) as described by Akbarian et al. (2014). Non-protein nitrogen (NPN) contents were determined by precipitating the true protein fraction with the use of trichloroacetic acid and calculating the difference between total CP and CP content of the precipitate (Licitra et al., 1996). To determine the soluble CP content, samples were incubated in bicarbonate-phosphate buffer and then filtered and the residue was analyzed for CP content. The soluble CP was calculated as the difference between total CP and residual CP. Milk samples were preserved using 2-bromo-2-nitropropane-1, 3- diol and analyzed for fat, true protein and lactose using the MilkoScan (134 BN Foss Electric, Hillerod, Denmark). Plasma samples were analyzed for albumin (BCG method), total protein (BIURET), creatinine (JAFFE), urea N (UV-TEST), and glucose (GOD-PAP) using commercial kits (Parsazmun Co. Lts., Karaj, Iran) by an auto-analyzer (Biotecnica, BT 15 00, Italy).

Statistical analysis

Chemical data analysis was performed using the GLM procedure of SAS (version 8.2, 2001; SAS Institute Inc., Cary, NC). In the production trial, repeated measurements on DMI, total-tract nutrient digestibility, milk yield and composition were reduced to period means for each cow. Data were analyzed using the MIXED procedure for a replicated 4 × 4 Latin square design according to the following model: $Y_{ijkl} = \mu + S_i + Pj + C_{k(i)} + T_l + \varepsilon_{ijkl}$, where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of square i, P_j is the fixed effect of period j, $C_{k(i)}$ is the random effect of cow k (within square i), T_l is the fixed effect of dietary treatment l, $S \times T_{il}$ is the interaction between square i and treatment l, and ε_{ijkl} is the residual error. The interaction effects of fixed variables (period by treatment and square by treatment) were not significant and did not influence the results. Results were reported as LSMEANS. Contrast statements were used to determine the effect of protein sources (SM vs. CM-17.7, CM-16.5 and CM-15.3), and the linear and quadratic effects of CP levels in CM diets. Treatment effects were considered significant at $P \leq 0.05$. A trend was considered to exist if $0.05 < P \leq 0.10$.

Results

Chemical composition of CM, SM, and experimental diets

The chemical composition and protein fractionation of SM and CM are presented in Table 2. Soybean meal contained lower soluble protein (A1+B) and cell wall bound protein (B3+C) fractions than CM. The highest fraction of protein in SM was B2. Contents of DM and NDF for the treated CM were greater than that of the untreated CM. Heat processing of CM decreased A and B1, and increased B3 and C fractions. No significant change was observed in fraction B2 due to heat-molasses processing of CM. Concentration of estimated RDP was 11% for the CM-17.7 diet and it was red-

Table 2. Chemical composition and protein fractions in soybean meal (SM), untreated canola meal (CM), and treated CM.

Item	SM	Untreated CM	Treated CM	SEM	P-value
DM[1], % of as-fed	89.0	94.3	96.1	-	-
NDF, %	34.7	33.1	40.4	-	-
ADF, %	22.8	20.2	21.7	-	-
Ash, %	6.7	8.6	9.3	-	-
CP, %	46.2	39.5	38.7	-	-
Soluble protein[2], % of CP	11.4^{c}	26.5^{a}	16.7^{b}	0.25	<0.01
True protein, % of CP	90.0^{a}	78.5^{c}	86.0^{b}	2.58	<0.01
A, % of CP	9.87^{c}	21.6^{a}	13.7^{b}	0.31	<0.01
B1, % of CP	1.52^{c}	4.90^{a}	2.98^{b}	0.13	<0.01
B2, % of CP	68.0^{a}	44.4^{b}	45.9^{b}	2.96	<0.01
B3, % of CP	16.7^{c}	23.3^{b}	26.6^{a}	0.71	<0.01
C, % of CP	3.90^{c}	5.73^{b}	10.8^{a}	0.13	<0.01

[1] DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber.

[2] Soluble protein= A+B1; True protein= 1000-A; A = non protein N (NPN) ×6.25; B1 = soluble true protein; B2 = CP-(A+B1+B3+C); B3 = cell wall associated and slowly degradable true protein; C = unavailable protein (Licitra et al., 1996).

Within a row, means with common superscripts are not different (P> 0.05).

Table 3. Chemical composition of experimental diets.

Item	Diets[1]			
	SM	CM-17.7	CM-16.5	CM-15.3
Dry matter, %	55.1	58.9	58.1	55.4
Crude protein, %	16.5	17.7	16.5	15.3
Rumen degradable protein, %	10.4	11.1	9.24	8.57
Rumen undegradable protein %	6.1	6.5	7.26	6.73
Metabolizable protein supply[2], g/d	2967	2808	2864	2547
Metabolizable balance[2], g/d	+119	+17	+67	-176
MP from bacteria[2], g/d	1400	1370	1412	1187
MP from RUP[2], g/d	1443	1318	1328	1244
Lysine[2], % MP	6.33	6.32	6.30	6.05
Methionine[2], % MP	1.79	1.89	1.88	1.86
Lysine: Methionine	3.54	3.34	3.35	3.25
Neutral detergent fiber, %	33.3	32.0	32.4	33.2
Non fibrous carbohydrate[3], %	40.2	39.8	40.9	41.7
Total carbohydrate fractions[4], %	73.5	71.8	73.3	74.9
Ether Extract, %	4.50	5.40	5.50	5.20
Net energy for lactation[2], Mcal/kg DM	1.59	1.62	1.59	1.60
Calcium, %	0.90	0.95	1.00	1.00
Phosphorous, %	0.50	0.51	0.50	0.40

[1] SM= diet containing 16.6 % CP based on soybean meal; CM-17.7= diet containing 17.7 % CP based on untreated canola meal and ground grains (barley and corn); CM-16.5=diet containing 16.5 % CP based on heated canola meal and ground grains (barley and corn), and CM-15.3= diet containing 15.3 % CP based on heated canola meal and steam flaked grains (barley and corn).

[2] Estimated using the NRC (2001) model based on observed dry matter intake, milk yield and composition, and BW of individual cows.

[3] NFC, % = 100 − EE − CP − NDF – ash.

[4] Total carbohydrate fractions = NDF+NFC.

uced to 10.1 and 8.9 % for the CM-16.5 and CM-15.3 diets, respectively (Table 3). The content of RUP remained constant at approximately 6.5 % for all diets. In addition to the SM and CM-17.7 diets, the estimated MP balance was positive for the CM-16.5 diet due to higher actual DMI (26.3 kg/d Table 4) than predicted DMI (25.0 kg/d). However, the estimated MP balance was negative for the CM-15.3 diet. The concentration of carbohydrate fractions (NFC and NDF) was increased as the content of dietary CP decreased in the CM diets. In the SM diet, Met and Lys were calculated to be 6.33 and 1.79 % MP, while Lys was slightly decreased and Met increased with the substitution of CM in the diets. Consequently, the estimated Lys: Met was lower in the CM diets (3.29) than the SM diet (3.53) and it was reduced by decreasing CP in the CM diets from 3.34 to 3.25.

Dry matter intake and nutrient digestibility

Voluntary feed intake, apparent digestibility, and digestible nutrient intake were similar between the SM diet and the CM diets (Table 4). The diets containing lower protein levels (CM-16.5 and CM-15.3) did not limit the DMI, or digestibility as compared with the CM-17.7 diet. Intake of NDF was quadratically affected by decreasing dietary CP with the highest amount observed in cows fed CM-16.5 (P<0.01). Intake of CP was similar between the SM and CM diets, and it was reduced linearly (P<0.01) with decreasing dietary CP in the CM diets. Total tract digestibility of DM and NDF was lower in cows fed CM-17.7 than cows fed either CM-16.5 or CM-15.3 (P=0.04).

Milk yield and nitrogen use efficiency

Milk production and energy corrected milk (ECM) did not differ between diets with different protein supplements (SM diet vs. CM diets) and averaged 45.1 kg/d and 43.5 kg/d for the SM and CM diets, respectively (Table 5). Milk protein and lactose percentage did not differ (P>0.05) between diets, whereas, milk fat tended to be greater (P=0.08) for the CM diets than the SM diet. Yield of milk, ECM, milk composition, and feed efficiency were not significantly affected by dietary CP concentrations. Apparent N efficiency (milk N/N in

Table 4. Nutrient intake, apparent digestibility and digestible nutrients intake in lactating cows fed different sources and concentrations of dietary crude protein

Item	Diets[1]				SEM	P-value[2]		
	SM	CM-17.7	CM-16.5	CM-15.3		SM vs. CM	L	Q
Nutrient intake, kg/d								
Dry matter	26.3	25.3	26.3	24.6	2.40	0.44	0.66	0.31
Crude protein	4.13	4.32	3.91	3.65	0.157	0.36	<0.01	0.70
Neutral detergent fiber	7.33	7.08	9.25	7.48	0.348	0.14	0.42	<0.01
Apparent digestibility coefficients								
Dry matter	0.72	0.64	0.72	0.72	0.024	0.28	0.04	0.22
Crude protein	0.74	0.69	0.67	0.68	0.033	0.16	0.88	0.79
Neutral detergent fiber	0.52	0.39	0.58	0.56	0.045	0.79	<0.01	0.02
Digestible nutrient intake, kg/d								
Dry matter	17.4	15.1	17.3	16.4	0.94	0.31	0.32	0.18
Crude protein	2.86	2.94	2.62	2.86	0.160	0.39	0.05	0.59
Neutral detergent fiber	3.72	2.78	5.37	4.17	0.425	0.33	0.02	<0.01

[1] SM: diet containing 16.6 % CP based on soybean meal; CM-17.7: diet containing 17.7 % CP based on untreated canola meal and ground grains (barley and corn); CM-16.5: diet containing 16.5 % CP based on heated canola meal and ground grains (barley and corn), and CM-15.3: diet containing 15.3 % CP based on heated canola meal and steam flaked grains (barley and corn).

[2] SM vs. CM: soybean meal vs. canola meal; L: linear effect of dietary CP in canola meal based diets; Q: quadratic effect of dietary CP in canola meal based diets.

take) was not different (P=0.59) between the SM and the CM diets. For CM-17.7, apparent N efficiency (milk N/N intake) was 0.285, and it was linearly increased up to 0.327 (P=0.02) with decreasing dietary CP in the CM diets. There was no significant treatment effect on BW during the study.

Blood metabolites and chewing activity

Plasma concentrations of urea N, creatinine, albumin, and total protein were not altered by either protein supplements or dietary CP content (Table 6). Concentration of glucose was lower in cows receiving CM-16.5 than those offered CM-17.7 and CM-15.3 (P=0.03). Total time spent ruminating did not exhibit any significant differences with either protein supplements or dietary CP. Cows fed the SM diet spent more time on eating (P=0.03) than those fed the CM diets. Cows fed CM-16.5 diets had higher time spent eating (P<0.01) and total chewing than cows fed either CM-

Table 5. Milk yield parameters, and milk and N efficiency in lactating cows fed different sources and concentrations of dietary crude protein.

Item	Diets[1]				SEM	P-value[2]		
	SM	CM-17.7	CM-16.5	CM-15.3		SM vs. CM	L	Q
Milk yield parameters								
Milk yield, kg/d	45.1	43.1	43.7	43.6	1.63	0.38	0.81	0.87
ECM[3], kg/d	43.0	41.7	42.5	44.0	2.24	0.88	0.32	0.84
Milk fat, %	3.05	3.13	3.17	3.20	0.025	0.08	0.81	0.80
Milk protein, %	3.09	3.19	3.11	3.05	0.069	0.78	0.17	0.93
Milk lactose, %	4.81	4.82	4.71	4.80	0.076	0.68	0.88	0.28
Body weight	580	581	590	583	20.7	0.64	0.84	0.50
Feed and N efficiency								
Milk/DMI	1.82	1.83	1.79	1.88	0.092	0.91	0.60	0.44
ECM/DMI	1.55	1.61	1.57	1.71	0.170	0.25	0.24	0.20
N Intake, g/d	720	756	690	640	65.3	0.44	<0.01	0.82
Milk N, g/d	219	217	214	208	5.76	0.91	0.26	0.79
N efficiency[4]	0.301	0.285	0.313	0.327	2.94	0.59	0.02	0.61

[1] SM= diet containing 16.6 % CP based on soybean meal; CM-17.7= diet containing 17.7 % CP based on untreated canola meal and ground grains (barley and corn); CM-16.5= diet containing 16.5 % CP based on heated canola meal and ground grains (barley and corn), and CM-15.3= diet containing 15.3 % CP based on heated canola meal and steam flaked grains (barley and corn).

[2] SM vs. CM: soybean meal vs. canola meal; L: linear effect of dietary CP in canola meal based diets; Q: quadratic effect of dietary CP in canola meal based diets. [3] ECM: energy-corrected milk calculated as ECM = (0.323 × kg milk) + (12.82 × kg milk fat) + (7.13 × kg milk protein).

[4] N efficiency = g milk protein/ g CP intake.

Table 6. Blood metabolites and chewing activity in lactating cows fed different sources and concentrations of dietary crude protein

Item	Diets[1]				SEM	P-value[2]		
	SM	CM-17.7	CM-16.5	CM-15.3		SM vs. CM	L	Q
Blood metabolites								
Glucose, mg/dL	65.3	65.5	59.6	65.9	2.18	0.50	0.90	0.03
Albumin, g/dL	3.32	3.38	3.41	3.41	0.043	0.12	0.68	0.81
Total protein, g/dL	7.04	6.69	6.99	6.93	0.132	0.55	0.88	0.74
BUN, mg/dL	11.7	12.4	11.6	10.9	1.00	0.96	0.30	0.99
Creatinine, mg/dL	0.98	0.90	1.02	0.91	0.055	0.52	0.87	0.09
Chewing activity								
Eating, min/d	308	246	315	261	20.0	0.03	0.43	<0.01
Ruminating, min/d	418	424	418	406	20.7	0.93	0.50	0.91
Total chewing, min/d	725	669	731	666	36.5	0.22	0.94	0.05

[1] SM: diet containing 16.6 % CP based on soybean meal; CM-17.7: diet containing 17.7 % CP based on untreated canola meal and ground grains (barley and corn); CM-16.5: diet containing 16.5 % CP based on heated canola meal and ground grains (barley and corn), and CM-15.3: diet containing 15.3 % CP based on heated canola meal and steam flaked grains (barley and corn).
[2] SM vs. CM: soybean meal vs. canola meal; L: linear effect of dietary CP in canola meal based diets; Q: quadratic effect of dietary CP in canola meal based diets.

17.7 or CM-15.3.

Discussion

In this study, diets based on SM or CM-17.7 were formulated to meet the NEL and MP requirements of a cow producing 46 kg milk/d, 3.3% fat and 3.0% CP with predicted DMI of 25 kg/d (NRC 2001). The NRC system (2001) regards a lower MP value for CM than SM on a CP basis, mainly due to the higher unavailable protein (C fraction, 7.0 vs. 0.7%) and ruminal degradability rate (10 vs. 7.5%/h) and lower digestibility of RUP (75 vs. 93%) in CM than SM. Martineau et al. (2013) also reported that estimated total MP supplies (g/cow/day), responded negatively to CM substitution based on iso-nitrogenous diets (17.2 % CP). In the present study, SM recorded better protein quality in terms of protein solubility. Truly insoluble protein (B2) in SM was greater and rapidly soluble (A and B1) and insoluble cell wall associated true protein (B3 and C) was lower than that of CM. The B2 fraction can be completely digestible in the rumen and/or intestine. The higher true protein observed in SM is in line with the previously reported data (NRC 2001). Heendeniya et al., (2012) observed that contents of soluble CP and protein fraction associated with the NDF and ADF were lower and true protein was higher in SM than that of CM. However, varying the RUP/RDP ratio in CM and their comparison with SM did not result in any differences in *in vitro* ruminal fermentation, nutrient digestion, and microbial growth using a dual-flow continuous culture system (Paula et al., 2017).

In this study, cows fed the SM diet had similar DMI and milk production as compared to cows fed the CM-17.7. However, feeding the CM diets at different dietary CP levels resulted in similar DMI, milk yield and milk composition and blood metabolites when compared to the SM diet. Further, N efficiency (milk N/N intake) was similar between CM and SM diets. Huhtanen et al. (2011) observed that milk and milk protein responses were higher with incremental levels of inclusion of CM in the diet compared with the inclusion of SM. Along with the increase in milk protein, Shingfield et al. (2003) also reported decreased milk urinary N concentration with CM treatments, indicating better utilization of feed N compared with SM. Positive responses in milk protein yield in CM diets might be related to a better quality of MP supply compared with SM (Huhtanen et al., 2011). In our study, the lack of response in milk protein when CM was substituted with SM may indicate that these protein sources were similarly utilized. Our findings are consistent with the results from another study (Maxin et al., 2013) that reported no changes in DMI and N intake, and milk production (isonitrogenous diet; 17.2 % CP) based on CM, dried distillers grain, or SM. In a meta-analysis by Martineau et al. (2013), positive responses in milk and milk protein yields were observed when CM replaced protein supplements other than SM (e.g., distillers grain, corn gluten meal, and cottonseed meal). Martineau et al. (2013) speculated that the essential AA profile of SM and CM might be more balanced or complementary to that of microbial protein compared with other protein sources. In this study, the CM diets supplied a better Lys: Met ratio in MP than the SM diet. Canola meal is similar to SM with regards to its AA profile containing 13.2% of essential AA of Lys but slightly higher Met at 4.39% of essential AA

(Huhtanen et al., 2011). This resulted to the closer ratio of absorbed Lys:Met in CM diets to 3:1 as recommended by NRC (2001). Milk fat tended to increase with substitution of SM with CM. This observation is in agreement with the result of Swanepoel et al. (2015) reporting that supplemental Met alone increased plasma Met levels and milk fat content. Martineau et al. (2013) did not find any change in milk fat percentage but reported a positive response in yields of milk protein and fat to CM substitution. In the present study, eating time was greater for cows fed SM and CM-16.5 diets than cows fed the CM-17.7 and CM-15.3 diets. Total chewing time was similar between the diets (SM vs. CM). This suggests that the increase in milk fat content observed with CM substitution may be related to metabolic pathways of Met. Methionine plays a role in the formation of choline which is essential for the synthesis of phospholipids, and consequently for that of chylomicrons and very low density lipoproteins (Zanton et al., 2014).

In our study, beet pulp was partly used instead of protein supplements for decreasing the dietary CP content. The estimated RUP content was almost similar across the diets based on CM; however, CM-15.3 diet had 2.1 percentage units lower RDP concentration (11 vs. 8.9 %) compared with CM-17.7 diet. The MP and the ratio of Lys:Met were also reduced by decreasing the dietary CP level in the CM diets. Feeding lower CP diets will decrease N excretion and reduce environmental concerns related to N pollution. In this study, cows fed CM-15.3 had lower CP intake compared to those fed the CM-17.7. However, the lower protein diets (CM-16.5 and CM-15.3) did not limit DMI, nutrients digestibility, milk yield and efficiency compared with the high protein group (CM-17.7); neither did we not observe a dilution of milk protein percentage with decreasing dietary CP. Moreover, blood urea N and proteins were not affected by the treatments. These results led to greater N efficiency by 14.7% in cows fed the low protein diet (CM-15.3) than those fed with the high protein diet (CM-17.7). In our study, decreasing the dietary CP level resulted in decreased estimated MP supply based on NRC (2001). Microbial protein flow was not measured in our study. However, increasing the RUP level of CM and/or ruminally fermentable carbohydrates (as energy source for microbial growth) could provide better duodenal AA profile and might be a possible explanation for no negative effects of CP reduction on milk yield in CM diets (Shingfield et al., 2003). Efficiency of MP has been improved by supplying adequate amounts of Lys and Met; the optimum Lys:Met ratio in MP to enhance milk protein yield is 3:1 (Schwab et al., 2007). Our results showed that when SM was replaced with CM the Lys:Met ratio was improved (decreased from 3.54 for SM diet to 3.25 for CM-15.3 diet). Further, treated CM diets (CM-16.5 and CM-15.3) reduced N intake but improved DM and NDF digestibility as compared with untreated CM diet. This observation supports the earlier results of Wright et al. (2005) who reported a greater NDF digestibility in cows fed lignosulfonate-treated CM. The reason for this observation is not clear or may be, in part, due to beet pulp substitution, but it may indicate that the RDP (8.9 %) did not limit the digestion of fiber. According to NRC (2001), the first emphasis in N nutrition is to meet sufficient RDP to support microbial growth. This is not only important for providing high-quality protein to the small intestine but also for fiber digestion, and greater DMI and performance. However, the current requirements for RDP (9.5-10.5 % of DM) seem to be high (Cyriac et al., 2008). Gressley and Armentano (2007) observed no changes in milk production when 10.1 and 7.4 % RDP diets were compared. Colmenero and Broderick (2006) did not find any difference in DMI with reducing dietary CP from 19.4 to 13.5 % in dairy cows (with 35 kg milk/d). Several trials (Colmenero and Broderick 2006; Bahrami-Yekdangi et al., 2014; Gidlund et al., 2015), testing various CP levels in diets formulated from typical feeds, showed no increase in yield of milk, fat corrected milk or protein with more than about 16-17% CP. In fact, over-feeding protein actually appeared to depress production (Broderick, 2006). There is a cost of 7.2 kcal of metabolizable energy per g of excess N excreted as urea. Colmenero and Broderick (2006) found that reducing dietary CP to 15.6 % by feeding RUP from heat-protected SM decreased milk yield. In our study, reducing dietary CP by increasing RUP from CM had no effect on milk yield. Wright et al. (2005) found that treatment of CM with lignosulfunate resulted in a 1.8 kg/d increase in fat corrected-milk. Shingfield et al. (2003) compared treated CM with solvent extracted SM in grass silage-based diets and reported increased milk and milk protein yields with the treated CM. These result indicated that CM was a more effective sources of RUP than heated soybean products. Maxin et al. (2013) concluded that the better quality of MP supply in CM as compared with other protein sources such as SM (low in Met), corn dried distillers grain (low in Lys), and wheat dried distillers grain (low in His) resulted in positive response in milk and milk protein yields to CM substitution. Treatment of CM increased His, Met, and branched chain AA concentrations in plasma (Shingfield et al., 2003). Therefore, reducing the ruminal degradability of CM without affecting its digestibility could be as a unique source of RUP. In this study, heating decreased soluble protein but increased true proteins in CM.

Conclusion

Among plant protein supplements, CM has a closer AA profile to that of milk protein, thereby including CM in the diet of dairy cows enhanced estimated Met supply and improved AA profile (Lys:Met: 3:1) of MP compared with SM. Inclusion of CM as the main protein source in dairy cow diets based on SM, resulted in similar DMI, digestibility, milk yield as well as apparent N efficiency. However, milk fat content responded positively to CM substitution. In addition, decreasing the dietary CP from 17.7 to 15.3 % by feeding a mixture of steam-flaked grains and heat-treated CM sources improved efficiency of dietary N without affecting the milk production.

Acknowledgments

We thank Fodeh Dairy Farm Co. personnel (Eng. Fodei and Eng. Soltani) for the providing and management of the animals. Isfahan University of Technology (IUT) is also acknowledged for their support. Finally, Dr. Ezzatollah Roustazadeh from ELC, IUT, is acknowledged for having edited the final English manuscript.

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Communicating editor: *Omid Dayani*

اثر جایگزینی کنجاله کلزا بجای کنجاله سویا و کاهش پروتئین خام جیره بر تولید و قابلیت استفاده از مواد غذایی در گاوهای شیرده آغاز شیردهی

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چکیده این مطالعه به منظور بررسی اثرات جایگزینی کنجاله کلزا بجای کنجاله سویا و همچنین کاهش پروتئین خام در جیره‌های بر پایه کنجاله کلزا هنگام استفاده از منابع مختلف غله و کنجاله کلزای فرآوری شده انجام شد. کنجاله کلزا در ۲ حالت بدون فرآوری و یا برشته شده در دمای ۱۳۰ درجه سلسیوس (۳۰ دقیقه) و دانه غلات (جو و ذرت) بصورت آسیاب یا پرک شده در جیره استفاده شدند. شمار ۸ راس گاو شیرده (دوبار زایش، روزهای شیردهی ۲± ۴۲ و وزن ۲۰±۶۰۰ کیلوگرم) در قالب طرح مربع لاتین ۴×۴ به: ۱) جیره بر پایه کنجاله سویا با پروتئین خام ٪۱۶/۵، ۲) جیره بر پایه کنجاله کلزای خام با پروتئین خام ٪۱۷/۷، ۳) جیره بر پایه کنجاله کلزا فرآوری شده با پروتئین خام ٪۱۶/۵ و ۴) جیره بر پایه کنجاله کلزا فرآوری شده–غله پرک شده با پروتئین خام ٪۱۵/۳ اختصاص یافتند. جیره‌ها از نظر انرژی خالص (۱/۶ مگاکالری در کیلوگرم) یکسان اما جیره دارای کنجاله سویا از نظر برآورد پروتئین قابل متابولیسم و نسبت لیزین: متیونین، بالاتر از جیره‌های بر پایه کنجاله کلزا بود و این فراسنجه‌ها با کاهش پروتئین خام در جیره‌های بر پایه کنجاله کلزا بطور تدریجی کاهش یافت. تغذیه جیره‌های بر پایه کنجاله کلزا با پروتئین متفاوت منجر به مصرف خوراک، تولید و کارآیی تولید شیر و همچنین بازده نیتروژن (نیتروژن شیر به نیتروژن مصرفی) مشابه با تغذیه جیره بر پایه کنجاله سویا شد. هرچند درصد چربی شیر در جیره‌های بر پایه کنجاله سویا کمتر از کنجاله کلزا بود. گوارش‌پذیری ماده خشک و الیاف شوینده خنثی در جیره بر پایه کنجاله کلزای بدون فرآوری کمتر از جیره‌های بر پایه کنجاله کلزای فرآوری شده و کنجاله سویا بود. کاهش پروتئین خام در جیره‌های بر پایه کنجاله کلزا تاثیری بر مصرف خوراک، تولید و ترکیب شیر نداشت اما بازده نیتروژن به شیوه خطی افزایش یافت. تیمارهای غذایی مختلف تاثیر بر فراسنجه های پلاسما نداشتند. نتایج این آزمایش نشان داد جیره های بر پایه کنجاله کلزا می توانند جایگزین جیره بر پایه کنجاله سویا در جیره‌های گاوی شیرده شوند. همچنین، کاهش پروتئین خام جیره از ۱۷/۷ به ۱۵/۳ درصد با فرآوری منابع غله و کنجاله کلزا تاثیر منفی بر عملکرد گاوهای شیرده نداشت اما بازده نیتروژن مصرفی را افزایش داد.