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Effects of soybean meal or canola meal on milk production and methane emissions in lactating dairy cows fed grass silage-based diets

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ABSTRACT

This study evaluated the effects of soybean meal (SBM) and heat-moisture-treated canola meal (TCM) on milk production and methane emissions in dairy cows fed grass silage-based diets. Twenty-eight Swedish Red cows were used in a cyclic change-over experiment with 4 periods of 21 d and with treatments in 2 × 4 factorial arrangement (however, the control diet without supplementary protein was not fed in replicate). The diets were fed ad libitum as a total mixed ration containing 600 g/kg of grass silage and 400 g/kg of concentrates on a dry matter (DM) basis. The concentrate without supplementary protein consisted of crimped barley and premix (312 and 88 g/kg of DM), providing 130 g of dietary crude protein (CP)/kg of DM. The other 6 concentrates were formulated to provide 170, 210, or 250 g of CP/kg of DM by replacing crimped barley with incremental amounts of SBM (50, 100, or 150 g/kg of diet DM) or TCM (70, 140, or 210 g/kg of diet DM). Feed intake was not influenced by dietary CP concentration, but tended to be greater in cows fed TCM diets compared with SBM diets. Milk and milk protein yield increased linearly with dietary CP concentration, with greater responses in cows fed TCM diets compared with SBM diets. Apparent N efficiency (milk N/N intake) decreased linearly with increasing dietary CP concentration and was lower for cows fed SBM diets than cows fed TCM diets. Milk urea concentration increased linearly with increased dietary CP concentration, with greater effects in cows fed SBM diets than in cows fed TCM diets. Plasma concentrations of total AA and essential AA increased with increasing dietary CP concentration, but no differences were observed between the 2 protein sources. Plasma concentrations of Lys, Met, and His were similar for both dietary protein sources. Total methane emissions were not influenced by diet, but emissions per kilogram

of DM intake decreased quadratically, with the lowest value observed in cows fed intermediate levels of protein supplementation. Methane emissions per kilogram of energy-corrected milk decreased more when dietary CP concentration increased in TCM diets compared with SBM diets. Overall, replacing SBM with TCM in total mixed rations based on grass silage had beneficial effects on milk production, N efficiency, and methane emissions across a wide range of dietary CP concentrations.

Key words: crude protein, dairy cow, methane emissions, nitrogen efficiency

INTRODUCTION

On dairy farms, finding the most efficient ways to convert plant protein into nutritious milk for human consumption is a key factor in improving farm profitability and decreasing environmental emissions. Soybean meal (SBM) is a common human food worldwide. It is also the most commonly used protein supplement for pigs and poultry and, in addition, is fed widely to dairy and beef cattle. However, marginal milk protein yield responses are small in cows fed grass silage-based diets (Huhtanen et al., 2011). Inclusion of canola meal (CM) in dairy rations increases DMI and yield of milk and milk protein compared with SBM (Huhtanen et al., 2011), and also compared with other protein sources (Martineau et al., 2013). The positive effects of CM on milk production have been attributed to increased His supply (Shingfield et al., 2003) and, more recently, to increased absorption of EAA (Martineau et al., 2014).

Most comparisons of CM and SBM in dairy cow diets performed to date have used separate feeding of forages and concentrates, although TMR systems have become more common. Feeding dairy cows with TMR or using frequent feedings compared with 2 feedings per day decreases rumen ammonia concentration (Carroll et al., 1988) and increases rumen pH (Robinson and McQueen, 1994). These results indicate better synchrony of ruminal degradation of protein and carbohydrates, which can improve the efficiency of microbial protein

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synthesis. It is possible that this improved efficiency of microbial protein synthesis in cows fed TMR frequently can reduce the quality requirement on the protein supplement, specifically with regard to effects on N utilization efficiency. The first aim of this study was therefore to investigate the production responses to graded levels of SBM or heat-moisture-treated CM (TCM) supplementation in dairy cows fed TMR based on grass silage and crimped barley. Higher levels of supplementation than in previous studies (Rinne et al., 1999; Shingfield et al., 2003) were also tested to identify the optimal dietary levels of CP from the 2 protein supplements used. The second aim was derived from Murphy et al. (1982) and Bannink et al. (2006) who with stoichiometric equations showed that it seems like ruminal fermentation of dietary CP result in less CH₄ production than fermentation of carbohydrates. Therefore, we found it to be of high interest to study this in a production trial comparing increased dietary CP concentration and the 2 most common protein supplements, SBM and CM.

MATERIALS AND METHODS

All animals were registered and cared for according to guidelines approved by the Swedish University of Agricultural Sciences Animal Care and Use Committee and the National Animal Research Authority, and the experiment was carried out in accordance with the laws and regulations controlling experiments performed with live animals in Sweden.

Experimental Design and Animals

The production trial was conducted at the Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, Umeå (63°45'N; 20°17'E). Twenty-eight lactating Swedish Red dairy cows at a mean 88 DIM (SD = 27) and yielding 34.9 kg milk per day (SD = 6.6) at the start of the trial were used. The experiment was conducted using a cyclic changeover design in 4 periods, with 2 replicates of 2 blocks (Davis and Hall, 1969). The treatments were provided in a 2 × 4 factorial arrangement consisting of 2 protein supplements (SBM and TCM) and 4 levels of CP concentration. The control diet without protein supplement was not repeated between SBM and TCM treatments, which resulted in a total of 7 experimental diets. Each period lasted for 21 d, divided into 14 d of adaptation and 7 d of data recording and sampling. The cows were assigned to blocks according to parity and milk yield. Within the block, the cows were randomly allocated to 1 of the 7 treatments. The cows were kept in an insulated loose-housing system and

were milked twice a day, at 0600 and 1500 h. They were fed TMR ad libitum, with free access to drinking water. A stationary feed mixer (Nolan A/S, Viborg, Denmark) processed the rations, which were then delivered with automatic feeder wagons into feed troughs 4 times a day (0330, 0800, 1300, and 1730 h). During the entire trial, each cow had access to the same 2 feed troughs. The amount of feed delivered to the feed troughs was monitored daily to allow ad libitum feeding, and the feed troughs were cleaned daily.

Diets

The formulation of the experimental diets is given in Table 1. The 7 dietary treatments consisted of 600 g/kg of DM of silage, supplemented with 100 g/kg of DM of a premix manufactured to meet mineral and vitamin requirements (Fodercentralen, Umeå, Sweden), and 300 g/kg of DM of crimped barley aiming for a concentrate CP concentration of 130 g/kg of DM. The concentrate CP was manipulated by replacing crimped barley with SBM or TCM to reach concentrate CP concentrations of 170, 210, and 250 g/kg of DM. The DM concentration of all feeds was determined once a week and the TMR was adjusted accordingly during the whole trial. The forage consisted of 2 silages (50:50 on DM basis) harvested from different primary growth grass swards in Umeå, both dominated by timothy along with some red clover. The silages were harvested with a mower conditioner and precision-chop forage harvester between June 10 and 15, 2012, and stored in bunker silos. They were preserved using an acid-based additive (PromyrTM XR 630, Perstorp, Sweden) provided at a rate of 3.5 L/t. The crimped barley (778 g/kg of DM) was rolled using a mill (Murska 1400 S2 × 2, Murska, Ylivieska, Finland) adjusted to 0.3 mm between the rollers, treated with 3.5 L/t of propionic acid, and stored in airtight bags (1.6 m × 60 m, Ltd. Rani Plast Oy, Terjärv, Finland). The SBM used in the study was solvent-extracted (Lantmännen, Umeå, Sweden). The TCM was the commercial product ExPro-00SF from AarhusKarlshamn Ltd. (Malmö, Sweden). ExPro-00SF consists of solvent-extracted CM containing low levels of glucosinolates and erucic acid treated by an industrial heat-moisture procedure.

Recordings and Sampling

Individual feed intake was recorded daily throughout the trial in Roughage Intake Control feeders (Insentec B. V., Marknesse, the Netherlands), but the data used for statistical analysis were limited to d 15 to 21 in every period. The cows were weighed before the start of the trial and subsequently after morning milking on

d 19 to 21 in every period. Body condition score was assessed according to Edmonson et al. (1989) before the start of the trial and on d 21 in each period.

Silage, crimped barley, premix, SBM, and TCM were sampled on d 16, 19, and 21 in each period and stored at -20°C until further processing. Frozen silage samples were milled in a cutter mill (SM 2000, Retsch Ltd., Haan, Germany) to pass through a 20-mm sieve, and a portion was returned to the freezer for later analysis of silage fermentation quality. All feed samples were oven-dried at 60°C for 48 h. The dried samples were milled by the same cutter mill to pass through a 2-mm and 1-mm sieve, for different analytical purposes. Silage and crimped barley samples from each period were analyzed separately for all 4 periods. Samples of premix, SBM, and TCM were pooled over the 4 periods before analysis.

Milk yield was recorded during all milkings with gravimetric milk recorders (SAC, S.A. Christensen and Co Ltd., Kolding, Denmark). Milk samples were collected at 4 consecutive milkings from the afternoon of d 19 until the morning of d 21 in every period. The 2 morning samples were pooled, as were the 2 evening samples, and then stored at 8°C until sent for analysis.

Blood sampling was performed after morning milking on d 19 in every period on the 14 cows in 2 blocks. One sample per cow was taken from the tail vein or artery (needle size 0.8×38 mm) using a 10-mL plastic evacuated blood collection tube spray-coated with K_2EDTA (BD Vacutainer, Franklin Lakes, NJ), and kept on ice until centrifugation. Blood plasma was separated out by centrifuging (EBA 8S, Hettich Lab Technology, Tuttingen, Germany) for 15 min at $2,100 \times g$ at room temperature, transferred to 2 separate Eppendorf tubes (2 mL), and stored at -80°C .

Spot samples of feces (300 mL) were collected from the rectum at 0600 and 1400 h on d 19 and 20. Directly after each sampling occasion, the samples were oven-dried at 60°C and milled using a hammer mill

(LM 3100, Danfoss Ltd., Linköping, Sweden) to pass through a 1-mm sieve.

Mass flux of CH_4 and CO_2 was measured by a portable open-circuit head chamber system (GreenFeed system, C-Lock Inc., Rapid City, SD) as described by Dorich et al. (2015) and Huhtanen et al. (2015). The animals are encouraged to visit the GreenFeed system by giving them small amounts of concentrate at each visit. In the present study, the system was programmed to allow each animal a visit every 5 h (i.e., when the cows used all opportunities to visit the GreenFeed, the time of visit advanced by 1 h each day). During each visit, they were given 6 servings of 50 g of concentrate at 40-s intervals. Airflow rates and gas concentrations are measured continuously in the system, and by using the gas sensor information, the volumetric flux (L/min) of gases emitted by the animal can be calculated. The system also records head position during the visit, so data with inappropriate head position can be filtered out. The cows in this study had access to the system only during the last 2 experimental periods because it was not installed in the barn before that time. The emissions data for 3 cows were missing as they did not visit the GreenFeed system.

Chemical Analysis

Dry matter concentration was determined for feeds and feces samples by drying at 105°C for 16 h and ash concentration by incinerating at 500°C for 4 h. Feeds were analyzed for CP (Nordic Committee on Food Analysis, 1979) using a 2020 Digester and a 2400 Kjeltac Analyzer Unit (Foss Analytical A/S, Hilleröd, Denmark), water-soluble carbohydrates (Larsson and Bengtsson, 1983) and crude fat according to Method B in the Official Journal of the European Communities (1984), and NDF with heat stable α -amylase and sodium sulfite (Mertens, 2002) using the filter bag technique in an Ankom²⁰⁰ Fiber analyzer (Ankom Technology Corp.,

Table 1. Formulation of the control diet and the 6 experimental diets (g/kg of DM)

Item	Diet ¹						
	Control	SL	SM	SH	CL	CM	CH
Silage	600	600	604	604	600	604	600
Premix ²	88	88	88	88	88	88	88
Barley ³	312	262	208	158	242	168	102
Canola meal	0	0	0	0	70	140	210
Soybean meal	0	50	100	150	0	0	0

¹Control = only crimped barley and no protein feed; SL = low level of soybean meal; SM = medium level of soybean meal; SH = high level of soybean meal; CL = low level of canola meal; CM = medium level of canola meal; CH = high level of canola meal.

²The premix (Fodercentralen, Umeå, Sweden) contained (g/kg of DM) sugar beet pulp (420), oats (416), calcium-fat (60), NaCl (36), mineral and vitamin concentrate (28), sugar beet molasses (20), calcium carbonate (20).

³Barley = crimped barley.

Macedon, NY). The concentration of indigestible NDF in feeds was determined following a 288-h in situ rumen incubation (Huhtanen et al., 1994) according to the procedure of Krizsan et al. (2012). The 2 lactating cows used for the incubation were fed a TMR of 600 g/kg of DM of silage and 400 g/kg of DM of a commercial concentrate mix (Solid 220, Lantmännen, Malmö, Sweden). For the concentrate feeds, starch was analyzed according to Larsson and Bengtsson (1983). The frozen silage samples were analyzed for ammonium-N using direct distillation after adding MgO with Kjeltac 2100 Distillation Unit (Foss Analytical A/S), and also analyzed for VFA and lactic acid according to Ericson and André (2010). Furthermore, samples of the frozen silage were thawed and pressed, and the pH in the liquid was measured with a pH meter (Metrohm, Herisau, Switzerland). Feed and feces samples were analyzed for acid insoluble ash according to Van Keulen and Young (1977) with a few modifications; 5 to 7 g of feces or 10 to 15 g of feed sample were weighed into the crucible, the mixture of sample and acid was boiled for 15 min, and the last ashing was performed at 600°C.

The milk samples were analyzed for concentration of fat, protein, lactose, and urea using a Fourier Transform infrared analyzer (CombiFoss 6000, Foss Electric, Hillerød, Denmark).

The concentration of AA in plasma was determined according to Haque et al. (2012) using an ultra-performance liquid chromatography-mass spectrometry system equipped with a UV detector and a mass detector for the co-eluting peaks for Arg and 3-methyl His.

Calculations

Metabolizable energy concentration in the silages was calculated following the procedure of Lindgren (1979) and that in the concentrates following Spörndly (2003). Metabolizable protein was calculated according to the Swedish feed evaluation system (Spörndly (2003)). Energy-corrected milk was calculated according to Sjaunja et al. (1990).

The efficiency of N utilization was calculated as (milk protein yield/6.38)/(dietary CP intake/6.25). Total-tract apparent digestibility of DM and OM was calculated according to Van Keulen and Young (1977) based on the acid insoluble ash concentration in feeds and feces from periods 3 and 4. Digestibility was only measured in the last 2 periods because the use of the Greenfeed units was limited to the last 2 periods.

Statistical Analysis

The data were analyzed by the MIXED procedure of SAS (release 9.3, SAS Inst., Inc., Cary, NC) with the statistical model:

$$Y_{ijkl} = \mu + B_i + C_j(B_i) + P_k + T_l + \varepsilon_{ijkl},$$

where Y_{ijkl} is the dependent variable, μ is the mean for all observations, B_i is the effect of block i , $C_j(B_i)$ is the effect of cow j within block i , P_k is the effect of period k , T_l is the effect of treatment l , and $\varepsilon_{ijkl} \sim N(0, \sigma^2_e)$ is the random residual error. For treatment comparisons, the following contrasts were used: comparison of TCM and SBM, linear and quadratic effects of dietary CP concentration, and interaction between protein supplement and dietary CP concentrations. As a first step, the interactions $B_j \times P_k$ and $B_j \times T_l$ were evaluated but found to be nonsignificant ($P \geq 0.1$) and therefore not included in the final model. Generally, differences were considered significant at $P \leq 0.05$.

RESULTS

Experimental Feeds

The chemical composition of the experimental dietary ingredients is given in Table 2. The average fermentation quality of the 2 experimental silages across all periods was pH 4.15 (SD = 0.22); lactic acid 76.3 (SD = 0.95) g/kg of DM; acetic acid 34.3 (SD = 0.71) g/kg of DM; butyric acid 4.71 (SD = 0.27) g/kg of DM; propionic acid 2.75 (SD = 0.05) g/kg of DM; ammonia-N 83.7 (SD = 2.03) g/kg of N. The chemical composition of the experimental diets is shown in Table 3. The TCM contained less CP than expected (351 g/kg of DM), which resulted in a numerically lower CP concentration in TCM diets compared with the corresponding SBM diets.

Nutrient Intake

Total DMI and silage DMI tended to increase for cows fed TCM diets compared with cows fed SBM diets ($P = 0.08$; Table 4). Intake of NDF increased more with increased dietary CP concentration when the cows were fed TCM diets ($P < 0.01$) compared with SBM diets, and intake of potentially digestible NDF was also greater ($P = 0.02$) with TCM diets. Intake of MP increased more as dietary CP concentration increased, when cows were fed TCM diets compared with SBM diets. Increasing dietary CP concentration did not affect DMI, but resulted in increased intake of CP and MP. Neither protein supplement nor increased diet CP concentration had any significant effect on apparent diet digestibility.

Milk Production, Nitrogen Efficiency, and Digestibility

Inclusion of TCM resulted in greater milk yield (Table 5) compared with SBM, and milk protein yield

Table 2. Chemical composition of experimental dietary ingredients (g/kg of DM unless otherwise stated)

Item	Feed ingredient				
	Silage ¹	Premix ²	Barley ³	TCM ⁴	Soybean meal
DM, g/kg of feed	229	886	778	872	855
OM	919	840	970	912	931
CP	174	103	128	351	518
EE ⁵	20	61	24	74	22
NDF	550	246	275	294	165
iNDF ⁶	75	58	45	94	2
Starch		213	583	32	17

¹Silage values are means from 2 different batches of silage.

²The premix (Fodercentralen, Umeå, Sweden) contained (g/kg of DM) sugar beet pulp (420), oats (416), calcium-fat (60), NaCl (36), mineral and vitamin concentrate (28), sugar beet molasses (20), calcium carbonate (20).

³Barley = crimped barley.

⁴TCM = heat-moisture-treated canola meal.

⁵EE = ether extract, values for the silage taken from tables (Spörndly, 2003), those for concentrate ingredients analyzed directly.

⁶iNDF = indigestible NDF.

increased more with increased diet CP concentration when cows were fed TCM diets compared with SBM diets ($P = 0.03$). Increased dietary CP concentration increased yields of ECM, milk protein, and lactose, whereas milk fat yield only tended to increase ($P = 0.06$). Increased dietary CP concentration increased MUN concentration more in cows on SBM diets than on TCM diets ($P < 0.01$). Similar interactions between protein supplement and dietary CP concentration were observed for N efficiency, which decreased more on SBM diets than on TCM diets when CP concentration increased ($P < 0.01$).

Plasma Amino Acids

Increased dietary CP concentration raised ($P \leq 0.05$) the plasma level of the individual AA Arg, His,

Ile, Leu, Lys, Phe, Val, Gln, 3-methyl His, Orn, and Tyr (Table 6). Compared with TCM diets, SBM diets increased Phe and Pro concentrations and tended to increase the concentrations of Asn ($P = 0.07$) and Tyr ($P = 0.08$). No differences were observed between the 2 protein supplements in terms of plasma His, Met, or Lys concentration. Increased dietary CP concentration resulted in higher ($P \leq 0.01$) plasma concentrations of branched-chain AA, EAA, and total AA.

Gas Emissions

Mean estimated total CH₄ emissions across all experimental diets were 455 g/d (SD = 48) or 23.0 g/kg of DMI (SD = 2.2; Table 7). Diet had no significant effect on total CH₄ emissions, but emissions

Table 3. Chemical composition of the experimental TMR (g/kg of DM unless stated otherwise)

Item	Diet ¹						
	Control	SL	SM	SH	CL	CM	CH
DM, g/kg of feed	464	468	469	473	471	475	484
OM	928	926	924	922	924	920	916
CP	153	173	190	210	170	184	201
NDF	437	432	428	422	439	441	441
pdNDF ²	373	370	368	364	371	370	367
iNDF ³	64	62	60	58	68	71	74
EE ⁴	24	23	22	21	25	27	28
ME, ⁵ MJ/kg of DM	11.66	11.74	11.79	11.87	11.55	11.45	11.35

¹Control = only crimped barley and no protein feed; SL = low level of soybean meal; SM = medium level of soybean meal; SH = high level of soybean meal; CL = low level of canola meal; CM = medium level of canola meal; CH = high level of canola meal.

²pdNDF = potentially digestible NDF. Calculated as NDF - iNDF.

³iNDF = indigestible NDF.

⁴EE = ether extract, values for the silage taken from tables (Spörndly, 2003), those for concentrate ingredients analyzed directly.

⁵ME concentrations in the silages determined following the procedure of Lindgren (1979), calculations for the concentrate ingredients following Spörndly (2003).

Table 4. Mean treatment effects (n = 16) on daily nutrient intake and digestibility between diets containing soybean meal or heat-moisture-treated canola meal

Item	Diet ¹										Significance ²		
	Control	SL	SM	SH	CL	CM	CH	SEM	S vs. C	Lin	PS × Lin		
Intake, kg/d													
Total DM	20.4	20.4	20.7	20.5	20.7	21.3	20.8	0.37	0.06	0.23	0.28		
Silage DM	12.2	12.2	12.5	12.3	12.4	12.9	12.5	0.23	0.08	0.14	0.48		
OM	18.9	18.9	19.1	18.9	19.1	19.6	19.0	0.34	0.15	0.63	0.50		
NDF	8.9	8.8	8.9	8.7	9.1	9.4	9.2	0.16	<0.01	0.73	<0.01		
pdNDF ³	7.6	7.6	7.6	7.5	7.7	7.9	7.6	0.14	0.02	0.82	0.16		
CP	3.1	3.5	3.9	4.3	3.5	3.9	4.2	0.07	0.29	<0.01	0.14		
MP, ⁴ g/d	1,583	1,666	1,759	1,818	1,794	1,997	2,127	33.9	<0.01	<0.01	<0.01		
ME, ⁵ MJ/d	241	244	248	246	244	249	241	4.4	0.63	0.27	0.34		
DMD, ⁶ %	75.2	74.6	76.6	75.9	74.9	74.3	76.3	0.64	0.31	0.18	0.70		
OMD, ⁷ %	77.2	76.5	78.5	77.7	76.8	76.2	78.1	0.66	0.29	0.28	0.58		

¹Control = only crimped barley and no protein feed; SL = low level of soybean meal; SM = medium level of soybean meal; SH = high level of soybean meal; CL = low level of canola meal; CM = medium level of canola meal; CH = high level of canola meal.

²S vs. C = probability of the effects due to protein supplement (soybean meal or canola meal); Lin = linear effect of dietary CP concentration; PS × Lin = interaction between protein supplement and linear dietary CP concentration. Quadratic effect of diet CP concentration was not significant ($P \geq 0.12$); interaction between protein supplement and quadratic dietary CP concentration was not significant ($P \geq 0.25$).

³pdNDF = potentially digestible NDF.

⁴Calculated according to Spörndly (2003).

⁵ME concentrations in the silages determined following the procedure of Lindgren (1979), calculations for the concentrate ingredients following Spörndly (2003).

⁶DMD = apparent DM digestibility (n = 8).

⁷OMD = apparent OM digestibility (n = 8).

Table 5. Mean treatment effects (n = 16) on milk production and N efficiency between diets containing soybean meal or heat-moisture-treated canola meal

Item	Diet ¹										Significance ²				
	Control	SL	SM	SH	CL	CM	CH	SEM	S vs. C	Lin	PS × Lin	SEM	S vs. C	Lin	PS × Lin
Yield															
Milk, kg/d	26.6	27.5	28.0	28.0	28.0	28.9	28.9	0.69	0.04	<0.01	0.14	0.69	0.04	<0.01	0.14
ECM, ³ kg/d	28.6	29.0	30.0	29.5	29.4	30.5	30.7	0.76	0.11	0.01	0.15	0.76	0.11	0.01	0.15
Fat, g/d	1,192	1,186	1,250	1,212	1,206	1,251	1,271	35.9	0.24	0.06	0.24	35.9	0.24	0.06	0.24
Protein, g/d	940	959	969	966	971	1,009	1,013	23.9	0.01	0.01	0.03	23.9	0.01	0.01	0.03
Lactose, g/d	1,272	1,322	1,343	1,351	1,337	1,386	1,378	34.9	0.17	<0.01	0.36	34.9	0.17	<0.01	0.36
Concentration															
Fat, g/kg	45.2	43.0	45.0	43.5	43.1	43.5	43.9	0.85	0.55	0.23	0.90	0.85	0.55	0.23	0.90
Protein, g/kg	35.5	35.0	35.1	34.8	35.0	35.0	35.3	0.53	0.45	0.24	0.24	0.53	0.45	0.24	0.24
Lactose, g/kg	47.9	48.0	48.0	48.2	47.8	47.9	47.6	0.32	0.09	0.79	0.08	0.32	0.09	0.79	0.08
MUN, mg/dL	8.50	10.0	12.6	14.6	9.30	11.0	12.0	0.42	<0.01	<0.01	<0.01	0.42	<0.01	<0.01	<0.01
N efficiency, ⁴ g/kg	301	273	246	225	277	258	244	5.8	<0.01	<0.01	<0.01	5.8	<0.01	<0.01	<0.01
BW, kg	633	632	636	635	632	637	637	12.0	0.47	0.17	0.47	12.0	0.47	0.17	0.47
BCS ⁵	3.2	3.1	3.2	3.2	3.1	3.2	3.1	0.06	0.52	0.90	0.16	0.06	0.52	0.90	0.16

¹Control = only crimped barley and no protein feed; SL = low level of soybean meal; SM = medium level of soybean meal; SH = high level of soybean meal; CL = low level of canola meal; CM = medium level of canola meal; CH = high level of canola meal.

²S vs. C = probability of the effects due to protein supplement (soybean meal or canola meal); Lin = linear effect of dietary CP concentration; PS × Lin = interaction between protein supplement and linear dietary CP concentration. Quadratic effect of diet CP concentration was not significant ($P \geq 0.24$); interaction between protein supplement and quadratic dietary CP concentration was not significant ($P \geq 0.24$).

³ECM calculated according to Sjaunja et al. (1990).

⁴N efficiency = milk N/N intake.

⁵BCS estimated according to Edmonson et al. (1989).

decreased quadratically ($P \leq 0.01$) when expressed per kilogram of DMI, with the lowest values obtained with the medium level of supplemental CP. A tendency for decreased emissions was also observed when total CH_4 emissions were expressed per kilogram of ECM (quadratic $P = 0.06$). Methane emissions per kg ECM decreased more ($P = 0.02$) as dietary CP concentration increased when TCM rather than SBM was used as the protein supplement. A numerical trend ($P = 0.11$) for a greater decrease in CH_4 as grams per kilogram of DMI as dietary CP concentration increased was observed with the TCM diets than the SBM diets. The CH_4/CO_2 ratio tended ($P = 0.08$) to decrease linearly with increased level of protein supplementation. The effects of diet on CO_2 emissions were generally small except for a quadratic effect on CO_2 per kilogram of DMI, with the lowest values with low and medium CP levels ($P = 0.01$).

DISCUSSION

Feed Intake

The aim of this study was to evaluate the production responses and CH_4 emissions in dairy cows fed grass silage-based diets supplemented with either SBM or TCM to give 4 incremental dietary CP concentrations. Intake of DM was not affected by increased dietary CP concentration. This is in agreement with Leonardi et al. (2003) and Olmos Colmenero and Broderick (2006), who increased the dietary CP level from 16.1 to 18.9% and 13.5 to 19.4% (5 levels), respectively. However, it contradicted findings by Broderick (2003), who showed increased DMI with increasing dietary CP concentration. When comparing the SBM and TCM supplements, the latter tended to increase DMI, which is in agreement with similar studies on cows fed grass silage-based diets (Shingfield et al., 2003; Vanhatalo et al., 2003; Rinne et al., 2006). Brito and Broderick (2007) reported significantly greater DMI (0.7 kg/d) for CM compared with SBM in cows fed diets based on alfalfa and corn silage. Huhtanen et al. (2011) suggested that increased DMI when feeding CM compared with SBM could be the result of greater energy demand with CM diets due to improved milk production. They also suggested that greater milk production responses with CM inclusion could be due to increased or more balanced AA supply, or both. Consistent with this, duodenal infusions of casein (Khalili and Huhtanen, 2002) and soybean protein isolates (Faverdin et al., 2003) have been shown to increase DMI. The results obtained with these duodenal infusions further indicate that the stimulating effect of protein on voluntary intake might be due to metabolic rather than physical factors.

Milk Production

Protein Level Response. In the present study, increased dietary CP concentration increased milk, ECM, and milk component yields. However, compared with the study by Shingfield et al. (2003) and the meta-analysis by Huhtanen et al. (2011), the milk and milk protein yield responses were numerically smaller. Although the quadratic effect of CP level was not significant, the responses seem to level out at the highest dietary CP concentration. This could possibly be explained by the greater concentrate CP concentrations fed in our study. Olmos Colmenero and Broderick (2006) reported that milk yield increased from 36.3 kg/d at 13.5% CP to 38.3 kg/d at 16.5%, and then declined to 36.6 and 37.0 kg/d at 17.9 and 19.4%, respectively. Our results also agree with predictions of diminishing milk yield responses with increased dietary CP concentration (NRC, 2001; Ipharraguerre and Clark, 2005).

Response to Protein Supplements. Increased yield of milk and milk protein for TCM diets compared with SBM diets is in agreement with Shingfield et al. (2003) and Vanhatalo et al. (2003) feeding grass-silage-based diets, whereas the parameters were unchanged in the study by Mustafa et al. (1997). Mustafa et al. (1997) fed barley-silage- and barley-grain-based diets supplemented with high-fiber CM, CM, or SBM. Brito and Broderick (2007) showed that supplementing dairy cows with SBM, cottonseed meal, or CM (true protein) instead of urea increased milk production, with CM being more effective than SBM, as also observed in the present study. In a meta-analysis, Martineau et al. (2013) compared the effects on milk production of substituting different feed protein sources, including SBM, with CM and found that when CM was substituted for SBM, milk and milk protein yield increased, as in this study.

One reason for the positive effects of TCM compared with SBM could be its lower ruminal CP degradability. According to tables presented by Spörndly (2003), the TCM used in our study has a ruminal in situ CP degradability value of 0.35, compared with 0.64 for SBM. As a result, calculated MP intake was greater for TCM diets compared with SBM diets. Tuori (1992) concluded that treating protein feeds for dairy cows theoretically improves the supply of RUP, although only a few production studies support this theory (Bertilsson et al., 1994; Jones et al., 2001). Others have showed no production response between CM and TCM (Huhtanen and Heikkilä, 1996; Rinne et al., 1999; Huhtanen et al., 2011). In the meta-analysis by Huhtanen et al. (2011), which was based on 108 (CM) and 39 (TCM) treatment means, milk protein yield responses were similar (136 and 133 g/kg incremental increase in CP intake for CM

Table 6. Mean treatment effects (n = 8) on plasma AA concentration (μmol/L) between diets containing soybean meal and heat-moisture-treated canola meal

Item	Diet ¹										Significance ²			
	Control	SL	SM	SH	CL	CM	CH	SEM	S vs. C	Lin	Quad	PS × Lin	PS × Q	
Arg	83	97	117	115	95	115	106	7.8	0.41	<0.01	0.07	0.41	0.81	
His	32	44	56	60	43	59	58	4.3	0.98	<0.01	0.07	0.86	0.54	
Ile	130	144	167	168	141	162	162	9.4	0.50	<0.01	0.36	0.60	0.93	
Leu	115	142	166	180	131	164	171	8.9	0.30	<0.01	0.39	0.66	0.85	
Lys	78	88	100	100	87	102	96	7.1	0.87	0.01	0.29	0.80	0.77	
Met	21	25	24	23	22	27	24	2.3	0.98	0.30	0.20	0.39	0.85	
Phe	51	60	62	61	52	58	57	2.8	0.02	0.01	0.16	0.41	0.26	
Thr	119	125	125	125	124	141	121	7.9	0.56	0.45	0.18	0.95	0.27	
Trp	45	50	48	44	45	49	47	2.9	0.70	0.73	0.15	0.29	0.28	
Val	236	262	307	333	264	313	326	13.5	0.95	<0.01	0.65	0.77	0.58	
Ala	239	259	229	238	230	245	227	16.7	0.39	0.54	0.56	0.81	0.95	
Asn	88	101	105	106	90	103	93	6.6	0.07	0.07	0.22	0.28	0.95	
Asp	21	20	19	20	20	20	21	0.7	0.28	0.42	0.17	0.49	0.59	
Cys	11	12	13	12	13	14	12	1.1	0.53	0.31	0.13	0.95	0.44	
Gln	300	276	278	272	258	264	271	11.9	0.22	0.05	0.05	0.99	0.25	
Glu	36	32	31	34	35	34	32	1.9	0.14	0.12	0.24	0.59	0.03	
Gly	367	380	349	324	358	347	396	30.1	0.43	0.66	0.80	0.05	0.11	
3-MH ³	5.7	5.7	5.1	4.5	5.5	5.5	4.7	0.26	0.43	<0.01	0.05	0.21	0.93	
Orn	42	45	54	57	45	57	54	3.5	0.99	<0.01	0.35	0.53	0.28	
Pro	99	107	111	111	96	107	98	4.3	<0.01	0.09	0.15	0.05	0.75	
Ser	99	111	103	106	102	100	96	6.5	0.10	0.96	0.35	0.39	0.82	
Tyr	45	55	60	61	47	59	53	4.1	0.08	0.01	0.16	0.35	0.91	
BCAA ⁴	480	549	639	681	536	641	659	29.0	0.60	<0.01	0.44	0.68	0.88	
EAA ⁵	911	1,044	1,178	1,226	1,008	1,206	1,172	50.5	0.60	<0.01	0.17	0.67	0.70	
NEAA ⁶	1,304	1,348	1,284	1,269	1,245	1,283	1,292	59.8	0.44	0.62	0.95	0.40	0.23	
TAA ⁷	2,214	2,393	2,474	2,487	2,254	2,482	2,467	93.5	0.45	0.01	0.39	0.82	0.58	

¹Control = only crimped barley and no protein feed; SL = low level of soybean meal; SH = high level of soybean meal; SM = medium level of soybean meal; CH = high level of canola meal; CM = medium level of canola meal; CL = low level of canola meal.

²S vs. C = probability of the effects due to protein supplement (soybean meal or canola meal); Lin = linear effect of dietary CP concentration; Quad = quadratic effect of dietary CP concentration; PS × Lin = interaction between protein supplement and linear diet CP concentration; PS × Q = interaction between protein supplement and quadratic dietary CP concentration.

³3-MH = 3-methyl histidine.

⁴BCAA = branched-chain amino acids (Ile + Leu + Val).

⁵EAA = essential amino acids (Arg + His + Ile + Leu + Lys + Met + Phe + Thr + Tyr + Val).

⁶NEAA = nonessential amino acids (Ala + Asn + Asp + Cys + Gln + Glu + Gly + Pro + Ser + Trp).

⁷TAA = total amino acids (EAA + NEAA).

Table 7. Mean treatment effects on methane and carbon dioxide emission between diets containing soybean meal or heat-moisture-treated canola meal

Item	Diet ¹										Significance ²			
	Control	SL	SM	SH	CL	CM	CH	SEM	S vs. C	Lin	Quad	PS × Lin		
n ³	7	8	6	7	7	7	7							
CH ₄	463	449	447	461	458	447	456	17.0	0.92	0.72	0.30	0.72		
g/d	24.1	23.0	21.9	24.1	22.6	22.6	22.5	0.63	0.31	0.12	<0.01	0.11		
g/kg of DMI	17.5	16.9	15.9	17.8	16.9	16.1	15.8	0.71	0.15	0.14	0.06	0.02		
g/kg of ECM														
CO ₂	11,300	11,100	11,100	11,400	11,300	11,500	11,700	280.0	0.14	0.28	0.21	0.26		
g/d	591	571	550	594	561	581	581	15.3	0.83	0.81	0.01	0.98		
g/kg of DMI	430	418	403	440	420	413	409	19.5	0.60	0.64	0.16	0.19		
g/kg of ECM	41.0	40.2	40.0	40.2	40.7	39.0	38.8	0.82	0.36	0.08	0.59	0.15		
CH ₄ /CO ₂ , g/kg														

¹Control = only crimped barley and no protein feed; SL = low level of soybean meal; SM = medium level of soybean meal; SH = high level of soybean meal; CL = low level of canola meal; CM = medium level of canola meal; CH = high level of canola meal.

²S vs. C = probability of the effects due to protein supplement (soybean meal or canola meal); Lin = linear effect of dietary CP concentration; Quad = quadratic effect of dietary CP concentration; PS × Lin = interaction between protein supplement and linear dietary CP concentration. Interaction between protein supplement and quadratic diet CP concentration was not significant ($P \geq 0.10$).

³Number of observations. Three cows did not visit the Greenfeed units.

and TCM, respectively). Additionally, Huhtanen and Hristov (2009) pointed out that rumen CP degradability probably has less effect on production than generally assumed. They evaluated diets in a meta-analysis and showed that TDN and CP intake predicted milk protein yield accurately, but on including CP degradability in the model as a third variable, the milk protein yield prediction was only slightly improved. Small production responses to increased RUP could also be related to potentially lower intestinal digestibility and decreased microbial protein flow. Broderick et al. (2010) compared regressions of observed omasal flow of RUP with flows predicted by the NRC (2001) model and found that the NRC predictions of RUP were 22% greater. They speculated that the overestimation of RUP in the NRC (2001) model may be related to shortcomings in the in situ methodology. Additionally, it seems likely that reduced ruminal protein degradability could decrease microbial protein synthesis, and hence decrease the feeding value. In a literature review, Santos et al. (1998) concluded that replacing SBM in dairy cow diets with high RUP supplements (treated SBM among others) decreases the flow of microbial N due to a suggested shortage of RDP limiting microbial synthesis. A meta-analysis by Ipharraguerre and Clark (2005) and a rumen metabolism study (S. J. Krizsan, H. Gidlund, F. Fatehi, P. Huhtanen, Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, Umeå, Sweden, unpublished) both reported that reduced ruminal CP degradability decreased the flow of microbial N to the small intestine. Earlier studies (Hoover and Stokes, 1991; Clark et al., 1992; Firkins, 1996) attributed this decrease to lack of energy, AA, peptides, or ammonia in the rumen inhibiting the microbial growth when RDP sources are replaced with RUP supplements.

Nitrogen Efficiency and Milk Urea Nitrogen

The decreased N efficiency with increasing dietary CP concentration was expected (Shingfield et al., 2003; Olmos Colmenero and Broderick, 2006). The increased MUN concentration due to incremental dietary CP concentrations in our study corresponded well to findings by Nousiainen et al. (2004) that dietary CP concentration is the most important nutritional factor influencing MUN. Nousiainen et al. (2004) also showed that MUN can predict urinary N excretion accurately, resulting in greater urinary N concentration with higher dietary CP concentration. From this, it can be concluded that urinary N losses would increase for the diets with increased CP concentration in the present study.

In this study, feeding TCM promoted greater N efficiency than feeding SBM. The range in N efficiency (23

to 27% for SBM diets and 24 to 28% for TCM diets) was wider than reported by Shingfield et al. (2003; 26 to 28% for SBM diets and 27 to 29% for heat-moisture canola expeller diets), probably because of the wider ranges of dietary CP concentration and small milk protein yield responses to supplementary protein in the present study. Brito and Broderick (2007), on the other hand, did not find differences in N efficiency between CM and SBM diets containing 16.6% CP. In our study, differences in N efficiency were closely related to MUN concentration, which was lower with TCM diets compared with SBM diets. A similar relationship was reported in studies by Shingfield et al. (2003) and Vanhatalo et al. (2003). In contrast, Brito and Broderick (2007) found no differences in MUN between CM and SBM diets. The greater N efficiency and lower MUN with TCM compared with SBM diets in the present study could be partly connected to the overall lower dietary CP concentration of the TCM diets used. However, with the TCM diets the increase in MUN and decrease in N efficiency per 1 g/kg of DM increase in dietary CP concentration was 67 and 86% of the corresponding values with the SBM diets. This suggests that the differences in MUN and N efficiency were not entirely related to the higher CP concentrations of the SBM diets. Lower concentrations of MUN and plasma urea with canola supplements compared with isonitrogenous SBM supplements (Shingfield et al., 2003) support this suggestion. Martineau et al. (2014) reported lower concentrations of MUN and BUN with CM compared with other protein supplements.

Plasma Amino Acids

As expected, increasing the dietary CP concentration by replacing grain with SBM or TCM increased the plasma concentrations of TAA, BCAA, EAA, and His, which agrees with similar studies (Shingfield et al., 2003; Vanhatalo et al., 2003). However the lack of difference observed here in plasma His and total EAA concentrations between the SBM and TCM diets contradict earlier observations (Shingfield et al., 2003; Vanhatalo et al., 2003; Martineau et al., 2014). Shingfield et al. (2003) found positive responses in EAA, BCAA, and most AA, including His, Leu, and Met, for heat-moisture-treated canola expeller compared with SBM. A meta-analysis by Martineau et al. (2014) concluded that a CM supplement increases plasma EAA concentrations, including His, Lys, and Met, more than other protein sources. Greater milk protein yield with CM feeding compared with SBM feeding has been associated with higher plasma His (Shingfield et al., 2003; Vanhatalo et al., 2003), suggesting that the supply of His increases when canola feeds are substituted for SBM. Histidine

is probably the first limiting AA in microbial protein (Fraser et al., 1991), and microbial protein is a more important source of AA for milk protein synthesis when feeding grass silage-based diets than, for example, corn-based diets. Postruminal infusion studies suggest that His, rather than Met or Lys, is the first limiting AA in cows fed grass-silage- and cereal-based diets (Vanhatalo et al., 1999). Because plasma EAA concentrations are direct measurements, whereas MP supply is estimated, it appears that only minor differences were present in the supply of absorbed AA between the 2 protein supplements. Infusion studies (Korhonen et al., 2000; Kim et al., 2001) have demonstrated that plasma His concentrations are very responsive to changes in the supply of AA from the small intestine. A close relationship was present between dietary CP concentration and plasma His concentration in the present study and in that by Shingfield et al. (2003; Figure 1). Interestingly, the slope regression line was lower in the present study (0.59 $\mu\text{mol/L}$ per g of CP/kg of DM) than reported by Shingfield et al. (2003; 0.93 $\mu\text{mol/L}$ per g of CP/kg of DM), but the corresponding slopes were similar for SBM. When the relationship between milk protein yield and plasma His concentration in our study was compared with that reported for diets supplemented with heat-moisture-treated canola expeller by Shingfield et al. (2003), the relationship was similar for the canola diets (Figure 1). Based on this indirect comparison of milk protein yield between the 2 studies, it can be concluded that feeding TCM rather than heat-moisture-treated canola expeller has no benefit. According to the tables of Spörndly (2003), the ruminal protein degradability of the TCM used in the present study is 0.35, whereas the estimated value (MTT, 2014) was much higher (0.63) for the heat-moisture-treated canola expeller used by Shingfield et al. (2003). Small milk protein yield (0.15 g/g) and plasma AA responses to incremental MP suggest that the MP value of TCM used in the present study was overestimated.

Gas Emissions

Methane emissions as a proportion of gross energy intake were similar to values reported by Ferris et al. (1999) and Yan et al. (2000) for cows fed highly digestible grass silage-based diets.

The most obvious reason for reduced CH_4 emissions per unit intake with increased dietary CP concentration is that ruminal fermentation of protein produces less CH_4 than ruminal fermentation of carbohydrates. According to stoichiometric equations developed by Bannink et al. (2006) and Sveinbjörnsson et al. (2006), protein fermentation produces approximately 30 to 50% less CH_4 than fermentation of carbohydrates. The

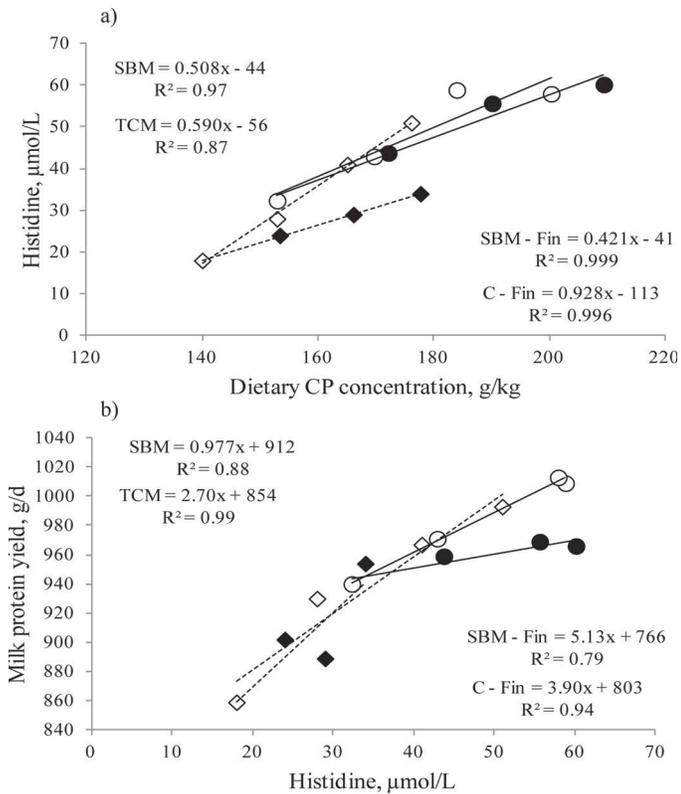


Figure 1. (a) Relationship between increasing diet CP concentration (g/kg) and plasma concentration of His ($\mu\text{mol/L}$). (b) Relationship between plasma His concentration ($\mu\text{mol/L}$) and daily milk protein yield (g/d). Comparison of diets (—) that included soybean meal (SBM; ●) or heat-moisture-treated canola meal (TCM; ○), with diets (---) from a similar study (Shingfield et al., 2003) including soybean meal (SBM - Fin; ◆) or heat-moisture-treated canola expeller (C - Fin; ◇).

decreases in CH_4 per kilogram of DMI were slightly greater for the TCM diets than predicted by the empirical model developed by Ramin and Huhtanen (2013) from respiration chamber data. A rumen metabolism study on cows fed 4 diets based on grass silage supplemented with crimped barley with graded levels replaced with TCM provide supporting evidence of reduced CH_4 emissions with increased dietary CP concentration (S. J. Krizsan, H. Gidlund, F. Fatehi, P. Huhtanen, Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, Umeå, Sweden, unpublished). The low (about 45%) ruminal CP degradability of TCM reduced the amount of substrate digested in the rumen. Reduced CH_4 emissions per kilogram of DMI with increased TCM supplementation can also partly be associated with increased molar proportion of propionate in rumen VFA (S. J. Krizsan, H. Gidlund, F. Fatehi, P. Huhtanen, Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, Umeå, Swe-

den, unpublished). When calculated according to the stoichiometric equation developed by Wolin (1960) CH_4 emissions decreased linearly from 366 to 352 mmol/mol VFA. On the other hand, reduced efficiency of microbial protein synthesis with increased dietary CP concentration (S. J. Krizsan, H. Gidlund, F. Fatehi, P. Huhtanen, Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, Umeå, Sweden, unpublished) could decrease the contribution of microbes as a H_2 sink.

Moss and Givens (2002) reported an approximately 10% decrease in CH_4 emissions per kilogram of OM apparently digested in sheep when grass silage was gradually replaced with SBM. Greater decreases in CH_4 emissions were reported by Blaxter et al. (1971) when the CP concentration of dried grass was manipulated by increased levels of N fertilization. However, in that case, part of the response may have been due to increased levels of nitrate in grasses with high CP concentration.

Greater decreases in CH_4 emissions were observed when expressed per kilogram of ECM. This is due to the positive effects of protein supplementation on dairy cow performance. The greater decreases with the TCM compared with the SBM diets are related to greater production responses to supplementary CP with the TCM diets relative to the SBM diets.

Emissions of CO_2 per kilogram of ECM were on average 4% lower at low and medium levels of protein supplementation, but the effect was not significant. This is consistent with the higher efficiency of utilization of incremental ME in protein supplementation studies compared with the mean response for all data (0.18 vs. 0.11 kg of ECM/MJ of ME) reported in a meta-analysis on milk production responses by Huhtanen and Nousiainen (2012). Energy balance calculations in a whole lactation study (Law et al., 2009) also indicated small improvements in the efficiency of ME utilization when feeding optimal levels of protein.

The decreased CH_4 emissions per kilogram of ECM, especially with TCM, were related to slightly smaller emissions per kilogram of DMI and partly to increased ECM yield. This agrees with Yan et al. (2010), who showed that CH_4 energy output as a proportion of energy intake and milk yield is reduced by increasing milk yield and energy efficiency. However, the overall potential to mitigate CH_4 and especially total greenhouse gas emissions by increased protein supplementation is small. With increased dietary protein supplementation, other negative environmental effects emerge due to increased N emissions. Increased protein supplementation is also often associated with increased output of manure P, as protein feeds generally have higher P concentrations than other dietary components.

CONCLUSIONS

Heat-moisture-treated canola meal increased milk and milk protein yield, decreased MUN concentration, and improved protein utilization compared with SBM supplementation. Compared with SBM, TCM supplementation also resulted in a greater decrease in CH₄ emissions per kilogram of ECM. The production responses to dietary CP concentration were smaller than expected, possibly partly as a result of increased ruminal microbial protein synthesis due to TMR feeding. Thus, the potential to decrease CH₄ emissions by increased protein supplementation can be considered small, and it risks increasing other environmental effects (e.g., N emissions). We concluded that SBM can be successfully replaced with CM in the diet of dairy cows fed grass-silage-based diets.

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