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Effect of substitution of soybean meal by canola meal or distillers grains in dairy rations on amino acid and glucose availability

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ABSTRACT

Canola meal (CM) or by-products of ethanol production (dried distillers grain, DDG) may offer an economical alternative to soybean meal (SBM) in North American dairy rations. These protein supplements can effectively replace SBM and, in 2 recent meta-analyses, CM had a positive effect on milk and milk protein yields compared with SBM. The objective of this study was to determine if the positive responses observed with inclusion of CM in dairy rations could be explained by an increased availability of His, Lys, Met, or glucose. Eight Holstein dairy cows were used in a replicated 4 × 4 Latin square with 14-d periods. Cows were fed isonitrogenous (17.2% crude protein) and isoenergetic (1.56 Mcal/kg of net energy of lactation) diets formulated to slightly exceed nutrient requirements. Diets contained 38% grass hay and 62% corn-based concentrate including SBM, CM, corn high-protein DDG (HPDDG), or wheat DDG plus solubles (WDDGS) as the single protein supplement. The effect of protein supplements on availability of His, Lys, Met, and glucose was estimated using variations in the whole-body (WB) flux of these nutrients, determined by isotopic dilution. As planned, dry matter intake and milk and milk protein yields were not affected by treatments and averaged 23.7, 31.4, and 1.14 kg/d, respectively. Lactose yield did not differ among diets although milk lactose content tended to be lower with CM and WDDGS diets than with SBM and HPDDG diets. Lysine availability was affected by treatments: the highest WB irreversible loss rate (ILR) was observed for the CM diet (371 g/d) and the lowest for HPDDG diet (290 g/d); values for SBM and WDDGS were intermediate (330 and 316 g/d, respectively). Availability of His and Met did not vary among diets and WB ILR averaged, respectively, 129 and 124 g/d; the CM diet, however, had numerically the highest His and Met ILR. Plasma concentrations of most of the essential AA were higher with the CM diet and lower with the HPDDG diet, the exception being Leu for which the concentration was highest for the HPDDG diet. Glucose WB rate of appearance was altered by diet, with the highest mean observed for SBM (3,036 g/d) and the lowest for CM (2,795 g/d); the 2 diets with the lowest WB glucose rate of appearance (CM and WDDGS) also had the lowest dietary starch concentration. Overall, this study suggested that positive responses in milk and milk protein yields observed with inclusion of CM in dairy rations could be linked to a greater supply of metabolizable protein, including some essential AA, especially His, Lys, and Met, as glucose availability was certainly not increased in cows fed the CM diet.

Key words: protein supplement, canola meal, amino acid, glucose

INTRODUCTION

Different protein supplements such as canola meal (**CM**) or dried distillers grains (**DDG**) are now widely used in North American dairy rations as an economical alternative to soybean meal (**SBM**). In western Canada, CM is the principal protein supplement included in dairy rations because it is locally available and contains adequate protein concentration and AA profile for dairy cattle nutrition (Hickling, 2008; Newkirk, 2009). With continued expansion of the ethanol industry in North America, DDG is becoming a common feed ingredient in cattle, used to supply both protein and energy (Schingoethe et al., 2009; Zhang et al., 2010). Moreover, changes in the fractionation process to improve the fermentation of corn to ethanol have resulted in new products, such as high-protein DDG, which is closer in nutrient composition (higher in CP and lower in fat) to CM and SBM than conventional DDG (Robinson et al., 2008; Schingoethe et al., 2009). Although corn is the most common grain used in ethanol production, wheat is predominantly used in western Canada. Wheat DDG usually has a composition similar to CM or high-protein DDG (approximately 40% CP and 4% fat; Boila and Ingalls, 1994; Li et al., 2012).

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Over the last few years, several studies have compared lactation performances of dairy cows fed rations including SBM, CM, corn high-protein DDG (**HP-DDG**), or wheat DDG with solubles (**WDDGS**; e.g., Kelzer et al., 2009; Christen et al., 2010; Chibisa et al., 2012). These studies concluded that feeding these protein supplements may be as effective as feeding SBM to lactating dairy cows. Moreover, a recent meta-analysis (Huhtanen et al., 2011) observed that milk and milk protein responses were higher with incremental levels of inclusion of CM in diets compared with inclusion of SBM. Another recent meta-analysis (Martineau et al., 2013) reported positive responses in milk and milk protein yields when CM replaced different protein supplements, the response in milk protein yield being higher in experiments in which CM replaced protein supplements other than SBM, as DDG. These positive responses in milk and milk protein yields with CM might be related to a more efficient N metabolism with improved microbial protein synthesis, a greater supply of MP from the RUP fraction, including some essential AA (especially His, Lys, and Met), or both. Indeed, Lys content in corn DDG is low and might limit milk and milk protein synthesis (Nichols et al., 1998), whereas Met is often the first-limiting AA in SBM-based diets (Illg et al., 1987).

The increase in milk yield is usually paralleled by an increase in lactose secretion because milk yield depends on mammary lactose synthesis through osmotic regulation (Linzell and Peaker, 1971). Indeed, Martineau et al. (2013) reported an increase in milk lactose yield with inclusion of CM in grass or legume forage-based diets. As glucose would be the main precursor for lactose (Bickerstaffe and Annison, 1974), increased milk lactose yield with inclusion of CM in dairy rations may reflect increased whole-body (**WB**) glucose availability.

We hypothesized that the positive responses of milk and milk protein yields to CM substitution were due to increased availability of His, Lys, Met, or glucose. Thus, the objective of this study was to compare the effects of feeding lactating cows with diets containing SBM, CM, HPPDG, or WDDGS as the single protein supplement, on N utilization and WB availability of His, Lys, Met, and glucose.

MATERIALS AND METHODS

Cows and Treatments

Eight rumen-fistulated Holstein cows, averaging 730 ± 43.2 kg of BW and 206 ± 29.4 DIM at the beginning of the study, were used for the experiment. The study was designed as a replicated 4 × 4 Latin square balanced for residual effects, with 14-d experimental periods. The cows were housed in tie-stalls and milked twice daily at 12-h intervals. The experimental protocol was approved by the Institutional Animal Care Committee of the Sherbrooke Dairy and Swine Research and Development Centre and animals were treated according to the Canadian Council on Animal Care (1993) guidelines.

Dietary treatments consisted of inclusion of different protein supplements in the diet: solvent-extracted SBM (Vita Plus, Lake Mills, WI), solvent-extracted CM (Bunge, Harrowby, MB, Canada), HPDDG (Poet's, Albert Lea, MN), and WDDGS (Terra Grain Fuel, Belle Plaine, SK, Canada). The chemical compositions of these protein supplements have been previously reported (Maxin et al., 2013). The 4 diets were formulated to be isonitrogenous at 17.2% CP and isoenergetic at 1.56 Mcal of NE_L/kg of DM (NRC, 2001), with a fixed forage-to-concentrate ratio of 62:38 (DM basis, Table 1). Importantly, all diets were formulated to meet or exceed the nutrient requirements according to NRC (2001), this being a prerequisite for the utilization of the method described below to assess variation in AA availability (Borucki Castro et al., 2008). Cows were individually fed at 95% of ad libitum intake, the latter being determined the week before the initiation of the experiment. To achieve steady-state conditions, the diets were offered in 12 equal meals (at 2-h intervals) using automated feeders (Ankom Technology, Fairport, NY).

Measurements and Sampling

Feed intake was recorded daily. Samples of hay, concentrates, and mixed diets were collected weekly and dried in a forced-air oven at 55°C for 24 h to determine DM content. Then, samples were pooled by period and ground to pass a 1-mm screen before analyses. Milk production was recorded at each milking, and milk samples were collected on the last 6 milkings of each period to analyze milk composition on each sample.

Total urine excretion was collected from d 10 to 13. Urine was collected in stainless steel containers via a Gooch tube (BF Goodrich Co., Kitchener, ON, Canada) attached to the vulva of the cow with nylon netting covered with neoprene (Spall Bowan Ltd., Guelph, ON, Canada). Urine was acidified twice a day with 88 mL/d of concentrated H_2SO_4, and representative daily samples were frozen until analyses.

The availability of His, Lys, and Met was estimated using the variation in the WB irreversible loss rate (**ILR**) of each AA, determined by the isotope dilution technique following a pulse dose of labeled AA. This technique offers a reliable and noninvasive method to estimate changes in the availability of essential AA, as

Table 1. Ingredients and chemical composition of the diets

Item	Diet[1]			
	SBM	CM	HPDDG	WDDGS
Ingredient (% of DM)				
Grass hay[2]	38.0	38.0	38.0	38.0
SBM[2]	13.7			
CM[2]		20.8		
HPDDG[2]			20.4	
WDDGS[2]				22.8
Wheat,[2] ground	6.4	5.9	5.9	5.9
Corn,[2] ground	28.7	24.1	24.8	21.9
Beet pulp[2]	7.2	6.2	6.2	6.2
Soybean hulls[2]	3.3	2.2	2.1	2.1
Ca-salts of fatty acids[3]	0.5	1.5	0.5	1.2
Vitamins and minerals	2.4	1.6	2.4	2.1
Chemical composition (% of DM)				
CP	17.1	17.5	17.4	17.4
NDF	32.5	36.3	35.1	35.8
ADF	19.1	21.8	19.8	20.4
Fat	2.6	3.9	3.0	4.0
Starch	20.6	17.5	19.0	16.5
NE_L[4] (Mcal/kg)	1.61	1.60	1.61	1.61

[1]SBM = soybean meal; CM = canola meal; HPDDG = high-protein corn dried distillers grain; WDDGS = wheat dried distillers grain with solubles.

[2]The detailed chemical composition of the 4 protein supplements was previously described in Maxin et al. (2013). Grass hay: 13.2% CP, 55.7% NDF, 33.2% ADF, and 2.2% fat; wheat: 20.3% CP, 39.2% NDF, 14.4% ADF, and 2.9% fat; corn: 8.7% CP, 9.9% NDF, 3.4% ADF, and 3.1% fat; beet pulp: 9.0% CP, 38.3% NDF, 27.9% ADF, and 0% fat; soyhulls: 10.9% CP, 67.4% NDF, 48.7% ADF, and 1.1% fat.

[3]Megalac (Church & Dwight Co. Inc., Princeton, NJ).

[4]Calculated according to NRC (2001).

previously validated by Borucki Castro et al. (2008). The glucose availability was estimated through WB rate of appearance (**Ra**) of glucose by isotopic dilution using a continuous infusion of labeled glucose (Lemosquet et al., 2004).

The tracer infusions were performed on the last day of each period. The day before the beginning of the infusions of the first period, 2 catheters were inserted into the 2 contralateral jugulars veins, one to perform infusions and the other to collect blood; these were replaced later only if patency was lost. On the day of sampling, a blood sample was collected 5 min before the 0800 h meal to determine the basal AA and glucose plasma concentrations and isotopic natural abundance. Then, to estimate the WB ILR of His, Lys, and Met, a mixture of 0.18 g of L-[$^{15}N_3$]His, 0.55 g of L-[α-^{15}N]Lys, and 0.12 g of L-[1-^{13}C]Met (Cambridge Isotopes Laboratories, Andover, MA), dissolved in 5 mL of sterile saline, was administered as a pulse dose into one jugular vein. Blood samples were then collected from the contralateral jugular vein at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 16, 19, 25, and 31 min after the bolus injection. Blood samples were kept on ice before being centrifuged at 1,800 × *g* for 15 min, and harvested plasma was frozen at −80°C for subsequent analysis of His, Lys, and Met isotopic enrichment (**IE**).

Starting at 1330 h, D-[6,6-$^2H_2$]glucose (Cambridge Isotope Laboratories) was infused continuously (3.12 ± 0.09 g/h) into one jugular vein for 120 min with a syringe pump, preceded by a priming dose of 1.52 g. Tracer was dissolved in sterile saline. Blood samples were collected at 3, 30, 60, 75, 90, 105, and 120 min after administration of the priming dose to determine plasma glucose concentration and IE. Additional blood was collected at 3, 30, 60, 90, and 120 min to determine plasma AA and urea concentrations. Blood samples were kept on ice before centrifugation at 1,800 × *g* for 15 min. To determine plasma AA concentration, 0.2 g of an internal standard consisting of a mixture of labeled AA (Doepel and Lapierre, 2010) was added to 1 g of plasma. The plasma was then stored at −80°C for further analysis.

To test the accuracy of the method used to determine AA availability, at the end of the main study, all cows were fed the SBM diet and received a 7-d continuous abomasal infusion of Lys (54.2 ± 2.0 g/d) and Met (20.0 ± 0.7 g/d). The AA were dissolved in hot tap water every 2 or 3 d and were infused continuously through a peristaltic pump in a daily volume of 6 L/cow for the duration of this extra period. On the last day of infusion, WB ILR of Lys and Met was determined using the procedure described above. Histidine

could not be included in the validation of the method because of budget constraints.

Analytical Methods

Samples of feed ingredients were analyzed for N, NDF, ADF, starch, and fat as described in Maxin et al. (2013). Milk composition (protein, fat, lactose, and urea) was determined by near infrared reflectance spectrometry (Valacta, Sainte-Anne-de-Bellevue, QC, Canada). Total urinary-N was determined by combustion (Dumas method, Rapid N Cube, Elementar Analysensystem GmbH, Hanau, Germany). Urinary purine derivatives (**PD**) and creatinine were analyzed by using an HPLC instrument (model 210, Varian ProStar, Agilent Technologies, Palo Alto, CA) according to the procedures of Shingfield and Offer (1999). Urinary PD excretion, including milk excretion assuming a concentration of 1 m*M*, was used to estimate the microbial CP duodenal flow according to the equation of Chen and Gomes (1992). Urinary urea-N concentration was determined colorimetrically with an automatic analyzer (Technicon Autoanalyser II, Technicon Instruments Corp., Tarrytown, NY) using the diacetyl monoxime method as described previously by Huntington (1984).

Glucose IE was analyzed through monitoring of ions 244/242, as described by Galindo et al. (2011). Glucose concentration was determined by colorimetry (Genzyme Diagnostics, BioPacific Diagnostic Inc., Vancouver, BC, Canada). The IE of the infused AA were analyzed through monitoring of ions 443/440, 432/431, and 321/320, for His, Lys, and Met, respectively, and the concentrations of plasma AA were determined by the isotope dilution method of Calder et al. (1999). For both IE and AA concentration samples, plasma were deproteinized with sulfosalicylic acid (38%) and centrifuged at 16,200 × *g* for 15 min. Supernatants were eluted through a poly-prep chromatography column (Resin 100-200 mesh H, Bio-Rad, Hercules, CA) and derivatized with *N*-(tert-butyldimethylsilyl)-*N*-methyltrifluoroacetamide and dimethylformamide (Sigma-Aldrich, Oakville, ON, Canada) in a 1:1 ratio. Measurements of IE in processed samples were performed using GC-MS (model CG6890-MS5973, Hewlett Packard Co., Wilmington, DE). Plasma urea-N concentrations were measured as described for urinary samples.

Calculations and Statistical Analysis

His, Lys, and Met WB ILR. The hypothesis—that variations in the WB ILR of an essential AA would reflect changes in absorbed essential AA and thus changes in essential AA availability among diets—was based on the assumption that, for adult animals fed to requirements under steady-state conditions, additional supply of one nonlimiting essential AA does not alter protein synthesis or protein breakdown. Then, based on the flux equation of Waterlow et al. (1978), if WB essential AA ILR = protein breakdown + absorption = protein synthesis + oxidation, variation in WB ILR would estimate variation in absorption. The WB ILR of His, Lys, and Met were calculated from plasma IE decay curve of the 3 AA as described by Holtrop et al. (2004; Table A1 and Figure A1 of Appendix). On 4 occasions (of a total of 40) when using the pulse dose technique (due to sampling difficulties associated with catheter patency and the short time intervals between the first blood samples), the first points of the IE decay curve were missing, preventing adequate estimation of 4 WB ILR of His, Lys, and Met. To complete the IE decay curve and allow calculation of ILR for these data, IE at time (t) = 0 were estimated for the 3 AA from plasma concentration of each AA, the dose of each AA administered, and the pool size of the cow of each AA using the following equations:

$$\mathrm{IE}_{t=0} = \frac{\mathrm{Dose}}{\mathrm{PS}},$$

$$\mathrm{PS} = [\mathrm{AA}] \times \mathrm{V},$$

where Dose is the quantity (mmol) of the labeled AA administered in the pulse dose; PS is the pool size (mmol) of the AA in the cow; [AA] is the AA plasma concentration (m*M*) measured for individual cow; and V is the distribution volume (L) of the respective AA, expressed as percent of BW, and was calculated as the average distribution volume of the cows with no missing blood samples.

Glucose WB Ra. The WB Ra of glucose was calculated using a non-steady-state model (Brockman, 1984) because, as expected, the plasma IE of glucose did not reach plateau at the first sampling times. The WB Ra of glucose for each cow × period was estimated as the average of the following equation calculated for each sampling interval, from 30 to 120 min of the glucose infusion period:

$$\mathrm{WB\ Ra} = \frac{\mathrm{F} - \mathrm{p} \times \mathrm{V} \times [\mathrm{G}] \times \Delta\mathrm{IE}/\Delta\mathrm{t}}{\mathrm{IE}} - \mathrm{F},$$

where WB Ra is expressed in mmol/h; F is the rate of infusion (mmol/h); [G] and IE are, respectively, the means of plasma concentration (m*M*) and IE of glucose over the time interval; ΔIE is the variation of plasma IE of glucose and Δt is the time interval for which Ra was calculated; V is the distribution volume of glucose

(L), and p is a pool fraction equal to 0.65, as proposed by Brockman (1984). The distribution volume was estimated from the priming dose (mmol), the IE at time = 3 min, and the glucose plasma concentration; the mean value of volumes across periods was used for each cow with the following equation:

$$V = \frac{\text{Priming dose}}{IE_{t=3\ min} \times [G]}.$$

Statistical Analysis. Dry matter intake and milk yield were averaged over the last 5 d of each period and milk composition over the last 6 milkings. One cow suffered from diarrhea during periods 1 and 4 and another had metabolic disorder during period 4, unrelated to treatments. Data from these cows were excluded from the statistical analysis. Data were analyzed as a replicated 4 × 4 Latin square using the MIXED procedure in SAS software (version 9.1, SAS Institute Inc., Cary, NC). The statistical model included cow (random effect), period, treatment (fixed effects), and residual error. Multiple comparisons of means were performed with an adjusted Tukey-Kramer test.

RESULTS

Diet Composition, Intake, Milk Production, and Composition

The analyzed nutrient composition of the experimental diets is presented in Table 1. All diets were slightly higher in net energy (1.61 Mcal/kg of DM) than formulated. The CP concentrations of the diets were slightly higher (17.4%) than anticipated (17.2%) except for the SBM diet (17.1%). The starch concentration of SBM and HPDDG diets (20.6 and 19.0%, respectively) was higher than that in the CM and WDDGS diets (17.5 and 16.5%, respectively). These 2 diets had higher fat concentrations (3.9 and 4.0% for CM and WDDGS diets vs. 2.6 and 3.0% for SBM and HPDDG diets), because of the addition of Ca-salts of fatty acids to obtain isoenergetic diets.

Dry matter and N intake, milk production, and ECM did not differ ($P > 0.40$) between diets and averaged 23.7 kg/d, 660 g/d, 31.4 kg/d, and 30.6 kg/d, respectively (Table 2). No significant effect of diets was observed for protein, fat, or lactose yields. Milk fat percentage was, however, higher ($P = 0.01$) with the WDDGS diet (3.96%) than with HPDDG diet (3.64%), with SBM and CM diets (3.70 and 3.75%) showing intermediate values. Milk protein percentage did not differ ($P = 0.39$) between diets, whereas milk lactose percentage tended ($P = 0.06$) to be higher with SBM and HPDDG diets than with the CM and WDDGS diets. Milk urea-N was lower for the CM diet (9.7 mg/dL; $P = 0.01$) than with the other diets (an average of 11.5 mg/dL). The overall efficiency of utilization of N (milk N/N intake ratio) was not affected ($P = 0.52$) by treatments and averaged 0.27.

Urinary Excretion

Total urinary-N excretion (expressed in g/d or in % of N intake, Table 3) did not differ ($P > 0.14$) among diets, even though values were numerically lower for CM diets. On average, 27% of N intake was excreted in urine. Urinary urea-N excretion (expressed in g/d, % of N intake, or % of total urinary N) was lower ($P < 0.05$) for the CM diet than for the other diets: 73% of total urinary-N and 18% of N intake for CM compared with an average of 77% of urinary-N and 21% of N intake for the other 3 diets. No differences ($P > 0.30$) were observed for urinary excretion of PD and creatinine, which averaged 473 and 125 mmol/d across diets. Therefore, microbial protein synthesis estimated from PD excretion also did not differ ($P = 0.30$) among diets and averaged 2,240 g/d.

His, Lys, and Met ILR

In cows fed the SBM diet, the abomasal infusions of Lys and Met performed after the main study increased the WB ILR of Lys by 51.4 g/d ($P = 0.02$) and of Met by 19.3 g/d ($P = 0.06$), which represented, on average, 95% of Lys and 96% of Met rate of infusion. For the main project, the WB ILR of Met and His did not differ among diets ($P > 0.18$; Table 4) and averaged, respectively, 124 ± 6.3 and 129 ± 5.5 g/d. The treatments affected ($P = 0.03$) the WB ILR of Lys: the highest WB ILR was obtained for the CM diet (371 g/d) and the lowest for the HPDDG diet (290 g/d). The values for SBM and WDDGS were intermediate, averaging respectively, 330 and 316 g/d.

WB Ra and Plasma Concentration of Glucose

Plasma concentration of glucose did not differ among diets and averaged 3.79 mM ($P = 0.74$; Table 4). The WB Ra of glucose varied across diets ($P = 0.02$): the highest value was observed with SBM diet (3,036 g/d) and the lowest with CM diet (2,795 g/d). The milk lactose yield on WB glucose Ra ratio was not affected ($P = 0.32$) by diets and averaged 0.49.

AA and Urea Plasma Concentration

All essential AA concentrations except His and Phe were affected ($P < 0.05$; Table 5) by diets. For the essential AA for which concentrations were modified by

Table 2. Dry matter and N intake, milk yield and composition of lactating dairy cows fed diets with different protein supplements

	Diet[1]					
Item	SBM	CM	HPDDG	WDDGS	SEM	*P*-value[2]
DMI (kg/d)	24.0	23.6	23.5	23.7	0.81	0.49
N intake (g/d)	659	660	657	665	22.7	0.87
Milk yield (kg/d)	31.9	30.9	32.2	30.8	2.57	0.42
ECM[3] (kg/d)	30.9	30.0	30.9	30.7	2.02	0.75
CP						
%	3.64	3.70	3.64	3.70	0.125	0.39
g/d	1,148	1,130	1,154	1,126	71.7	0.83
Fat						
%	3.70[ab]	3.75[ab]	3.64[b]	3.96[a]	0.191	0.01
g/d	1,120	1,086	1,107	1,146	83.8	0.53
Lactose						
%	4.56	4.48	4.58	4.49	0.078	0.06
g/d	1,454	1,383	1,476	1,389	122.1	0.21
MUN (mg/dL)	11.4[a]	9.7[b]	11.6[a]	11.6[a]	0.52	0.01
N milk[4] (% of N intake)	27.4	27.0	27.7	26.4	1.24	0.52

[a,b]Values in the same row with different superscript are different ($P < 0.05$).

[1]SBM = soybean meal; CM = canola meal; HPDDG = high-protein corn dried distillers grain; WDDGS = wheat dried distillers grain with solubles.

[2]Probability corresponding to the null hypothesis.

[3]Estimated with the equation of Sjaunja et al. (1991): ECM (kg) = kg milk yield × [(383 × fat % + 248 × CP % + 164 × lactose % + 20.7)/3,140].

[4]Calculated assuming N milk = 6.34 × milk CP (Karman and van Boekel, 1986).

treatments, the CM diet had the highest concentrations, except for Leu, which was highest for the HPDDG diet. Consequently, total essential AA plasma concentration was numerically higher with the CM diet. The lowest AA concentrations were observed with the HPDDG diet, except Met and Leu, which were the lowest for the SBM diet. Concentrations of total nonessential AA and individual nonessential AA did not differ between diets, except for Glu ($P < 0.01$), Gly ($P = 0.03$), and Tyr ($P = 0.04$). Compared with the HPDDG diet, Glu concentration was higher with WDDGS diet and Gly concentration with the CM diet; Tyr concentration was higher with the HPDDG diet than with the SBM diet. Plasma urea-N concentration was lower with CM diet than with DDG diets (7.8 vs. 8.9 m*M* on average for the DDG diets; $P = 0.02$), the value being intermediate for SBM (8.5 m*M*).

DISCUSSION

In this study, experimental diets were formulated to be isonitrogenous at 17.2% and isoenergetic at 1.56

Table 3. Urinary nitrogen excretion of lactating dairy cows fed diets with different protein supplements

	Diet[1]					
Item	SBM	CM	HPDDG	WDDGS	SEM	*P*-value[2]
Urinary excretion						
Total N (g/d)	180	159	175	186	8.0	0.14
Total N (% of N intake)	27.4	24.3	26.8	28.3	1.54	0.15
Urea-N (g/d)	139[ab]	116[b]	137[ab]	142[a]	6.2	0.02
Urea-N (% of N urine)	77.4[a]	72.8[b]	78.2[a]	76.5[a]	1.10	<0.01
Urea-N (% of N intake)	21.6[ab]	17.9[b]	20.9[ab]	21.7[a]	1.30	0.04
PD[3] (mmol/d)	496	501	434	462	30.7	0.30
Creatinine (mmol/d)	126	132	118	123	7.5	0.43
Microbial CP flow[4] (g/d)	2,363	2,386	2,030	2,181	165.2	0.30

[a,b]Values in the same row with different superscripts are different ($P < 0.05$).

[1]SBM = soybean meal; CM = canola meal; HPDDG = high-protein corn dried distillers grain; WDDGS = wheat dried distillers grain with solubles.

[2]Probability corresponding to the null hypothesis.

[3]Urinary purine derivatives (allantoin plus uric acid).

[4]Estimated from urinary PD excretion according to the procedure of Chen and Gomes (1992).

Table 4. Estimated His, Lys, Met and glucose whole-body flux, and plasma glucose concentration in lactating dairy cows fed diets with different protein supplements

	Diet[1]					
Item	SBM	CM	HPDDG	WDDGS	SEM	*P*-value[2]
WB ILR of AA[3] (g/d)						
His	126	139	130	122	5.5	0.18
Lys	330[ab]	371[a]	290[b]	316[ab]	18.3	0.03
Met	118	131	129	117	6.3	0.23
Glucose						
Concentration (m*M*)	3.83	3.80	3.77	3.77	0.050	0.74
WB Ra[4] (g/d)	3,036[a]	2,795[b]	2,984[ab]	2,844[ab]	107.9	0.02
Milk lactose:WB Ra	0.48	0.51	0.50	0.49	0.032	0.32

[a,b]Values in the same row with different superscripts are different ($P < 0.05$).

[1]SBM = soybean meal; CM = canola meal; HPDDG = high-protein corn dried distillers grain; WDDGS = wheat dried distillers grain with solubles.

[2]Probability corresponding to the null hypothesis.

[3]Whole-body (WB) irreversible loss rate (ILR) of His, Lys, and Met measured after a bolus injection of labeled AA (L-[$^{15}N_3$]His, L-[1-^{13}C]Met, and L-[α-^{15}N]Lys).

[4]Whole-body glucose rate of appearance (WB Ra) measured using a D-[6,6-$^2H_2$]glucose continuous infusion.

Mcal, with a constant forage:concentrate ratio (38:62), and to meet or exceed the nutrient requirements of the cows based on NRC (2001). Our objective was to compare the effects of feeding lactating dairy cows with diets containing SBM, CM, HPDDG, or WDDGS as the single protein supplement on the WB availability of His, Lys, Met, and glucose. As required when using the WB ILR method to estimate essential AA availability, no difference between treatment in milk and milk protein yields was observed and the milk N:N intake ratio was similar between diets, suggesting that dietary treatments did not alter protein synthesis or protein

Table 5. Plasma concentrations of AA and urea in lactating dairy cows fed diets with different protein supplements[1]

	Diet[2]					
Item	SBM	CM	HPDDG	WDDGS	SEM	*P*-value[3]
Essential AA (μ*M*)						
His	65.3	67.7	63.3	62.0	3.10	0.77
Ile	158.6[ab]	168.9[a]	148.5[b]	163.8[a]	3.16	<0.01
Leu	213.3[b]	260.0[ab]	321.4[a]	230.6[b]	17.10	<0.01
Lys	92.0[a]	98.9[a]	75.4[b]	93.1[a]	2.61	<0.01
Met	32.4[b]	40.5[a]	38.1[ab]	38.0[ab]	1.60	0.02
Phe	60.8	67.6	70.4	63.2	3.69	0.26
Thr	111.5[ab]	125.2[a]	103.4[b]	109.5[ab]	5.11	0.04
Trp	49.8[ab]	54.5[a]	43.8[b]	51.1[a]	1.68	<0.01
Val	312.3[b]	345.2[a]	307.1[b]	315.6[ab]	8.06	0.02
Total	1,096	1,229	1,171	1,130	38.2	0.13
Nonessential AA (μ*M*)						
Ala	255.1	263.6	250.4	260.5	9.20	0.72
Asn	53.3	53.6	51.0	52.1	2.35	0.83
Asp	15.7	16.5	17.8	15.3	1.48	0.65
Gln	249.7	270.2	274.9	269.2	11.24	0.40
Glu	42.6[ab]	42.6[ab]	39.1[b]	44.9[a]	1.02	<0.01
Gly	243.2[ab]	253.6[a]	217.4[b]	239.1[ab]	8.14	0.03
Pro	100.6	108.4	136.7	105.5	9.67	0.05
Ser	84.8	84.4	83.4	82.5	4.26	0.98
Tyr	63.5[b]	78.6[ab]	82.5[a]	69.5[ab]	4.70	0.04
Total	1,112	1,164	1,150	1,140	42.0	0.84
Urea-N (m*M*)	8.5[ab]	7.8[b]	8.8[a]	8.9[a]	0.42	0.02

[a,b]Values in the same row with different superscripts are different ($P < 0.05$).

[1]Mean plasma values obtained from individual analysis of 5 samples harvested on the last day of each period.

[2]SBM = soybean meal; CM = canola meal; HPDDG = high-protein corn dried distillers grain; WDDGS = wheat dried distillers grain with solubles.

[3]Probability corresponding to the null hypothesis.

breakdown. The starch content of the SBM diet was slightly higher than anticipated but in agreement with the highest corn inclusion in this diet.

AA and Urea

The use of the WB ILR method to estimate essential AA availability was first reported in cows by Borucki Castro et al. (2008). In that study, the method was proven to be accurate to estimate the changes in Lys availability induced by an abomasal infusion of Lys and yielded similar results compared with other methods evaluated (i.e., plasma Lys response and digesta flow). In the current study, the WB ILR of Lys and Met increased, respectively, by 51.4 g/d and 19.3 g/d following an abomasal infusion of 54.2 g/d of Lys and 20.0 g/d of Met, confirming that this method is suitable to estimate changes in essential AA supply and availability.

The diet containing CM yielded the highest WB ILR for the 3 essential AA evaluated. However, the difference reached significance only for WB Lys ILR, with the highest WB Lys ILR observed with the CM diet and the lowest with the HPDDG diet. This result suggests a lower Lys availability with the HPDDG diet compared with the CM diet, in agreement with the known low concentration of Lys in corn products (Kelzer et al., 2010; Mjoun et al., 2010) and indicates that Lys supply could limit the milk and milk protein secretion in corn DDG-based diets. Results on WB Lys ILR with the WDDGS diet were intermediary between CM and HPDDG diets, suggesting that Lys would be less limiting in a wheat DDG diet than in a corn DDG diet. These findings agree with the higher concentration of Lys in the residues of 16-h rumen incubation in WDDGS compared with HPDDG, despite a lower concentration in the original feed ingredient (Maxin et al., 2013). The WB ILR of His and Met did not differ statistically between diets, suggesting similar availability of these 2 AA across diets. However, the WB ILR method might not be sensitive enough to reveal differences in His and Met availability between dietary treatments, as suggested by the fact that the increment in Met WB ILR in response to the 20.0 g/d abomasal infusion only tended ($P = 0.06$) to be significant. Therefore, because dietary differences might be smaller and probably more variable than those induced by an abomasal infusion of AA, significance in the treatments might be hard to reach. However, WB ILR of Met was numerically lower for SBM and WDDGS, protein supplements that showed low Met concentrations or high disappearance of Met after 16 h of incubation in the rumen, respectively (Maxin et al., 2013).

The variations in AA absorption assessed by changes in WB ILR can be compared with the changes in digestive flows estimated with NRC (2001). This comparison, however, cannot include the CM diet, because NRC (2001) seems to underestimate MP supply and therefore digestive flows of AA (Martineau et al., 2013) with CM inclusion in dairy rations. For example, the SBM diet was estimated to yield a Lys digestive flow of 172 g/d compared with 146 g/d for the HPDDG diet—the difference was 26 g/d, whereas the difference in Lys WB ILR was 40 g/d. On the other hand, the difference in Lys digestive flow between SBM and WDDGS diets was estimated to be 27 g/d by NRC (2001) and the change in Lys WB ILR was 14 g/d. Although variations were not identical, they were in the same range and same direction. The large standard error of the mean values associated with WB ILR measurements with the pulse dose may be, however, a technical limitation to the use of this method to detect small changes in AA digestive flow.

Variations in plasma AA concentration were consistent with variations observed in the WB ILR, with no effect on His concentrations, the lowest Lys concentration for the HPDDG diet, and the highest Met concentration with CM diet. Mulrooney et al. (2009) and Oba et al. (2010) also observed a lower plasma Lys concentration when comparing corn DDG and CM. However, in contrast to the current study, other authors have observed no difference in plasma Lys concentration between corn DDG-based diets and those based on wheat (Abdelqader and Oba, 2012) or triticale DDG (Oba et al., 2010). The plasma concentration of the other essential AA, except Leu and Phe, was also lower with the HPDDG diet than with the CM diet, suggesting that the CM diet would offer a higher supply of these AA than the HPDDG diet. High concentrations of Leu with HPDDG are directly related to the high Leu concentrations in corn products (NRC, 2001). Results for WDDGS diet were again intermediary between CM and HPDDG diets, suggesting that the AA digestive flows would be slightly higher with wheat DDG diets than with corn DDG diets. The lowest Met concentration in plasma was obtained with the SBM diet, confirming that SBM-based diets could be a limiting source of Met (Illg et al., 1987; Christen et al., 2010).

Overall, the CM diet presented the highest plasma concentration of most essential AA compared with the 3 other diets and thus seemed to supply more of these AA. This is probably linked to the RUP fraction of each protein supplement rather than to an effect on microbial protein synthesis, because the plasma essential AA concentrations were consistent with the essential AA concentrations measured in the 4 protein supple-

ment residues after 16 h of ruminal incubation (Maxin et al., 2013).

Determination of urinary PD excretion is considered a noninvasive, indirect method for estimating differences in rumen microbial protein flow (Moorby et al., 2006). The observed PD excretion was similar between diets and resulted in a predicted flow of 2,240 g/d of microbial protein using the equation of Chen and Gomes (1992). Similarly, no difference in PD production and microbial CP flow was observed when comparing SBM and CM (Brito and Broderick, 2007), SBM and corn DDG (Janicek et al., 2008), or SBM and HPDDG (Kelzer et al., 2009). This confirms that alterations of AA supply could be first explained by difference in RUP fractions rather than an effect on microbial protein synthesis.

The urea-N concentrations in milk, plasma, and urine were lower with the CM diet than with the other diets. No significant changes in MUN concentration or plasma urea-N were previously observed when comparing SBM and CM (Sánchez and Claypool, 1983; Brito and Broderick, 2007), or SBM+CM and WDDGS (Chibisa et al., 2012), or when SBM, CM, and HPDDG were compared (Christen et al., 2010). However, Shingfield et al. (2003) reported lower urea-N concentrations in milk and plasma with a CM diet compared with an SBM diet. In the current study, the lower MUN, plasma urea-N, and urinary urea-N observed with the CM diet could be linked to a lower ruminal protein degradation of this protein supplement. Indeed, the estimated CP ruminal degradation was greater for SBM (58%) and WDDGS (61%) compared with CM when determined in sacco (47%: Maxin et al., 2013). The higher urinary and plasma urea concentrations with SBM and WDDGS diets suggested that the higher ruminal ammonia production was not captured for microbial protein formation. For the HPDDG diet, the high urinary and plasma urea concentrations could not be related to higher ruminal degradation because CP degradation in the rumen was low for HPDDG (36%: Maxin et al., 2013). The lower urinary and plasma urea concentrations with the CM diet could also be explained by a lower N digestibility, which would divert excess N from urine to feces.

Glucose

Similarly to milk yield, milk lactose yield did not differ across diets, but milk lactose concentration tended to be lower with CM and WDDGS diets than with SBM and HPDDG diets. Most of the authors comparing CM with DDG or with SBM did not observe differences in milk lactose yield or concentration (Brito and Broderick, 2007; Mulrooney et al., 2009; Oba et al., 2010). Only Abdelqader and Oba (2012) reported that milk lactose concentration tended to decrease with CM compared with corn and wheat DDG. Overall, in a recent meta-analysis, Martineau et al. (2013) reported a slight negative response in milk lactose concentration when CM replaced protein supplements other than SBM. In the current study, the decrease in milk lactose concentration was paralleled by a decrease in glucose WB Ra: the lowest value being observed with the CM diet and the highest with the SBM diet. This decrease in glucose availability, as assessed by glucose WB Ra, could explain the slight decrease in milk lactose concentration, although the proportion of glucose available directed for lactose secretion did not differ among diets. In this study, the higher glucose WB Ra observed with SBM and HPDDG diets could be attributed to the greater starch concentration in these diets, 20.6 and 19.0% for SBM and HPDDG diets, respectively, compared with 17.5 and 16.5% for CM and WDDGS diets. Indeed, the observed difference in WB Ra of glucose was consistent with the variations in the flow of starch digestible in the small intestine estimated with the empirical model of Offner and Sauvant (2004), averaging 1,090, 921, 998, and 867 g/d for the SBM, CM, HPDDG, and WDDGS diets, respectively.

Milk Fat

Milk fat concentration was unexpectedly affected by diets and was lower with the HPDDG diet than with the WDDGS diet. Previous studies reported no difference in milk fat concentration between corn and wheat or triticale DDG (Oba et al., 2010; Abdelqader and Oba, 2012), or when comparing HPDDG with SBM or CM (Hubbard et al., 2009; Kelzer et al., 2009; Christen et al., 2010) and WDDGS with CM or SBM+CM (Abdelqader and Oba, 2012; Chibisa et al., 2012). Schingoethe et al. (2009) indicated that up to 40% DDG can be included in dairy rations without causing milk fat depression, providing that diets contain an adequate amount of fiber. The quantity of fiber was sufficient in this study, and the difference observed between HPDDG and WDDGS diets could be attributed to the dietary fat content of the 2 diets, 3.0% for HPDDG and 4.0% for WDDGS and mainly linked to the quantity of Ca-salts of fatty acids added in diets to maintain similar energy content between diets. The dilution effect caused by a numerically greater milk production in HPDDG could also be part of the difference observed in milk fat concentration.

CONCLUSIONS

Feeding a CM diet resulted in a better quality of MP supply compared with SBM, HPDDG, and WDDGS

diets, and presented no deficiency in a single essential AA, as SBM did for Met, HPDDG did for Lys, and, although nonsignificant, WDDGS seemed to do for His supply. This could explain the previously reported positive response in milk and milk protein yields to CM substitution. Glucose availability did not seem to be involved in these positive responses, as the lowest glucose availability was observed with the CM diet.

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REFERENCES

Abdelqader, M. M., and M. Oba. 2012. Lactation performance of dairy cows fed increasing concentrations of wheat dried distillers grains with solubles. J. Dairy Sci. 95:3894–3904.

Bickerstaffe, R., and E. F. Annison. 1974. The metabolism of glucose, acetate, lipids and amino acids in lactating dairy cows. J. Agric. Sci. 82:71–85.

Boila, R. J., and J. R. Ingalls. 1994. The post-ruminal digestion of dry matter, nitrogen and amino acids in wheat-based distillers dried grains and canola meal. Anim. Feed Sci. Technol. 49:173–188.

Borucki Castro, S. I., H. Lapierre, L. E. Phillip, P. W. Jardon, and R. Berthiaume. 2008. Towards non-invasive methods to determine the effect of treatment of soya-bean meal on lysine availability in dairy cows. Animal 2:224–234.

Brito, A. F., and G. A. Broderick. 2007. Effects of different protein supplements on milk production and nutrient utilization in lactating dairy cows. J. Dairy Sci. 90:1816–1827.

Brockman, R. P. 1984. Validation of equation for calculation of glucose appearance during nonsteady state in sheep. Can. J. Physiol. Pharmacol. 62:341–344.

Calder, A. G., K. E. Garden, S. E. Anderson, and G. E. Lobley. 1999. Quantification of blood and plasma amino acids using isotope dilution electron impact gas chromatography/mass spectrometry with U-^{13}C amino acids as internal standards. Rapid Commun. Mass Spectrom. 13:2080–2083.

Canadian Council on Animal Care. 1993. Guide to the Care and Use of Experimental Animals. Vol. 1. 2nd ed. E. D. Olfert, B. M. Cross, and A. A. McWilliam, ed. Canadian Council on Animal Care, Ottawa, ON, Canada.

Chen, X. B., and M. J. Gomes. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives—An overview of the technical details. Int. Feed. Res. Unit, Occas. Publ. Rowett Res. Inst., Bucksburn, Aberdeen, UK.

Chibisa, G. E., D. A. Christensen, and T. Mutsvangwa. 2012. Effects of replacing canola meal as the major protein source with wheat dried distillers grains with solubles on ruminal function, microbial protein synthesis, omasal flow, and milk production in cows. J. Dairy Sci. 95:824–841.

Christen, K. A., D. J. Schingoethe, K. F. Kalscheur, A. R. Hippen, K. K. Karges, and M. L. Gibson. 2010. Response of lactating dairy cows to high protein distillers grains or 3 other protein supplements. J. Dairy Sci. 93:2095–2104.

Doepel, L., and H. Lapierre. 2010. Changes in production and mammary metabolism of dairy cows in response to essential and nonessential amino acid infusions. J. Dairy Sci. 93:3264–3274.

Galindo, C. E., D. R. Ouellet, S. Lemosquet, I. Ortigues-Marthy, and H. Lapierre. 2011. Effect of amino acid or casein supply on whole-body, splanchnic and mammary glucose kinetics in lactating dairy cows. J. Dairy Sci. 94:5558–5568.

Hickling, D. 2008. Maximized utilization of canola co-products in livestock industry. Pages 3–14 in Proc. 29th Western Nutrition Conference, Edmonton, AB, Canada. University of Alberta, Edmonton, AB, Canada.

Holtrop, G., H. Lapierre, and G. E. Lobley. 2004. Modelling transport of amino acid into the red blood cells of sheep. J. Agric. Sci. 142:577–588.

Hubbard, K. J., P. J. Kononoff, A. M. Gehman, J. M. Kelzer, K. Karges, and M. L. Gibson. 2009. The effect of feeding high-protein distillers dried grains on milk production of Holstein cows. J. Dairy Sci. 92:2911–2914.

Huhtanen, P., M. Hetta, and C. Swensson. 2011. Evaluation of canola meal as a protein supplement for dairy cows: A review and a meta-analysis. Can. J. Anim. Sci. 91:529–543.

Huntington, G. B. 1984. Net absorption of glucose and nitrogenous compounds by lactating Holstein cows. J. Dairy Sci. 67:1919–1927.

Illg, D. J., J. L. Sommerfeldt, and D. J. Schingoethe. 1987. Lactational and systemic responses to the supplementation of protected methionine in soybean meal diets. J. Dairy Sci. 70:620–629.

Janicek, B. N., P. J. Kononoff, A. M. Gehman, and P. H. Doane. 2008. The effect of feeding dried distillers grains plus solubles on milk production and excretion of urinary purine derivatives. J. Dairy Sci. 91:3544–3553.

Karman, A. H., and A. J. S. van Boekel. 1986. Evaluation of the Kjeldahl factor for conversion of the nitrogen content of milk and milk products to protein content. Neth. Milk Dairy J. 40:315–336.

Kelzer, J. M., P. J. Kononoff, A. M. Gehman, L. O. Tedeschi, K. Karges, and M. L. Gibson. 2009. Effects of feeding three types of corn-milling coproducts on milk production and ruminal fermentation of lactating Holstein cattle. J. Dairy Sci. 92:5120–5132.

Kelzer, J. M., P. J. Kononoff, L. O. Tedeschi, T. C. Jenkins, K. Karges, and M. L. Gibson. 2010. Evaluation of protein fractionation and ruminal and intestinal digestibility of corn milling co-products. J. Dairy Sci. 93:2803–2815.

Lemosquet, S., J. N. Thibault, A. Thomas, E. Debras, and C. Hurtaud. 2004. Validation of the measurement of glucose appearance rate with [6,6-$^{2}H_2$]glucose in lactating dairy cows. Reprod. Nutr. Dev. 44:17–27.

Li, C., J. Q. Li, W. Z. Yang, and K. A. Beauchemin. 2012. Ruminal and intestinal amino acid digestion of distiller's grain vary with grain source and milling process. Anim. Feed Sci. Technol. 175:121–130.

Linzell, J. L., and M. Peaker. 1971. Mechanism of milk secretion. Physiol. Rev. 51:564–597.

Martineau, R., D. R. Ouellet, and H. Lapierre. 2013. Feeding canola meal to dairy cows: A meta-analysis on lactational responses. J. Dairy Sci. 96:1701–1714.

Maxin, G., D. R. Ouellet, and H. Lapierre. 2013. Ruminal degradability of dry matter, crude protein and amino acids in soybean meal, canola meal, corn and wheat dried distillers grains. J. Dairy Sci. 96:5151–5160.

Mjoun, K., K. F. Kalsheur, A. R. Hippen, and D. J. Schingoethe. 2010. Ruminal degradability and intestinal digestibility of protein and amino acids in soybean and corn distillers grains products. J. Dairy Sci. 93:4144–4154.

Moorby, J. M., R. J. Dewhurst, R. T. Evans, and J. L. Danelon. 2006. Effects of dairy cow diet forage proportion on duodenal nutrient supply and urinary purine derivative excretion. J. Dairy Sci. 89:3552–3562.

Mulrooney, C. N., D. J. Schingoethe, K. F. Kalscheur, and A. R. Hippen. 2009. Canola meal replacing distillers grains with solubles for lactating dairy cows. J. Dairy Sci. 92:5669–5676.

Newkirk, R. 2009. Canola Meal: Feed Industry Guide. 4th ed. Canadian International Grains Institute, Winnipeg, MB, Canada.
Nichols, J. R., D. J. Schingoethe, H. A. Maiga, M. J. Brouk, and M. S. Piepenbrink. 1998. Evaluation of corn distillers grains and ruminally protected lysine and methionine for lactating dairy cows. J. Dairy Sci. 81:482–491.
NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academies Press, Washington, DC.
Oba, M., G. B. Penner, T. D. Whyte, and K. Wierenga. 2010. Effects of feeding triticale dried distillers grains plus solubles as a nitrogen source on productivity of lactating dairy cows. J. Dairy Sci. 93:2044–2052.
Offner, A., and D. Sauvant. 2004. Prediction of in vivo starch digestion in cattle from in situ data. Anim. Feed Sci. Technol. 111:41–56.
Robinson, P. H., K. Karges, and M. L. Gibson. 2008. Nutritional evaluation of four coproduct feedstuffs from the motor fuel ethanol distillation industry in the Midwestern USA. Anim. Feed Sci. Technol. 146:345–352.
Sánchez, J. M., and D. W. Claypool. 1983. Canola meal as a protein supplement in dairy rations. J. Dairy Sci. 66:80–85.
Schingoethe, D. J., K. F. Kalscheur, A. R. Hippen, and A. D. Garcia. 2009. Invited review: The use of distillers products in dairy cattle diets. J. Dairy Sci. 92:5802–5813.
Shingfield, K. J., and N. W. Offer. 1999. Simultaneous determination of purine metabolites, creatinine and pseudouridine in ruminant urine by reversed-phase high-performance liquid chromatography. J. J. Chromatogr. B Biomed. Sci. Appl. 723:81–94.
Shingfield, K. J., A. Vanhatalo, and P. Huhtanen. 2003. Comparison of heat-treated rapeseed expeller and solvent-extracted soya-bean meal as protein supplements for dairy cows given grass silage-based diets. Anim. Sci. 77:305–317.
Shipley, R. A., and R. E. Clark. 1972. Compartment analysis: Two-pool open systems. Pages 21–44 in Tracer Methods for In Vivo Kinetics: Theory and Applications. Academy Press Inc., New York, NY.
Sjaunja, L. O., L. Baevre, L. Junkkarinen, J. Pedersen, and J. Setälä. 1991. A Nordic proposal for an energy corrected milk (ECM) formula. Pages 156–157 in Performance Recording Animals: State of the Art, 1990: Proc. 27th Biennial Session of the International Committee for Animal Recording. P. Gaillon and Y. Chabert, ed. EEAP Publication No. 50. Wageningen Academic Publishers, Wageningen, the Netherlands.
Waterlow, J. C., P. J. Garlick, and D. J. Milward. 1978. Protein Turnover in Mammalian Tissues and in Whole Body. Elsevier-North Holland, Amsterdam, the Netherlands.
Zhang, S. Z., G. B. Penner, W. Z. Yang, and M. Oba. 2010. Effects of partially replacing barley silage or barley grain with dried distillers grains with solubles on rumen fermentation and milk production of lactating dairy cows. J. Dairy Sci. 93:3231–3242.

APPENDIX

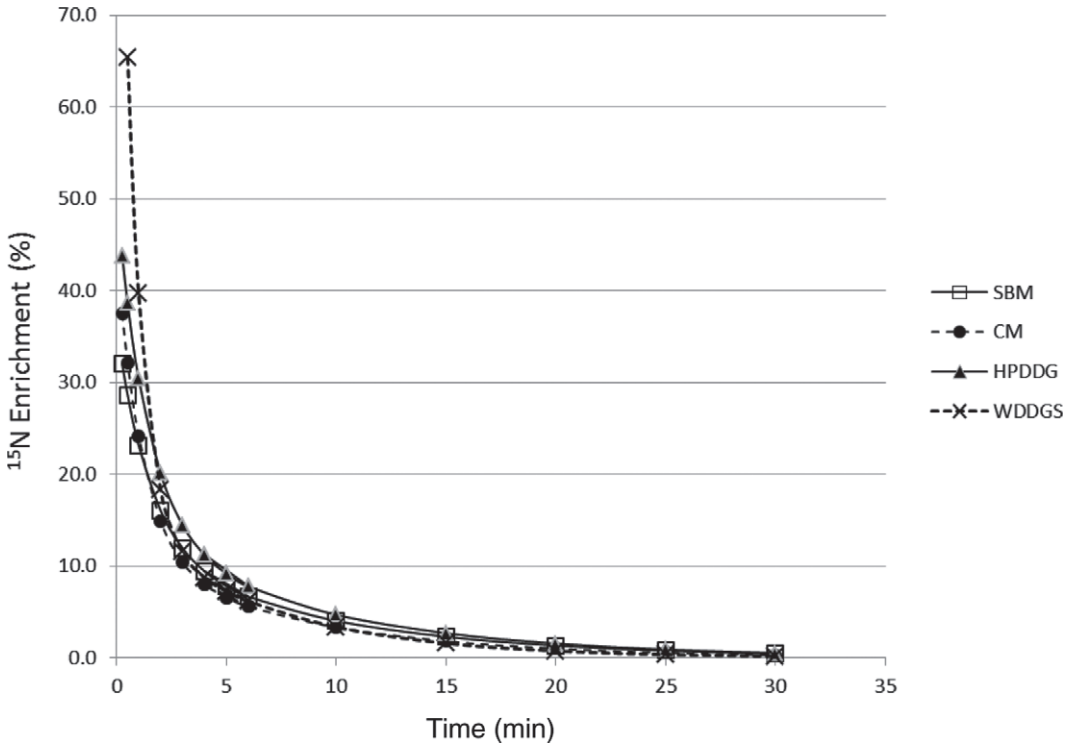

Figure A1. Plasma ^{15}N Lys enrichment in dairy cows fed diets with different protein supplements (SBM = soybean meal, CM = canola meal, HPDDG = corn high-protein dried distillers grain, WDDGS = wheat dried distillers grain with solubles).

Table A1. Mean values of parameters obtained by fitting a model[1] of plasma enrichment for His, Lys, and Met in dairy cows fed diets with different protein supplements

	Diet[2]				
Item	SBM	CM	HPDDG	WDDGS	SEM
His enrichment					
α	0.24	0.21	0.21	0.25	0.027
β	0.06	0.06	0.06	0.06	0.004
k1	0.79	0.86	0.83	0.79	0.075
k2	0.05	0.05	0.05	0.05	0.003
Lys enrichment					
α	0.24	0.33	0.36	0.99	0.288
β	0.12	0.11	0.14	0.15	0.023
k1	0.66	0.83	0.70	1.31	0.313
k2	0.11	0.12	0.11	0.15	0.018
Met enrichment					
α	0.43	0.37	0.37	0.43	0.038
β	0.10	0.11	0.13	0.10	0.019
k1	0.75	0.90	0.88	0.77	0.137
k2	0.12	0.13	0.14	0.11	0.014

[1]Model equation: Enrichment = $\alpha e^{-k1 \times t} + \beta e^{-k2 \times t}$, where t = time in minutes (Shipley and Clark, 1972; Holtrop et al., 2004). When the data are plotted on a semi-log scale, the 2-compartment model yields 2 straight lines, where α and β correspond to isotopic enrichment at time = 0, and k1 and k2 correspond to the slopes of each line, respectively.

[2]SBM = soybean meal; CM = canola meal; HPDDG = high-protein corn dried distillers grain; WDDGS = wheat dried distillers grain with solubles.