Potential of Fermentation Byproducts as Nitrogen Supplements for Lactating Dairy Cows[1]

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ABSTRACT

Two feeding trials evaluated several byproducts from commercial amino acid fermentations as N supplements for lactating cows. Trial 1 was a replicated 5 × 5 Latin square that used 2-wk periods and 25 Holstein cows (five with ruminal cannulae) fed diets containing [dry matter (DM) basis] 28% alfalfa silage, 31% corn silage, 28% high moisture ear corn plus 4 percentage units of crude protein (CP) from: soybean meal, urea, commercial fermentation byproduct 1 or 2, or a blend of fermentation byproducts plus wheat middlings. Diets averaged 15.1% CP and 32% neutral detergent fiber. Intake of DM, body weight (BW) gain, and yield of milk and milk components were greatest for cows fed soybean meal; animal performance was similar with urea, byproduct 1 and the byproduct blend. Intake, BW change, and yield of milk and protein when cows were fed byproduct 2 were lower than when fed urea. Urine output (estimated with creatinine in spot urine samples) was greater on fermentation byproduct 1 and the byproduct blend. There were no differences due to N source in microbial synthesis (based on estimated purine derivative excretion), in situ digestion of alfalfa hay DM, or molar proportions of ruminal volatile fatty acids. Trial 2 was a replicated 5 × 5 Latin square using 2-wk periods and 10 Holstein cows fed diets containing (DM basis) 37% alfalfa silage, 28% corn silage, 29% high moisture ear corn plus 2 percentage units of CP from urea, fermentation byproduct 1, or one of three blends of fermentation byproducts plus wheat middlings. Except for greater DM intake in cows fed the byproduct blends, performance and urinary metabolite excretion did not differ because of N supplement. Relative to other fermentation byproducts and urea, byproduct 1 resulted in reduced milk urea N in both trials. Under the conditions of these trials, fermentation byproducts were less effective than soybean meal, and no more effective than urea, as N supplements.
(**Key words:** urea, soybean meal, fermentation byproducts)

Abbreviation key: AS = alfalfa silage, **CB1** = commercial fermentation byproduct 1, **CB2** = commercial fermentation byproduct 2, **DCAD** = dietary cation-anion difference, **FB** = fermentation byproduct mixture, **HMEC** = high moisture ear corn, **MUN** = milk urea N, **SBM** = solvent soybean meal.

INTRODUCTION

The addition of non-NH_3 N to ruminal in vitro incubations as free AA (1) or peptides (10) has been reported to increase fiber digestion and microbial protein formation. Van Kessel and Russell (24) showed that mixed ruminal bacteria grow more rapidly and efficiently in vitro when provided with N from enzymatic hydrolysates (i.e., peptide mixtures) of casein and soy protein versus NH_3. In the Cornell model, the presence of peptides and AA are assumed to increase protein yield from bacteria fermenting NSC by as much as 18% (17). A number of fermentation byproducts are available that supply NPN as a mixture of NH_3 and non-NH_3 N. In vitro results suggested that these byproducts may improve microbial protein synthesis in the rumen (W. H. Hoover, personal communication). The objective of the research reported here was to use animal performance data to evaluate several fermentation byproducts as N sources relative to standard supplements of NPN (urea) and true protein (solvent extracted soybean meal; **SBM**), for lactating dairy cows. One supplement was a commercial byproduct often fed to pregnant nonlactating cows during the last 2 to 4 wk before calving to alter dietary cation-anion balance; the second was a commercial byproduct with similar N composition that is recommended for feeding to lactating cows. The four other fermentation byproducts tested were experimental mixtures of various N sources and wheat middlings.

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MATERIALS AND METHODS

Trial 1

Twenty-five lactating Holstein cows, 20 multiparous cows, including five fitted with permanent ruminal cannulae [BW, 647 ± 59 kg; milk yield, 38 ± 5 kg/d; parity, 3.2 ± 1.3; and DIM, 187 ± 60 (mean ± SD)], plus five primiparous cows [BW, 514 ± 36 kg; milk yield, 39 ± 1 kg/d; and DIM, 96 ± 15 (mean ± SD)], were blocked by DIM into five groups (three of multiparous cows, one of cannulated cows, and one of primiparous cows) and randomly assigned to five 5 × 5 Latin squares with 2-wk periods (total 10 wk). Five different sources of supplemental N were added to supply 4.0 percentage units of CP (4, 6) in each experimental diet (Table 1): urea, SBM, commercial fermentation byproduct 1 (**CB1**; Bio-Chlor; Biovance Technologies, Omaha, NE), commercial fermentation byproduct 2 (**CB2**; Fermenten; Biovance Technologies), or a mixture of fermentation byproducts (**FB1**). The supplemental N sources added to diet FB1 were Proteferm (a byproduct from monosodium glutamate production; Ajinomoto USA, Eddyville, IA) and CMS (a byproduct from lysine production; Heartland Lysine, Eddyville, IA) and wheat middlings. All diets (Table 2) contained similar amounts of alfalfa silage (**AS**), corn silage, and rolled high moisture ear corn (**HMEC**) fed as TMR. The AS was second-cutting forage that was field-wilted to about 40% DM, chopped to a theoretical length of 2.9 cm and ensiled in a bunker silo in July, 1996. The corn silage and HMEC also were from the 1996 crop year. Corn silage was rolled at the time of ensiling (M. Bal, 1997, personal communication) and HMEC was rolled when removed from the silo to reduce particle size and to increase ruminal fermentability (8). Diets contained an estimated 1.67 Mcal of NE_I/kg of DM (14) and averaged 15.1% CP. All cows were injected with bST (500 mg of Posilac/d; Monsanto, St. Louis, MO) on d 1 of each 14-d period throughout the trial. Cows were weighed on 3 consecutive days at the start of the trial and the last 3 d of each period.

Cows were housed in tie stalls, had free access to water throughout the trial, and were offered their respective TMR once daily at about 1000 h. Orts were collected and recorded daily at 0800; feeding rate was adjusted daily to yield orts of about 5% of intake. Weekly composites of each TMR, type of orts, AS, corn silage, and HMEC were collected from daily subsamples of about 0.5 kg and stored at –20°C. Weekly samples of SBM, CB1, CB2, and wheat middlings were collected and stored at 21 to 24°C; weekly samples of CMS and Proteferm, which were liquids, were collected and stored at 4°C. Samples of TMR and orts were analyzed for DM (60°C, 48 h); DMI was computed on this basis. The proportions of dietary DM from each ingredient on an as-fed basis were adjusted weekly based on DM

Table 1. Composition of fermentation byproduct supplements fed during the lactation trials.

Item	CB1[1]	CB2[2]	FB1[3]	FB2[4]	FB3[4]	FB4[4]	SE[5]	$P > F$[6]
NDF, % of DM	21.3	21.8	19.3	24.2	24.1	19.7	0.5	0.08
Total N, % of DM	8.76^{a}	8.74^{a}	8.02^{c}	8.23^{b}	8.21^{b}	6.67^{d}	0.04	<0.01
DM from byproduct, % of DM[7]	33.6^{bc}	32.2^{c}	40.1^{a}	24.7^{d}	25.0^{d}	38.6^{ab}	1.6	0.04
DM from wheat middlings, % of DM[7]	66.4^{bc}	67.8^{b}	59.9^{d}	75.3^{a}	75.0^{a}	61.4^{cd}	1.6	0.04
N from byproduct, % of total N[7]	77.0^{a}	76.4^{a}	77.3^{a}	72.1^{b}	72.2^{b}	72.0^{b}	0.7	0.03
N from wheat middlings, % of total N[7]	23.0^{b}	23.6^{b}	22.7^{b}	27.9^{a}	27.8^{a}	28.0^{a}	0.7	0.03
NPN, % of total N	56.6^{cd}	52.2^{d}	78.7^{a}	57.3^{c}	58.3^{c}	73.2^{b}	1.1	0.02
NH_3 N, % of total N	29.8^{c}	29.6^{c}	48.3^{a}	30.5^{bc}	31.1^{b}	1.3^{d}	0.2	<0.01
Non-NH_3 NPN, % of total N	26.8^{b}	22.5^{c}	30.4^{b}	26.8^{b}	27.2^{b}	71.9^{a}	1.0	<0.01
Na, % of DM	1.31^{c}	0.37^{d}	1.71^{b}	1.27^{c}	1.29^{c}	3.36^{a}	0.02	<0.01
K, % of DM	1.52^{a}	1.08^{c}	1.37^{b}	1.57^{a}	1.60^{a}	0.89^{d}	0.03	<0.01
Cl, % of DM	8.86^{b}	0.48^{e}	10.06^{a}	8.26^{c}	8.22^{c}	3.75^{d}	0.09	<0.01
S, % of DM	2.48^{c}	5.79^{a}	2.61^{b}	2.27^{d}	2.35^{d}	0.14^{e}	0.04	<0.01
DCAD,[8] meq/kg of DM	–3087^{c}	–3310^{d}	–3368^{d}	–2793^{b}	–2813^{b}	545^{a}	34	<0.01

^{a,b,c,d,e}Means in columns without common superscripts are different ($P < 0.05$).

[1]Bio-Chlor (Biovance Industries, Omaha, NE).

[2]Fermenten (Biovance Industries, Omaha, NE).

[3]An experimental blend of (% of DM) 24% Proteferm (Ajinomoto USA, Eddyville, IA), 16% CMS (Heartland Lysine, Eddyville, IA) and 60% wheat middlings.

[4]Experimental blends of fermentation components and wheat middlings prepared by Ajinomoto Co. (Tokyo, Japan).

[5]SE = Standard error.

[6]Probability of a significant difference due to source of supplement.

[7]Proportions of total DM and total N contributed from fermentation byproducts were computed assuming that wheat middlings was the only source of NDF in each supplement.

[8]Dietary cation-anion difference in milliequivalents of $(Na^+ + K^+) - (Cl^- + S^{2-})$ (22).

Table 2. Composition of diets fed in trails 1 and 2.[1]

	Trial 1					Trial 2				
Ingredient	Urea	SBM	CB1	CB2	FB1	Urea	CB1	FB2	FB3	FB4
	(% of DM)									
Alfalfa silage	28.2	28.2	28.4	28.4	28.2	37.2	37.3	37.3	37.3	36.8
Corn silage	30.5	30.5	30.7	30.7	30.6	28.2	28.3	28.3	28.3	27.9
High moisture corn	32.2	32.2	32.4	32.4	32.2	29.4	29.5	29.4	29.4	29.0
Wheat middlings	7.2	...	...	...	4.7	3.5	...	...	...	...
Urea	0.9	...	...	...	...	0.5	...	...	...	...
Soybean meal	...	8.0	...	...	...	...	...	...	...	...
Bio-Chlor[2]	...	...	7.5	...	...	...	3.8	...	...	...
Fermenten[2]	...	...	...	7.5	...	...	...	...	...	...
Proteferm[3]	...	...	...	...	1.8	...	...	...	...	...
CMS[4]	...	...	...	...	1.3	...	...	...	...	...
FB2[5]	...	...	...	...	...	...	...	3.9	...	...
FB3[5]	...	...	...	...	...	...	...	...	4.0	...
FB4[5]	...	...	...	...	...	...	...	...	...	5.3
Dicalcium phosphate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium bicarbonate	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Trace mineral salt[6]	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitamins ADE mix[7]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Composition										
CP	15.3	14.8	15.2	15.2	15.0	14.8	14.8	14.7	14.7	14.7
NDF	32	31	32	32	32	34	34	34	34	34
Na	0.28	0.27	0.34	0.29	0.36	0.29	0.35	0.33	0.35	0.51
K	1.50	1.63	1.51	1.53	1.54	1.80	1.85	1.84	1.81	1.66
Cl	0.35	0.41	1.05	0.46	1.08	0.44	0.82	0.78	0.82	0.65
S	0.19	0.19	0.33	0.65	0.44	0.20	0.30	0.31	0.31	0.20
DCAD,[8] meq/kg of DM	285	303	34	–16	–31	339	207	202	194	338

[1]CB = Commercial fermentation byproduct, FB = experimental blends of fermentation byproducts, SBM = solvent soybean meal.

[2]Biovance Industries (Omaha, NE).

[3]Ajinomoto USA (Eddyville, IA).

[4]Heartland Lysine (Eddyville, IA).

[5]Experimental blends of fermentation byproducts and wheat middlings prepared by Ajinomoto Co. (Tokyo, Japan).

[6]Provided (per kg of DM): Mn, 27 mg; Zn, 27 mg; Fe, 17 mg; Cu, 7 mg; I, 0.40 mg; Se, 0.30 mg; and Co, 0.10 mg.

[7]Provided (per kg of DM): vitamin A, 3880 IU; vitamin D, 730 IU; and vitamin E, 0.73 IU.

[8]Dietary cation-anion difference in milliequivalents of $(Na^+ + K^+) - (Cl^- + S^{2-})$ (22).

determined by drying weekly composites at 60°C (48 h) for AS, corn silage, HMEC, and TMR and at 105°C (24 h) for SBM, CB1, CB2, wheat middlings, CMS, and Proteferm. Ingredients and TMR dried in this way were ground through a 1-mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA), and five composites of each were prepared, one for each 2-wk period of the trial, by mixing equal amounts of DM from the two weekly samples. Ingredient composites were analyzed for DM at 105°C (2), total N (Leco 2000; Leco Instruments, Inc., St. Joseph, MI), and for NDF (9) using heat stable α-amylase (16) and Na_2SO_3 (11). Byproduct and TMR composites were assayed for Na, K, Cl, and S (Dairyland Laboratories, Arcadia, WI) and dietary cation-anion differences (**DCAD**) were computed with the equation: $DCAD = (Na + K) - (Cl + 2 \times S)$, using elemental concentrations expressed in milliequivalents per kilogram of DM (22). Aqueous extracts were made (13) from weekly samples of fresh AS and unheated byproduct feeds (CB1, CB2, CMS, Proteferm, and wheat middlings); extracts were analyzed for NH_3N (4) and NPN (13). The proportion of non-NH_3 NPN, an estimate of N present as free and peptide-bound AA, was computed by subtracting NH_3 N from total NPN.

Cows were milked twice daily and individual milk yields were recorded at each milking. Milk samples were collected at two consecutive milkings (1 p.m. and 1 a.m.) midway through wk 2 of each period and analyzed for fat, protein, lactose, and SNF by infrared analysis (AgSource, Menomonie, WI). Milk samples also were deproteinized and analyzed for milk urea N (**MUN**) by a colorimetric assay (8). Mean daily milk yield was computed for each cow over the last 7 d of each period. Yield of 3.5% FCM (19) and concentrations and yields of fat, protein, lactose, and SNF were computed as the weighted means from p.m. and a.m. milk yields on test days.

On d 13 of each period, six in situ bags—four containing 5.0 g of ground (2.0-mm screen; Wiley mill; Arthur H. Thomas, Philadelphia, PA) alfalfa hay (17.3%

CP and 54% NDF, DM basis), and two blanks—were attached to rubber stoppers within mesh bags (27) and placed into the ventral rumen of each cannulated cow at 1800 h. In situ bags were removed from the rumen after 16 h and rinsed in a commercial washing machine, and DM disappearance was determined (27). On d 14, spot urine samples were obtained from each cow between 2 and 5 h postfeeding when cows urinated spontaneously; 10-ml aliquots were diluted immediately with 90 ml of 0.036 N H_2SO_4 and stored at –20°C for later analysis. After the trial, all urine samples were thawed and analyzed for allantoin, uric acid, and creatinine as described earlier (23). Daily urine volume and urinary allantoin and uric acid outputs were estimated assuming a daily creatinine excretion of 0.256 mmol/kg of BW; this mean (SE = 0.004) was not influenced by diet over a range of 35 to 80% dietary DM from alfalfa silage (23). Microbial CP was computed from estimated excretion of allantoin and uric acid using the equation of Vagnoni et al. (21) assuming CP: purine ratio of ruminal microbes was 3.99 g/mmol (20). Also on d 14, ruminal samples were taken from the ventral sac of each cannulated cow at 0 (just before feeding), 1, 2, 3, 4, and 6 h after feeding. Ruminal fluid was prepared by straining contents through two layers of cheesecloth. After pH was measured, two subsamples were preserved by adding 0.2 ml of 50% (vol/vol) H_2SO_4 to 10 ml of ruminal fluid for later analysis of NH_3 and total free AA, and by adding 5 ml of formic acid to 5 ml of ruminal fluid for later analysis of VFA (7). These samples were stored at –20°C. Later, ruminal samples were thawed, centrifuged (15,000 × *g*, 4°C, 15 min), and analyzed for NH_3 and total free AA (5) and for VFA (7).

Trial 2

Ten multiparous, lactating Holstein cows [BW, 653 ± 64 kg; milk yield, 33 ± 1 kg/d; parity, 2.5 ± 0.7; and DIM, 264 ± 59 (mean ± SD)] were blocked by DIM into two groups of five cows and randomly assigned to one of two diet sequences in 5 × 5 Latin squares with 2-wk periods (total 10 wk). Animal care, feeding protocols, and sampling, and analytical methods were the same as in trial 1 except that there were no ruminal fluid samplings or in situ incubations. Diets contained more AS and less HMEC than trial 1 (Table 2), an estimated 1.62 Mcal NE_L/kg of DM (14), and an average 14.7% CP. Five N supplements were fed to supply 2.1 percentage units (DM basis) of CP (Table 2): urea or CB1 (also fed in trial 1), or one of three experimental fermentation byproducts (FB2, FB3, and FB4) that were blends of wheat middlings plus differing purified N sources. These three materials were prepared by Ajinomoto Co. (Tokyo, Japan) and are not available commercially.

Statistical Analysis

The general linear models procedure of SAS (18) was used. The model used to analyze compositions of the period composites of the six N supplements included period and supplement; period was used as the error term. The lactation studies were analyzed as 5 × 5 Latin squares, replicated five times (trial 1) or two times (trial 2); data analyzed were BW change; means from the last 7 d of each period for DMI, yield of milk and 3.5% FCM, and DM efficiency (milk yield: DMI); and milk composition, yield of milk components, MUN, N efficiency [(milk protein yield/6.38): N intake], and urinary metabolites. The model included square, cow-within-square, period, treatment, and period × treatment interaction. Period × treatment was significant for milk urea N ($P = 0.02$) in trial 1 and approached significance for DMI ($P = 0.07$) in trial 2, so these interactions were included in the models for these variables. Period × treatment interactions were not significant for any other variable tested ($P \geq 0.14$, trial 1; $P \geq 0.31$, trial 2), so these were pooled with the residual. Means for ruminal in situ DM digestibility and metabolite concentration were analyzed as a 5 × 5 Latin square, replicated once, using a model that included cow, period, and treatment. When dietary treatment effects were significant ($P < 0.05$), mean separation was by least significant difference at the 5% level of probability.

RESULTS AND DISCUSSION

Trial 1

Total N content of CB1 and CB2 was greater than that of the blend of components that made up FB1 (Table 1), so somewhat more of the FB1 blend was incorporated into the diet (Table 2). The five supplemental N sources provided an average of 27% of dietary CP in this trial. This was similar to the proportion of supplemental CP we fed in earlier studies that assessed the lactation response, relative to urea, of several sources of true protein (e.g., 5). The NPN contents of CB1, CB2, and FB1 were, respectively, 57, 52, and 79% (total N basis); FB1 also contained more NH_3 N than the other two supplements (Table 1). Non-NH_3 NPN content (a measure of N in free AA and peptides) of the three byproduct supplements averaged about 27% of total N (Table 1); CB2 contained a lower proportion of non-NH_3 NPN. Although not analyzed in this trial, solvent SBM was earlier found to contain about 2% NPN (6). Urea may be assumed to contain 100% NPN.

There were significant effects of supplemental N source on a number of variables in this study (Table 3). Intake of DM, yield of milk, FCM, protein, and SNF, and N efficiency all were greatest with SBM in the diet;

Table 3. Effect of feeding supplemental N as urea, solvent soybean meal (SBM), one of two commercial fermentation byproducts (CB1 and CB2), or an experimental mixture of fermentation byproducts (FB1) on performance of lactating dairy cows (trial 1)[1].

	Supplemental N source						
Item	Urea	SBM	CB1[2]	CB2[3]	FB1[4]	SE[5]	$P < F$[6]
DMI, kg/d	23.7[b]	24.9[a]	23.0[bc]	22.9[c]	23.7[b]	0.3	<0.01
BW gain, kg/d	0.65[ab]	1.13[a]	0.27[b]	–0.48[c]	0.52[ab]	0.25	<0.01
Milk yield, kg/d	32.3[b]	34.4[a]	31.1[cd]	30.3[d]	32.0[bc]	0.4	<0.01
DM efficiency,[7]	1.38	1.39	1.36	1.34	1.36	0.02	0.45
N efficiency,[8]%	27.9[b]	30.2[a]	28.4[b]	27.9[b]	28.7[b]	0.5	0.01
3.5% FCM, kg/d	33.6[b]	35.3[a]	32.0[b]	32.0[b]	32.3[b]	0.6	<0.01
Fat, %	3.75[ab]	3.67[b]	3.65[b]	3.89[a]	3.54[b]	0.07	0.03
Fat, kg/d	1.21[ab]	1.25[a]	1.14[b]	1.17[b]	1.13[b]	0.03	0.01
Protein, %	3.19[b]	3.29[a]	3.24[ab]	3.25[ab]	3.23[ab]	0.02	0.03
Protein, kg/d	1.03[b]	1.13[a]	1.01[bc]	0.98[c]	1.04[b]	0.02	<0.01
SNF, %	8.77	8.84	8.72	8.70	8.70	0.04	0.06
SNF, kg/d	2.84[b]	3.05[a]	2.72[bc]	2.63[c]	2.80[b]	0.04	<0.01
Milk urea N, mg/dl	14.5[a]	11.3[c]	10.8[c]	12.7[b]	12.7[b]	0.3	<0.01

[a,b,c,d]Means in rows without common superscripts are different ($P < 0.05$).

[1]Trial 1 was a replicated 5 × 5 Latin square that used 25 lactating cows.

[2]Bio-Chlor, Biovance Industries (Omaha, NE).

[3]Fermenten, Biovance Industries (Omaha, NE).

[4]An experimental blend of Proteferm (Ajinomoto USA, Eddyville, IA), CMS® (Heartland Lysine, Eddyville, IA), and wheat middlings (See Table 2).

[5]SE = Standard error.

[6]Probability of a significant effect of diet.

[7]Milk yield/DMI.

[8]Milk N yield/N intake.

BW gain and fat yield also were greater on SBM than with all other N supplements except urea. In addition to serving as an effective source of degradable protein for ruminal microbial growth (12), protein in SBM also has ruminal escape values ranging from 20 (15) to 35% (14). Compared with that of cows fed diets with SBM, DMI was lower for cows fed the diets containing urea, CB1, and the FB1 blend; BW gains of cows fed these three diets reflected DMI. Yields of milk and milk components also tended to be similar on these three diets, although milk yield of cows on CB1 was lower than on urea (Table 3). Yield of FCM and fat was not different among the four diets supplemented with other than SBM. However, performance was poorest when supplemental N was provided by CB2: DMI and yield of milk, protein, and SNF were lower than on all other diets except CB1. Loss of BW was observed only for cows fed the diet supplemented with CB2. Low DCAD values of CB1 and CB2 (Table 2) may have depressed DMI and contributed to reduced milk production of cows fed diets containing those N supplements (25, 26). However, DMI and yield of milk and milk components on FB1 were similar to that on urea (Table 3), even though FB1 had the lowest DCAD (Table 2). That diet CB2 gave rise to the greatest milk fat concentration, as well as FCM and fat yields similar to the other NPN diets, may have reflected fat mobilization occurring due the substantial BW loss of cows fed this diet. Body weight changes were measured over periods of only 2 wk in this Latin square study and results likely were influenced by gut fill. However, each value was the mean of 25 observations and, although DMI (and presumably the effects of intake on gut fill) were the same on CB1 and CB2 diets, BW change was significantly different between these treatments (Table 3). Concentrations of MUN were typical for the levels of dietary CP fed in this trial (3); MUN was greatest on urea, intermediate on CB2 and FB1 diets, and lowest on SBM and CB1 diets (Table 3). Similar MUN on SBM and CB1 was surprising. It seems possible that MUN was influenced by urine volume. Increased urine output may have resulted in greater removal of urea N from the animal; this likely would reduce MUN concentration (23). Urine volume, estimated from creatinine concentration, averaged nearly 6 L/d greater on CB1 and FB1 than on SBM, urea and CB2 (Table 4). However, MUN concentration was not depressed in cows fed the FB1 diet (Table 3) despite the apparently high urine excretion.

Urinary concentrations of purine derivatives and creatinine, and estimated urine volume, purine derivative excretion, and microbial protein synthesis in the rumen determined in spot urine sampling are shown in Table 4. Concentrations and proportions of these four urinary metabolites were typical of lactating dairy cows (23).

Allantoin averaged 89% of the total purine derivative concentration, which was similar to earlier reports: mean proportions of allantoin in total urinary purine derivatives were observed to be 91 (23), 87 (20), and 91% (21). Urinary concentrations of allantoin and total purines were higher in cows fed supplemental N as urea, SBM, and CB2. However, the differences may be explained by the lower urine volumes of cows fed diets with these N supplements. Although creatinine concentration was not affected by diet ($P = 0.11$), urine output computed from creatinine concentration (23) averaged nearly 6 L/d greater on CB1 and FB1. Dietary concentrations of Cl were greater on CB1 and FB1 than on the other three diets (Table 2). Vagnoni and Oetzel (22) speculated that feeding Cl, as NH_4Cl, may increase urinary output in some way unrelated to its effect on DCAD. Although there were numeric differences among the microbial protein yields computed from total purine derivative excretion (21), effects due to diet were not significant ($P = 0.62$); estimated yield of microbial CP averaged 1974 g/d. Mean yields of microbial CP and true protein, computed from mean NE_L intake (14), were 2620 and 2098 g/d. Using the Cornell model (15, 17), mean yields of microbial CP and true protein were computed to be 2620 and 1572 g/d.

The supply of ruminally available protein relative to that required for microbial protein synthesis (14) on these diets was computed to assess the adequacy of RDP. Degraded intake protein values used in these computations were (15): 92 (AS), 75 (corn silage), 62 (HMEC), and 77% (wheat middlings). Recycled N was assumed to equal 15% of total N intake and ruminal availability of degraded intake protein plus recycled N was assumed to be 90% (14). The basal diets (i.e.,

Table 4. Effect of feeding supplemental N as urea, solvent soybean meal (SBM), one of two commercial fermentation byproducts (CB1 and CB2), or experimental mixtures of different fermentation byproducts (FB1, FB2, FB3, and FB4) on urinary excretion of purine derivatives (PD), creatinine, and on estimated urine volume and microbial CP synthesis (trials 1 and 2)[1].

	Supplemental N source						
Item	Urea	SBM	CB1[2]	CB2[3]	FB1[4]	SE[5]	$P > F$[6]
Trial 1							
Allantoin, m*M*	30.2[a]	31.7[a]	22.8[b]	30.6[a]	23.7[b]	1.2	0.07
Uric acid, m*M*	3.36	3.87	2.73	3.38	2.92	0.18	0.19
PD,[7] m*M*	33.5[a]	35.6[a]	25.5[b]	34.0[a]	26.6[b]	1.3	0.06
Creatinine, m*M*	10.6	10.6	8.2	10.3	8.3	0.4	0.11
Urine volume,[8] L/d	16.2[b]	16.3[b]	21.8[a]	16.5[b]	22.4[a]	1.1	0.02
PD excretion, mmol/d	523	553	511	515	531	12	0.62
Microbial CP,[9] g/d	1957	2095	1901	1918	1998	54	0.62
	Supplemental N source						
	Urea	CB1[2]	FB2[10]	FB3[10]	FB4[10]	SE[5]	$P > F$[6]
Trial 2							
Allantoin, m*M*	25.7	27.7	26.3	23.4	23.5	0.7	0.73
Uric acid, m*M*	2.75	3.22	3.03	2.74	2.45	0.12	0.50
PD,[7] m*M*	28.5	30.9	29.4	26.1	26.0	0.8	0.67
Creatinine, m*M*	10.6	10.4	10.1	9.0	9.0	0.4	0.70
Urine volume,[8] L/d	17.3	17.0	17.5	20.9	20.2	0.8	0.42
PD excretion, mmol/d	466	527	507	503	501	15	0.51
Microbial CP,[9] g/d	1693	2096	1884	1865	1853	68	0.63

[a,b]Means in rows without common superscripts are different ($P < 0.05$).

[1]Trials 1 and 2 were replicated 5 × 5 Latin squares that used, respectively, 25 and 10 lactating cows.

[2]Bio-Chlor, Biovance Industries (Omaha, NE).

[3]Fermenten, Biovance Industries (Omaha, NE).

[4]An experimental blend of Proteferm (Ajinomoto USA, Eddyville, IA), CMS (Heartland Lysine, Eddyville, IA), and wheat middlings (See Table 2).

[5]SE = Standard error.

[6]Probability of a significant effect of diet.

[7]Allantoin plus uric acid.

[8]Urine volume estimated from creatinine concentration, assuming daily creatinine excretion equal to 0.256 mmol/kg BW (23).

[9]Microbial CP computed from estimated PD excretion using the equation of Vagnoni et al. (21) assuming CP:purine ratio of ruminal microbes was 3.99 g/mmol (20).

[10]Experimental blends of fermentation byproducts and wheat middlings prepared by Ajinomoto (Tokyo, Japan) (See Table 2).

Table 5. Effect of feeding supplemental N as urea, solvent soybean meal (SBM), one of two commercial fermentation byproducts (CB1 and CB2), or an experimental mixture of fermentation byproducts (FB1) on in situ DM digestion (DMD), and on ruminal pH, NH_3, total AA and VFA (Trial 1).[1]

	Supplemental N source						
Item	Urea	SBM	CB1[2]	CB2[3]	FB1[4]	SE[5]	$P > F$[6]
In situ DMD, %	59.8	59.8	56.1	59.4	60.0	1.3	0.14
pH	6.07[ab]	6.16[a]	5.97[b]	6.00[b]	5.98[b]	0.05	0.06
NH_3, m*M*	13.53[b]	8.84[c]	13.88[ab]	14.52[ab]	17.35[a]	1.18	<0.01
Total AA, m*M*	0.99	1.56	1.48	1.53	1.80	0.28	0.38
Total VFA, m*M*	140.5[a]	131.9[ab]	130.0[b]	128.3[b]	127.5[b]	3.0	0.07
mol/100 mol of total VFA							
Acetate (A)	60.7	62.1	60.4	61.4	59.6	0.9	0.34
Propionate (P)	21.7	19.8	21.2	20.4	22.4	1.0	0.44
A:P[7]	2.86	3.14	3.03	3.03	2.71	0.15	0.36
Butyrate	12.8	13.3	14.1	13.8	13.7	0.6	0.62
Isobutyrate	1.04[bc]	1.10[a]	0.98[c]	1.07[ab]	1.01[bc]	0.02	0.01
IVal + 2MeBu,[8]	1.82	1.77	1.50	1.51	1.48	0.11	0.12
Valerate	1.95	1.89	1.82	1.84	1.86	0.05	0.43

[a,b]Means in rows without common superscripts are different ($P < 0.05$).

[1]Trial 1 was a 5 × 5 Latin square that used five ruminally cannulated cows.

[2]Bio-Chlor, Biovance Industries (Omaha, NE).

[3]Fermenten, Biovance Industries (Omaha, NE).

[4]An experimental blend of Proteferm (Ajinomoto USA, Eddyville, IA), CMS (Heartland Lysine, Eddyville, IA), and wheat middlings (See Table 2).

[5]SE = Standard error.

[6]Probability of a significant effect of diet.

[7]Ratio of acetate:propionate.

[8]Isovalerate plus 2-methylbutyrate.

including wheat middlings but without other supplemental N) provided ruminally available protein sufficient for 74 (SBM, CB1, CB2, and FB1 diets) or 81% (urea diet) of the microbial protein synthesis that would have been supported by NE_L intake (14). Assuming an RDP of 80% for SBM (15) and the other N sources indicated that diets with N supplementation supplied ruminally available protein ranging from 114 (SBM diet) to 124% (urea diet) of that required to support microbial protein synthesis. The manufacturer's recommended feeding levels for CB1 and CB2 were, respectively, 1.1 and 0.9 kg/d; actual consumption in Trial 1 averaged 1.7 kg/d for both products. Nevertheless, the computations suggested that our diet formulations were appropriate for testing the relative effects of these N supplements on microbial protein synthesis in the rumen.

If supplemental N sources stimulated microbial activity, then forage digestion in the rumen also may be increased. However, there were no differences among diets in DM digestion of alfalfa hay incubated for 16 h in the rumen in situ (Table 5). Ruminal pH was greatest on SBM, intermediate on urea, and lowest on the three fermentation byproducts (Table 5). Feeding acidified fermentation byproducts, including CB1, was found to reduce urinary pH (22); depressed ruminal pH may be related to reduced urinary pH. That ruminal NH_3 concentrations were greatest on the four diets supplemented with N sources that were either all NPN (urea) or more than 50% NPN (Table 1) was not surprising. Ruminal NH_3 was substantially lower on SBM. It is not apparent why total VFA concentration was greater on urea than on the byproduct diets; however, total VFA concentrations were in excess of 128 m*M* and similar among the four diets other than that containing urea (Table 4). Although small but significant differences were detected in the molar proportion of ruminal isobutyrate, there were no other shifts in ruminal VFA patterns.

Overall, animal performance was better when supplemental N was fed as true protein (SBM) than as any of the four N sources (Table 3). Yield of milk and milk components was no greater on any of the three fermentation byproducts than on urea; indeed, production actually was reduced on one byproduct N source (CB2). The lack of significant effects on in situ digestion (Table 4) and estimated yield of microbial protein (Table 5) suggested that the improved performance with feeding SBM partly resulted from its RUP contribution.

Trial 2

Total CP (14.7%) and the proportion of CP from supplemental N (14% of dietary CP) were lower, and DCAD

was more positive, in the diets fed in this trial than in trial 1 (Table 2). Consumption of CB1 in trial 2 averaged 0.8 kg/d compared with the manufacturer's recommended feeding level of 1.1 kg/d. Supplies of RDP from the basal diets, computed as described above, ranged from 90 to 93% of that required for the microbial protein synthesis that would have been supported by NE_L intake in this trial (14). The addition of these N supplements increased these values to 102 to 106% of the required RDP. Content of total N, NPN, NH_3 N and non-NH_3 NPN for three of the four byproduct supplements fed in this trial were similar (Table 1). However, FB4, which was elaborated from wheat middlings and polypeptone, an enzymatic hydrolysate of isolated soy protein (N. Usui, 1997, personal communication), was lower in total N and much higher in total NPN and non-NH_3 NPN than the other three byproducts fed in this trial, and contained very little NH_3 N (Table 1). Thus, FB4 supplied greater amounts of peptides for microbial protein formation in the rumen (10, 24).

Generally, DMI was greater on the byproduct blends FB3 and FB4 than on urea or CB1. Although both protein and SNF contents were lower in milk from cows fed supplemental urea, there was no significant effect of dietary N supplement on yield of milk and milk components in this study (Table 6). The concentration of MUN was greatest on urea, intermediate on the three byproduct blends, and lowest on CB1 (Table 6). In trial 1, MUN was depressed and urine volume was increased in cows fed CB1. However, estimated urine output was numerically lowest on CB1 in this study (Table 4), so increased urinary urea excretion probably does not explain the lower MUN in either trial. Concentrations and proportions of these four urinary metabolites were typical of lactating dairy cows (23). There were no significant effects ($P \geq 0.42$) of dietary N supplement on any urinary variable. Overall, there was little difference between supplementing dairy cows with N as urea or as commercial (CB1) or experimental (FB2, FB3, and FB4) fermentation byproducts. No advantage was detected for feeding a supplement containing large amounts of peptides.

SUMMARY

The performance of lactating dairy cows was better when supplemental N was fed as true protein from solvent SBM than when fed N as urea or fermentation byproducts. The yield of milk and milk components was no greater on commercial byproduct CB1 (two short-term trials) than on urea; production was significantly poorer on commercial byproduct CB2 (one short-term trial) than on urea. The lactation performance of four artificial mixtures of fermentation byproducts and wheat middlings was also similar to urea. Lack of significant effects on in situ digestion and estimated in vivo yield of microbial protein suggested that improved yields of milk and milk components on SBM resulted

Table 6. Effect of feeding supplemental N as urea, a commercial fermentation byproduct (CB1), or one of three experimental mixtures of different fermentation byproducts (FB2, FB3, and FB4) on DMI, BW gain, DM and N efficiencies, and yield of milk and milk components (trial 2).[1]

	Supplemental N source						
Item	Urea	CB1[2]	FB2[3]	FB3[3]	FB4[3]	SE	$P > F$[4]
DM intake, kg/d	21.4[b]	21.3[b]	22.2[ab]	22.8[a]	22.9[a]	0.5	0.01
BW gain, kg/d	0.66	0.02	0.69	0.35	0.64	0.47	0.54
Milk yield, kg/d	26.0	25.1	25.2	24.7	26.1	1.0	0.61
DM efficiency[5]	1.22	1.19	1.13	1.08	1.15	0.05	0.09
N efficiency,[6] %	27.7	28.4	27.0	26.1	26.9	1.2	0.40
3.5% FCM, kg/d	29.6	28.0	28.7	27.6	30.0	1.4	0.43
Fat, %	4.33	4.22	4.27	4.16	4.48	0.18	0.51
Fat, kg/d	1.12	1.05	1.09	1.03	1.16	0.07	0.37
Protein, %	3.42[b]	3.57[a]	3.57[a]	3.59[a]	3.55[a]	0.05	0.02
Protein, kg/d	0.89	0.89	0.90	0.89	0.92	0.03	0.92
SNF, %	8.89[b]	9.09[a]	9.03[a]	9.10[a]	9.09[a]	0.07	0.03
SNF, kg/d	2.32	2.28	2.30	2.27	2.36	0.09	0.90
Milk urea N, mg/dl	14.2[a]	12.3[c]	13.5[ab]	13.1[bc]	13.0[bc]	0.5	0.01

[a,b,c]Means in rows without common superscripts are different ($P < 0.05$).

[1]Trial 2 was a replicated 5 × 5 Latin square that used 10 lactating cows.

[2]Bio-Chlor, Biovance Industries (Omaha, NE).

[3]Experimental blends of fermentation byproducts and wheat middlings prepared by Ajinomoto (Tokyo, Japan) (See Table 2).

[4]Probability of a significant effect of diet.

[5]Milk yield/DMI.

[6]Milk N yield/N intake.

from the RUP contributed by that protein. Under the conditions of these experiments, fermentation byproducts were less effective than SBM, and no more effective than urea, as N supplements.

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