

# Potential of Biochlor and Fermenten for Improving Nitrogen Utilization in Lactating Dairy Cows

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## Introduction

A number of fermentation byproducts are reputed to improve microbial protein synthesis in the rumen. One that has received considerable attention in this regard is Biochlor, a byproduct of microbial fermentations producing MSG. The objective of this research was to determine whether the commercial fermentation byproducts Biochlor and Fermenten (a product similar to BioChlor but with perhaps a “better” ion balance) can serve as effective N sources, relative to standard supplements for lactating dairy cows. Urea served as the standard NPN source and solvent soybean meal (SBM), known to be an effective N source for microbial protein formation that also provides about 35% ruminal escape protein, was used as the standard protein N source. A third N source, a mixture of three fermentation products from Ajinomoto Co. (API), also was fed.

## Materials and Methods

Twenty-five Holstein cows, 20 multiparous (five of which were fitted with ruminal cannulae) and five primiparous cows, with mean ( $\pm$  SD) 627 ( $\pm$  76) kg BW, parity 2.7 ( $\pm$  1.4) and 39 ( $\pm$  5) kg/d of milk yield were blocked by days-in-milk and randomly assigned to five 5 x 5 Latin squares with 2-wk periods (total 10 wk). Five diets that differed only in source of supplemental N but with approximately equal energy were fed as TMR (Table 1). All cows were injected with bST. Milk yield from wk-1 was discarded and data analyzed from wk-2 of each period. Milk samples were collected on d-11 of each period and analyzed for fat, protein, lactose, SNF and milk urea N (MUN). On d-13 of each period, ruminal samples were taken at 0, 1, 2, 3, 4 and 6 h after feeding from cannulated cows for determination of pH, NH<sub>3</sub>, total AA and VFA. Spot urine samples also were taken on d-13 and analyzed for creatinine, allantoin and uric acid to estimate microbial N from output of purine derivatives assuming a daily creatinine excretion of 0.256 mmol/kg BW (R.F.D. Valadares, unpublished).

Results were analyzed as a replicated 5 x 5 Latin square using the general linear models procedure of SAS. When dietary treatment effects were significant ( $P \leq 0.05$ ), mean separation was by LSD at the 5% level of probability.

## Results and Discussion

Alfalfa silage fed in this trial averaged 22.1% CP and 45.4% NDF (DM basis), pH 5.2 and 49% NPN (% of total N); corn silage and high moisture corn contained, respectively, 7.3 and 8.0% CP and 39.6 and 16.4% NDF (DM basis). Thus, the composition of major ingredients in these diets was typical for dairy cattle rations in the U.S. Diets were about isonitrogenous and supplements supplied nearly equal proportions of the total dietary CP: 28, 26, 27, 27 and 26% of CP in, respectively, diets A, B, C, D and E (Table 1). Intake of DM, BW gain, DM and N efficiencies, and milk yield and composition data are in Table 2. Intake of DM and BW gain were greatest on the diet containing SBM, with generally lower intakes and weight gains on urea, Biochlor and API (a mixture of Protoferm, CMS and wheat middlings). Intake was lower and BW loss occurred on the Fermenten diet. Yield of milk, FCM, fat, protein, lactose and SNF all were highest on the SBM containing diet. As for intake and BW gain, yields of milk and milk components were intermediate on urea, Biochlor and API, and lowest on Fermenten. Efficiency of capture of dietary N as milk N also was greatest with cows fed the diet with SBM; however, this observation was confounded by the fact that diet B had slightly lower CP content (Table 1).

Generally, milk protein yield in lactating cows is a function of the absorbable protein supplied by microbial protein synthesized in the rumen plus feed protein that escapes the rumen. The SBM diet provided the most absorbable protein, possibly because about 35% of the protein in SBM can escape the rumen (NRC, 1989). That protein yields on Biochlor and API were similar to urea suggested that

neither of these two fermentation products gave rise to greater ruminal microbial protein and neither was superior to urea as an NPN source. The very low milk protein yield on Fermenten indicated that it was an inferior source of NPN for the dairy cow. Several interesting effects were noted on concentrations of milk components and MUN. Milk fat content was highest on Fermenten; this may have been related to mobilization of body fat because this was the only diet on which there was BW loss (Table 2). It was surprising that MUN content was lowest, and not different from SBM, on Biochlor; MUN was intermediate on AP1 and Fermenten and highest on urea (Table 2). Levels of MUN possibly were influenced by urine volume, which was estimated to be numerically greatest on the Biochlor diet (Table 4). Milk protein, despite supplemental N coming from NPN sources on four out five diets, was relatively high, ranging from 3.19 to 3.29%. Average milk protein concentration found in 25 trials at the Dairy Forage Center in cows fed 80 different diets was 2.99%. Increased protein content of milk is now more valuable in the U.S. with the advent of component pricing to determine the farm price of bulk milk. Rather than being constant, lactose concentration in milk from cows fed urea and SBM both were higher in milk from cows fed the other NPN sources (Table 2).

Ruminal pH was lower on Biochlor, Fermenten and AP1 than on SBM; this may reflect the acid load coming from these three fermentation byproducts (Table 3). Total VFA concentration was greater on the urea diet than on other NPN containing diets; this may explain why ruminal pH on urea was intermediate between SBM and the other three diets. As expected, ruminal NH<sub>3</sub> was greater on the four NPN sources than on SBM. Ruminal NH<sub>3</sub> concentrations are the resultant of production and absorption; absorption is reduced at lower ruminal pH. It is interesting that feeding AP1 gave rise to higher ruminal NH<sub>3</sub> than feeding comparable levels of NPN as urea, Biochlor and Fermenten at similar ruminal pH (Table 3). Ruminal concentrations of isobutyrate were greater on the diet containing SBM than on the NPN supplemented diets; this likely was due to catabolism of valine released from degradation of SBM true protein. Urinary excretion of the purine derivatives allantoin and uric acid by dairy cows arises largely from body catabolism of absorbed purines originating from microbial growth in the rumen. Concentrations of allantoin and purine derivatives (allantoin plus uric acid) were greater in spot urine samples taken from cows fed urea, SBM and Fermenten than in urine from cows fed Biochlor and AP1 (Table 4). However, creatinine concentrations also were greater in these

Table 1. Diet composition.

Ingredient	A Urea	B SBM	C Biochlor (% of DM)	D Fermenten	E AP1
Alfalfa silage	28.2	28.2	28.4	28.4	28.2
Corn silage	30.5	30.5	30.7	30.7	30.5
High moisture corn	32.2	32.2	32.4	32.4	32.2
Wheat middlings	7.15	0	0	0	4.71
Urea	0.94	0	0	0	0
Solvent SBM	0	7.95	0	0	0
Biochlor	0	0	7.45	0	0
Fermenten	0	0	0	7.48	0
Protoferm	0	0	0	0	1.85
CMS	0	0	0	0	1.30
DiCal	0.30	0.30	0.30	0.30	0.30
Bicarb	0.39	0.39	0.40	0.40	0.39
TMS	0.30	0.30	0.30	0.30	0.30
Vitamins ADE conc.	0.10	0.10	0.10	0.10	0.10
<b>Composition (DM basis)</b>					
CP, %	15.3	14.8	15.2	15.2	15.0
NE <sub>L</sub> , Mcal/kg	1.66	1.68	1.66	1.66	1.67
NDF, %	33	31	32	32	33

The corn silage was rolled before ensiling and the high moisture ear corn was rolled just before feeding.

same urine samples, indicating urine from cows fed the other diets was more diluted. Because urinary creatinine excretion per unit BW is constant, volume of urine output was estimated to be greater in cows fed the Biochlor and AP1 diets (Table 4). Although there were no significant differences in estimated purine derivative excretion and microbial protein synthesis, both were numerically lower on Biochlor and Fermenten. This also suggested that, in this trial, ruminal microbial growth was not greater on these two commercial fermentation byproducts than on urea.

(AP1), in lactating dairy cows. The true protein N source SBM was superior to all NPN sources. Similar milk protein yields on Biochlor and AP1 as on urea suggested that neither gave rise to greater microbial protein production in the rumen than urea. The same yields of ruminal microbial protein were computed from estimated urinary excretion of purine derivatives for cows fed Biochlor and Fermenten. In these studies, Fermenten was the least effective NPN source for lactating dairy cows.

## Summary and Conclusion

Feeding the fermentation NPN sources Biochlor and Fermenten did not result in better utilization of their N, relative to urea and a third fermentation product

## References

- Vagnoni, D.B., G.A. Broderick, M.K. Clayton, and R.D. Hatfield. 1997. Excretion of purine derivatives by Holstein cows abomasally infused with incremental amounts of purines. *J. Dairy Sci.* 80:1695-1702.
- Vagnoni, D.B. and G.A. Broderick. 1997. Effects of supplementation of energy or ruminally undegraded protein to lactating cows fed alfalfa hay or silage. *J. Dairy Sci.* 80:1703-1712.

Table 2. Effect of feeding supplemental CP as urea, solvent soybean meal (SBM), Biochlor, Fermenten, or a mixture of Ajinomoto fermentation products (AP1) on DM intake, BW change, DM and N efficiencies, and yield of milk and milk components.

Item	Diet					SEM <sup>1</sup>	P > F <sup>2</sup>
	A Urea	B SBM	C Biochlor	D Fermenten	E AP1		
DM intake, kg/d	23.7 <sup>b</sup>	24.9 <sup>a</sup>	23.0 <sup>bc</sup>	22.9 <sup>c</sup>	23.7 <sup>b</sup>	0.3	< 0.001
BW gain, kg/d	0.65 <sup>ab</sup>	1.13 <sup>a</sup>	0.27 <sup>b</sup>	-0.48 <sup>c</sup>	0.52 <sup>ab</sup>	0.25	< 0.001
Milk yield, kg/d	32.3 <sup>b</sup>	34.4 <sup>a</sup>	31.1 <sup>cd</sup>	30.3 <sup>d</sup>	32.0 <sup>bc</sup>	0.4	< 0.001
DM efficiency <sup>3</sup>	1.38	1.39	1.36	1.34	1.36	0.02	0.450
N efficiency <sup>4</sup>	27.9 <sup>b</sup>	30.2 <sup>a</sup>	28.4 <sup>b</sup>	27.9 <sup>b</sup>	28.7 <sup>b</sup>	0.5	0.012
3.5% FCM, kg/d	33.6 <sup>b</sup>	35.3 <sup>a</sup>	32.0 <sup>b</sup>	32.0 <sup>b</sup>	32.3 <sup>b</sup>	0.6	< 0.001
Fat, %	3.75 <sup>ab</sup>	3.67 <sup>b</sup>	3.65 <sup>b</sup>	3.89 <sup>a</sup>	3.54 <sup>b</sup>	0.07	0.025
Fat, kg/d	1.21 <sup>ab</sup>	1.25 <sup>a</sup>	1.14 <sup>b</sup>	1.17 <sup>b</sup>	1.13 <sup>b</sup>	0.03	0.012
Protein, %	3.19 <sup>b</sup>	3.29 <sup>a</sup>	3.24 <sup>ab</sup>	3.25 <sup>ab</sup>	3.23 <sup>ab</sup>	0.02	0.029
Protein, kg/d	1.03 <sup>b</sup>	1.13 <sup>a</sup>	1.01 <sup>bc</sup>	0.98 <sup>c</sup>	1.04 <sup>b</sup>	0.02	< 0.001
Lactose, %	4.83 <sup>a</sup>	4.78 <sup>ab</sup>	4.73 <sup>b</sup>	4.70 <sup>b</sup>	4.72 <sup>b</sup>	0.03	0.029
Lactose, kg/d	1.56 <sup>b</sup>	1.65 <sup>a</sup>	1.48 <sup>cd</sup>	1.42 <sup>d</sup>	1.52 <sup>bc</sup>	0.02	< 0.001
SNF, %	8.77	8.84	8.72	8.70	8.70	0.04	0.056
SNF, kg/d	2.84 <sup>b</sup>	3.05 <sup>a</sup>	2.72 <sup>bc</sup>	2.63 <sup>c</sup>	2.80 <sup>b</sup>	0.04	< 0.001
MUN, mg/dl	14.53 <sup>a</sup>	11.31 <sup>c</sup>	10.82 <sup>c</sup>	12.74 <sup>b</sup>	12.68 <sup>b</sup>	0.28	< 0.001

<sup>a,b,c,d</sup>Means in rows with no common superscripts are different ( $P < 0.05$ ).

<sup>1</sup>SEM = Standard error of the mean.

<sup>2</sup>Probability of a significant effect of diet.

<sup>3</sup>Milk yield : DMI.

<sup>4</sup>Milk N yield : N intake.

Table 3. Effect of feeding supplemental CP as urea, solvent soybean meal (SBM), Biochlor, Fermenten, or a mixture of Ajinomoto fermentation products (AP1) on ruminal pH, NH<sub>3</sub>, total AA and VFA patterns.

Item	Diet					SEM <sup>1</sup>	P > F <sup>2</sup>
	A Urea	B SBM	C Biochlor	D Fermenten	E AP1		
pH	6.07 <sup>ab</sup>	6.16 <sup>a</sup>	5.97 <sup>b</sup>	6.00 <sup>b</sup>	5.98 <sup>b</sup>	0.05	0.059
NH <sub>3</sub> , mM	13.53 <sup>b</sup>	8.84 <sup>c</sup>	13.88 <sup>ab</sup>	14.52 <sup>ab</sup>	17.35 <sup>a</sup>	1.18	0.004
Total AA, mM	0.99	1.56	1.48	1.53	1.80	0.28	0.383
Total VFA, mM	140.5 <sup>a</sup>	131.9 <sup>ab</sup>	130.0 <sup>b</sup>	128.3 <sup>b</sup>	127.5 <sup>b</sup>	3.0	0.065
<u>Molar proportion, mol/100 mol of total VFA</u>							
Acetate (A)	60.7	62.1	60.4	61.4	59.6	0.9	0.338
Propionate (P)	21.7	19.8	21.2	20.4	22.4	1.0	0.437
A: P ratio	2.86	3.14	3.03	3.03	2.71	0.15	0.357
Butyrate	12.8	13.3	14.1	13.8	13.7	0.6	0.622
Isobutyrate	1.04 <sup>bc</sup>	1.10 <sup>a</sup>	0.98 <sup>c</sup>	1.07 <sup>ab</sup>	1.01 <sup>bc</sup>	0.02	0.013
IV+ 2MB <sup>3</sup>	1.82	1.77	1.50	1.51	1.48	0.11	0.122
Valerate	1.95	1.89	1.82	1.84	1.86	0.05	0.433

<sup>a,b</sup>Means in rows with no common superscripts are different ( $P < 0.05$ ).

<sup>1</sup>SEM = Standard error of the mean.

<sup>2</sup>Probability of a significant effect of diet.

<sup>3</sup>Isovalerate plus 2-Methylbutyrate.

Table 4. Effect of source of supplemental CP on urinary excretion of purine derivatives (PD) and creatinine, and estimated urine volume, PD excretion and microbial protein formation.

Item	Diet					SEM <sup>1</sup>	P > F <sup>2</sup>
	A Urea	B SBM	C Biochlor	D Fermenten	E AP1		
Allantoin, mM	30.2 <sup>a</sup>	31.7 <sup>a</sup>	22.8 <sup>b</sup>	30.6 <sup>a</sup>	23.7 <sup>b</sup>	1.2	< 0.01
Uric acid, mM	3.36 <sup>b</sup>	3.87 <sup>a</sup>	2.73 <sup>c</sup>	3.38 <sup>ab</sup>	2.92 <sup>bc</sup>	0.18	< 0.01
PD, <sup>3</sup> mM	33.5 <sup>a</sup>	35.5 <sup>a</sup>	25.5 <sup>b</sup>	34.0 <sup>a</sup>	26.6 <sup>b</sup>	1.3	< 0.01
Allantoin, % of PD	89.8	88.8	89.0	89.8	88.9	0.5	0.21
Creatinine, mM	10.6 <sup>a</sup>	10.78 <sup>a</sup>	8.07 <sup>b</sup>	10.83 <sup>a</sup>	8.15 <sup>b</sup>	0.38	< 0.01
Urine volume, <sup>4</sup> L/d	16.2 <sup>b</sup>	16.3 <sup>b</sup>	22.2 <sup>a</sup>	16.2 <sup>b</sup>	22.8 <sup>a</sup>	1.1	< 0.01
PD excretion, <sup>5</sup> mmol/d	529	559	517	517	537	11	0.12
Microbial CP, <sup>6</sup> g/d	1984	2125	1928	1930	2025	50	0.12

<sup>a,b,c</sup>Means in rows with no common superscripts are different ( $P < 0.05$ ).

<sup>1</sup>SEM = Standard error of the mean.

<sup>2</sup>Probability of a significant effect of diet.

<sup>3</sup>Allantoin plus uric acid.

<sup>4</sup>Urine volume estimated from creatinine concentration, assuming daily creatinine excretion equal to 0.256 mmol/kg BW (R.F.D. Valadares, unpublished, 1998).

<sup>5</sup>Urinary PD excretion estimated using the equation of Vagnoni et al. 1997.

<sup>6</sup>Microbial CP computed from estimated PD excretion assuming CP: purine ratio in ruminal microbes equal to 3.99 g/mmol (Vagnoni and Broderick 1997).