

Effects of a Liquid Byproduct Nitrogen Source on Nitrogen Utilization by Ruminal Microbes in Continuous Culture Fermenters

¹G.I. Crawford, ²M.D. Stern, ¹R.L.K. Hulbert, ¹K.A. Caperoon and ³P.G. Summer

¹Department of Animal Science, University of Minnesota, Saint Paul, MN 55108, USA

²Department of Animal Science, 180A Haecker Hall, 1364 Eckles Avenue, Saint Paul, MN 55108-6118, USA; ³Ajinomoto USA, Inc., Eddyville, IA 52553, USA

Abstract: A liquid byproduct of monosodium glutamate (MSG) production containing 13.7% N and 8.3% NH₃N (DM basis) was evaluated as a N source for ruminal microbes using eight dual-flow continuous culture fermenters. Two dietary treatments were utilized, with the primary N source being provided by soybean meal (SBM) in the first treatment and by the MSG byproduct (MSGN) in the second treatment. Each treatment was formulated to contain approximately 17% CP, 30% NDF, 17.5% ADF and 5% fat (DM basis). The experiment consisted of one 10-d experimental period, including a 7-d stabilization period followed by 3 d of sampling. Fermenter pH was allowed to fluctuate between 5.8 and 6.5 throughout the experiment. Measured pH was greater ($P < 0.05$) for SBM (5.83) than for MSGN (5.82). Digestion of ADF was 63.4 and 69.3% for SBM and MSGN, respectively, and tended to be greater ($P = 0.06$) with MSGN. Organic matter, CP, and NDF digestion were not influenced ($P > 0.05$) by treatment. Efficiency of bacterial synthesis averaged 20.5 g of N/kg of OM truly digested and was not different ($P > 0.05$) between treatments. Effluent concentration of NH₃N was greater ($P < 0.05$) for MSGN (13.5 mg/100 mL) compared with SBM (2.4 mg/100 mL). Molar proportions of acetate ($P = 0.07$) tended to be greater with MSGN, while molar proportions of propionate ($P = 0.07$) and valerate ($P = 0.08$) tended to be greater with SBM. Total amino acid input and flow were greater ($P < 0.05$) with SBM and total amino acid flow relative to input was greater ($P < 0.05$) with MSGN. Results from this experiment indicate that the liquid byproduct of MSG production supported similar *in vitro* microbial growth as SBM, and also supported greater ADF digestion. However, the MSG byproduct was unable to support AA flows to match those produced when SBM was the primary N source.

Key words: Liquid Byproduct, Nitrogen, Continuous Culture

Introduction

The ruminant animal is unique in its ability to utilize nonprotein nitrogen (NPN) sources to produce microbial protein. Crude protein consists of various sources such as ammonia, peptides, free amino acids (AA) and true protein. Ammonia is recognized as the main source of N for protein synthesis by ruminal microbes (Atasoglu *et al.*, 1999). However, maximal microbial growth and production may occur with mixtures of ammonia, peptides and AA (Hoover and Stokes, 1991 and Wallace *et al.*, 1999). This is due to differing affinity for different N sources exhibited by the mixed rumen microbial population (Griswold and Mackie, 1997). Argyle and Baldwin (1989) observed increased microbial growth when either peptides or AA were added to ammonia, and the greatest growth was observed when peptides and AA were added together to ammonia. Greater ADF digestion was observed when peptides or AA were supplied to *in vitro* cultures in place of ammonia (Griswold *et al.*, 1996).

Monosodium glutamate (MSG) is a common flavor enhancer found in a variety of foods and is produced through the fermentation of corn glucose. The resulting fermentation broth or byproduct contains a mixture of ammonium chloride, bacterial cell mass, and a small amount free AA and peptides. Hoover and Miller (1993) supplemented diets with either a fermentation byproduct or NH₄Cl and observed greater efficiency of microbial synthesis and greater DM and nonstructural carbohydrate digestion with addition of the fermentation byproduct to attain a dietary CP level of 22%. However, a reduction in milk production (Broderick *et al.*, 2000) and a reduction in ADG and DMI (Trenkle, 2002) were observed when a fermentation byproduct was fed to dairy cows and beef steers, respectively.

The objective of this study was to assess the viability of a liquid fermentation byproduct as a N source for ruminal microbes to support normal microbial growth and feed digestion in a dual flow continuous culture fermenter system.

Materials and Methods

Treatments: Two dietary treatments were utilized in this experiment, with each treatment assigned to four fermenters, resulting in four replications for each treatment. The two dietary treatments were: 1) a control containing soybean meal as the primary nitrogen source (SBM), and 2) nitrogen supplied as a liquid byproduct of monosodium glutamate production (MSGN). Composition of the liquid byproduct is presented in Table 1 and

dietary treatment compositions are shown in Table 2. Each diet contained approximately 17% CP, which supplied approximately 2 g of N/d. Chemical composition of each dietary treatment is presented in Table 3.

Continuous culture system and operation: The continuous culture fermenter system was a modification of the system of Hoover *et al.* (1976), as described by Hannah *et al.* (1986). The system consisted of eight fermenter flasks, each of which was inoculated with ruminal fluid at 0900 h on day 1 of each period. Ruminal fluid was collected from a cannulated Holstein cow, strained through four layers of cheesecloth and immediately added to the fermenter flasks. Flask volume for the eight flasks ranged from 1,060 to 1,100 mL.

Artificial saliva (Weller and Pilgrim, 1974) containing 0.4 g/L of urea was continuously infused into the culture flasks. Urea was added to simulate N recycling. Temperature of each fermenter was maintained at 38.6° C and anaerobic conditions were maintained by continuous infusion of N₂ into each flask at a rate of 40 mL/min. Fermenter contents were monitored for pH using an Omega pre-amp electrode; pH was recorded once every 10 min using a DasyLab pH recording software program. Fermenter pH was allowed to fluctuate between 5.80 and 6.50 by adding either 3 N HCl or 5 N NaOH to the fermenter flasks. Liquid and solids were removed separately by filtrate flow and overflow, respectively. Liquid dilution rate was maintained at 0.10/hr, and solids dilution rate was maintained at 0.06/hr. Liquid and solids overflows were measured daily at 0900, 1200, 1500, and 2100 h to determine dilution rates and make necessary adjustments.

Dietary ingredients were ground through a 2 mm screen, mixed, and pelleted to a final dimension of 6 mm in diameter x 10 mm in length. Fermenters were supplied with 75 g DM daily by an automatic feeding mechanism adjusted to deliver pelleted diets in eight equal portions over a 24 h period.

Sample collection and analyses: The experiment consisted of one 10-d experimental period, including a 7-d stabilization period followed by a 3-d sampling period. During the sampling period, effluent collection containers were maintained in a 2° C cold-water bath to suspend microbial function. Solids and liquid effluents were combined and homogenized after the 0900 h weighing and a 500-mL sample from each fermenter was then taken by aspiration. Fermenter effluent from the three sampling days was composited and frozen; a 500-mL sample was freeze-dried for further analyses.

On d 10 of the experimental period, fermenter contents were strained through two layers of cheesecloth. The remaining fluid was used to isolate bacteria by differential centrifugation at 1,000 x *g* for 10 min to remove particulate matter, and then centrifuged again at 20,000 x *g* for 20 min to separate bacteria from supernatant. Isolated bacteria were then freeze-dried for further analyses.

Nutrient composition of diets and chemical analyses of bacterial and effluent samples were determined using AOAC (1995) procedures. Organic matter content of dietary treatments, dried effluent, and bacteria were determined after samples were ashed in a muffle furnace at 550° C for 24 h. Dry matter content of bacteria and effluent were determined by freeze-drying the samples and then drying a sub-sample in a 100° C forced-air oven for 24 h.

All bacterial analyses, along with effluent analyses of DM, OM, NDF, ADF, amino acids (AA), total nonstructural carbohydrates (TNC) and purines were completed using freeze-dried samples. Analyses of N, NH₃N and VFA were completed using homogenized, fresh liquid samples of effluent. Ammonia-N was determined by steam-distillation using a Kjeltac 2300 Autoanalyzer (Foss Tecator AB, Hoganas, Sweden), and total N of both effluent and diet were determined via the Kjeldahl method (AOAC, 1995). Volatile fatty acid samples were prepared according to procedures of Erwin *et al.* (1961) and analyzed using a Hewlett Packard 5880 gas chromatograph (Hewlett Packard, Palo Alto, CA). Total nonstructural carbohydrates were determined according to Smith (1969) using potassium ferricyanide as a colorimetric indicator. Analyses for NDF and ADF were performed following procedures of Van Soest *et al.* (1991). Purine concentrations were determined following procedures of Zinn and Owens (1986), with the resulting purine to N ratio used to determine flow of bacterial N and OM in the effluent samples. Amino acids were analyzed using a Beckman System Gold® high-pressure liquid chromatograph (Beckman Instruments, Inc., Palo Alto, CA).

Dacron polyester bags (6 cm x 10 cm with a mesh pore size of approximately 52 µm) containing 0.5 g of ground (2 mm) sample were attached to weights and placed in the rumen of a Holstein cow to determine in situ DM digestion and CP degradation of dietary treatments. After 2, 4, 8, 16, 24, 48 and 72 h of incubation, triplicate bags were removed and rinsed thoroughly with cold tap water until the rinse water was clear. Bags were then placed in a 60° C forced-air oven for 48 h. After determining DM loss, bags and residue were analyzed for Kjeldahl N. Rate of disappearance of potentially digestible CP was determined using the equation of Mathers and Miller (1981).

Statistical analyses: Statistical analyses were completed using the GLM procedure of SAS (1999). Treatments were arranged in a completely randomized design. The model used for analysis was $Y_i = \mu + D_i + \epsilon_i$, with μ equal to the treatment mean, D representing the treatment effect, and ϵ representing the error term. Fisher's protected

($P < 0.05$) F-test was used to compare least square means for treatments.

Results and Discussion

Chemical composition of the dietary treatments is presented in Table 3. Each dietary treatment contained approximately 17% CP, 91% OM, 30% NDF, 17.5% ADF and 27% TNC. Soybean meal accounted for 42% of the N in the SBM treatment, and the liquid byproduct of monosodium glutamate production accounted for 39% of the N in the MSGN treatment. The SBM treatment contained 11.3 g/100 g of AA (DM basis), while the MSGN treatment contained 8.8 g/100 g of amino acids (DM basis). Glutamic acid was the most abundant individual AA in both treatments. Glutamic acid concentrations were 2.14 and 2.38 g/100 g of DM when N was supplied primarily as SBM or MSGN, respectively.

Results for nutrient digestion are shown in Table 4. Apparent and true DM and OM digestion were not influenced ($P > 0.05$) by treatment. Likewise, in situ DM degradation was not affected ($P > 0.05$) by treatment, and averaged 64.8% and 63.5% for the SBM and MSGN treatments, respectively. Digestion of NDF was not affected ($P > 0.05$) by treatment, and averaged 56.4% across treatments. Digestion of ADF was 63.4% and 69.3% for SBM and MSGN treatments, respectively, and tended ($P = 0.06$) to be greater for the MSGN treatment. Griswold *et al.* (1996) observed greater OM digestion with in vitro cultures supplemented with either peptides or AA compared with soybean meal. Use of urea as an NPN source had no effect on ADF, NDF, or OM digestibility when supplemented to dairy cows at 0.75% of the diet (Cameron *et al.*, 1991). Organic matter and ADF digestibility increased by 37% and 15%, respectively compared with treatments without urea when urea provided supplemental NPN in continuous culture (Griswold *et al.*, 2003). In beef steers, urea supplemented at 0%, 0.5%, 1.0% or 1.5% of the diet had no effect on OM or ADF digestibility (Milton *et al.*, 1997).

Total non-structural carbohydrate (TNC) digestion averaged 80.3% and was not influenced ($P > 0.05$) by treatment (Table 4). Russell *et al.* (1992) stated that ammonia is the sole N source for structural carbohydrate-degrading bacteria, while nonstructural carbohydrate (NSC)-degrading bacteria are able to utilize ammonia, peptides, and amino acids. Addition of urea to in vitro cultures improved NSC digestibility compared with treatments without urea (Griswold *et al.*, 2003). In the Cornell model, presence of AA and peptides are assumed to increase protein yield from NSC-fermenting bacteria by as much as 18% (Russell *et al.*, 1992).

Measurements for pH, CP degradation, ammonia-N concentration, and efficiency of bacterial synthesis are presented in Table 5. Fermenter contents exposed to the SBM treatment had an average pH of 5.83, which was greater ($P < 0.05$) than those exposed to the MSGN treatment (5.82). Although the pH difference was significant, there was likely minimal biological impact due to the small numerical difference. In a study by Vagnoni and Oetzel (1998), a lower ruminal pH was observed when a basal dairy cow diet was supplemented with 8% of a fermentation byproduct. The authors attributed the reduction in pH to an alteration in the dietary cation-anion difference.

Degradation of CP was 48.1% and 52.2% for SBM and MSGN, respectively, and was not influenced ($P > 0.05$) by treatment. However, feeding the MSGN treatment led to greater ($P < 0.05$) in situ CP degradation (76.3% vs 65.5%). Crude protein degradation was not affected when diets containing 3.0% SBM were replaced with diets containing 0.4% urea or 3.3% of a fermentation byproduct in continuous culture (Ariza Nieto, 1998). In vitro cultures with supplemental protein from SBM had the lowest CP degradation compared with cultures containing ammonia, peptides or AA as supplemental protein (Griswold *et al.*, 1996). Low CP degradation in the study by Griswold *et al.* (1996) resulted in decreased microbial growth and OM digestion.

Ammonia-N concentration was greater ($P < 0.05$) for the MSGN treatment, at 13.5 mg/100 mL compared with 2.4 mg/100 mL with the SBM treatment. The greater ammonia-N concentration with the MSGN treatment was expected because of the high ammonia content of the liquid byproduct; however, the ammonia concentration observed with the SBM treatment was lower than expected. Broderick *et al.* (2000) observed an increase in ruminal ammonia-N concentration when fermentation byproducts replaced SBM in dairy cow diets. Results from research with dairy cows (Satter and Roffler, 1975), beef steers (Slyter *et al.*, 1979) or in vitro (Satter and Slyter, 1974) indicate that maximal microbial protein production occurs at an ammonia-N concentration of approximately 2 mg/100 mL. However, other researchers found maximal microbial growth and efficiency at higher ammonia-N concentrations. Windschitl and Stern (1988) reported improved efficiency of bacterial protein synthesis as ammonia-N concentration increased from 5.4 to 18.5 mg/100 mL. They also noted that OM digestibility was maximized at an ammonia-N concentration of 10.1 mg/100 mL, which supported statements by Hoover (1986), who suggested that ammonia-N levels for optimal microbial growth and optimal nutrient fermentation and digestion may be different. Microbial protein production in beef steers was maximized at a ruminal ammonia-N concentration of 2.2 mg/100 mL, while DM digestion was maximized at a ruminal ammonia-N concentration of 4.5 mg/100 mL (Slyter *et al.*, 1979). Despite a much greater ammonia-N concentration observed with the MSGN treatment in the current experiment, OM digestion and efficiency of bacterial synthesis were not influenced ($P > 0.05$) by treatment. Similar results showing a lack of effect on OM digestion or efficiency of bacterial synthesis were

Table 1: Composition of liquid byproduct of monosodium glutamate production

| Item | Concentration |
|-------------|-------------------|
| DM, % | 44.9 |
| | -----% of DM----- |
| Nitrogen | 13.7 |
| Ammonia-N | 8.3 |
| Chloride | 20.4 |
| Sodium | 3.0 |
| Potassium | 1.8 |
| Sulfur | 1.2 |
| Phosphorous | 0.8 |
| Calcium | 1780 ^a |
| Magnesium | 400 ^a |

^aConcentration in ppm

Table 2: Ingredient composition of dietary treatments provided as substrate to ruminal microbes in continuous culture fermenters

| | Nitrogen source (% of DM) | |
|----------------------|---------------------------|-------------------|
| | SBM ^a | MSGN ^b |
| Corn silage | 31.3 | 31.8 |
| Alfalfa hay | 18.5 | 17.9 |
| Ground corn | 24.4 | 28.9 |
| Soybean meal, 44% CP | 14.2 | ---- |
| Soybean hulls | 7.3 | 9.8 |
| Vitamin/mineral mix | 2.2 | 2.6 |
| Tallow | 2.1 | 1.8 |
| Liquid byproduct | ---- | 7.4 |

^aNitrogen supplied primarily by soybean meal^bNitrogen supplied primarily by liquid byproduct of monosodium glutamate production

Table 3: Chemical composition of dietary treatments provided as substrate to ruminal microbes in continuous culture fermenters

| Item | Nitrogen source (% of DM) ^a | |
|------------------|--|------|
| | SBM | MSGN |
| CP | 16.9 | 17.1 |
| OM | 91.1 | 91.0 |
| NDF | 29.3 | 30.0 |
| ADF | 17.2 | 17.5 |
| TNC ^b | 26.5 | 28.4 |
| Fat | 5.0 | 5.0 |
| AA, g/100g of DM | 11.3 | 8.8 |
| Essential | 5.7 | 4.1 |
| Nonessential | 5.6 | 4.7 |
| Alanine | 0.73 | 0.60 |
| Arginine | 0.65 | 0.44 |
| Aspartic acid | 0.81 | 0.52 |
| Glutamic acid | 2.14 | 2.38 |
| Glycine | 0.65 | 0.50 |
| Histidine | 0.33 | 0.30 |
| Isoleucine | 0.64 | 0.45 |
| Leucine | 1.15 | 0.84 |
| Lysine | 0.73 | 0.46 |
| Methionine | 0.20 | 0.19 |
| Phenylalanine | 0.65 | 0.43 |
| Proline | 0.55 | 0.25 |
| Serine | 0.34 | 0.25 |
| Threonine | 0.69 | 0.48 |
| Tyrosine | 0.37 | 0.28 |
| Valine | 0.65 | 0.50 |

^aSBM = nitrogen supplied primarily by soybean meal; MSGN = nitrogen supplied primarily by liquid byproduct of monosodium glutamate production^bTotal nonstructural carbohydrate

Table 4: Effect of nitrogen source on nutrient digestion in continuous culture^a.

| Digestion (%) | Nitrogen source ^b | | SE ^c | P-value |
|-------------------------|------------------------------|------|-----------------|---------|
| | SBM | MSGN | | |
| Apparent DM | 39.0 | 40.5 | 40.5 | 0.53 |
| True Dm ^d | 56.0 | 58.0 | 58.0 | 0.36 |
| In situ Dm ^e | 63.5 | 64.8 | 64.8 | 0.64 |
| Apparent OM | 48.4 | 50.2 | 50.2 | 0.32 |
| True Om ^d | 63.3 | 65.2 | 65.2 | 0.20 |
| ADF | 63.4 | 69.3 | 69.3 | 0.06 |
| NDF | 55.2 | 57.6 | 57.6 | 0.40 |
| TNC | 81.9 | 78.6 | 1.1 | 0.42 |

^aEach value is the mean of four fermentation^bSBM = nitrogen supplied primarily by soybean meal; MSGN = nitrogen supplied primarily by liquid by product of monosodium glutamate production.^cStandard error of the mean^dTrue digestibility was corrected for contribution of bacterial matter^eDetermined using calculations of Mthers and Miller (1981)Table 5: Effect of nitrogen source on pH N metabolism in continuous culture^a.

| Item | Nitrogen source ^b | | SE ^c | P-value |
|--|------------------------------|------|-----------------|---------|
| | SBM | MSGN | | |
| pH | 5.83 | 5.82 | <0.01 | 0.02 |
| NH ₃ N, mg/100 mL | 2.4 | 13.5 | 0.6 | <0.01 |
| CP degradation, % | 48.1 | 52.2 | 3.9 | 0.48 |
| In situ CP degradation, % ^d | 65.5 | 76.3 | 1.7 | 0.01 |
| Bacterial synthesis, g of N/kg OM truly digested | 20.4 | 20.6 | 1.0 | 0.89 |

^aEach value is the mean of four fermentations.^bSBM = nitrogen supplied primarily by soybean meal; MSGN = nitrogen supplied primarily by liquid byproduct of monosodium glutamate production^cStandard error of the mean^dDetermined using calculations of Mathers and Miller (1981)Table 6: Effect of nitrogen source on total N flow and partition of effluent N in continuous culture^a.

| Nitrogen flow (g/d) | Nitrogen source ^b | | SE ^c | P-value |
|---------------------|------------------------------|------|-----------------|---------|
| | SBM | MSGN | | |
| Total | 2.02 | 2.27 | 0.07 | 0.04 |
| Ammonia | 0.06 | 0.35 | 0.01 | <0.01 |
| Dietary | 1.05 | 0.98 | 0.08 | 0.56 |
| Bacterial | 0.90 | 0.94 | 0.04 | 0.61 |

^aEach value is the mean of four fermentations.^bSBM = nitrogen supplied primarily by soybean meal; MSGN = nitrogen supplied primarily by liquid byproduct of monosodium glutamate production.^cStandard error of the mean

reported when a diet with 3.3% of a fermentation byproduct was compared to diets supplemented with 3.0% SBM or 0.4% urea in continuous culture (Ariza Nieto, 1998). In the current experiment, efficiency of bacterial synthesis was lower than expected for both treatments, but the lack of a difference between the treatments suggests that efficiency of bacterial synthesis was maximized at the lower ammonia-N concentration observed with the SBM treatment.

Total N flow and partition of N flow are shown in Table 6. Bacterial N flow was 0.90 and 0.94 g/d for the SBM and MSGN treatments, respectively, and was not affected ($P > 0.05$) by treatment. The lack of treatment effect on bacterial N flow again suggests that bacterial protein synthesis may have been maximized at an ammonia-N concentration of 2.4 mg/100 mL, the concentration observed with the SBM treatment. Lamothe *et al.* (2003)

Table 7: Effect of nitrogen source on volatile fatty acid concentration in continuous culture^a.

| Item | Nitrogen source ^b | | SE ^c | P-value |
|---------------------|------------------------------|-----------------------|-----------------|---------|
| | SBM | MSGN | | |
| Total VFA (mM) | 120.1 | 127.7 | 6.9 | 0.46 |
| | | -----mol/100 mol----- | | |
| Acetate | 50.8 | 56.8 | 2.0 | 0.07 |
| Propionate | 34.2 | 26.6 | 2.5 | 0.07 |
| PButyrate | 11.0 | 12.9 | 0.9 | 0.20 |
| Valerate | 2.8 | 2.1 | 0.2 | 0.08 |
| Isovalerate | 0.1 | 0.1 | 0.1 | 0.64 |
| Isobutrate | 0.3 | 0.3 | 0.03 | 0.51 |
| 2-methylbutyrate | 0.8 | 1.2 | 0.2 | 0.15 |
| Acetate: Propionate | 1.5 | 2.2 | 0.2 | 0.05 |

^aEach value is the mean of four fermentations.^bSBM = nitrogen supplied primarily by soybean meal; MSGN = nitrogen supplied primarily by liquid byproduct of monosodium glutamate production^cStandard error of the meanTable 8: Effect of nitrogen source on amino acid flow in continuous culture^a.

| Amino acid flow (g/d) | Nitrogen source ^b | | SE ^c | P-value |
|-----------------------|------------------------------|------|-----------------|---------|
| | SBM | MSGN | | |
| Total flow | 7.12 | 5.99 | 0.13 | <0.01 |
| Essential | 3.82 | 3.33 | 0.07 | <0.01 |
| Nonessential | 3.30 | 2.67 | 0.08 | <0.01 |
| Individual flow | | | | |
| Alanine | 0.47 | 0.42 | 0.02 | 0.21 |
| Arginine | 0.41 | 0.31 | 0.02 | 0.01 |
| Aspartic acid | 0.53 | 0.44 | 0.02 | 0.04 |
| Glutamic acid | 1.10 | 0.83 | 0.06 | 0.02 |
| Glycine | 0.50 | 0.47 | 0.03 | 0.46 |
| Histidine | 0.17 | 0.18 | 0.01 | 0.60 |
| Isoleucine | 0.47 | 0.41 | 0.01 | 0.01 |
| Leucine | 0.71 | 0.59 | 0.02 | <0.01 |
| Lysine | 0.49 | 0.48 | 0.01 | 0.78 |
| Methionine | 0.22 | 0.22 | 0.01 | 0.42 |
| Phenylalanine | 0.43 | 0.36 | 0.01 | <0.01 |
| Proline | 0.19 | 0.05 | 0.04 | 0.04 |
| Serine | 0.20 | 0.17 | 0.01 | 0.15 |
| Threonine | 0.46 | 0.37 | 0.01 | <0.01 |
| Tyrosine | 0.32 | 0.29 | 0.01 | 0.01 |
| Valine | 0.46 | 0.41 | 0.01 | 0.01 |

^aEach value is the mean of four fermentations^bSBM = nitrogen supplied primarily by soybean meal; MSGN = nitrogen supplied primarily by liquid byproduct of monosodium glutamate production^cStandard error of the mean

found an increase in microbial protein production when urea replaced corn gluten feed as the primary protein source for gestating beef cows. In contrast, the addition of 0.75% urea to a dairy cow diet increased ruminal ammonia-N concentration from 6.3 to 14 mg/100 mL, but had no effect on microbial N flow or efficiency of microbial protein synthesis (Cameron *et al.*, 1991). A greater ammonia-N concentration in the current experiment led to greater ammonia-N flow, which contributed to greater total N flow for the MSGN treatment (2.27 g/d) compared with the SBM treatment (2.02 g/d). In accordance with the lack of effect on CP degradation, dietary N flow was not influenced ($P > 0.05$) by treatment.

Concentration of total VFA and molar proportions of individual VFA are given in Table 7. Total VFA concentration was not affected by treatment ($P > 0.05$), and averaged 120.1 and 127.7 mM for the SBM and MSGN treatments,

Table 9: Effect of nitrogen source on amino acid flow relative to input in continuous culture^a.

| Digestion (%) | Nitrogen source ^b | | SE ^c | P-value |
|---------------|------------------------------|-------|-----------------|---------|
| | SBM | MSGN | | |
| Amino Acid | -----% of input----- | | | |
| Total | 84.3 | 90.4 | 1.5 | 0.03 |
| Essential | 89.6 | 108.5 | 1.9 | <0.01 |
| Nonessential | 78.8 | 74.8 | 2.0 | 0.21 |
| Individual | | | | |
| Alanine | 85.4 | 95.1 | 4.4 | 0.17 |
| Arginine | 84.7 | 93.0 | 4.6 | 0.25 |
| Aspartic acid | 86.7 | 113.4 | 4.5 | 0.01 |
| Glutamic acid | 68.4 | 46.5 | 3.4 | <0.01 |
| Glycine | 102.0 | 125.6 | 6.5 | 0.04 |
| Histidine | 69.7 | 80.8 | 4.4 | 0.12 |
| Isoleucine | 97.9 | 120.8 | 2.9 | <0.01 |
| Leucine | 81.7 | 92.9 | 2.1 | 0.01 |
| Lysine | 89.2 | 140.5 | 3.9 | <0.01 |
| Methionine | 151.9 | 153.0 | 3.6 | 0.85 |
| Phenylalanine | 89.0 | 109.8 | 2.3 | <0.01 |
| Proline | 46.2 | 25.0 | 18.6 | 0.45 |
| Serine | 76.9 | 92.5 | 6.0 | 0.12 |
| Threonine | 88.4 | 104.4 | 2.1 | <0.01 |
| Tyrosine | 117.1 | 137.7 | 3.1 | <0.01 |
| Valine | 94.4 | 110.0 | 2.1 | <0.01 |

^aEach value is the mean of four fermentations^bSBM = nitrogen supplied primarily by soybean meal; MSGN = nitrogen supplied primarily by liquid byproduct of monosodium glutamate production^cStandard error of the mean

respectively. Addition of MSGN in place of SBM tended ($P = 0.07$) to produce a greater molar proportion of acetate. Molar proportion of propionate tended ($P = 0.07$) to be greater for the SBM treatment at 34.2 mol/100 mol compared with 26.6 mol/100 mol for the MSGN treatment. The acetate:propionate ratio (A:P) was greater ($P = 0.05$) for MSGN at 2.2 compared with 1.5 for SBM. Of the remaining VFA, there were no effects ($P > 0.05$) due to treatment other than for valerate, which tended ($P = 0.08$) to be greater for the SBM treatment compared with the MSGN treatment. Responses in acetate, propionate and A:P are likely due to greater ADF digestion observed with the MSGN treatment. Cameron *et al.* (1991) observed greater total VFA concentration when urea was supplemented to dairy cow diets. A fermentation byproduct fed to dairy cows reduced total VFA from 131 to 121 mM compared with a high-forage diet with no fermentation byproduct (Vagnoni and Oetzel, 1998). Amino acid flows are shown in Table 8. Total flow of AA measured 7.12 and 5.99 g/d for SBM and MSGN, respectively, and was greater ($P < 0.05$) with the SBM treatment. Essential and nonessential AA flows were also greater ($P < 0.05$) with the SBM treatment. These results were somewhat expected due to greater concentrations of AA contained within the SBM dietary treatment than within the MSGN dietary treatment (Table 3), but also indicate that greater concentrations of ammonia-N from MSGN did not stimulate greater bacterial AA production to match the total AA flow of the SBM treatment. Flows of lysine and methionine, recognized as the first limiting AA acids for lactating dairy cows (NRC, 2001), were not influenced ($P > 0.05$) by treatment, and averaged 0.48 g/d and 0.22 g/d for lysine and methionine, respectively. Total and essential AA flow relative to input was greater ($P < 0.05$) for the MSGN treatment than for the SBM treatment (Table 9). Essential AA flow relative to input was 108.5% for the MSGN treatment, indicating a net synthesis of AA associated with this treatment. Greater ($P < 0.05$) individual AA flows relative to input due to MSGN supplementation were observed for aspartate, glycine, isoleucine, leucine, lysine, phenylalanine, threonine, tyrosine, and valine, while the only improvement ($P < 0.05$) due to SBM supplementation was observed with glutamate. Methionine flow, which measured 152% and 153% relative to input for the SBM and MSGN treatments, respectively, had the greatest flow relative to input for both treatments. This supports research by Chalupa (1976), who reported methionine to be the least degradable essential AA *in vitro*.

Conclusions

Greater ammonia-N concentrations observed in this experiment when a MSG byproduct replaced SBM may have

stimulated an increase in ADF digestion, but had no effect on OM digestion or efficiency of bacterial synthesis. The low ammonia-N concentrations (2.4 mg/100 mL) observed when SBM was the primary N source appeared to be adequate to support normal bacterial growth and nutrient fermentation; adding ammonia-N from MSGN was not effective in improving bacterial N flow from fermenters. Despite having a higher AA flow relative to input, using MSGN as a N source did not match the total AA flow observed with the SBM treatment. Therefore the liquid byproduct of MSG production may be included in a lactating dairy cow diet at levels similar to those in this experiment without causing a detrimental effect on microbial fermentation, however, it may not produce total AA flows to match those produced when using SBM as the primary N source. Additional research should be conducted *in vivo* to determine effects on milk production and efficiency when using a MSG byproduct in dairy cow diets at levels similar to those used in this experiment.

Acknowledgements

This research has been supported in part by the Minnesota Agric. Exp. Sta. The authors are grateful to Ajinomoto USA for partial support of this research.

References

- AOAC. 1995. Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists, Arlington, Va.
- Argyle, J. L., R. L. Baldwin, 1989. Effects of amino acids and peptides on rumen microbial growth yields. *J. Dairy Sci.*, 72: 2017-2027.
- Ariza Nieto, P., 1998. Effects of nonprotein nitrogen and protein source on microbial growth in continuous culture fermenters. M.S. Thesis. Univ. of Minnesota, St. Paul.
- Atasoglu, C., C. J. Newbold, R. J. Wallace, 1999. Influence of ammonia concentration on [¹⁵N] ammonia uptake and *de novo* synthesis of different amino acids by mixed rumen microorganisms from the sheep rumen *in vitro*. Page 212 in *Proc. Br. Soc. of Anim. Sci.* Edinburgh, Scotland.
- Broderick, G. A., N. De Leon, Y. Nakamura, 2000. Potential of fermentation byproducts as nitrogen supplements for lactating dairy cows. *J. Dairy Sci.*, 83: 2548-2556.
- Cameron, M. R., T. H. Klusmeyer, G. L. Lynch, J. H. Clark, D. R. Nelson, 1991. Effects of urea and starch on rumen fermentation, nutrient passage to the duodenum, and performance of cows. *J. Dairy Sci.*, 74: 1321-1336.
- Chalupa, W., 1976. Degradation of amino acids by the mixed rumen microbial population. *J. Anim. Sci.*, 43: 828-834.
- Erwin, E. S., G. T. Marco, E. M. Emery, 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *J. Dairy Sci.*, 44: 1768-70.
- Griswold, K. E., G. A. Apgar, J. Bouton, J. L. Firkins, 2003. Effects of urea infusion and ruminal degradable protein concentration on microbial growth, digestibility, and fermentation in continuous culture. *J. Anim. Sci.*, 81: 329-336.
- Griswold, K.E., W. H. Hoover, T. K. Miller, W. V. Thayne, 1996. Effect of form of nitrogen on growth of ruminal microbes in continuous culture. *J. Anim. Sci.*, 74: 483-491.
- Griswold, K. E., R. I. Mackie, 1997. Degradation of protein and utilization of the hydrolytic products by a predominant ruminal bacterium, *Prevotella ruminicola* B.4. *J. Dairy Sci.*, 80: 167-175.
- Hannah, S. M., M. D. Stern, F. R. Ehle, 1986. Evaluation of a dual flow continuous culture system for estimating bacterial fermentation *in vivo* of mixed diets containing various soya bean products. *Anim. Feed Sci. Technol.*, 16: 51-62.
- Hoover, W. H., 1986. Chemical factors involved in ruminal fiber digestion. *J. Dairy Sci.*, 69: 2755-2766.
- Hoover, W. H., B. A. Crooker, C. J. Sniffen, 1976. Effects of differential solid-liquid removal rates on protozoa numbers in continuous cultures of rumen contents. *J. Anim. Sci.*, 43: 528-34.
- Hoover, W. H., T. K. Miller, 1993. Evaluation of Proteferm as a nitrogen source for rumen microbes. *J. Dairy Sci.*, 76.
- Hoover, W. H., S. R. Stokes, 1991. Balancing carbohydrates and proteins for optimum rumen microbial yield. *J. Dairy Sci.*, 74: 3630-3644.
- Lamothé, M., T. Klopfenstein, D. Adams, J. Musgrave, G. Erickson, 2003. Microbial protein production in gestating cows supplemented with different sources of rumen degradable protein grazing dormant range. Nebraska Beef Cattle Report. MP 80-A, 10-12. Univ. of Nebraska, Lincoln.
- Mathers, J. C., E. L. Miller, 1981. Quantitative studies of food protein degradation and the energetic efficiency of microbial protein synthesis in the rumen of sheep given chopped lucerne and rolled barley. *Br. J. Nutr.*, 45: 587-604.
- Milton, C. T., R. T. Brandt, Jr., E. C. Titgemeyer, 1997. Urea in dry-rolled corn diets: Finishing steer performance, nutrient digestion, and microbial protein production. *J. Anim. Sci.*, 75: 1415-1424.

- NRC, 2001. Nutrient Requirements of Dairy Cattle (7th Ed.). National Academy Press, Washington, DC.
- Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, C. J. Sniffen, 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J. Anim. Sci.*, 70: 3551-3561.
- SAS Institute, 1999. SAS: Statistical Analysis System, Version 8.02. SAS Institute Inc., Cary, NC.
- Satter, L. D., R. E. Roffler, 1975. Nitrogen requirement and utilization in dairy cattle. *J. Dairy Sci.*, 58: 1219-1237.
- Satter, L. D., L. L. Slyter, 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. *Br. J. Nutr.*, 32: 199-208.
- Slyter, L. L., L. D. Satter, D. A. Dinius, 1979. Effect of ruminal ammonia concentration on nitrogen utilization by steers. *J. Anim. Sci.*, 48: 906-912.
- Smith, D., 1969. Removing and analyzing total nonstructural carbohydrates from plant tissue. *Wisconsin Agric. Exp. Sta. Res. Rep.* 41: 1.
- Trenkle, A., 2002. Evaluation of Proteferm® as a nitrogen supplement for finishing steers. Iowa State Univ. Beef Research Report. A.S. Leaflet R1776. Iowa State Univ., Ames.
- Vagnoni, D. B., G. R. Oetzel, 1998. Effects of dietary cation-anion difference on the acid-base status of dry cows. *J. Dairy Sci.*, 81: 1643-1652.
- Van Soest, P. J., J. B. Robertson, B. A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in animal nutrition. *J. Dairy Sci.*, 74: 3583-3597.
- Wallace, R. J., C. Atasoglu, C. J. Newbold, 1999. Role of peptides in rumen microbial metabolism: Review. *Asian-Australasian J. Anim. Sci.*, 12: 139-147.
- Weller, R. A., A. F. Pilgrim, 1974. Passage of protozoa and volatile fatty acids from the rumen of the sheep and from a continuous in vitro fermentation system. *Br. J. Nutr.*, 32: 341-351.
- Windschitl, P. M., M. D. Stern, 1988. Effects of urea supplementation of diets containing lignosulfonate-treated soybean meal on bacterial fermentation in continuous culture of ruminal contents. *J. Anim. Sci.*, 66: 2948-2958.
- Zinn, R. A., F. N. Owens, 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. *Can. J. Anim. Sci.*, 66: 157-166.