Effects of Diet Fermentability on Efficiency of Microbial Nitrogen Production in Lactating Dairy Cows

M. Oba¹ and M. S. Allen

Department of Animal Science,
Michigan State University,
East Lansing 48824-1225

ABSTRACT

Effect of diet fermentability on efficiency of microbial N production was evaluated. Eight ruminally and duodenally cannulated Holstein cows (55 ± 15.9 days in milk; mean $\pm$ SD) were used in a duplicated 4×4 Latin square design with a 2×2 factorial arrangement of treatments. Experimental diets contained either ground high moisture corn (HM) or dry ground corn (DG) at two dietary starch concentrations (32 vs. 21%). All diets were formulated for 18% CP, and the sources of dietary protein were alfalfa silage (50% of forage at DM basis), soybean meal, distillers grain, and blood meal. The amount of OM truly fermented in the rumen varied from 7.7 (DG at 21% dietary starch) to 11.3 kg/d (HM at 32% dietary starch) among treatments, and was greater for high starch diets and HM treatments compared with low starch diets and DG treatments, respectively. Microbial N flow was greater for high starch diets compared with low starch diets, but was not affected by corn grain treatment. Microbial efficiency was lower for HM compared with DG treatment (39.7 vs. 48.4 g of microbial N/kg of true ruminally degraded OM), but was not affected by dietary starch concentration. Microbial efficiency was positively correlated with rate of passage for OM and starch ($r = 0.77$ and 0.75 , respectively). Rapid passage rate may have decreased microbial turnover in the rumen, enhancing microbial efficiency. Microbial efficiency was negatively correlated with rate of starch digestion ($r = -0.55$), consistent with the energy spilling theory. However, energy spilling did not appear to be from lack of ammonia or low ruminal pH. Microbial efficiency was not related to ruminal ammonia concentration, daily mean ruminal pH, or minimum ruminal pH. Rate of starch availability and rates of passage for starch and OM from the rumen are important determinants of efficiency of microbial protein synthesis *in vivo*.

(**Key words:** conservation method of corn, energy spilling, microbial N, microbial efficiency, rate of passage)

Abbreviation key: TRDOM = true ruminally degraded OM, HM = high moisture corn, DG = dry ground corn, NANMN = nonammonia nonmicrobial nitrogen.

INTRODUCTION

Maximum milk production of high yielding dairy cows is often limited by supply of energy and metabolizable protein. Metabolizable protein consists of microbial protein synthesized in the rumen, dietary protein that escapes ruminal degradation, and endogenous protein (NRC 2001). Microbial protein has the most significant impact on both quantity and quality of metabolizable protein absorbed from the small intestine. Dietary protein with low ruminal degradation can have lower digestibility than microbial protein in the small intestine and is more expensive than that readily degraded in the rumen. Microbial protein is highly digestible in the small intestine, and its amino acid profile is close to that which ruminants require (O'Connor et al., 1993). Therefore, diets that maximize microbial protein production often increase milk yield and reduce diet cost.

Although microbial protein production is often limited by energy supplied from OM fermentation in the rumen, efficiency of microbial N production varies significantly. Microbial efficiency, defined as g of microbial N passing to the duodenum per kilogram of true ruminally degraded OM (TRDOM), ranges more than twofold (Clark et al., 1992). Clark et al. (1992) found a quadratic relationship between TRDOM and microbial efficiency, suggesting factors other than TRDOM affect microbial protein synthesis. Energy spilling or energy uncoupling refers to energy that is wasted for nongrowth functions, and increases with low ruminal pH (Strobel and Russell, 1986) or lack of degradable N (Ricke and Schaefer, 1996). Bacteria fermenting nonstructural carbohydrates may decrease microbial efficiency when peptides or amino acids are insufficient (Russell and Sniffen, 1984). Faster rate of passage was also shown to be positively related to microbial efficiency (Oba and Allen, 2000).

Received March 18, 2002.

Accepted June 11, 2002.

Corresponding author: M. S. Allen; e-mail: allenm@pilot.msu.edu.

¹Current address: Department of Animal and Avian Sciences, University of Maryland.

Although various factors affecting microbial efficiency *in vitro* have been reported, the effect of diet fermentability on microbial efficiency *in vivo* has not been consistent (Overton et al., 1995; Plascencia and Zinn, 1996; Crocker et al., 1998; Knowlton et al., 1998; Callison et al., 2001). Production of microbial N in the rumen is often limited by fermentable energy, but diet fermentability might directly and indirectly affect microbial efficiency by altering ruminal pH or rate of passage. Fermentability of diets is primarily affected by the concentration and fermentability of starch. Corn grain is the major source of dietary starch for lactating dairy cows in the United States. It is often fed as high moisture corn (HM) or dry ground corn (DG), and HM has greater starch degradability in the rumen (Ying et al., 1998). The objective of this experiment was to evaluate the effect of diet fermentability altered by feeding HM and DG at two concentrations of dietary starch on efficiency of microbial N production.

MATERIALS AND METHODS

This paper is the one of three papers in series from one experiment that evaluated effects of corn grain conservation method at two dietary starch concentrations. This paper discusses treatment effects on efficiency of microbial nitrogen production, and the companion papers focus on feeding behavior and productivity (Oba and Allen, 2003a) and ruminal digestion kinetics (Oba and Allen, 2003b). Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University.

Treatments and Cows

Eight multiparous Holstein cows (55 ± 15.9 DIM; mean $\pm$ SD) from the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to treatment sequence within a duplicated 4×4 Latin square balanced for carryover effects with a 2×2 factorial arrangement of treatments. Factors evaluated were dietary starch concentration (21 vs. 32%) and conservation method of corn grain (HM vs. DG). Treatment periods were 21 d, with the final 10 d used to collect samples and data. Cows were cannulated ruminally and duodenally before calving. Duodenal cannulas were soft gutter type made of Tygon and vinyl tubing (Crocker et al., 1998). The duodenum was fistulated between the pylorus, and the pancreatic duct and cannulas were placed between 10th and 11th ribs as described by Robinson et al. (1985). Surgeries were performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University. At the beginning

Table 1. Nutrient composition of corn grains used to formulate experimental diets.¹

	HM ²	DG ³	SE
DM% ⁴	63.2	89.7	0.2
	% of DM		
Starch	67.0	70.5	1.3
NDF ⁴	7.1	10.3	0.2
ADF	2.4	3.0	0.2
Crude protein	9.1	9.1	0.1
Ether extract ⁵	6.3	7.3	0.2
Ash ⁵	1.6	1.3	0.1
Lactate	2.4	—	—
Ethanol	0.2	—	—
VFA	0.8	—	—
Other organic acids ⁶	3.9	—	—
Mean particle size (μm) ⁴	1863	885	40

¹Nutrient composition of corn grains sampled each period (n = 4 for each grain) were analyzed by ANOVA.

²HM: High moisture corn.

³DG: Dry ground corn.

⁴Differed significantly between high moisture corn and dry ground corn ($P < 0.001$).

⁵Differed significantly between high moisture corn and dry ground corn ($P < 0.05$).

⁶Citrate, formate, malate, oxalate, and succinate.

of the experiment, empty BW (ruminal digesta removed) of cows were 565.5 ± 58.5 kg (mean $\pm$ SD).

One corn hybrid (Pioneer 3730; Pioneer Hi-bred International, Inc., Johnston, IA) was grown in 1998, and half of the field was harvested as HM at a DM concentration of 63.2%. The HM was ground to mean particle size of 1863 μm and ensiled in a 2.4×9.0 -m silage bag (Ag Bagger, Ag Bag Corp., Blair, NE). The remaining half of the field was harvested as dry corn at 89.7% of DM. Dry corn was finely ground to a mean particle size of 885 μm . Nutrient composition for corn grain treatments is shown in Table 1. Experimental diets contained HM corn or DG corn, corn silage (50% of forage DM), alfalfa silage (50% of forage DM), a premix of protein supplement (soybean meal, distillers grains, and blood meal), and a premix of minerals and vitamins (Table 2). All diets were formulated for 18% dietary CP concentration, and fed as a TMR.

Data and Sample Collection

Throughout the experiment, cows were housed in tie stalls, fed once daily (1400 h) at 110% of expected intake, and milked twice daily. The amount of feed offered andorts were weighed for each cow daily during the collection period. Samples of all dietary ingredients (0.5 kg) and orts (12.5%) were collected daily and composited into one sample per cow per period. The rumen was evacuated manually through the ruminal cannula at 1800 h (4 h after feeding) on d 19 and at 1000 h (4 h before feeding)

Table 2. Ingredients and nutrient composition of experimental diets (% of dietary DM).¹

	High starch		Low starch		SE
	HM ²	DG ³	HM	DG	
Diet ingredients					
Dry ground corn	—	31.6	—	10.8	—
High-moisture corn	32.0	—	11.0	—	—
Corn silage ⁴	20.8	20.9	31.8	32.0	—
Alfalfa silage ⁵	22.2	22.3	34.0	34.1	—
Protein mix ⁶	21.4	21.5	19.5	19.5	—
Mineral and vitamin mix ⁷	3.6	3.7	3.7	3.6	—
Protein source (% of dietary CP)					
Dry ground corn	—	16.1	—	5.4	—
High-moisture corn	16.2	—	5.5	—	—
Corn silage	8.2	8.2	12.4	12.4	—
Alfalfa silage	23.3	23.3	35.1	35.1	—
Soybean meal	36.1	36.2	32.4	32.4	—
Distillers grain	13.8	13.9	12.4	12.4	—
Blood meal	1.5	1.5	1.3	1.3	—
Mineral and vitamin mix	0.8	0.8	0.8	0.8	—
Nutrient composition					
DM	48.8 ^b	53.0 ^a	42.8 ^d	43.8 ^c	0.2
OM	93.4 ^a	93.5 ^a	92.2 ^b	92.3 ^b	0.1
Starch	31.1 ^a	32.2 ^a	21.0 ^b	21.3 ^b	0.5
NDF	23.1 ^b	24.2 ^b	30.1 ^a	30.5 ^a	0.2
ADF	15.2 ^b	15.4 ^b	20.8 ^a	20.9 ^a	0.2
Lignin	2.2 ^b	2.2 ^b	3.3 ^a	3.3 ^a	0.1
Indigestible NDF ⁸	10.9 ^b	11.2 ^b	14.6 ^a	14.7 ^a	0.2
CP	18.0	18.0	18.3	18.3	0.1
Ether extract	5.2 ^b	5.5 ^a	4.8 ^c	4.9 ^c	0.1
Forage NDF	16.5 ^b	16.5 ^b	25.3 ^a	25.4 ^a	0.1
Treatment corn grain starch (% of dietary starch)	68.8 ^a	69.8 ^a	35.0 ^b	36.2 ^b	0.6

¹Actual nutrient composition of experimental diets were calculated for each period (n = 4 for each treatment) and analyzed by ANOVA. Means for nutrient composition in same row followed by different superscript letters differ significantly ($P < 0.05$).

²HM: High-moisture corn.

³DG: Dry ground corn.

⁴Concentrations of NDF and CP were 39.1 and 7.2 (% of DM), respectively.

⁵Concentrations of NDF and CP were 38.2 and 19.0 (% of DM), respectively.

⁶Protein mix contained 70.2% soybean meal, 26.9% distillers grain, and 2.9% blood meal.

⁷Mineral and vitamin mix contains 50.0% DG, 15.6% sodium bicarbonate, 11.3% limestone, 10.4% dicalcium phosphate, 4.9% salt, 4.5% magnesium oxide, 2.5% trace mineral premix, and 0.85% vitamin ADE premix.

⁸ Indigestible NDF: estimated after 120-h in vitro ruminal fermentation.

on d 21 of each period. Total ruminal content mass and volume were determined. During evacuation, a 10% aliquot of digesta was separated for ease of subsampling. Aliquots were squeezed through a nylon screen (pore size: 1 mm) to separate into primarily solid and liquid phases. Samples were taken from both phases for determination of pool size of digesta components in the rumen.

Ruminal pH was monitored from d 16 through 19 (96 h) of each period by a computerized data acquisition system (Dado and Allen, 1993). Ruminal pH data were recorded for each cow every 5 s. Electrodes for ruminal pH determination were checked daily and calibrated as needed, and ruminal pH data were deleted for the entire day if pH drifted more than 0.1 unit at pH 7 and 4. The system successfully collected 90.6% of the total ruminal pH data (69,120 observations per cow per period). Daily

mean, minimum, maximum, variation range, time below pH 6.0, 5.8, and 5.5, and area below the pH 6.0, 5.8, and 5.5 were calculated. Response variables were averaged over 4 d for each period.

Chromic oxide was used as a marker to estimate nutrient digestibility in the rumen and in the total tract. Gelatin capsules (1.5 oz. Tropac Inc., Airfield, NJ) containing 5 g of chromic oxide and ground spelt hulls (Wiley mill, 2-mm screen; Authur H. Thomas, Philadelphia, PA) were dosed through the rumen cannula at 0600, 1400, and 2200 h (total of 15 g of Cr₂O₃/d) from 7 to 14 d with the priming dose of 3X on d 7. Duodenal samples (1000 g per a cow) and fecal samples (500 g) were collected every 9 h from 12 to 14 d so that eight samples were taken for each cow, representing every 3 h of a 24-hour period to account for diurnal variation. Ruminal contents

were sampled near the reticulo-omasal orifice every 4 h on d 15 to obtain a microbial pellet. Samples were immediately frozen at -20°C .

Sample and Statistical Analysis

All samples were composited to one sample per cow per period before drying. Duodenal samples were composited and separated into primarily solid and liquid phases with four layers of cheesecloth. Both phases were weighed, the ratio of each phase was determined, and subsamples were taken from both phases according to the ratio determined for each sample to minimize sampling errors due to segregation of samples into solid and liquid phases. Diet ingredients, orts, and feces were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. Ruminal digesta, duodenal samples, and microbial pellets obtained after differential centrifugation of ruminal fluid (Overton et al., 1995) were lyophilized (Tri-Philizer MP; FTS Systems, Stone Ridge, NY). All samples were ground with a Wiley mill (1-mm screen; Authur H. Thomas). Samples were analyzed for ash, NDF, ADF, lignin, indigestible NDF, CP, and starch. Ash concentration was determined after 5 h of oxidation at 500°C in a muffle furnace. Concentrations of NDF and ADF were determined [(Van Soest et al., 1991); method A for NDF]. Crude protein was analyzed according to Hach et al. (1985). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide, and glucose concentration was measured using glucose oxidase (Glucose kit #510; Sigma Chemical Co., St. Louis, MO) and absorbance was determined with micro-plate reader (SpectraMax 190; Molecular Devices Corp., Sunnyvale, CA). Indigestible NDF was estimated as NDF residue after 120-h in vitro fermentation (Goering and Van Soest, 1990). Ruminal fluid for the in vitro incubations was collected from a nonpregnant dry cow fed alfalfa hay only. Concentrations of all nutrients except for DM were expressed as percentages of DM determined by drying at 105°C for more than 8 h in a forced-air oven. Corn grain treatments were dry sieved through eight sieves (sieve apertures: 4750, 2360, 1180, 600, 300, 150, 75 μm and bottom pan), using a sieve shaker (model RX-86; W.S. Tyler Inc., Gastonia, NC) for approximately 20 min until the bottom pan weight was constant, and mean particle size of corn grain was calculated (ASAE, 1968).

Duodenal digesta and fecal samples were analyzed for concentrations of Cr. Samples were digested with phosphoric acid (Williams, 1962), and quantified by flame atomic absorption spectrometry (SpectraAA 220; Varian, Victoria, Australia) according to manufacturer's recommendation. Ruminal pool sizes (kg) of DM, OM, NDF, indigestible NDF, and starch were determined by

multiplying the concentration of each component by the ruminal digesta DM weight (kg) except for DM. Turnover rate in the rumen, passage rate from the rumen, and ruminal digestion rate of each component (%/h) were calculated (Oba and Allen, 2003b).

Purines concentration was used as a microbial marker, and total purines were measured by spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) at 260 nm (Zinn and Owens, 1986). Ratios of purines to microbial N, starch, and OM were determined for microbial pellets. Duodenal digesta were analyzed for purine concentration, and duodenal flow of microbial N, starch, and OM were calculated by multiplying the ratios obtained from microbial pellets. Duodenal samples were analyzed for ammonia concentration to estimate nonammonia N and nonammonia nonmicrobial N flow to the duodenum. Dried duodenal digesta samples were hydrated for 20 min to extract ammonia N retained in samples. Ammonia concentration was determined for the duodenal samples and the centrifuged rumen fluid according to Broderick and Kang (1980).

All data were analyzed using the fit model procedure of JMP (version 4, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + C_i + P_j + T_k + e_{ijkl}$$

where

- μ = overall mean,
- C_i = random effect of cow ($i = 1$ to 8),
- P_j = fixed effect of period ($j = 1$ to 4),
- T_k = fixed effect of treatment ($k = 1$ to 4), and
- e_{ijkl} = residual, assumed to be normally distributed.

Period $\times$ treatment interaction was originally evaluated, but it was removed from the statistical model because interaction was not significant. Orthogonal contrasts were made for the effect of dietary starch concentration, conservation method of corn grain, and interaction of dietary starch concentration and conservation method. Treatment effects and their interaction were declared significant at $P < 0.05$ and $P < 0.10$, respectively, and tendency for treatment effects were declared at $P < 0.10$. When interactions of main effects were significant, treatment means were compared using Student's *t*-test and differences were declared significant at $P < 0.05$.

RESULTS AND DISCUSSION

Nitrogen Metabolism

The TRDOM was greater for high starch diets compared with low starch diets ($P < 0.001$; Table 3) and for

Table 3. Effects of corn grain conservation method at two dietary starch concentrations on N metabolism.

	High starch		Low starch		SE	<i>P</i> value		
	HM ¹	DG ²	HM	DG		Starch ³	Corn ⁴	INT ⁵
TRDOM ⁶ (kg/d)	11.3	10.3	9.3	7.7	0.6	<0.001	0.03	0.60
N Intake (g/d)	583 ^b	631 ^a	563 ^b	561 ^b	12.5	<0.01	0.09	0.06 ⁹
Ammonia in the rumen (mg/dl)								
Daily mean	5.1	6.5	9.3	7.8	1.1	0.02	0.94	0.20
Daily minimum	1.4	1.5	1.4	1.6	0.2	0.87	0.53	0.92
Passage to duodenum								
Ammonia N (g/d)	24.5	26.3	28.2	30.2	2.2	0.08	0.38	0.96
NAN ⁷ (g/d)	571	607	518	538	24.7	0.03	0.30	0.69
(% of intake)	98.1	96.4	92.7	95.9	4.4	0.55	0.92	0.62
NANMN ⁸ (g/d)	138	123	164	191	34.3	0.16	0.93	0.61
(% of intake)	23.9	20.2	29.9	34.2	5.7	0.08	0.97	0.53
(% of duodenal NAN ⁷)	22.1	20.1	31.9	35.2	5.5	0.03	0.97	0.71
Microbial N (g/d)	434	484	354	347	30.7	<0.01	0.48	0.37
(% of duodenal NAN ⁷)	77.9	79.9	68.1	64.8	5.5	0.03	0.97	0.71
(g/kg of TRDOM ⁶)	39.3	47.3	40.0	49.4	0.39	0.81	0.03	0.76
NAN ⁷ digested in the intestines (g/d)	429	418	369	382	24.0	0.07	0.93	0.71
(% of duodenal passage)	74.3 ^a	68.7 ^b	71.2 ^{ab}	70.6 ^b	1.3	0.69	0.02	0.07
N apparently digested in total tract (g/d)	441	442	414	403	13.1	0.02	0.70	0.62
(%)	75.5 ^a	70.0 ^c	73.3 ^{ab}	71.9 ^{bc}	1.0	0.87	<0.01	0.05

¹HM: High-moisture corn.²DG: Dry ground corn.³Starch: Effect of dietary starch concentration.⁴Corn: Effect of conservation method of corn grain.⁵INT: Interaction of dietary starch concentration and conservation method of corn grain.⁶TRDOM: True ruminally degraded organic matter.⁷NAN: nonammonia nitrogen.⁸NANMN: nonammonia and nonmicrobial nitrogen.⁹Student's *t*-test was conducted if $P < 0.10$ for interactions of main effects. Treatment means in a same row followed by different superscript letters differ significantly ($P < 0.05$).

HM treatment compared with DG treatment ($P < 0.03$). Treatment means for TRDOM varied from 7.7 kg/d for DG treatment in low starch diets to 11.3 kg/d for HM treatment in high starch diets, which is within the range reported by Allen (1997) for lactating dairy cows: 9.8 ± 2.1 kg/d. Ammonia concentration in the rumen was greater for low starch diets compared with high starch diets ($P < 0.02$), although N intake was less for low starch diets compared with high starch diets ($P < 0.01$). This cannot be attributed to decreased ruminal degradation of feed protein for high starch diets. Nonammonia nonmicrobial N (NANMN) flow to the duodenum as a percentage of N intake tended to be lower for high starch diets compared to low starch diets ($P < 0.08$), and NANMN flow (g/d) was numerically lower for high starch diets ($P = 0.16$). The lower ruminal ammonia concentration observed for high starch diets in this experiment can be attributed to increased utilization of ruminally degraded N for microbial protein synthesis for high starch diets. Microbial N flow was

greater for high starch diets compared with low starch diets ($P < 0.01$). Microbial N production was probably limited by the energy available from fermentable substrates for low starch diets.

A significant interaction of dietary starch concentration and conservation method of corn grain for intestinal NAN digestibility ($P < 0.07$) indicated that DG treatment decreased intestinal NAN digestibility to a greater extent when cows were fed high starch diets (68.7 vs. 74.3%) compared with low starch diets (70.6 vs. 71.2%). Similar results were observed for total tract N digestibility; DG treatment decreased total tract N digestibility compared with HM treatment, and the reduction in digestibility was greater for cows fed high starch diets (70.0 vs. 75.0%) compared with low starch diets (71.9 vs. 73.0%). The NANMN from DG might be more resistant to digestion than that from HM. These observations can be also attributed to greater fecal excretion of microbial N synthesized in the large intestine. Starch digestion in the large intestine might be greater

for DG treatment compared with HM treatment because cows fed DG shifted site of starch digestion from the rumen to the intestines (Oba and Allen, 2003b). Our results agree with similar observations by others. Knowlton et al. (1998) compared dry corn with HM processed either ground or rolled, and found total tract N digestibility was lower for cows fed dry corn compared with HM treatments. They reported that starch disappearance in the large intestine was greater for dry corn compared with HM treatments with a tendency for greater microbial N production in the large intestine and greater microbial N flow in the feces for dry corn treatment. Greenfield et al. (2001) and Tine et al. (2001) reported that feeding brown midrib corn silage tended to decrease urinary N excretion and increase fecal N excretion. Because diets with brown midrib corn silage increase starch passing to the duodenum (Oba and Allen, 2000; Greenfield et al., 2001), it is speculated that greater intestinal starch digestion might stimulate urea N absorption from the blood circulation to the large intestine for microbial N production (Orskov, 1982). Substitution of DG for HM might alter site of N excretion from urine to feces and decrease apparent N digestibility.

Microbial Efficiency

Microbial N flow to the duodenum was greater for high starch diets compared with low starch diets, which can be explained by greater TRDOM for high starch diets. Microbial N flow was positively related to TRDOM across cow-period observations for this experiment (Figure 1). Although efficiency for microbial N production, defined as grams of microbial N flow at the duodenum per kilogram of TRDOM, was not affected by dietary starch concentration, it was greater for DG treatment compared with HM treatment ($P < 0.03$). Cows fed DG maintained a similar flow of microbial N as cows fed HM corn despite lower TRDOM. Although energy from OM fermentation in the rumen limits maximum microbial N production, microbial efficiency varies greatly (Clark et al., 1992). In this experiment, microbial efficiency decreased as TRDOM increased (Figure 2), indicating that factors other than availability of energy limited efficiency of microbial N production and that energy from OM fermentation was uncoupled from microbial growth.

Energy spilling can occur when ruminal pH is low (Strobel and Russell, 1986). Strobel and Russell (1986) proposed that energy used to maintain intracellular pH decreases the energy available for microbial growth. However, this does not appear to be the reason for the lower microbial efficiency observed for HM compared with DG treatment because ruminal pH was not af-

Figure 1. Relationship between true ruminally degraded OM (TRDOM) and microbial N flow at the duodenum. Microbial N flow = $174.7 + 23.8 \times \text{TRDOM}$ ($r^2 = 0.35$; $P < 0.001$). Closed circle denotes high moisture corn in high starch diets, closed triangle denotes dry ground corn in high starch diets, open circle denotes high moisture corn in low starch diets, and open triangle denotes dry ground corn in low starch diets.

Figure 2. Relationship between true ruminally degraded OM (TRDOM) and microbial efficiency. Microbial efficiency = $67.9 - 2.49 \times \text{TRDOM}$ ($r^2 = 0.31$; $P < 0.001$). Closed circle denotes high moisture corn in high starch diets, closed triangle denotes dry ground corn in high starch diets, open circle denotes high moisture corn in low starch diets, and open triangle denotes dry ground corn in low starch diets.

Table 4. Effects of corn grain conservation method at two dietary starch concentrations on ruminal pH.

	High starch		Low starch		SE	P value		
	HM ¹	DG ²	HM	DG		Starch ³	Corn ⁴	INT ⁵
Ruminal pH								
Mean	6.12	6.13	6.25	6.32	0.05	<0.01	0.41	0.48
Variance	0.120	0.121	0.109	0.070	0.016	0.08	0.23	0.20
Maximum	6.74	6.74	6.81	6.79	0.03	0.07	0.69	0.63
Minimum	5.52	5.55	5.69	5.82	0.08	<0.01	0.24	0.44
Range	1.22	1.19	1.13	0.97	0.07	0.05	0.18	0.33
Hours								
<pH 6.0	9.4	9.0	6.0	4.7	1.4	0.02	0.51	0.71
<pH 5.8	6.3	5.7	3.3	2.1	1.1	<0.01	0.40	0.75
<pH 5.5	2.0	1.6	0.9	0.2	0.4	0.02	0.19	0.65
Area ⁶								
<pH 6.0	361.5	333.5	199.9	117.8	65.3	<0.01	0.38	0.64
<pH 5.8	173.9	159.9	91.5	36.8	36.6	<0.01	0.33	0.54
<pH 5.5	33.2	34.8	18.3	5.5	10.1	0.05	0.53	0.44

¹HM: High moisture corn.²DG: Dry ground corn.³Starch: Effect of dietary starch concentration.⁴Corn: Effect of conservation method of corn grain.⁵INT: Interaction of dietary starch concentration and conservation method of corn grain.⁶Area was calculated by the time under a specific ruminal pH weighed by the deviation from it.

ected by corn grain treatments. In this experiment, ruminal pH was recorded every 5 s continuously for 4 d, allowing us to calculate daily minimum pH, daily variation of ruminal pH, time and area below pH 6.0, 5.8, and 5.5. However, none of these response variables were affected by corn grain treatments (Table 4). No relationships were observed between microbial efficiency and daily mean ruminal pH (Table 5; Figure 3), daily minimum ruminal pH (Figure 4) or other expressions of ruminal pH (data not shown). Therefore, lower microbial efficiency for HM compared with DG treatment was because of factors unrelated to ruminal pH in this experiment. Russell and Wilson (1996) proposed that low ruminal pH decreases microbial growth by accumulation of intracellular VFA, but no relationship was observed between microbial efficiency and total VFA concentration in the rumen ($P = 0.49$) or undissoci-

ated total VFA concentration in the rumen (calculated from daily means of ruminal pH and VFA concentration; $P = 0.18$) for this experiment.

Ammonia concentration was not affected by conservation method of corn grain, but ammonia concentration was relatively low in our experiment especially for cows fed HM corn in high starch diets (5.1 and 1.4 mg/dl for daily mean and daily minimum, respectively). Although ruminal bacteria utilize ammonia N very efficiently (Schaefer et al., 1980), this ammonia concentration might be thought to limit maximum microbial N production in the rumen. Microbial N production was maximized at 5 mg/dl of ammonia N concentration in ruminal fluid, and further increases in ammonia concentration did not increase microbial N production (Satter and Slyter, 1974). This concentration is slightly higher than the intracellular concentration of ammonia

Table 5. Pearson correlation coefficients for microbial efficiency and related variables.¹

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
(1) Microbial efficiency, g MN ² /kg TRDOM ³	—	—	—	—	—	—	—
(2) Daily mean for ruminal pH	0.21	—	—	—	—	—	—
(3) Daily minimum for ruminal pH	0.26	0.89 ¹	—	—	—	—	—
(4) Hours below pH 6.0	-0.27	-0.99 ¹	-0.87 ¹	—	—	—	—
(5) Area below pH 6.0 ⁴	-0.25	-0.95 ¹	-0.86 ¹	0.96 ¹	—	—	—
(6) Ruminal VFA concentration, mM	-0.13	-0.46 ¹	-0.53 ¹	0.47 ¹	0.49 ¹	—	—
(7) Ruminal ammonia concentration, mg/dl	-0.09	-0.52 ¹	-0.33	0.51 ¹	0.40 ¹	0.33	—

¹Correlation is significant ($P < 0.05$).²MN: Microbial nitrogen.³TRDOM: Truly ruminally digested OM.⁴Area was calculated by the time under ruminal pH 6.0 weighed by the deviation from it.

Figure 3. Relationship between daily mean ruminal pH and microbial efficiency. ($r^2 = 0.05$; $P > 0.24$). Closed circle denotes high moisture corn in high starch diets, closed triangle denotes dry ground corn in high starch diets, open circle denotes high moisture corn in low starch diets, and open triangle denotes dry ground corn in low starch diets.

Figure 5. Relationship between ruminal ammonia concentration and microbial efficiency. ($r^2 = 0.01$; $P > 0.62$). Closed circle denotes high moisture corn in high starch diets, closed triangle denotes dry ground corn in high starch diets, open circle denotes high moisture corn in low starch diets, and open triangle denotes dry ground corn in low starch diets.

N required to maximize glutamine synthesis that fixes ammonia N to amino acids (Harrison and McAllan, 1980). However, within the dataset of cow-period obser-

vations for this experiment, there was no relationship between microbial efficiency and ruminal ammonia concentration (Figure 5). Decreased microbial efficiency for HM treatment was probably not from a deficiency of ammonia N in the rumen.

Figure 4. Relationship between daily minimum ruminal pH and microbial efficiency. ($r^2 = 0.07$; $P > 0.15$). Closed circle denotes high moisture corn in high starch diets, closed triangle denotes dry ground corn in high starch diets, open circle denotes high moisture corn in low starch diets, and open triangle denotes dry ground corn in low starch diets.

Decreased efficiency of microbial N production also occurs with a deficiency of available amino acids or peptides (Van Kessel and Russell, 1996). Ruminal bacteria fermenting nonstructural carbohydrates grow faster and more efficiently when incorporating amino acids and peptides into microbial protein (Russell and Sniffen, 1984). Ruminal microorganisms fermenting nonstructural carbohydrate can obtain approximately two thirds of their N from amino acids or peptides (Russell et al., 1983), while fiber digesting bacteria derive all of their N from ammonia (Bryant, 1973). Maeng and Baldwin (1976) reported that iso-nitrogenous replacement of urea with a mixture of amino acids increased microbial growth from 19.3 to 24.4 mg of microbial cells per 100 mg of glucose. Although concentrations of amino acids and peptides in ruminal fluid were not determined in our experiment, HM treatment might have decreased microbial efficiency because the availability of amino acids or peptides in the rumen limited maximum utilization of energy for microbial protein synthesis. However, diets in this experiment were formulated for sufficient quantity of RDP; animals were fed 18% dietary CP (% of DM) with observed RDP ranging from 66 to 80% (NANMN ranged from 20.2 to 34.2%

Table 6. Effects of corn grain conservation method at two dietary starch concentrations on digestion kinetics in the rumen.

	High starch		Low starch		SE	P value		
	HM ¹	DG ²	HM	DG		Starch ³	Corn ⁴	INT ⁵
Ruminal digestion rate (%/h)								
Starch	28.2 ^a	14.6 ^{bc}	16.8 ^b	12.2 ^c	1.2	<0.001	<0.001	<0.01 ⁶
Potentially digestible NDF	2.25	2.91	3.15	3.44	0.23	<0.01	0.05	0.44
Ruminal passage rate (%/h)								
OM	3.98	5.04	4.05	5.11	0.32	0.88	<0.01	0.94
Starch	16.8	21.2	13.9	18.2	2.3	0.20	0.07	0.95
NDF	3.27	3.61	3.48	3.87	0.22	0.31	0.11	0.89
Potentially digestible NDF	2.59	3.01	2.84	2.67	0.33	0.84	0.67	0.41
Indigestible NDF	3.33	3.58	3.38	3.71	0.22	0.16	0.03	0.29

¹HM: High-moisture corn.²DG: Dry ground corn.³Starch: Effect of dietary starch concentration.⁴Corn: Effect of conservation method of corn grain.⁵INT: Interaction of dietary starch concentration and conservation method of corn grain.⁶Student's *t*-test was conducted if *P* < 0.10 for interactions of main effects. Treatment means in a same row followed by different superscript letters differ significantly (*P* < 0.05).

of N intake) and soybean meal was the primary protein supplement (32 to 36% of total dietary CP). Soybean meal increases microbial N flow compared with other protein supplements (Clark et al., 1992). If our experimental diets failed to maximize microbial efficiency due to lack of amino acids or peptides, it is unlikely that conventional diet ingredients supply sufficient amino acids and peptides to achieve maximum microbial growth in vivo without excessive excretion of nitrogen. Lower microbial efficiency for HM treatments compared with DG treatments cannot be explained by low ruminal pH or availability of ruminal degraded N, and the existing literature does not offer a good explanation for our observation.

Digestion Kinetics and Microbial Efficiency

Rate of starch digestion in the rumen was greater for HM treatments compared with DG treatments, and passage rate of OM and starch was greater for cows fed DG than for cows fed HM (Table 6). Microbial efficiency was positively correlated with rate of passage of OM and starch and negatively correlated with TRDOM and digestion rate of starch (Table 7) but was not correlated with digestion rate or passage rate of the potentially digestible NDF fraction. Although many microbial organisms flow from the rumen attached to fibrous particles, passage rate of NDF was not related to microbial efficiency possibly because differences in dietary starch sources and concentrations were the primary factors affecting microbial efficiency in this experiment. Microbial efficiency increased as digestion rate of starch decreased (Figure 6; three outliers were not influential observations for this relationship; DFFITS <0.8), indi-

cating that energy from starch fermentation might not have been efficiently utilized for microbial growth as rate of starch digestion increased. As discussed above, this might be because of a deficiency of amino acids, peptides, or any unknown growth factors. In addition, maximum rate of fermentation might have exceeded the maximum growth rate of the microbial population.

A positive relationship was observed between microbial efficiency and starch passage rate (Figure 7), indicating that energy from ruminal fermentation was more efficiently utilized for microbial growth as passage rate for starch and OM increased. This is consistent with previous observations in vitro. Isaacson et al. (1975) evaluated efficiency of microbial growth in a glucose medium at dilution rates of 0.02, 0.06, and 0.12 h⁻¹, and found *Y*_{ATP} (g of microbial cell production per mole of ATP) increased as the dilution rate increased. Greater dilution rate decreased degradation and turnover of microbes in the rumen (Stouthamer and Bettenhausen, 1973; Kennedy and Milligan, 1978), and greater passage rate of ruminal digesta possibly decreases microbial lysis and turnover in the rumen. Extensive microbial turnover in the rumen due to predation by protozoa also reduces microbial efficiency (Wallace and McPherson, 1987). Microbial efficiency can be divided into two factors: efficiency of microbial N production in the rumen and efficiency of microbial N passage.

$$\begin{aligned} &\text{Microbial N flow at the duodenum (g)/TRDOM (kg)} \\ &= \text{Microbial N production (g)/TRDOM (kg)} \\ &\times \text{Microbial N flow at the duodenum (g)/Microbial N} \\ &\quad \text{production (g)} \end{aligned}$$

Table 7. Pearson correlation coefficients for microbial efficiency and related variables.¹

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(1) Microbial efficiency, g MN ² /kg TRDOM ³	—	—	—	—	—	—	—	—	—
(2) TRDOM ³ , kg/d	-0.56 ¹	—	—	—	—	—	—	—	—
(3) Duodenal MN ² flow, g/d	0.27	0.59 ¹	—	—	—	—	—	—	—
(4) Passage rate for OM, %/h	0.77 ¹	-0.64 ¹	0.06	—	—	—	—	—	—
(5) Passage rate for starch, %/h	0.75 ¹	-0.32	0.40 ¹	0.77 ¹	—	—	—	—	—
(6) Passage rate for PDNDF ⁴ , %/h	0.24	-0.19	0.05	0.37 ¹	0.20	—	—	—	—
(7) Passage rate for IDNDF ⁵ , %/h	0.62 ¹	-0.28	0.33	0.73 ¹	0.55 ¹	0.07	—	—	—
(8) Digestion rate for starch, %/h	-0.55 ¹	0.57 ¹	0.11	-0.41 ¹	-0.39 ¹	-0.05	-0.10	—	—
(9) Digestion rate for PDNDF ⁴ , %/h	0.20	0.10	0.38 ¹	0.23	0.35	-0.39 ¹	0.57 ¹	-0.16	—

¹Correlation is significant ($P < 0.05$).²MN: Microbial nitrogen.³TRDOM: True ruminally digested OM.⁴PDNDF: Potentially digestible NDF.⁵IDNDF: Indigestible NDF.

Because host animals can absorb microbial N only if it flows to the duodenum, rate of microbial N flow from the rumen is an important factor determining microbial efficiency. If rate of passage is decreased, energy supplied from OM fermentation in the rumen is wasted due to microbial turnover in the rumen.

Our results related to microbial efficiency for this experiment should be interpreted with caution. Microbial efficiency was greater than 70 g of microbial N per kilogram of TRDOM for three observations, but these high values of microbial efficiency might not be physiologically possible as discussed below. These three obser-

Figure 6. Relationship between rate of starch digestion in the rumen and microbial efficiency. Microbial efficiency = $56.8 - 0.72 \times$ rate of starch digestion ($r^2 = 0.30$; $P < 0.001$). Closed circle denotes high moisture corn in high starch diets, closed triangle denotes dry ground corn in high starch diets, open circle denotes high moisture corn in low starch diets, and open triangle denotes dry ground corn in low starch diets.

Figure 7. Relationship between rate of starch passage in the rumen and microbial efficiency. Microbial efficiency = $23.9 + 1.13 \times$ rate of starch passage ($r^2 = 0.56$; $P < 0.0001$). Closed circle denotes high moisture corn in high starch diets, closed triangle denotes dry ground corn in high starch diets, open circle denotes high moisture corn in low starch diets, and open triangle denotes dry ground corn in low starch diets.

variations were all from DG treatments (one from high starch diet and two from low starch diet). Therefore, if these high values of microbial efficiency are caused by sampling error or problems associated with a single marker method to calculate duodenal flow, they might have caused bias that exaggerates treatment effects on microbial efficiency. Treatment means for observed microbial efficiency were also high in this experiment, ranging from 39.3 to 49.4 g of microbial N per kilogram of TRDOM. Our data fit within the range of variation reported in the literature (Clark et al., 1992), which is from less than 20 to 50 g of microbial N flow at the

duodenum per kg of TRDOM. However, our methods might have overestimated microbial N flow at the duodenum because our values were greater than the theoretical maximum microbial efficiency, calculated as 31.3 g of microbial N/kg of TRDOM. The theoretical calculation requires partitioning of microbial efficiency into two factors: efficiency of microbial ATP production and efficiency of microbial N production.

$$\begin{aligned} &\text{Microbial N production (g)/TRDOM (kg)} \\ &= \text{ATP production (moles)/TRDOM (kg)} \\ &\times \text{Microbial N production (g)/ATP (mole)} \end{aligned}$$

One mole of hexose equivalent TRDOM can generate up to 4 moles of ATP when it ferments to VFA (Harrison and McAllan, 1980), and theoretical maximum Y_{ATP} was shown to be approximately 26 g of microbial cell/mole of ATP based on biochemical calculations (Hespell and Bryant, 1979). Assuming microbial cells are 8% N (Clark et al., 1992), one mole of hexose equivalent TRDOM can produce up to 8.3 g of microbial N (which equals to 51 g of microbial N/kg of TRDOM: 1 kg of TRDOM/(162 g of glucose per mole before hydrolysis) $\times$ 8.3 g/mole). However, maximum microbial efficiency can be limited by the efficiency of microbial ATP production (moles of ATP/kg of TRDOM). Less substrate is available for microbial ATP production as microbial cell production increases because TRDOM is conserved as fermentation end products and microbial cells. The partitioning of TRDOM into microbial cell synthesis and fermentation end product synthesis by which microbial ATP is generated at a maximum microbial efficiency is expressed in the following equation. Solving this equation for MN gives the theoretical maximum microbial efficiency of 31.3 (g of microbial N/kg of TRDOM).

$$((1000 - (\text{MN}/0.08))/162) \times 4 \times 26 = \text{MN}/0.08$$

where

MN = microbial N, g

1000 = TRDOM (g)

0.08 = N concentration in microbial cells

162 = molecular weight of hexose equivalent (g/mole)

4 = ATP production per mole of hexose (moles/mole of hexose)

26 = microbial cell production per mole of ATP (g/mole of ATP)

We observed high microbial efficiency that is close to 50 g of microbial N/kg of TRDOM for DG treatments, but this high level of microbial efficiency would require approximately 8 moles of ATP production from one mole of hexose-equivalent TRDOM. This is not theoretically

acceptable, and the reasons for disagreement between the observed data and the theoretical maximum values are not known. However, efficiency of microbial ATP production is not well established because measured microbial ATP production has not been reported in the literature. Microbial production of VFA is associated with formation of reducing equivalents as well as ATP production. Although it has been generally assumed that anaerobic bacteria do not possess phosphorylation systems linked to electron transport, this has been argued (Russell and Wallace, 1997). Utilization of energy conserved in reducing equivalents for microbial growth might explain our observations. Further research is needed to understand energy metabolism of anaerobic bacteria.

Purines were used as a microbial marker in this experiment, and microbial N flow was determined by N:purines ratio in microbial pellets and concentration of purines in duodenal digesta. The N:purines ratio in our experiment (1.16) is close to the mean value of 1.06 reported in the literature by Clark et al. (1992), but microbial efficiency could be overestimated if the N:purines ratio in microbial pellets is higher than actual MN:purines ratio in duodenal digesta. In addition, contamination of purines from other sources, if occurred, possibly increased analyzed microbial N flow because our methods assumed that duodenal digesta contains no purines originating from either feeds or epithelial cells of the digestive tract. We do not know whether theoretical calculation underestimated actual microbial efficiency or whether our analytical methods overestimated microbial efficiency.

Another possible explanation for relatively high microbial efficiency observed in this experiment is overestimation of duodenal DM flow. In a companion paper, we mentioned the possibility of underestimation in ruminal NDF digestibility from overestimation of duodenal NDF flow, which might have been because of an overestimate of duodenal DM flow because of potential marker flow problems. Overestimated duodenal DM flow (including NDF and microbial cells) relative to flow of chromic oxide would result in an underestimate of ruminal DM (including NDF) digestibility, and an overestimate of microbial N yield and efficiency of microbial N production. To minimize this problem, we sampled from the duodenum fewer times (but distributed evenly throughout the day) and took larger samples than in some other studies. Although it is impossible to identify whether sampling errors occurred, we acknowledge this possibility.

CONCLUSION

Microbial N flow was greater for high starch diets than for low starch diets, suggesting that microbial N

production was limited by energy from OM fermentation in the rumen for low starch diets. However, microbial efficiency as grams of microbial N flow at the duodenum per kilogram of TRDOM was greater for DG compared with HM treatment. Within the dataset of cow-period observations, microbial efficiency was negatively correlated with TRDOM and rate of starch digestion, consistent with the theory that energy spilling decreases microbial efficiency. However, energy spilling did not appear to be from lack of ammonia or low ruminal pH because microbial efficiency was not related to ruminal ammonia concentration, daily mean ruminal pH, or minimum ruminal pH. Microbial efficiency was positively correlated with passage rate for OM and starch. A more rapid passage rate decreases microbial turnover in the rumen, resulting in increased microbial efficiency. Rate of starch availability and rates of passage for starch and OM from the rumen are important determinants of microbial protein synthesis in vivo.

ACKNOWLEDGMENTS

Acknowledgment is made to the Corn Marketing Program of Michigan and the Michigan Agricultural Experiment Station for financial support of this research. The authors thank N. K. Ames for performing duodenal and ruminal cannulation surgery, and thank R. E. Kreft, R. A. Longuski, C. S. Mooney, D. G. Main, R. J. Tempelman, and Y. Ying for technical assistance.

REFERENCES

- Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physical effective fiber. *J. Dairy Sci.* 80:1447–1462.
- ASAE. 1968. Method of determining and expressing fineness of feed material by sieving. ASAE standard S319. St. Joseph, MI.
- Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* 63:64–75.
- Bryant, M. P. 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. *Fed. Proc.* 32:1809.
- Callison, S. L., J. L. Firkins, M. L. Eastridge, and B. L. Hull. 2001. Site of nutrient digestion by dairy cows fed corn of different particle sizes or steam-rolled. *J. Dairy Sci.* 84:1458–1467.
- Clark, J. H., T. H. Klusmeyer, and M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. *J. Dairy Sci.* 75:2304–2323.
- Crocker, L. M., E. J. DePeters, J. G. Fadel, H. Prez-Monti, S. J. Taylor, J. A. Wyckoff, and R. A. Zinn. 1998. Influence of processed corn grain in diets of dairy cows on digestion of nutrients and milk composition. *J. Dairy Sci.* 81:2394–2407.
- Dado, R. G., and M. S. Allen. 1993. Continuous computer acquisition of feed and water intake, chewing reticular motility, and ruminal pH of cattle. *J. Dairy Sci.* 76:1589–1600.
- Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handb. No. 379. ARS-USDA, Washington, DC.
- Greenfield, T. L., R. L. Baldwin VI, R. A. Erdman, and K. R. McLeod. 2001. Ruminal fermentation and intestinal flow of nutrients by lactating cows consuming brown midrib corn silages. *J. Dairy Sci.* 84:2469–2477.
- Hach, C. C., B. K. Bowden, A. B. Lopelove, and S. V. Brayton. 1987. More powerful peroxide Kjeldahl digestion method. *J. AOAC* 70:783–787.
- Harrison, D. G., and A. B. McAllan. 1980. Factors affecting microbial growth yields in the reticulo-rumen. Pages 205–226 in *Digestive Physiology and Metabolism in Ruminants*. Y. Ruckebusch Y., and P. Thivend. Avi Publishing Company, Westport, CT.
- Hespell, R. B., and M. P. Bryant. 1979. Efficiency of rumen microbial growth: Influence of some theoretical and experimental factors on YATP. *J. Anim. Sci.* 49:1640–1659.
- Isaacson, H. R., F. C. Hinds, M. P. Bryant, and F. N. Owens. 1975. Efficiency of energy utilization by mixed rumen bacteria in continuous culture. *J. Dairy Sci.* 58:1645–1659.
- Karkalas, J. 1985. An improved enzymatic method for the determination of native and modified starch. *J. Sci. Food Agric.* 36:1019–1027.
- Kennedy, P. M., and L. P. Milligan. 1978. Effects of cold exposure on digestion, microbial synthesis and nitrogen transformations in sheep. *Br. J. Nutr.* 39:105–117.
- Knowlton, K. F., B. P. Glenn, and R. A. Erdman. 1998. Performance, ruminal fermentation, and site of starch digestion in early lactation cows fed corn grain harvested and processed differently. *J. Dairy Sci.* 91:1972–1984.
- Maeng, W. J., and R. L. Baldwin. 1976. Factors influencing rumen microbial growth rates and yields: Effects of urea and amino acids over time. *J. Dairy Sci.* 59:643–647.
- National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington DC.
- Oba, M., and M. S. Allen. 2000. Effect of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two levels of dietary NDF: 3. Digestibility and microbial efficiency. *J. Dairy Sci.* 83:1350–1358.
- Oba, M., and M. S. Allen. 2003a. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. *J. Dairy Sci.* 86:174–183.
- Oba, M., and M. S. Allen. 2003b. Effects of corn grain conservation method on ruminal digestion kinetics for lactating dairy cows at two dietary starch concentrations. *J. Dairy Sci.* 86:184–194.
- O'Connor, J. D., C. J. Sniffen, D. G. Fox, and W. Chalupa. 1993. A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. *J. Anim. Sci.* 71:1298–1311.
- Orskov, E. R. 1982. Protein Nutrition in Ruminants. Academic Press, New York.
- Overton, T. R., M. R. Cameron, J. P. Elliott, J. H. Clark, and D. R. Nelson. 1995. Ruminal fermentation and passage of nutrients to the duodenum of lactating cows fed mixtures of corn and barley. *J. Dairy Sci.* 78:1981–1998.
- Plascencia, A., and R. A. Zinn. 1996. Influence of flake density on the feeding value of steam-processed corn in diets for lactating dairy cows. *J. Anim. Sci.* 74:310–316.
- Ricke, S. C., and D. M. Schaefer. 1996. Growth and fermentation responses of *Selenomonas ruminantium* to limiting and non-limiting concentrations of ammonium chloride. *Appl. Microbiol. Biotechnol.* 46:169–175.
- Robinson, P. H., C. J. Sniffen, and D. F. Smith. 1985. Development of a one-piece reentrant cannula for the proximal duodenum of dairy cows. *J. Dairy Sci.* 68:986–995.
- Russell, J. B., and C. J. Sniffen. 1984. Effect of carbon-4 and carbon-5 volatile fatty acids on growth of mixed rumen bacteria in vitro. *J. Dairy Sci.* 67:987.
- Russell, J. B., and D. B. Wilson. 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? *J. Dairy Sci.* 79:1503–1509.
- Russell, J. B., and R. J. Wallace. 1997. Energy-yielding and energy-consuming reactions. Pages 246–282 in *The Rumen Microbial Ecosystem*. P. N. Hobson and C. S. Stewart, eds. Blackie Academic & Professional, London.
- Russell, J. B., C. J. Sniffen, and P. J. Van Soest. 1983. Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. *J. Dairy Sci.* 66:763.

- Stouthamer, A. H., and C. Bettenhausen. 1973. Utilization of energy for growth and maintenance in continuous and batch cultures of microorganisms. *Biochim. Biophys. Acta* 301:53–70.
- Strobel, H. J., and J. B. Russell. 1986. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. *J. Dairy Sci.* 69:2941–2947.
- Satter, L. D., and L. L. Slyter. 1974. Effect of rumen ammonia concentration on rumen microbial production in vitro. *Br. J. Nutr.* 32:199–208.
- Schaefer, D. M., C. L. Davis, and M. P. Bryant. 1980. Ammonia saturation constants for predominant species of rumen bacteria. *J. Dairy Sci.* 63:1248.
- Tine, M. A., K. R. McLeod, R. A. Erdman, and R. L. Baldwin. 2001. Effects of brown midrib corn silage on the energy balance of dairy cattle. *J. Dairy Sci.* 84:885–895.
- Van Kessel, J. S., and J. B. Russell. 1996. The effect of amino nitrogen on the energetics of ruminal bacteria and its impact on energy spilling. *J. Dairy Sci.* 79:1237–1243.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Ying, Y., M. S. Allen, M. J. VandeHaar, and N. K. Ames. 1998. Effects of fineness of grinding and conservation method of corn grain on ruminal and whole tract digestibilities and ruminal protein production of Holstein cows in early lactation. *J. Dairy Sci.* 81(Suppl. 1):339.
- Williams, C. H., D. J. David, and O. Iismaa. 1962. The determination of chromic oxide in feces samples by atomic absorption spectrophotometry. *J. Agric. Sci.* 59:381–385.
- Wallace, R. J., and C. A. McPherson. 1987. Factors affecting the rate of breakdown of bacterial protein in rumen fluid. *Br. J. Nutr.* 58:313–323.
- Zinn, R. A., and 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.