



Effects of Degree of Processing and Nitrogen Source and Level on Starch Availability and In Vitro Fermentation of Corn and Sorghum Grain

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Abstract

Ruminal fluid from two heifers (384 ± 2 kg) fed an 85% concentrate diet was used to determine effects of degree of processing and N source and level on in vitro DM disappearance (DMD), starch availability, pH changes, and NH₃ concentrations of corn and sorghum. In Exp. 1, treatments (2 × 4 factorial) were unprocessed corn and sorghum (UP) and each grain steam-flaked to bulk densities (BD) of 0.38, 0.33, and 0.28 kg/L (C38, B33, A28, respectively). Treatments in Exp. 2 (2 × 2 × 3 factorial within grain type) were UP and A28 with 0, 10, or 20 mg of N/g provided by either urea (U) or casein hydrolysate (CH). Starch availability of both grains increased linearly (P<0.001) as BD decreased. In Exp. 1, DMD of sorghum was greater (P<0.10) than that of corn at 24 and 48 h of incubation. For both grains, DMD increased linearly (P<0.02) as BD decreased at 4 and 8 h. Corn culture NH₃ was less (P<0.10) than sorghum at 8 h, and as BD decreased at 4, 8, and 12 h, NH₃ decreased linearly (P<0.05).

Culture pH was less for corn than for sorghum at 8 h and decreased linearly (P<0.04) with decreasing BD at 12, 18, and 24 h. In Exp. 2, source of N did not affect (P>0.10) DMD of corn or sorghum beyond 4 h, but DMD increased linearly (P<0.04) as N level increased at 8, 12, 18, and 24 h for corn and at all incubation times for sorghum. At 8 through 24 h, CH and U increased NH₃ of UP and A28 corn linearly (P<0.001), but the magnitude was greater (P<0.001) for U than for CH; the same trends were evident (P<0.001) at 12, 18, and 24 h for NH₃ of sorghum treatments. Increasing N level increased pH of corn linearly (P<0.05) at 4, 8, 18, and 24 h, with a greater increase for U than for CH. Increasing N level increased pH of sorghum linearly (P<0.03) at 1 through 24 h, but to a greater extent for U than CH at 1, 2, 12, and 18 h. Degree of processing and level of supplemental N were positively related to in vitro DMD, whereas no benefit was evident from supplementing amino acid N compared to U.

(Key Words: Steam Flaking, Ammonia, Starch Availability, pH.)

Introduction

The degree of processing of steam-flaked sorghum supporting optimum performance by finishing cattle has been variable. Available data suggest a range of optimum bulk densities from 0.28 to 0.36 kg/L for sorghum (16, 26, 30, 31, 35) and approximately 0.36 kg/L for corn (36). The sensitivity of bulk density in describing the degree of processing has been questioned (34), and many recent experiments have included some type of laboratory measure of starch availability to assess the degree of processing. However, comparisons of starch availability are often precluded by either differences in the type of assay used or by variation in the conditions used within a particular type of assay. In light of the input costs associated with grain processing (26) and the paradox between the desire to maximize energetic efficiency and minimize the potential for acidosis in cattle (24), further study of the relationship between starch availability and ruminal fermentation seems warranted.

Increasing the degree of grain processing may alter ruminal N needs

through increasing the rate of fermentation (9) and/or potential changes in grain CP digestibility (35, 36). Industry recommendations for feedlot cattle fed diets with a high extent of ruminal fermentation call for dietary CP levels in excess of established requirements and favor ruminally degraded N sources (8). Our objective was to determine the effect of degree of processing and the interaction between degree of processing and level of supplemental nonprotein N (NPN) and amino acid N on measurements of starch availability and in vitro fermentation of corn and sorghum grain.

Materials and Methods

Two ruminally cannulated heifers (384 ± 2 kg) were used as ruminal fluid donors in two, replicated in vitro fermentation experiments. Heifers were adapted to (14 d), and fed at 1.7% of BW thereafter, an 85% concentrate diet. Composition of the diet (DM basis) was: sudangrass hay, 7.92%; alfalfa hay, 7.51%; rolled corn, 70.19%; soybean meal, 2.49%; molasses, 4.86%; yellow grease, 3.04%; limestone, 0.74%; dicalcium phosphate, 0.49%; salt, 0.35%; urea, 0.91%, ammonium sulfate, 0.5%; and premix, 1.00%. Premix supplied trace minerals, vitamins A and E, monensin (33 mg/kg), and tylosin (8.8 mg/kg). Heifers were handled and cared for according to a protocol approved by the New Mexico State University Institutional Animal Care and Use Committee.

Experimental Design and Treatments. In Exp. 1, effects of grain type and degree of processing on enzymatic starch availability, in vitro DM disappearance (DMD), pH, and NH_3 concentrations were evaluated. Treatments were arranged in a 2×4 factorial replicated in time, and consisted of unprocessed corn and sorghum (UP = 0.72 kg/L bulk density) and each grain steam-flaked to bulk densities (BD) of 0.38, 0.33, and 0.28 kg/L (C38, B33, and A28, respectively). Densities of the steam-flaked grains were selected on the

basis that they reflect the upper, middle, and lower points of the range currently used in Great Plains cattle feedlots. In Exp. 2, effects of the degree of processing and N source and level on in vitro DMD, pH, and NH_3 were evaluated. Treatments were arranged in a $2 \times 2 \times 3$ factorial within grain type. Treatments consisted of UP and A28 corn or sorghum supplemented with 0, 10, and 20 mg of N/g of substrate provided by either casein hydrolysate (CH; no. A2427, Sigma Chemical Co., St. Louis, MO) or urea (U; no. 4202-01, J. T. Baker, Inc., Phillipsburg, NJ). Stock solutions of U and CH were formulated to contain 5 or 10 mg of N/mL (DM basis). One milliliter of stock solution was added to tubes containing 0.5 g of substrate at the time of culture inoculation, whereas the control tubes (0 mg of N) received 1 mL of distilled water. Stock solutions contained 10.75 and 21.5 mg of U, and 39.1 and 78.2 mg of CH for 5 and 10 mg of N/mL solutions (DM basis), respectively.

Grain Processing. The experimental steam-flaked grains were prepared by the Texas Tech University Department of Animal and Food Technology, Lubbock, TX. Grains were cleaned and then processed using a pilot steam-flaking unit (no. 18 \times 12 HYD, Ferrell-Ross, Oklahoma City, OK) with a steam chest capacity of 33.98 kg of grain. The steam chest measured 25.5 cm in diameter and 8 m in length. Four steam lines, spaced 2 m apart, spanned the total length of the steam chamber. Steam was allowed to enter the chest by opening the valve on each of the steam lines, and 1.76 kg/cm² (25 psi) of pressure was maintained on the steam lines. Grain was steamed (internal steam chest temperature = 96.1°C) for 40 min. Cooked grain was moved to the two 30.5 cm \times 45.7 cm rollers by a variable speed magnetic feeder (No. BF2AFMC, Syntron, Philadelphia, PA). The rollers had four corrugations per centimeter in a Stevens cut. Aliquots of 22.72 kg of grain for each desired BD (0.38, 0.33, and 0.28 kg/L) were flaked by adjust-

ing the distance between the rolls. A 2-kg subsample was taken as the grain exited the rolls and stored at -20°C . The remainder was allowed to air-dry before use. Each treatment grain (substrate) was prepared for in vitro fermentation, enzymatic starch availability assay, and chemical analyses by grinding in a Wiley mill to pass a 1-mm screen.

Laboratory Methods. Chemical analyses of grain treatments included DM, ADF, ash, Kjeldahl N (1), and total starch content (18). An enzymatic starch availability assay using an amyloglucosidase (No. A7255, Sigma Chemical Co., St. Louis, MO) incubation at 50°C for 1 h was used to determine glucose (No. G5767, Sigma Chemical Co., St. Louis, MO) release (34).

Batch culture in vitro fermentation procedures were used (7) with McDougall's artificial saliva (19) as the buffer, followed by a 48-h incubation with acidified pepsin (No. C4351R, Sargent-Welch, Buffalo Grove, IL; 33). Briefly, two 0.5-g aliquots of each substrate were weighed into 50-mL plastic centrifuge tubes. Ruminal fluid collection took place 3 h after feeding as outlined by Duff et al. (5). Culture inoculation was performed by flushing the tube plus substrate with CO_2 , adding 7 mL of strained ruminal fluid and 28 mL of McDougall's artificial saliva, refluxing with CO_2 , capping, and shaking the tube initially and every 3 h thereafter (7). Samples were incubated for various incubation periods in a water bath at 39°C.

Following incubation, samples were centrifuged at 1500 \times g for 15 min to pellet the remaining DM, and the supernatant fluid was aspirated. Samples were then incubated for 48 h with 35 mL of acidified pepsin solution (33). After 48 h, samples were filtered onto ashless filter paper, the residue was dried in a forced-air oven at 100°C for 24 h, and the dry weight was determined. Duplicate treatment culture tubes and blanks (ruminal fluid + buffer only) were represented within each incubation time, with the run replicated on a

second day. For both experiments, DMD was determined at culture incubation times of 0, 4, 8, 12, 24, and 48 h, each followed by a 48-h pepsin digest.

Because in vitro fermentation cultures are highly buffered, McDougall's artificial saliva (19) was diluted with distilled water (25:75 buffer:water) to develop an assay for measuring in vitro pH changes. The goal was to allow pH changes over time, yet not hinder microbial activity as measured by DMD. The in vitro fermentation procedure (7) was modified as follows: 1) add 1 L of McDougall's artificial saliva to 3 L of distilled water in a 4-L Erlenmeyer flask, and stir; 2) place diluted buffer solution in a 39 °C water bath and bubble with CO₂ while collecting ruminal fluid; and 3) after collection, raise the tubing supplying CO₂ above the surface of the buffer solution so that CO₂ flushes the flask but does not bubble, and continue to do so throughout inoculation (this prevented an increase in the pH of the diluted buffer solution while cultures were inoculated). Culture inoculation followed the procedure of Galyean (7).

Ammonia concentrations and pH were measured following 0, 1, 2, 4, 8, 12, 18, and 24 h incubation using the buffer:water mixture in both experiments, whereas DMD of the buffer:water media was determined at 0, 4, 8, 12, and 24 h for assay validation. After incubation and centrifugation, 1 mL of supernatant fluid was removed from each tube, dispensed into a microcentrifuge tube with 0.1 mL of 7.2 N H₂SO₄, and frozen at -20 °C until later NH₃ analysis (3). The pH of the remaining supernatant fluid was determined using a pH meter (No. 01048, Denver Instrument Co., Denver, CO) and combination electrode with automatic temperature compensation. Each run measuring NH₃, pH, and diluted-buffer DMD in both experiments contained duplicate treatment culture tubes and blanks at each incubation time and was replicated on a second day.

Data Analyses. Data were analyzed by analysis of variance for a completely random design (28). Ammonia concentrations, pH, and DMD data were analyzed within incubation time. For Exp. 1, the model included effects for grain type, degree of processing, and grain type × degree of processing. For Exp. 2, the model included effects for degree of processing, N source, N level, and all possible interactions. Duplicate treatment tubes were averaged within each time for each run; therefore, the residual (replicate within treatment) served as the error term. Main-effect means were separated following a significant ($P < 0.10$) *F* test using orthogonal contrasts of unprocessed vs steam-flaked (UP vs C38, B33, and A28), and linear and quadratic effects among steam-flaked grains in Exp. 1. Main-effect mean separation tests in Exp. 2 involved orthogonal contrasts of UP vs A28, U vs CH, and linear and quadratic effects of N level. In both experiments, the nature of interactions was determined using multiple orthogonal contrasts (Table 1).

Results and Discussion

Chemical Composition. Percentage of ash, ADF, and total starch were relatively constant for each grain (Table 2), as was CP of the sorghum BD. Greater variation in CP was evident among corn BD than among sorghum BD. The mean of CP for cracked corn according to NRC (22) is 9.8%. Ash and ADF of corn BD, and ash content of sorghum BD agree well with NRC (22). Conversely, CP and ADF of sorghum BD were less than NRC (22) values (12.6 and 6.4%, respectively).

Starch availability (SA; milligrams of glucose per gram of grain) depended on grain type and degree of processing ($P < 0.03$; Table 2). Both UP corn and sorghum had the least ($P < 0.0001$) amount of glucose liberated enzymatically, whereas SA increased linearly ($P < 0.0001$) as BD decreased from C38 to A28. Xiong et al. (34, 35) used the same SA assay and reported that 0.28 kg/L sorghum had SA of 678 and 559 mg/g, respectively; both studies indicated a linear effect of the degree of processing on SA. Preston et al. (25) reported that

TABLE 1. Multiple orthogonal contrasts used to determine the nature of main-effect interactions.

Experiment 1 ^a	Experiment 2 ^b
Corn vs sorghum	Unprocessed vs steam-flaked (SF)
Unprocessed vs SF	Urea (U) vs casein hydrolysate (CH)
Degree of processing	N level
Linear	Linear
Quadratic	Quadratic
Grain × unprocessed vs SF	Degree of processing × U vs CH
Linear	Degree of processing × N level
Quadratic	Linear
	Quadratic
	U vs CH × N level
	Linear
	Quadratic
	Degree of processing × U vs CH × N level
	Linear
	Quadratic

^aMain effects were grain type and degree of processing (2 × 4 factorial).

^bMain effects were degree of processing, N source, and N level (2 × 2 × 3 factorial).

TABLE 2. Dry matter chemical composition of unprocessed and steam-flaked corn and sorghum grain.

Item	Corn density, kg/L				SE ^b	Sorghum density, kg/L				SE ^b
	0.72 ^a	0.38	0.33	0.28		0.72 ^a	0.38	0.33	0.28	
Ash, %	1.5	1.5	1.6	1.5	—	1.8	1.8	2.0	1.8	—
ADF, %	4.0	4.0	3.9	3.9	—	4.5	4.5	4.3	4.4	—
CP, %	9.6	8.4	9.0	9.3	—	11.3	11.6	11.7	11.3	—
Total starch, %	66.9	66.7	67.8	67.0	—	71.1	71.3	71.1	71.9	—
SA ^c	168 ^d	390 ^e	475	578	10	187 ^d	475 ^e	548	633	10
SA, % ^f	23	53	64	78	—	24	61	70	80	—

^aUnprocessed corn or sorghum grain.

^bStandard error of the least squares mean, n = 8

^cSA = starch availability, mg of glucose/g of grain. Grain x processing interaction ($P < 0.03$).

^dUnprocessed vs average of steam-flaked ($P < 0.0001$).

^eLinear effect of density among steam-flaked grains ($P > 0.0001$).

^fPercent available starch = (SA, mg.g⁻¹/(1100 mg glucose/g of starch)(Total starch, %/100)).

the percentage of available starch of unprocessed sorghum grain was 25% of the total starch content (calculated SA = 185 mg/g). Zinn (36) evaluated the degree of processing of steam-flaked corn in beef cattle finishing diets; however, SA comparisons with present data are precluded by differences in enzymatic (amylglucosidase) assay conditions.

Sorghum SA was greater than corn at each BD. These SA values are influenced by both the chemical and physical changes that result from processing, as well as grain starch content; however, by calculating the percentage of available starch (Table 2), one can account for the variation in total starch content. Comparing the calculated percentage of available starch, UP corn and sorghum as well as A28 corn and sorghum were processed to a similar degree. Conversely, C38 and B33 corn seemed to be processed to a lesser degree than C38 and B33 sorghum, respectively. This particular SA assay was chosen because it directly measures the monomer substrate that is rapidly fermented in vivo to organic acids. In practical situations, potential variation in degree of processing resulting from roller wear, kernel size, steaming time (14), and grain starch

content may be poorly represented by measurements of BD. Conversely, this SA measure would seem to account for how such extraneous

TABLE 3. Effect of the degree of processing on in vitro dry matter disappearance (%) of corn and sorghum grain (Exp. 1).

Item	Incubation time, h				
	4	8	12 ^a	24	48
Grain type					
Corn	42.4	58.4	67.4	80.4 ^e	88.6 ^e
Sorghum	43.1	56.9	68.2	83.1 ^f	90.1 ^f
SE ^b	1.2	1.5	0.8	0.9	0.5
Density, kg/L					
0.72	36.1	51.7	65.6	80.4	89.5
0.38	40.5	54.5	65.4	78.3	89.7
0.33	44.3	60.8	68.7	84.5	89.3
0.28	50.2	63.8	71.5	83.9	88.9
SE ^c	1.7	2.1	1.1	1.2	0.7
Contrasts ^d					
0.72 kg/L vs others	0.002	0.01	—	—	—
Steam-flaking					
Linear	0.004	0.02	—	—	—

^aGrain type x degree of processing at 12 h ($P < 0.07$). Main-effect means are presented, and simple-effect means provided in the text.

^bStandard error of the least squares mean, n = 8.

^cStandard error of the least squares means, n = 4.

^dOnly highest order observed significance level ($P < 0.10$) reported.

^{e,f}Column values differ ($P < 0.10$).

factors influence the amount of starch available for rapid fermentation.

Dry Matter Disappearance. In Exp. 1, grain type did not influence DMD at 4 and 8 h of incubation, whereas DMD of sorghum was 3.3 and 1.7% greater ($P<0.10$) than that of corn at 24 and 48 h (Table 3). For both grains, DMD was less ($P<0.01$) for UP than for steam-flaked grains and increased linearly ($P<0.02$) as BD decreased at 4 and 8 h. A grain type \times degree of processing interaction occurred ($P<0.07$) at 12 h; thus, simple-effect means will be discussed for 12-h data. Corn treatments did not differ in DMD at 12 h (68.3, 63.4, 67.6, and 69.8%, SE = 1.6, for UP, C38, B33, and A28 corn, respectively), whereas DMD increased linearly ($P<0.10$) as sorghum BD decreased, with UP sorghum less ($P<0.02$) than other sorghum BD treatments (62.9, 67.5, 69.8, and 72.8%, SE = 1.6, for UP, C38, B33, and A28 sorghum, respectively). The reason for the numerically lower DMD of C38 corn at 12 h is not evident; however, Zinn (36) indicated a tendency for lower ruminal digestion of feed N for 0.36 kg/L corn. At 24 and 48 h, degree of processing did not affect ($P>0.10$) DMD.

Galyean et al. (9) reported that steam-flaked corn (BD = 0.45 kg/L) had an in vitro DMD of 33.7% at 12 h of incubation, or approximately half that of C38 corn; however, a 48-h pepsin digest was not used as in the present experiment. Duff et al. (5) reported 61.9, 72.4, and 79.0% DMD of control cultures at 12, 24, and 48 h using a 90% concentrate diet based on steam-flaked sorghum (degree of processing not specified) as the substrate. These values are less than our UP pooled mean, which may be explained by the larger particle size used (2-mm screen) by Duff et al. (5). Indeed, particle size effects seem to be much more profound for UP than for steam-flaked corn or sorghum (10).

The similar DMD of corn and sorghum in the present study is

somewhat surprising. The presence of prolamine crosslinks in the more extensive protein matrix of UP sorghum (27) decreases the digestibility of both the protein and embedded starch granules compared with corn (32). Following steam flaking, ruminal digestibility of sorghum grain protein (0.36 kg/L) has been estimated to be 30 percentage points less (37) than that of steam-flaked corn grain protein (0.30 kg/L). Using steers fed a 60% concentrate diet based on steam-flaked sorghum, Herrera-Saldana et al. (12) reported that in situ DMD of UP corn was greater (approximately 10 percentage points at 12 h) than UP sorghum. Present data indicate that UP corn had a numerically higher DMD than UP sorghum at 12 h (5.4 percentage points). This fact, coupled with convergence in corn DMD across BD

at 12 h, is interpreted to reflect less barriers to digestion in corn than sorghum.

In vivo results of Lozano et al. (17) showed a linear effect of degree of processing (measured by BD) of sorghum on ruminal and total tract DMD by steers. Grain was processed to 0.44, 0.36, and 0.28 kg/L, which allows the 0.36 and 0.28 kg/L data to be compared with our results. Ruminal DMD was 57.6 and 63.1% for 0.36 and 0.28 kg/L sorghum, respectively. Interpolation to derive DMD for 0.36 kg/L sorghum from our 8-h data pooled across grain type (Table 3), indicates 57.7 and 63.8% DMD for 0.36, and 0.28 kg/L sorghum, respectively. Moreover, total tract DMD for 0.36 and 0.28 kg/L sorghum (78.2 and 81.4%, respectively) of Lozano et al. (17) compares reasonably well with the present 24-h in

TABLE 4. Effect of N source and level on in vitro dry matter disappearance (%) of unprocessed and steam-flaked corn grain (Exp. 2).

Item	Incubation time, h				
	4 ^a	8	12	24	48
Density, kg/L					
0.72	24.3	37.9 ^f	51.0 ^g	81.3 ^g	87.7 ^g
0.28	28.8	51.8 ^g	64.9 ^h	84.3 ^h	88.5 ^h
N source ^b					
CH	29.2	44.6	57.8	82.9	88.2
Urea	23.9	45.1	58.1	82.7	88.0
SEc	0.6	1.6	0.9	0.8	1.0
N level ^d					
0	23.4	41.1	54.0	80.1	87.3
10	27.9	45.6	58.6	83.6	88.3
20	28.4	47.8	61.3	84.7	88.7
SEe	0.8	2.0	1.0	1.0	1.2
Contrasts ^f					
N level (linear)	0.04	0.0001	0.001	0.007	0.004

^aMain effect interactions ($P<0.05$) at 4 h; processing \times N level, N source \times N level, processing \times N source \times N level. Main-effect means are presented, and simple-effect means discussed in the text.

^bCH = casein hydrolysate.

^cStandard error of the least squares mean, n = 12.

^dSupplemented to provide 0, 10, or 20 mg of N/g of substrate.

^eStandard error of the least squares mean, n = 8.

^fOnly highest order observed significance level ($P<0.10$) reported.

^{g,h}Column values differ ($P<0.03$).

vitro DMD (Table 3, 80.8% from interpolation for 0.36 kg/L and 83.9% for 0.28 kg/L sorghum). Zinn (36) observed a numerical increase in ruminal organic matter digestibility for 0.30 kg/L corn vs 0.42 and 0.36 kg/L corn; total tract organic matter digestibility increased linearly as the degree of processing increased.

In Exp. 2, degree of processing \times N level, N source \times N level, and degree of processing \times N source \times N level interactions ($P < 0.05$) for corn occurred at 4 h of incubation. Therefore, simple-effect means will be discussed for 4-h data, and main-effect means for other times are shown in Table 4. Increasing CH level linearly increased ($P < 0.02$) DMD of UP (19.8, 27.0, and 32.4%, SE = 1.6, for 0, 10, and 20 mg of N, respectively) and A28 corn (27.2, 31.9, and 37.3%, respectively), whereas increasing U level linearly increased ($P < 0.10$) DMD of UP (18.9, 22.9, and 25.0%, respectively) but linearly decreased ($P < 0.01$) DMD of A28 corn (27.8, 29.7, and 19.0%, respectively) at 4 h. The DMD of UP corn was 27, 21, and 3% less ($P < 0.03$) than the DMD of A28 corn at 8, 12, and 24 h (Table 4). Source of N did not affect ($P > 0.10$) DMD of corn, whereas DMD increased linearly ($P < 0.04$) as N level increased at 8, 12, and 24 h.

The linear decrease in DMD of A28 corn supplemented with U at 4 h was unexpected. The N levels used (0, 10, and 20 mg of N/g of substrate) correspond to 0, 21, and 42 mg of U/g of substrate. Assuming the average CP concentration of corn is 9% (Table 2), these U levels equate to approximately 9, 15, and 21% CP equivalent (CPE). Mendoza and Britton (21) observed a linear increase in starch disappearance in vitro of UP corn and sorghum following 1 h of incubation with 0, 5, 10, 20, and 30 mg of U/g of substrate. As will be discussed later, NH_3 concentration was 14.8 mM for A28 corn with the highest U level at 4 h, which is much less than Satter and Slyter (29) found to be nontoxic (47 mM) in continuous cultures.

To our knowledge, no reports in the literature describe in vitro evaluation of supplemental N sources (NPN vs ruminally degraded amino N) or levels with substrates comparable to high-concentrate diets based on steam-flaked grains. Chester-Jones et al. (4) found no difference in DMD between U- or soybean meal-supplemented (15% CP) continuous cultures. Garrett et al. (11), also using a continuous culture system, observed numerically higher DMD when U was supplemented to an 85% UP corn-based concentrate substrate compared with soybean meal (12% CP; 57.4 vs 52.0%, respectively). These findings agree with our results for corn in Exp. 2, indicating that DMD is not improved by introduction of supplemental amino N compared with U N. Furthermore, the BD of each grain responded similarly, except A28 corn supple-

mented with U at 4 h, to increasing N level by increasing DMD linearly.

Our data indicating increased DMD with increasing N level agree with in situ trials using graded levels of U up to 1% of the dietary DM. Erdman et al. (6) reported that UP corn in situ DMD increased linearly with U level (7.4 to 14% CPE) with cows fed a 50% ground corn diet. Similarly, Mehrez et al. (20) indicated that barley in situ DMD increased with U level (13 to 16% CPE) in sheep fed a UP barley diet. Although the relative N levels among trials differ, the highest level of N in each trial allowed maximum DMD. As will be discussed, NH_3 concentrations that supported maximum DMD also were similar among these trials.

The DMD of A28 sorghum was 14, 28, 24, and 6% greater ($P < 0.04$) than UP sorghum at 4, 8, 12, and 24 h (Table 5). Supplementation with CH

TABLE 5. Effect of N source and level on in vitro dry matter disappearance (%) of unprocessed and steam-flaked sorghum grain (Exp. 2).

Item	Incubation time, h				
	4	8	12	24	48
Density, kg/L					
0.72	30.1 ^f	40.8 ^f	54.2 ^f	83.8 ^f	91.4 ^h
0.28	35.0 ^g	56.8 ^g	71.5 ^g	88.6 ^g	92.0 ⁱ
N source ^a					
CH	35.3 ^f	50.1	62.6	85.7	92.0
Urea	29.8 ^g	47.5	63.1	86.7	91.5
SE ^b	1.5	1.1	1.3	1.0	0.2
N level ^c					
0	29.8	42.5	57.5	83.0	91.0
10	32.4	50.5	64.0	87.0	91.8
20	35.6	53.4	67.0	88.6	92.4
SE ^d	1.8	1.4	1.6	1.2	0.3
Contrasts ^e					
N level (linear)	0.04	0.0001	0.001	0.007	0.004

^aCH = casein hydrolysate.

^bStandard error of the least squares mean, n = 12.

^cSupplemented to provide 0, 10, or 20 mg of N/g of substrate.

^dStandard error of the least squares mean, n = 8.

^eOnly highest order observed significance level ($P < 0.10$) reported.

^{f,g}Column values differ ($P < 0.05$).

^{h,i}Column values differ ($P < 0.10$).

increased ($P<0.04$) DMD of sorghum by 18% at 4 h, but was not different from U thereafter. The DMD of sorghum increased linearly ($P<0.04$) with increasing N level (approximately 11, 17, and 23% CPE) at all incubation times. Similarly, Mendoza and Britton (21) reported a linear increase in starch disappearance of UP sorghum in vitro with increasing urea level (up to 30 mg of U/g of substrate).

Degree of processing and level of supplemental N were positively related to in vitro DMD of corn and sorghum grain. Our Exp. 2 in vitro DMD results for corn and sorghum indicate that NPN and ruminally degraded amino acid N were equally effective N sources. Furthermore, UP and A28 corn displayed maximum DMD at approximately 21% CPE, whereas UP and A28 sorghum had maximum DMD at approximately 23% CPE.

It is noteworthy that the 25:75 buffer:water media that was used for NH_3 and pH measures provided greater separation in DMD among BD at 24 h than conventional (100% buffer) media. In Exp. 1, DMD of UP, C38, B33, and A28 corn and sorghum was 52.7, 60.3, 64.4, and 67.8%, respectively, with diluted buffer, whereas DMD with undiluted buffer at 24 h was 80.4, 78.3, 84.5, and 83.9%, respectively. The 25:75 buffer:water media also revealed DMD differences ($P<0.02$) in Exp. 1 among BD, pooled across grain type, at 8 through 24 h. Conventional media revealed similar DMD separation ($P<0.02$) at 4 and 8 h, but resulted in convergence of DMD among BD at 12, 24, and 48 h.

Changes in NH_3 . In Exp. 1, NH_3 was less for corn ($P<0.10$) than for sorghum at 8 h of incubation (Table 6). At 1, 2, 4, 8, 12, 18, and 24 h, NH_3 of UP was greater ($P<0.02$) than NH_3 for the average of C38, B33, and A28 (pooled across grain type). As BD decreased from C38 to A28 at 4, 8, and 12 h, NH_3 decreased linearly ($P<0.05$).

Corn NH_3 , pooled across BD, was less than sorghum NH_3 at 8 h (Table

6). Zinn (37) estimated ruminal digestibility of steam-flaked sorghum protein to be 30 percentage points less than steam-flaked corn protein (20 vs 50%). Herrera-Saldana et al. (12) indicated that grain protein disappearance paralleled DMD trends of UP corn and sorghum (within grain type). Thus, numerically lower NH_3 of corn cultures than sorghum at 12 h seems to support our 12-h DMD data, suggesting less barriers to digestion of corn DM than of sorghum.

Ruminal feed N digestibility with 0.36 kg/L steam-flaked corn tended to be less than 0.42 and 0.30 kg/L corn in steers fed an 88% concentrate diet (36). Lozano et al. (17) observed a similar tendency for 0.44, 0.36, and 0.28 kg/L steam-flaked sorghum. However, Xiong et al. (37) indicated a linear decrease in grain protein disappearance in vitro (61.4, 56.6, and 42.2%) as sorghum BD decreased (0.44, 0.36, and 0.28 kg/L, respectively). As a result, it is difficult to discern the extent to which increased

DMD vs possible decreases in grain CP digestibility with decreasing BD influenced NH_3 (Table 6). Nonetheless, the linear decrease in NH_3 as BD decreased suggests an increased N demand. That is, increasing the degree of processing resulted in progressively more rapid NH_3 utilization relative to NH_3 liberation.

Microbial uptake of NH_3 occurs by the action of glutamate dehydrogenase, a constitutive enzyme that does not require ATP, when NH_3 is greater than 2 to 3 mM (15). Below this concentration, glutamine synthase uses 1 mol of ATP /mol of NH_3 fixed (15). Based on NH_3 concentrations for Exp. 1, all cultures except UP corn and sorghum at 12, 18, and 24 h would have experienced energetic uncoupling resulting from low NH_3 within the microbial population. Any associated decrease in the rate of fermentation should become more evident as degree of processing increases.

In Exp. 2, main-effect interactions for corn culture NH_3 were N source \times

TABLE 6. Effect of the degree of processing on in vitro ammonia concentrations (mM) of unprocessed and steam-flaked corn and sorghum grain (Exp. 1).

Item	Incubation time, h						
	1	2	4	8	12	18	24
Grain type							
Corn	1.9	1.6	1.5	1.2 ^d	1.2	1.6	1.9
Sorghum	1.8	1.8	1.5	1.5 ^e	1.5	1.6	1.9
SE ^a	0.1	0.1	0.1	0.1	0.1	0.2	0.2
Density, kg/L							
0.72	2.2	2.1	2.2	2.8	3.1	4.0	4.5
0.38	1.8	1.6	1.3	1.2	1.0	1.2	1.6
0.33	1.8	1.6	1.2	0.9	0.7	0.9	1.0
0.28	1.8	1.6	0.9	0.7	0.4	0.6	0.9
SE ^b	0.1	0.1	0.1	0.1	0.1	0.2	0.3
Contrasts ^c							
0.72 kg/L vs others	0.02	0.001	0.001	0.001	0.001	0.001	0.001
Steam-flaking							
Linear	—	—	0.05	0.07	0.03	—	—

^aStandard error of the least squares mean, n = 8.

^bStandard error of the least squares mean, n = 4.

^cOnly highest order observed significance level ($P<0.10$) reported.

^{d,e}Column values differ ($P<0.10$).

N level ($P<0.05$) at all times, degree of processing \times N source \times N level ($P<0.10$) at 2 h, and degree of processing \times N level ($P<0.05$) at 4 h (Table 7). There was a general trend for NH_3 of UP corn to be greater ($P<0.001$) than A28 corn at 4 h and thereafter, and for U supplementation to increase NH_3 compared with CH ($P<0.001$) at all incubation times. At 1 h, NH_3 increased quadratically ($P<0.01$) with increasing N level. At 2 h, A28-CH, A28-U, and UP-CH increased NH_3 quadratically ($P<0.07$), whereas UP-U increased linearly ($P<0.001$) with increasing N level. At 4 h, A28-CH and UP-CH increased NH_3 quadratically ($P<0.01$), whereas A28-U and UP-U increased NH_3 linearly ($P<0.01$) with N level. At 8, 12, 18, and 24 h, CH and U increased NH_3 linearly ($P<0.001$), but the magnitude was greater ($P<0.001$) for U than for CH.

Results of Ørskov et al. (23) illustrate that ruminal NH_3 needed for maximum DMD does not necessarily coincide with maximum microbial CP yield. Generally, NH_3 required for maximum microbial CP yield is much less than NH_3 required for maximum DMD. Satter and Slyter (29) reported that 3 mM of NH_3 supported maximum microbial growth, above which NH_3 began to accumulate. Garrett et al. (11) reported that maximum microbial growth occurred at 3.5 mM using an 85% UP corn-based concentrate substrate, whereas Kang-Meznarich and Broderick (13) indicated that 5 mM permitted maximum microbial CP production in dairy cows fed an 80% concentrate diet.

In Exp. 2, NH_3 results for corn indicated that UP corn DMD was increased up to at least 18 mM (21% CPE), whereas A28 corn DMD was increased up to at least 15 mM (21% CPE). Urea proved to be more effective at increasing NH_3 than CH. Again, results from in situ trials support our findings. Erdman et al. (6) reported a linear effect of urea level (up to 1% of DM) on UP corn DMD using dairy cows, and maximum DMD was associated with a

mean ruminal NH_3 of 15 mM (14% CPE). Mehrez et al. (20) noted that maximum DMD of barley in barley-fed sheep occurred with ruminal NH_3 of 14 mM (16% CPE). Current industry recommendations for finishing cattle call for dietary CP levels (12.5 to 14.4% CP; .5 to 1.5%

urea) in excess of established requirements (8). It seems likely that a portion of the perceived benefits in animal performance of cattle fed rapidly fermented steam-flaked grains supplemented with higher CP levels may arise from increased grain DMD afforded by higher ruminal NH_3 .

TABLE 7. Effect of the degree of processing and N source and level on in vitro ammonia concentrations (mM) of unprocessed and steam-flaked corn (Exp. 2)^a.

Item	Incubation time, h						
	1	2 ^b	4 ^b	8	12	18	24
0.72 kg/L							
CH level ^c							
0	3.6	3.6	3.7	4.0	4.5	4.2	4.0
10	3.7	4.3	5.1	6.7	7.8	7.9	8.3
20	3.9	4.2	5.1	7.3	9.8	10.3	11.2
U level ^c							
0	3.9	3.7	3.6	3.9	4.8	4.3	4.0
10	8.5	7.8	10.1	10.4	11.3	10.7	10.7
20	8.8	12.5	16.0	16.9	18.0	17.2	17.2
0.28 kg/L							
CH level ^c							
0	3.3	2.8	1.5	0.9	0.9	0.7	0.9
10	3.6	3.9	3.5	3.3	4.2	3.9	4.2
20	3.6	4.0	3.9	4.2	5.4	6.3	7.2
U level ^c							
0	3.3	2.8	1.6	1.0	1.0	0.7	1.0
10	8.2	11.0	8.2	7.6	7.6	7.3	7.2
20	9.8	11.2	14.8	14.0	15.1	13.9	14.6
SE ^d	0.3	0.6	0.1	0.4	0.6	0.6	0.6
Contrasts ^e							
0.72 kg/L vs others	—	—	0.001	0.001	0.001	0.001	0.001
U vs CH	0.001	0.001	0.001	0.001	0.001	0.001	0.001
N level							
Linear	—	—	—	0.001	0.001	0.001	0.001
Quadratic	0.002	0.07	0.005	—	—	—	—
Processing \times N level							
Linear	—	—	0.008	—	—	—	—
Quadratic	—	0.07	—	—	—	—	—
U vs CH \times N level							
Linear	—	0.001	—	0.001	0.001	0.001	0.001
Quadratic	0.003	—	0.09	—	—	—	—
Processing \times U vs CH \times N level							
Quadratic	—	0.07	—	—	—	—	—

^aN source \times N level ($P<0.05$) at all incubation times.

^bProcessing \times N source \times level ($P<0.10$) at 2 h; processing \times N level ($P<0.05$) at 4 h.

^cCH = casein hydrolysate; U = urea; N added to provide 0, 10, or 20 mg of N/g of substrate.

^dStandard error of the least squares mean, $n = 2$.

^eOnly highest order observed significance levels ($P<0.10$) reported.

TABLE 8. Effect of the degree of processing and N source and level on in vitro ammonia concentrations (mM) of unprocessed and steam-flaked sorghum (Exp. 2)^a.

Item	Incubation time, h						
	1 ^b	2 ^b	4	8	12	18	24
0.72 kg/L							
CH level ^c							
0	3.1	3.3	3.6	4.0	4.9	5.2	5.4
10	3.4	3.9	4.9	6.6	8.2	9.4	10.0
20	3.6	4.2	5.2	7.6	10.6	11.5	13.1
U level ^c							
0	3.3	3.4	3.6	4.0	4.9	5.2	5.1
10	7.5	9.5	10.6	10.6	11.8	12.1	12.2
20	7.6	10.0	16.3	16.4	18.8	18.3	19.7
0.28 kg/L							
CH level ^c							
0	2.8	2.2	0.9	0.1	0.4	0.4	1.0
10	3.4	3.4	3.1	3.0	2.8	3.6	3.9
20	3.6	3.6	3.7	4.0	5.7	6.3	7.6
U level ^c							
0	3.1	2.2	0.9	0.1	0.9	0.6	1.2
10	7.8	9.1	7.6	6.4	7.0	6.9	7.6
20	7.9	11.9	14.0	13.0	13.7	13.7	15.2
SE ^d	0.1	0.3	0.3	0.2	0.6	0.5	0.5
Contrasts ^e							
0.72 kg/L vs others	—	—	0.001	0.001	0.001	0.001	0.001
U vs CH	0.001	0.001	0.001	0.001	0.001	0.001	0.001
N level							
Linear	—	—	—	—	0.001	0.001	0.001
Quadratic	0.001	0.001	0.09	0.008	—	—	—
Processing x N level							
Linear	0.02	0.03	—	—	—	—	—
U vs CH x N level							
Linear	—	—	0.001	—	0.001	0.001	0.001
Quadratic	0.001	0.005	—	0.04	—	—	—

^aN source x N level ($P < 0.05$) at all incubation times.
^bProcessing x N level ($P < 0.05$) at 1 and 2 h.
^cCH = casein hydrolysate; U = urea; N added to provide 0, 10, or 20 mg of N/g of substrate.
^dStandard error of the least squares mean, $n = 2$.
^eOnly highest order observed significance level ($P < 0.10$) reported.

Main-effect interactions for NH_3 of sorghum were N source x N level at all times, and degree of processing x N level at 1 and 2 h ($P < 0.05$; Table 8). Like corn, UP sorghum had consistently higher ($P < 0.02$) NH_3 than A28 sorghum at 4 h and beyond, and NH_3 of U exceeded CH ($P < 0.02$) at all incubation times. At 1 h, NH_3 of UP-CH increased linearly ($P < 0.02$), whereas UP-U, A28-CH, and A28-U

responded quadratically ($P < 0.001$) with increasing N level. At 2 h, NH_3 of A28-U increased linearly ($P < 0.03$), and the remaining treatments increased NH_3 quadratically ($P < 0.001$) with N level. At 4 and 8 h, NH_3 continued to increase quadratically ($P < 0.10$ and 0.01 , respectively), and increased to a greater extent for U than for CH with increasing N level. At 12, 18, and 24 h, NH_3 of all

sorghum treatments increased linearly with increasing N level ($P < 0.001$), whereas U-supplemented cultures exceeded CH. Comparing Exp. 2 sorghum DMD (Table 5) relative to NH_3 results indicates that UP sorghum DMD was increased up to at least 19.5 mM (23% CPE), whereas A28 sorghum DMD was increased up to at least 15 mM (23% CPE) in vitro. As was the case with corn, U supplementation increased NH_3 more than CH.

Changes in pH. In Exp. 1, culture pH was less for corn ($P < 0.10$) than for sorghum (5.54 vs 5.65) at 8 h, but not different at other incubation times (Table 9). Culture pH did not differ with BD up to 4 h, but pH of UP was greater ($P < 0.02$) than C38, B33, and A28 at 8, 12, 18, and 24 h and decreased linearly ($P < 0.04$) with decreasing BD at 12, 18, and 24 h. It is generally accepted that corn is fermented more rapidly than sorghum grain (2). However, the similar Exp. 1 DMD of corn and sorghum is reflected in our pH data. At 12 h and beyond, pH decreased linearly with decreasing BD. Zinn (36) observed a linear decrease in average ruminal pH of steers as corn BD decreased (0.42, 0.36, and 0.30 kg/L). Reinhardt et al. (26) induced acidosis in steers fed 0.36, 0.32, and 0.28 kg/L steam-flaked sorghum and found that total concentrations of volatile fatty acids and lactate did not differ, whereas 0.36 kg/L sorghum resulted in higher ruminal pH than 0.32 and 0.28 kg/L sorghum.

In Exp. 2, main-effect interactions for pH of corn cultures were N source x N level ($P < 0.05$) at 1 through 18 h, and degree of processing x N level ($P < 0.05$) at 12 h (Table 10). There was a general trend for UP corn to have higher pH ($P < 0.001$) than A28 at 2 h and later, and U supplementation resulted in higher pH than CH ($P < 0.002$) at 1 through 18 h. Increasing N level quadratically increased ($P < 0.05$) pH at 1 and 2 h, and linearly increased pH at 4, 8, 18, and 24 h, with the magnitude of increase greater for U than for CH. As N level increased at 12 h, pH of A28-CH

TABLE 9. Effect of degree of processing on in vitro pH changes of unprocessed and steam-flaked corn and sorghum grain (Exp. 1).

Item	Incubation time, h						
	1	2	4	8	12	18	24
Grain type							
Corn	6.32	6.24	6.04	5.54 ^d	5.32	5.20	5.10
Sorghum	6.39	6.30	5.95	5.65 ^e	5.37	5.21	5.12
SE ^a	0.03	0.04	0.06	0.04	0.02	0.02	0.02
Density, kg/L							
0.72	6.33	6.28	6.05	5.75	5.54	5.37	5.24
0.38	6.37	6.28	6.01	5.58	5.34	5.20	5.11
0.33	6.37	6.27	5.98	5.57	5.28	5.15	5.06
0.28	6.36	6.25	5.94	5.50	5.23	5.08	5.02
SE ^b	0.04	0.05	0.12	0.06	0.03	0.03	0.03
Contrasts ^c							
0.72 kg/L vs others	—	—	—	0.02	0.001	0.001	0.001
Steam-flaking							
Linear	—	—	—	—	0.04	0.02	0.03

^aStandard error of the least squares mean, $n = 8$.

^bStandard error of the least squares mean, $n = 4$.

^cOnly highest order observed significance level ($P < 0.10$) reported.

^{d,e}Column values differ ($P < 0.09$).

linearly increased the least ($P < 0.001$), followed by UP-CH, A28-U, and UP-U corn.

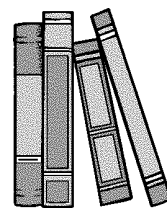
Despite an increase in the rate of fermentation with N supplementation, both CH and U addition resulted in net buffering compared with control cultures. The in vitro system is a closed system, and the dynamics of NH_3 flux into and out of the rumen that occur in vivo are not represented. The ionization constant (pKa) of NH_3 is approximately 9.2 (38), suggesting that NH_3 may act as a buffer in the rumen. Erdman et al. (6) found mean ruminal pH was unchanged in cows fed a 50% corn-based concentrate diet when 0, 0.33, 0.66, or 1.0% dietary U was infused. The extent to which increased NH_3 would contribute to buffering of ruminal organic acids in finishing cattle fed diets based on steam-flaked grains remains to be determined.

A higher pH ($P < 0.02$) for UP than for A28 sorghum was noted at 2, 12, 18, and 24 h (Table 11). Supplementation of U increased pH ($P < 0.02$) greater than CH at 1, 2, 12, and 18 h, but sources did not differ at 24 h. Changes in pH of sorghum resulted in a N source \times N level interaction ($P < 0.05$) at 4 and 8 h (data not shown). Increasing N level linearly increased ($P < 0.01$) pH at all incubation times, but to a greater extent ($P < 0.03$) for UP-U and A28-U than for UP-CH and A28-CH at 4 and 8 h. As with corn, U increased pH more than CH, and to a greater extent at 4 and 8 h incubation. Also as noted for corn, increasing N level resulted in net buffering of sorghum cultures.

Implications

Determining the BD for steam-flaked corn and sorghum may not necessarily reflect the quantity of

substrate available for rapid fermentation, which may explain, in part, the variation in the degree of sorghum processing associated with optimal animal performance. It seems likely that a portion of the perceived benefits associated with higher dietary CP levels on performance by animals fed steam-flaked grains may arise from DMD afforded by higher ruminal NH_3 concentrations. Moreover, dietary CP levels currently used in industry may measurably contribute to physiologically buffering the ruminal environment, but further research is needed.



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TABLE 10. Effect of N source and level on in vitro pH changes of unprocessed and steam-flaked corn (Exp. 2)^a.

Item	Incubation time, h						
	1	2	4	8	12 ^b	18	24
0.72 kg/L							
CH level ^c							
0	6.40	6.27	6.06	5.70	5.43	5.22	5.11
10	6.44	6.34	6.15	5.75	5.54	5.33	5.20
20	6.48	6.41	6.18	5.81	5.65	5.45	5.31
U level ^c							
0	6.44	6.33	6.08	5.69	5.51	5.24	5.11
10	6.61	6.54	6.31	5.98	5.74	5.41	5.25
20	6.64	6.61	6.49	6.15	5.90	5.60	5.39
0.28 kg/L							
CH level ^c							
0	6.40	6.23	5.83	5.26	5.12	4.98	4.93
10	6.45	6.26	5.88	5.34	5.21	5.06	5.00
20	6.51	6.32	5.88	5.39	5.26	5.13	5.10
U level ^c							
0	6.44	6.21	5.84	5.33	5.13	4.99	4.94
10	6.62	6.46	6.12	5.53	5.28	5.13	5.04
20	6.57	6.50	6.28	5.82	5.44	5.30	5.19
SE ^d	0.03	0.02	0.08	0.05	0.03	0.03	0.05
Contrasts ^e							
0.72 vs 0.28 kg/L	—	0.001	0.001	0.001	0.001	0.001	0.001
U vs CH	0.001	0.001	0.002	0.001	0.001	0.001	—
N level							
Linear	—	—	0.001	0.001	0.001	0.001	0.001
Quadratic	0.05	0.03	—	—	—	—	—
Processing x U vs CH	—	—	—	—	0.04	—	—
U vs CH x N level							
Linear	—	—	0.01	0.001	0.004	0.009	—
Quadratic	0.04	0.01	—	—	—	—	—

^aN source x N level ($P < 0.05$) at 1 through 18 h.

^bDegree of processing x N level ($P < 0.05$) at 12 h.

^cCH = casein hydrolysate, U = urea. N added to provide 0, 10, or 20 mg of N/g of substrate.

^dStandard error of the least squares mean, $n = 2$.

^eOnly highest order observed significance level ($P < 0.10$) reported.

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TABLE 11. Effect of N source and level on in vitro pH changes of unprocessed and steam-flaked sorghum (Exp. 2)^a.

Item	Incubation time, h						
	1	2	4 ^a	8 ^a	12	18	24
Density, kg/L							
0.72	6.38	6.30	6.11	5.78	5.52	5.30	5.14
0.28	6.38	6.21	5.68	5.23	5.06	4.97	4.92
OSL ^b	—	0.02	—	—	0.001	0.001	0.001
N source							
Casein hydrolysate	6.33	6.20	5.82	5.44	5.24	5.10	5.01
Urea	6.43	6.31	5.97	5.57	5.34	5.16	5.05
SE ^c	0.01	0.03	0.04	0.03	0.02	0.02	0.02
OSL ^b	0.001	0.01	—	—	0.01	0.02	—
N level ^d							
0	6.32	6.19	5.77	5.39	5.18	5.05	4.94
10	6.39	6.26	5.91	5.51	5.29	5.13	5.03
20	6.43	6.32	6.01	5.62	5.40	5.22	5.12
SE ^c	0.02	0.03	0.05	0.04	0.03	0.02	0.02
Contrasts ^f							
N level							
Linear	0.001	0.01	—	—	0.001	0.001	0.001

^aN source x N level ($P < 0.01$) at 4 and 8 h.

^bOSL = observed significance level.

^cStandard error of the least squares mean, $n = 12$.

^dNitrogen added to provide 0, 10, or 20 mg of N/g of substrate.

^eStandard error of the least squares mean, $n = 8$.

^fOnly highest order observed significance level ($P < 0.10$) reported.

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