Nutrient and Microstructural Indices of Maize and Sorghum Responses to Ammonia Pressurization/Depressurization[1]

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ABSTRACT

A pressurized ammonia (NH_3) technique (TAME) to enhance nutrient availability from maize and sorghum for cattle feeds was investigated. Treatment conditions evaluated were NH_3 loading rate and temperature. Starch, enzyme susceptible starch, in vitro dry matter digestibility (IVDMD), in situ (ISDMD) dry matter digestibility, and structure were determined. Grain treated at 50°C or higher temperatures exhibited popped characteristics and had no intact starch granules. Enzyme susceptible starch was 3.6- and 4.6-fold higher than controls in treated maize and sorghum. The IVDMD increased above control maize (87.8 ± 2.3%) and sorghum (80.0 ± 0.65%) with all NH_3 levels, and it averaged 91.5 ± 1.9% for maize and 87.8 ± 1.0% for sorghum. At 12 and 24 hr, ISDMD was at least 31.8 and 9.3% higher than controls in treated maize, respectively, and 52.9 and 46.6% higher in treated sorghum, respectively. This is the first process to make sorghum more valuable than maize as an energy/protein feedstuff in cattle diets. Because TAME treatment increased ISDMD and rate of digestion, this procedure is expected to increase total nutrient availability from these grains in common beef cattle diets.

In recent years, aflatoxin has contaminated grain throughout the United States. In Texas, grain was destroyed or discounted because of high aflatoxin levels. As a result, ammoniation (Muirhead 1990, Muschen and Frank 1990) was approved for use on contaminated maize in March 1991 (Texas Grain & Feed Association 1991). An existing pressurized ammonia process has been used successfully to increase nutrient digestibility of lignocellulosic feedstuffs (Hagevoort et al 1990, Turner et al 1991). A new treatment system involving ammonia and pressurized conditions (TAME) was developed to process grains by modifying the existing process.

The objective of this research was to determine the physical and chemical effects of TAME conditions (ammonia loading or processing temperature) on maize and sorghum for high-energy feeds for beef cattle. Assessment of the affects of treatment conditions on nutrient availability with time of digestion was a primary focus.

MATERIALS AND METHODS

TAME Process

Samples of whole, feed-grade maize and sorghum grain acquired from the local producers' cooperative were used for both experiments. Samples (100 g) were equilibrated with water (1:1 w/w) for 20 min before being placed into the reaction vessel. Liquid anhydrous ammonia was weighed from an ammonia tank into the vessel. Temperature was controlled by heating tape placed around the outside of the vessel. Internal temperature and pressure probes measured these variables continuously, and the data were displayed on digital readouts. When the desired temperature and pressure were reached and maintained for 15 min, a valve was opened, allowing immediate volatilization of the ammonia. Ammonia was recovered in another tank connected to the reaction vessel. The specific TAME conditions used for these experiments are listed in Table I. Two main criteria in the process, the quantity of ammonia and the temperature necessary for optimal responses, were the factors evaluated in these experiments. Vessel pressure, resulting from a combination of ammonia loading rate and temperature, is also presented in Table I.

Dry Matter Digestibility

Treated samples were removed, air dried, and frozen. Composites from multiple processes of the same treatment conditions were combined, and aliquots were ground in a Wiley mill to pass a 2-mm screen. Ruminal digestibility of the control and treated samples was estimated using in vitro dry matter digestibility (IVDMD) and in situ dry matter digestibility (ISDMD) determinations of dry matter disappearance. All treatments of both grains were run at the same time in both the in vitro and in situ analyses.

In vitro analyses were conducted using ruminal fluid strained through four layers of cheesecloth. Ruminal fluid was placed in a test tube along with a buffer solution and the grain and incubated at 39°C in a shaking water bath (Tilley and Terry 1963). Ruminal fluid was obtained from two steers consuming diets consisting of 90% or more grain (mixed maize and sorghum). After 48 hr of incubation, the fermentation was stopped by addition of 2 ml of 6*N* HCl to each tube. Then 0.5 g of pepsin was added and the tubes were incubated for an additional 48 hr to simulate total tract digestion. The fermented samples were stored frozen until filtered through coarse sintered glass crucibles, dried, and weighed to determine dry matter loss.

To measure ISDMD, 2-g samples were prepared in triplicate for each sampling time. Samples were weighed into 7.25- × 12.25-cm nylon bags. All bags to be removed at each sampling time were placed into a single 24- x 35-cm Dacron bag with a 1- × 1.5-mm mesh to allow easy removal and free access to the feed by ruminal bacteria (Zinn et al 1981). All samples were placed into a steer that was consuming a diet consisting of >90% grain (mixed maize and sorghum). After 0, 4, 8, 12, 24, and 48 hr, samples were removed, washed, dried, and weighed to determine DMD. Rates of digestion were derived as the first derivative of DMD vs. time.

Microscopy

Samples of control and treated maize and sorghum were observed using a low-power dissecting microscope (Tessovar, 6×) and environmental scanning electron microscopy (ESEM) (20 kV

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accelerating voltage on an Electroscan model E4 ESEM; Electroscan Corp., Wilmington, MA). Preparation for ESEM involved slicing each of the samples in half along the same plane with a razor blade, with no further sample preparation (fixing, drying, or coating) required.

TABLE I
TAME Treatment Conditions Used to Evaluate the Effects of Ammonia Loading Rate[a] and Temperature

Grain	Temperature (°C)	NH_3 Loading Rate	Time (min)	Pressure (psi)	Heat Up Time (min)
Ammonia loading experiment					
Maize	60	1.0	15	235	10
	60	1.5	15	280	10
	60	2.0	15	320	10
	60	2.5	15	360	10
Sorghum	60	1.0	15	210	10
	60	1.5	15	285	10
	60	2.0	15	340	10
	60	2.5	15	350	10
Temperature experiment					
Maize	32	1.5	15	185	0
	50	1.5	15	250	12
	78	1.5	15	370	20
	90	1.5	15	420	26
Sorghum	31	1.5	15	150	0
	50	1.5	15	240	5
	78	1.5	15	320	18
	90	1.5	15	420	26

[a] Ammonia loading rate is kilograms of ammonia per kilogram of grain dry matter.

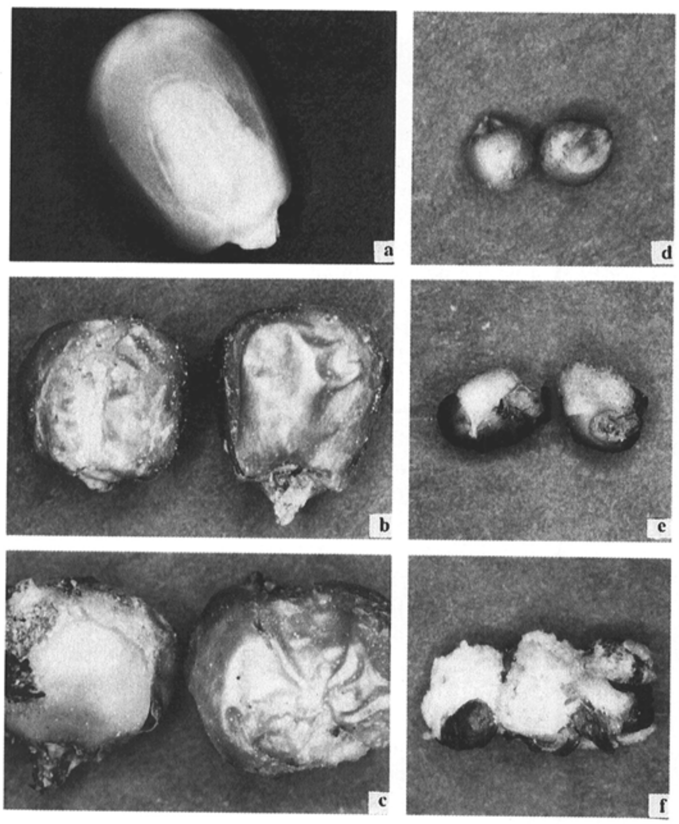

Fig. 1. Views (6×) of the control and treated maize (left) and sorghum (right). Panels a and d are the control samples, b and e are 32°C treated maize and 31°C treated sorghum, and c and f are 50°C treated maize and sorghum. Puffing occurred with TAME treatment, and the extent of puffing was related to the temperature. Sorghum was affected more than maize at the same temperatures.

Analysis of Starch Characteristics

Ground samples were used to determine the quantity of total and enzyme susceptible starch (ESS). Both total starch and ESS were determined using the glucose hexokinase procedure (Technicon Industrial Systems, method SF4-0046FA8, Tarrytown, NY). Measurement of ESS included a modification of the total starch procedure whereby samples were not gelatinized prior to adding the enzyme. Therefore, only that fraction of starch that was enzyme susceptible in the control or in TAME-treated samples was measured.

Statistical Analyses

Chemical composition and digestibility data were analyzed using ANOVA procedures of SAS (SAS 1985) and nonlinear regression procedures to define the response to TAME treatment conditions. Nonlinear regression functions were fitted to ISDMD with grain (class) and treatment temperature (continuous) effects and interactions in models. Rate of digestion was derived from the first derivative of these functions vs. time of digestion.

RESULTS AND DISCUSSION

Structure

Both the maize and sorghum had a puffy, popped grain texture (i.e., similar to popcorn) and appearance after TAME treatment, with the extent of puffing dependent on the temperature used (Fig. 1). TAME processing modified the starch in both maize and sorghum. At each treatment level, sorghum was more extensively modified than was maize, probably reflecting sorghum's greater surface area to mass ratio. However, with high pressures and treatment temperatures of 50°C or above, starch in both grains was completely gelatinized; no intact starch granules remained (Fig. 2). Physical appearance of the 50°C and higher treatments

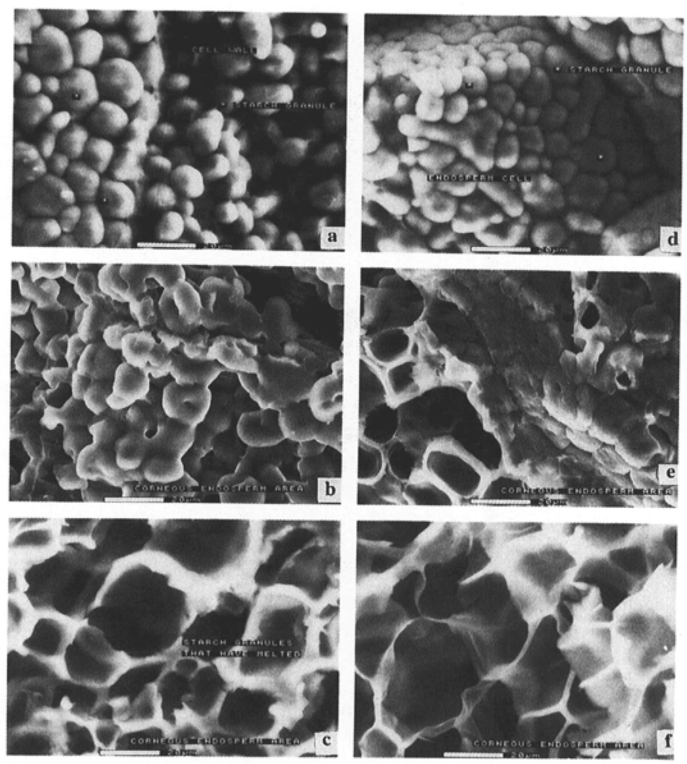

Fig. 2. Environmental scanning electron microscopic views (800×) of the maize (left) and sorghum (right) samples. Panels a and d are the control samples, b and e are 32°C treated maize and 31°C treated sorghum, and c and f are 50°C treated maize and sorghum. As the temperature increased in both the maize and sorghum samples, the extent of starch gelatinization increased, with nearly all of the starch granules being gelatinized with the 50°C temperature.

was similar, thus only the 50°C samples are presented in the figures. The ESEM micrographs indicated that as TAME temperature increased and greater degrees of starch disruption occurred, an amorphous starch/protein phase was formed (Fig. 2). Similar development of an amorphous phase in cereal products under extreme heat conditions (deep-fat frying) was reported by Gomez et al (1992) and McDonough et al (1993).

Both the degree of starch gelatinization and flake density can affect diet intake and performance of cattle (Reinhardt et al 1993). Isolated starch from maize and sorghum are equally digestible (Hibberd et al 1982). Thus, the slow rates of digestion that occur with sorghum must be due to characteristics other than those of the starch itself, such as reduced accessibility of starch to microbial or enzymatic degradation (Rooney and Pflugfelder 1986). Previous research has demonstrated that the protein matrix that encapsulates sorghum starch granules (Rooney and Pflugfelder 1986) is responsible for reduced sorghum digestibility (Lichtenwalner et al 1978; Theurer 1986). Physical disruption, as occurs in steam flaking (Theurer 1986) and TAME processing (Figs. 1 and 2), probably accounts for a substantial part of the increased accessibility of the starch and protein in grain to attack by ruminal microorganisms.

Ammonia Loading Experiment

The IVDMD of the control maize (87.8%) was higher ($P < 0.03$) than that of sorghum (80.0%) (Fig. 3). However, treatment of grain with only 1 kg of ammonia increased ($P < 0.03$) the 48-hr IVDMD to 86.5% for sorghum and to 92.2% for maize. Maize IVDMD did not increase with the 1.5-kg ammonia loading rate, yet sorghum IVDMD was further increased to 90.0% with the 1.5-kg loading rate. Any further addition of ammonia did not produce a significant increase or decrease in IVDMD in either grain. A single loading rate was chosen for the subsequent experiment, which allowed maximum DMD for both grains (1.5 kg ammonia/kg of dry matter).

Variable Temperature Experiment

Starch properties. Total starch ranged from 79.3 to 82.1% for sorghum and from 73.5 to 75.5% for maize but was not different between treated and control samples. The amount of ESS (percentage of total starch) increased ($P = 0.0002$) dramatically in all TAME-treated samples (Fig. 4). Mean control ESS content was only 16.4%. However, with the lowest TAME temperature, ESS content increased to 76.3 and 80.6% for maize and sorghum, respectively. Increasing temperatures above 50°C had no additional effect on the percentage of ESS. Across all TAME temperatures, ESS was increased by 362% in maize and by 459% in sorghum. Increased ESS of steam-flaked maize is probably the reason Lee et al (1982) found increased ruminal starch digestion of maize diets when steam-flaked maize replaced whole, shelled maize. Micronizing also produces increased levels of ESS in sorghum (Rusnak et al 1980).

In situ dry matter digestibility. Solubility (0 hr digestibility) of dry matter from control sorghum (12.5%) was 47% less ($P < 0.0001$) than that of control maize (23.6). TAME treatment decreased ($P < 0.0001$) solubility of maize dry matter with all temperatures tested (6.7, 10.2, 11.6, and 11.7% for 32, 50, 78, and 90°C, respectively). However, only TAME treatment of sorghum at 31, 50, and 78°C decreased ($P < 0.001$) solubility (1.9, 6.8, and 8.8%, respectively). Dry matter solubility of sorghum treated at

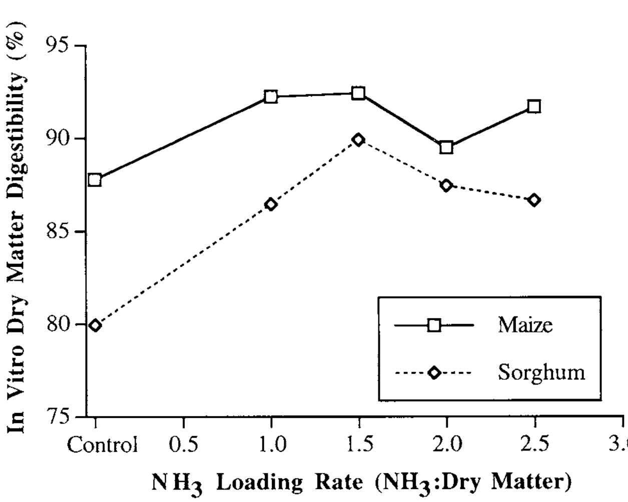

Fig. 3. Ruminal in vitro dry matter digestibility (after 48 hr) of maize and sorghum in response to variable NH_3 loading rates during TAME treatment (SEM = 1.3).

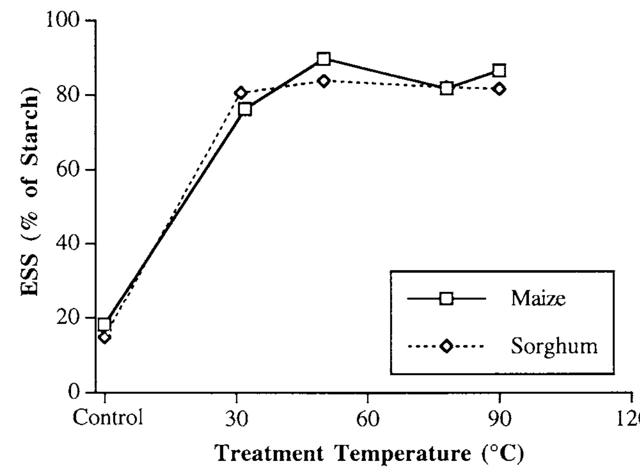

Fig. 4. Effect of TAME temperature on enzyme susceptible starch (ESS) content (percent total starch) of maize and sorghum. * = Maize was treated at 32°C and sorghum was treated at 31°C. ** = TAME-treated sample ESS differs from controls ($P < 0.0002$, SEM = 1.4).

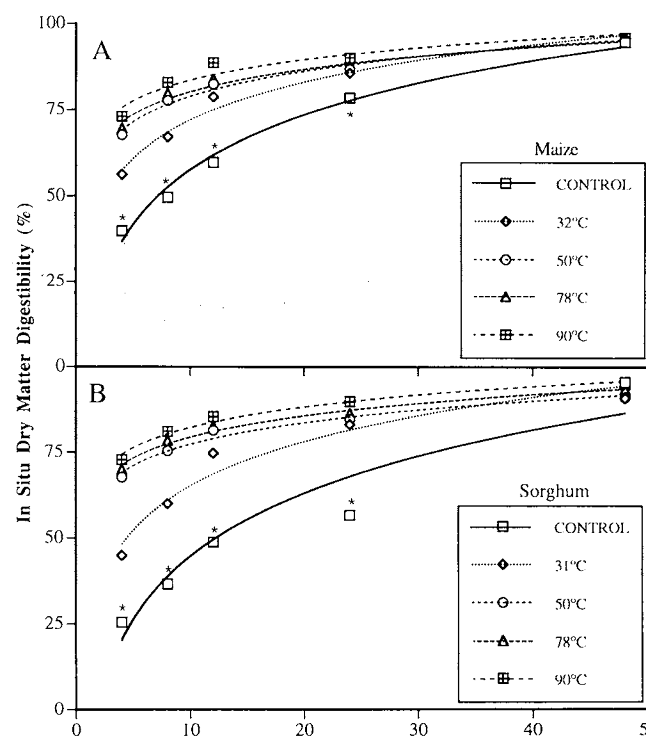

Fig. 5. Ruminal in situ dry matter digestibility of maize (**A**) and sorghum (**B**) in response to TAME treatment temperatures. * = Control differs from treated ($P < 0.0001$, SEM = 0.78).

90°C was 10.4%, which did not differ from the control.

The ISDMD of control maize and sorghum was less ($P < 0.001$) than TAME treated maize and sorghum after 4, 8, 12, and 24 hr of fermentation (Fig. 5). After 24 hr of fermentation, the control maize ISDMD was 78.6%, which was only 87.0–91.5% of the ISDMD of TAME-treated maize. Sorghum, which typically is less digestible than maize, was only 56.7% digestible by 24 hr when it was not TAME treated. The TAME-treated sorghum, in contrast, was between 83.1 and 89.8% digestible, which was not different from the ISDMD achieved with maize treated at 78 or 90°C. Thomas et al (1988) discussed the problem of starch retrogradation (Rooney and Pflugfelder 1986) when steam-flaked maize or sorghum cools as an explanation for the reduced rate of DM disappearance observed in their studies. All TAME samples were air-dried after treatment and were frozen before use in these studies. This would imply that starch retrogradation is not an inherent problem for TAME-treated maize and sorghum, especially because the level of ESS was so high in the treated samples.

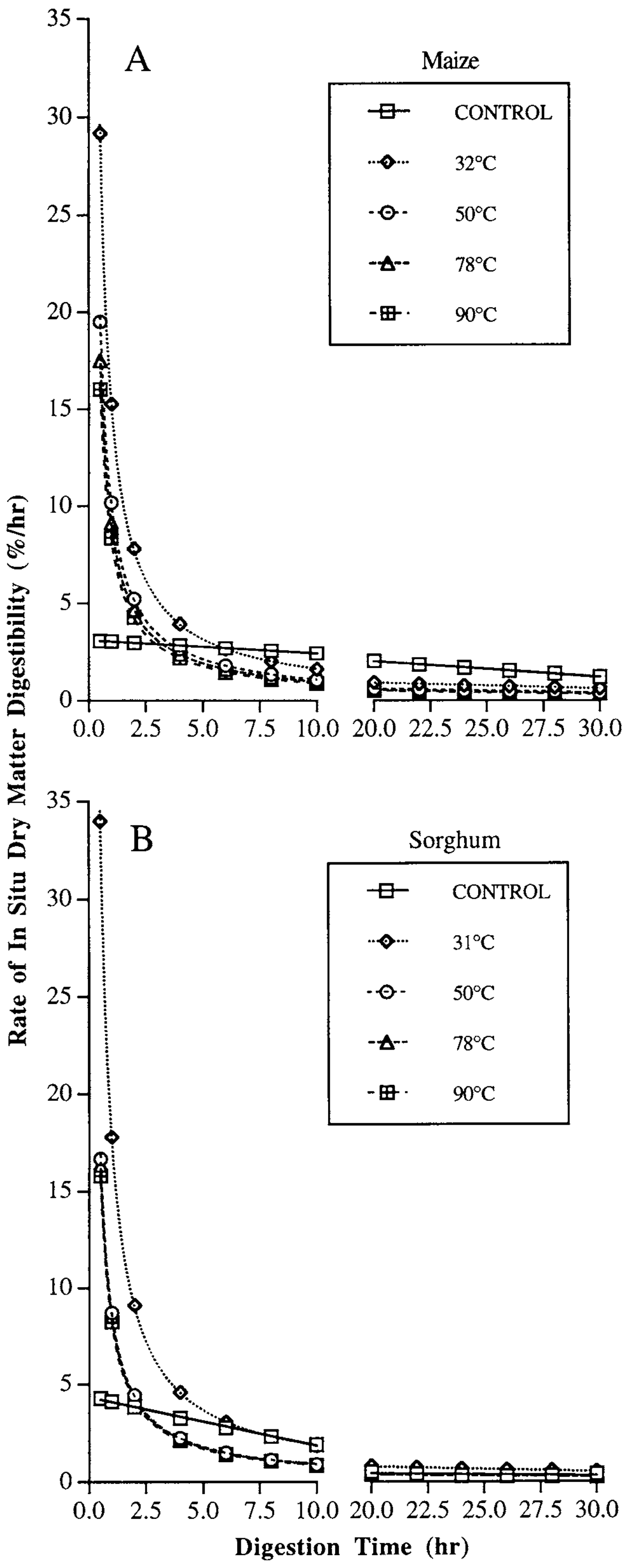

Fig. 6. Rate of in situ dry matter digestibility of maize (**A**) and sorghum (**B**) in response to TAME treatment temperatures (first derivative of dry matter digestibility values).

By 48 hr, all samples had reached their maximum percent digestibility. Even though the 48-hr digestibility data in our study were similar ($P > 0.59$) for control and treated grains, it is important to note that grain would not be retained in the rumen of an animal consuming a primarily grain diet for this length of time. Ørskov (1986) indicated that the key to efficient utilization of starch in grains is improving ruminal digestion. Most grain exits the rumen within 24 hr, and the average length of retention is between 10 and 24 hr (Galyean et al 1979). Therefore, the ISDMD measured at 24 hr would represent the maximum degree to which nutrients from maize and sorghum would be ruminally available. As demonstrated by Lee et al (1982), replacing whole, shelled maize in a steer diet with steam-flaked maize resulted in increased total tract dry matter, organic matter, and starch digestion. McAllister et al (1993) found that pretreatment of maize with protease increased starch digestion after 16 hr, but the difference was reduced after 24 hr. Their data suggest that the protein matrix is a limiting factor in ruminal starch digestibility. Therefore, to achieve maximal nutrient availability in the total tract, both availability in the rumen and the rate of passage from the rumen are critical.

Initial rates of DMD (0 to 4 hr) were higher ($P < 0.0001$) for TAME-treated maize (3.5 times control) and sorghum (4.4 times control) than control samples (Fig. 6). Rates of DMD declined most rapidly in maize and sorghum samples TAME-treated at 50, 78, and 90°C, whereas the rates for control and samples TAME treated at the lowest temperature retained similar rates of DMD from 4 to 12 hr of fermentation.

As noted earlier, maize is preferentially used in feedlots because of its higher feed value, as compared to sorghum. Brandt et al (1989) estimated that sorghum has only 90–95% of the feed value of maize, and extensive processing is required to achieve even this level of nutrient utilization (Rusnak et al 1980; Hibberd et al 1982; Anderson 1994). The data in Figure 5 also demonstrate that processing is required, but, with the TAME process, nutrients in similarly processed sorghum and maize were equally available. Rooney and Pflugfelder (1986) stated that sorghum must be much more vigorously processed than maize to maximize digestibility. The TAME process improved nutrient availability of sorghum sufficiently to make it feasible to use sorghum instead of maize, even if priced the same. The combined TAME physical and chemical effects (e.g., gelatinization, increased surface area) increased enzyme susceptibility of starch, and physically disrupted the protein matrix, which otherwise inhibits microbial accessibility to starch granules (McNeill et al 1975, McAllister et al 1993). Owens et al (1986) suggested that to successfully increase digestion in the rumen and small intestine would require a process that involved an alteration in the protein matrix as well as a change in physical characteristics. The additive effects of changes in starch granule structure and physical changes in the grain (Theurer 1986) causes the TAME process to improve digestion.

Because gut fill limits feed intake, rate of passage becomes an important factor in determining nutrient availability of feeds in cattle. Steam flaking results in increased rates of digestion and rate of passage (Ramirez et al 1985), which enables cattle to consume more of the treated diets than of a diet containing whole, shelled maize. Rate of passage through the animal found with a typical whole maize diet is approximately 66 hr, however, the time was reduced to 41 hr by steam-flaking maize (Ramirez et al 1985). Considering the initial rapid rate of digestion observed with TAME-treated samples, we would expect that the rate of passage in vivo would also be increased.

Wester et al (1992) indicated that the feeding value of sorghum was influenced by the rate of ruminal starch digestion. Based on the current data, nutrients would be equally available from TAME-treated maize and sorghum, resulting in similar feed energy values and an increased value for sorghum in some diets because of its greater protein content. Potter et al (1971) indicated that ruminal digestion of sorghum protein is essential if cattle amino acid requirements are to be met because the amino acid content of sorghum is not in balance with mammalian tissue needs. With current feedstuff prices, TAME-treated sorghum in a common least cost feedlot diet (11.5% protein, 1.60 kg/day gain) with equal net energy and 1.5% more protein than maize, would be worth $4.31/t (4%) more than maize. The projected cost of treating a ton of feedstuff with the TAME process is approximately $10/t. Schake et al (1982) reported that the cost of steam flaking ranged from $5 to $10/t, depending on the size of feedlot operation and the source of energy used.

CONCLUSIONS

An ammonia loading rate of 1.5 kg/kg dry matter produced the greatest increase in ISDMD for sorghum and maize. The lowest TAME temperature used in this experiment was sufficient to modify grain structure and increase ISDMD. ISDMD at 12 and 24 hr was greater for all treated grains than for the control grains. This is critical because feed grains are retained in the ruminal section of the digestive tract of cattle for 10–24 hr, making material not digested within that time much less available. The increase in digestibility was a result of both chemical and structural changes that occurred with TAME treatment. Sorghum treated with the TAME process had a similar predicted feed energy value and greater protein value than TAME-treated maize. Processing grains with this procedure will increase the quantity of nutrients available from grains by increasing rate and extent of nutrient digestion. Further research must be conducted to determine the minimum TAME treatment temperature necessary to maximize digestibility and to optimize TAME process conditions.

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