ESTIMATING STARCH AVAILABILITY AND PROTEIN DEGRADATION OF STEAM-FLAKED AND RECONSTITUTED SORGHUM GRAIN THROUGH A GAS PRODUCTION TECHNIQUE

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ABSTRACT

Five steam-flaked sorghum grain (SFSG) samples with bulk densities of 476, 412, 347, 309 and 283 g/liter made by adjusting tension between mill rollers and three reconstituted sorghum grain (RSG) samples with reconstitution times of 10, 20 and 30 d and a control sample were analyzed for gas production kinetics (rumen liquor fermentation) and enzymatic glucose release (amyloglucosidase). Protein degradation was estimated from 6-h gas production and residual ammonia in the liquid. Gas production followed first-order kinetics ($k > .98; P < .01$) and was used to describe rate and extent of digestion kinetics. Rate of gas production increased as processing degree increased. The magnitude of increase in gas production, however, was much less for RSG than for SFSG. Linear relationships were observed between enzymatic glucose release and the gas production rate constant $k$ as well as gas production at 4, 6 and 8 h ($r^2 > .98; P < .01$). Protein degradation decreased with processing degree of SFSG but increased with reconstitution time. A technique based on 6-h gas production and residual ammonia in the liquid is proposed to estimate both ruminal starch availability and ruminal protein degradability for processed sorghum grain.

(Key Words: Sorghum, Processing, Starch, Protein.)

Introduction

Several gas production procedures have been used to estimate starch availability of processed grain for beef cattle (Tre et al., 1970; Hibberd et al., 1982; McLeod and Richardson, 1986). Menke et al. (1979) developed a gas production procedure that includes calibration of rumen liquor activity with standard hay and starch, and a device with piston-syringes in a rotating thermo-controlled (39 ± .5°C) oven. Huang (1986) reported that gas production of hay, grain and cotton fiber measured by Menke’s procedure followed first-order reaction kinetics.

Raab et al. (1983) and Bartle et al. (1986) estimated feed protein degradation and microbial protein synthesis using modifications of the gas production procedure. Simultaneously, a procedure based on 6-h gas production and residual ammonia in a rumen liquor-buffer solution was developed at Beijing Agricultural University (Meng, 1985). The correlation between in vitro estimates and in vivo values (37 samples with known in vivo protein degradation were obtained from laboratories in the U.S. and Australia) across sample source was .56 ($P < .01$), and within-sample source correlations were .98 ($P < .01$; Meng, 1985; Zhang, 1987).

Little attention has been given to the interaction between protein and starch avail-
ability of sorghum grain. The objectives of the present research were to evaluate the above gas production technique to quantitatively measure starch availability in processed sorghum grain and to measure possible changes in protein degradation.

Materials and Methods

Five steam-flaked sorghum grain (SFSG) samples with bulk densities of 476, 412, 347, 309 and 283 g/liter (bushel weight of 37 [B37], 32 [B32], 27 [B27], 24 [B24] and 22 lb/ bu [B22], respectively) were made by adjusting the tension between mill rollers. Sample collection was completed within 1 h. Roller mill electrical load was determined using an ammeter. Three reconstituted sorghum grain samples (RSG) with reconstitution times of 10 (R10), 20 (R20) and 30 d (R30) were made by adding water to bring the grain moisture to 27% and storing at 24°C in sealed glass bottles for specified times. A control sample was collected from the same sorghum source. All samples were air-dried and ground to pass through a 1-mm screen.

Enzymatic glucose release and corresponding starch gelatinization were determined according to Xiong et al. (1989). Corresponding samples were sent to Beijing Agricultural University for gas production, residual ammonia and protein degradation analysis. Gas production at 2, 4, 6, 8, 12, 24, 36 and 48 h were determined as described by Menke et al. (1979). Gas production values over time for each sample were fit to a one-component model, \( Y = A(1 - e^{-kx}) \). Respective \( k \) values and 4-, 6- and 8-h gas production values then were correlated with corresponding glucose release values. Residual ammonia at 6 h was measured and protein degradation was estimated according to Meng (1985). That procedure is detailed in the Appendix.

Relationships among variables were evaluated using linear and nonlinear regression procedures of SAS (1985).

Results and Discussion

Because SFSG samples all were collected within 1 h, the effects of variables such as kernel size, grain moisture and roller wear should be negligible. Bulk density should reflect processing degree of SFSG.

Enzymatic glucose release, a sensitive and reproducible procedure to measure the effects of processing on SFSG (Xiong et al., 1989), and the estimated starch gelatinization values of steam-flaked and reconstituted sorghum grain are shown in Table 1. Glucose release increased with increased degree of processing for SFSG but not with days of reconstitution. The glucose release data suggest that steam flaking results in greater total tract starch digestion by increasing ruminal fermentation. The performance and overall utilization of RSG by cattle, however, has been shown to be similar to that of SFSG (Hale, 1973), although ruminal fermentation is slower. Assuming ruminal retention times are similar, reconstituted sorghum grain must, therefore, result in more starch digestion in the lower tract. McNeill et al. (1971) observed that ruminal starch digestion was 67 and 83% for RSG and SFSG, respectively, and the amount of starch digested in the small intestine for RSG was twice that for SFSG. These data support the hypothesis that starch gelatinization, which increases ruminal starch digestion, as the only evaluation of processed grain (including reconstitution) has limitations (Hale, 1973; Rooney and Pflugfelder, 1986).

Intercepts (A), rate constants (k) and \( r^2 \) values of the regression equations describing gas production are listed in Table 2. Gas production over time followed first-order reaction kinetics and, therefore, can be used to describe rate and extent of digestion kinetics similar to other in vitro or in situ procedures. Figure 1 shows gas production changes over time of control, B22, B34 and R30 samples, respectively. Gas production rate increased with degree of steam flaking and with days of reconstitution. Gas production, however, was much less for reconstitution than for steam flaking (Table 2; Figure 1). This finding is in contrast with that of McLeod and Richardson, (1986), who reported a greater gas production from reconstituted than from steam-flaked sorghum grain. Their experiment differed in that amyloglucosidase and yeast were used for fermentation rather than rumen liquor and their samples were collected from a commercial scale facility.

Estimates of ruminal starch availability using the enzymatic and gas production procedures were compared. Because it would be easier and faster to determine a single gas production time rather than k, and because the half-maximum gas production was about 6 h and gas production increased linearly from 2 to
TABLE 1. ENZYMATIC GLUCOSE RELEASE, ESTIMATED GELATINIZATION AND PROTEIN DEGRADATION OF PROCESSED SORGHUM GRAIN

<table>
<thead>
<tr>
<th>Item</th>
<th>Steam-flaked</th>
<th>Reconstituted</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roller mill load, amps</td>
<td>B37, B32, B27, B24, B22, R10, R20, R30</td>
<td>21, 28, 36, 48, 52</td>
<td></td>
</tr>
<tr>
<td>Glucose release, mg/g</td>
<td>213b, 422c, 512d, 587e, 618f, 678g</td>
<td>199b, 215b, 219b</td>
<td>7.8</td>
</tr>
<tr>
<td>Gelatinization, %</td>
<td>0b, 30c, 44d, 56e, 60f, 68g</td>
<td>0b, 0b, 0b</td>
<td>3.5</td>
</tr>
<tr>
<td>Protein degradation, %</td>
<td>65.9b, 46.8d, 38.1c, 39.3c, 38.9c, 33.9b</td>
<td>73.1f, 77.7f, 79.7f</td>
<td>1.73</td>
</tr>
</tbody>
</table>

*Treatment abbreviations: B37, B32, B27, B24 and B22 = steam-flaked sorghum grain with bulk densities of 467, 412, 347, 309 and 283 g/liter, respectively.

Eight hours (Figure 1), the relationships between glucose release and 4-, 6- and 8-h gas production and k were determined ($r^2 = .96, .97, .95, \text{ and } .97 (P < .01))$.

Figure 2 shows the relationship between 6-h gas production and enzymatic glucose release. The agreement between these procedures indicates that rumen liquor-based gas production, like glucose release, can be used as a quantitative measurement of processing effects on ruminal starch availability. The preferred index for routine analysis would be 6-h gas production because it has the highest $r^2$ value and can measure protein degradation simultaneously.

Estimated ruminal protein degradation of SFSG and RSG samples based on 6-h gas production and residual ammonia are presented in Table 1. The lowest degree of steam flaking decreased protein degradation 19 percentage units compared to unprocessed sorghum (B37 vs control), suggesting that steam exposure and minimal flaking decreases protein degradation. A decrease in protein degradation occurred from B37 to B32 (21 to 28 amps); protein degradation then remained relatively unchanged to B24 (48 amps). A second drop in protein degradation was noted with further processing intensity (B22; 48 amps). The additional heat created during roller mill flaking may explain the decrease in protein degradation with increasing processing degree. McLeod and Richardson (1986) reported decreased protein solubility of SFSG. Matras et al. (1989) reported decreased apparent protein digestibility in lambs for SFSG vs dry-rolled sorghum grain diets. In contrast to our results, however, Potter et al. (1971) and Rahnema et al. (1987) reported that SFSG had a higher ruminal protein digestion coefficient than did dry-rolled sorghum grain. Further investigation is needed to clarify the direction and extent of the effect of steam flaking on protein degradation.

Reconstitution, as shown in Table 1, increased protein degradation. The longer the reconstitution time, the more extensive the protein degradation; McLeod and Richardson

**TABLE 2. KINETIC PARAMETERS FOR GAS PRODUCTION FROM PROCESSED SORGHUM GRAIN ($Y = A[1 - e^{-kt}]$)**

| Item | Control | B37 | B32 | B27 | B24 | B22 | R10 | R20 | R30 |
|------|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A    | 79.4    | 74.3| 74.0| 74.2| 74.6| 74.0| 78.3| 77.3| 76.0|
| k    | .0599   | .0878| .0995| .1059| .1087| .1125| .0641| .0648| .0745|
| $r^2$| .996    | .98 | .98 | .98 | .98 | .98 | .99 | .99 | .99 |

*Treatment abbreviations: B37, B32, B27, B24 and B22 = steam-flaked sorghum grain with bulk densities of 476, 412, 347, 309 and 283 g/liter, respectively.

$b$ R10, R20 and R30 = reconstituted sorghum grain stored for 10, 20 and 30 d, respectively.

$e$ All $r^2 (P < .01)$. 
(1986) found an increase in protein solubility for reconstituted sorghum grain. These data support the theory that reconstitution solubilizes the protein in the protein-starch matrix, allowing enzymatic attack of the starch to be more effective (Hale, 1973; McNeill et al., 1975; Rooney and Pflugfelder, 1986).

**Implications**

Estimated ruminal starch availability of sorghum grain was increased more by steam flaking than by reconstitution. The two processing methods had opposite effects on estimated protein degradation; steam flaking decreased ruminal protein degradation, but reconstitution increased ruminal protein degradation. These results indicate that these two processing methods improve sorghum feeding value through different mechanisms. The gas production procedure appears to be a useful method to measure the effects of sorghum grain processing on ruminal starch availability. This same procedure may prove useful to estimate the effects of processing on ruminal protein degradation.

**Appendix: In Vitro Procedure for Estimation of Ruminal Protein Degradation Based on 6-Hour Gas Production and Residual Ammonia**

**Apparatus and Reagents**

1. Rotor, thermo-controlled oven, syringes and reagents according to Menke et al. (1979).
2. Pure starch, \((\text{NH}_4)_2\text{SO}_4\), standard casein and 3% HgCl$_2$. 

Figure 1. Gas production over time for control, steam-flaked sorghum (B37 and B22) and reconstituted sorghum grain (R30) samples.
Figure 2. Relationship between 6-h gas production (GP) and glucose release. C = control; B37, B32, B27, B24 and B22 = steam-flaked sorghum grain with bulk densities of 476, 412, 347, 309 and 283 g/liter, respectively; R10, R20 and R30 = reconstituted sorghum grain stored for 10, 20 and 30 d, respectively.

Procedure

1. Construction of standard curve: to syringes containing 40, 80, 120, 160 and 200 mg starch add 30 ml rumen liquor-buffer solution and 42.7 mg (NH₄)₂SO₄. Six-hour gas production (GP₆) is measured according to Menke et al. (1979). Fermentation is stopped at 6 h by adding 2 ml 3% HgCl₂ and mixing. Residual ammonia in the fermentation liquid is determined (Searle, 1984). The reduction in residual ammonia is a measure of microbial protein-N (MCP-N) synthesis. The linear equation relating GP₆ with the residual ammonia is established for estimation of synthesized MCP-N from gas production: residual ammonia = a - b(GP₆), where b represents the reduction in residual ammonia in mg (or MCP-N synthesis) per ml of GP₆. The r² should be > .97.

2. Estimation of 6-h in vitro protein degradation (IVPD₆): GP₆ and residual ammonia of 200 mg feed sample are measured according to step 1 and IVPD₆ is calculated as follows: IVPD₆ = residual ammonia + b(GP₆)/total N in sample, where residual NH₃ N plus b(GP₆) represent the N released from the sample.

3. Estimation of ruminal protein degradation: IVPD₆ of casein is determined on a mixture of 65.5 mg casein and 134.5 mg of starch according to step 2. Estimated ruminal protein degradation (ERPD) of the feed sample is calculated as follows (assuming ruminal degradation of casein is 100%): ERPD = IVPD₆ x (100/IVPD₆ of casein).

Note

Steps 1, 2, 3, of this procedure should be done in one run, which is possible in the rotor described by Menke et al. (1979).

Literature Cited


PROCESSING OF SORGHUM GRAIN


