Influence of corn processing provided in the diet on the ruminal dynamics of dairy steer

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ABSTRACT - The objective of the present study was to evaluate the ruminal dynamics of dairy steer fed diets containing whole corn grains, grains ground into grits or whole grain treated with urea. Thus, six rumen-fistulated animals were kept in confinement and fed diets with similar contents of energy and protein. The diet was formulated with 40:60 roughage:concentrate ratio in the dry matter using sorghum silage as roughage. The experiments followed a 3 × 3 latin square design, with three animals and three periods and they were repeated either two or four times depending on the studied parameter, totaling six or 12 replicates per diet. Corn grain treatment did not affect the pH of the ruminal fluid neither the ruminal degradability of dry matter, acid detergent fiber and cellulose. All diets provided a concentration of amoniacal nitrogen suitable for ruminal microbial growth; however, for animals fed whole corn grain treated with urea, this concentration was significantly lower. Bacterial activity was lower in animals fed diets containing ground corn and they do not differ among animals fed whole corn grain or corn grains treated with urea.

Key Words: bacterial activity, bovine, in situ degradability

Introduction

The oldest and most commonly used methods for grain processing are those that cause physical destruction of cells by mechanical means. As a consequence, corn grain, because of its lining layer, is usually ground to improve starch digestibility, by making the starch more exposed to the action of ruminal microorganisms and to the impact of enzymes from the digestive tract.

According to Vargas Junior et al. (2008), the treatment of corn grains with urea is of interest because, in addition to allowing the storage of the cereal on the farm under high humidity conditions and free from pests, it also acts in the pre-processing of the cereal, inasmuch as urea, because of being diluted in water and mixed with corn in a sealed environment, causes microfissures in the grain lining layers, thus facilitating the access to bacteria and the degradation of starch granules. However, Alcade & Andrade (1997)
underlined that, despite being a common practice, the processing of corn grains often does not have the expected efficacy.

The rate of ruminal microbial activity depends directly on the amount and availability of carbohydrates and nitrogen in the rumen (Theurer, 1986) therefore on the type of food and the treatment to which this food is subjected to (Potter, 1971). According to Huntington (1989), diets with high grain content, consequently with a higher proportion of readily fermentable carbohydrates, tend to reduce ruminal pH, favoring bacteria that are less sensitive to low pH, such as amylolytic and saccharolytic bacteria, increasing the use of ammonia and decreasing its concentration in the rumen. In addition, the number and the activity of ureolytic bacteria attached to the wall of the rumen are increased. Thus, ruminal pH reflects the balance between the production rates of volatile fatty acids, the input of buffers through the saliva, and the release of gases from ruminal fermentation, via eructation (Hobson & Steward, 1997). According to Silva & Leão (1979), the highest absorption of volatile fatty acids occurs under low pH, i.e. from 5.6 to 5.8. In addition, according to Allen (1997), an exceedingly low pH reduces appetite, ruminal motility, microbial growth, and fiber digestion, possibly leading to laminitis, ruminal ulcerations, hepatic abscesses or even to the death of the animal. According to Owens & Goetsch (1993), diets with a high proportion of grains tend to reduce the pH of the ruminal fluid and to decrease the ruminal degradability of fiber due to the lower survivorship of cellulolytic and hemi-cellulolytic bacteria.

According to Rooney & Pflugfelder (1986), depending on the degree of grinding of the corn grain, the velocity of ruminal degradation will vary and, as a consequence, the amplitude in the concentrations of volatile fatty acids and of the ammoniacal nitrogen will be higher in the ruminal environment. Therefore, the goal of this experiment was to test the effect of the method of provisioning of corn in the diet (whole grain, grains ground into grits, or whole grains treated with urea) in the ruminal dynamics of dairy steer.

**Material and Methods**

This study was carried out in the Setor de Nutrição Animal, Departamento de Zootecnia, Universidade Federal de Santa Maria (UFSM), in Santa Maria, state of Rio Grande do Sul, southern Brazil. Six castrated dairy steer, with an average initial live weight of 219 kg and fitted with ruminal canula, were used in the experiments. The animals were kept in confinement, fed sorghum silage based diet with forage:concentrate ratio of 40:60.

All diets had similar levels of energy and protein and differed only with respect to the type of corn grain (ground or whole), or whether it was treated or not with urea. Three types of corn provisioning were assessed: whole grain, grain ground into grits, and whole grains treated with urea (Table 1).

Corn grains were ground into grits by using a hammer mill without a sieve. For whole grains treated with urea, corn grains were treated with a 40% aqueous solution, so urea accounted for 2% of the total corn dry weight to be treated. To facilitate the application and to ensure complete homogenization, grains were mixed to the urea solution in a horizontal mixer and then covered with a plastic tarp to prevent volatilization of ammonia and finally, the solution was stored for four weeks in a brick shed.

The method used for investigating ruminal disappearance of dry matter (DM), acid detergent fiber (ADF) and sorghum silage cellulose was based on *in situ* degradability according to the procedure adopted in Hernandez et al. (1998). The experimental period consisted of three 21-day periods: on the 15\textsuperscript{th} day of each period, two 5 × 10-cm polyester bags with 48-mm mesh size were introduced into the rumen of each animal for every incubation interval. Each bag was

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Whole grain</th>
<th>Ground grain</th>
<th>Whole grain treated with urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum silage, kg</td>
<td>40.0</td>
<td>40.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Whole corn grain, kg</td>
<td>40.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ground corn grain, kg</td>
<td>-</td>
<td>40.6</td>
<td>-</td>
</tr>
<tr>
<td>Whole corn grain treated with urea, kg</td>
<td>-</td>
<td>-</td>
<td>41.2</td>
</tr>
<tr>
<td>Soybean meal, kg</td>
<td>17.6</td>
<td>17.6</td>
<td>17.6</td>
</tr>
<tr>
<td>Urea, kg</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Salt (NaCl), kg</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Caletic limestone, kg</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Crude protein (CP), %</td>
<td>14.60</td>
<td>14.60</td>
<td>14.89</td>
</tr>
<tr>
<td>Digestible energy, %</td>
<td>63.14</td>
<td>65.37</td>
<td>61.98</td>
</tr>
</tbody>
</table>

\(^7\) Vargas Junior et al. (2008).
identified with insoluble ink and received 15 mg/cm² of silage sorghum dry matter previously ground in a 4-mm mesh size willy mill. Each bag was sealed with an automatic heat sealer.

Incubation procedures followed the guidelines of AFRC (1992), following the method of sequential incubation, i.e. the bags first inserted into the rumen were the first to be removed. The bags were placed in a polyamide bag (12.5 × 21 cm, 1-mm mesh size) that was attached to an anchor with lead weights and an average total weight of 250 g. The anchor was tied to a 50-cm nylon thread to ensure the immediate immersion and movement of the bag in the ruminal contents and to facilitate its removal, inasmuch as the end of the thread was fixed near the lid of the canule. Incubation times, following the order of the placement of the bags in the rumen, were 96, 72, 48, 24, 12, 8, and 0 h, respectively. At the end of the assay, all bags were removed from the rumen and, together with the zero-time bags, were washed by vortexing until the water was completely clear. After washing, the bags were placed on a 65°C incubator for 72 h. Analyses were later conducted to determine the contents of dry matter, acid detergent fiber, and cellulose in silage samples that were incubated in the rumen and of the respective residues that remained in the bag after ruminal incubation, following the guidelines described in the AOAC (1984) and by Goering & Van Soest (1970).

The determination of the percentage of DM, ADF, and cellulose that disappeared in the rumen at each interval was obtained as follows: ruminal disappearance of dry matter (DM), acid detergent fiber (ADF) or cellulose = [(DM, ADF or incubated cellulose (g) – residual DM, ADF or cellulose after ruminal incubation (g)) / g of incubated DM, ADF or cellulose] × 100. Following the estimates of parameters for ruminal degradation of DM, ADF and cellulose, data on disappearance at different intervals were analyzed. Partial degradation data were adjusted according to the model of Orskov & McDonald (1979) Dg = a + b (1 - exp -ct), in which a = soluble fraction degraded at time zero; b = insoluble, potentially degradable fraction; c = fractional rate of degradation of b fraction per unit of time; and t = incubation time. The effective degradability (Ede) for each food type was calculated using the formula Ede = a + (b * c) / (c + k), in which k = the passage rate, as described by McDonald (1981).

The experimental design used to assess the effect of the diets on the ruminal degradability of DM, ADF and sorghum silage cellulose and their respective effective degradabilities were conducted in a 3 × 3 latin square with two replicates, such that columns represented the animals (six animals) and lines represented the intervals (three intervals), for a total of six replicates per mode of corn processing. The data were subjected to analysis of variance and to LSMEANS mean comparison test using the statistical package SAS (2003).

On the 21st day of each experimental interval during the degradability assay, samples were also obtained from the ruminal liquid to determine its pH, the level of amoniacal nitrogen, and bacterial activity. A vacuum pump were used to remove an aliquot of 50 mL of ruminal liquid, which was filtered with a cotton fabric and kept heated at approximately 39°C, as well as the material that came into contact with the liquid.

Six samples of ruminal liquid were obtained at 1.5-h intervals to determine its pH by using a digital potentiometer: the first sample was removed before the morning feeding (zero time) and the remaining samples at 1.5, 2.5, 3.5, 5.5, and 8.5 h after feeding. Food and water were provided in abundance, although the time for solid diet feeding was restricted to 1.5 h to decrease the effects of a constant input of food into the rumen.

The concentration of amoniacal nitrogen was determined immediately after filtration by removing a 10-mL aliquot of ruminal liquid and centrifugation at 5000 rpm for 15 min. After that, the concentration of amoniacal nitrogen was measured (mg N/100 mL), using the technique described by Fenner (1965).

Bacterial activity was measured by reduction of methylene blue immediately after the filtration of the ruminal liquid, following the procedures described by Dirksen (1981). A 19-mL volume of ruminal liquid was pipetted at a constant temperature (38 to 40°C) in a test tube and added to 1 mL of a 0.03% solution of methylene blue. The sample was then homogenized and the reduction time was measured using as a control another test tube with 20 mL of ruminal fluid. Time was measured between the moment of homogenization of ruminal liquid with methylene blue and the time for it to become similar to the standard as an indication of the number of seconds necessary for reduction. Reduction times were classified into three classes: up to 180 s, from 180 to 360 s, and above 360 s, representing a highly active, moderate or poorly active flora, respectively.

The experimental design used to assess the influence of the diet on the pH, amoniacal nitrogen and bacterial activity in the ruminal liquid was a 3 × 3 latin square, repeated four times (12 replicates) for pH and amoniacal nitrogen, and twice for bacterial activity (3 replicates). The obtained data were submitted to analysis of variance as a function of time and to the LSMEANS test for comparison of means using statistical package SAS (2003).
Results and Discussion

Corn grain treatment did not influence (P>0.05) the disappearance of dry matter, acid detergent fiber or cellulose from sorghum silage in any of the ruminal incubation periods (Table 2). Therefore, the hypothesis of a possible reduction in ruminal pH, associated with a higher rate of degradation of processed corn grains, would influence fiber degradation by ruminal microorganisms was not corroborated. Vargas Junior et al. (2008) also did not find evidence that the processing of corn grains influenced the total digestibility of these diet components.

However, in this experiment, the ruminal degradability of sorghum silage increased with incubation time, stabilizing after 72 hours (Table 2). Disappearance values of DM and ADF, when compared to the ways of corn processing in each time interval, were similar to each other and did not differ in any of the intervals. But, for cellulose disappearance, these values were not as homogeneous, but still did not differ among experimental diets. Hernandez et al. (1998), studying *Pennisetum purpureum* and *Pennisetum glaucum*, found values similar to those in the present study for DM disappearance, particularly for longer incubation periods.

In the present experiment, fractions “a” and “b”, the degradation rate “c”, the degradation potential (a+b), the lag time and the different effective degradations of sorghum silage were not significantly influenced by corn grain treatment, regardless of whether they were assessed in terms of DM, ADF, or cellulose (Tables 3, 4 and 5). These results indirectly show that the ruminal environment was not affected by the processing of corn to the point of influencing ruminal degradation. Beauchemin et al. (1994), using the same level of concentrate, but with ground barley as the forage, and Oliveira et al. (2003), in a study on sorghum silage with the same forage:concentrate ratio and an equal proportion of ground corn in the diet, observed values of “a”, “b”, and effective degradabilities similar to dry matter. Bürger et al. (2000), working with the same level of concentrate and ground corn in the diet, but using coastcross hay, found lower values of dry matter, and the influence of the forage properties on the degradability of dry matter was highlighted.

The “a” fraction estimated for the degradability of ADF and cellulose, which was expected to be null inasmuch as none of these fractions is theoretically soluble in water, showed relatively high values, with an average of 7.74%
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Table 4 - Disappearance potential, degradation potential, time estimated for the beginning of degradation (lag time - hours) and effective degradability of fiber in acid detergent and sorghum silage cellulose

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Potential disappearance</th>
<th>Potential degradation</th>
<th>Lag time</th>
<th>Effective degradability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>a+b</td>
</tr>
<tr>
<td>Fiber in detergent acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole corn</td>
<td>8.53</td>
<td>52.25</td>
<td>0.02108</td>
<td>60.78</td>
</tr>
<tr>
<td>Ground corn</td>
<td>7.05</td>
<td>54.20</td>
<td>0.02152</td>
<td>61.25</td>
</tr>
<tr>
<td>Whole corn treated with urea</td>
<td>7.65</td>
<td>58.19</td>
<td>0.01806</td>
<td>65.84</td>
</tr>
<tr>
<td>Mean</td>
<td>7.74</td>
<td>54.88</td>
<td>0.02022</td>
<td>62.62</td>
</tr>
<tr>
<td>Cellulose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole corn</td>
<td>6.88</td>
<td>67.62</td>
<td>0.01789</td>
<td>74.50</td>
</tr>
<tr>
<td>Ground corn</td>
<td>5.80</td>
<td>60.93</td>
<td>0.01970</td>
<td>66.73</td>
</tr>
<tr>
<td>Whole corn treated with urea</td>
<td>7.83</td>
<td>54.55</td>
<td>0.01832</td>
<td>62.38</td>
</tr>
<tr>
<td>Mean</td>
<td>6.84</td>
<td>61.03</td>
<td>0.01864</td>
<td>67.87</td>
</tr>
</tbody>
</table>

(Table 4). However, Petit (1994) also reported positive values for ADF disappearance for hay (*Phleum pratense*) and corn silage, with values close to 8 and 15%, respectively.

In the present study, the lag time period, which is the time necessary to begin ruminal degradation by microorganisms after incubation, was expected for all measured parameters, despite of being inferred only for ADF and cellulose but not for DM and indicating the beginning of degradation immediately after incubation. There was a long estimated duration for the lag time period for cellulose, averaging 9.02 hours, which was also recorded by Grant (1994) for the digestion of neutral detergent fiber, in which pH was controlled *in vitro* to 5.5 and a decrease for 2 hours was recorded when pH approached 6.8. Mertens & Loften (1980) observed a linear increase in lag time in relation to fiber degradation following an increasing addition of starch in an *in vitro* experiment, but did not observe any effect on the degradation rate – only a decrease in degradation potential with the addition of starch. This same effect could have occurred in the present experiment, not because of the amount of starch, but because of the possible differences in the velocity of degradation of cornstarch, which accounted for nearly 77% of the total starch in the tested diets.

In this experiment, because pH of the ruminal liquid was not significantly influenced by experimental diets (Table 5), it can be inferred that corn treatment did not affect rumination and saliva production, which was sufficient to buffer ruminal fluid. The diet containing whole starch grain led to an average pH higher than that of other diets, reaching 0.12 pH units higher than that associated with the diet with grains ground into grits.

At time zero (initial sampling), pH was above 6.8 in all diets and became lower after feeding (Figure 1). Nevertheless, Galyean et al. (1976) showed the influence of the mode of processing corn grains on ruminal pH, which averaged 6.2 possibly because of the high level of concentrate in the diet (85%). Galyean et al. (1979) compared whole corn grains to

<table>
<thead>
<tr>
<th>Parameter</th>
<th>pH</th>
<th>N-amoniacal (mg N/100 mL)</th>
<th>Bacterial activity (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ground corn</td>
<td>Whole corn</td>
<td>Whole corn treated with urea</td>
</tr>
<tr>
<td>pH</td>
<td>6.62</td>
<td>6.74</td>
<td>6.66</td>
</tr>
<tr>
<td>N-amoniacal (mg N/100 mL)</td>
<td>17.13a</td>
<td>17.99a</td>
<td>13.29b</td>
</tr>
<tr>
<td>Bacterial activity (seconds)</td>
<td>230.34a</td>
<td>124.11b</td>
<td>113.91b</td>
</tr>
</tbody>
</table>

Table 5 - pH, N-amoniacal and bacterial activity in the ruminal fluid of dairy steer fed experimental diets

a,b Different letters in the same line indicate significant difference at the 5% level by the LSMEANS test.
grains ground to three different granulometries and did not find any significant differences in the ruminal fluid in the tested animals.

The pH curve of the ruminal fluid of the animals right after feeding showed irregular behavior, with a peak at 3.5 hours, differing from the expectation of a constant drop, followed by a new increase over time. The explanation for the tendency for the pH to be maintained from 6.60 to 6.65 after feeding, from 1.5 to 5 hours after the beginning of the experiments, with a peak at 3.5 hours, could be a possibly higher rumination in that interval, which kept buffered the ruminal fluid. Murphy et al. (1994) showed that, immediately after feeding, ruminal pH was lower for steer fed diets containing whole corn grains (5.95) compared to those fed laminated corn grains (6.26), for until two hours (the lowest value). Later, there was a significant inversion until nine hours after feeding.

The processing of corn grains did not affect the concentration of amoniacal nitrogen in the rumen, with a peak of 5 mg N/100 mL of fluid one hour after feeding. Coombe et al. (1979) also did not detect any influence of the diet on ruminal pH, although the concentration of amoniacal nitrogen was influenced by the application of NaOH in the forage, resulting in a low level of ruminal fluid (0.7-1.3 mgN/100mL).

The levels of amoniacal nitrogen obtained for each treatment curve were similar ($P>0.05$), starting from around 12.17 mg N/100 mL of ruminal fluid at zero time, to a peak of 24.42 mg N/100 mL after 1.5 hours after feeding, and decreasing to 9.91 mg N/100mL after 8.5 hours. Animals were not fed after morning feeding, which reduced the amount of protein and urea available in the rumen therefore limiting the level of degraded amoniacal nitrogen, particularly the readily degradable fraction, whose degradation was significant until 1.5 hours after feeding. After that period, ruminal absorption and the use by microorganisms reduced the concentration of amoniacal nitrogen. The animals fed diet containing whole corn grains always showed the lowest levels of ammonia ($P<0.05$) (Figure 2).

The observed levels of ammonia in the present experiment were similar to those reported by Kang-Meznarich & Broderick (1981) and Murphy et al. (1994) and considered as excellent for the adequate digestion of food in the rumen and, therefore for maximal microbial growth. According to Kang-Meznarich & Broderick (1981), from 3.3 to 8.50 mgN/100 mL of ruminal fluid, there are not any differences in the ruminal digestion of DM, in the digestion rate and in the maximal microbial growth in diets containing 74% of corn grain. Ludden & Cecava (1995) also showed this tendency when analyzing amoniacal nitrogen and ruminal pH in the presence of different protein sources with ground corn and did not find any effect of the diet on pH and amoniacal concentration, which remained 3.46 mg N/100 mL of ruminal fluid.

The measured bacterial activity was very sensitive. There was a significant difference from the average activity in ground corn when compared to whole corn and corn treated with urea (Table 5). The bacterial activity remained at an average that was classified as moderate for the ground corn and active for both whole and corn treated with urea. For diets with whole grains and grains treated with urea, the activity remained from active to moderate, whereas in the case of the diet based on ground corn, the activity ranged from reduced to active (Figure 3), which is expected and easy to be explained if the ruminal pH had

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**Figure 1-** Ruminal fluid pH in dairy steers after feeding.

**Figure 2-** Amoniacal nitrogen in the ruminal fluid after feeding whole corn grain (WCG), ground corn grains (GCG), and whole corn grain treated with urea (WCGU).
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Ground corn grain diet was always associated with a lower bacterial activity in comparison to the other diets. In general, the peaks of maximal bacterial activity coincided with the lowest values of pH, confirming that, during those collection times, the highest concentration levels of fermentation products due to the higher bacterial activity, as well as the lower pH occurred. This was also found for the level of amoniacal nitrogen, inasmuch as the maximal peak coincided with the first peak in the maximal bacterial activity and to the first pH reduction right after feeding. Therefore, the large amount of substrate that reached the rumen was rapidly fermented due to the high bacterial activity resulting from a possible elevation in the concentration of fermentation products (volatile fatty acids) and the decrease in pH.

The similarity in the results of different diets might have happened because of the ability of young ruminants to chew corn grains, causing the natural rupture of the pericarp – the layer that surrounds the seed – exposing the starch granules and the remaining nutrients from the grain to microbial fermentation and to the action of digestive enzymes present in the gastrointestinal tract of the animal. These data corroborate the information provided by Orskov (1990) on the higher ability for chewing in young bovines up to 150 kg of live weight. Accordingly, in those cases, it would be advantageous to provide whole corn grains to the animals.

The amount and availability of carbohydrates and nitrogen are directly related with microbial activity (Theurer, 1986), confirming that, based on the results of the present study, these parameters were kept balanced in spite of corn treatment. This also influenced the concentration of free hydrogen-ion concentration, which resulted in a ruminal pH, which was similar among the tested diets, with no influence on the fiber degradation of the forage, as it was expected in any of the studied post-feeding intervals.

Conclusions

For dairy steer, the grinding of corn grains and the treatment of whole grains with urea do not interfere with the pH of the ruminal fluid nor with the ruminal degradability of dry matter, acid detergent fiber and cellulose in sorghum silage. The concentration of amoniacal nitrogen in the ruminal fluid is lower in animals fed whole corn grains treated with urea, whereas microbial activity is lower when the animals are fed corn grains ground into grits.

References


Figure 3 - Bacterial activity in the ruminal fluid when measured by the time for the reduction of methylene blue, adjusted according to the time after feeding and to the experimental diets: whole corn grain (WCG), ground corn grains (GCG), and whole corn grain treated with urea (WCGU).


