

**Strategies to Improve the Digestibility of Distiller's Grains**

**Final Report to Texas Corn Producer's Board**

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## STRATEGIES TO IMPROVE THE DIGESTIBILITY OF DISTILLER'S GRAINS

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### Summary

Commodity wet distiller's grains (WDG; minimum 85% corn-based) was treated with sodium hydroxide (NaOH), limestone + enzyme (LS), and enzyme without buffer (ENZ) followed by exposure to pressure (PS) to determine if the rumen digestibility of WDG could be improved. The resulting 10 samples were incubated in the rumen of four steers for 0, 4, 8, 12, 16, 24, and 96 hours. The sample residues of a selected group of samples were refluxed in acid detergent fiber (ADF) solution. An exponential model was developed to determine the rate and extent of digestibility of dry matter and ADF. The addition of LS increased the effective ruminal digestibility of WDG by 10% over untreated WDG. This was accomplished by increasing the rapidly degradable fraction of the WDG, presumably by solublizing hemicellulose. The addition of ENZ also increased the rapidly soluble fraction of WDG, but did not significantly increase the effective rumen digestibility over untreated WDG. The addition of PS did not further improve the ruminal digestibility of WDG when other treatments were used. However, applying pressure to untreated WDG significantly improved the rumen digestibility compared to untreated WDG with no PS. This suggests that PS treating WDG may be a viable option for improving the digestibility of WDG when no other treatment is used. The addition of NaOH did not appear to improve the ruminal digestibility of distiller's grains unless used in combination with the ENZ. However, the response to NaOH and ENZ was similar to the addition of ENZ alone, and less than the addition of LS and ENZ. Therefore, we observed no benefit of using NaOH over the other treatments. We conclude that treating WDG with a buffer such as LS and ENZ, or applying heat and PS to WDG are two viable methods of improving the ruminal digestibility of WDG. The addition of LS and ENZ appears to provide the greatest benefit.

### Introduction

Wet distiller's grains (WDG) is becoming an increasingly important feed commodity in the Texas High Plains Region. However, WDG available in this region may have a lower energy value compared to WDG available in the Northern Plains. Research from our laboratory suggest that WDG from the Northern Plains has an energy value similar to steam-flaked corn whereas WDG produced in the Texas High Plains reduces feed efficiency in a steam-flaked corn-based diet. Metabolism data suggests the addition of WDG into steam-flaked corn-based diets reduces ruminal digestibility of the diet. Therefore, improving the ruminal digestibility of WDG may improve its feeding value and maintain the competitive advantage of feeding steam-flaked corn-based diets in the Texas High Plains Region. We have previously reported that treating WDG with a buffered enzyme, sodium hydroxide, or steam improves the digestibility of WDG in vitro. Additional data are necessary to determine if the improvement in ruminal digestibility are maintained in situ. Therefore, the objective of this project was to evaluate the in situ digestibility of WDG that had been treated with combinations of enzyme, limestone, sodium hydroxide, and steam.

### Experimental Procedures

Four ruminally and duodenally cannulated steers (1000 lbs) were used to determine the in situ digestibility of WDG treated with combinations of enzyme, limestone, sodium hydroxide, and steam to improve ruminal digestibility. Ten samples were generated from WDG produced in the Texas High Plains Region. The WDG was derived from a minimum of 85% corn and was 32% crude protein, 38% neutral detergent fiber, 25% acid detergent fiber, and

10.8% ether extract. The WDGS was treated using five strategies: 1) untreated WDGS (UNT); 2) WDGS treated with a commercial enzyme provided by Biozyme Inc. (St. Joseph, MO) added at a rate of 0.227 ml / kg (1 L / US ton) diluted into water and added at a volume of 265 ml / kg (240 L / US ton; ENZ); 3) WDGS buffered with 10% (DM-basis) limestone followed by addition of ENZ (LS); 4) WDGS treated with 3% sodium hydroxide (NaOH); and 5) WDGS treated with NaOH followed by the addition of ENZ (NaOH+ENZ). After sample preparation, the treatments were packed in mini-silos constructed from 8 inch PVC pipe (8 inches long) were packed by placing a 50 lb weight on top of a cylinder constructed to fit inside of the PVC diameter. Samples were collected after 14-d and analyzed for DM, and pH. A portion of each sample was then exposed to 10 PSI of steam for 60 minutes (PS). All samples were then frozen (-5 deg. C) for further analysis. Samples were thawed and approximately 2 g (DM-basis) of sample was weighed into an in situ bag (5 cm X 10 cm; 50 micron porosity; Ankom Technologies, Macedon, NY) which was pre-weighed and labeled. Bags were sealed and frozen until all samples could be prepared for incubation. Seventy-five bags per sample were prepared as follows: three bags per observation X four animals X 6 time points plus three bags for a common washout observation.

Steers were limit fed a diet (2% BW) once daily consisting of 25% dried distiller's grains, 23.5% steam-flaked corn, 20% alfalfa hay, 20% cottonseed hulls, 9% molasses, and 2.5% supplement. Samples (3 bags per observation) were incubated for 96, 24, 16, 12, 8, and 4 hours. Bags were enclosed in a nylon mesh bag and inserted into the ventral sac of the rumen in reverse order so all bags were removed at the same time. After removal, bags were washed in a washing machine and dried in a 60 degree forced air oven for 48-hours. Bags were cooled in a desiccator and weighed. Percent disappearance was calculated for the average of three bags for each observation.

Data were fitted to the following non-linear regression model:

$$Y = A + B * (1 - \exp^{-c * t})$$

Where:

A = the rapidly soluble fraction

B = the potentially degradable fraction which degrades at a constant rate

C = rate of digestion (% per hour)

T = time

The effective rumen degradability (ERD) was then calculated as:

$$ERD = A + [(B * C) / (C + 0.05)]$$

Where: 0.05 is the assumed passage rate of 5% per hour.

Extent of digestion was the % disappearance at 96-hours. The resulting digestibility coefficients (A, B, C, ERC, and Extent) were analyzed using the mixed procedures of SAS. Steer was the experimental unit and was considered a random effect. Data were analyzed as a 2X5 factorial arrangement of treatments where the five WDGS treatments (UNT, ENZ, LS, NaOH, and NaOH+ENZ) served as one factor and non-exposure or exposure to PS served as the second factor.

Following the analysis of DM, four of the ten treatments which showed the greatest promise to improve digestibility were selected and the samples and residues were analyzed for acid detergent fiber (ADF). The same non-linear regression model use for DM digestibility coefficients was developed for ADF and the resulting coefficients were analyzed in the mixed procedure of SAS as a completely randomized design. Steer was the experimental unit and was considered a random effect.

## Results and Discussion

Dry matter in situ digestion parameters and pH are shown in Table 1. Similar to our previous results, the addition of ENZ or exposure to PS did not have a large influence on the pH of treated WDGS after 14-d of storage in oxygen limited environment. However, the addition of either LS or NaOH increased the pH of WDGS from 3.27 to approximately 5.0. The premise of this project has been that increasing the pH of distiller's grains would allow enzymes to more effectively degrade the fiber in WDGS. These data are consistent that our buffering techniques may allow that to happen. We also hypothesize that a major impact of the buffered enzyme is to solubilize a portion of the fiber into simple sugars that would be more easily digested in the rumen. In our analysis, this would be expressed in a larger A fraction and smaller B fraction. All of the strategies that we tested significantly increased the A fraction over UNT except for the combination of NaOH+ENZ that had been exposed to PS. The LS treatment had the largest impact on the shift of DM from the B fraction to the A fraction. The LS treatment resulted in the largest A fraction and lowest B fraction of any treatment ( $P < 0.08$ ). Pressure exposure also significantly reduced the B fraction compared to samples not exposed to PS ( $P < 0.01$ ). The concomitant increase in the A fraction and reduction of the B fraction generally coincided with a reduction in the rate of digestion for the remaining material. This is consistent with our hypothesis that the enzyme is solubilizing fiber into simple sugars. The remaining fiber that has not been solubilized appears to have reduced rates of digestion. The variable of greatest interest is the effective rumen degradability (ERD) because it accounts for both the shift from the B fraction to the A fraction and the rate and extent of digestion. Treatment of LS resulted in a 10% increase in ERD compared to UNT ( $P < 0.001$ ). NaOH + ENZ also improved ERD over the UNT, but less than LS. The addition of ENZ alone did not significantly improve ERD over the UNT. This is consistent with the hypothesis the moderation of pH is important for enzyme activity. The exposure of UNT to PS also increased the ERD compared to UNT samples not exposed to PS. Interestingly, exposure to PS did not further enhance the ERD of samples that had been treated with other techniques. This suggests that PS is a may be a viable option to improve the digestibility of WDGS instead of using other techniques, but the advantages do not appear to be additive. We were surprised that the addition of NaOH did not have a larger impact on ERD given our previous in vitro results which suggested a 15% improvement in fiber digestibility due to 3% NaOH addition. Both PS and NaOH treatment appear to reduce the extent of DM digestibility which would have negatively affected ERD. While we still suspect that the addition of NaOH may improve rumen digestibility, these data are not supportive. However, these data do suggest that LS has a large potential to improve ruminal digestibility of WDGS.

The digestion parameters for ADF of selected samples are shown in Table 2. Based on the DM digestion characteristics, we selected three samples that had the greatest potential to improve digestion to more closely evaluate the digestion of fiber. Those samples included ENZ, LS, and UNT + PS. Those three samples were compared to UNT to evaluate the improvement in ADF digestion over commodity WDGS. Exposure of UNT to PS did not significantly change the digestion parameters of ADF. This suggests that any improvement in ERD we observed for DM (Table 1) was due to improved digestion of hemicellulose, or non-fiber components. Both ENZ and LS increased the A fraction of ADF compared to UNT ( $P < 0.05$ ). The use of LS also reduced the ADF fraction of the sample by 18% whereas the addition of ENZ slightly increased or did not change the ADF fraction. It is important to acknowledge that the addition of 10% limestone will dilute the ADF fraction of the sample. While this does not account for the entire 18% reduction in ADF content, at least a portion of the reduction may be due to a dilution effect. Nevertheless, we still hypothesize that at least a portion of the ADF fraction was solubilized by LS. However, the addition of ENZ increased the ERD compared to UNT whereas LS did not. An indices of indigestible ADF may be a more appropriate method of evaluating changes in ADF digestibility when a portion has been solubilized. When expressed on an indigestible ADF basis, ENZ resulted in a 9% reduction in indigestible ADF whereas LS resulted in a 24% reduction. We suspect that LS treatment of WDGS would have the greatest potential to improve digestibility of WDGS. However, it is difficult to surmise from these data if the addition of LS would

result in animal performance greater than ENZ alone as both appear to improve the digestion of fiber in WDGS and LS would cause a dilution effect from the addition of buffer. Therefore, an animal performance trial is necessary to determine if LS is more effective than ENZ in improving animal performance.

### **Implications**

The potential to improve the digestibility of distiller's grains by enzymatic activity appears to be large if the distiller's grains are first buffered to moderate pH to approximately 5.0. The improvement in digestibility appears to be due to an increase in the rapidly soluble fraction of the distiller's grains, likely due to a breakdown of hemicellulose and cellulose into soluble sugars. The apparent enzymatic activity may result in a 10% improvement in ruminal digestibility of dry-matter and a 24% reduction in indigestible acid detergent fiber.

Table 1. Dry matter in situ degradation parameters, effective digestibility, and pH of treated disitiller's grains.

Item	Non – Pressure Treated					Pressure Treated					SEM
	Untreated	Enzyme	Enzyme +Buffer	NaOH	NaOH +Enzyme	Untreated	Enzyme	Enzyme +Buffer	NaOH	NaOH +Enzyme	
pH	3.27	3.25	4.71	4.96	4.46	3.62	3.58	5.15	5.10	4.67	-
Dry Matter											
a (%)	47.7 <sup>f</sup>	54.5 <sup>e</sup>	60.7 <sup>a</sup>	49.8 <sup>e</sup>	54.3 <sup>c</sup>	52.3 <sup>d</sup>	55.3 <sup>c</sup>	57.5 <sup>b</sup>	50.3 <sup>e</sup>	47.3 <sup>f</sup>	0.6
b (%) <sup>gh</sup>	45.2	40.9	38.0	40.5	39.3	38.1	37.1	33.4	38.3	38.7	5.5
c (%) <sup>ij</sup>	2.76	1.77	1.89	3.09	2.37	3.90	2.24	2.74	2.55	2.88	0.59
ERD (%)	63.3 <sup>de</sup>	65.1 <sup>cd</sup>	69.6 <sup>a</sup>	64.0 <sup>dc</sup>	66.1 <sup>bc</sup>	67.6 <sup>ab</sup>	67.1 <sup>bc</sup>	68.9 <sup>a</sup>	62.5 <sup>e</sup>	60.5 <sup>f</sup>	0.9
Extent (%) <sup>kl</sup>	89.1	87.9	88.5	86.4	88.2	88.3	88.6	87.9	84.1	82.4	3.5

<sup>abcdef</sup>Means within row differ ( $P < 0.01$ ) when treatment by pressure interaction is significant ( $P < 0.001$ ).

<sup>g</sup>Main effect of pressure significant ( $P < 0.01$ ) where Non – Pressure Treated > Pressure Treated.

<sup>h</sup>Tendency for main effect of treatment ( $P = 0.07$ ) where Enzyme + Buffer is less than Untreated, NaOH, and NaOH + Enzyme ( $P < 0.10$ ) and Enzyme is intermediate ( $P > 0.10$ ).

<sup>i</sup>Tendency for main effect of pressure ( $P = 0.08$ ) where Non – Pressure Treated < Pressure Treated.

<sup>j</sup>Main effect of treatment ( $P = 0.05$ ) where Untreated is greater than Enzyme, Enzyme+Buffer, NaOH + Enzyme, and NaOH is intermediate ( $P > 0.10$ ).

<sup>k</sup>Main effect of pressure ( $P = 0.03$ ) where Non – Pressure Treated > Pressure Treated.

<sup>l</sup>Main effect of treatment ( $P = 0.01$ ) where Untreated, Enzyme, Enzyme+Buffer is greater than NaOH, and NaOH + Enzyme ( $P < 0.03$ ).



Table 2. Acid detergent fiber in situ degradation parameters and effective digestibility of treated disitiller's grains.

Item	Untreated	Enzyme	Enzyme +Buffer	Untreated + Pressure	SEM
ADF, %	25.1	27.3	20.5	29.3	-
a (%)	31.7 <sup>b</sup>	44.1 <sup>a</sup>	40.1 <sup>a</sup>	30.8 <sup>b</sup>	0.2
b (%)	61.1	44.9	53.3	50.9	6.7
c (%)	2.64	2.62	2.28	3.39	6.64
ERD (%)	51.3 <sup>b</sup>	59.3 <sup>a</sup>	54.4 <sup>b</sup>	53.0 <sup>b</sup>	1.5
Extent (%)	85.1	85.4	79.7	80.4	4.6
Indigestible ADF	12.2	11.1	9.3	13.8	-

<sup>ab</sup>Means within row differ ( $P < 0.05$ ).