# The influence of dry-rolled corn particle size and dried corn distillers grains plus solubles inclusion levels on rumen pH, ammonia and VFA concentration, total in vitro ruminal gas production and enteric methane emission

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*The objectives of this study were to determine the influence of dry-rolled corn particle size and dried distillers grains with solubles (DDGS) inclusion level on ruminal pH, ammonia (NH3) and volatile fatty acid (VFA) concentrations and in vitro ruminal gas production and methane (CH4) emission. No differences in rumen pH were seen among treatments. Rumen ammonia was greater in steers receiving 20 percent DDGS, while steers fed fine-rolled corn had greater concentrations of butyric acid than steers fed coarse-rolled treatments. Total gas production and methane concentration were unaffected by treatment.*

## Summary

Eight cannulated Holstein steers (1,159 ± 8 pounds) were used in a 4 x 4 Latin square design to examine the impact of coarse (2.5 millimeter [mm]) vs. fine-rolled corn (1.7 mm) and 20 vs. 40 percent DDGS inclusion on ruminal pH, ammonia and VFA concentrations, in vitro ruminal gas production and enteric methane emission. Steers were housed in individual tie stalls (3.3 by 7.2 feet) in a temperature-controlled environment at the NDSU Animal Nutrition and Physiology Center. Dietary treatments (Table 1) were offered for ad libitum intake and consisted of 1) 65 percent coarse-rolled corn and 20 percent DDGS, 2) 45 percent coarse-rolled corn and 40 percent DDGS, 3) 65 percent fine-rolled corn and 20 percent DDGS and 4) 45 percent fine-rolled corn and 40 percent DDGS. Steers were provided experimental diets for 14 days (seven days of diet adaptation and seven days of data collection). Results indicate no differences among dietary treatments in overall rumen pH. The concentration of $NH_3$ was greater ($P = 0.02$) in cattle consuming 20 percent DDGS. Butyric acid concentration was greater ($P = 0.02$) in cattle fed fine-rolled corn, while no other VFAs differed among treatments. No differences were observed in the amount and rate of total gas produced or concentration of methane emitted.

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## Introduction

Ethanol is a commonly produced alternative fuel that largely is manufactured using corn grown in the Midwestern U.S. The production of ethanol also supplies a byproduct known as dried corn distillers grains plus solubles (DDGS), which provides a valuable feed source for ruminants. Ensuring the amount of corn grown to produce ethanol also allows much of the crop to be fed to cattle, which has proven to be extremely beneficial in regards to animal efficiency and environmental sustainability.

Methane is a greenhouse gas produced during enteric fermentation of feed in ruminants and can be influenced by feed intake, type of carbohydrate in the diet, feed processing methods and changes in ruminal microflora. The high levels of starch found in corn-based rations have been shown to be beneficial to the environment because cattle fed these diets produce less methane due to reduced hydrogen production in the rumen. In addition, a greater feed efficiency provides a shorter time to market, allowing less opportunity of methane emission to occur (Swanson et al., 2014).

Unfortunately, in regard to distillers grains and methane production, variable results have been observed. For example, distillers grains contain greater concentrations of fat and fiber. The fat found in these byproducts may reduce or eliminate protozoa as well as methanogenic bacteria in the rumen, helping mitigate CH4 emissions by altering the hydrogen sink through bio-hydrogenation via propionate production (Massé et al., 2014). Fiber, however, is concentrated nearly three-fold during ethanol production and possesses greater methanogenic potential than that of starch (Behlke et al., 2008).

While a diet containing corn and DDGS offers a desirable nutrient profile, careful consideration must be taken when formulating such rations. Corn distillers grains commonly are mixed with cattle rations in ranges from 10 to 50 percent (dry-matter [DM] basis), depending on the goal of supplementation. When used as a protein source, 10 to 15 percent inclusion usually is the most desirable, while an addition of 20 to 30 percent is more common when used as an energy source, with approximately 40 to 50 percent generally the upper limit (Klopfenstein et al., 2008).

The available information on particle size reduction of rolled corn is limited, but Loe et al. (2006) reported an increase in intake when offering finely vs. coarsely rolled corn. Grain particle size, level of rumen carbohydrate fermentation and level of neutral detergent fiber (NDF) also will impact rumen pH and may cause acidosis. With this in mind, developing feeding strategies is important to determine the optimum corn processing method and DDGS inclusion rates to obtain the greatest benefit from each ration.

## Experimental Procedures

All procedures involving animals were approved by the NDSU Animal Care and Use Committee. Eight cannulated Holstein steers (1,159 ± 8 pounds) were used in a 4 x 4 Latin square-designed experiment to examine the impact of dry-rolled corn processing and DDGS inclusion rate on ruminal pH, ammonia concentration, VFA profile, ruminal gas production and in vitro enteric methane emission in cattle.

Steers were housed in individual stalls in a temperature-controlled environment at the NDSU Animal Nutrition and Physiology Center. Dietary treatments (Table 1) were offered to ensure ad libitum intake and approximately 6 percent feed refusal daily. Treatments consisted of 1) coarse-rolled (2.5 millimeters [mm]) and 20 percent DDGS, 2) coarse-rolled corn and 40 percent DDGS, 3) fine-rolled corn (1.7 mm) and 20 percent DDGS and 4) fine-rolled corn and 40 percent DDGS.

Diets were formulated to meet or exceed National Research Council (NRC) recommendations for degradable intake protein (DIP), metabolizable protein (MP), vitamins and minerals (NRC, 1996). Before the initiation of the experiment, steers were adapted to a high-grain diet during a period of 21 days. A preliminary period of seven days on the animals' respective treatment preceded seven days of sample collection for each period. This was followed by a three-day rest period in which steers were offered an intermediate diet to allow all animals to return to a basal level.

Ruminal pH was determined using a wireless pH sensor (Kahne Ltd., Auckland, New Zealand), with measurements taken every five minutes from days 3 to 5 of the collection period. Sensors were calibrated with 7 and 4 pH solutions before each period and were inserted manually into the rumen and placed in the liquid phase of the ventral sac.

Ammonia and VFA concentrations were quantified using a subsample of approximately 200 milliliters (mL) of rumen fluid collected from days 3 to 5 (at 2 a.m., 8 a.m., 2 p.m. and 8 p.m. on day 3; 4 a.m., 10 a.m., 4 p.m. and 10 p.m.

**Table 1. Dietary composition and analyzed nutrient concentration of diets (DM basis).**

| | Coarse-rolled corn | | Fine-rolled corn | |
|---|---|---|---|---|
| **Dietary component, % of DM** | **20% DDGS** | **40% DDGS** | **20% DDGS** | **40% DDGS** |
| Coarse-rolled corn | 65.0 | 45.0 | – | – |
| Fine-rolled corn | – | – | 65.0 | 45.0 |
| Dried corn distillers grains with solubles | 20.0 | 40.0 | 20.0 | 40.0 |
| Grass-legume hay | 5.0 | 5.0 | 5.0 | 5.0 |
| Corn silage | 5.0 | 5.0 | 5.0 | 5.0 |
| Limestone | 1.56 | 1.90 | 1.56 | 1.90 |
| Urea | 0.85 | – | 0.85 | – |
| Salt | 0.20 | 0.20 | 0.20 | 0.20 |
| Vitamin premix | 0.01 | 0.01 | 0.01 | 0.01 |
| Trace mineral premix | 0.05 | 0.05 | 0.05 | 0.05 |
| Rumensin/Tylan premix | 0.03 | 0.03 | 0.03 | 0.03 |
| Fine-ground corn | 2.05 | 2.56 | 2.05 | 2.56 |
| Chromium oxide | 0.25 | 0.25 | 0.25 | 0.25 |
| **Feed Analysis** | | | | |
| Dry matter, % of as fed | 82.2 | 82.9 | 82.4 | 83.6 |
| Organic matter, % of DM | 94.9 | 93.7 | 95.1 | 93.8 |
| Crude protein, % of DM | 16.3 | 17.9 | 15.9 | 17.4 |
| Neutral detergent fiber, % of DM | 27.1 | 30.2 | 24.5 | 30.5 |
| Acid detergent fiber, % of DM | 9.02 | 11.1 | 8.47 | 11.0 |
| Fat, % of DM | 4.45 | 4.92 | 3.77 | 4.86 |
| Calcium, % of DM | 0.794 | 0.929 | 0.757 | 1.00 |
| Phosphorus, % of DM | 0.408 | 0.537 | 0.409 | 0.538 |

on day 4; and 6 a.m., noon, 6 p.m. and midnight on day 5 to represent every other hour in a 24-hour cycle.

After collection, subsamples were taken to the lab and stored frozen (minus 20 C) until the end of the collection period, at which point they were thawed, equally composited and used for ammonia and VFA analysis.

Gas production was determined using Ankom's gas pressure flasks, wireless system and analysis software (Gas Pressure Monitor, Ankom Technology Corp., Macedon, N.Y.). After the addition of ruminal fluid and buffer, the vials were flushed with carbon dioxide. The flasks then were screwed tightly to the pressure monitor caps and placed in an oscillating water bath (Northwest Scientific Incorporated) at 39 C for 24 hours, with the oscillation set at 125 revolutions per minute.

Data obtained from this system were converted from pressure units to volume units (mL) using the formula reported by López et al. (2007). Gas production was examined on days 1 and 7 of the collection period for approximately 24 hours.

Data were analyzed as a 2 × 2 factorial using the Mixed procedure of SAS (SAS Inst. Inc., Cary, N.C.). The model included the effects of animal, period, degree of dry-roll processing (coarse vs. fine), DDGS inclusion (20 vs. 40 percent DDGS) and the interaction between the degree of dry-roll processing × DDGS inclusion rate. Statistical significance was declared at $P \leq 0.05$.

## Results and Discussion

No differences were observed in ruminal pH among dietary treatments ($P > 0.05$). Rumen NH3 was increased ($P = 0.02$) in diets containing 20 percent DDGS. Urea was added to rations with 20 percent DDGS to meet the NRC's DIP requirement. This urea likely was rapidly hydrolyzed to ammonia by bacterial urease. Volatile fatty acids were generally unaffected by dietary treatment; however, the level of butyric acid was greater ($P = 0.02$) in cattle consuming fine-rolled corn (Table 2).

In vitro gas production and enteric methane emission were not different among treatments ($P \geq 0.44$; Table 3). Acetate has been shown to increase methane production, while propionate has the opposite effect (Moss et al., 2000). As no changes to the acetate:propionate ratio were found in the current study, we were not surprised that methane concentrations did not differ between the variable rations.

Dietary treatments did not affect rumen pH or VFA concentration in a way that would affect gas production or enteric methane emission significantly. This would indicate that the digestive tracts of the cattle tested were not strongly influenced by the degree of corn processing or inclusion rate of DDGS.

**Table 2. Ruminal pH and VFA profiles of steers fed coarse- vs. fine-rolled corn with 20 vs. 40 percent dried distillers grains with solubles.**

| | Coarse-rolled corn | | Fine-rolled corn | | | *P*-Values | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 20% DDGS | 40% DDGS | 20% DDGS | 40% DDGS | SEM[a] | Corn | Distiller's | Corn * Distiller's | Hour |
| Rumen pH | 5.96 | 5.68 | 5.88 | 5.70 | 0.134 | 0.85 | 0.12 | 0.72 | <0.001 |
| Minimum | 5.31 | 5.04 | 5.22 | 5.10 | 0.197 | 0.94 | 0.32 | 0.68 | - |
| Maximum | 6.69 | 6.77 | 7.04 | 6.78 | 0.214 | 0.44 | 0.69 | 0.49 | - |
| Time < 5.5, h/d | 3.02 | 11.1 | 4.66 | 5.82 | 2.125 | 0.40 | 0.07 | 0.14 | - |
| Rumen $NH_3$, mM | 13.3 | 10.4 | 13.4 | 9.8 | 13.31 | 0.87 | 0.02 | 0.80 | <0.001 |
| Total VFA, mM | 184 | 183 | 197 | 198 | 9.7 | 0.14 | 0.99 | 0.91 | <0.001 |
| | | | | | VFA, mol/100 mol | | | | |
| Acetic | 33.1 | 34.1 | 32.0 | 32.7 | 1.44 | 0.41 | 0.56 | 0.92 | 0.18 |
| Propionic | 22.0 | 24.7 | 25.2 | 22.2 | 2.19 | 0.87 | 0.96 | 0.20 | <0.001 |
| Isobutyric | 2.79 | 2.77 | 2.48 | 2.58 | 0.159 | 0.12 | 0.82 | 0.73 | <0.001 |
| Butyric | 17.4 | 15.3 | 19.6 | 21.4 | 1.68 | 0.02 | 0.93 | 0.24 | 0.45 |
| Isovaleric | 16.5 | 14.7 | 11.9 | 10.9 | 2.07 | 0.06 | 0.50 | 0.84 | <0.001 |
| Valeric | 8.24 | 8.51 | 8.83 | 10.2 | 0.642 | 0.10 | 0.23 | 0.42 | 0.05 |
| Acetate:Propionate | 1.65 | 1.52 | 1.39 | 1.70 | 3.4 | 0.84 | 0.66 | 0.28 | 0.001 |

[a]Data are presented as least square means per treatment ± SEM, n = 8

**Table 3. Gas production and methane emission of steers fed coarse- vs. fine-rolled corn with 20 vs. 40 percent dried distillers grains with solubles.**

| | Coarse-rolled corn | | Fine-rolled corn | | | *P*-Values | | |
|---|---|---|---|---|---|---|---|---|
| | 20% DDGS | 40% DDGS | 20% DDGS | 40% DDGS | SEM[a] | Corn | Corn * Distiller's | Distiller's |
| Gas production, mLs | | | | | | | | |
| A | 195 | 176 | 183 | 198 | 30.5 | 0.86 | 0.96 | 0.56 |
| C | 0.075 | 0.063 | 0.082 | 0.094 | 0.0234 | 0.38 | 0.10 | 0.55 |
| d | 0.019 | 0.128 | -0.068 | -0.132 | 0.1013 | 0.09 | 0.82 | 0.38 |
| L | 1.19 | 1.07 | 1.65 | 0.804 | 0.2890 | 0.71 | 0.08 | 0.17 |
| Methane, % of gas | 10.8 | 12.1 | 11.5 | 12.6 | 12.64 | 0.68 | 0.44 | 0.94 |

[a]Data are presented as least square means per treatment ± SEM, n = 4 per treatment. A = asymptote, C = rate, d = degradation rate, L = Lag


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