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# Effect of Supplementation Using Distillers-Dried Grains (DDGS) on *in situ* Ruminal Fermentation in Cattle Grazing on Forage Sorghum (*Sorghum* spp)

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## ABSTRACT

**Aim.** To evaluate the effect of supplementation with distillers-dried grains (DDGS) on *in situ* ruminal fermentation parameters by cattle grazing on forage sorghum. **Materials and methods:** Four Hereford heifers (793 ± 73 kg live weight) using a ruminal cannula, grazing on forage sorghum, with day-in enclosure and artificial shade, were part of a randomized crossover design study with two treatments: without supplementation (WTS) and with supplementation (WS), fed on DDGS. The experimental design comprised two 14-day periods (10 days for diet transition, and 4 days for measurements). **Results:** Supplementation did not affect DM consumption ($P > 0.05$), though it increased total consumption ($P < 0.05$). The WS treatment caused lower ruminal pH (6.17 vs. 6.55; ($P < 0.05$), and greater $N\_NH_4$ concentration ($P < 0.05$). The effective degradability of DM was 51.25%, the same as the other treatment ($P > 0.05$), whereas the effective NDF was lower in the WS treatment ($P < 0.05$). DM digestibility in WS was lower (65.75 vs. 60.75%), whereas NDF digestibility was 68.50 vs. 62.50%. **Conclusion:** DDGS supplementation decreased ruminal pH and raised $N\_NH_4$ concentrations, reducing the fiber' s effective degradability of DM and NDF.

**Keywords:** DDGS, heifers, supplementation (*Source*: *AGROVOC*)

## INTRODUCTION

Forage sorghum (*Sorghum* spp) provides cattle raising systems with high forage volumes during the summer. This C4 species is productive after a short cycle since it adjusts to high water shortages and raised temperatures (Moyano et al., 2021). However, these pastures have little

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protein and high fiber content (Vargas, 2005 y Murray et al., 2010), which might be a nutritional issue for growing cattle (Aduli et al., 2022). Supplementation as an alternative could lift the nutritional restrictions of pastures, increasing live weight gains and feed conversion.

The commonly used supplements are based on grains. They have high starch content, which in the rumen, may raise volatile fatty acids, decreasing ruminal pH with lower nitrogen capture and use (Raposo et al., 2015; Chibisa et al., 2016), thus reducing grass fiber degradability (Firkins, 1996).

Distillers-dried grains (DDGS) are soluble, they are a sub-product from ethanol production obtained after milling, hydrolysis, and fermentation of starch from grains (Liu, 2011; Aristizabal, 2016). They have high protein, energy (30.9% PC, 3.2 EM Mcal/ kg) (BCNRM, 2016) content, and highly digestible fiber (Westreicher-Kristen et al., 2013; De Boever et al., 2014). Few papers have evaluated the effect of supplemented fiber and dry matter (DM) digestibility and degradability of forage sorghum. This paper aims to evaluate the effect of supplementation with distillers-dried grains (DDGS) on *in situ* ruminal fermentation parameters in cattle grazing on forage sorghum (*Sorghum* spp).

# MATERIALS AND METHODS

This study was done on the west coast of Uruguay (32.5º south latitude, 58º west longitude), between 1/30/19 and 2/26/19, covering 6hr of forage sorghum (hybrid ADV 2800, 5959± 420 kg DM/ha, 91 ± 45,2 cm high), planted on 12/1/2018, at 25 kg/ha, using 60 kg/ha of fertilizer (18-46-0).

## Animals, treatments, and experimental design

Four Hereford heifers (793 ± 73 kg LW) using a ruminal cannula, grazing (4’ ’ silicone; KEHL®) on forage sorghum, with day-in enclosure and artificial shade (10:00 h-16:00 h), were part of a randomized crossover design study with two treatments: without supplementation (WTS) and with supplementation (WS), on DDGS (40% corn + 60% wheat) at 1 kg of DM every 100 kg LW. The experimental design comprised two 14-day periods (10 days for diet transition, and 4 days for measurements).

## Experimental management

Grazing was designed for separate lots by animal, administered 8 kg DM every 100 kg LW. The lots were occupied for 10 days during the transition, followed by daily stripe grazing lots for the 4 days of sampling in each period. Stripe lot changes were performed each morning following supplementation. The grazing area was limited by electric wires.

Supplementation took place at 7:00hr using the feeders placed in each lot, depending on the DM and most recent LW. The animals were withdrawn from the grazing areas between 10:00 and

16:00 h, which had been limited by electric wires, and included water *ad libitum* and artificial shade (80% sun radiation blocking net; 2.75 m high; 3.5 $m^2$/animal; east-west orientation).

**Sampling, fodder, and supplement measurements**

LW was recorded every 14 days. The available and residual fodder biomass was determined using the double sampling technique (Haydock and Shaw, 1975), and a three-point scale was used for marking, including two repetitions; the fodder scored 100 random points per lot. The samples in the scale were collected by cutting the biomass at soil level in a 0.3 x 0.3 square m. then they were placed in a forced-air circulation stove (60 ºC until a constant weight was achieved) to determine the dry weight and storing for further analysis.

The DM from the fodder was estimated according to the forage gone from the grazing lot (Macoon et al., 2003). Supplementation ingestion was measured daily as the difference between the amounts supplied and rejected. Samples from the supplement supplied and rejected were collected in each period and placed in a stove to determine the dry weight and preserve them for further analysis.

The grazing behavior was observed directly and recorded (%) every 20 minutes day-in (7:00-19:00 h), on a day corresponding to every transition period (end of the period) between diets, including the estimation of occurrence of grazing (effective and search), rumination, rest access to feeders with supplementation, and water consumption (Forbes, 1988). The bite rate was estimated as the number of bites per minute (Gregorini et al., 2007; Gregorini et al., 2009) in two moments: before lot changes (morning grazing), and the first grazing session after freeing the animals (afternoon grazing session).

**Parameters of ruminal fermentation and *in situ* DM and NDF ruminal degradability**

*In situ* DM and NDF ruminal degradability (DEG) of the fodder selected by the animal were determined, along with effective degradability (ED) considering a fixed weighing rate of 5% (Orskov and McDonald, 1979). The incubated fodder was selected by *hand clipping* simulating the fodder selected by the animals in the grazing areas (Coates and Penning, 2000). The samples were dried in an air-forced circulation stove at 60 °C for 48 h until a constant weight was reached, then they were milled to 1 mm using WILEY MIL equipment. Later, a single sample was made for drying in the stove at 105 °C for 24 h.

A dry fodder sample was placed in each previously labeled bag of the filter (1.5 mg/$cm^2$), then they were incubated by duplicate (2 bags/time) in the ventral sac of the rumen, simultaneously, at 7:00 h before supplementation (0 h) on day 11 (first day of measurement). Later, the bags were withdrawn from the rumen after 3, 6, 9, 12, 24, 48, and 72 h, and stored at -18 °C. After the experimental period, they were washed using conventional detergent and dried at 60 °C for 48 hours (until reaching constant weight). The loss of dry matter was calculated as the weight difference in the incubated bags and expressed as the proportion of the initial weight.

PH and initial ammonium were determined by collecting the ruminal fluid directly from the rumen' s ventral sac at 0, 3, 6, 9, 12, and 24 h on day 11 (time 0 corresponds to the moment

before supplementation). Upon removing the ruminal fluid, it was filtered using a cheese-making mesh to eliminate the remaining ruminal content, and the pH was determined with a digital pH meter (OAKLON). Then, the ammonium concentration was determined by diluting 40 ml of the fluid in 2 ml of pure sulfuric acid, and stored at -18 °C until additional analysis in the lab was performed (Bremmer, 1960).

The diet' s apparent digestibility (DM, OM, and NDF) was estimated using the concentration of ashes insoluble in acid (AIA) (Van Keulen and Young, 1977). Feces samples were collected daily, a sample/animal/day, at three different times: 7:00; 12:00; and 16:00 h, on days 12, 13, and 14, respectively. The samples were collected directly from the soil (fresh), avoiding contamination with forage or soil. Then they were stored at -18 °C. Finally, they were thawed at room temperature, mixed in a sample made up of animal and period, and dried in a stove (60 °C for seven days). The samples of ingested fodder were collected daily on days 11, 12, and 13 by *hand clipping* (Coates and Penning, 2000), simulating grazing in the area adjacent to each previously grazed lot.

The digestibility of DM (DMD), MO, and NDF were calculated using the following equations:

**DMD= [ 1- (FMC / SMC)] * 100**

where FMC: feed marker concentration, and SMC: stools marker concentration.

Nutrient digestibility or fraction (Di):

**Di=[100*(Y-X) + X*DMD]/y**

where X and Y are " i" concentrations in the feces and feeds, respectively.

## Chemical analysis

From the samples containing DDGS, supplied and incubated fodder (a sample/period), the DM (method 934.01), organic matter (OM, method 942.05), crude protein (CP; N × 6.25; method 984.13), and ethereal extract (EE, method 920.39), were determined, according to AOAC (1990) and AOAC (2007). The N content insoluble in acid detergent, NDF using α-amylase, and correcting by ash contamination (aNDFMO) and acid detergent fiber (ADFMO) was determined as described by Goering and Van Soest (1970). The feed samples in feces collected for estimating apparent digestibility were combined into a sample per animal and period to determine DM, OM, ADFMO, and ashes insoluble in acid (Van Keulen and Young, 1977). The DM and aNDF contents were determined from the samples from the incubation residues (a sample per animal per period and incubation time). The ruminal fluid samples were analyzed for ammonium concentration of (μgN-NH4/ml) (AOAC, 2015; method 984.13).

## Statistical analysis

The experiment was analyzed through SAS, 9.4 (SAS Institute, Cary, NC, 2012) linear models, using a crossover design with the animal as the experimental unit. To determine the effect of treatments on ingestion, bite rate, pH, ammonium, and digestibility, a linear model with repeated

measures was used through the MIXED procedure. The grazing behavior was analyzed by adjusting linear models with a binomial distribution through the GLIMMIX procedure.

The Orsksov and McDonald models were adjusted for degradability (1979):

$$Y_i = a + b\,(1-\exp^{(-kt)}) + e_i$$

where: " $Y_i$" is the missing fraction in " *t" hours;* " a" is the soluble fraction; " b" is the slowly degradable fraction; " k" is the degradation rate of " b" ; " $e_i$" is the experimental error.

# RESULTS AND DISCUSSION

Table 1 shows the effect of supplementation on dry matter ingestion and grazing behavior.

**Table 1. Effect of supplementation with distillers' grains on dry matter ingestion and grazing behavior**

| | Without supplementation | With supplementation | SE | P value |
|---|---|---|---|---|
| FDMI (kg/d) | 19.31 | 15.73 | 1.32 | 0.1145 |
| SDMI (kg/d) | - | 8.23 | 0.50 | - |
| TDMI (kg/d) | 19.31 | 23.96 | 1.22 | 0.0430 |
| Daily grazing (%) | 41.0 | 36.0 | 1.0 | 0.0218 |
| Daily rumination (%) | 27.0 | 26.0 | 3.0 | 0.8901 |
| Daily rest (%) | 27.0 | 29.0 | 3.0 | 0.5759 |
| Bite rate (bite/min) | 12.38 | 12.25 | 0.37 | 0.8182 |

**FDMI: Forage dry matter ingestion (kg/a/d); SDMI: Supplementation dry matter ingestion (kg/a/d); TDMI: Total dry matter ingestion (kg/a/d); Daily activity (7:00 - 19:00 h); SE: Standard error.**

As reported by MacDonald et al. (2007), Isla and Soto-Navarro (2011), and Larson et al. (2019) supplementation with DDGS did not affect the ingestion of dry matter from fodder, though it increased total dry matter ingestion. The supplemented animals grazed less than the rest, with no differences in rumination and rest, having the same bite rate ($P > 0.05$). It appeared that DDGS could meet the daily requirements of energy and metabolizable energy (Gregorini et al. (2009), Chilibroste et al. (2012, Marco and Aello (2001) with a lower energy consumption associated with less harvest activity.

Table 2 shows the chemical composition of the feeds supplied and incubated, as well as the resulting diet in each treatment, conforming to the experimental period average.

**Table 2. Chemical composition of forage sorghum (available and incubated), DDGS, and diet, during the experimental period.**

| Chemical composition (% dry base) | Fodder supplied | Fodder Incubated | DDGS | $Diet^{WTS}$ | $Diet^{WS}$ |
|---|---|---|---|---|---|
| Dry matter | 19.10 | 88.78 | 94.63 | 19.10 | 45.05 |
| Ashes (%) | 12.93 | 10.89 | 4.57 | 12.93 | 10.05 |
| Crude protein | 5.31 | 8.07 | 33.59 | 5.31 | 15.02 |
| Neutral detergent fiber | 63.78 | 64.71 | 61.45 | 63.78 | 62.98 |
| Acid detergent fiber | 34.74 | 32.83 | 26.09 | 34.74 | 31.77 |

| | | | | | |
|---|---|---|---|---|---|
| Crude energy (Mcal/kg) | 4.25 | _ | 5.25 | 4.25 | 4.59 |
| Ethereal extract | 2.21* | - | 6.25 | 2.21 | 3.60 |
| Fiber-adhered nitrogen | 0.45* | - | 13.68 | 0.45 | 4.99 |

**[I] Incubated fodder; [WTS] Without supplementation; [WS] With supplementation. The diet was estimated from the grazed fodder proportion and supplementation ingestion in the total diet.**
*** Values obtained from chart BCNRM (2016).**

The low protein level (5.31% CP) and high fiber level (63.78% NDF; 34.74% ADF) of forage sorghum associated with protein contribution (33.59% CP) and energy (5.25% SE) from DDGS could improve the ruminal environment. Because the rumen conditions vary depending on the environmental conditions of the rumen itself, and because of the characteristics of the diet (Newbold and Ramos-Morales, 2020).

Volatile fatty acids are the product of carbohydrate fermentation (Raposo et al., 2015), which influences the characteristics of the ruminal pH and, in turn, they change the ruminal environment and its dynamic (Chibisa et al., 2016).

## Ruminal pH and ammonium

DDGS has been used in enclosure diet, favoring optimum conditions for fermentation (Al-Suwaiegh et al., 2002) without affecting ruminal pH when it was used as a grazing supplement in winter (Islas and Soto-Navarro, 2011). However, in this study, the average ruminal pH was lower in the animals with supplementation (6.17 vs. 6.55; P = 0.030) (Table 3). Although the average lower, major unfavorable effects on the ruminal microflora were unlikely to occur due to the range of variation, since cellulolytic bacteria may have a serious impact when the pH drops below 6.0 (Church, 1988).

**Table 3 Effect of DDGS supplementation on ruminal pH and N_NH4 concentration in heifers grazing on forage sorghum**

| | pH | | N_NH4 *(µgN-NH₄/mL)* | |
|---|---|---|---|---|
| **Time** | **Without supplementation** | **With supplementation** | **Without supplementation** | **With supplementation** |
| **0 h** | 6.7 | 6.4 | 61.5 | 192.0 |
| **3 h** | 6.5 | 5.9 | 59.1 | 291.9 |
| **6 h** | 6.5 | 6.2 | 55.2 | 206.8 |
| **9 h** | 6.5 | 6.2 | 55.5 | 192.0 |
| **12 h** | - | - | 65.6 | 213.5 |
| **24 h** | - | - | 51.8 | 159.0 |
| **Average** | 6.55 | 6.17 | 58.1 | 209.2 |
| **SE** | 0.90 | | 6.75 | |
| **P value** | 0.0302 | | <0.0001 | |

**$N\_NH_4$: Ammonia nitrogen ppm (µgN-$NH_4$/mL); SE: Standard error.**
**Time 0 and 24: the animals were in the lot, corresponding to the moment before supplementation and entry to a different stripe (7:00 h). Time 3: the animals were in the lot, corresponding to the moment prior to daily enclosure. Time 6: the animals were in daily enclosure. Time 9: the animals were in the lot, corresponding to the moment after daily enclosure. Time 12: the animals were in the lot.**

Ruminal pH was affected by the sampling times (P = 0.003), but not by the interaction between the times and treatments (with vs without supplementation) (P = 0.164). There was a decrease in the pH at three hours (h3) of sampling compared to the time 0, but the pH is usually minimum between one and four hours after feed ingestion due to the acidification of the ruminal environment caused by the release of volatile fatty acids (Raposo et al., 2015). Then, at time 3 (h3), there was an increase in pH stability, possibly due to the balance between the acid production rate and the saliva buffer activity (Church, 1988). The concentration of ammonia nitrogen in the rumen was affected by supplementation (P= <0.0001), with higher levels of animals under supplementation (Table 3). Likewise, the interaction time x treatment was significant (P= 0.0137) with or without supplementation.

A study done by Pancini et al. (2021) showed that DDGS supplementation did not affect $N\_NH_4$ concentration. However, in this paper, though the ammonium concentration increased in the two treatments, the ammonia nitrogen was within the concentrations that needed ruminal bacteria (between 35 and 290 ppm), for normal performance (Church, 1988). The $N\_NH_4$ concentration increased after the first hours upon ingesting the supplementation, with a greater value three hours later. Possibly, excess in the contribution of degradable protein in the rumen for the synthesis of microbial protein in accordance with the availability of fermentable energy in the rumen would be consistent with an increase in the concentration of the ammonium observed.

### *In situ* apparent ruminal degradability and digestibility

Table 4 shows the effects of DDGS supplementation on *in situ* ruminal degradability of dry matter and NDF, as well as the apparent digestibility of DM, OM, and NDF of the diet in heifers grazing on forage sorghum.

**Table 4 Effects of supplementation on the degradability potential of dry matter and NDF, as well as diet digestibility**

| | ***Without supplementation*** | ***With supplementation*** | ***SE*** | ***P value*** |
|---|---|---|---|---|
| *DM_DE (%)* | 52.65 | 49.8 | 0.85 | 0.064 |
| *a+b (%)* | 78.17 | 81.01 | 2.229 | 0.409 |
| *a (%)* | 27.76 | 24.67 | 1.73 | 0.156 |
| *b (%)* | 50.41 | 56.34 | 3.05 | 0.394 |
| *k (h)* | 0.05 | 0.04 | 0.006 | 0.349 |
| *NDF_DE (%)* | 52.2 | 48.1 | 0.48 | 0.002 |
| *a+b (%)* | 76.9 | 88.92 | 7.44 | 0.305 |
| *a (%)* | 22.6 | 25.6 | 1.23 | 0.156 |
| *b (%)* | 54.3 | 63.4 | 6.91 | 0.394 |
| *k (h)* | 0.06 | 0.03 | 0.01 | 0.021 |
| *DM_DIG (%)* | 65.75 | 60.75 | 1.00 | 0.026 |
| *NDF_DIG (%)* | 68.5 | 62.5 | 1 | 0.011 |
| *ADF_DIG (%)* | 66.75 | 63.75 | 0.9 | 0.078 |

**DM_DE: Effective degradability of dry matter; NDF_DE: Effective degradability of neutral detergent fiber; *a+b*: potentially degradable fraction (%); *a*: soluble fraction (%); *b:* slowly degradable fraction (%); *k*: b degradation rate (%). DM_DIG: Organic matter digestibility (%); DM_DIG: Dry matter digestibility (%); NDF_DIG: Neutral detergent fiber digestibility (%); ADF-DIG: Acid detergent fiber digestibility (%).**

Supplementation did not affect the potentially degradable fraction, (*a* + *b*), soluble (*a*), and slowly DM and NDF degradable(*b*) (Table 4). Because the NDF degradation rate of (b) was slower in the animals without supplementation (6% vs. 3%). When estimating the effective degradability from a fixed passage rate (5%), greater degradability could be expected in the animals without supplementation than in the supplemented animals. This response is significant, considering that the feed degradability depends on the degradation speed of the slowly degradable fraction and the ruminal transit rate (Orskov and McDonald, 1979; Calsamiglia, 1997; NRC, 2000).

The DM effective degradability average in forage sorghum was 51.25% (52.65 vs 49.80 (whereas fiber degradability was 50.15% (52.20 vs 48.10). Vargas (2005) reported a lower average (48.90%) of DM degradability with the same transit rate. Meanwhile, in previous studies, Jiménez (1995) with a transit rate of 4% found values between 53.3% and 48.1% for DM degradability, and 40.0% and 33.2% for NDF degradability in animals fed different feedstuff levels.

The pH of normal concentrations, high ammonium concentration in the rumen, and higher degradation speed of the slowly degradable fraction could have led to a greater transit rate than the animals without supplementation (Orskov and McDonald, 1979; Raposo et al., 2015). Besides, a drop in apparent digestibility, however, could be explained in part by the greater consumption of total dry matter in the animals without supplementation through a rise in the feed transit rate (Galyean and Hubbert, 2014).

Even when supplementation reduced DM and NDF digestibility, the values were higher than the ones reported by Lagrange (2009), who found lower DM digestibility in the animals without supplementation than the animals that grazed on sorghum grains. Montossi et al. (2017) noted that for many years they found digestibility values between 49.9% and 61.4%, on diets containing different types of supplements (protein and energy) in animals grazing on forage sorghum.

The nutritional value of forage sorghum improves when compared to perennial grass in the summer (Ademosum et al., 1968). Even so, digestibility tends to be low, as described by Vargas (2005), in a study of 15 genotypes of bi-color forage sorghum found 57.83% DM digestibility on average. DM digestibility in this study was 65.7%, above the findings of Vargas (2005), because sorghum is considered to have brown central veins (BMR) with less lignification (McCuistion et al., 2005; Porter et al., 1978), offering greater digestibility than the sorghum lacking this gene. However, DM digestibility was lower than the ones reported by McCuistion et al. (2011) in two consecutive years on sorghum BMR (87.3%). Meanwhile, NDF digestibility (68.5%) was found within the values (55%-74%) reported by Porter *et al*. (1978) in different BMR genotypes. Both DM and NDF digestibility values found in this paper were consistent with the potential degradability values found *in situ.*

Moreover, concentrations greater than 8% of fat in the diet, might reduce digestibility and affect fiber degradability (Zinn, 1989; Hess et al., 2008). Because oil reduces the surface exposure to

the action of the microbial population, and due to the toxic effect, microbial growth and development are inhibited (Plascencia et al., 2003). Although DDGS contains high fat concentrations (6.25%), these concentrations would be diluted in the diet (3.60%), supplying (Ku Vera et al., 2014), a high energy density with low caloric increases, which might lead to higher efficiency.

## CONCLUSIONS

DDGS supplementation increased $N\_NH_4$ and reduced ruminal pH, as well as the effective fiber degradability and DM and NDF digestibility.

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## AUTHOR CONTRIBUTION STATEMENT

Research conception and design: DC, AS, VB, OB, EMT, MT; data analysis and interpretation: DC, AS, VB, OB; redaction of the manuscript: DC, AS, VB.

## CONFLICT OF INTEREST STATEMENT

The authors state there are no conflicts of interest whatsoever.