



Dried distiller's grains plus solubles supplementation improves low-quality tropical grass utilization on beef steers

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ABSTRACT: *This study was designed to evaluate the effect of corn dried distiller's grains (DDGS) supplementation on feed intake, total tract digestibility, and ruminal fermentation of beef steers fed low-quality Guinea grass (*Megathyrsus maximus*, cv. *Gatton panic*). Twelve Braford crossbred steers were housed in individual pens ($n = 4$ steers/treatment), provided with three levels of DDGS supplement: 0%, 0.6%, or 1.2% BW. Steers were blocked by live weight and randomly assigned to treatments within the block. Corn DDGS supplementation increased total OM intake (21.55, 40.23, and 56.69 g/kg BW^{0.75}) and tract OM digestibility (46.33, 49.03, and 72.39 % DM). Total tract digestible OM, CP, NDF and EE intake also increase in response to DDGS supplementation. Forage OM intake decreased when supplementation level reached 1.2 % BW. Also, ruminal pH decreased with DDGS supplementation level (6.88, 6.47, and 6.27). No differences were observed in total volatile fatty acids (VFA) concentration; however, the molar proportion of acetate decreased (77.98, 73.90, and 67.29 % Total VFA) as well as acetate: propionate ratio (4.38, 3.48, and 2.74). On the contrary, propionate proportions increased (18.32, 21.86, and 24.81 % Total VFA). Levels of ammonia and lactate were within suggested values for optimal fermentation and bacterial growth. Low-quality grass supplementation with corn DDGS increased total OM intake and digestibility. Also, DDGS inclusion favorably altered volatile fatty acids profile by reducing the acetate to propionate ratio regarding forage-only diets.*

Key words: *distiller's grains plus solubles, forage, beef cattle, intake, supplementation.*

Suplementação de grãos de destilação secos com solúveis melhora a utilização de grama tropical de baixa qualidade para novilhos de corte

RESUMO: *O objetivo deste estudo foi avaliar o efeito da suplementação com grãos de destilação secos de milho com solúveis (DDGS) no consumo, digestão e fermentação ruminal de novilhos de corte alimentados com capim-da-índia de baixa qualidade (*Megathyrsus maximus*, cv. *Gatton panic*). Doze novilhos mestiços Braford foram alojados em baias individuais ($n = 4$ novilhos / tratamento), fornecidos com três níveis de suplemento de DDGS: 0%, 0,6% ou 1,2% PV. Os novilhos foram bloqueados pelo peso vivo e atribuídos aleatoriamente aos tratamentos dentro do bloco. A suplementação com DDGS de milho aumentou o consumo de matéria orgânica total (21,55, 40,23 e 56,69 g / kg PV^{0,75}), a digestibilidade da matéria orgânica (46,33, 49,03 e 72,39% MS) e o consumo de todos os nutrientes digestíveis. O consumo de matéria orgânica da forragem diminuiu quando o nível de suplementação atingiu 1,2% PV. Além disso, o pH ruminal diminuiu com o nível de suplementação com DDGS (6,88, 6,47 e 6,27). Não foram observadas diferenças na concentração de ácidos graxos voláteis totais, no entanto, a proporção molar de acetato diminuiu (77,98, 73,90 e 67,29% de AGV total), bem como a relação acetato: propionato (4,38, 3,48 e 2,74). Pelo contrário, as proporções de propionato aumentaram (18,32, 21,86 e 24,81% Total de AGV). Os níveis de amônia e lactato estavam dentro dos valores sugeridos para fermentação ideal e crescimento bacteriano. A suplementação de gramíneas de baixa qualidade com DDGS de milho aumentou o consumo e a digestibilidade da MO total. Além disso, a inclusão de DDGS alterou favoravelmente o perfil de ácidos graxos voláteis, reduzindo a proporção de acetato para propionato em relação às dietas apenas com forragem.*

Palavras-chave: *grãos de destilaria mais solúveis, forragem, gado de corte, ingestão, suplementação.*

INTRODUCTION

Bioethanol co-products (e.g., dried distiller's grains plus solubles [DDGS]) to feed livestock had had a substantial expansion during the last decade in Argentina. However, the use of

bioethanol co-products has been mainly focused on high concentrate finishing diets rather than as protein and energy supplements to enhance animal performance in mid or low-quality forage-fed diets (MORRIS et al., 2005). In the northwest of Argentina, livestock is mainly fed with tropical pastures (i.e.,

Guinea grass; *Megathyrus maximums* cv. Gatton panic) under grazing conditions. More than 60% of forage production of these pastures is concentrated during the summer season, following by a winter season of null forage production and poor feeding value. In winter forages, the main limiting factors for animal production are low forage intake and low fiber digestion (VALENTE et al., 2011). Dried distillers' grains are high in protein, fat, and readily digestible fiber but low in starch (KLOPFENSTEIN et al., 2008). With this regard, DDGS might be a good source of supplementation to improve growing and reproduction performance in beef cattle for medium-low quality forages (DELCURTO et al., 2000; MORRIS et al., 2005; MARTÍNEZ-PÉREZ et al., 2013) by supplying digestible protein and energy (MACDONALD et al., 2007). However, the level of DDGS supplementation to optimize mid- or low-quality forage utilization is still not widely documented (ALAVA et al., 2019).

Thus, this study evaluated the effect of increasing levels of DDGS supplementation on total and forage intake, total tract digestibility, and ruminal fermentation parameters on beef steers fed low-quality Guinea grass (*Megathyrus maximus* cv. Gatton panic).

MATERIALS AND METHODS

Animals, housing and management

Twelve Braford crossbred steers (9 rumen-cannulated and 3 non-cannulated; 383 ± 100 kg BW) were used in a randomized block experimental design. Before initiation of the study, steers were blocked by BW and randomly assigned within the block to treatments and pens. Steers were allocated in individual pens (3 × 4 m).

Treatments and diet composition

Treatments consisted of Guinea grass (*Megathyrus maximus* cv. Gatton panic) hay with three levels of supplementation with DDGS: 0DDGS or not supplemented (R:C 100:1), 2) DDGS offered at 0.6% BW (0.6DDGS), and 3) DDGS offered at 1.2% BW (1.2DDGS), as-fed basis. Nutritional composition for hay and DDGS are shown in table 1. The steers were fed once daily (06:00 h) with free access to water and mineral mix. Dry distiller's grains plus solubles were offered once daily before feeding hay both in the same trough. Refusals of DDGS and hay were weighted and sampled individually. The experimental period lasted 23 d, and it was divided into 4 intervals: 1) 14 d for treatment adaptation, 2) 5 d for *ad libitum* intake, 3) 3 d for digestibility evaluation, 4) 1 d for ruminal fermentation profile.

Every morning refusals were collected, weighed, and recorded to estimate daily feed intake. Acid detergent insoluble ash (ADIA) was utilized as an internal marker and determined on the supplement, hay, orts, and fecal samples by combusting ADF residues in Ankom bags for 8 h at 450°C in a muffle furnace. Fecal production was estimated from ADIA values using procedures as described by COCHRAN & GALYEAN (1994) including corrections for orts composition. During 3 d (20 to 22 d of the experiment), fecal grab samples were collected every 6 h advancing the sampling time 3 h each day to represent a 3-h sampling interval for a whole period of 24 h, to minimize diurnal variation in marker excretion. Sampling procedure consisted of removing the cannula lid and collecting whole ruminal content samples with a gloved hand from four locations in the rumen: the ventral sac, the atrium, or reticulum, and two samples from the feed mat. Approximately 200 g of contents were collected

Table 1 - Chemical composition of Guinea grass (*Megathyrus maximus* cv. Gatton panic) hay and dried distiller's grains plus solubles (DDGS).

| Item | Hay* | DDGS |
|-------------------------------------|-------|-------|
| Dry matter (%) | 93.00 | 90.00 |
| Organic matter (% DM) | 87.84 | 97.36 |
| Crude protein (% DM) | 7.00 | 25.80 |
| Neutral detergent fiber (% DM) | 74.50 | 59.00 |
| Acid detergent fiber (% DM) | 45.00 | 19.10 |
| Ether extract (% DM) | 1.50 | 6.50 |
| Acid detergent insoluble ash (% DM) | 3.08 | 0.61 |

* Guinea grass (*Megathyrus maximus* cv. Gatton panic) hay.

from each location; contents were thoroughly mixed, squeezed, and filtered using two layers of cheesecloth, and subsamples were used for further processing and analyses. On day 23, ruminal liquor samples were taken from rumen-cannulated steers at 0, 3, 6, and 12 h after feeding for pH, VFA, N-NH₃, and lactate determinations. Ruminal fluid pH was determined using a portable pH meter (Orion Research, Boston, MA, USA) immediately following each collection. For VFA, 8 mL of ruminal liquor sample was diluted in 2 mL of metaphosphoric acid 25% (w/v). For N-NH₃ and lactate analysis, a 2 mL ruminal liquor sample was diluted in 8 mL hydrochloric acid (16 N). Samples of ruminal fluid were kept frozen until analysis.

Laboratory analyses

Hay, DDGS, Orts, as well as feces samples were dried at 60 °C in an air forced-air oven, then grounded through a Wiley mill (1-mm screen; TS3375E15, Thomas Scientific, Swedesboro, New Jersey, USA) for chemical analysis. Partially dried samples of feed, Orts, and feces were dried for 24 h at 105 °C for DM determination and then ashed for 3 h at 600 °C to determine organic matter (OM) and ash content (NRC, 2000). Crude protein [CP] was analyzed by Kjeldahl (AOAC, 1990), and neutral detergent fiber [NDF] and acid detergent fiber [ADF] were analyzed according to ROBERTSON & VAN SOEST (1981) as was described by (KOMAREK, 1993) in an Ankom 200 Fiber Analyzer (Ankom Technology Corp.). Then, ADF residue was ashed in a muffle furnace for 3 h at 600 °C to obtain ADIA. The ether extract was determined using the method described by PALMQUIST & JENKINS (2003). Ruminal liquor samples were centrifuged (30000 × g) for 10 min at 4 °C and stored in a freezer. Ruminal VFA were measured by a gas chromatographer (Konik HRGC-3000 C, Barcelona, Spain) equipped with a Zebtron ZB-FFAP Capillary GC Column (15 m × 0.32 mm i.d., 0.25 μm film thickness; Phenomenex, Inc. (Torrance, CA). The oven temperature was programmed at 100 °C, hold for 3 min, and increasing at 8 °C/min from 100 to 230 °C. The carrier gas was N at 1.2 mL/min. The split ratio was 30:1. The N-NH₃ concentration in the rumen fluid was determined by using the colorimetric procedure of BRODERICK & KANG (1980). Finally, ruminal lactic acid was quantified by using the colorimetric method proposed by BARKER & SUMMERSON (1941).

Feed intake was calculated by the difference between offered (kg) and refusal feed (kg). To calculate nutrient intake, feeds and refusals

were corrected by nutrient concentration. Total tract digestibility was estimated by using ADIA as an internal marker following the procedure described by COCHRAN & GALYEAN (1994).

Statistical analysis

Intake and digestion were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The pen was the experimental unit, and the BW block was used as the random effect. The model includes the fixed effects of the level of DDGS in diet (2 degrees of freedom) and the random effect of live weight (block; 3 degrees of freedom). The following model was fitted to the data set for all variables intake, digestibility, and digestible nutrients intake:

$$Y_{ij} = \mu + B_i + D_j + \varepsilon_{ij}$$

Where Y_{ij} is the response to diet, μ is the overall mean, B_i is the random effect of block i , D_j is the fixed effect of diet j and ε_{ij} is the experimental error (6 degrees of freedom).

The model for traits with repeated measures (pH, N-NH₃, lactate, acetate, propionate, butyrate, valerate, isovalerate, total VFA, and acetate: propionate ratio) was:

$$Y_{ijk} = \mu + B_i + D_j + t_k + (D_j \times t_k) + \varepsilon_{ijk}$$

Where Y_{ijk} is the dependent variable, μ is the overall mean, B_i is the random effect of block i , D_j is the fixed effect of diet j , t_k is the fixed effect of time k , $(D_j \times t_k)$ is the fixed effect of the interaction between treatment j and time k , and ε_{ijk} is experimental error (24 degrees of freedom).

Orthogonal polynomial contrasts were used to characterize the response to the level of DDGS. Multiple comparisons among means were performed using the LSD Fisher test ($P < 0.05$).

RESULTS

Intake

Total organic matter (TOMI), crude protein (CPI), neutral detergent fiber intake (NDFI), and ether extract intake (EEI) increased linearly in response to DDGS supplementation (Table 2; $P < 0.01$). On the contrary, forage organic matter intake (FOMI) decreased when the DDGS supplementation level reached 1.2 % BW ($P = 0.02$).

Digestibility and digestible nutrients intake.

Total tract OM digestibility (TTOMD; $P = 0.01$), total tract CP digestibility (TTCPD; $P = 0.04$), total tract NDF digestibility (TTNDFD; $P = 0.03$), and total tract EE digestibility (TTEED; $P < 0.01$; Table 2) increased linearly.

Table 2 - Effect of DDGS supplementation level on total OM intake (TOMI), forage OM intake (FOMI), CP intake (CPI), NDF intake (NDFI), EE intake (EEI), total tract digestible OM intake (TTDOMI), total tract digestible CP intake (TTDCPI) total tract NDF intake (TTDNDFI), total tract OM digestibility (TTOMD), total tract CP digestibility (TTCPD), total tract NDF digestibility (TTNDFD), and total tract EE digestibility (TTEED) in beef steers fed low-quality hay.

| Item | -----Treatments----- | | | SEM | -----P-value----- | |
|----------------------------------|----------------------|---------|---------|------|-------------------|------|
| | 0DDGS | 0.6DDGS | 1.2DDGS | | L | Q |
| TOMI, g/kg BW ^{0.75} | 21.55 | 40.23 | 56.69 | 2.77 | <0.01 | 0.75 |
| FOMI, g/kg BW ^{0.75} | 21.55 | 18.72 | 12.78 | 2.02 | 0.02 | 0.55 |
| CPI, g/kg BW ^{0.75} | 1.51 | 8.12 | 15.47 | 0.58 | <0.01 | 0.62 |
| NDFI, g/kg BW ^{0.75} | 16.49 | 39.26 | 66.54 | 3.03 | <0.01 | 0.56 |
| EEI, g/kg BW ^{0.75} | 0.10 | 1.66 | 3.32 | 0.09 | <0.01 | 0.42 |
| TTDOMI, g/kg BW ^{0.75} | 12.61 | 27.27 | 48.83 | 3.35 | <0.01 | 0.40 |
| TTDCPI, g/kg BW ^{0.75} | 0.91 | 4.61 | 10.51 | 1.09 | <0.01 | 0.44 |
| TTDNDFI, g/kg BW ^{0.75} | 6.54 | 15.64 | 26.86 | 3.08 | <0.01 | 0.77 |
| TTDEEI, g/kg BW ^{0.75} | 0.05 | 1.48 | 2.92 | 0.05 | <0.01 | 0.87 |
| TTOMD, % | 40.82 | 50.60 | 70.17 | 5.82 | 0.01 | 0.54 |
| TTCPD, % | 39.49 | 51.67 | 69.70 | 7.99 | 0.04 | 0.77 |
| TTNDFD, % | 27.56 | 40.62 | 57.18 | 7.39 | 0.03 | 0.85 |
| TTEED, % | 53.45 | 89.12 | 87.99 | 5.21 | <0.01 | 0.03 |

Treatments: DDGS supplementation: 0DDGS = non-supplemented, 0.6DDGS = DDGS offered at 0.6% BW, and 1.2DDGS = DDGS offered at 1.2% BW. n = 4 observations per treatment. SEM: standard error of media. DDGS: corn dried distiller's grains plus solubles. BW: body weight. OM: organic matter. CP: crude protein. NDF: neutral detergent fiber. EE: ether extract. Orthogonal polynomial contrast coefficients were used to determine linear (L) and quadratic (Q) effects of increasing concentrations of DDGS.

Total tract digestible OM intake (TTDOMI; $P < 0.01$), CP (TTDCPI), NDF (TTDNDFI), as well as ether extract (TTDEEI) intake increased linearly ($P < 0.01$) in response to DDGS supplementation (Table 2).

Ruminal fermentation profile

The interaction treatment \times sampling time was not significant ($P > 0.10$) for most ruminal parameters, except for ruminal pH ($P = 0.07$) and butyrate ($P = 0.03$). All ruminal variable means are reported in table 3. While ruminal pH and butyrate are shown in figures 1 and 2, respectively. Ruminal pH decreased linearly as the DDGS supplementation level increased ($P < 0.01$; table 3). In contrast, there were no effects on total VFA, $N-NH_3$, and lactate concentration in ruminal fluid among treatments. Nonetheless, molar proportions of propionate ($P < 0.01$; linear), butyrate ($P < 0.01$, Linear), isovalerate ($P < 0.01$, quadratic), and valerate ($P < 0.01$, quadratic) increased as increasing DDGS supplementation. While acetate molar proportion, as well as acetate to propionate ratio, decreased linearly ($P < 0.01$) in response to DDGS supplementation.

DISCUSSION

This study evaluated the effect of DDGS supplementation on feed intake, total tract digestibility, and ruminal fermentation profile of beef steers fed low-quality Guinea grass. With this regard, our results showed that DDGS supplementation at levels of 12 g/kg BW (23:77 forage: concentrate ratio) reduced forage intake, while total tract diet digestibility and digestible nutrients intake increased (*i.e.* OM, CP, fibers, and fat), improving the nutritional status compared to non-supplemented hay. Also, DDGS supplementation switched for a favorably molar proportion of propionate and decreased acetate to propionate ratio in the rumen, which could enhance energy utilization compared to forage-only diets. The reduction of forage intake matches with data reported in previous researches (LOY et al., 2007; MACDONALD et al., 2007) where they reported a decrease in forage intake because of DDGS supplementation. Besides, MORRIS et al. (2005) on weaned female calves evaluated increasing DDGS supplementation levels on low (Bromegrass hay; 53 % digestibility) or high-quality (alfalfa hay +

Table 3 - Effect of DDGS supplementation level on beef steers fed low-quality Guinea grass (*Megathyrsus maximus* cv. Gatton panic) hay on ruminal fermentation patterns.

| Item | -----Treatments----- | | | SEM | -----P-value----- | | | | |
|-----------------------------------------------------------|----------------------|---------|---------|------|-------------------|-------|-------|--------|-------|
| | 0DDGS | 0.6DDGS | 1.2DDGS | | T | H | T × H | L | Q |
| pH | 6.88 | 6.47 | 6.27 | 0.09 | <0.01 | <0.01 | 0.07 | <0.01 | 0.26 |
| N-NH ₃ (mM) | 11.61 | 7.13 | 9.31 | 1.76 | 0.14 | <0.01 | 0.74 | 0.30 | 0.09 |
| Lactate (mM) | 0.71 | 2.86 | 2.15 | 1.03 | 0.34 | 0.08 | 0.55 | 0.33 | 0.27 |
| Total VFA (mM) | 115.22 | 129.28 | 114.60 | 5.63 | 0.14 | 0.22 | 0.92 | 0.94 | 0.05 |
| -----Molar proportion (moles/100 moles of total VFA)----- | | | | | | | | | |
| Acetate | 77.98 | 73.90 | 67.29 | 0.90 | <0.01 | 0.05 | 0.10 | <0.01 | 0.26 |
| Propionate | 18.32 | 21.86 | 24.81 | 0.83 | <0.01 | 0.39 | 0.20 | <0.001 | 0.77 |
| Butyrate | 3.02 | 3.69 | 6.14 | 0.28 | <0.01 | <0.01 | 0.03 | <0.001 | <0.01 |
| Isovalerate | 0.41 | 0.27 | 0.88 | 0.08 | <0.01 | <0.01 | 0.26 | <0.001 | <0.01 |
| Valerate | 0.27 | 0.28 | 0.88 | 0.05 | <0.01 | 0.23 | 0.55 | <0.01 | <0.01 |
| A:P ¹⁾ | 4.38 | 3.48 | 2.74 | 0.17 | <0.01 | 0.44 | 0.15 | <0.01 | 0.69 |

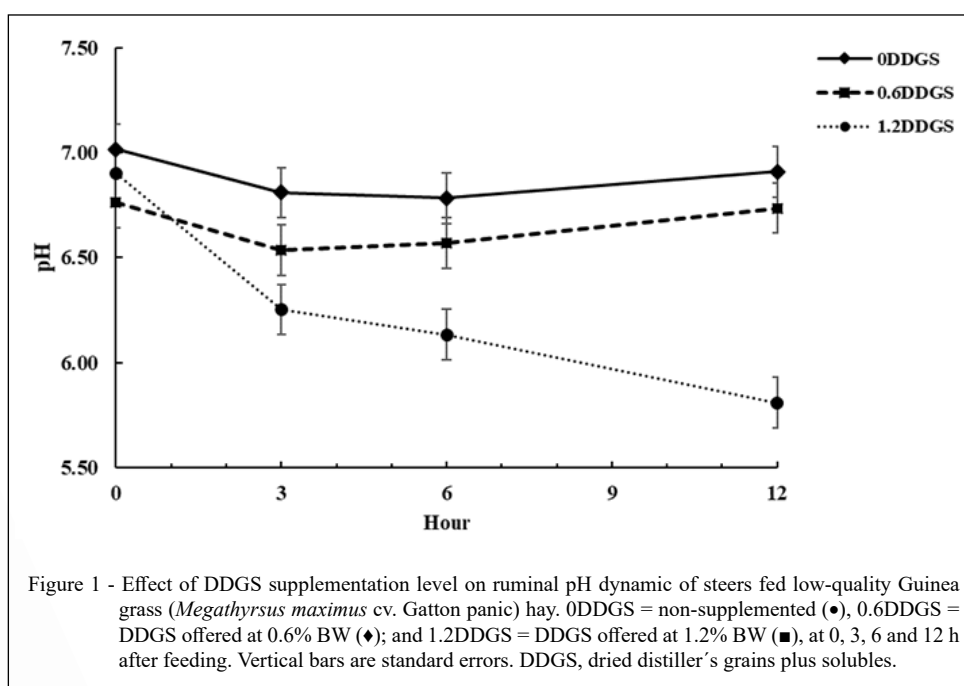
Treatment: DDGS supplementation: 0DDGS = non-supplemented, 0.6DDGS = DDGS offered at 0.6% BW, and 1.2DDGS = DDGS offered at 1.2% BW. n= 3 observations per treatment. SEM: Standard error of media. T: treatment. H: sampling moment. T x H: interaction between treatment and sampling moment. DDGS: corn dried distiller's grains plus solubles. N-NH₃: ammonia. VFA: volatile fatty acid.

Orthogonal polynomial contrast coefficients were used to determine linear (L) and quadratic (Q) effects of increasing concentrations of DDGS.

¹⁾ Acetate: Propionate ratio.

sorghum silage; 65 % digestibility) forages. In both treatments, these authors observed that forage intake decreased as DDGS increased, and hypothesize that this response is due to an “addition and substitution

effect”. In this sense, MACDONALD et al. (2007) and LOY et al. (2007) observed a decrease in forage intake with levels of DDGS supplementation higher than 4 g DM/kg BW. Although, in our study, no



differences in forage intake were observed between 0DDGS (100:0 forage to concentrate ratio) and 0.6DDGS (46:54 forage to concentrate ratio) but decreased when DDGS supplementation level raised to 12 g DM DDGS/ kg BW. Therefore, this discrepancy among this study compared with others could be due to differences in forage quality, particularly in CP content. Comparing forage intake (g OM/kg BW^{0.75}) in control with the highest level of inclusion was almost halved (from 21.55 to 12.78, respectively).

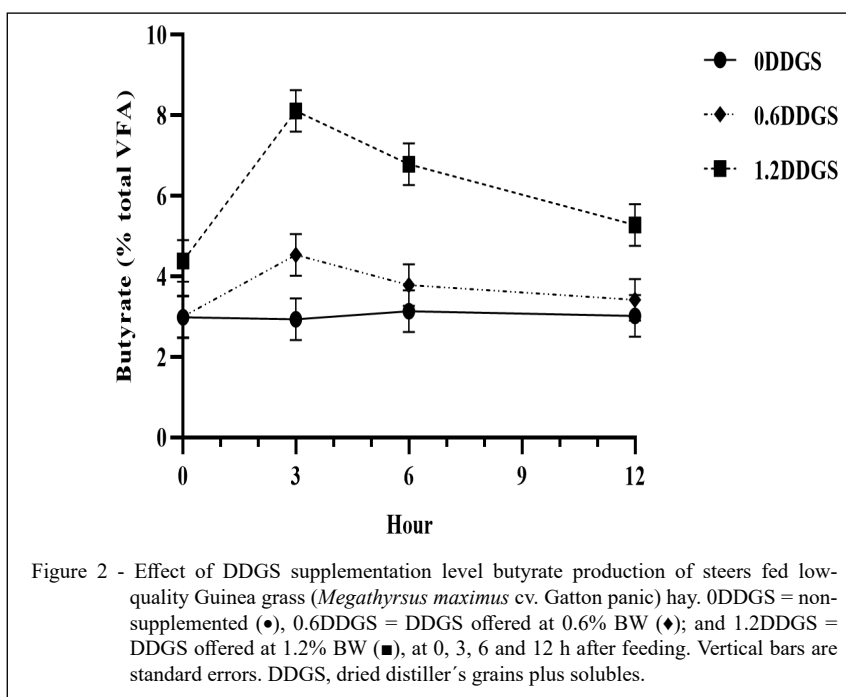
The increase in total OM intake in response to DDGS supplementation was also reported by other researchers (LOY et al., 2007; MURILLO et al., 2016; MCCANN et al., 2017). With this regards, previous research showed that increasing protein supplementation improves forage digestion and accelerates passage rate in low-quality forages (i.e., < 6 to 8% CP; BOHNERT et al., 2011). However, MARTÍNEZ-PÉREZ et al. (2013) did not find any improvement in DMI evaluating four DDGS supplementation levels (0, 0.2, 0.4, and 0.6 % BW) on calves grazing growing pastures, maybe due to forage was a medium-low quality (9.31% CP). Crude protein intake increased as DDGS supplementation level increased as was reported in other studies (MARTÍNEZ-PÉREZ et al., 2013; MURILLO et al., 2016; MCCANN et al., 2017) because of the higher content of CP of DDGS. Besides, NDF intake

increased with supplementation as was described by other works (MURILLO et al., 2016; MCCANN et al., 2017); although, some others (ISLAS & SOTO-NAVARRO, 2011; MARTÍNEZ-PÉREZ et al., 2013) did not find differences among treatments.

Also, TOMI and TTDOMI increased with DDGS supplementation, similar to the pattern of response observed in previous studies with low-quality forages (WINTERHOLLER et al., 2009; MCCANN et al., 2017).

Regarding TTOMD, as was seen by others (MARTÍNEZ-PÉREZ et al., 2013; MURILLO et al., 2016), increases with the level of DDGS in the diet. This observation could be supported by the high rate of forage substitution by DDGS enhancing total diet digestibility (MACDONALD et al., 2007). However, with mid to high-quality forage (> 7 % CP) as was reported in other trials (ISLAS & SOTO-NAVARRO, 2011; VAN DE KERCKHOVE et al., 2011), there were no differences among treatments in OMD. Total tract crude protein digestibility (TTCPD) increased with DDGS supplementation in agreement with other studies (VAN DE KERCKHOVE et al., 2011; MURILLO et al., 2016). VAN DE KERCKHOVE et al. (2011) observed responses to CP supplementation in forages with < 7 % CP, whereas its response decreases when CP concentration in forage is > 7 %.

Besides, there was an increase in TTNDFD with the level of DDGS in the diet, as well as was



previously described in other studies (ISLAS & SOTO-NAVARRO, 2011; MARTÍNEZ-PÉREZ et al., 2013; MURILLO et al., 2016). It can be expected an increase in the extent of NDF digestibility with DDGS supplementation due to its high proportion of rapidly digestible NDF (MARTÍNEZ-PÉREZ et al., 2013).

Conversely, the increase in EE digestibility with DDGS supplementation observed in our trial agrees with previous studies (ISLAS & SOTO-NAVARRO, 2011; MARTÍNEZ-PÉREZ et al., 2013). Concerning digestible nutrient intake, MCCANN et al. (2017) reported a linear increase in DOMI and DNDFI, as well as it was observed in this study, due to an increase in both intake and digestibility as DDGS increased in the diet.

Ruminal pH decreased in response to DDGS supplementation alike to results obtained in previous studies (LOY et al., 2007; ALAVA et al., 2019), and it was more highlighted over sampling time for the highest level of supplementation (1.2DDGS). DIJKSTRA et al. (2012) explain this response by suggesting that a reduction in ruminal pH is associated with decreased acetate to propionate ratio. While ruminal pH for 0DDGS and 6DDGS ranged within the normal values for a suitable performance of cellulolytic bacteria (MOULD et al., 1983). The lowest pH value (5.8 at 12 h; data not shown) recorded for 1.2DDGS was below the threshold (6.0) to limiting fiber digestion (CALSAMIGLIA et al., 2008). However, it was not reflected on total tract NDFD, perhaps because ruminal pH was below the threshold only after 12 h from feeding. Whereas in prior sampling times (0, 3, and 6 h) it was above the value for limiting fiber digestion. In this sense, MOURIÑO et al. (2001) –*in vitro* studies– suggested that once bacteria-feed particle association is established (covered by glycocalyx) makes stable the cellulolytic activity even when pH falls below 6. In this experiment, DDGS supplementation did not affect ruminal N-NH₃; because N-NH₃ ruminal concentrations were already high for all treatments. Besides, all treatments had values above the minimal suggested values for optimal fermentation and bacterial growth (3.57 mM; SATTER & SLYTER, 1974). Low RDP proportion in DDGS and hay might limit ruminal ammonium availability, which supports the small differences among treatments (KLEINSCHMIT et al., 2006). Additionally, highly fermentable carbohydrates – such as digestible fiber in DDGS – stimulate N capture by ruminal microbial (NOVIANDI et al., 2014), and it could explain similar values of ruminal ammonium among treatments.

Ruminal lactate was low and similar among treatments, as was observed in other studies (SCHOONMAKER et al., 2010). All treatments reached values within the normal lactate range (1-20 mM; MØLLER, 1969). Since most starch is removed during ethanol production, it was expected a decrease in lactate concentrations in steers fed diets with DDGS. In addition, some lactate possibly was metabolized to propionate (UWITUZE et al., 2010), which could also explain the greater propionate concentrations relative to acetate concentrations resulted in the reduced acetate: propionate ratio.

Total VFA concentration was similar among treatments, in agreement with prior research (ISLAS & SOTO-NAVARRO, 2011; MARTÍNEZ-PÉREZ et al., 2013). However, previous data are conflicting with some authors (SCHOONMAKER et al., 2010) where they observed a linear decrease as DDGS supplementation increased while others (LOY et al., 2007; MCCANN et al., 2017) reported an increase in total VFA with DDGS supplementation.

Similar to other researches (MURILLO et al., 2016; MCCANN et al., 2017), acetate molar proportion decreased to increasing DDGS, and conversely, propionate increased. There was a linear increment in butyrate proportions as same as was reported by previous studies (MCCANN et al., 2017; WANG et al., 2020). On the contrary, others did not find effects (ISLAS & SOTO-NAVARRO, 2011; MARTINEZ-PEREZ et al., 2013; MURILLO et al., 2016). Acetate: propionate ratio linearly decreased as DDGS supplementation increase, similar responses were observed in previous reports (MARTÍNEZ-PÉREZ et al., 2013; MCCANN et al., 2017). This response could be due to lipid hydrolysis in the rumen produces glycerol, which is readily metabolized by ruminal bacteria to propionic acid (JENKINS et al., 1993; ARRIGONI et al., 2016). In contrast, ISLAS & SOTO-NAVARRO (2011) did not observe differences in acetate to propionate ratio. It is important to highlight that in our study forage:concentrate ratio in the diet shifted from 100:0 to 23:77 for 0DDGS and 1.2DDGS, respectively. In this sense, it is reasonable to expect that as the proportion of concentrate in the diet increases, acetate molar proportion decreases while propionate increases (Angle et al., 2010; MCCANN et al., 2017). Conversely, variables like TOMI, TTOMD as was observed in other researches (MURILLO et al., 2016; MCCANN et al., 2017) increased with higher proportions of concentrate in the diet. Although, supplementation with starch decreases organic matter digestibility (BOWMAN & SANSON, 1996), DDGS are low in starch (< 7 %) because it is removed from

corn grain for ethanol production (KLOPFENSTEIN et al., 2008). Thus, energy input is supplied mainly by fat and readily digestible fiber. It is reasonable that TTOMD digestion increase with DDGS supplementation due to both additional protein and energy supply.

In conclusion, readily digestible fiber and crude protein supply throughout DDGS supplementation improved TTOMI, TTOMD, rumen fermentation parameters. Also, a higher propionic acid proportion and lesser acetate: propionate ratio might enhance the energy efficiency of the diet compared with forage-only diets. Because of these, we can infer that DDGS is a suitable alternative of supplementation in beef steers fed low-quality forages.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The experimental animals were managed according to the institutional protocols approved by the Instituto Nacional de Tecnología Agropecuaria (INTA, National Institute of Agricultural Technology) for Experimental Animal Care and Use (INTA, 2013).

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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