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Dried distillers grains in supplements for pasture-fed cattle

Grãos secos de destilaria em suplementos para bovinos a pasto

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Abstract: The objective was to evaluate the inclusion of increasing levels of DDG on nutrient intake and digestibility and ruminal fermentation in cattle kept on pasture. Five castrated male steers, fitted with a ruminal cannula, weighing 450±50kg, 18 months old, kept on Marandu grass pasture were used; and distributed in a 5x5 Latin square. DDG was included in the supplements in the following proportions: 0, 100, 150, 200 and 300 g/kg DM. The data were analyzed and subjected to analysis of variance, at a significance level of 5%, being evaluated by simple polynomial regression. The total availability of pasture and green matter presented averages of 2.0 Ton/ha and 1.3 Ton/ha, respectively, allowing selectivity by animals. The consumption of pasture (P=0.032), MS (P=0.041), MO (P=0.022), and PB (P=0.035) showed quadratic behavior with the inclusion of DDG in the supplements, where the highest consumption for both corresponded to the supplements with 100 and 200g/kg inclusion. The digestibility of NDF (P=0.001) and OM (P=0.046) also suffered a quadratic effect (P<0.05), noting that at levels above 200g/kg it can be reduced. N-consumed also suffered a quadratic effect (P=0.032) with an increase in the inclusion of 100, 200 and 300g/kg of DDG, being linked to the protein fraction of DDG. The levels of Isovalerate (P=0.0001), AGCR (P=0.004) and CH4 production (P=0.022) decreased linearly, indicating a decrease in the energy levels of the animals. Therefore, levels between 150 and 200g/kg of DDG are recommended in supplements for cattle kept on pasture.

Keywords: DDG; microbial synthesis; ethanol co-product.

Resumo: Objetivou-se avaliar a inclusão de níveis crescentes de DDG sobre consumo e digestibilidade de nutrientes e fermentação ruminal em bovinos mantidos a pasto. Foram utilizados 5 novilhos, machos, castrados, providos de cânula ruminal pesando 450±50kg, com 18 meses de idade, mantidos em pasto de capim Marandu; e distribuídos em quadrado latino 5x5. O DDG foi incluído nos suplementos nas seguintes proporções 0, 100, 150, 200 e 300 g/kg de MS. Os dados foram analisados e submetidos à análise de variância, ao nível de significância de 5%, sendo avaliados por regressão polinominal simples. A disponibilidade total de pasto e de matéria verde apresentaram médias de 2,0 Ton/ha e 1,3 Ton/ha, respectivamente, permitindo a seletividade pelos animais. O consumo de pasto (P=0,032), MS (P=0,041), MO (P=0,022), e PB (P=0,035) apresentaram comportamento quadrático com a inclusão do

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DDG nos suplementos, onde os maiores consumos para ambos correspondeu aos suplementos com 100 e 200g/kg de inclusão. A digestibilidade da FDN (P=0,001) e MO (P=0,046) também sofreu efeito quadrático (P<0,05), constatando que em níveis acima de 200g/kg pode ser diminuída. O N-consumido também sofreu efeito quadrático (P=0,032) com aumento na inclusão de 100, 200 e 300g/kg de DDG, estando ligado a fração proteica do DDG. Os níveis de Isovalerato (P=0,0001), AGCR (P=0,004) e a produção de CH4 (P=0,022) diminuíram linearmente, indicando decréscimo nas perdas energéticas pelos animais. Com isso, recomenda-se níveis entre 150 e 200g/kg de DDG em suplementos para bovinos mantidos a pasto.

Palavras-chave: DDG; síntese microbiana; coproduto do etanol.

1. Introduction

Grazing supplementation strengthens forage intake to meet animals' nutritional requirements, providing beneficial additives to the rumen environment that increase the use of the food consumed and animal production. In animal production, 70% of costs come from nutrition, justifying the increasing search for alternatives to reduce these costs ([1]).

Brazil has studied the use of dried distillers grains (DDG), a dietary alternative, as a supplement for grazing livestock. DDG is a co-product of ethanol production from grains (corn and sorghum) and has high nutritional value ([2]). Corn is the most widely used grain in this process and is the raw material for obtaining DDG ([3]).

Notably, DDG has been used frequently by livestock farmers in the United States, Paraguay, and Argentina, and is available in international and national markets owing to a huge demand for corn ethanol in Brazil ([4]). The nutritional composition of DDG varies greatly based on the type of grain, fermentation efficiency, and differences in practical ethanol production protocols used by suppliers, which is also a relevant reason to study the dietary potential of this co-product and analyze samples from different suppliers to achieve consistent DDG quality ([5]). Different types of DDG are obtained from grain processing, with fermentation and distillation residue resulting in oil, solubles, and wet distillers grains (WDG). The WDG with solubles (WDGS) is created by incorporating solubles into WDG. Moreover, WDG and WDGS can be dried to produce DDG and DDG with solubles (DDGS) ([6]).

Hernández et al. ([7]) used corn DDGS to supplement grazing steers to reduce the impact of low forage quality and reported that DDGS is a suitable supplementation alternative because it improved rumen fermentation parameters. In the same study, the authors reported that the inclusion of 12 g/kg of DDGS in the supplement reduced forage consumption and increased digestibility and digestible nutrient intake.

Moreover, DDG is rich in bypass protein (protein not degraded in the rumen) and is useful in increasing weight, particularly in young animals with higher protein requirements ([2]). In addition, its effect on nutrient physiology, metabolism, and digestibility in ruminants remains poorly understood, with most studies focusing on monogastric animals.

Although DDG has many advantages in cattle farming, most studies analyzing confinement production have been conducted in other countries, with few studies focusing on grazing

animals. In this study, we aimed to assess the effects of the inclusion of increasing DDG levels in the diet of grazing cattle on consumption, digestibility, rumen fermentation, and nitrogen (N) balance.

2. Material and methods

The current experiment agrees with the principles established by the Ethics Committee from Federal University of Grande Dourados (approval protocol: (023/2015 CEUA / UFGD). This study was performed between October 2020 and January 2021 at the Ruminant Nutrition facility and Animal Nutrition Laboratory from the School of Agrarian Sciences of Federal University of Grande Dourados, Dourados, Brazil. The analyzes were carried out in the Animal Nutrition laboratory of the Faculty of Agricultural Sciences and in the Oilseed Co-products Assessment Laboratory, of the Research Center for Agroenergy and Environmental Conservation (LAPAC/FINEP), of the Federal University of Grande Dourados (UFGD). UFGD is located in the city of Dourados in the State of Mato Grosso Do Sul-MS, with a geographic location located at coordinates 22°11'43.49" south latitude and 54°55'77" west latitude.

2.1 Location, animals and treatments

Five cannulated crossbreed steers were used, with an average age of 18 months and 450±50kg. The animals were randomly distributed in a Latin square design (5x5). In each experimental period, the supplements were rotated between the animals, causing them to consume all the different treatments. The five experimental periods lasted 18 days, of which 8 days were spent adapting the animals and 10 days were spent collecting data. The animals were distributed in five individual paddocks of approximately 0.2 hectares, equipped with a trough, drinking fountain and pasture Urochloa brizantha, cv. Marandu (Syn Brachiaria).

The supplements used were made up of corn, soybean meal, urea and mineral core, and formulated according to NRC (8) to contain 18% CP (Table 2). The DDG (Dry distilled grains) used was the one without solubles, it was inserted in the following proportions: 0, 100, 150, 200 and 300 g/kg DM. The amount of supplement provided daily was 1% of the animals' body weight, once a day, in the morning (08:00). The chemical composition of the ingredients used in the supplements is described in Table 1. The proportion of ingredients and chemical chemical composition of the supplements are shown in Table 2.

Table 1 Bromatological composition of DDG, corn and soybean meal used in the supplement provided to animals

Item	DDG	Corn	Soybean meal
DM%	86.19	88.23	89.09
CP%	45.19	9.46	47.74
NDF%	46.72	30.88	22.14
ADF%	27.68	5.85	10.50

Ash%	2.23	1.13	6.62
*TDN%	64.3	70.91	74.56

*DM= Dry matter. CP= Crude protein (% of DM). NDF= Neutral detergent fiber (% of DM); ADF= acid detergent fiber (% of DM). TDN= total digestible nutrients (% of DM). DDG= distillers dry grains. *%TDN = 83.79 – 0.4171*NDF, Capelle et al., [9].*

Table 2 Proportion (%) and bromatological chemical composition of experimental supplements (MS basis) containing increasing levels of DDG and proportion of ingredients

	DDG inclusion levels (g/kg)				
Ingredient	0	100	150	200	300
Corn	79.90	79.40	75.90	72.90	64.80
Soybean meal	13.0	3.50	2.00	1.00	0.00
DDG	0.00	10.00	15.00	20.00	30.00
Urea controlled release	3.00	3.00	3.00	2.00	1.00
Mineral mix	4.1	4.1	4.1	4.1	4.2
DM%	82.08	81.79	81.68	82.45	83.03
CP%	20.66	20.60	21.81	21.01	21.99
NDF%	27.55	29.97	30.89	32.08	34.03
ADF%	6.04	7.78	8.80	9.91	12.09
*TDN%	66.35	65.34	64.96	65.30	65.24

*DM= Dry matter. CP= Crude protein (% of DM). NDF= Neutral detergent fiber (% of DM); ADF= acid detergent fiber (% of DM). TDN= total digestible nutrients. *%TDN = 83.79 – 0.4171*NDF, Capelle et al., [9].*

2.2 Pasture availability and chemical composition

The available pasture was determined at the first day of each period by cutting forage at ground level from 10 areas (0.25 m^2) in each paddock. Samples were individually weighed and composited in one sample from each paddock. Then, the composited samples were analyzed for morphological characteristics (Table 3). The ingested forage (extrusa) collection was performed on day 21 of each period after ruminal evacuation as described by Dubbs et al. (2003). Before the extrusa collection, animals were submitted to 12 h fasting to ensure total forage intake, then animals were allocated in the paddocks. After 30 min grazing, rumen was evacuated and a subsample of digesta (400 g) was frozen for chemical composition analysis.

Samples of extrusa and concentrate were analyzed for DM (method 930.15), CP (N × 6.25; method 984.13) and ether extract (EE; method 920.39) according to AOAC (2000). Ash was determined after 4 h at 600 °C in a muffle furnace. Neutral detergent fiber and ADF were determined in a fiber analyzer (TE-149 fiber analyzer, Tecnal Equipment for Laboratory Inc., Piracicaba, Brazil) using alpha amylase and no sodium sulfite (Van Soest et al., 1991). Potentially digestible DM ($_{pd}$DM) was estimated according to (Paulino et al., 2006). Samples of extrusa and concentrate were ground (2-mm particle size), placed in non-woven textile bags (5 × 5 cm, 20 mg DM/cm^2) and incubated in the rumen of two crossbred steers adapted to a similar diet of this experiment. After 288h, bags were removed from the rumen and washed in running tap water and analyzed for NDF concentration (Casali et al., 2008), in order to further estimate nutrient total apparent digestibility.

2.3 Nutrient intake and total apparent digestibility

Dry matter intake was estimated based on the total DM fecal excretion, the $_{i}$NDF content of feces, pasture and concentrate. To determine daily DM fecal excretion, titanium dioxide (TiO_2) was supplied from day 11 to day 20 of each period (10 g/d at 08h00), in which the first 5 days were allowed to stabilize TiO_2 fecal excretion (Ferreira et al., 2009a). Fecal samples (200 g) were collected directly from rectum of each animal from day 16 to day 20 of each experimental period in different times (08h00, 10h00, 12h00, 14h00 and 16h00) and composited (wet-basis) to form a sample for each animal within period and frozen for posterior chemical analyses (Ferreira et al., 2009b). The fecal titanium dioxide content was determined by a colorimetric method and reading performed by spectrophotometer (Myers et al., 2004).Fecal samples were analyzed for DM, CP, NDF, EE and $_{i}$NDF as previously described to determine the apparent total digestibility of each nutrient. Nutrient digestibility was estimated based on the nutrient intake (kg/d) and its excretion in feces (kg/d).

2.4 Ruminal fermentation

Ruminal digesta samples (from five sites from rumen) were collected on day 21 of each experimental period immediately after concentrate was supplied (time 0) and 2, 4, 6 and 8 h relative to supplementation. Ruminal digesta samples were composited and strained in four layers cheesecloth, and ruminal juice pH was measured using a potenciometer (PH 1500, Instrutherm, Sao Paulo, Brazil). Aliquots of 1600 µL of these samples were mixed with 400 µL methanoic acid (98-100% H_2CO_2), being centrifuged at 7,000 × g for 15 min at 4°C, and the supernatant of each sample was frozen for posterior short-chain fatty acid (SCFA) analysis. Additionally, aliquots of 2 mL were mixed with 1 mL of sulfuric acid (0.5 Mol/L H_2SO_4) and stored at -20°C for subsequent analysis of ammonia nitrogen by the colorimetric phenol-hypochlorite method (Broderick and Kang, 1980).

Ruminal SCFA concentrations were measured using a gas chromatograph (model GC-2104, Shimadzu, Tokyo, Japan) according to the method described by Erwin et al. (1961) and adapted by Getachew et al. (2002). The gas chromatograph was equipped with a split injector and dual flame ionization detector temperature at 250ºC and with a capillary column (Stabilwax, Restek, Bellefonte, PA, USA) at 145ºC. The gases used in the analyses were helium as the carrier gas (8.01 mL/min flow), hydrogen as the fuel gas (pressure of 60 kPa), and synthetic air as the oxidiser gas (pressure of 40 kPa). An external standard was prepared with acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids (Chem Service, Inc., West Chester, PA, USA). The software GCSolution (Shimadzu) was used for calculation of SCFA concentrations. The estimate of methane production (mM/L) was carried out according to the formula proposed by Moss et al. (13), CH4 = 0.45 (C2) – 0.275 (C3) + 0.4 (C4); with C2 being the concentration of acetic acid, C3 the concentration of propionic acid and C4 the concentration of butyric acid.

2.5 Microbial protein synthesis

Urine samples (100 mL) were collected by preputial stimulation on day 14 of each experimental period four hours relative to the supplementation (Chizzotti et al., 2006). Aliquots

(10 mL) of urine were diluted into sulphuric acid (40 mL, 0.036 N) to avoid purine derivatives degradation and uric acid precipitation, and then were analyzed to creatinine, urea, uric acid and allantoin as described by Valadares et al. (1999). The leftover of urine samples were frozen to determine total nitrogen. Allantoin in urine was assessed according to Fujihara et al. (1987), creatinine and uric acid were determined by colorimetric methods using commercial kits (Labtest, Lagoa Santa, Brazil; Gold Analisa Diagnostica Ltda, Belo Horizonte, Brazil), and readings were performed using a semi-automatic biochemical analyzer (BIO-200, Bioplus, Barueri, Brazil). The total excretion of purine derivatives (PD) was calculated as the sum of allantoin and uric acid amounts excreted in urine. The absorbed microbial purines (P_{abs}) were calculated using the equation of Verbic et al. (1990). The daily urinary excretion volume was determined according to Rennó et al. (2000).

2.6 Determination of urea and creatinine levels

On day 14 of each experimental period, blood samples (20 mL) were collected by puncture of coccygeal vessels after 4 hours of supplementation. Then, samples were centrifuged 2,700 × *g* for 20 min (4ºC), serum supernatant was collected and analyzed for urea and creatinine by colorimetric method using commercial kits (Gold Analisa Diagnostica Ltda) and readings performed by a semi-automatic biochemical analyzer (BIO-200, Bioplus). The plasmatic depuration or clearance of creatinine and urea were obtained by the ratio between the urinary excretion during 24 hours and the plasmatic concentration of each substance. The excretion fraction of urea was determined by the relation between the depurations of plasmatic urea and creatinine as described by Gandra et al. (2016b).

2.7 Analysis for bromatological composition

Samples of feces, supplements, extrude (forage obtained by ruminal emptying) and pasture were evaluated for dry matter (DM; # 934.01), crude protein (CP) content obtained by determining total nitrogen (N) using the micro technique. Kjeldahl (#920.87, Nx6.25); ash (CZ; #924.05; AOAC, 1990); and organic matter (100-CZ). Acid detergent fiber (ADF) was determined as described by Van Soest and Robertson (19). Neutral detergent fiber (NDF) analyzes were carried out according to Mertens (20) with some adaptations, where the autoclave apparatus was used without the use of sodium sulfite. Cellulose analysis was carried out according to Klason apud CRUZ et al. (21), which uses determination by acid hydrolysis (72% sulfuric acid). Based on the cellulose value, the calculation was carried out to obtain lignin from the pasture and extrusion. The forage TDN content was calculated based on the NDF content, according to the equation proposed by Capelle et al. (9): %TDN = 83.79 - 0.4171*NDF.

2.8 Statistical analysis

The data obtained were analyzed using SAS (Version 9.2. SAS Institute, Cary, NC 2009), where the normality of the residuals and the homogeneity of the variances were verified using the PROC UNIVARIATE command.

For the purposes of evaluating inclusion levels, the following model was adopted:

$$Yijl = \mu + Ai + Pj + Dl + erijl;$$

Where Yijl = dependent variable, μ = overall average, Ai = animal effect (i = 1 to 5), Pj = period effect (j = 1 to 5), Dl = DDG level effect (l = 1 to 5); and eijl = experimental error.

The random effect of the model (random) was characterized by: Ai and Pj. The degrees of freedom were corrected by DDFM= kr. The data obtained was subjected to analysis of variance using the PROC MIXED command of SAS, version 9.0, adopting a significance level of 5%, being evaluated by simple polynomial regression using PROC REG of SA, adopting a significance level of 5. %.

3. Results

The mean total pasture feed availability and mean total green matter availability were 2.0 and 1.3 t/ha, respectively. Crude protein (CP) and neutral detergent fiber (NDF) contents were 4.3 and 77,6%, respectively. The mean ratio of total digestible nutrients (TDN) and CP (TDN:CP) was 12.0 (Table 3).

Table 3 Availability of total dry matter and green matter, morphological characteristics and bromatological composition of *Urochloa brizantha*, syn. *Brachiaria brizantha cv. Marandu*

	DDG inclusion levels (g/kg)					
	0	100	150	200	300	Average
Total DM (t/ha)	2.07	2.14	2.35	1.88	1.60	2.0
Total green DM (t/ha)	1.18	1.27	1.51	1.32	1.03	1.3
Height (cm)	29.2	28.26	35.00	32.38	32.16	31.4
Culm %	19.0	14.8	18.6	19.7	20.0	18.4
Sheet %	43.21	47.03	46.20	49.32	46.24	46.4
Senescent material %	37.78	38.21	35.19	31.00	33.73	35.2
DM%	39.06	36.86	39.34	34.57	36.28	37.2
OM%	91.66	91.74	91.61	91.40	91.10	91.5
CP%	4.60	4.08	4.02	4.64	4.26	4.3
NDF %	78.73	76.69	77.39	77.69	77.29	77.6
ADF %	43.04	40.37	41.84	41.00	41.85	41.6
Lignin %	10.94	9.11	10.20	11.69	9.68	10.3
Ash %	8.34	8.26	8.39	8.60	8.90	8.5
*TDN%	50.95	51.80	51.51	51.39	51.55	51.4
TDN:CP	11.08	12.71	12.81	11.07	12.11	12.0

*DDG0 = Dried distillers grains with 0g/kg inclusion in the supplementation; DDG100 = Dried distillers grains with 100 g/kg inclusion in the supplementation; DDG150 = Dried distillers grains with 150 g/kg inclusion in the supplementation; DDG200 = Dried distillers grains with 200 g/kg inclusion in the supplementation; DDG300 = Dried distillers grains with 300 g/kg inclusion in the supplementation. DM = dry matter, MO = organic matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, TDN = total digestible nutrients. *%NDT = 83.79 – 0.4171*FDN, Capelle et al. (9).*

Notably, the dietary inclusion of DDG exhibited a quadratic effect on the intake of pasture feed (P = 0.032), dry matter (DM) (P = 0.041), organic matter (OM) (P = 0.022), and CP (P = 0.035) and on NDF (P = 0.001) and OM (P = 0.046) digestibility (Table 4).

Table 4 Average daily intake and nutrient digestibility of pasture-raised cattle supplemented with increasing levels of DDG

	DDG inclusion levels (g/kg)					SEM	P-Value		
	0	100	150	200	300		Treat	Linear	Quad
Intake (kg/dia)									
Pasture[A]	15.81	16.68	13.95	18.82	14.65	0.585	0.030	0.214	0.032
Supplement	4.10	4.08	4.09	4.12	4.15	0.003	0.958	0.899	0.902
Dry matter[B]	19.92	20.77	18.03	22.94	18.80	0.585	0.029	0.541	0.041
Organic matter[C]	17.26	18.30	16.05	19.65	15.81	0.522	0.042	0.654	0.022
Crude proteinD	2.60	3.21	2.43	3.09	2.40	0.111	0.041	0.577	0.035
NDF	11.11	12.70	11.22	13.29	11.41	0.420	0.173	0.624	0.239
Digestibility (g/kg)									
Dry matter	488.90	492.40	510.62	521.91	471.21	9.054	0.343	0.647	0.161
Organic matter[E]	551.92	557.56	576.21	602.74	496.71	13.121	0.044	0.547	0.046
Crude protein	450.82	507.62	454.22	464.52	462.45	17.503	0.557	0.657	0.778
NDF[F]	495.31	533.81	577.12	550.23	515.28	12.256	0.015	0.296	0.001

DDG0 = Dried distillers grains with 0g/kg inclusion in the supplementation; DDG100 = Dried distillers grains with 100 g/kg inclusion in the supplementation; DDG150 = Dried distillers grains with 150 g/kg inclusion in the supplementation; DDG200 = Dried distillers grains with 200 g/kg inclusion in the supplementation; DDG300 = Dried distillers grains with 300 g/kg inclusion in the supplementation. NDF= neutral detergent fiber. Treat = treatment effect; Quad= quadratic effect; SEM = Standard error of the mean. $^{A}Y = 15.596 + 0.1289X - 0.00475X^2$; $r^2 = 0.69$; $^{B}Y = 19.698 + 0.12630X - 0.00461X^2$; $r^2 = 0.52$; $^{C}Y = 17.108 - 0.1514X - 0.00604X^2$; $r^2 = 0.35$; $^{D}Y = 2.6385 + 0.0445X - 0.0017X^2$; $r^2 = 0.34$; $^{E}Y = 542.954 + 6.5468X - 0.2584X^2$; $r^2 = 0.34$; $^{F}Y = 492.754 + 8.0657X - 0.2433X^2$; $r^2 = 0.21$.

The variables showed no statistically significant effects on microbial protein synthesis and nitrogen balance, except for N-intake, which exhibited a quadratic effect (P = 0.032) with the dietary inclusion of DDG (Table 5).

Table 5 Nitrogen balance and microbial protein synthesis of experimental supplements with increasing levels of DDG

Item	DDG inclusion levels (g/kg)						P-Value		
	0	100	150	200	300	SEM	Treat	Linear	Quad
(g/dia)									
N-Intake	410.66	514.98	390.25	495.37	495.37	17.919	0.041	0.254	0.032
N-Feces	84.83	82.52	74.62	93.54	102.81	3.625	0.086	0.214	0.154
N-Urine	73.11	68.86	41.88	74.42	65.52	8.428	0.587	0.846	0.395
N-absorbed	331.82	432.46	315.62	401.83	282.50	19.270	0.065	0.314	0.122
N-retained	258.71	363.60	273.74	327.40	216.98	18.863	0.105	0.355	0.064
Microbial protein synthesis									
Total purines (mmol/day)	219.36	336.18	335.06	197.82	299.51	72.594	0.382	0.209	0.456

Ab purines (mmol/day)	243.04	382.12	380.78	217.39	302.60	86.422	0.382	0.209	0.456
N-microbial (g/day)	176.70	277.82	276.85	158.06	278.73	62.833	0.382	0.209	0.456
CP- microbial (g/day)	1104.38	1736.35	1730.30	987.85	1728.56	392.707	0.382	0.209	0.456
Blood urea N (mg/dL)	12.96	14.87	15.49	12.15	11.31	0.128	0.315	0.243	0.125

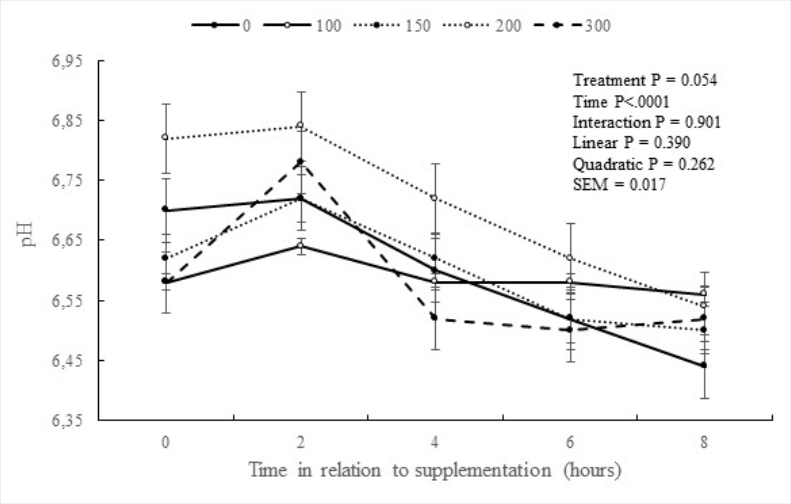
DDG0 = Dried distillers grains with 0g/kg inclusion in the supplementation; DDG100 = Dried distillers grains with 100 g/kg inclusion in the supplementation; DDG150 = Dried distillers grains with 150 g/kg inclusion in the supplementation; DDG200 = Dried distillers grains with 200 g/kg inclusion in the supplementation; DDG300 = Dried distillers grains with 300 g/kg inclusion in the supplementation. NDF= neutral detergent fiber. Treat = treatment effect; Quad= quadratic effect; SEM = Standard error of the mean.

The impact of time (P <0.05) was limited to a few parameters, including pH and the levels of ammoniacal N (N-NH_3), acetate, and branched-chain fatty acids (BCFA), including isobutyrate and isovalerate. Increasing DDG levels in the diets resulted in a linear decrease in total BCFA content (P = 0.004) and isovalerate content (P = 0.0001) and in the production of enteric methane (CH_4) (P = 0.022). The other variables were not affected by the treatments tested in this study (Table 6). Time impacted the pH value and N-NH_3 levels in rumen fluid at the different DDG supplementation levels (Graphs 1 and 2).

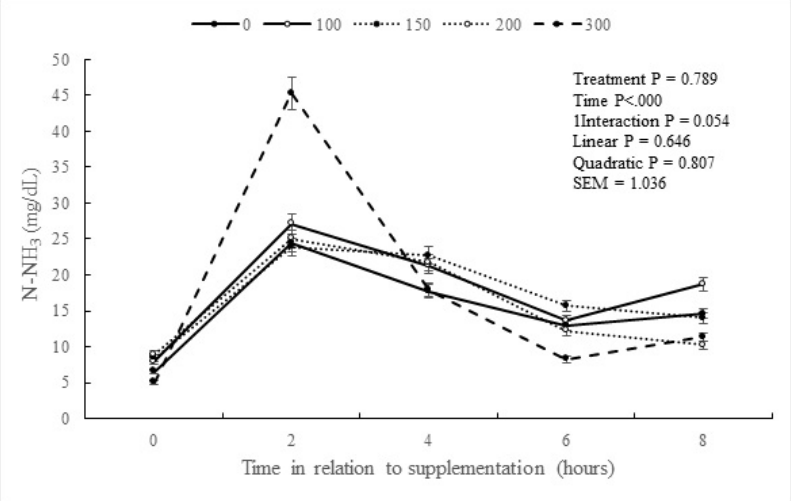
Table 6 Concentrations of short-chain fatty acids and rumen fermentation parameters of experimental supplements with increasing levels of DDG

Item	DDG inclusion levels (g/kg)						P-Value				
	0	100	150	200	300	EPM	Treat	Time	Interaction	Linear	Quad
pH	6.59	6.58	6.59	6.70	6.58	0.017	0.054	<.0001	0.901	0.90	0.262
N-NH_3 (mg/dL)	15.28	17.74	16.96	15.64	17.59	1.036	0.789	<.0001	0.054	0.646	0.807
					mmol/L						
Acetate	60.69	56.92	54.17	54.56	52.50	1.428	0.538	0.015	0.024	0.106	0.626
Propionate	17.53	15.49	15.43	15.44	15.19	1.771	0.450	0.164	0.036	0.142	0.331
Butyrate	10.02	8.60	8.47	8.36	8.66	0.491	0.292	0.077	0.049	0.125	0.132
Isobutyrate	1.040	0.907	0.891	0.925	0.821	0.041	0.370	0.007	0.068	0.088	0.678
valerate	0.830	0.785	0.688	0.702	0.671	0.319	0.266	0.860	0.094	0.085	0.529
Isovalerato[A]	1.76	1.43	1.41	1.32	1.28	0.070	0.012	<.0001	0.015	0.001	0.178
AGCR[B]	3.64	3.12	2.99	2.95	2.77	0.027	0.040	<.0001	0.017	0.004	0.304
Total	91.89	84.15	81.07	81.34	79.13	2.587	0.440	0.019	0.020	0.088	0.455
Acetate/propionate	3.47	3.66	3.52	3.53	3.49	0.123	0.526	0.005	0.883	0.735	0.357
Methane (g/day) [C]	26.49	24.79	23.47	23.65	22.91	0.776	0.010	0.015	0.021	0.022	0.567

DDG0 = Dried distillers grains with 0g/kg inclusion in the supplementation; DDG100 = Dried distillers grains with 100 g/kg inclusion in the supplementation; DDG150 = Dried distillers grains with 150 g/kg inclusion in the supplementation; DDG200 = Dried distillers grains with 200 g/kg inclusion in the supplementation; DDG300 = Dried distillers grains with 300 g/kg inclusion in the supplementation. NDF= neutral detergent fiber. Treat = treatment effect; Quad= quadratic effect; SEM = Standard error of the mean. $^{A}Y = 1.68063 - 0.01570X$; $r^2 = 0.54$; $^{B}Y = 3.51681 - 0.02785X$ - $r^2 = 0.52$. $^{C}Y = 24.246 - 0.0354X$; $r^2 = 0.55$.



Graph 1 Ph values of ruminal liquid of steers supplemented with increasing levels of DDG



Graph 2 Ammonia values in the ruminal liquid of steers supplemented with increasing levels of DDG

4. Discussion

Total pasture feed and green matter availability resulted in forage selectivity by the animals as, according to Silva et al. ([22]), the concentration of forage needs to be in the range of 1,200–4,500 kg DM/ha to induce selectivity (Table 3). The mean TDN:CP ratio was 12.0. According to Moore & Kunkle ([23]), protein supplementation can increase voluntary pasture feed consumption when this ratio exceeds the value of 7 because such a high ratio indicates protein deficiency in the pasture feed, leading to the need for supplementation ([24]). Therefore, the TDN:CP ratio found in this study corroborates this scenario, interfering with pasture feed and DM consumption.

Grazing intake had a quadratic effect with the inclusion of DDG supplementation (P = 0.032), increasing when the animals consumed supplements added with 100 g/kg (16.68 kg/day) and 200 g/kg (18.82 kg/day) of DDG, but decreasing with the other supplements (Table 4). Increased effective DM degradability may have occurred, because the more degraded the food is, the less time it stays in the rumen, resulting in more space for food consumption. The consumption may also have been impacted by the TDN:CP ratio, which was higher than 7.

Similar trends were observed for DM, OM, and CP intake, which increased at 100 and 200 g/kg of the dietary inclusion of DDG, with both concentrations exhibiting a quadratic effect. Notably, the DDG levels above 200 g/kg resulted in decreased DM, OM, and CP intake. Based on the quadratic equations for OM, CP and DM intake, the optimal DDG levels for maximum OM, CP, and DM intake are 14.03, 7.85, and 16.82%, respectively.

Supplementation can modulate consumption through additive, substitutive, or combined associative effects ([25]). Therefore, a substitution and addition effect may have occurred, with increased pasture feed consumption with the supplement. However, for some treatments, roughage consumption is reduced, but not to the same extent as supplement incorporation into the diet. The quadratic equation for pasture feed intake showed that the optimum DDG level for maximum intake is 16.55%. Higher DDG levels result in reduced forage consumption.

As DDG inclusion increased, OM and NDF digestibility also had a quadratic effect. The inclusion of 150 and 200 g/kg increased OM digestibility, which was reduced with quantities above 200 g/kg. As a result, the optimum DDG level for maximum OM intake is around 10.63% in the supplement, according to the quadratic OM equation. However, NDF digestibility peaked with 150 g/kg (577.12 g/kg) of inclusion, while the others had digestibility below this value. Based on the quadratic equation for NDF digestibility, the optimal proportion of DDG needed for maximum NDF digestibility is 10.29%. Benchaar et al. ([26]) included DDGS in the diet of dairy cows and reported that NDF digestibility slightly increased with the dietary inclusion of up to 20% DDGS and decreased with the inclusion of 30% DDGS, causing a quadratic effect (P = 0.05) in the treatment. These authors also stated that this increased NDF digestibility reflects the high degradability of DDGS NDF. Consequently, the passage rate may have increased because the main cause of digestibility variation in the diet is the time of particle retention in the rumen (27). An increase in digestible fiber content may have stimulated consumption by increasing this passage rate, opening relatively more space in the rumen to be filled with food. Leite et al. ([24]) assessed the effects of the inclusion of DDG as a 50% and 100% substitute for corn and cottonseed meal in the diet of grazing cattle and reported different results. These authors reported that the dietary inclusion of DDG did not affect the DM, OM, and CP contents and NDF digestibility in the treated animals.

The dietary inclusion of DDG exhibited a quadratic effect (P <0.05) on N-intake (P = 0.032), resulting in increased N-intake at 100 g/kg (514.98 g/day), 200 g/kg (495.37 g/day), and 300 g/kg (495.37 g/day) of DDG inclusion (Table 5) because it is a high-protein ingredient. These values can be compared to those shown in Table 4, with CP intake also showing quadratic behavior.

There was no effect on the rate of fecal N excretion (N-feces), N-retained per unit N intake, N-absorbed per unit N intake, and the levels of N-urea in the blood. However, numerical data indicate an increase in N-feces value at the highest levels of the dietary inclusion of DDG, reaching 102.81 g/day (300 g/kg inclusion). According to Zhu et al. ([28]), the amount of excreted N reflects protein use and N deposition efficiency. Increased N-retained values at a DDG intake of 100 and 200 g/kg suggest better N use. However, the CP content and microbial

protein synthesis were not impacted by the dietary inclusion of DDG. Similar results were reported by Silva et al. ([29]), where increasing DDG levels (0%, 31.5%, 63.0%, and 94.5%) in diets were tested as a substitute for corn. These authors reported no impact of these treatments on microbial protein yield (P >0.05). Microbial protein is essential in ruminant nutrition, as it is a source of high-quality amino acids available for absorption. Numerical data show decreased N-urea values at high levels of DDG inclusion in the diet. The metabolism of nitrogenous compounds is extremely important for ruminant nutrition, as reduced N levels in the urine and low plasma and urea concentrations in the urine increase the efficiency of the use of nitrogenous compounds, which mainly contribute to CP sources in the diet ([30]).

The pH value and N-NH_3 levels varied with the dietary inclusion of DDG and were impacted by the collection time. These findings are in agreement with those reported by Araújo et al. ([31]), who analyzed the use of DDG as a substitute for cottonseed meal in the diet of grazing bulls and reported the impact of time on pH value and N-NH_3 levels. These results correlate with the data presented in Graphs 1 and 2, which clearly show the impact of time on these parameters. Table 6 shows that pH value and N-NH_3 levels can change throughout the day with DM intake. However, these two parameters were not affected by the treatments analyzed in this study. The N-NH_3 values ranged between 15.28 and 17.59 mg/dL. According to Detmann et al. ([32]), N-NH_3 levels below 8 mg/dL negatively affect rumen fermentation and are usually observed with diets containing around 10% CP. However, we did not encounter such problems in the present study. Notably, the N-NH_3 levels represent the balance between its production and rumen absorption rates. A positive balance indicates that the quantity of N-NH_3 in the rumen is sufficient for microorganism growth and microbial protein production ([33]).

A linear decrease in BCFA concentrations (P = 0.004) with the dietary inclusion of DDG may be a result of NH_3 and BCFA production during protein degradation, which are substrates for fibrolitic bacteria. Specifically, rumen-degradable protein is made of nonprotein N and true protein. Degraded true protein results in peptides and amino acids, which are deaminated into N and NH_3 and can be degraded by microorganisms producing BCFA in the rumen ([34]). Benchaar et al. ([26]) also reported a linear decrease in BCFA concentrations (P <0.01) as the dietary proportion of DDGS increased. Isovalerate concentration decreased linearly (P = 0.0001) with the dietary inclusion of DDG. Its concentration in the rumen indicates amino acid fermentation, showing a direct correlation with protein degradation ([35]).

In grazing animals, diets containing DDG tend to reduce enteric CH_4 emissions, as shown in our results, which corroborate the findings of Benchaar et al. ([26]), who also reported a linear decrease in CH_4 levels with increasing DDGS levels in the diet. Enteric CH_4 production is directly associated with ruminal fiber degradation because it produces acetate, responsible for releasing hydrogen used to form CH_4 ([36]). Thus, acetate content decreased with increasing DDG levels, reducing the amount of substrate available for CH_4 formation. The use of DDGS to replace corn and soybean meal in the diet of dairy cows also reduces CH_4 production per unit of DM intake ([37]). Methane is a greenhouse gas that causes damage to the environment and economic losses in the production system in the form of energy released in the rumen fermentation process. Enteric CH_4 emissions in grazing animals are impacted by dietary

manipulation, mainly by forage consumption. The methanogenic potential varies based on the plant species consumed ([38]). Considering these conditions, DDG is an alternative that can be supplemented in the diet of grazing cattle to reduce enteric CH4 emissions, consequently reducing energy losses by the animals.

5. Conclusion

The inclusion of up to 200 g/kg of DDG in the diet of grazing cattle led to increased pasture feed, DM, OM, and CP consumption, also increasing DM and NDF digestibility. The N balance was not impacted by the dietary inclusion of the DDG supplements tested in this study; however, ruminal fermentation parameters showed decreased enteric CH_4 production, reducing energy losses by the animals. Considering the results of the consumption, digestibility, and rumen fermentation analyses, the recommended level of the inclusion of DDG supplements in the diet of grazing cattle ranges between 150 and 200 g/kg, representing 1% of the weight of the live animal.

Declaration of conflict of interest

The authors declare that there are no conflicts of interest

Data availability

Data is available upon request

Author contributions

Conceptualization: R.H.T.B. Goes. Data curation: Y. S. Picanço and D. P. Barbosa. Formal Analysis: J. R. Gandra. Acquisition of financing: R. H. T. B. Research: Y. S. Picanço, D. P. Barbosa, J. P. S. Vale, N. G. Silva and R. T. Oliveira. Methodology: R. H. T. B. Goes. Project administration: R. H. T. B. Goes. Resources: R. H. T. B. Goes and J. R. Gandra. Programs: J. R. Gandra. Supervision: R. H. T. B. Goes and Y. S. Picanço. Visualization: Y. S. Picanço. Writing – original draft: Y. S. Picanço. Writing – review and editing: Y. S. Picanço and R. H. T. B. Goes.

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