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Effect of dried distillers' grains on nutrients digestibility and nitrogen metabolism of Nellore cattle fed non-forage diets

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ABSTRACT - This study was carried out to evaluate the effects of corn dried distillers' grains (DDG) levels in non-forage diets by *in vitro*, *in situ* (0 to 50%), and *in vivo* (0 to 40%) trials on kinetic parameters of gas production (GP) on rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) as well as on nitrogen (N) metabolism in Nellore cattle. For *in vitro* and *in situ* studies, three rumen cannulated Nellore males with body weight of 340.48 ± 22.22 kg were used. For the *in vivo* study, five non-castrated male Nellore cattle with an initial body weight of 355.20 ± 35.28 kg and 24 ± 3 months old were used to evaluate the effect of increasing DDG levels in non-forage diets on N metabolism by a 5 × 5 Latin square design. Experimental diets were based on ground corn, cottonseed cake, urea, mineral supplement, and increasing levels of DDG replacing ground corn. The DDG levels caused a linear decrease in GP and *in vitro* dry matter digestibility. The DDG presented a lower dry matter digestibility (DMD) and RDP and higher RUP than corn grain and cottonseed cake, making DDG diets present lower DMD and RDP than control. The DDG levels linearly increased the intake of crude protein, neutral detergent fiber, and ether extract, whereas non-fiber carbohydrates intake decreased and tended to decrease the DMD and organic matter digestibility. The DDG levels caused a linear increase in N intake and in total, fecal, and urinary N excretion, which was the major N excretion route, causing a quadratic drop in retained N and caused a quadratic effect on alanine aminotransferase. In contrast, triglycerides were cubically affected, and total blood protein increased. Thus, DDG levels negatively affect *in vitro* and *in situ* digestibility, increasing N intake but increasing urinary N excretion.

Keywords: gas production, nitrogen balance, ruminal fermentation, ruminant

1. Introduction

Corn used for the ethanol industry also produces dried distillers' grains (DDG), which have been used as strategic dietary ingredients in ruminants diets in North America. However, DDG availability in Brazil is recent since the ethanol industry in this country was only based on sugar cane use until 15 years ago. Although there is considerable literature regarding DDG composition and feeding value in North

America, which theoretically could be used to guide nutritionists in Brazil, because of differences related to corn type and specific characteristics of ethanol production, the DDG produced in Brazil has been reported to present some differences in terms of chemical composition compared to those produced in the Canada and USA (Rosa e Siva et al., 2022), where DDG produced in USA usually presents higher lipid (10.70 × ~4.0%) and lower crude protein (CP; 30.80 × ~33%) (NASEM, 2016) than Brazilian DDG.

Replacing corn with DDG in feedlot diets helps decrease the risk of ruminal acidosis (Klopfenstein et al., 2008) since it increases neutral detergent fiber and decreases starch content in the diets, which favors the increase of ruminal pH (Alhadas et al., 2023). A better ruminal environment caused by a higher rumen pH can help animals to present higher dry matter intake in challenging periods such as during adaptation to high-grain diets (Rosa e Silva et al., 2022). Considering DDG inclusion, acidosis is the second problem related to animal health in Brazilian feedlots, and this is important for animals fed grain-based diets (Silvestre and Millen, 2021).

However, distiller's grain-based diets present higher CP levels than corn-based diets when DG are included at 20 to 40% of DM, according to American (Klopfenstein et al., 2008) and Brazilian literature (Rosa e Silva et al., 2022), which has created some debate regarding of impacts of these on N excretion by the animal (Benchaar et al., 2013; Hunerberg et al., 2013). High CP diets are associated with N losses, mainly through urinary excretion, which may cause soil and water contamination. However, the literature has shown results that confirm that diets based on distillers' grain increase the efficiency of animal feed (Klopfenstein et al., 2008), which, when combined with good management, can help to reduce the negative environmental impact of such CP diets.

Despite the advantages of including DDG in finished cattle diets, mainly with low or non-forage diets (Rosa e Silva et al., 2022), it is still necessary to determine the optimal levels that optimize feed efficiency and N use. In this way, we hypothesized that replacing ground corn by corn distillers' grains could improve the nutrient use efficiency by the animals. Therefore, this study aimed to evaluate the increasing levels of DDG in non-forage-based diets evaluated by *in vitro*, *in situ*, and *in vivo* trials.

2. Material and Methods

The protocol used in this experiment was conducted according to the Ethical Principles for Animal Research established by the National Council for the Control of Animal Experimentation (CONCEA). Research on animals was conducted according to the institucional committee on animal use (protocol 23108.060964/13–6).

The study was carried out in Santo Antônio de Leverger (15°47'5" S, 56°04' W, and 140 m above sea level), Mato Grosso, Brazil. The climate is classified as tropical (Aw in the Köppen international system). During the study, the average maximum temperature was 32.27 °C, and the average minimum temperature was 18.01 °C, whereas the total precipitation in the period was 43.9 mm.

2.1. *In vitro* study

Experimental diets were composed of ground corn, cottonseed cake, urea, and mineral supplement, in which the corn was replaced by DDG levels (0, 10, 20, 30, 40, and 50% on dry matter [DM] basis; Tables 1 and 2). The DDG evaluated in this study was produced in an ethanol plant where condensed solubles are not added to DDG.

The *in vitro* study was conducted during three successive runs. The incubations were performed with 0.5 g samples of substrate weighed into 120 mL serum bottles in triplicate, which were represented by dietary ingredients (corn, cottonseed cake, DDG, and mineral mixture + urea).

Into the bottles containing substrates were added 40 mL of reduced buffer solution (McDougall, 1948) and 10 mL of the ruminal inoculum. Immediately after, the bottles were stopped with butyl rubber stoppers sealed with aluminum seals and kept at 39 °C in the shaking water bath (Dubnoff Agi. Orbital SL-158 Solab, Piracicaba, SP, Brazil).

Table 1 - Chemical composition of corn and dried distillers' grains (DDG) used in this study and found in the NRC (2016)

Item	In this study			NRC (2016)		
	Corn	DDG	Concentration	Corn	DDGS	Concentration
DM[1]	86.86	91.59		87.20	90.00	
OM[2]	98.70	98.20	0.99			
EE[2]	3.58	4.70	1.31	3.80	10.70	2.82
NFC[2]	73.02	19.96	0.27	73.90	6.30	0.07
CP[2]	8.61	34.35	3.99	8.80	30.80	3.50
NDFap[2]	13.49	39.19	2.91	9.70	33.70	3.47
ADFap[2]	3.01	11.65	3.87	-	-	-
iNDF[2]	5.25	18.70	3.56	-	-	-
RDP[3]	58.25	35.39	0.61	34.70	32.10	0.93
RUP[3]	41.74	64.60	1.55	65.30	67.90	1.04
NDICP[3]	14.87	55.14	3.71	-	-	-
ADICP[3]	13.71	23.18	1.69	3.10	27.80	8.97

DM - dry matter; OM - organic matter; EE - ether extract; NFC - non-fiber carbohydrates; CP - crude protein; NDFap - neutral detergent insoluble fiber corrected for ash and protein; ADFap - acid detergent insoluble fiber corrected for ash and protein; iNDF - indigestible neutral detergent insoluble fiber; RDP - rumen degradable protein; RUP - rumen undegradable protein; NDICP - neutral detergent insoluble crude protein; ADICP - acid detergent insoluble crude protein.
[1] Percentage on feed basis.
[2] Percentage on dry matter basis.
[3] Percentage on crude protein basis.

Table 2 - Ingredient and chemical composition of treatment diets (dry matter basis, except DM)

Item	DDG level (% of diet DM)					
	0	10	20	30	40	50
Ingredients in the experimental diets						
Ground corn	75.00	65.00	55.00	45.00	35.00	25.00
DDG	-	10.00	20.00	30.00	40.00	50.00
Cottonseed cake[1]	20.00	20.00	20.00	20.00	20.00	20.00
Urea	1.00	1.00	1.00	1.00	1.00	1.00
Mineral supplement[2]	4.00	4.00	4.00	4.00	4.00	4.00
Chemical composition (% on DM basis)						
DM (% on feed basis)	88.43	88.90	89.37	89.85	90.32	90.79
OM	95.83	95.78	95.73	95.68	95.63	95.58
CP	14.89	17.47	20.04	22.62	25.19	27.77
EE	4.47	4.59	4.70	4.81	4.92	5.03
NDF	23.95	29.95	35.95	41.95	47.95	53.95
ADF	11.25	12.15	13.05	13.95	14.85	15.75
NDFap	19.51	22.65	25.80	28.94	32.09	35.24
NFC	58.11	52.23	46.35	40.47	34.59	28.71
iNDF	11.81	13.34	14.88	16.41	17.95	19.49

DM - dry matter; OM - organic matter; CP - crude protein; EE - ether extract; NDF - neutral detergent fiber; NDFap - neutral detergent fiber corrected for ash and protein; ADFap - acid detergent insoluble fiber corrected for ash and protein; NFC - non-fiber carbohydrates; iNDF - indigestible neutral detergent insoluble fiber.
[1] Chemical composition of cottonseed cake: 98.20% OM, 28.31% CP, 8.46% EE, 61% NDF, 37% ADF, 49.46% NDFap, 32.94% ADFap, 11.97% NFC, 38.60% iNDF.
[2] Guaranteed levels (kg): 156 g calcium, 7 mg cobalt, 344 mg copper, 5 mg chrome, 15 g sulfur, 231 mg iron, 8100 mg phosphorus, 17 mg iodine, 1034 mg manganese, 748 mg monensin, 40 g potassium, 2 mg selenium, 37 mg sodium, 1383 mg zinc, 68,794 IU vitamin A, 89.72 IU vitamin D, 103 IU vitamin E.

The ruminal inoculum was obtained from three rumen-cannulated bulls with body weight (BW) of 340.48 ± 22.22 kg housed in individuals pens (14 m^2 each) equipped with feed bunks and water fountains. The inoculum donors were fed a non-forage diet (ground corn, cottonseed cake, mineral mixture, and urea). The diet was provided daily at 08:00 and 16:00 h, strictly in the amount of 1.8%

of the animal's BW. The inoculum was obtained at 07:00 h, filtered through cheesecloth, subsequently stored in pre-warmed thermo containers (39 °C) without leaving empty spaces (Yáñez-Ruiz et al., 2016).

The gas pressure readings were recorded at 6, 12, 24, 36, and 48 h from the beginning of incubation, using semiautomatic pressure transducers (Data Logger GN200, MPL, Piracicaba, SP, Brazil) according to Theodorou et al. (1994) and Mauricio et al. (1999).

In determining dry matter digestibility (DMD), all bottles were removed from the water bath at 48 h of incubation and then placed in cold water to cease substrate fermentation. The residuals of each bottle were filtered in woven non-woven bags (WNW), washed with distilled water, dried in an oven at 55 °C for 72 h and 105°C for 12 h, and weighed, allowing the undigested DM.

2.2. *In situ* study

The diet was the same as in the *in vitro* trial. The *in situ* study was carried out simultaneously with the *in vitro* experiment using the same three rumen-cannulated male Nellore by three successive incubations. In this study, 2 g of corn, DDG, and cottonseed cake ground to 2 mm were placed in separate WNW bags (weighing 100 g/cm^2) to keep the ratio of 10-20 mg.cm^2 (Vanzant et al., 1998). The bags were incubated in the rumen for 24 h in triplicate.

After being removed from the rumen, the bags were washed in tap water until the wash water became clear. Afterward, the bags were pre-dried in an air-forced oven at 55 °C for 72 h and at 105 °C for 16 h to determine the DM and CP residual. The residual DM was used to calculate the DMD, and the residual CP was used to calculate the rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) of ingredients. The RDP was considered CP, which disappeared from bags during 24 h of ruminal incubation when RUP was the residual CP in the bags. The DMD, RDP, and RUP of experimental diets were estimated based on the concentration of these items in the ingredients and their respective inclusion.

2.3. *In vivo* study

Experimental diets were composed of ground corn, cottonseed cake, urea, and mineral supplement, in which the corn was replaced by DDG levels (0, 10, 20, 30, and 40% on a DM basis) (Table 2) and cottonseed cake was used as the source of physically effective neutral detergent insoluble fiber (peNDF). The study was carried out at a covered barn with a concrete floor, where the animals were housed individually in pens (14 m^2 each) equipped with a feed bunk, water fountain, and artificial shade (4 m^2) for 105 days. Granulometric evaluation of experimental diets was presented in Rosa e Silva et al. (2022).

Five non-castrated male Nellore cattle with an initial body weight of 355.20 ± 35.28 kg and 24 ± 3 months old were used to evaluate the effect of increasing DDG levels in non-forage diets on N metabolism by a 5 × 5 Latin square design. The experimental periods lasted 21 days each, in which the first 16 days were used for animal adaptation and the last five days for sampling and data collection.

At the beginning of the study, the animals were dewormed and vaccinated by application of Exceller® (Doramectina 1%), Fertiguard® (inactivated suspension of antigens from Bovine Infectious *Rhinotracheitis*, Bovine Viral Diarrhea, Bovine Parainfluenza type 3, Bovine Respiratory Syncytial Virus, and bacterins from *Leptospira pomona*, *L. wolfii*, *L. hardjo*, *L. icterohaemorrhagiae*, *L. canicola* and *L. grippotyphosa*; *Campylobacter fetus fetus*, *Campylobacter fetus venerealis*; Sodium selenate 4.8 mg/mL and thimerosal), and Poli-star® (inactivated culture of *Clostridium chauvoei* e toxoides of *Clostridium botulinum* type C and D, *Clostridium septicum*, *C. novyi*, *C. perfringens* type B, C, and D, and *C. sordelli*, plus immunostimulant).

The control diet (0% DDG) was formulated, aiming for an average gain of 1.5 kg/day (Valadares Filho et al., 2016). The diet was provided twice daily (08:00 and 16:00 h), strictly in the amount of 1.8% of BW, which was adjusted to BW in each experimental period.

2.4. Experimental procedures and samplings

The animals were weighed on the first experimental day of each period at 06:00 h as well as on the last day of each period. Sample collections of ingredients and diet were performed weekly.

Dry matter and nutrient digestibility were obtained by fecal sample collection twice daily between day 17 and day 20. After sampling, fecal samples were dried and proportionately composited throughout the day for each animal. Fecal excretion was obtained according to the following equation, using indigestible neutral detergent insoluble fiber (iNDF) as an internal marker:

$$FE = \frac{DMI \times \text{diet iNDF}}{\text{fecal iNDF}} \quad (1)$$

in which FE = fecal excretion (kg/day); DMI = dry matter intake; fecal iNDF = iNDF in the feces (%); diet iNDF = iNDF in the diet (%).

Samples of ingredients, diets, and feces were dried in a forced circulation oven at 60 °C for 72 h and ground with a knife mill using 1- and 2-mm sieves for further analysis.

To estimate daily urine excretion, spot urine samples were collected manually using a plastic container after spontaneous urination on the same days and times of fecal sampling. After collection, urine samples were filtered through a paper filter, and 40 mL of pure urine was immediately frozen at −20 °C and subsequently used to determine the concentration of urea and creatinine.

Urine volume was estimated by multiplying animal live weight (kg of BW) by the daily creatinine excretion (mg/kg of BW) and dividing the product by the creatinine concentration (mg/L) in the urine (Chizzotti et al., 2008). Urinary creatinine excretion (UCE; g/d) was related to the BW and estimated according to the equation proposed by Costa e Silva et al. (2012):

$$UCE = 0.0345 \times BW^{0.9491} \quad (2)$$

Urea daily excretion was calculated as the product of the urea concentration and the urinary volume after 24 h, which was then multiplied by 0.466, corresponding to the nitrogen content in the urea (Rennó et al., 2000).

The amount of N absorbed was obtained from the difference between the N ingested and the N excreted in the feces, whereas the N balance (N retained; g/d) was calculated as:

$$N \text{ retained} = N \text{ consumed} - (N \text{ feces} + N \text{ urine}) \quad (3)$$

in which N retained = N retained in the organism (g/day), N consumed = N consumed by the animal (g/day), N feces = N excreted in the feces (g/day), and N urine = N excreted in the urine (g/day).

In addition, to estimate plasma urea, triglycerides, aspartate transaminase, alanine aminotransferase (ALT), albumin, globulin, and total protein, blood samples were collected from the caudal vein on the 21st day. After that, the blood was centrifuged at 4000 rpm for 15 min to extract the serum, which was stored at −20 °C.

Along with blood collection, to obtain information about rumen pH, rumen fluid samples were manually collected 3 h after the morning feeding via oroesophageal tube; the liquid was collected with a vacuum pump. Immediately after sampling, the rumen liquid was passed through four layers of gauze tissue; an aliquot was used for pH measurement using a digital pH meter.

2.5. Chemical composition analysis

Chemical analyses were performed on ground samples (1 mm). The contents of DM (method INCT-CA no. G-003/1), organic matter (OM) (method INCT-CA no. M 001/1), CP (Kjeldahl procedure; method INCT-CA no. N-001/1), ether extract (EE) (method INCT-CA no. G-005/1), and neutral detergent insoluble fiber corrected for ash and protein (NDFap) (omitting sodium sulfite, and correcting for residual ash and protein; method INCT-CA no. F-002/1) were quantified according to the standard

analytical procedures of the Instituto Nacional de Ciência e Tecnologia de Ciência Animal (INCT-CA) (Detmann et al., 2012). The nonfiber carbohydrates (NFC) were quantified according to Hall (2000).

For iNDF determination in the feces and ingredients, 0.5 g of each sample was weighted into non-woven textile bags (5 × 5 cm, weighing 100 g/m^2) in triplicate, maintaining a ratio of the 20 mg de DM/cm^2 of surface area (Vanzant et al., 1998). Finally, the bags were incubated in the rumen of a cannulated Nellore bull for 288 h and, after that, were removed from the rumen and washed in the tap water until the water was clear (Valente et al., 2011).

2.6. Statistical analysis

The data from the *in vitro* study were analyzed considering a randomized design by PROC MIXED of SAS (Statistical Analysis System, version 9.3.1), in which the DDG levels were considered fixed effects, and runs were considered random effects, whereas, in the *in situ* study, DDG levels were considered fixed effects and animals as random effects, and statistical differences were considered when P<0.05.

The data from the *in vivo* study were analyzed by PROC MIXED of SAS by a 5 × 5 Latin square design, in which DDG levels were considered fixed effects, period and animals were considered random effects, and statistical differences were considered when P<0.05, while tendency was considered when 0.05 ≤ P≤0.1.

The results of the research were subjected to analysis of variance, and the averages were compared using Tukey's test, according to the statistical model:

$$yijkl = \mu + qli + dj + pk + dixpk + \varepsilon ijkl, \quad (4)$$

in which *yijkl* = observation referring to animal *l* in the period *k* receiving diet *j* within Latin square *i*; μ = constant inherent to all observations; *qli* = effect of the Latin square *i*; *dj* = effect of diet *j*; *pk* = effect of period *k*; *dixpk* = interaction effect of diet *j* with period *k*; and $\varepsilon ijkl$ = random error associated with each observation.

3. Results

In the *in vitro* study, DDG levels in the diet presented tendency to have a linear decrease on gas production at 6 h of incubation (P = 0.06), while linearly decreased (P<0.01) the accumulated *in vitro* gas production from 12 to 36 h and showed a quadratic effect (P = 0.03) on gas production at 48 h (Table 3). A linear decrease (P<0.01) in DMD was also observed according to DDG levels.

When avaluated *in situ*, a greater (P<0.001) 24 h DMD was observed for ground corn and cottonseed cake than for DDG. The DDG presented a lower RDP and a higher RUP than corn and cottonseed cake (P<0.001) (Table 4).

Table 3 - *In vitro* gas production (GP) at different incubation times and dry matter digestibility (DMD) of feedlot cattle fed increasing levels of dried distillers' grains (DDG) (*in vitro* study)

Item	DDG level (% of diet DM)						SEM	P-value	
	0	10	20	30	40	50		L	Q
In vitro GP (mL/g DM)									
6 h	67.76	65.63	64.77	60.46	54.64	52.79	6.00	0.06	0.74
12 h	143.47	138.29	132.36	122.36	109.57	103.35	7.28	<0.01	0.63
24 h	279.56	266.46	253.04	231.21	207.33	197.29	6.76	<0.01	0.67
36 h	368.57	351.89	346.28	309.97	279.32	260.74	7.05	<0.01	0.12
48 h	408.78	394.09	386.33	352.14	321.88	298.44	6.08	<0.01	0.03
In vitro DMD (g/g)									
48 h	0.803	0.740	0.703	0.636	0.600	0.566	0.02	<0.01	0.36

SEM - standard error of the means; L - linear effect; Q - quadratic effect.

In the *in vivo* study (Table 5), there were no effects of DDG levels in the diets on intake of DM (P = 0.97) and OM (P = 0.76); however, the inclusion of DDG in the diet resulted in a linear increase (P<0.01) on the intake of CP, EE, NDF, NDFap, iNDF, peNDF, and linearly decreased NFC intake.

Regarding nutrient digestibility (Table 6), increasing DDG levels did not affect the digestibility of CP (P = 0.19) and NFC (P = 0.28). However, DDG levels tended to have a quadratic effect (P = 0.08) on DMD

Table 4 - *In situ* dry matter digestibility (DMD), rumen degraded protein (RDP), and rumen undegradable protein (RUP) of dietary ingredients and experimental diets in function of levels of dried distiller grains (DDG)

Item	*In situ* digestibility (%)				SEM	P-value
	Corn	DDG	CSC	MM + Urea		
DMD 24 h	56.55b	41.03d	47.67c	67.89a	1.179	<0.001
RDP (% CP)	58.25c	35.39d	77.63b	98.16a	1.558	<0.001
RUP (% CP)	41.74b	64.60a	22.36c	1.83d	1.558	<0.001
Item[1]	DDG level (% of diet DM)					
	0	10	20	30	40	50
DMD 24 h	55.34	53.79	52.24	50.69	49.14	47.59
RDP (% CP)	64.12	61.83	59.55	57.27	54.98	52.69
RUP (% CP)	35.87	38.18	40.44	42.73	45.02	47.31

CSC - cottonseed cake; MM - mineral mixture; SEM - standard error of the mean.
[1] Diet calculated by the values of the ingredients.

Table 5 - Intake of dietary components by feedlot cattle (kg/day) fed increasing levels of dried distillers' grains (DDG)

Item	DDG level (% of diet DM)					SEM	P-value	
	0	10	20	30	40		L	Q
DM	7.87	7.74	7.73	7.88	8.04	0.15	0.97	0.37
OM	7.57	7.42	7.39	7.51	7.64	0.15	0.76	0.37
CP	1.17	1.35	1.55	1.78	2.02	0.02	<0.01	0.26
EE	0.35	0.36	0.36	0.38	0.39	<0.01	<0.01	0.36
NDF	1.51	1.78	2.07	2.42	2.77	0.03	<0.01	0.22
NDFap	1.32	1.56	1.84	2.15	2.47	0.03	<0.01	0.26
iNDF	0.55	0.61	0.68	0.77	0.86	0.01	<0.01	0.24
NFC	4.73	4.15	3.65	3.20	2.75	0.11	<0.01	0.52
peNDF	0.91	1.12	1.15	1.40	1.64	0.04	<0.01	0.61

DM - dry matter; OM - organic matter; CP - crude protein; EE - ether extract; NDF - neutral detergent fiber; apNDF - neutral detergent fiber corrected for ash and protein; iNDF - indigestible NDF; NFC - non-fiber carbohydrates; peNDF - physically effective NDF; SEM - standard error of the mean; L - linear effect; Q - quadratic effect.

Table 6 - Digestibility of dietary components by feedlot cattle fed increasing levels of dried distillers' grains (DDG)

Digestibility (%)	DDG level (% of diet DM)					SEM	P-value	
	0	10	20	30	40		L	Q
DM	73.39	73.23	74.01	71.04	72.02	0.74	0.08	0.08
OM	77.34	77.14	77.73	75.11	75.88	0.63	0.05	0.08
CP	71.87	72.79	75.10	73.29	77.29	1.05	0.19	0.22
EE	88.59	90.65	87.45	84.58	84.88	1.52	<0.04	0.13
NDF	37.64	46.54	52.09	53.93	55.16	2.30	<0.01	0.15
NFC	87.70	87.19	88.65	85.79	88.87	0.84	0.28	0.19

DM - dry matter; OM - organic matter; CP - crude protein; EE - ether extract; NDF - neutral detergent fiber; NFC - non-fiber carbohydrates; SEM - standard error of the mean; L - linear effect; Q - quadratic effect.

and organic matter digestibility (OMD), a linear increase in (P<0.01) NDF, and a linear decrease in EE digestibility (P = 0.04).

Regarding N utilization (Table 7), increasing DDG in the diet caused a linear increase in N intake (P<0.01) and in fecal N excretion (P<0.01), while causing a quadratic response (P = 0.02) for urinary N excretion. In this study, there was a quadratic effect of DDG levels (P<0.01) on fecal N excretion as a percentage of total N excretion, and there was no effect (P = 0.19) on fecal N excretion as a percentage of N intake. Urinary N excretion as the percentage of total N excretion and as the percentage of N intake were affected quadratically (P<0.01) by DDG inclusion in the diets. The increasing levels of DDG in the diet quadratically affected the N retention (P<0.01) as a percentage of N intake.

Ruminal pH was linearly increased (P<0.001, SE = 0.0565) by DDG levels (Figure 1). In the blood parameters, a cubic effect of DDG levels was observed on triglycerides (P = 0.02) and a quadratic effect on blood urea (P = 0.03) and ALT (P<0.01), whereas for the other parameters, the DDG levels did not have any effect (Table 8).

Table 7 - Nitrogen use pattern of feedlot cattle fed increasing levels of dried distillers' grains (DDG)

Item	DDG level (% of diet DM)					SEM	P-value	
	0	10	20	30	40		L	Q
			N intake					
g/d	187.63	216.23	247.97	285.29	324.00	3.62	<0.01	0.25
			Fecal N					
g/d	52.79	59.13	60.99	76.49	73.42	2.63	<0.01	0.11
% N excretion	60.30	46.48	40.90	44.38	32.49	1.95	<0.01	<0.01
% N intake	28.13	27.21	24.90	26.71	22.71	1.06	0.19	0.22
			Urinary N					
g/d	34.50	68.08	90.03	95.19	153.22	5.51	<0.01	0.02
% N excretion	39.70	53.52	59.10	55.62	67.51	1.95	<0.01	<0.01
% N intake	18.62	31.40	36.03	33.46	47.38	1.65	<0.01	<0.01
			Retained N					
g/d	100.34	89.02	96.95	113.62	97.36	3.50	0.01	<0.01
% N intake	53.26	41.38	39.07	39.82	29.91	1.49	<0.01	<0.01

SEM - standard error of the mean; L - linear effect; Q - quadratic effect.

Table 8 - Blood parameters of feedlot cattle fed increasing levels of dried distillers' grains (DDG)

Item	DDG level (% of diet DM)					SEM	P-value		
	0	10	20	30	40		L	Q	C
Triglycerides (mg/dL)	15.87	18.98	16.52	15.88	17.67	0.92	0.87	0.82	0.02
Urea (mg/dL)	19.24	24.97	30.98	42.74	51.40	1.25	<0.01	0.03	0.41
ALT (U/L)	17.80	16.36	16.84	17.22	19.36	0.49	0.02	<0.01	0.92
AST (IU/L)	85.54	87.38	81.78	84.38	96.42	4.34	0.20	0.10	0.24
Albumin (g/dL)	3.33	3.17	3.33	3.42	3.40	0.07	0.13	0.49	0.10
Globulin (g/dL)	3.33	3.22	3.27	3.41	3.46	0.09	0.12	0.23	0.35
TP (g/dL)	6.66	6.39	6.60	6.84	6.86	0.15	0.09	0.28	0.16
A:G	1.01	0.99	1.03	1.03	0.99	0.02	0.98	0.32	0.17

ALT - alanine aminotransferase; AST - aspartate transaminase; TP - total protein; A:G - albumin:globulin ratio; SEM - standard error of the mean; L - linear effect; Q - quadratic effect; C - cubic effect.

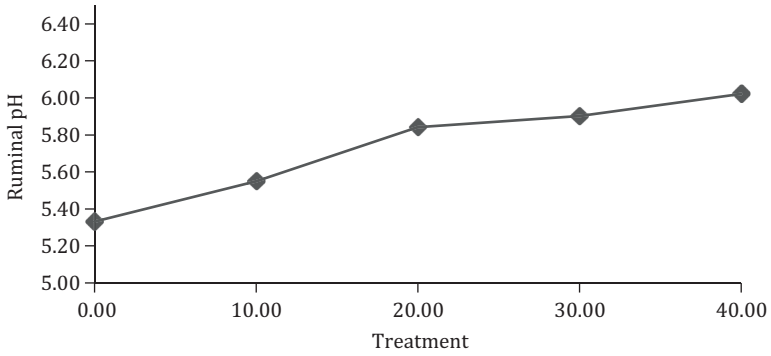

Figure 1 - Ruminal pH of feedlot cattle fed increasing levels of dried distillers' grains.

4. Discussion

The chemical composition found in DDG used in this study is corroborated by data found in literature since during ethanol production, the major proportion (around 95%) of corn starch is fermented to ethanol, making CP, EE, and NDF concentrated about three-fold (Spiehs et al., 2002). However, it must be highlighted that during the production of DDG evaluated in this study, the condensed solubles are not included in the final product, making this DDG higher in CP and NDF and lower in fat compared with dried distillers' grain with solubles (DDGS; NASEM, 2016; Rosa e Silva et al., 2022). For example, Hunerberg et al. (2013) evaluated corn DDGS that presented 31.3% CP, 10% fat, and 37.3% NDF, which means that our DDG was 9.74% higher in NDF and 113% lower in fat than that used by those authors. Thus, diets containing DDG tend to present higher CP, NDF, and fat content but lower starch content than corn-based diets, which would be expected to cause many changes in ruminal fermentation.

Schingoethe et al. (2009) emphasized that although distiller grains present a considerably high NDF content, which usually ranges from 38 to 40% of DM basis, considering that its NDF contains low amounts of lignin, DDG can be considered as having an NDF highly digestible (62 to > 71% digestible).

The lower DMD observed for DDG compared with ground corn (Table 3) can be explained by its higher NDF content and very-low starch content in DDG, since NDF represents a fraction of feeds of slow and incompletely digestion as well as occupies space in the gastrointestinal tract. In contrast, starch is a non-fiber carbohydrate, presenting fast and almost total digestibility in the digestive tract (Mertens, 1996). Additionally, DDG presents higher RUP and lower RDP content than ground corn and cottonseed cake, making DDG-based diets a good source of RUP (Table 4). This has been frequently reported in the literature, since McDonald (1954) showed that zein, the primary protein in corn, was only 40% degraded in the rumen, value very close to that found in this study (35.39% of RDP). In this respect, Klopfenstein et al. (2008) reported in their review that RUP usually ranges between 47 and 64% of the CP for higher-quality distillers' grains with solubles (DGS), with wet DGS presenting 5 to 8% lower in RUP than dried DGS. The low content of RDP of DDG-based diets would be interesting in high CP diets, aiming to decrease ammonium concentration in the rumen and prevent high N urinary excretion.

Lower starch content and higher NDF content observed in DDG-based diets, which reduces the amount of OM available for rumen microbe fermentation, helps to explain a linear reduction *in vitro* gas production and DMD. Although methane data was not obtained in our *in vitro* study, one could infer that lower methane would be produced according to DDG levels in the diets, which was observed by Hunerberg et al. (2013), who obtained a 19% drop in emission (184 vs. 228 g/animal/day) as well as on intensity (21.5 vs. 25.3 g/kg of DMI) of methane by feedlot growing beef cattle fed diets containing 40% of corn DDGS replacing barley grain.

Dry matter intake is the most important factor affecting animal performance and, in this way, DDG levels did not affect this variable in the present study due to having fixed the supply at 1.8% of the animal's BW, but in function of higher content of CP, fat, and NDF, the DDG levels promoted an increase in the intake of these dietary components. Similar behavior of DDG on DMI has been reported in the literature (Leupp et al., 2009; Felix et al., 2012; Schoonmaker et al., 2013), although some authors have found negative (Hunerberg et al., 2013) or positive effects of DDG levels on DMI (Alhadas et al., 2022; Rosa e Silva et al., 2022).

The low content of starch and high content of NDF in DDG can help to prevent a drop in DMI or bunker refusal as well as the occurrence of ruminal acidosis in feedlot cattle fed high grain diets or non-forage-based diets, such as was observed by Rosa e Silva et al. (2022), in which increasing DDG levels improved DMI during adaptation period (first 34 days) of feedlot growing cattle. When we compare NDF content among experimental diets, we can see that 40% of DDG in the diet increases NDF by 75%, compared with the control diet, which can bring important effects on ruminal fermentation, considering that slower fermentation of NDF compared with starch helps to keep rumen pH higher ($P<0.001$) (Figure 1). Thus, the linear increase of rumen pH can be explained by the low content of starch and high content of NDF according to DDG levels in the diets.

A tendency of linear decrease in DMD (down 1.37% from 0 to 40% DDG) and OM apparent digestibility according to DDG levels in the diets can be explained by higher NDF content in DDG than in corn, which is less digestible than starch from corn, since starch is a non-fiber carbohydrate. At the same time, NDF is the fiber portion of the diet, which presents slower and lower digestibility than NFC (Mertens, 1996). Negative effects of DDG levels in diets on DMD and OMD have been reported in other studies (Felix et al., 2012; Hunerberg et al., 2013; Alhadas et al., 2022).

However, the linear increase in NDF digestibility can be associated with a better ruminal environment characterized by a higher pH in animals fed DDG diets, which probably helps to keep higher abundance and activity of fibrolytic organisms compared with the control diet, which was higher in starch content and poor in NDF, promoting a drop in rumen pH. Similar improvements in NDF digestibility according to the DDG levels have also been observed in the literature (Felix et al., 2012; Hunerberg et al., 2013; Alhadas et al., 2022). In addition, NDF from DDG is more digestible than that from cottonseed cake, which also helps to explain that NDF digestibility was increased according to DDG levels.

Similar effects of DDG levels on N intake and N metabolism have been observed in the literature (Walter et al., 2012; Hunerberg et al., 2013; Alhadas et al., 2023), wherein N intake was increased, but there also was an increase in N excretion, especially by urinary excretion. This effect on N intake was expected since DDG presents higher CP content than corn (8.61 vs. 34.25%) and therefore, the diet with 40% DDG inclusion contained 69.17% more CP than the control diet (Table 2). Alhadas et al. (2023) observed that the 40% DDG diet promoted 29% higher N intake than the control diet, while Walter et al. (2012) observed 35% higher N intake in cattle fed 40% DDGS diets compared with control (262 vs. 193 g N/d).

The linear increase in fecal N excretion and the quadratic increase in urinary N excretion are directly related to the higher N intake according to DDG levels in the diets. In this study, we observed that fecal N excretion was 39.07% higher and urinary N excretion was 344.11% higher for the diet containing 40% DDG compared with the control diet (0% DDG). Similarly to this study, Alhadas et al. (2023) also reported a 39.15% increase in N excretion in feces (g/d) in Nellore cattle consuming 45% DDGS in the diet compared with a control diet. However, the same authors observed a less pronounced increase in urinary N excretion (28.60%) compared with what was found in this study (344.11%). It needs to be highlighted those authors, as mentioned earlier, evaluated diets containing lower CP levels compared with those evaluated in this study. Because of increased N excretion, DDG levels linearly decrease N retained by animals expressed in g/day or as a percentage of N intake.

Urinary N excretion became the predominant form of N excretion according to DDG levels, representing more than 50% of total N excretion until achived 67% of total N excretion when DDG level in the diet was 40%. Probably if urea was omitted in diets containing DDG, the urinary N excretion was lower than was observed and thus beef cattle diets containing more than 20% of DDG urea can be omitted

securately or signicantly reduced. However, it needs to be determined in future studies, since although DDG inclusion in the diets increases substantially CP levels, DDG presents higher proportion of its CP as rumen-undegradable protein (RUP), which might limit N supply to rumen microbial growth.

In this study, N retained by animals expressed in g/day or as a percentage of N intake exhibited a quadratic response to the levels of DDG inclusion in the diet. This behavior differs from the findings of Alhadas et al. (2023), who did not observe any significant differences, and from the finding of Benchaar et al. (2013), who reported a linear increase.

The reduction in the RDP content found in distillery grains can cause a decrease in the concentration of urea in the serum, especially when this ingredient is added to replace soybean meal and urea (May et al., 2009). In this study, DDG was added to replace corn and, given the large proportion of protein that passes through, DDG increased the levels of ureic N in the blood.

We did not observe any effect of DDG levels on concentration of triglycerides in the blood, possibly due to the low EE content of the DDG in the study, and the markers of liver function were unreliable.

5. Conclusions

Although the inclusion of DDG in diets by up to 40% improves the intake of CP, EE, NDF, and peNDF as well as increases EE and NDF digestibility, it causes a substantial increase of N excretion, primarily through urine, which needs to be considered when formulating diets for ruminants aiming to achieve high efficiency of N use. Probably urea addition in the diets presenting more than 20% of DDG can be omitted to reduce urinary N excretion and increase the N use efficiency by the animal.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

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