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Rumen fermentation, nitrogen balance and microbial responses to DDGS supplementation in sheep fed low-quality forages

Fermentação ruminal, balanço de nitrogênio e respostas microbianas à suplementação com DDGS em ovinos alimentados com forragens de baixa qualidade

Fermentación ruminal, balance de nitrógeno y respuestas microbianas a la suplementación con DDGS en ovejas alimentadas con forrajes de baja calidad

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Abstract
The objective of this study was to evaluate the fermentative parameters and rumen microbial population of sheep fed with low quality forage (rhodes grass hay; *Chloris gayana*, Kunth) supplemented or not with distiller's dried grains with solubles (**DDGS**). Eight adult sheep (*Ovis aries*) were used and distributed into two treatments according to the diet received: 1) Hay (**H**), Rhodes grass hay alone, and 2) H + DDGS, Rhodes grass hay + DDGS (proportion 64:36 on a dry basis), both diets were offered *ad libitum*. During the experimental stage, feed intake was recorded, and rumen fluid samples were collected to analyze pH, volatile fatty acids (**VFA**) and rumen ammonia nitrogen (**N-NH$_3$**). Animals supplemented with DDGS showed higher N-NH3 concentrations ($P = 0.0007$), while no significant differences were observed in VFA concentrations ($P = 0.466$) or rumen pH ($P = 0.809$) compared to those fed only rhodes grass hay. Animals supplemented with DDGS had higher concentrations of methanogenic archaea ($P = 0.017$) and sulfate-reducing bacteria ($P > 0.009$) in the rumen but did not present differences in the concentration of total bacteria ($P=0.061$). Although supplementation with DDGS promoted an improvement in nitrogen balance, no significant differences were detected between treatments ($P = 0.094$). In conclusion, animals supplemented with DDGS showed improvements in rumen fermentative activity without changes in pH and VFA compared to animals that received hay alone.
Keywords: Ruminants; Agro-industrial by-products; Protein concentrate.

Resumo

O objetivo deste estudo foi avaliar os parâmetros fermentativos e a população microbiana ruminal de ovinos alimentados com forragem de baixa qualidade (feno de capim Rhodes; *Chloris gayana*, Kunth), suplementados ou não com burlanda seca de milho (**DDGS**). Foram utilizados oito ovinos adultos (*Ovis aries*) distribuídos em dois tratamentos de acordo com a dieta recebida: Feno (**H**), apenas feno de capim-Rhodes; e H + DDGS, feno de capim-Rhodes + DDGS (proporção 64:36 com base na matéria seca). Ambas as dietas foram fornecidas *ad libitum*. Durante a etapa experimental, foi registrado o consumo de alimento e foram coletadas amostras de líquido ruminal para análise de pH, ácidos graxos voláteis (**AGV**) e nitrogênio amoniacal ruminal (**N-NH₃**). Os animais suplementados com DDGS apresentaram maiores concentrações de N-NH₃ (P = 0.0007), enquanto não foram observadas diferenças significativas nas concentrações de AGV (P = 0.466) ou no pH ruminal (P = 0.809) em comparação com os animais alimentados apenas com feno de capim-Rhodes. Os animais suplementados com DDGS apresentaram maiores concentrações de arqueias metanogênicas (P = 0.017) e de bactérias redutoras de sulfato (P = 0.009) no rúmen, mas não apresentaram diferenças na concentração de bactérias totais (P = 0.061). Embora a suplementação com DDGS tenha promovido uma melhora no balanço de nitrogênio, não foram detectadas diferenças significativas entre os tratamentos (P = 0.094). Em conclusão, os animais suplementados com DDGS apresentaram melhorias na atividade fermentativa ruminal sem alterações no pH e nos AGV em comparação com os animais que receberam apenas feno.
Palavras-chave: Ruminantes; Coprodutos agroindustriais; Concentrados proteicos.

Resumen

El objetivo de este estudio fue evaluar los parámetros fermentativos y la población microbiana ruminal de ovejas alimentadas con forraje de baja calidad (heno de grama Rhodes; *Chloris gayana*, Kunth), suplementadas o no con burlanda seca de maíz (**DDGS**). Se utilizaron ocho ovinos adultos (*Ovis aries*) distribuidos en dos tratamientos según la dieta recibida: Heno (**H**), únicamente heno de grama Rhodes; y H + DDGS, heno de grama Rhodes + DDGS (proporción 64:36 en base seca). Durante la etapa experimental se registró el consumo de alimento y se recolectaron muestras de líquido ruminal para analizar pH, ácidos grasos volátiles (**AGV**) y nitrógeno amoniacal ruminal (**N-NH₃**). Los animales suplementados con DDGS presentaron mayores concentraciones de N-NH₃ (P = 0.0007), mientras que no se observaron diferencias significativas en las concentraciones de AGV (P = 0.466) ni en el pH ruminal (P = 0.809) en comparación con los animales alimentados únicamente con heno de grama Rhodes. Los animales suplementados con DDGS mostraron mayores concentraciones de arqueas metanogénicas (P = 0.017) y de bacterias reductoras de sulfato (P = 0.009) en el rumen, pero no presentaron diferencias en la concentración de bacterias totales (P = 0.061). Aunque la suplementación con DDGS promovió una mejora en el balance de nitrógeno, no se detectaron diferencias significativas entre tratamientos (P = 0.094). En conclusión, los animales suplementados con DDGS mostraron mejoras en la actividad fermentativa ruminal sin cambios en el pH y en los AGV en comparación con los animales que recibieron únicamente heno.
Palabras clave: Rumiantes; Subproductos agroindustriales; Concentrados proteicos.

1. Introduction

Pastures composed of megathermal and native forage species form the foundation of livestock feeding systems in tropical and subtropical regions across many countries in the Americas, Asia, Africa, and Oceania. Megathermal forages are known for their high productivity, hardiness, and ability to adapt to a wide range of environmental conditions. However, from a nutritional standpoint, these species typically exhibit low to moderate digestibility and nitrogen (**N**) content, along with high fiber levels. Limited N availability restricts the development of ruminal microbiota, thereby reducing ruminal digestive efficiency, forage intake, and the supply of microbial protein to the animal. The positive effect of N supplementation (*e.g.*, urea, protein concentrates) on forage intake is most evident when the crude protein (**CP**) content of the forage falls below 6–8%, as is commonly observed in tropical species (NASEM, 2016). Additionally, the anatomical structure and chemical composition of the forage influence both its digestibility and intake. The proportion of tissues with high to moderate digestibility (*e.g.*, mesophyll, phloem), in combination with the degree of lignification and silica deposition, is known to modulate the digestion rate (Dryden, 2008).

Rhodes grass (*Chloris gayana* Kunth) is a forage species capable of growing in saline-alkaline soils and has demonstrated adaptability to a wide range of climatic conditions, from subtropical to warm environments (Sacido & Cicetti, 2016). Rhodes grass exhibits a pre-flowering CP concentration ranging from 7.5% to 9%. However, when harvest is delayed, the protein content declines to below 4%, although the forage maintains good palatability and relatively soft stems (Balbuena

et al., 2008).

In pastoral systems, proper management of forage resources is essential to reduce production costs. When low- to medium-quality forages are used, industrial by-products can provide an opportunity to supplement deficient nutrients while simultaneously helping to mitigate negative environmental impacts.

Distiller's dried grains with solubles (**DDGS**) are the main by-product of ethanol production and have become widely available in many regions of the world in recent decades. During the ethanol production process, a large portion of the grain's starch is consumed through hydrolysis and subsequent fermentation. The resulting residue contains approximately 4.5% to 6.1% starch, 30.8% to 37.6% neutral detergent fiber (**NDF**), 7.5% to 12.5% lipids, and 30.2% to 39.0% CP, of which about 88% is degradable in the rumen (NASEM, 2021). Due to its increasing availability and high energy and protein content, DDGS has become widely used in ruminant diets worldwide.

When N supply is inadequate—either in type or quantity—bacterial growth and fermentative activity in the rumen are limited (Stern *et al*., 1994). However, microbial protein synthesis efficiency may remain unaffected (Tebot *et al*., 2002). The amount of rumen degradable protein (**RDP**) required to achieve maximum growth of ruminal microorganisms ranges from 10% to 15% of dry matter (**DM**) (Hoover & Stokes, 1991; Stern *et al*., 1994). When the rate of protein degradation exceeds that of carbohydrate (**CHO**) fermentation, large amounts of N are lost as $N\text{-}NH_3$, reducing N utilization efficiency (Nocek and Russell, 1988). The use of DDGS in animals consuming low-quality forages can enhance the release of ruminal $N\text{-}NH_3$, thereby improving the ruminal environment to benefit microbial growth and activity. This promotes microbial protein synthesis and ultimately increases protein supply to the host ruminant. Consequently, improvements in both forage digestion and intake are expected.

The objective of this study was to evaluate the effect of supplementing Rhodes grass hay with DDGS on the fermentative parameters and rumen microbial population of sheep. Complementary results of this work regarding methane (**CH_4**) emissions were previously published (Gere *et al*., 2022).

2. Methodology

A mixed research was carried out, partly in the field and partly in the laboratory, in a qualitative and a quantitative study (Pereira *et al*., 2018) and using simple descriptive statistics with values of relative percentage frequencies (Shitsuka *et al*., 2014) and statistical analysis (Vieira, 2021).

2.1 Experimental design and procedures with animals

The experiment was carried out in the Department of Animal Science of the School of Agriculture (University of Buenos Aires) between October and December 2018 with the participation of the Ruminal Microbiology Laboratory (National Institute of Agricultural Technology (INTA); Argentina) for microbiota characterizations, and from the National Technological University for gas measurements. The experimental protocols were approved by the animal's ethics committee (N° 5229/2017; University of Buenos Aires, Argentina).

Eight adult sheeps (*Ovis aries*) with an average body weight (**BW**) of 64 ± 8 kg. Four of these sheep, fitted with permanent rumen cannulas, were housed in individual pens, while the other 4 (non-cannulated) were kept in individual metabolic cages. The experimental animals received one of the following treatments: 1) Hay (**H**), Rhodes grass hay alone; 2) H + DDGS, 64:36 proportion based on dry matter (0.5% BW). Both groups were fed ad libitum once a day (8 am) with free access to water.

The study lasted 54 days, divided into two periods according to a "*cross-over*" design: 17 days of adaptation and

customization, and 10 days for the collection of experimental data. During the adaptation stage, the animals were familiarized with the daily management and the experimental diet to achieve a steady state during the measurement period. This included a daily recording of consumption, collection of feces and urine, monitoring ruminal environment, and measuring CH_4 emissions.

2.2 Chemical analysis of feed, refusals and ovine feces

All procedures were adjusted according to the "Standardized Operative Procedures" of the "Program for the Improvement of forage and food assessment" (PROMEFA, "Programa para el mejoramiento de la evaluación de forrajes y alimentos"; Jaurena and Wawrzkiewicz, 2018). Feeds, orts and fecal samples were dried (65ºC) and ground (1 mm; Willey-type mill) before characterization. All results were reported on a dry matter (**DM**) basis (105°C for 4 h, AOAC, 2005; No 934.01). The ash content was determined after complete ignition at 550°C for 4 h (AOAC, 2005; No 942.05). The protein content was measured by Kjeldahl method with a Pro-Nitro distiller (Selecta J.P., Barcelona, Spain) and the ether extract (**EE**) with petroleum ether in Soxhlet equipment (AOAC, 1990; No 920.39). Neutral detergent fiber (NDFom; without sodium sulfite and using thermostable amylase) and Acid detergent fiber (**ADF_{OM}**) were reported ash-free, and acid detergent lignin (**ADL**) was performed in an ANKOM® equipment (Model 220; Goering and Van Soest, 1970).

Voluntary dry matter intake (**DMI**) was calculated for all animals as the difference between the amount of feed offered and the refusals, using pooled samples composed of daily collected aliquots for each period (days 18 to 26). At the end of each period, pooled samples were frozen for subsequent drying and chemical analysis. Total feces and urine collections were performed in metabolic cages to determine energy and nitrogen balances.

2.3 Nitrogen balance

During the measurement week (days 18 to 26), animals housed in metabolic cages were monitored daily for feces and urine output. Total daily urine production was collected into containers with a 10% H_2SO_4 solution to maintain its pH below 3. Each day, the volume of urine was individually measured, and a representative sample (*ca*. 10%) was collected and stored at -18ºC to create a pooled sample per animal and week. At the end of each experimental period, feed, orts, and feces samples were dried at 65ºC for 48 hours for subsequent chemical analysis.

Nitrogen balances were calculated individually for each animal housed in the metabolic cages as the difference between nitrogen intake and excreted (feces and urine) in every measurement period:

$$\text{N Balance (g/day)} = \text{Ingested N (g/day)} - \text{Fecal N (g/day)} - \text{Urine N (g/day)}$$

2.4 Ruminal environment and methane emission

On the last day of each period (day 27), rumen fluid samples were collected from the ventral sac of the rumen of the cannulated animals to measure pH, VFA, N-NH_3, and microbial populations. For the quantification of enteric CH_4 emissions, the sulfur hexafluoride (SF_6) tracer gas technique (Johnson *et al*., 1994) was used on the non-cannulated animals, and the results have already been published (Gere *et al*., 2022).

2.5 Determinations of ruminal fermentation parameters

Ruminal fluid samples were taken from the ventral sac, filtered with a gauze (× 4) and the liquid was stabilized in an acid solution (25% orthophosphoric acid, 1:5 ratio, acid:sample), stored in Eppendorf tubes (2 mL) and frozen at -20ºC for subsequent VFA analysis (Friggens *et al*., 1998). The pH was measured on a representative aliquot with a portable pH meter (ALTRONIX Mod. EZDO-PC pH-mV-Cond.-TDS).

The VFAs were measured by gas chromatography (Nukol capillary column [30 mt × 0.32 mm diameter × 0.25 µm fineness]; Perkin Elmer - Elite FFAP; Part N9316354). Hydrogen was used as a carrier with a flow rate of 2.4 ml/min. For the calibration curve, a standard Supelco VFA standard was used [Cat. No. 46975-U]. For the determination of N-NH_3, another aliquot was prepared and stabilized with 0.02 N sulfuric acid (1:1 ratio, sample: acid) and a uremia kit (Wiener® Laboratories) was used for its determination.

2.6 DNA extraction and microbial quantification of specific populations

At the end of each experimental period (day 27), ruminal contents samples were collected from each canulated animal for the quantification of total bacteria (**TB**) and sulfate-reducing bacteria, and methanogenic archaea. Filtered ruminal fluid samples were stored at -80ºC until microbiota analysis. Total DNA was extracted from 200 mg of each thawed sample using the commercial QIAmp DNA Stool Mini kit (Qiagen GMBH, Germany) according to the manufacturer's instructions. The quality and quantity of DNA were evaluated using 0.8% (w/v) agarose gel electrophoresis and UV-Vis spectrophotometer (Nanodrop ND-1000, Thermo Fisher Scientific, USA). Total bacteria, sulfate-reducing, and methanogenic were determined by qPCR (quantitative real-time), by comparison serial dilutions (101 to 108) of specific DNA standards. The methodology for qPCR conditions was followed as described by Ortiz-Chura *et al*., (2018). The qPCR was performed using StepOne $V_{2.3}$ software (Applied Biosystems). A total of 2 µL of template DNA (10 ng/uL) was added to the amplification reaction (20 uL total volume) containing 20 pmol of each primer, 4 µL 5 × HOT FIREPol EvaGreen qPCR Mix Plus (Solis BioDyne, Estonia) and water-free DNA/RNA adjusted to a total volume. The amplification procedure briefly consisted of a cycle of 95°C for 15 min, followed by 40 cycles of 95°C for 30 s for denaturation; hybridization at 60°C for 30 s, but which varied according to the primer (Table 1), and 72°C for 1 min for the extension.

Table 1. Sequences of primers for real-time PCR assay.

Target group	Primer	Sequence (5'-3')	Amplicom size (bp)	Annealing (°C)
Total bacteria (*16S* rRNA)[1]	F	CGGCAACGAGCGCAACCC	130	60
	R	CCATTGTAGCACGTGTGTAGCC		
Desulfobulbus propionicus [2]	F	GYGAGTGGKCCTGCTAYGA	172	54
	R	CCAGGTGCCGATAACRGC		
Total methanogens (mcrA)[3]	F	TTCGGTGGATCDCARAGRGC	140	60
	R	BARGTCGWAWCCGTAGAATCC		

Sources: [1]Denman y McSweeney (2006); [2]Adapted from Spence *et al*. (2008); [3]Denman *et al*. (2007).

2.7 Statistical analysis

Results were analyzed according to a double Latin square experimental design (one Latin square with cannulated animals and one with non-cannulated animals; taking individual animals and periods as lines and columns for the analysis). The proc Mixed procedure was used (SAS Version 8.0, SAS Institute Inc. Cary, NC, USA), and differences were declared significant when $P< 0.05$.

3. Results

Supplementation with DDGS increased CP (74 g kg^{-1} vs. 149 g kg^{-1}) and EE (15 g kg^{-1} vs. 54 g kg^{-1}) concentrations in the diet, improving DM digestibility (DMD, 310 g kg^{-1} *vs*. 450 g kg^{-1}; Table 2).

Table 2. Proportion of ingredients, chemical composition and *in vivo* dry matter digestibility of the experimental diets.

	Experimental feeds[1]		
	Hay	**DDGS**	**Hay + DDGS**
Proportion of diet ingredients (% DM)			
Rhodes grass hay	100	---	64
DDGS	0	---	36
Chemical composition of the experimental diets (g kg^{-1})			
Dry matter (g kg^{-1} as fed)	806	796	806
Ash	136	49	118
Crude protein	74	285	149
Neutral detergent fiber	737	440	616
Acid detergent fiber	401	120	293
Acid detergent lignin	72	22	51
Ether extract	15	120	54
Water-soluble carbohydrates	40	71	49
Starch	78	96	83
Dry matter digestibility (*in vivo*)	310	---	450
Gross energy (MJ.kg^{-1})	17	21	19

[1]Hay: Rhodes grass hay; Hay + DDGS: Rhodes grass hay + DDGS, proportion 64:36 based on dry matter. Source: Authors.

In concordance with the DDGS supplementation an increase in ruminal N-NH_3 concentration was recorded (Table 3), though no alterations in pH and VFA (P = 0.47) were observed. In addition, the diet with DDGS increased the number of total bacteria (P = 0.061), as well as the population of methanogens (P = 0.017) and sulfate-reducing bacteria (P = 0.009; Table 3).

Table 3. Ruminal response variables and microbiota descriptors of sheep fed Rhodes grass hay alone or supplemented with DDGS.

	Hay	**Hay + DDGS**	**SEM[1]**	***P*-Value**
Ruminal descriptors				
pH	6.9	7.0	0.11	0.809
N-NH_3 (mg N/100 ml)[2]	8.1	18.8	1.57	0.0007
VFAt (m*M*/L)[3]	112.3	121.9	17.21	0.466
Acetate (m*M*/L)	87.1	94.4	15.00	0.496
Propionate (m*M*/L)	21.4	23.6	2.98	0.509
Butyrate (m*M*/L)	3.9	3.6	0.74	0.760
Acetate/Propionate	4.3	4.0	0.53	0.587
Microbiological Descriptors (qPCR, Log_{10} copies/g)				
Total bacteria	10.9	11.1	0.08	0.061
Total methanogens	7.1	7.8	0.18	0.017
Sulfate-reducing bacteria	7.2	8.1	0.20	0.009

[1]SEM: standard error of the mean. [2]N-NH_3: Ammoniacal nitrogen. [3]VFAt: Total volatile fatty acids. Source: Authors.

Nitrogen intake was noticeably higher in animals receiving DDGS (from 9 to 22.7 g N d^{-1}; P = 0.036), but fecal losses were not affected (P = 0.099). Urinary losses increased from 3.6 to 10.2 g day^{-1} (P = 0.057), total excretions from 10.1 to 18.8

g day^{-1} (P = 0.070), and nitrogen balance from -0.9 to 4.0 g day^{-1} (P = 0.094) but none of them reached statistical significance (Table 4).

Table 4. Nitrogen balance of sheep fed Rhodes grass hay alone or supplemented with DDGS.

	Hay	Hay + DDGS	SEM[1]	*P*-Value
Nitrogen, g day^{-1}				
Intake	9.0	22.7	2.10	0.036
Excretion in feces	6.5	8.7	0.81	0.099
Excretion in urine	3.4	9.9	1.16	0.057
Total excretion	9.9	18.6	1.70	0.070
Balance	-0.9	4.0	1.27	0.094

[1]SEM: standard error of the mean. Source: Authors.

4. Discussion

Dietary changes can influence the ruminal microbiota, fermentative profiles, and pH, potentially altering rumen digestibility and fermentation efficiency. Ruminal pH was not affected by DDGS supplementation (average 6.95; P = 0.809; Table 3), in accordance with previous reports (Leupp *et al*., 2009; Martínez-Pérez *et al*., 2013). According to Ørskov and Ryle (1990) a minimum ruminal pH of 6.2 is necessary for optimal fiber digestion. The removal of grain starch during the industrial production of DGGS results in reduced glycan content, which helps maintain stable ruminal pH. This, in turn, creates favorable conditions for microbiota activity and consequent cell wall digestion (NRC, 1985).

Ammonia (**N-NH_3**) levels in the rumen are important for microbial protein synthesis. Ruminal N-NH_3 concentration increased in animals supplemented with DDGS. This observation was expected as a direct consequence of N intake raise from 9 to 22.7 g day^{-1} (Table 4), driving N-NH_3 from 8.10 to 18.8 mg.100 mL^{-1} (P = 0.0007; Table 3). Therefore, the higher N-NH_3 availability in the rumen could have contributed to improving microbial indicators, as well as protein synthesis and digestibility. It has been shown for nitrogen-limiting conditions that ammonia concentration "in excess of 5 mg 100 mL^{-1} of rumen fluid had no effect on microbial protein production" (Satter and Slyter, 1974; Satter and Roffler, 1975). In contrast, Mehrez and Ørskov (1976) recommended that achieve maximum microbial synthesis requires ammonia concentration around 23 mg / 100 mL^{-1}.

Similar trends were observed by Leupp *et al*. (2009), who found a quadratic response in ruminal N-NH_3 concentration in steers fed Bromus hay (*Bromus inermis*; 10.6% CP) and supplemented with different levels of DDGS. Besides, Loy *et al*. (2007) reported that heifers supplemented with either dry-rolled corn or DDGS had greater ruminal ammonia concentrations when compared with no-supplemented controls.

No changes were observed in total VFA productions or individual VFAs when animals were supplemented with DDGS (P = 0.466; Table 3). Similarly, no differences in total VFA production were found when steers were supplemented with DDGS at 0.2 to 0.6% BW (on *Bouteloua curtipendula* and *Bouteloua gracilis* pastures; Martínez-Pérez *et al*., 2013), and even up to 1.2% BW on Bromus (Leupp *et al*., 2009) and *Megathyrsus maximus*, cv. *Gatton panic* (Hernández *et al*., 2021). The acetate: propionate ratio decreased from 4.50 to 3.34 as DDGS supplementation changed from 0 to 0.6% BW (Martínez-Pérez *et al*., 2013).

Diet composition can also alter the rumen and gastrointestinal microbiota (Gouws and Kistner, 1965; Russell, 1984). In the present study, total bacterial population in rumen fluid was not significantly affected by DDGS supplementation (P = 0.06; Table 3), consistent with previous findings by Li *et al*. (2012) who reported no changes when replacing barley grain with

DDGS (at 30%) in a RUSITEC system.

However, methanogens (P = 0.017) and sulfate-reducing bacteria (P = 0.009) increased when DDGS was added to the diet. Callaway *et al*. (2010) found changes in the relative proportions of the microbial population in the rumen of cattle fed 0, 25, or 50% of the concentrated portion of the ration replaced by DDGS. The same authors found reductions in the populations of the genus *Succinivibrio*, and an increase in the populations of *Bacteroides* when fed with 50% DDGS. A previous study carried out by Fron *et al.* (1996) found *ca*. 2 to 7 fold increase in total, amylolytic, and lactic acid utilizing bacteria with 15% DDGS inclusion in the diet. An increase in the population of *Ruminococcus* (a bacterial genus that digests fiber) and no modification in the rest of the bacterial families monitored (*Prevotellaceae*, *Lachnospiraceae*, *Veillonellaceae*, *Spirochaetaceae* and *Paraprevotellaceae*) was found in dairy cattle supplemented with DDGS and low-fat DDGS (Castillo-Lopez *et al*., 2017). In a previous experiment with sheep fed with low-quality grasses (6.6% CP) an increment in cellulolytic bacteria proportion (*Fibrobacter succinogenes*, *R. flavefaciens* and *R. albus*) was detected in the treatment with 30% DDGS, though total bacteria numbers and methanogens were similar between treatments (Fernández Pepi *et al*., 2019).

As previously stated, DDGS supplementation increased N consumption, apparently covering the daily CP requirements of the animals (60 to 70 kg BW; 76 to 85 g CP day^{-1}; NRC, 2007). Despite this, no changes were found in nitrogen balance, fecal and urinary N losses. However, supplementation with DDGS increased N retention to 18% of N intake, as consequence of an apparent digestibility of 62% and a retention: digested ratio of 29% (both efficiency coefficients were negative for animals fed with hay alone). The supplemented animals increased noticeably the excretion of N through urine (Hay= 34%; Hay+DDGS= 53%).

The DDGS can increase by-pass protein in the diet, which may increase duodenum N flow, metabolizable protein, and nitrogen efficiency usage, as well as the N excretion route Hoffmann *et al*., (2021). evaluated the N balance of young grazing Nellore bulls supplemented with DDGS (0.3% PV) and found no significant differences in N intake, N excreted in feces, N excreted in urine and retained N. The absence of effects on N-balances of DDGS inclusion in the diets disagrees with the studies of Hünerberg *et al*. (2013a; 2013b) who found a dramatic increase in N intake and excretion.

Additionally, supplementing low-quality roughage with DDGS resulted in a reduction of approximately 35% in daily CH_4 emissions. This reduction was associated with the higher feed intake (+22%), digestibility (**DMD**) (+45%; Table 2), and ether extract content (+260%) when comparing DDGS supplementation versus hay alone (Gere *et al*., 2022).

5. Conclusion

Supplementation with DDGS in sheep fed low-quality forages proved to be a nutritionally beneficial strategy, as it increased ruminal N-NH_3 concentrations and promoted the growth of specific microbial groups (methanogens and sulfate-reducing bacteria), without altering ruminal pH, total bacterial counts, or VFA concentrations. Although N balance remained unaffected, the improvement in nitrogen availability likely supported enhanced ruminal fermentation. Moreover, as previously reported by the same research group, DDGS supplementation under similar dietary conditions also led to reductions in daily enteric CH_4 emissions, methane yield, and Ym, suggesting an added environmental benefit. Collectively, these findings support the use of DDGS as a promising alternative for improving the nutritional and environmental efficiency of ruminant production systems based on low-quality forages.

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