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Enteric Methane Emission from Sheep Fed with Rhodes Grass Hay (*Chloris gayana*) Alone or Supplemented with Dried Distillers' Grains with Solubles

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Abstract: Livestock systems based on subtropical and tropical pastures are characterized by the low productivity of livestock due to the poor nutritional value of the forage (low nitrogen concentration and digestibility, and high fiber and lignin concentrations). These conditions lead to low productivity and, consequently, high absolute emissions of methane (CH_4) per unit of product. Dry distilled grains with solubles (DDGS) are the main by-product resulting from ethanol production, and they are characterized by their high-energy fibrous and protein content, thus becoming an option for the supplementation of low-quality forage. This research investigated the effects of dietary DDGS inclusion on dry matter digestibility (DMD) and enteric CH_4 emission. Eight adult sheep of 64 ± 8 kg live weight were used. The duration of the study was 54 days, divided into two periods (changeover design), which comprised a 17-day pre-experimental period and 10 days for experimental data collection. Animals were allocated to one of two treatments used: hay (H) as a control treatment, where animals were fed with Rhodes grass hay alone; and H + DDGS, where animals were fed with H supplemented with DDGS. CH_4 emissions were estimated using the sulfur hexafluoride (SF_6) tracer technique. Diets containing DDGS increased DMI by 22% ($p < 0.05$) and reduced daily CH_4 emissions by 24% (g/d), the CH_4 yield by 35% (g/kg DMI), and the average value of CH_4 energy per gross energy intake (Ym) by 44%, compared to the control treatment ($p < 0.05$). The experiment demonstrated that supplementation with DDGS in low-quality roughage reduced daily CH_4 emissions, yields, and Ym.

Keywords: sheep; agro-industry by-products; enteric methane emission; SF_6 tracer technique

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1. Introduction

The agricultural sector faces the challenge of feeding a growing human population by 2050 [1], while meeting the social and environmental obligations of reducing greenhouse gas (GHG) emissions; the sector itself is responsible for producing 10 to 12% of the total global anthropogenic emissions [2]. However, the increased demand for protein-rich foods is leading to intensification of animal production and, hence, a likely increase in GHG emissions [2].

The production of enteric methane (CH_4) is a significant loss of the energy contained in feed [3], and although its persistence in the atmosphere is about 10 years, it has a warming effect about 28 times greater than that of CO_2 [4]. Of all animal production, enteric fermentation from ruminants is a major source of GHG emissions, accounting for 39% of all GHG emissions from the livestock sector [5] and between 11 and 13% of global CH_4 emissions [6]. Ruminant livestock represent one of the few sources that can be manipulated. This source is, furthermore, an attractive target for manipulation, since the reduction in CH_4 is usually associated with improved productivity [7–9].

Enteric CH_4 losses depend on several factors including feed intake, carbohydrate type, forage processing, lipid addition, and ruminal microbiota manipulation [3]. Feed intake is the most important predictor of CH_4 production [10,11]. Dry matter intake (DMI) is significantly and positively related to CH_4 production in adult sheep, with slopes ranging from 13.8 to 20.4 g CH_4 kg^{-1} DMI, and this relationship demonstrates that methanogenesis increases when more substrate is available for microbial fermentation in the rumen [11].

The quality of forage affects the activity of ruminal microbiota and CH_4 production in the rumen. Forage species, forage processing, the proportion of forage in the diet, and the source of the grain also influence CH_4 production in ruminants [8,12]. Total CH_4 production ($g{\cdot}d^{-1}$) tends to decrease as the protein content of feed increases, and it increases as the fiber content increases [8,13]

Ruminants produce proteins of high nutritional value, transforming fibrous forage resources that are not edible for humans. The symbiotic relationship with the rumen anaerobic microorganisms yields a high amount of hydrogen ions (H^+) that must be eliminated to keep the system functional. The main mechanism for the elimination of these ions is ruminal methanogenesis, which involves the reduction in carbon dioxide (CO_2) through the uptake of H^+ by Archaeobacteria [14]. Although there other metabolic pathways channel hydrogen, methanogenesis is the primary route of elimination.

The productivity of livestock in tropical and subtropical areas is usually low due to the low nutritional value of the available forages due to their highly lignified cell walls, low digestibility, and poor nitrogen content [15,16]. Under these conditions, supplementation with protein concentrates is an alternative having recognized productive benefits [17].

Dry distilled grains with solubles (DDGS) are a by-product from ethanol production, and due to their high energy and protein content, they can mostly replace grains [18] and, to a lesser extent, forages [19]. DDGS is a concentrate rich in crude protein (CP) (between 27% and 30%) and lipids (between 8% and 11%), and it also contributes with fiber, phosphorus, and lower concentrations of starch compared to the grain from which it is derived [20–22].

The use of industrial by-products of animal production systems leads to a reduction in the environmental impact and may also cause a reduction in the cost of waste treatment. Therefore, these reductions generate an economic benefit in terms of the added value given to by-products and waste [23]. In agricultural systems, it is necessary to modify the resource inputs and flows by increasing on-farm and farm-to-farm recycling, by redirecting current outputs into inputs for other production systems, and by reducing input costs and recovering income from resources that would otherwise be wasted and could harm the environment [24].

The aim of this study was to assess the effect of adding DDGS to Rhodes grass hay on dry matter digestibility and enteric CH_4 emissions from sheep.

2. Results

Table 1 shows the chemical composition of diet for both treatments. The inclusion of DDGS improved the quality of feed offered by increasing the CP (from 74 to 149 $g{\cdot}kg^{-1}$ DM), EE (from 15 to 54 $g{\cdot}kg^{-1}$ DM), WSC (from 40 to 49 $g{\cdot}kg^{-1}$ DM), starch (from 78 to 83), and DMD (from 310 to 450 $g{\cdot}kg^{-1}$ DM), and reduced the NDF (from 737 to 616 $g{\cdot}kg^{-1}$ DM), the ADF (from 401 to 293 $g{\cdot}kg^{-1}$ DM), and the lignin content (72 to 51 $g{\cdot}kg^{-1}$ DM) for H and H + DDGS, respectively.

Table 1. Feedstuffs chemical composition (g/kg dry matter, except stated otherwise).

Chemical Fraction	Feed		
	Hay	DDGS	Hay + DDGS
Dry matter ($g \cdot kg^{-1}$ as fed)	806	796	806
Ash	136	49	118
Crude Protein	74	285	149
Neutral detergent fibre	737	440	616
Acid detergent fibre	401	120	293
Lignin	72	22	51
Ether extract	15	120	54
Water soluble carbohydrates	40	71	49
Starch	78	96	83
Dry matter digestibility	310	-	450
Gross energy ($MJ \cdot kg^{-1}$)	17	21	19

The results obtained when evaluating the daily CH_4 emissions, the DMI, and the emissions related to DMI are shown in Table 2. Animals on the H + DDGS treatment presented a significantly higher DMI (827 vs. 679 $g \cdot d^{-1}$) and lower CH_4 emissions (16 vs. 21 $g \cdot d^{-1}$) than those on the H treatment and, consequently, they emitted less CH_4 when evaluating the CH_4 yield (20 vs. 31 $g \cdot kg^{-1}$ DMI). The H + DDGS-fed animals presented a CH_4 energy loss through eructation (Ym) of 5.7%, and the H-fed animals showed 10.1%.

Table 2. Dry matter intake and enteric CH_4 production of sheep fed hay alone or supplemented with DDGS.

	Treatments		SEM [1]	*p* Value
	H	H + DDGS		
Dry matter intake ($g \cdot d^{-1}$)				
Hay	679	535	65	0.054
DDGS	0	292	-	-
Total	679	827	69	0.035
Total (% liveweight)	1.2	1.5	0.14	0.049
CH_4 emission				
CH_4 ($g \cdot d^{-1}$)	21	16	1.1	0.014
CH_4 ($g \cdot kg^{-1}$ dry matter intake)	31	20	1.9	0.005
Ym (%) [2]	10.1	5.7	0.6	0.002

[1] Standard error of the mean. [2] Energy loss through eructation.

3. Materials and Methods

3.1. Experimental Treatments, Study Location and Animal Procedures

The experiment was carried out in the Animal Production Department of the School of Agriculture (University of Buenos Aires; Buenos Aires, Argentina), in conjunction with the Rumen Microbiology Laboratory (National Institute of Agricultural Technology; Hurlingham, Argentina) and the National Technological University (Regional School of Buenos Aires; Buenos Aires, Argentina). The experimental protocols, procedures, and the care of the animals were approved by the Ethics and Animal Welfare Committee (N° 5229/2017) of the University of Buenos Aires, Argentina.

Eight adult Friesian sheep (*Ovis aries*) having a 64 ± 8 kg live weight were used, of which four had permanent ruminal cannulas. The duration of the study was 54 days, divided into two periods (changeover design), which comprised a 17-day pre-experimental period and 10 days for experimental data collection. The pre-experimental phase entailed the adaptation of the animals to the canisters, the placement, and monitoring of the permeation tubes, and adaptation to the experimental diet. The experimental stage involved daily collection of feces, urine, and feed intake. The measurement of enteric CH_4 emissions was carried out in the last 5 days of this period.

Two treatments were used: (1) hay (H), where animals were fed with Rhodes grass hay alone; and (2) Hay + DDGS (H + DDGS), where animals were fed with Rhodes grass hay with added DDGS (ratio of 64:36 on a dry matter basis; Table 1). Cannulated animals were housed in individual pens, and the remainder of the animals were housed in metabolic cages. Both groups were fed ad libitum once a day (8 a.m.) with free access to water.

The voluntary DMI of all animals was calculated as the difference between the offered and rejected ingredients for each period (pool samples of daily collected aliquots). At the end of each period, the pool sample was frozen until subsequent drying and preparation for chemical analysis. Total collections of feces and urine from animals in metabolic cages were conducted to compute the energy and nitrogen balances, which will be reported in a subsequent article.

3.2. Measurement of Enteric Methane Emissions

For the quantification of enteric CH_4, the sulfur hexafluoride (SF_6) tracer gas technique proposed by Johnson et al. was used [3]. At the beginning of the acclimatization period, the sheep were orally dosed with brass permeation tubes containing SF_6 (ca. 1 g of SF_6 per tube; mean permeation rate 2.26 ± 0.56 mg·d^{-1}), which were prepared at the Institute of Pathobiology (INTA) 2 months before the experiment and calibrated for 4 weeks. The sampling period of collected exhaled air was 5 consecutive days. The sample system consisted of a polyvinyl chloride (PVC) yoke-shape collection device (1.25 L volume) with a sample flow regulated by a capillary system. The concentrations of CH_4 and SF_6 were analyzed using a gas chromatograph (Perkin Elmer 600, Kansas City, USA), as described by Gere et al. [25].

3.3. Chemical Analysis

All procedures were adjusted according to the standardized protocols of the Program for the Improvement of the Evaluation of Forages and Feeds [26]. The feed, refusals, and feces samples were dried (65 °C, 48 h) and ground (1 mm; Willey-type mill) before characterization. All results are reported on a dry matter (DM) basis (105 °C for 4 h, AOAC, 1991; No. 976.63). The ash content was determined after complete ignition at 500 °C for 4 h (AOAC, 1995; No. 942.05). The content of Pro-Nitro® protein (Selecta J.P., Barcelona, Spain) and the ether extract was determined in Soxhlet with petroleum ether [27]. The neutral detergent fiber (NDF) was reported ash-free and determined according to the Van Soest et al. methodology without sodium sulfite and using thermostable amylase [28]. Subsequently, the NDF was corrected for ashes (aNDFmo). The acid detergent fiber (ADF_{MO}) and the lignin contents (LDA_{MO}) were reported ash-free according to Goering and Van Soest [29] and Van Soest et al. [28] and determined using ANKOM® equipment (Model 220). The total starch content was measured using the AA/AMG Megazyme enzyme kit (Megazyme Ltd., Neogen, Ireland). The content of water-soluble carbohydrates (WSC) was also determined by colorimetry using the Antrona method [30]. The gross energy (GE) was determined using a bomb calorimeter (PARR 1261, Parr Instrument Company, Moline, IL, USA).

3.4. Statistical Analysis

Results were analyzed according to a double Latin square experimental design (one Latin square with cannulated animals, and the other with non-cannulated animals; feed, period), using proc Mixed (SAS Version 8.0, SAS Institute Inc. Cary, NC, USA). Differences were declared significant when $p < 0.05$.

4. Discussion

Supplementing diets with DGGS as a source of protein increased digestibility and total DMI, as previously found by McCollum et al. [31], Beaty et al. [32], and Mathis et al. [33]. Hence, the ration with DDGS increased the total DMI by 22% compared to hay alone (Table 2; $p = 0.035$), increasing the DMI from 1.2% to 1.5% of body weight ($p = 0.049$). This result agrees with the results previously reported by Winterholler et al. [34], who used low quality hay (PC = 5.9%) and a range of DDGS supplementation levels (0.3, 0.75, 1.20, and 1.65% PV) in a beef steer diet and observed a linear increase in total DMI; and Morris et al. [35], who found in low-quality forage a substitution rate of 0.32 kg for every additional kilogram of DDGS fed. Schauer et al. [36] and Felix et al. [37] reported that lambs could be fed with 60% DDGS (DM basis), without affecting DMI and animal performance, and the optimum dietary inclusion of DDGS for lambs occurred at 20% of the DM [37].

The average CH_4 emissions (21 and 16 $g \cdot d^{-1}$ for H and H + DDGS, respectively) were in the range of reported values of a database of individual sheep records from CH_4 emission studies conducted in the Latin America and Caribbean (LAC) region (219 individual sheep records from 11 studies) [11]. The summary report for mature animals (n = 79, mean BW of 52.4 kg, DMI 1.35 $kg \cdot d^{-1}$) showed values between 14 and 57 $g \cdot d^{-1}$, with a mean value of 30 $g \cdot d^{-1}$ [11].

Diets containing DDGS reduced the CH_4 yield by 35% ($g \cdot kg^{-1}$ DMI, $p = 0.005$; Table 2) compared to the control treatment (hay alone). The decrease in CH_4 emissions agreed with the increase in DMI and DMD. Similarly, McGinn et al. observed a reduction in CH_4 yield when Hereford steers were supplemented with DDGS receiving a base diet of barley silage [38].

In addition, it should be noted that the H + DDGS ration was 54 g $EE \cdot kg^{-1}$ DM (Table 1), which could have contributed to the reduction in CH_4 emissions. It has been noted that EE can negatively affect the emissions of CH_4. The lipid content of DDGS (120 g $EE \cdot kg^{-1}$ DM; Table 1) increased the crude fat content from 15 to 54 g $EE \cdot kg^{-1}$ DM for H and H + DDGS, respectively. As result of a meta-analysis, Beauchemin et al. [39] concluded that, for each 1% of lipid added in the diet, there was a 5.6% reduction in the production of enteric CH_4 ($g \cdot kg^{-1}$ DMI). Benchaar et al. [40] worked with dairy cattle fed increasing levels of DDGS in the diet (10, 20, and 30% of the DM replacing flaked corn and soybean meal) and found that enteric CH_4 yield decreased by 0.5 $g \cdot kg^{-1}$ DMI (0.5, 0.4, and 0.7 for 10%, 20%, and 30% of the DM, respectively). The reduction observed in our experiment was higher, and closer to the prediction reported by Beauchemin et al. [39], who signaled considerable variation in the CH_4 reductions observed among fat sources.

Several studies have reported decreases in enteric CH_4 emissions when cattle diets were supplemented with unprotected fat [41–43]. It has been argued that a decrease in CH_4 emissions is due to the reduction in organic matter fermented in the rumen and by the toxic effects on cellulolytic bacteria, methanogen activity, and number of protozoa [39,44].

The production of alcohol and carbon dioxide from maize grain requires starch removal from the grain; hence, the remaining nutrient concentration in the DDGS increases approximately three-fold [20]. Several factors such as the original grain quality and industrial process, among others, can influence the nutritional and physical properties of DDGS, which is usually considered a highly variable by-product (e.g., residual starch, WSC, lipids). Aside from the lipid content, starch and WSC can also contribute to the reduction in CH_4 production [5]. The literature reports average values for Ym of 5.4% of gross energy intake (GEI) for grazing sheep [45]. For growing lambs, Savian et al. [46] reported an average value of 7.3% (grazing ryegrass), and Amaral et al. [47] reported an average value of 5% (grazing pearl millet). The summary report of Congio et al. [11] showed values from 4.4% to 11.6%, with a mean value of 7%. The average values of Ym in this study were 10.1% and 5.7% for the H and H + DDGS treatments, respectively, agreeing with the results found in the literature (Table 2).

5. Conclusions

These results showed that supplementation with DDGS on Rhodes grass hay reduced CH_4 emissions from sheep. This effect was associated with a greater DMI and higher DMD and EE concentration in the diet. These results suggest industrial by-products as supplements for low-quality diets may be a promising CH_4 emission mitigation strategy.

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