Watermelon Plant Silage: A Viable Alternative to Alfalfa Hay for Feeding Murciano-Granadina Goats

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Article

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Abstract: The study aimed to evaluate the nutritional value of watermelon plant silage (WPS) and to assess the effect of replacing alfalfa hay (AH) with WPS in a diet formulated for dairy goats on the basis of two *in vitro* trials. The study used four goats as donors of rumen microbiota to analyze *in vitro* fermentation parameters. The chemical composition analysis revealed that WPS and AH had similar protein content (21.1 *vs.* 18.9 g CP/100 g DM), but WPS had lower contents of organic matter (77.4 *vs.* 89.6 g/100 g DM), neutral detergent fiber (36.3 *vs.* 49.4 g/100 g DM), acid detergent fiber (26.4 *vs.* 34.6 g/100 g DM), acid detergent lignin (6.40 *vs.* 8.01 g/100 g DM), and amino acids (716 *vs.* 874 g AA/kg N) compared to AH. Conversely, WPS had higher crude fat content (3.09 *vs.* 1.29 g/100 g DM) and different macro- and micromineral content values than AH. In a first *in vitro* trial, WPS and AH were incubated independently to compare their fermentation behaviour, indicating that the metabolizable energy tended to be higher for WPS compared to the AH diet (6.67 *vs.* 5.72 MJ/kg DM; $P = 0.058$). WPS fermentation produced higher concentrations of total volatile fatty acids than AH (66.6 *vs.* 53.3 mM; $P = 0.037$), lower proportions of propionate and valerate ($P \leq 0.022$), higher proportions of isobutyrate ($P = 0.001$), and showed similar gas production (GP) kinetics, as degradation rate and its potential release, and estimated organic matter digestibility. In a second *in vitro* trial, a formulated goat diet (consisting of commercial concentrate and AH in a 1:1 ratio) was used as a control to assay the impact of replacing 25 and 50 % of AH by WPS. Trends were observed in several parameters, such as a linear increase in GP rate ($P = 0.088$), organic matter digestibility ($P = 0.067$), and CH_4 concentration in the gas produced ($P = 0.094$) as AH was replaced by WPS. The study concluded that WPS could serve as a viable fodder to replace AH in conventional dairy goat diets while simultaneously reducing agricultural waste and serving as a regenerative model for implementing the circular economy strategy in the affected agronomic sectors.

Keywords: watermelon; silage; fermentation; rumen; goats; byproducts

Introduction

In recent years, the idea of the circular economy has gained importance to meet the requirements of sustainable livestock farming. In March 2020, the European Commission adopted a new Circular Economy Action Plan to increase the growth of sustainability that includes a "waste and recycling" policy aimed to protect the environment and human health [1]. The main targets of this policy are to stimulate innovation in recycling and improve waste management. These policies become increasingly more important because the world's population is expected to increase by 33 % over the current population by 2050 [2], which will increase the demand for agricultural products [3], while the natural resources and provision of services from agriculture will not grow [4]. Furthermore, in light of the increasing global population feeding animals with ecological leftovers, instead of human-edible products is currently proposed as a means to increase the food supply [5]. In parallel, livestock is suffering from the negative effects of the warmer climate, which generates the scarcity of forage availability and, in consequence, the increasing of the prices, causing the need for searching alternative strategies to feed animals must be sought for the sector's economic sustainability [6]. Thus,

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including by-products in livestock feeding could help address the challenges of this emerging scenario by reducing the cost of feed and improving sustainability on-farm, both from the economic and environmental point of view [7].

Specifically, the biomass generated in the case of the watermelon plant (*Citrullus lanatus*) was 100 million tons/year in 2019 worldwide [8]. Watermelon fruit and even its seeds have been assayed [9] but, to our knowledge the study of Hassan et al. [10], is the only one published on the suitability of using the watermelon plant as forage, and this has been carried out *in vitro* using sheep as an animal model for meat production systems. However, several studies concluded that there were substantial interspecific differences between goats and sheep in their ability to digest and utilize the nutrients of a number of by-products (e.g. [11, 12]). Another aspect to consider is that, the high moisture content may be the main challenge for the practical use and conservation of watermelon plants, because it usually contains certain amounts of discarded fruits left on the plant after harvesting. Nonetheless, ensiling is a technique usually chosen to address this problem and, in addition, it can also enhance the substrate's nutritional value and increase its palatability [13]. We hypothesized that watermelon plant silage, because of their intrinsic properties, that resemble those of the medium-good quality forage [10], could be beneficial for dairy goats feeding. Alfalfa hay (AH) is the most widely available high-quality forage used in intensive dairy goat feeding. In the southern Iberian Peninsula, due to the region's climate, AH is typically dried as hay rather than ensiled, with hay being the preferred form of feed for this livestock typology. However, AH faces an additional challenge: its availability and consequently its price are subject to significant variability and uncertainty, largely dependent on interannual climatic fluctuations and the impacts of global warming. This work aimed to study the nutritional value of watermelon plant silage (WPS), as well as to assess the impact of replacing AH with WPS on different rumen *in vitro* fermentation parameters using goats as donors of inoculum of the rumen microbiota.

Materials and Methods

Animals and Feeding

Animal procedures and care were conducted in accordance with the Spanish regulations (RD 53/2013) that transpose the European Directive (2010/63/EU) on the protection of animals used in experiments or alternative scientific purposes. Experimental protocols were approved previously by the CSIC Ethical Committee for Experimental Animal Protection and authorized by the regional Andalusian government (procedure 06/07/2023/61) as the competent body in the matter. Four Murciano-Granadina goats (46 ± 4 kg body weight) fitted with rumen cannula were used in this study as donors of the inoculum to be used in the experimental procedures of *in vitro* ruminal fermentation. The animals were fed a diet consisting of a 50:50 ratio of concentrate to forage. This diet was administered in two equal meals at 08:00 and 14:00 h, providing sufficient quantities to meet their daily metabolic requirements.

Silage Preparation

The study utilized the entire watermelon plant at its post-harvest phenological stage, encompassing branches and leaves but excluding roots. This plant material is typically considered agricultural waste and left in the field after fruit harvesting. In our case, the collected plant matter included small quantities of unharvested fruits that remained as surplus, deemed commercially insignificant. The WPS was prepared by pressing and wrapping with four to six layers of "bale wrap plastic" (25 μm stretch film). This was performed using a bale wrapper machine with a front-loader (Vicon RF 135 Balepack 3D Opticut 23, Brazil). Formic acid (0.45 % of fresh matter) was previously added to enhance the pH drop. Bales were opened after 68 days of ensiling and pH was measured (3.61 ± 0.53). When opened, the colour, aroma and lack of mould were checked as an indicator of the quality of the ensiling process and some samples were squeezed to obtain separate juice fractions for the determination of the concentration of volatile fatty acids (VFA) as indicators of proper fermentation that ensure the absence of proliferation of clostridia or enterobacteria. Thus, the silage

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juice showed values of 2.86 ± 0.002 and 0.171 ± 0.005 g/100 g DM of WPS, respectively for acetate and propionate (Mean ± SD), while the values for the rest of the VFA were negligible (< 0.03 g/100 g DM). Representative samples of watermelon plant and WPS were freeze-dried and ground before chemical analysis and in vitro experiments.

Experimental Design and Samplings

Two *in vitro* trials were carried out using the methodology based in the non-renewed culture system of ruminal microorganisms [14] inoculated with rumen fluid from goats. In the first experiment, samples of WPS and AH were incubated independently to compare their fermentative behaviour.

In the first trial, 300 mg of each dried forage (AH and WPS) were carefully weighed into 120 ml Wheaton bottles. Approximately 500 ml of rumen content was collected from the rumen of 4 fistulated fasting (12 h) goats and filtered through four layers of cheesecloth. The rumen fluid of each goat (n = 4) was mixed separately in 1:3 proportion with buffer solution [15]. One bottle per buffered inoculum was incubated as blank (without diet). Then, 40 ml of culture media in bottles containing either AH or WPS were added and incubated in triplicate for each donor animal. The bottles were incubated at 39 ˚C for 72 h and the gas pressure in each bottle was measured using a wide range pressure meter (Sper Scientific LTD, Scottdale, AZ, USA) at 2, 4, 6, 8, 12, 24, 48 and 72 h after the start of incubation. Gas production (GP, ml) was measured using a system consisting of a 40 ml capacity syringe coupled to the pressure meter via a three-way stopcock. This apparatus was connected to a needle inserted into the bottle's headspace. Gas was extracted using the syringe until the system's pressure equalized with atmospheric pressureml. After 24 h of incubation, one of the three initially prepared set of bottles was opened, the pH was measured and samples (0.8 ml) of each bottle were obtained for VFA determination. The experiment was repeated three times to corroborate the results.

In the second *in vitro* trial, the effect of replacing AH with WPS in different proportions was evaluated. The samples weighted into the Wheaton bottles were: 1) Control diet consisting in 300 mg of dairy goats-formulated diet of AH and commercial concentrate (50:50 ratio), 2) the same diet as before diet but where 25 % of the AH was replaced by WPS, 3) a diet where 50 % of the AH was replaced by WPS. One bottle per buffered inoculum was incubated as blank (without diet). *In vitro* incubations were prepared following the same protocol as in the first trial. Each bottle contained 300 mg DM of the respective diet and 40 ml of incubation solution. The bottles were incubated at 39 °C for 72 hours. Gas pressure measurements were taken at the same time intervals and following the same procedure as in the initial experiment. Additionally, after 24 h of incubation, one bottle from each triplicate set was opened to measure pH, and samples were collected for VFA quantification.

Chemical Analyses and Calculations

The dry matter (DM) (method 934.01), organic matter (OM) (method 942.05) and crude fat (CF) (method 920.39) in both WPS and AH were evaluated in triplicate according to the AOAC procedures [16]. The nitrogen (N, AOAC method 990.03) was determined by Dumas procedure (Leco TruSpec CN®, St. Joseph, Michigan, USA) to obtain the crude protein (CP = total N g/100 g DM × 6.25). Neutral (NDF) and acid (ADF) detergent fibre were analyzed following the sequential procedure of Van Soest [17] using the Ankom 220 Fiber Analyzer (ANKOM Technology). The cellulose was solubilized with 72 % sulfuric acid for acid detergent lignin (ADL) determination. These fibre fractions were expressed excluding residual ash.

Heavy metals and mineral contents were determined by atomic spectroscopy inductively coupled plasma-optical emission spectrometry (ICP-OES) analysis. The analyses were carried out in duplicate. The content of some essential and non-essential minerals was estimated. The method was based on the addition of a mixture of Milli-Q water and 65 % HNO_3 to the sample (1:3), followed by digestion in an ultra-wave digester at 220 ˚C for 15 min and cooling to 60 ˚C under high pressure. The product was then transferred to a volumetric flask and diluted with Milli-Q water. Determinations were conducted using an ICP-OES 720-ES system with a sea spray nebulizer and axial torch. Two spectral lines per element were selected, with linearity (correlation coefficient ≥ 0.995) ensured by

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utilizing standard solutions. Each analytical result was obtained by calculating the average of the two spectral line readings.

The amino acid (AA) composition of forages was determined by high-performance liquid chromatography using a Waters Lambda-Max LC Spectrophotometer detector (Waters Corporation, USA) applying the Waters® Pico-Tag method, which involves precolumn derivatisation with phenylisothiocyanate. Protein hydrolysis was carried out with 6 N HCl using evacuated tubes at 110 °C for 24 h [18].

Rumen fluid samples were analysed for total VFA concentration as well as their individual molar proportions. Immediately after opening the bottles at 24 h of incubation, a 0.8 ml aliquot of incubation fluid was mixed with an equal amount of an acid solution consisting of metaphosphoric acid (20 % wt/vol in 0.5 N HCl) and crotonic acid (0.8 g/L, internal standard), then the samples were centrifuged at 2700 g for 20 min. The VFA concentration was determined using a gas chromatography (GC) system coupled with a Flame Ionization Detector (Autosystem Perkin-Elmer Cor., Norwalk, Connecticut, USA).

To measure the methane (CH_4) production, 4 ml of headspace gas were taken at 24 h at atmospheric pressure and stored in an evacuated tube (Terumo Europe N.V., Leuven, Belgium) at 4 °C until the determination by GC using a HP Hewlett 5890 Packard Series II gas chromatograph (Waldbronn, Germany) equipped with a flame ionization detector and with the methodology described by Kheddouma et al. [19].

Statistical Analysis and Calculations

Data analysis was performed using the SPSS software (IBM Corp. IBM SPSS Statistics for Windows, Version 29.0.0.0 Armonk, New York USA). The comparison between AH and WPS fermentation parameters was analysed by a one-way analysis of variance (ANOVA). The effect of substituting AH with WPS was also analysed with ANOVA, but following an orthogonal contrast. This analysis allowed us to study the effect of the type of forage and the substitution of AH by WPS on the parameters of degradation kinetics related to GP, ME, OMD, pH, VFA and CH_4 production. Fisher's Least Significant Difference LSD test was used to compare mean values and data were considered significant differences at $P < 0.05$, and higher P values but lower than 0.10 were considered as trends.

The gas dynamics generated throughout the incubation was adjusted using the exponential model $y = A \cdot (1 - e^{-c \cdot t})$ described by France et al. [20], where "y" represents the gas production (GP, ml/g); "A" represents the asymptote (ml); "c" represents the rate of gas production (h^{-1}) and "t" represents the time of incubation (h). Feed chemical composition (CP and OM) and fermentation GP data were used for the estimation of the metabolizable energy (ME) and organic matter digestibility (OMD) following the models described by Menke and Steingass [15]:

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136GP + 0.0057CP + 0.00029\ CP^2$$

$$OMD\ (g/kg\ OM) = 148.8 + 8.89GP + 0.448CP + 0.651Ash$$

Results

Chemical and Amino Acid Composition

The chemical and AA composition of WPS and AH are shown in Tables 1-2. The DM content was low, both in WPS and in the starting material used for the ensiling process (15.7 and 14.7 g/100 g fresh matter (FM), respectively), compared to the value obtained for the properly hayed alfalfa (92.8 g/100 g FM) used in this comparative study. The OM varied from 77.4 (WPS) to 89.6 g/100 g DM (AH). The CP was also variable, with values from 21.1 (WPS) to 18.9 g /100 g DM (AH). In the same way, CF varied widely, from 3.09 (WPS) to 1.29 g/100 g DM (AH). The content in NDF varied, from 36.3 (WPS) to 49.4 g/100 g DM (AH), ADF from 26.4 (WPS) to 34.9 g/100 g DM (AH) and ADL from 6.40 (WPS) to 8.01 g/100 g DM (AH). Consequently, hemicellulose ranged from 9.91 (WPS) to 14.6 g/100 g DM (AH) and cellulose from 20 (WPS) to 26.9 g/100 g DM (AH). Total carbohydrates changed from 53.2 (WPS) to 69.4 g/100 g DM (AH) and crude energy (CE) from 15.5 (WPS) to 17.6 MJ/kg DM (AH).

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A large amount of variation between diets was observed in the macrominerals content, with WPS having a higher Ca content (44.7 g/kg) compared to AH (4.40 g/kg). Furthermore, the Mg and P content was higher for WPS (6.07 and 2.91 g/kg, respectively) compared to AH (2.30 and 2.00 g/kg, respectively).

Table 1. Nutrient composition of the materials used in the study.

Composition	Watermelon plant	Watermelon plant silage	Alfalfa hay	Concentrate
DM [1], g/100 g FM [2]	14.7	15.7	92.8	91.3
Nutrients, g/100 g DM				
OM [3]	79.8	77.4	89.6	91.6
CP [4]	22.6	21.1	18.9	19.7
CF [5]	1.40	3.09	1.29	3.29
NDF [6]	36.7	36.3	49.4	30.3
ADF [7]	24.2	26.4	34.9	15.3
ADL [8]	7.86	6.40	8.01	4.75
Hemicellulose	12.6	9.91	14.6	15.0
Cellulose	16.3	20.0	26.9	10.6
Total Carbohydrates	55.8	53.2	69.4	68,0
Non Fibrous Carbohydrates	19.1	16.9	20.0	38.3
CE [11], MJ/kg DM	16.0	15.5	17.6	17.1
Macrominerals, g/kg DM				
Ca		44.7	4.40	
K		22.5	11.1	
Mg		6.07	2.30	
P		2.91	2.00	
S		2.28	1.30	
Microminerals, mg/kg DM				
Na		198	40.0	
Fe		119	180	
Al		103	227	
Mn		39.6	43.1	
Zn		14.6	46.8	
Cu		5.62	5.33	
Ti		3.42	10.6	
As		n.d. [12]	0.340	
B		n.d. [12]	11.9	
Si		n.d. [12]	129	
Sr		n.d. [12]	46.0	

[1] DM: Dry matter; [2] FM: Fresh matter; [3] OM: Organic matter; [4] CP: Crude protein; [5] CF: Crude fat; [6] NDF: Neutral detergent fiber; [7] ADF: Acid detergent fiber; [8] ADL: Acid detergent lignin; [9] Total Carbohydrates = (100-(CP+CF+Ash); [10] Non Fibrous Carbohydrates = Total Carbohydrates - NDF; [11] CE: Crude energy; [12] n.d.: not detected.

Regarding the AA composition, the content in relation to the DM was lower for WPS compared to AH (151 and 165 g AA/kg DM, respectively) and up to 18 % lower with respect to the total N content (716 and 874 g AA/kg N, respectively for WPS and AH). The WPS had a higher content of glutamic acid, phenylalanine, serine, threonine and leucine, while the content in glycine + histidine, aspartic acid, proline, lysine, tyrosine, arginine, isoleucine and valine were higher for AH. However, the sum of essential (EAA) and non-essential (NEAA) amino acids was balanced and similar for both forages (48.8 and 50.3 g EAA/100 g AA and 51.2 and 49.7 g NEAA/100 g AA, respectively for WPS and AH).

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Table 2. Amino acid (AA) composition of forage protein compared.

g AA/100 g AA	Watermelon plant silage	Alfalfa Hay
Aspartic acid	8.56	12.5
Serine	5.86	4.69
Glutamic acid	14.9	9.66
Glycine+Histidine*	4.96	8.15
Arginine	5.67	6.15
Threonine*	5.38	4.63
Alanine	8.10	6.20
Proline	8.10	10.5
Tyrosine*	3.22	3.92
Valine*	6.61	6.67
Lysine*	5.88	7.40
Isoleucine*	4.59	4.84
Leucine*	9.16	8.66
Phenylalanine*	9.03	5.98
EAA [1]	48.8	50.3
NEAA [2]	51.2	49.7
g AA/kg DM	151	165
g AA/kg N	716	874

[1] EAA: essential amino acids (Hou and Wu et al, 2018; Cys, Met and Trp were not determined), [2]NEAA: non-essential amino acids.

Comparison between both forages based on in vitro ruminal fermentation parameters

The results of the fermentation parameters obtained from the first *in vitro* trial with AH and WPS incubated independently are shown in Table 3. In the present study, we found no significant differences ($P = 0.506$) between the forages in the GP, the asymptotic value of total gas production (A, ml) and the rate of degradability (c, h^{-1}) ($P = 0.679$ and $P = 0.901$, respectively). However, differences were found in pH of the medium after 24 h of incubation, being higher for AH compared to WPS (7.04 and 7.00, respectively; $P = 0.017$). Furthermore, the metabolisable energy (ME) tended to be higher for WPS compared to the AH (6.67 and 5.72 MJ/kg DM, respectively; $P = 0.058$). The organic matter digestibility (OMD) was also numerically higher for the WPS than for the AH, but the difference was not significant (471 and 410 g/kg, respectively; $P = 0.126$).

Table 3. Fermentation parameters, after 24 h of *in vitro* culture of rumen microorganisms, of the compared forages.

	Watermelon plant silage	Alfalfa hay	SEM [1]	*p*-Value
A [2], ml	135	125	6.71	0.679
c [3], h^{-1}	0.108	0.107	0.006	0.901
pH	7.00	7.04	0.015	0.017
GP [4] 24h, ml/g DM	120	111	5.07	0.506
ME [5], MJ/kg DM	6.67	5.72	0.167	0.058
OMD [6], g/kg	471	410	13.8	0.126
CH_4 ml/l GP	94.2	86.8	2.54	0.287
Total VFA [7], mM	66.6	53.3	2.44	0.037
Acetate, %	70.1	70.2	0.809	0.963
Propionate, %	17.3	19.0	0.283	0.022
Butyrate, %	7.40	6.17	0.381	0.170
Isobutyrate, %	1.78	1.17	0.042	0.001
Valerate, %	1.35	1.57	0.047	0.014
Isovalerate, %	2.07	1.77	0.102	0.210

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Acetate/Propionate	4.08	3.51	0.103	0.057

[1] SEM: Standard Error of Mean, [2] A: Asymptotic value of total gas production, [3] c: the rate of degradability, [4] GP: gas production, [5] ME: Metabolizable energy, [6] OMD: Organic matter digestibility, [7] VFA: Volatile fatty acids.

The CH_4 production was not different between WPS and AH (94.2 and 86.8 ml/l GP, respectively; $P = 0.287$).

According to the total VFA concentration, the AH was less fermentable compared to WPS (53.3 and 66.6 mM, respectively; $P = 0.037$). In terms of VFA molar proportions, no differences were detected in the acetate, whereas propionate was lower for WPS than AH (17.3 and 19.0 %, respectively; $P = 0.022$). Conversely, isobutyrate was higher for WPS compared to AH (1.78 and 1.17 %, respectively; $P < 0.001$). The valerate proportion was lower with the WPS incubation compared to AH (1.35 and 1.57 %, respectively; $P = 0.014$). No significant differences were found in the molar proportions of butyrate and isovalerate ($P = 0.183$). Consequently, the acetate:propionate ratio tended to be higher in WPS fermentation compared to AH (4.08 and 3.51 %, respectively; $P = 0.057$).

The graph of the accumulated GP for both WPS and AH forages is represented in Figure 1. The WPS had the highest cumulative GP compared to AH, regarding the asymptote of the kinetics curve. Initially, the GP slope was similar for both WPS and AH forages, but after 20 hours of fermentation, the WPS GP extent exceeded the threshold of the AH one.

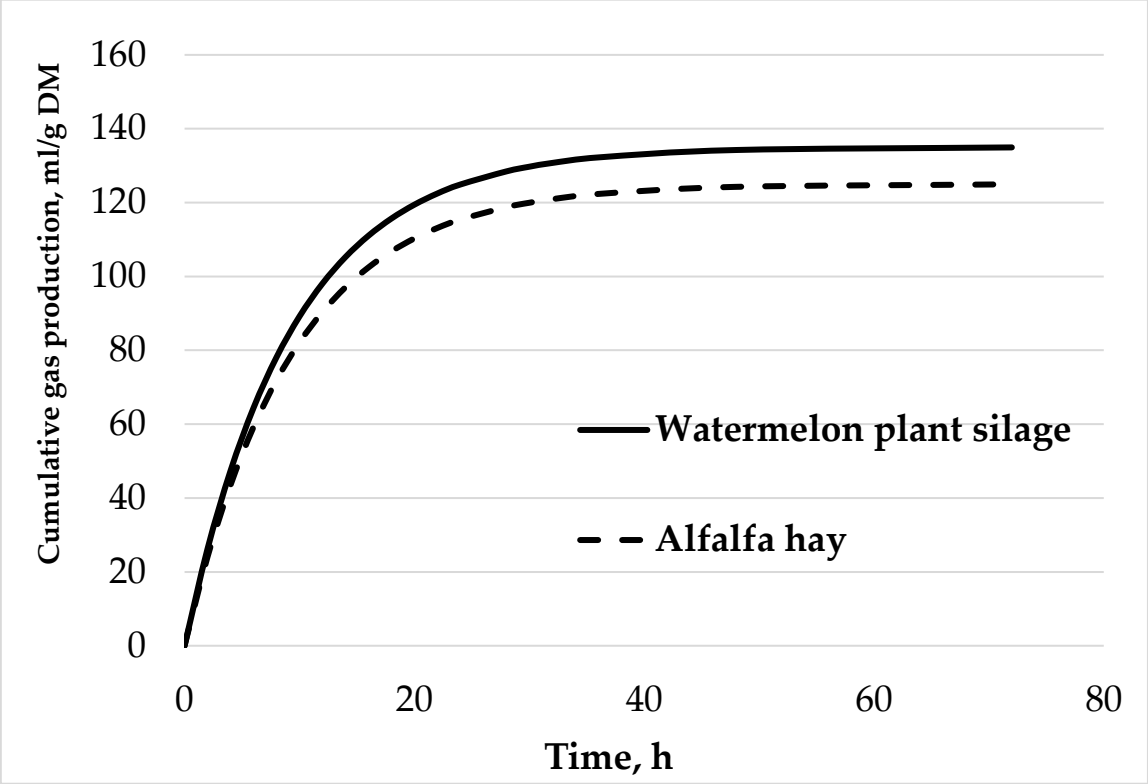

Figure 1. Graphical representation of the cumulative gas production during 72 h using the equation $y = A \cdot (1 - e^{-c \cdot t})$ and the values of A and c obtained for WPS and AH from the *in vitro* incubation.

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Effects of Replacing Alfalfa Hay with Watermelon Plant Silage on the Ruminal Fermentation Parameters of the Experimental Diet

The results of the second trial, in which the replacement of AH by WPS was evaluated, are depicted in Table 4. No significant differences were found in the parameters of rumen fermentation kinetics nor the total VFA or their molar proportions when 25 or 50 % of AH was replaced by WPS. However, we did find trends in different parameters such as an increase in the degradability rate (c, h^{-1}; $P = 0.088$), the OMD, ($P = 0.067$) and the CH_4 concentration in the gas produced ($P = 0.094$). Such trends seemed to follow a linear response to the replacement level of AH by WPS performed.

Table 4. Effect of substituting different percentages of alfalfa hay (0, 25 and 50 %) with watermelon plant silage on fermentation parameters after 24 h *in vitro* rumen microorganisms culture.

Substitution rate, %	0	25	50	SEM [1]	*p*-value
A [2], ml	179	175	170	9.01	0.549
c [3], h^{-1}	0.116	0.123	0.127	0.01	0.088
pH	6.71	6.72	6.72	0.16	0.469
GP [4] 24h, ml/g DM	162	169	170	4.65	0.558
ME [5], MJ/kg DM	6.81	6.80	6.80	0.28	0.436
OMD [6], g/kg	645	692	769	59.9	0.067
CH_4 ml/l GP	96.6	109	110	2.00	0.094
CH_4, ml/mol VFA	1.62	2.01	1.74	0.088	0.319
Total VFA [7], mM	59.7	54.3	63.1	2.38	0.304
Acetate, %	66.3	66.6	66.5	0.33	0.594
Propionate, %	20.5	20.7	20.9	0.24	0.183
Butyrate, %	8.16	7.89	7.73	0.32	0.895
Isobutyrate, %	1.42	1.39	1.38	0.03	0.345
Valerate, %	1.53	1.52	1.51	0.02	0.183
Isovalerate, %	1.86	1.82	1.81	0.05	0.531
Acetate/Propionate	3.22	3.22	3.18	0.03	0.138

[1] SEM: Standard Error of Mean, [2] A: Asymptotic value of total gas production, [3] c: the rate of degradability, [4] GP: gas production, [5] ME: Metabolizable energy, [6] OMD: Organic matter digestibility, [7] VFA: Volatile fatty acids.

The effect of replacing different percentages of AH with WPS in GP is graphically represented in Figure 2. Diets with 25 and 50 % substitution of AH with WPS showed a slightly lower GP asymptote (A), while the rate of GP until 20 h of incubation resulted quite similar among treatments.

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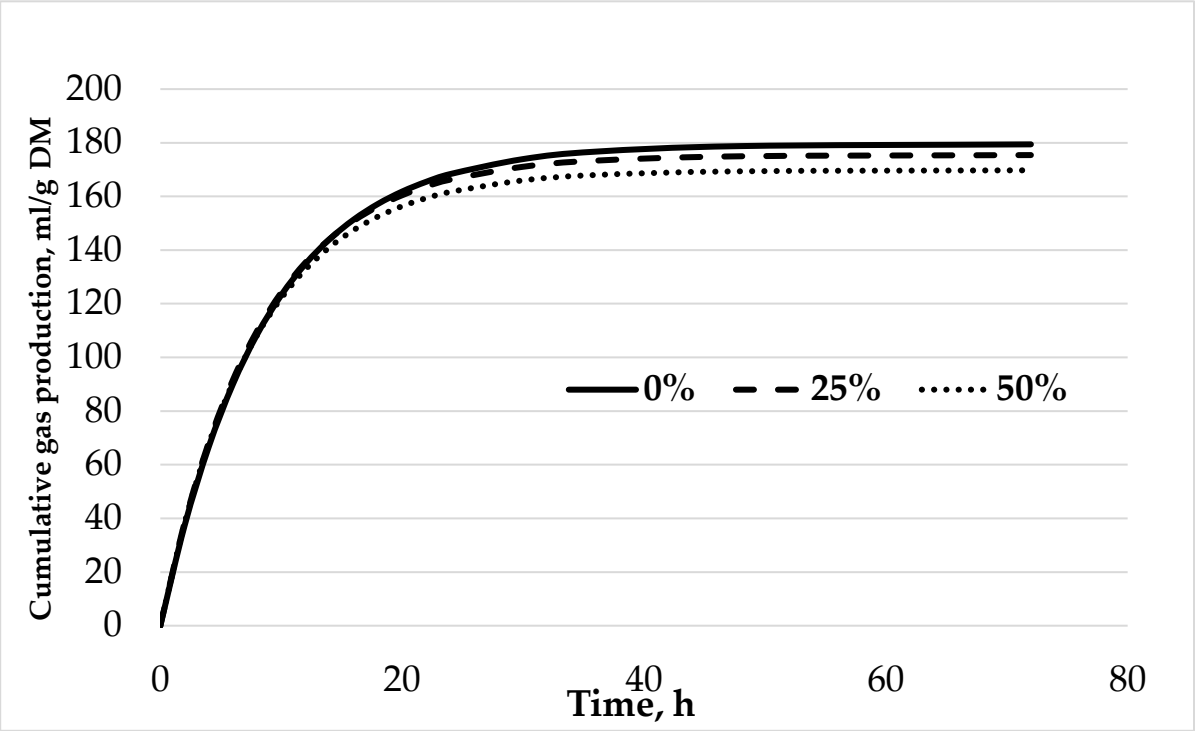

Figure 2. Graphical representation of the cumulative gas production during 72 h using the equation $y = A \cdot (1-e^{-c \cdot t})$ and the values of *A* and *c* obtained in the *in vitro* incubation where different percentages of alfalfa hay were replaced by watermelon plant silage.

Discussion

The use of plant silages as an alternative source in animal feed has been considered during the last decades, showing a wide variation in its chemical composition due to several factors such as plant species, climatic conditions during cultivation and treatments to improve ensiling processing. The principle of ensiling is based on preserving green fodder under anaerobic conditions to support the growth of lactic acid-producing bacteria, which generate lactic acid and cause a decrease in the pH of the conserved material [21].

Several studies have shown promising results when using plant silages to feed ruminants [22, 23], but is scarce the scientific literature on the suitability and adequacy of the watermelon plant ensiling, especially considering the entire plant and not only surplus fruits. According to the literature [26], maintaining upper limits of 3% acetate and 0.5% propionate in the squeezed silage liquid has been established as a good indicator of adequate fermentation of the silage and the absence of clostridia or enterobacteria proliferation. In our case, these values were 2.86 and 0.171 % of WPS DM, respectively for acetate and propionionate. These authors also argued that high concentrations of these acids are related to a low voluntary feed intake of silage in cows, although it may not be directly attributable to those compounds, rather because they also can be considered indicators of an incorrect conservation or storage. In any case, VFA concentrations in silage are proportional to the moisture content, that in the case of WPS (almost 85 %, conferred by the presence of watermelon fruits in the pre-silage mixture) was much higher than the average of the studies reviewed in the cited work (75 % upper limit).

Similar to what Lin et al. [27] found when analyzing the impact of AH silage on the its chemical composition, the nutrient composition of the WPS hardly changed concerning what the plant had before the ensiling process. They reported a significant reduction in water-soluble carbohydrates, probably for a reason that can explain the decrease in hemicellulose in exchange for the increase in cellulose (in a magnitude close to 20 %) observed in our study. The main explanation could be that hemicellulose could be susceptible to being used to a greater extent by certain microorganisms involved in the fermentation that occurred during the ensiling process.

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Some authors reported variations in the saturation of fatty acids due to the ensiling, but there is no available information on the impact of this process on the plant CF content that could explain the increase (by more than 100 %) of this nutrient observed in the WPS. However, Hassan et al. [10], reported a WPS CF content similar to that in our product (2.80 *vs.* 3.09 g/100 g DM, respectively), whereas Ibrahim et al. [28] found a range of CF content in the intact watermelon plant (from 1.19 to 1.82 g/100 g DM) which covered the value of our observation (1.40 g/100 g DM) before ensiling.

Alfalfa stands out among forage crops due to its high levels of CP and energy, which minimize the necessity for additional supplements in feed and makes it ideal for inclusion in the diets of high-yield dairy ruminants. The comparison between the composition of WPS and AH used in our trial reflected that the former showed a higher content of minerals, CP and CF, while AH contained higher NDF, ADF and ADL proportions. Considering this, and that the NDF content (nearly 50%) of the AH used in our study, it can be regarded as a medium-quality forage legume, while WPS could be expected to offer a higher potential for providing ME. The ADF represents the percentage of highly indigestible and slowly digestible components in forages, including cellulose, lignin, pectin, and ash. A lower ADF value in WPS, approximately 25% less than in AH, suggests that this forage might be more digestible and thus suitable for dairy goats.

The lower OM and carbohydrates content in WPS likely resulted from the presence of earth in the obtained plant samples, suggesting that the collection method used in this experiment may have been suboptimal. This same reason would explain the very high calcium (Ca) content since the WPS cultivation soil in this study was made up of quaternary deposits of the Ca luvisol type. Minerals are necessary for metabolic processes in small ruminants and Ca is required for lactation, maintenance and growth in small ruminants. Those requirements have been reported to be between 1.2 to 2.6 g/kg DM [29] and, while AH is considered rich in Ca source (4.40 g Ca/kg DM), the concentration of this element in WPS exceeded almost twenty times the upper threshold of the requirements range (44.7 g Ca/kg DM in WPS). However, if the Ca is in the form of carbonates of soil contamination, the bioavailability of this element may be lower and, adversely, it could depress the absorption of Fe [30]. The WPS showed higher concentrations of K, Mg and Na, and lower of Fe, Al, Zn, Ti, As, B, Si and Sr than AH. In comparison with AH, and contrarily to described by Hassan et al. [10], the low heavy metals content of WPS draws attention, which would constitute it as a safe fodder for animals.

The AA analysis plays a crucial role in examining the make up of proteins, as well as in studying the constituents of foods and animal feeds and their potential to cover the protein requirements of the different animal species. Moreover, EAA is defined as AA whose carbon skeletons are not synthesized *de novo* by animal cells or AA that are insufficiently synthesized *de novo* by animal cells relative to metabolic needs [31]. Although many species of rumen bacteria are capable of *de novo* synthesizing AA, ruminants' diets must provide sufficient protein and EAA when high rates of growth or lactation are required [32]. Ibrahim et al. [33] studied the AA composition of different parts of the watermelon plant (*Citrullus vulgaris*) and their results regarding proportions of AA in stem and leaves can be considered equivalent to those found in our study, including the observation related to a proportion higher in NEAA than in EAA. They also concluded that most of the AA values of the watermelon plant are comparable with those of most vegetable proteins, an observation that can also be extended to the comparison of WPS with AH performed in our study. Nevertheless, it should be noted that the ratio of AA-N in WPS was 18 % lower than in AH, probably indicating a worse protein value for WPS. This limitation could potentially be offset by the higher total N content (exceeding 11.5%) of this alternative fodder, which might be suitable for promoting microbial synthesis in the rumen.

The *in vitro* gas technique, based on volume and pressure measurements, has been used for decades to assess the degree of fermentation of diets in ruminants [34] and monogastric animals [35]. Using the *in vitro* gas technique allows to reduce the cost, time and use of *in vivo* experiments [36] and is considered a replacement and reduction system to implement two of the three Rs (replacement, reduction and refinement) principles to address the ethical protection of experimental animals.

When evaluating the quality of silage, it is important to consider the rumen fermentation parameters, which adequacy indicates that the silage can be conveniently degraded by the animal

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and supplies the required nutrients. In the present study, in terms of rumen fermentation dynamics, the WPS was shown to lead a fermentative process similar to AH regarding the rate (c), the asymptotic limit (A) of GP, indicating the suitability of its inclusion in the diet of goats. Although the range of ME values for medium quality AH reported in the literature, determined through *in vivo* tests, typically falls between 8 and 9 MJ/kg DM, and differs from the 5.72 MJ/kg DM found in this study, it is important to recognize that the *in vitro* values obtained are still valid for comparing the energy availability of AH and WPS. This allows for an assessment of the suitability of WPS as a potential substitute for AH. Moreover, the ME calculations following the model proposed by Menke and Steingass [15], which considered the GP and the chemical composition (CP and OM) of the substrates, revealed a value favourable to WPS in comparison to AH. Thus, the underestimation obtained in the calculation of ME was due to the low GP records, which resulted from a lower ruminal inoculum to buffer ratio in our study (1:3) compared to that used in the work of mentioned authors (1:2).

The end products of dietary carbohydrate fermentation in ruminants are VFA, mainly acetate, propionate and butyrate [37]. All of them are the main source of energy in ruminants, accounting for more than 70 % of total metabolisable energy [37]. Acetate and propionate are essential for fat synthesis and gluconeogenesis [38]. In the present study, when comparing WPS to AH *in vitro* fermentation, we found that WPS fermentation promoted similar acetate and butyrate proportions to the AH but lower propionate rate. Considering the total VFA production, the energy potential showed by the rumen fermentation of the WPS was equivalent to AH. Total VFA production is typically expressed in relation to digested OM as an indicator of fermentation efficiency. Although the OM content was lower in WPS, its OMD was numerically higher. Therefore, this calculation did not affect the VFA production potential, which was 25.6 and 25.9 mmol/g of degraded OM for WPS and AH, respectively. When examining the CH_4 proportion in the total GP, no significant variation was observed in the methanogenic potential of WPS compared to AH. The isobutyrate and isovalerate proportions were higher for WPS compared to AH. These VFAs are iso-acids and are involved in the stimulation of microbial protein synthesis [39]. The lower proportion of valerate promoted by WPS fermentation could indicate *per se* a decreased possibility of protein degradation of this forage compared to AH.

The second trial did not reflect a greater impact at the levels of 25 and 50% replacement of HA by WPS on rumen fermentation parameters, except for trends to linearly increase the rate of degradation (c), the OMD and the methanogenic activity of the diet. Contrary to what observed in the first trial, no effect was found on the ME and VFA molar proportions because the substitution of AH by WPS. These differences could probably be explained by synergistic interactions of the microbial utilization of WPS nutrients together with those of the other dietary components (AH and concentrate), which did not occur when the forages were individually incubated.

In an *in vivo* trial, Soliman et al. [40] used a mixed ration for lactating cows (40:60 forage to concentrate ratio) to study the replacement of berseem (leguminous plant) hay by dried watermelon vine in different percentages (0, 25, 50, 75 and 100 %) and observed, contrary to our observations, that OMD and total VFA production decreased from a 50 % of hay replacement. From this substitution level, they also observed a detrimental effect on productive parameters (i.e. milk yield and its dry extract). Nevertheless, it is necessary to consider that the quality of the watermelon plant used by these authors had significantly lower nutritional characteristics compared to the WPS in our study, as it had lower protein contents and higher cellulose and, especially, hemicellulose contents.

Recently, Hassan et al. [10], studied the impact of the replacement (10, 20 and 30 %) of sunflower meal (protein concentrate that represented 6 % of the diet) with WPS using an *in vitro* approach with rumen inoculum from sheep and no effect was observed on GP or total and molar proportions of VFA. This agrees with our results, although the total proportion of inclusion of WPS in the diet was much higher in our case, which indicates that a considerable replacement of the standard diet with WPS has no harmful effects on rumen fermentation.

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Conclusions

The present study shows that WPS could be used as a suitable ingredient in the formulation of diets for dairy goats, given the positive effects observed on ruminal fermentation and the ability to provide nutrients and energy. There are few results in the literature on the use of this source in the feeding of small ruminants. Therefore, the evaluation of the use of this by-product in animals, including the evaluation of WPS voluntary feed intake, is crucial in the feeding of lactating animals, would be favoured by further studies under practical farm conditions.

Given that conventional forages in many cases present problems because the demand of livestock farmers exceeds existing production or because prices are excessive, the use of watermelon plant silage could be an alternative to improve livestock sustainability through a strategy that meets the axioms of the circular bio-economy.

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