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In Vitro Rumen Fermentation Kinetics Determination and Nutritional Evaluation of Several Non-Conventional Plants with Potential for Ruminant Feeding

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Abstract: Using invasive plants as non-conventional forage in livestock production can contribute to meeting climate change targets and provide a competitive advantage in a global market. This study evaluated the nutritional potential of several non-conventional plants: *Pennisetum setaceum*, *Ricinus communis*, *Arundo donax*, *Acacia melanoxylon*, *Opuntia ficus-indica*, *Agave americana*, *Pittosporum undulatum*, and *Hedychium gardnerianum*. Chemical composition, in vitro digestibility, gas production, and energy estimates were determined. Opuntia showed the lowest DM value (6.65%), while the highest was found for Acacia (39.43%). Ricinus recorded the highest levels of CP (23.56% DM), RFV (273.86), and in vitro gas production at 24 h (43.49 mL/200 mg DM). The highest NDF (80.39% DM), HEM (39.03% DM), and CEL (36.81% DM) values were observed for Pennisetum. Agave produced the highest amount of gas from the start to the end of incubation (22.68 to 48.99 mL/200 mg DM), while Acacia produced the least (3.83 to 14.78 mL/200 mg DM). The highest ME (8.72 MJ/kg DM) and NEL (5.06 MJ/kg DM) estimates were obtained for Agave. Correlations between the chemical compositions and feed quality indices of the plants were observed, showing strong negative correlations between ADF and DMD, OMD, and DMI ($r > 0.86$, $p < 0.01$). We can conclude that all these non-conventional plants have potential as an alternative feed for ruminants when there are fodder shortages.

Keywords: invasive plants; non-conventional forage; alternative feed sources; ruminant nutrition; sustainability; digestibility; gas

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1. Introduction

Livestock production, the main economic engine of the Azores, is currently facing multiple challenges at a global level, including the need to respond to human population growth and food security, but also environmental pollution and accelerating climate change [1].

Considering the context of the limited agricultural area of the archipelago of the Azores, obtaining a sustainable productive increase, capable of responding to an increasingly demanding and globalized market, is a challenge. In this context, the answer must necessarily come from the optimization of available resources and the valorization of the specific potential of the territory [2].

As nutrition is one of the factors that has the greatest impact on animal production and can represent over 50% of production costs, meeting the growing competitive challenges must necessarily involve improving the quality, quantity, and availability of the used fodder [3].

In the Azores, ruminant production depends heavily on pastures as a food source. During periods of greater food scarcity, forage is preserved as silage, prepared from pasture surplus produced in the spring [4]. It is, thus, a system highly dependent on climate and very susceptible to climate change [5]. Consequently, the need to research non-conventional animal feed sources has been increasing, not only in the Azores but also in the rest of the world [6]. Taking advantage of invasive plants in this context is particularly beneficial, as it can not only result in a competitive advantage from an economic perspective, which is an increasingly important aspect in a globalized market, but also make livestock production more eco-sustainable [7].

Invasive plants are indeed a problem that, to a greater or lesser extent, affects the entire region of the Azores, constituting a threat to the endemic flora and fauna of the region. From a resource optimization perspective, making use of them as a non-conventional animal feed source makes particular sense, as it not only constitutes a complementary means of combating their spread, as suggested by [4], but also provides an alternative coarse feed, given that they are rich in fiber, and because they typically present higher levels of protein and minerals than the straw fed to ruminants [8]. Furthermore, their use can contribute to meeting climate change targets [9].

This study aims to assess the viability from a nutritional standpoint of several invasive plants as non-conventional animal feed sources: *Pennisetum setaceum*, *Ricinus communis*, *Arundo donax*, *Acacia melanoxylon*, *Opuntia ficus-indica*, *Agave americana*, *Pittosporum undulatum*, and *Hedychium gardnerianum*.

2. Materials and Methods

2.1. Study Area

This study was conducted on Terceira Island, Azores. The Azores are located in the North Atlantic and form part of Macaronesia (Figure 1). Just like the remaining archipelagos of Macaronesia, the Azores are of volcanic origin, and its soils can be characterized as andosols, having evolved under temperate and humid Atlantic climate conditions. The average temperature is 17.5 °C, with little variation throughout the year. The minimum temperature is reached in February and the maximum in August. Relative atmospheric humidity is typically high, reaching up to 95% in high-altitude native forests. The rainfall regime usually peaks in January–February and has its minimum in July [10].

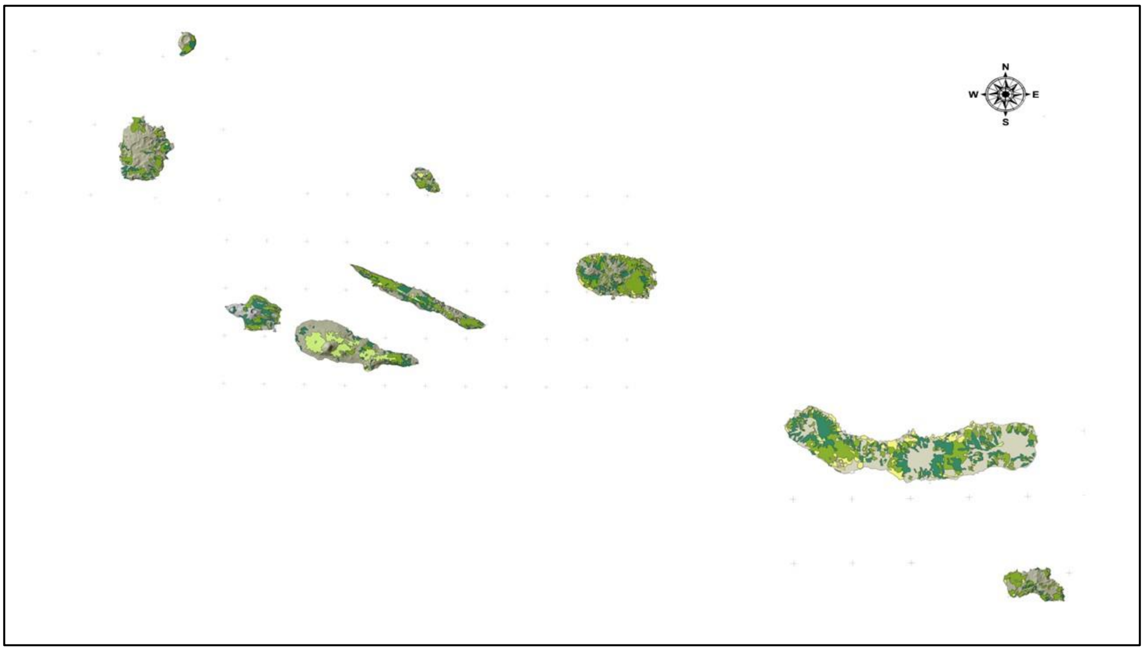

Figure 1. The Azorean archipelago.

2.2. Sample Collection and Preparation

Eight different non-conventional invasive plant species were studied. For each species, eight plants were manually harvested from different locations between the autumn and spring.

For *Pennisetum setaceum*, *Ricinus communis*, *Arundo donax*, *Acacia melanoxylon*, *Pittosporum undulatum*, and *Hedychium gardnerianum*, only the leaves were harvested. In the case of Opuntia, cladodes were collected from the middle of the plant, while the whole blade was used for Agave.

The plant samples were dried in a forced-air oven at 65 °C until they reached a constant weight. They were then ground through a 1 mm screen using a Retsch mill for further analytical analysis of the remaining chemical and biological parameters.

2.3. Chemical Analyses

2.3.1. Determination of the Chemical Parameters

For analytical characterization, the Weende scheme [11] was used to determine the DM (method 930.15), ash (method 942.05), EE (method 920.39), and CP (method 954.01). NDF, ADF, and ADL were measured by the methods proposed by [12]. Both NDF and ADF were expressed without residual ash. Hemicellulose (HEM) and cellulose (CEL) contents were estimated as follows:

$$HEM(\%DM) = NDF(\%DM) - ADF(\%DM) \tag{1}$$

$$CEL(\%DM) = ADF(\%DM) - ADL(\%DM) \tag{2}$$

2.3.2. Determination of Biological Parameters

Three biological parameters were determined in the scope of this study: In vitro dry matter digestibility, in vitro organic matter digestibility, and gas production. The first two were measured according to the method of [13], modified by [14], while the gas production was determined according to [15], which consists of incubating two hundred milligrams of sampled dry matter, weighed in triplicate and placed in a 100 mL calibrated glass syringe to which 30 mL of a mixture of rumen juice and inoculant medium (Menke medium mixture) are added in a ratio of 1:2 v/v and held in CO_2. The inoculant medium was prepared using the buffer solutions (reduced and mineral solutions) as described by Menke [15].

Afterwards, the glass syringe was incubated at 39 ± 0.5 °C in an electrically heated isothermal oven equipped with a rotor, which rolled continuously at 1–2 rpm. The gas production was measured manually, directly from the syringe, at 4, 8, 12, 24, 48, 72, and 96 h, after the onset of incubation. Differences in the composition and activity of the rumen liquor were controlled by three parallel measurements, a blank test, and incubation of a roughage and a concentrate standard as described by [15].

The gas production constants used were based on the model of [16] and fitted to the gas production kinetics curve of [17]:

$$y = a + b(1 - e^{(-ct)}) \tag{3}$$

where y is the gas production at time t; a is the gas production of the immediately soluble fraction (mL 200 mg^{-1} DM); b is the gas production of the insoluble fraction (mL 200 mg^{-1} DM); c is the gas production rate constant for the insoluble fraction (mL h^{-1}); and t is the incubation time (in hours).

The rumen fluid for each digestibility and gas production experiment was collected in the local slaughterhouse as described in [18]. The same conditions were observed for each experiment. Rumen was collected from 5 healthy dairy cows. Each cow had been fed ryegrass (*Lolium multiflorum*) and silage-based corn in the preceding days. Their rumen fluid was collected within 10 min of them being slaughtered. The rumen was filtered with a cheesecloth, preserved at 39 °C under anaerobic conditions, and delivered to the animal nutrition laboratory within 30 min of being collected [19].

2.4. Energy Estimates

Metabolizable energy (ME) and net energy for lactation (NEL) were estimated according to the following equations:

$$ME\left(\frac{MJ}{kgDM}\right) = 2.20 + 0.136GP + 0.057CP + 0.002859EE^2 \quad (4)$$

$$NEL\left(\frac{MJ}{kgDM}\right) = 0.101GP + 0.051CP + 0.11EE \quad (5)$$

where GP is the gas production at 24 h (mL/200 mgDM), CP is the crude protein (% DM), and EE is the ether extract (% DM).

2.5. Forage Quality Indices

Dry matter intake (DMI), digestible dry matter (DDM), and relative feed value (RFV) were estimated using the following equations:

$$DMI(\%LW) = \frac{120}{NDF(\%DM)} \quad (6)$$

$$DDM(\%) = 88.9 - (0.779 \times ADF(\%DM)) \quad (7)$$

$$RFV = \frac{DMD \times DMI}{1.29} \quad (8)$$

The forage quality grading standard assigned by the Hay Marketing Task Force of the American Forage and Grassland Council, the RFV, was assessed as roughages based on prime (>151), premium (151–125), good (124–103), fair (102–87), poor (86–75), and reject (<75) [20].

2.6. Statistical Analyses

The statistical analyses were performed using SPSS v.27 software (IBM Inc., Armonk, NY, USA). All data were tested for normality, with the aim of fulfilling the ANOVA assumptions, using the Kolmogorov–Smirnov test to assess the normality distribution of the different variables, and Levene's statistic was performed to test the equal homogeneity. All comparisons between means were tested using the Tukey method, with a significance level for acceptance of less than 5%. The plant species served as the experimental units. The evaluated parameters were considered the response (variables), while the plant species under evaluation were the factors (fixed term), using the three different sampling locations as statistical replications. Correlational analysis was conducted by Pearson Correlations using the same version of SPSS software. The mean of the obtained results is presented, as is the standard error of the mean (SEM).

3. Results

Table 1 presents the results obtained for the chemical composition and the dry and organic matter digestibility (DMD and OMD). The lowest dry matter (DM) value was observed for *Opuntia ficus-indica* (6.65%), while the highest was found for *Acacia melanoxylon* (39.43%). *Ricinus communis* was observed to have the highest crude protein (CP) value (24.51% DM), while *Opuntia ficus-indica* had the lowest recorded value (4.94% DM). The high level of ash present in *Opuntia ficus-indica* (17.66% DM) was also noteworthy, considering the chemical parameters tested in this study.

Table 1. Chemical composition of the different plants.

Samples	DM (%)	CP (% DM)	EE (% DM)	Ash (% DM)	DMD (%)	OMD (%)
Pennisetum setaceum	18.44 [a]	16.61 [a]	1.26 [a]	16.10 [a]	60.19 [a]	52.38 [a]
Ricinus communis	19.16 [a]	24.51 [b]	2.34 [b]	9.29 [b]	79.19 [b]	77.14 [b]
Arundo donax	23.56 [c]	15.69 [a]	1.82 [a,b]	10.76 [b,c]	58.16 [a]	55.43 [a]
Acacia melanoxylon	39.43 [d]	16.99 [a]	2.05 [a,b]	5.26 [d]	27.32 [c]	25.19 [c]
Opuntia ficus-indica	6.65 [e]	4.94 [c]	1.26 [a]	17.66 [a]	84.41 [b]	72.53 [b]
Agave americana L.	11.15 [f]	5.69 [c]	1.58 [a,b]	11.34 [b,c]	77.52 [b]	75.33 [b]
Pittosporum undulatum	33.37 [b]	7.84 [c]	3.24 [c]	8.14 [b]	39.54 [d]	35.27 [d]
Hedychium gardnerianum	11.89 [f]	8.78 [c]	1.57 [a,b]	11.62 [c]	31.37 [c,d]	23.50 [c,d]
SEM	2.21	1.36	0.14	0.80	4.89	4.34
p-Value	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001

DM: dry matter; CP: crude protein; EE: ether extract; DMD: dry matter digestibility; OMD: organic matter digestibility. SEM: standard error of the mean. Different letters next to the respective value indicate significant differences in the nutritive parameters among sampling dates. $p < 0.05$ significant differences were found.

Regarding the study of dry matter digestibility, it was noted that the results varied between 27.32% for *Acacia melanoxylon* and 84.41% for *Opuntia ficus-indica*. The same was observed in the determination of organic matter digestibility (OMD), with values varying between 23.50% *for Acacia melanoxylon* and 77.14 for *Ricinus communis*.

Most plants showed digestibility values greater than 50%; however, *Acacia melanoxylon*, *Pittosporum undulatum*, and *Hedychium gardnerianum* obtained lower percentages.

As summarized in Table 2, the maximum value found for NDF was for *Pennisetum setaceum* (80.39% DM), and it is also this plant that contains the maximum value found in this study for HEM (39.03% DM) and the maximum value of CEL (36.81% DM). Conversely, the minimum value found for NDF was for *Opuntia ficus-indica* (21.23% DM), which was also the plant to contain the minimum value found in this study for ADF (14.31% DM), while the maximum value for ADF was observed with *Acacia melanoxylon* (64.01% DM).

Table 2. Analytical detergent fiber fractions of the different plants.

Samples	NDF (% DM)	ADF (% DM)	ADL (% DM)	HEM (% DM)	CEL (% DM)
Pennisetum setaceum	80.39 [a]	41.36 [a]	4.55 [a]	39.03 [a]	36.81 [a]
Ricinus communis	24.91 [b]	20.07 [b]	4.45 [a]	4.84 [b]	15.62 [b]
Arundo donax	71.93 [c]	35.66 [a]	3.76 [a]	36.26 [a]	31.91 [a]
Acacia melanoxylon	64.01 [d]	52.22 [c]	41.26 [c]	11.79 [c]	10.96 [b]
Opuntia ficus-indica	21.23 [b]	14.31 [b]	3.95 [a]	6.92 [b]	10.36 [b]
Agave americana L.	28.55 [b]	23.88 [b]	4.37 [a]	4.67 [b]	19.51 [b]
Pittosporum undulatum	39.31 [e]	35.72 [a]	17.42 [d]	3.60 [b]	18.30 [b]
Hedychium gardnerianum	72.39 [c]	42.48 [a]	9.41 [a]	29.91 [d]	33.07 [a]
SEM	4.76	2.53	2.57	3.00	2.11
p-Value	<0.001	<0.001	<0.001	<0.001	<0.001

NDF: neutral detergent insoluble fiber; ADF: acid detergent insoluble fiber; ADL: acid detergent lignin; HEM: hemicellulose; CEL: cellulose. SEM: standard error of the mean. Different letters next to the respective value indicate significant differences in the nutritive parameters among sampling dates. $p < 0.05$ significant differences were found.

The ADL values found ranged from 3.76% DM for *Arundo donax* to 17.42 for *Pittosporum undulatum*, this plant being the one that also contained the minimum value of HEM (3.60% DM) found in this study.

The values for estimated dry matter intake (DMI), digestible dry matter (DDM), and relative feed value (RFV) are shown in Table 3, with the highest DMI value (5.75%) found for *Opuntia ficus-indica* being very similar to that found for *Ricinus communis* (4.82%) and *Agave americana* (4.24%). The minimum value was 1.49 for *Pennisetum setaceum*, which is comparable to *Hedychium gardnerianum*, *Arundo donax*, and *Acacia melanoxylon*.

Table 3. Estimated feed quality indices and forage quality grading of the different plants.

Samples	DMI (%LW)	DDM (%)	RFV	Forage Quality Grading
Pennisetum setaceum	1.49 [a]	56.68 [a]	65.60 [a]	Reject
Ricinus communis	4.82 [b]	73.27 [b]	273.86 [d]	Premium
Arundo donax	1.67 [a]	61.12 [a]	79.23 [a]	Poor
Acacia melanoxylon	1.88 [a]	48.22 [c]	70.07 [a]	Reject
Opuntia ficus-indica	5.75 [b]	77.75 [b]	346.79 [c]	Premium
Agave americana L.	4.24 [b]	70.30 [b]	231.33 [d]	Premium
Pittosporum undulatum	3.06 [c]	61.08 [a]	144.62 [b]	Good
Hedychium gardnerianum	1.66 [a]	55.81 [a]	71.89 [a]	Reject
SEM	0.33	1.97	21.79	
p-Value	<0.001	<0.001	<0.001	

DMI: dry matter intake; DDM: digestible dry matter; RFV: relative feed value. SEM: standard error of the mean. Different letters next to the respective value indicate significant differences in the nutritive parameters among sampling dates. $p < 0.05$ significant differences were found.

The maximum estimated for DDM was 77.75% for *Opuntia ficus-indica,* similar to the value estimated for *Ricinus communis* (73.27%) and *Agave americana* L. (70.30%). In contrast, the minimum estimated value was 48.22 for *Acacia melanoxylon.* The highest RFV value found in this study was 346.79 for *Opuntia ficus-indica*, followed by *Ricinus communis* (273.86) and *Agave americana* L. (231.33).

The in vitro gas production recorded over the tested incubation hours (4, 8, 12, 24, 48, 72, and 96 h) varied considerably ($p < 0.05$). As shown in Table 4, *Agave americana* L. produced the highest amount of gas from the start of incubation to the end (22.68 to 48.99 mL/200 mgDM), contrasting with *Acacia melanoxylon*, which produced the least gas (3.83 to 14.78 mL/200 mgDM).

Table 4. Cumulative fitted values of gas production (mL/200 mgDM).

Samples	Incubation Time (h)						
	4	8	12	24	48	72	96
Pennisetum setaceum	0.48 [a]	5.58 [a]	10.77 [a]	22.25 [a]	33.96 [a]	38.56 [a]	40.37 [a]
Ricinus communis	8.00 [b]	16.92 [b]	23.60 [b]	35.16 [b]	42.06 [b]	43.28 [b]	43.49 [b]
Arundo donax	5.28 [c]	11.76 [c]	17.25 [c]	29.12 [a]	40.53 [b]	44.84 [b]	46.38 [b,c]
Acacia melanoxylon	3.83 [d]	5.43 [a]	6.80 [d]	9.86 [c]	13.02 [c]	14.28 [c]	14.78 [d]
Opuntia ficus-indica	0.40 [a]	5.70 [a]	10.44 [a]	21.23 [a]	32.89 [a]	37.88 [a]	40.02 [a]
Agave americana	22.68 [d]	32.68 [d]	38.29 [e]	45.29 [d]	48.42 [d]	48.91 [d]	48.99 [c]
Pittosporum undulatum	0.99 [e]	1.15 [e]	3.12 [f]	8.15 [c]	15.14 [c]	19.41 [c,e]	22.02 [e]
Hedychium gardnerianum	1.66 [e]	3.31 [a,f]	4.88 [d,f]	9.21 [c]	16.30 [c]	21.74 [e]	25.90 [e]
SEM	1.55	2.37	2.89	3.54	2.98	3.47	2.15
p-value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

SEM: standard error of the mean. Different letters next to the respective value indicate significant differences in the nutritive parameters among sampling dates. $p < 0.05$ significant differences were found.

Figure 2 shows the cumulative patterns of in vitro gas production of the different plants over the hours of incubation.

The in vitro gas production kinetics parameters are shown in Table 5. All of *Acacia melanoxylon*, *Agave americana* L., and *Hedychium gardnerianum* showed lag times of 0 h. This means that the fermentation started right as the element was incubated. The *a* constant of the reaction kinetics varied between a value of −7.56 mL/200 mgDM pa for *Pennisetum setaceum* and 1.96 mL/200 mgDM for *Acacia melanoxylon. Arundo donax* produced the highest insoluble fraction, *b* (49.65 mL), at the degradation rate *c* (0.0389 mL/h), which is similar to the value obtained for *Penisetum setaceum*, which produced *b* (49.10 mL) at a rate of 0.0419 mL/h. In contrast, *Acacia melanoxylon* generated *b* (13.16 mL) at a degradation rate *c* of 0.0382 mL/h.

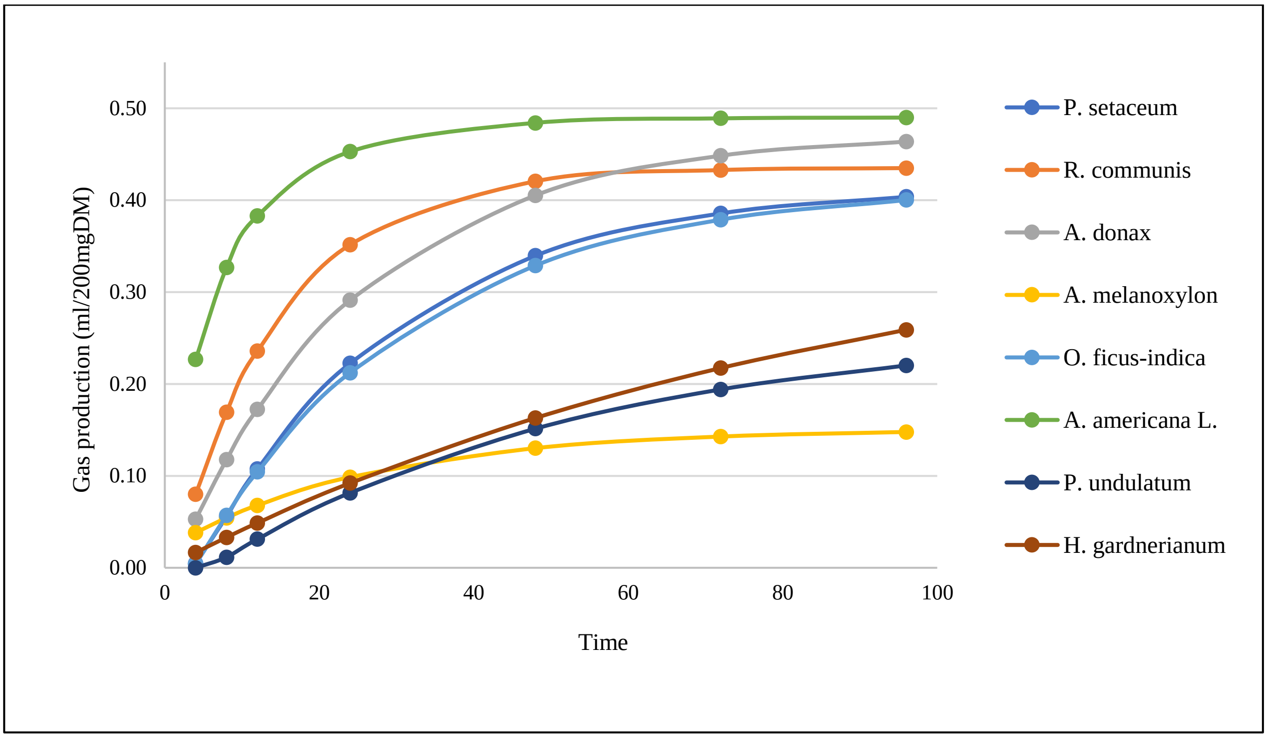

Figure 2. Cumulative fitted values of gas production of the different plants.

Table 5. In vitro gas production kinetics parameters of the different plants.

Samples	a	b	c	tlag	rsd
Pennisetum setaceum	−7.56 [a]	49.10 [a]	0.0389 [a,e]	4.3 [a]	2.35
Ricinus communis	−3.91 [b]	47.45 [b]	0.0722 [b]	1.2 [b]	1.38
Arundo donax	−2.39 [c]	49.65 [a]	0.0419 [a]	1.2 [b]	1.24
Acacia melanoxylon	1.96 [d]	13.16 [c]	0.0382 [a,e]	0 [c]	0.31
Opuntia ficus-indica	−4.96 [e]	41.64 [d]	0.0356 [e]	3.20 [d]	1.54
Agave americana L.	3.36 [f]	45.83 [b]	0.174 [c]	0 [c]	1.39
Pittosporum undulatum	−3.31 [b]	29.41 [e]	0.0205 [d]	5.8 [e]	1.10
Hedychium gardnerianum	0.06 [g]	39.54 [d]	0.0111 [d]	0 [c]	0.05
SEM	0.26	1.45	0.015	0.56	
p-value	<0.05	<0.05	<0.05	<0.05	

a: gas production of the immediately soluble fraction (mL/0.2 g DM); b: gas production of the insoluble fraction (mL/0.2 g DM); c: gas production rate constant for the insoluble fraction (mL/h); Lag t: time it takes to produce gas (h); RSD: residual standard deviation. SEM: standard error of the mean. Different letters next to the respective value indicate significant differences in the nutritive parameters among sampling dates. $p < 0.05$ significant differences were found.

Table 6 shows the values of the metabolizable energy and net energy lactation of the different plants. The highest estimates of ME (8.72 MJ/kgDM) and NEL (5.06 MJ/kgDM) were obtained for *Agave Americana*, while *Ricinus communis* recorded the second highest estimates of ME (8.40 MJ/kgDM), and NEL (5.08 MJ/kgDM). On the other hand, *Pittosporum undulatum* and *Hedychium gardnerianum* had similar ME (3.81 vs. 3.99 MJ/kgDM) and NEL (1.60 vs. 1.57 MJ/kgDM) values, which were the lowest estimated values.

Table 6. Metabolizable energy and net energy lactation.

Samples	ME (MJ/kgDM)	NEL (MJ/kgDM)
Pennisetum setaceum	6.21 [a]	3.26 [a]
Ricinus communis	8.40 [b]	5.08 [b]
Arundo donax	7.08 [c]	3.96 [c]
Acacia melanoxylon	4.55 [d]	2.11 [d]
Opuntia ficus-indica	5.39 [e]	2.54 [e]
Agave americana L.	8.72 [f]	5.06 [b]
Pittosporum undulatum	3.81 [g]	1.60 [f]
Hedychium gardnerianum	3.99 [g]	1.57 [f]
SEM	2.94	0.99
p-value	<0.001	<0.001

ME: metabolizable energy (MJ/kg/DM); NEL: net energy lactation (MJ/kgDM); SEM: standard error of the mean. Different letters next to the respective value indicate significant differences in the nutritive parameters among sampling dates. $p < 0.05$ significant differences were found.

It is possible to observe in Table 7 the existing correlations between the chemical compositions and the feed quality indices of the studied plants. In general, the results showed strong negative correlations between ADF and DMD, OMD, and DMI ($r > 0.86$, $p < 0.01$). There was also a very strong negative relationship between ADF and RFV ($r = 0.91$, $p < 0.01$), and a perfect negative correlation between ADF and DDM ($r = 1.00$, $p < 0.01$). We can also highlight a very strong relationship between NDF, DMI, and RFV, showing a value of $r > 0.92$ ($p < 0.01$).

Table 8 shows us the existing association between chemical composition and gas production. We observe that there are not very strong correlations in this association; the strongest positive links found were between digestibility (dry matter and organic matter) and gas production at 24, 48, 72, and 96 h, with the r ranging between 0.791 and 0.899 with a $p < 0.01$.

Table 9 shows the relationships between chemical compositions and in vitro degradation kinetics (b, c, ME, and NEL). Only the ADL content is strongly negatively correlated with *c* ($r = 0.93$, $p = 0.01$), with all other correlations being moderate, weak, or very weak.

Table 7. Pearson correlation coefficients among chemical composition and feed quality indices.

	CP	NDF	ADF	ADL	EE	Ash	HEM	CEL	DMD	OMD	DMI	DDM	RFV
DM	0.385	0.296	0.640 **	0.789 **	0.646 **	−0.788 **	−0.071	−0.195	−0.654 **	−0.566 **	−0.468 *	−0.640 **	−0.525 **
CP		0.235	0.179	0.122	0.192	−0.348	0.221	0.066	−0.025	0.055	−0.212	−0.179	−0.207
NDF			0.830 **	0.210	−0.221	−0.067	0.883 **	0.742 **	−0.646 **	−0.656 **	−0.953 **	−0.830 **	−0.929 **
ADF				0.660 **	0.074	−0.476 *	0.472 *	0.396	−0.886 **	−0.863 **	−0.890 **	−1.000 **	−0.917 **
ADL					0.345	−0.696 **	−0.224	−0.429 *	−0.704 **	−0.661 **	−0.332	−0.660 **	−0.393
EE						−0.683 **	−0.412 *	−0.333	−0.340	−0.258	0.001	−0.074	−0.058
Ash							0.295	0.279	0.583 **	0.454 *	0.289	0.476 *	0.359
HEM								0.841 **	−0.276	−0.311	−0.758 **	−0.472 *	−0.697 **
CEL									−0.205	−0.229	−0.664 **	−0.396	−0.622 **
DMD										0.986 **	0.758 **	0.886 **	0.795 **
OMD											0.734 **	0.863 **	0.764 **

DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent insoluble fiber; ADF: acid detergent insoluble fiber; ADL: acid detergent lignin; HEM: hemicellulose; CEL: cellulose; DMD: dry matter digestibility; OMD: organic matter digestibility; DMI: dry matter intake; DDM: digestible dry matter; RFV: relative feed value, significant * $p < 0.05$; ** $p < 0.01$.

Table 8. Pearson correlation coefficients of chemical composition, feed quality indices with in vitro incubation times.

	Incubation Time (h)						
	4	**8**	**12**	**24**	**48**	**72**	**96**
MS	−0.253	−0.354	−0.398	−0.468 *	−0.546 **	−0.607 **	−0.655 **
PB	−0.100	−0.012	0.044	0.131	0.139	0.095	0.053
NDF	−0.380	−0.426 *	−0.436 *	−0.415 *	−0.348	−0.303	−0.274
ADF	−0.316	−0.451 *	−0.516 **	−0.604 **	−0.650 **	−0.663 **	−0.666 **
ADL	−0.200	−0.352	−0.439 *	−0.583 **	−0.715 **	−0.791 **	−0.841 **
EE	−0.113	−0.177	−0.207	−0.270	−0.349	−0.391	−0.413 *
CB	−0.162	−0.031	0.049	0.206	0.385	0.484 *	0.536 **
DMS	0.412 *	0.581 **	0.668 **	0.791 **	0.858 **	0.864 **	0.853 **
DMO	0.509 *	0.670 **	0.751 **	0.859 **	0.899 **	0.888 **	0.866 **
HEM	−0.335	−0.295	−0.256	−0.148	−0.003	0.079	0.128
CEL	−0.136	−0.111	−0.084	−0.013	0.092	0.170	0.226
DMI	0.292	0.378	0.412 *	0.439 *	0.422 *	0.399	0.380
DDM	0.316	0.451 *	0.516 **	0.604 **	0.650 **	0.663 **	0.666 **
RFV	0.272	0.372	0.414 *	0.460 *	0.461 *	0.448 *	0.433 *

DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent insoluble fiber; ADF: acid detergent insoluble fiber; ADL: acid detergent lignin; HEM: hemicellulose; CEL: cellulose; DMD: dry matter digestibility; OMD: organic matter digestibility; DMI: dry matter intake; DDM: digestible dry matter; RFV: relative feed value, significant * $p < 0.05$; ** $p < 0.01$.

Table 9. Pearson correlation coefficients between chemical composition and in vitro degradation kinetics, b, c, ME, and NEL.

	b	**c**	**ME**	**NEL**
MS	−0.245	−0.653 **	−0.365	−0.315
PB	0.108	0.130	0.326	0.373
NDF	−0.418 *	−0.015	−0.347	−0.346
ADF	−0.409 *	−0.518 **	−0.533 **	−0.516 **
ADL	−0.231	−0.926 **	−0.528 **	−0.503 *
EE	−0.085	−0.339	−0.223	−0.172
Ash	0.042	0.652 **	0.123	0.075
HEM	−0.317	0.413 *	−0.100	−0.112
CEL	−0.210	0.509 *	0.005	−0.006

DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent insoluble fiber; ADF: acid detergent insoluble fiber; ADL: acid detergent lignin; HEM: hemicellulose; CEL: cellulose, significant * $p < 0.05$; ** $p < 0.01$.

4. Discussion

Agriculture is an activity that is highly dependent on climatic factors such as temperature, rainfall, soil moisture, and solar radiation. Climate change can affect agricultural production due to the increase in temperature and the reduction of precipitation, resulting in longer periods of drought. The demand for using plants found in the natural flora as unconventional fodder for animal production has increased, not only in areas of the globe where the drought is increasingly severe and where fodder is scarce but also in developed countries where this type of food can make the activity more economically and environmentally sustainable [21]. Investigating how to best use the plants of a region is an important step towards finding a balance between the highest nutritional value and the lowest emission of polluting agents [22]. There have been several studies carried out with the aim of deepening the understanding of non-conventional forages [4], namely their interference with reproduction [23], their nutritional value, and their potential in combating the emission of greenhouse gases [19,24].

As animal feed is one of the aspects that most influence production [3], it is important to understand and know the specific characteristics of forages regarding their nutrients and digestibility [21,25], which allow supporting the design of a diet that meets the nutritional needs of animals [26,27].

Forages can have their chemical composition influenced by several factors, which include their genotype, maturity stage, and harvest time [28].

A routine system of chemical characterization of forages should include the determination of fiber, crude protein, and crude ash, among others. For this to be possible, the accurate determination of dry matter, which represents the percentage of plant tissue that remains after drying, is fundamental [5].

Opuntia ficus-indica (6.65%) and *Agave americana* (11.15%) showed the lowest dry matter values among the plants being studied. This can be justified by the fact that these are the only CAM plants (crassulacean acid metabolism) considered in this study. These species usually have a high-water retention capacity and, compared to other species, can produce up to 5 times more DM per millimeter of rainfall [29], making them a viable complementary source of water for ruminants.

In addition, *Hedychium gardnerianum* obtained a low value of dry matter (11.89%), similar to that found by [5], and is also considered a good source of water, especially during the dry season.

The highest value of dry matter found was 39.43% (*Acacia melanoxylon*), similar to that referenced by [5]. According to [30], Acacia is distributed in most parts of the world, occupying vast areas of land due to its phenotypic plasticity, which allows it to adapt and establish itself successfully in changing environments.

The lowest crude protein content found in these plants was for *Opuntia ficus-indica* (4.94% DM) and *Agave americana* (5.69% DM), which is below the threshold of 7%, typically seen as the required minimum value for normal ruminal microorganism function [31,32]. Regarding *Opuntia*, it should be noted that [33] reports variable amounts of CP across several plant varieties and that some clones from Brazil could exceed 11% DM of CP. In the case of *Agave americana L.* [7], crude protein values were also similar to those found in this study, ranging between 5.16% DM and 6.30% DM.

Moreover, for *Pittosporum undulatum* and *Hedychium gardnerianum*, low crude protein values were found, 7.84% DM and 8.78% DM respectively, which are similar to the values found in a study by [19], although in this study the crude protein of *Pittosporum undulatum* did not exceed 6.11% DM. On the other hand, [5] references crude protein values for *Pittosporum undulatum* of 7.96% DM and for *Hedychium gardnerianum* of 12.03% DM.

Crude protein values above 15% DM, required for optimum growth and lactation of dairy cattle [34], were found for the remaining plants in the study.

For *Pennisetum setaceum*, *Acacia melanoxylon*, and *Arundo donax*, the crude protein values were not very different (16.61% DM, 16.99% DM, and 15.69% DM, respectively). [35] found crude protein values between 13.0% DM and 16.9% DM for different types of Acacia, while [5] reported crude protein values of 16.86% DM for the same Acacia species in our study.

In addition, [5] reported slightly higher crude protein values for *Arundo donax* (16.86% DM), while [36] reported protein values of 7.61% DM lower than those found in our study.

The highest crude protein value found in this study, 24.51% DM, for *Ricinus communis*, was very similar to that reported by [37]. However, the plant contains ricin, a highly toxic compound in the seeds that has a potent cytotoxic effect that promotes severe gastroenteritis [38]. Ricin is a toxic alkaloid present in the leaves, stems, and pericarps of *Ricinus communis* seeds that can induce severe neurological disorders [38,39]. Although there are now studies that suggest ways for the plant's toxicity to be overcome [40], in particular, a number of chemical and physical methods have been tested. The chemical methods consisted of the use of formaldehyde, ammonia, lime, tannic acid, sodium chloride, and sodium hydroxide at different concentrations. The physical methods were boiling, immersion, steaming, autoclaving, and heating. The conclusion was that, from the means studied, the calcium hydroxide treatment was the only effective method for eliminating the ricin [41] determined that the thermal detoxification process is able to degrade toxic molecules present in ricin, making it a practical and efficient method to be used on a large scale.

According to [8], in vitro dry matter digestibility is one of the main criteria for assessing the usefulness of feeds used in livestock production since it reflects the amount of plant material that can be digested by the ruminants. In this study, *Acacia melanoxylon* (27.32%), *Pittosporum undulatum* (39.54%), and *Hedychium gardnerianum* (31.37%) showed DMD values below 50%, which, according to [42], is the minimum DMD value for the maintenance needs of animals [5] also found similar DMD values but admits, citing [43], that the digestibility of shrubs and trees is underestimated, possibly due to secondary metabolites, such as tannins or saponins, being present. These can have a negative impact on in vitro digestibility values.

Of all the plants studied, *Opuntia ficus-indica* was the one that obtained the highest DMD of 84.41%, which is consistent with the value reported by [44]. According to [45], *Opuntia ficus-indica* is highly digestible. It was also found that *Ricinus communis* has a high digestibility value (79.19%), similar to that observed by other authors [46,47].

Table 2 shows the analytical detergent fiber (ADF) of the different plants. Fiber is made up of complex polysaccharides and represents the plant fraction that is partially digestible in the gastrointestinal tract of herbivorous animals. By determining the NDF, ADF, and ADL, information about different cell wall fractions can be obtained, such as cellulose, hemicellulose, and lignin. Non-conventional plants are characterized by having a higher cell wall-to-cell content ratio than other forage types. Plant quality is not solely influenced by the species, however, as the state of maturity, handling, and soil quality all play a role as they impact the leaf area and photosynthetic capacity of the plants [48]. Dry seasons usually lead to an increase in cellulose, hemicellulose, and lignin. This, in turn, increases the forage resistance due to the existence of more cellulose and lignin connections, which results in a harder process of ingestion, rumination, and fermentation by microorganisms [28].

According to [49], when a diet's NDF content is above 55%, the feed intake is lower. This was corroborated in this trial, where it was found that there was a negative correlation between plant NDF values and dry matter intake. There were several plants that obtained values higher than 55%, such as *Pennisetum setaceum*, which presents the highest NDF value in this study (80.39% DM) and also has high contents of HEM and CEL (39.03% DM and 38.81% DM, respectively), comparable to the results found by [37]. *Hedychium gardnerianun* also obtained a high value of NDF (72.39% DM), which is a very different result from that found by [5] (38.02% DM). These differences in data between previous studies and the present one for the same plant can be attributed to variations in soil characteristics and different leaf maturation stages when these studies were conducted [50]. *Ricinus communis*, *Opuntia ficus-indica*, *Agave Americana* L., and *Pittosporum undulatum* had NDF values below 50% DM.

The in vitro evaluation of these plants (Tables 4–6) provides further insights into the nutritional value and energy contents of these plants, leveraging on the relation between the amount of gas produced in in vitro fermentation, the extent of fermentation and digestibility of a forage [51], and the rate at which the substrate is degraded [8].

The highest gas production and energy estimates recorded were for *Ricinus communis*, *Arundo donax*, and *Agave americana* L., which were also the ones that obtained the highest DMD values.

As shown in Table 5, *Acacia melanoxylin*, *Agave americana*, and *Hedychium gardnerianum* have a lag time of 0, which means the process of fermentation initiates just as the element is incubated. This plant has a high saponin content, as noted by some authors [52]. It would, therefore, be of interest to determine anti-nutritive substances in future works. These plants were also the only ones for which a positive value for *a* was obtained. This indicates the component started to degrade quickly. Conversely, a negative value would have meant that there was an initial period during which no cell wall degradation occurred, called the lag phase [53].

According to the Quality Rating Standard assigned by the Hay Marketing Working Group of the American Forage and Grassland Council, *Pennisetum setaceum*, *Acacia melanoxylon,* and *Hedychium gardnerianum* were considered of no interest for animal feed. *Arundo donax* is also considered of poor quality. *Ricinus communis*, *Opuntia ficus-indica,* and *Agave americana* L. were classified as premium. *Pittosporum undulatum* was rated good.

5. Conclusions

With the results obtained in this work and based on the chemical composition, gas production, and other estimates of nutritional importance evaluated, we can conclude that all these unconventional invasive plants show promising nutritional qualities that would make them a viable alternative feed for ruminants when there is a shortage of forage.

Among the species that were studied, *Opuntia ficus-indica*, *Agave americana* L., and *Hedychium gardnerianum* were the ones that presented the lowest dry matter values, meaning they are rich in water and can be used to complement diets during dry periods. *Pittosporum undulatum*, *Acacia melanoxylon*, and *Arundo donax* showed high fiber values and can therefore be used as roughage during the winter.

Pennisetum setaceum, *Ricinus communis,* and *Opuntia ficus-indica* were the plants with the highest digestibility, whereas *Pittosporum undulatum* showed low digestibility values despite having a low protein content. *Pittosporum* also achieved a good RFV fodder grade, which means it can be used as an alternative source of fiber fodder for ruminants.

Overall, *Ricinus communis* gave the best results, although further studies are needed to suggest ways for the plant's toxicity to be overcome.

Regarding gas production, *Agave americana* obtained the highest values from the beginning to the end of the incubation, while *Acacia melanoxylon* produced the least amount of gas.

However, further research is needed to identify the presence of secondary metabolites as well as in vivo animal response tests to determine their acceptability and impact on animal health and performance.

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