

Nutritional valorisation of cane (*Arundo donax*) by treatment with sodium hydroxide

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Abstract

This study evaluated the effects of sodium hydroxide (NaOH) treatment on the nutritional value of *Arundo donax* (*A. donax*). Its ultimate goal was to develop an environmentally friendly animal feed alternative that could be produced sustainably while combatting the spread of *A. donax*. Plants were collected and dried at 60 °C in an oven with controlled air circulation to determine the dry matter (DM) content. The dry plant material was then sprinkled with an NaOH solution of 2%, 4%, 6%, or 8% and placed in leak-proof containers for four weeks. The chemical and in vitro digestibility properties of treated and untreated *A. donax* samples were analysed in triplicate. The treatment led to significant decreases in neutral detergent fibre (NDF) from 81.06% DM to 69.39% DM, acid detergent fibre (ADF) from 46.99% DM to 43.19% DM, and ether extract (EE) from 2.06% DM to 1.39% DM, in the untreated samples compared with those treated with 8% NaOH, respectively. In addition, DM digestibility increased from 24.61% to 33.78%, ash content from 11.75% DM to 19.92% DM, and ADL from 7.43% M to 15.38% DM. Thus, treatment of *A. donax* with an 8% solution of NaOH concentration improved its nutritional value.

Keywords: digestion kinetics, gas production, in vitro digestibility

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Introduction

Minimizing concentrate feeds and maximizing the use of forages have become increasingly important considerations for the diets of ruminant animals. However, the low digestibility of forages relative to concentrates can compromise animal production.

The feeding of ruminants in the Azores is based mainly on grass, specifically Gramineae. Each year the production of grass is insufficient to meet the nutritional requirements of the animals in the dry months. Thus, the dry season causes nutritional stress and decreases animal productivity. A forage deficiency makes it necessary to import feedstuffs (Borba, 2015). However, supplementation with concentrates during the dry season may not be a profitable practice owing to high costs. A potential strategy to increase the quality and availability of food for ruminants in the dry season may be through feeding unconventional bushes and forages (Pezo, 1991) such as *Pittosporum undulatum*, *Hedychium gardnerianum*, *Morella faya* and *Ilex perado* (Borba, 2007). Shrubs and non-conventional forages are low-quality fibrous products that are characterized by cellulose, hemicellulose, and lignin contents of around 70%, and low concentrations of soluble carbohydrates, protein, minerals, and vitamins. Such products do little to stimulate the cellulolytic flora of the reticulum-rumen, making their fibrous structure inadequately degradable. Given that these foods have low food value, low digestibility, and low voluntary intake, they do not satisfy the maintenance needs of ruminants (Borba, 2006; Rocha, 2016).

Physical, chemical and biological treatment of low-quality roughages could provide quality fodder, meeting the nutritional requirements of the animals and mitigating the effects of the scarcity of high-quality forage (Bertipaglia *et al.*, 2005). In recent years, several studies have been carried out on the valorization of *Hedychium gardnerianum* (Borba *et al.*, 2015). However, treatment of green *Hedychium gardnerianum* fodder with urea did not have the same effect as similar treatment of low-quality fibrous straws, because the green fodder had a much higher crude protein content than the straws. Rocha *et al.* (2019) studied *A. donax*

treated with urea and concluded that treatment with urea at 3.5% of DM had no significant effect on its nutritional value and postulated that other treatments might be more effective.

Arundo donax, commonly known as giant cane or elephant grass, is a robust perennial herb that can be found from coastal dunes, cliffs, and coastal wetlands to bushes, permanent and semi-natural pasture, as well as in agricultural areas and anthropic vegetation. It is one of most widespread Macaronesian invasive plants. *Arundo donax* infestations create serious physical and biological problems. Its biomass creates obstacles during storm events, and can lead to flooding of adjacent lands, erosion of stream banks, and change in natural flow patterns. Therefore, it was proposed to study the effect of treatment of *A. donax* with NaOH on the chemical composition and nutritional value of the residue to see if it could be used by Azorean farmers to feed livestock. If such treatment were found to make a useful feedstuff, then the vegetative propagation of *A. donax* might be reduced.

Material and Methods

The present study was conducted in Prof. Doutor Gourlay Young do Amaral Animal Nutrition Laboratory, Department of Agricultural Sciences, University of the Azores, Angra do Heroísmo, Terceira, Azores, Portugal. The plant, which was harvested manually in full vegetative state in Terceira Island was chopped to pieces that were approximately 10 cm long. The samples were sprayed with NaOH solution (control group, 2%, 4%, 6%, and 8%). For each treatment, 1 kg samples were placed in plastic boxes with lids and observed for seven days. After treatment, the samples were dried in a forced air oven at 65 °C for 72 hours.

Dried samples were then ground through a 1-mm screen using a Retsch mill (GmbH, 5657 Haan, Germany). These ground samples were analysed for DM (method 930.15), crude protein (CP) (method 954.01) and total ash (method (42.05), according to AOAC (1995). Briefly, the dry matter content was determined by placing samples in a forced air oven at 105 °C for 24 hours. Ash was measured by burning samples in a muffle furnace at 500 °C for 12 hours. Crude protein was determined by the standard micro-Kjeldahl method, using digestion equipment (Kjeldatherm System KT 40, Gerhart Laboratory Instruments, Bonn, Germany) and an automated Kjeltac 2300 auto-analyser apparatus for distillation and titration (Foss Electric, Copenhagen, Denmark). Acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF) were determined following the methods of Goering and Van Soest (1970). In vitro digestibility was determined using the Tilley and Terry (1963) method as modified by Alexander & McGowan (1966). The rumen fluid used in assaying in vitro digestibility was obtained from a local slaughterhouse.

Each assay was repeated three times using the in vitro gas production (GP) technique which simulates rumen fermentation. This technique is used to evaluate the potential for ruminant animals to produce greenhouse gases when fed a particular feedstuff. Blanks were used for each inoculum to measure the fraction of total gas production because of substrate in the inocula and these values were subtracted from the total to obtain net GP. All treatments were incubated simultaneously (Menke *et al.*, 1979). The preparation of buffer solutions and rumen inocula was as described by Menke and Steingass (1988). Gas production was recorded after 4, 8, 12, 24, 48, 72, and 96 hours of incubation. Kinetics of the in vitro gas production were described using an exponential model (McDonald, 1981; Ørskov & McDonald, 1979):

$$p = a + b(1 - \exp^{-ct})$$

where: p was the gas production at time t ;

the values of a and b were constant values in the exponential equation with their sum predicting total potential gas production (ml/g DM), and

c was the rate constant.

The parameters were estimated using the Neway software program (Rowett Research Institute, Aberdeen, UK), which was developed by Chen (1997).

Effects of the treatments on the parameter estimates were assessed using SPSS (IBM SPSS Statistics for Windows, version 24.0., IBM Corp., Armonk, New York, USA). Normality of the data was evaluated with the Shapiro-Wilk test and ANOVA was performed, followed with post-hoc Tukey least significant difference test. For all analyses, a P -value less than 0.05 was considered to indicate a difference.

Results and Discussion

Owing to its high fibre and low protein contents, *A. donax* foliage is digested poorly. In a study by Rocha *et al.* (2019) urea was applied to *A. donax*, but the concentration that was used did not produce favourable results. That motivated the use of NaOH in the current study. Also, Pires *et al.* (2006) concluded that NaOH and calcium hydroxide were more efficient than urea in degrading the cell wall and increasing

digestibility. In the current study, chemical treatment of *A. donax* with sodium hydroxide (NaOH) affected some of its nutritional components (Table 1). There was a difference in DM between treatments with 2% and 8% NaOH. Treatment with 2% NaOH decreased ($P < 0.05$) the amount of CP relative to both the untreated control and the 4% NaOH treatment. However, treatment of the forage with higher levels of NaOH did not alter the CP content significantly. The changes in NDF and ADF were similar across treatments, with the fibre contents being reduced as the level of NaOH applied was increased. Treatment with 8% NaOH reduced the EE content relative to the other treatments, which performed similarly. The ash content increased with increased levels of NaOH. However, these effects did not manifest themselves in changes of dry matter digestibility (DMD).

Table 1 Chemical composition and nutritive value of *Arundo donax* when augmented with various levels of sodium hydroxide

Nutrient	Treatment				
	Control	2% NaOH	4% NaOH	6% NaOH	8% NaOH
DM, %	33.66 ^{ab} ± 0.95	34.84 ^b ± 0.77	33.19 ^{ab} ± 1.25	33.35 ^{ab} ± 0.51	31.73 ^a ± 0.58
DMD, %	24.61 ^a ± 3.41	24.66 ^a ± 0.45	25.02 ^a ± 0.84	26.66 ^a ± 0.80	33.78 ^b ± 1.39
CP /100g DM	7.61 ^a ± 0.14	6.18 ^b ± 0.19	7.57 ^a ± 0.34	6.63 ^{ab} ± 0.91	7.37 ^{ab} ± 0.51
NDF /100g DM	81.06 ^a ± 4.55	75.90 ^{ab} ± 0.30	72.17 ^b ± 0.81	72.43 ^b ± 1.12	69.39 ^b ± 3.05
ADF /100g DM	46.99 ^a ± 0.58	45.07 ^{ab} ± 1.33	43.99 ^b ± 0.72	42.80 ^b ± 0.60	43.19 ^b ± 1.04
ADL /100g DM	7.43 ^a ± 0.40	7.59 ^a ± 0.26	9.29 ^{ab} ± 2.93	13.12 ^{bc} ± 1.14	15.38 ^c ± 2.59
EE /100g DM	2.06 ^a ± 0.24	2.02 ^a ± 0.01	2.32 ^a ± 0.18	2.03 ^a ± 0.12	1.39 ^b ± 0.38
Ash /100g DM	11.65 ^a ± 1.07	12.49 ^{ab} ± 0.43	15.63 ^{ab} ± 1.67	16.08 ^{bc} ± 1.49	19.92 ^c ± 2.22

DM: dry matter, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, EE: extract ether, DMD: in vitro dry matter digestibility; NaOH: sodium hydroxide

^{a,b,c} Within a row, means with a common superscript were not different with probability $P = 0.05$

Crude protein showed a small decrease when the NaOH treatment was applied. This observed decrease was consistent with previous studies and was a disadvantage of this treatment when compared with others, such as treatment with ammonia or urea, which are sources of non-protein nitrogen (Madrid *et al.*, 1998). The decrease in NDF was most evident with the 8% concentration of NaOH. Similar results were reported by Pereira Filho *et al.* (2003), who indicated that increasing NaOH from 2% to 8% in black Jurema hay decreased its NDF content. The main effect of NaOH on cell wall components is the alkaline hydrolysis of covalent ester-like bonds between lignin and structural carbohydrates (Tarkov & Feist, 1969). Thus, hemicellulose becomes soluble with a consequent decrease in the NDF content (Wannapat *et al.*, 1985; Chaudhry, 2000). This facilitates fibre digestion by rumen microbes. In general, there was no discernible trend in ADF content resulting from the NaOH treatments. Many other studies have shown that the use of NaOH to treat fibrous foods does not alter ADF (Manzano *et al.*, 2000; Chaudhry, 1997; Sundstøl & Coxworth, 1984). In the work of Nolte *et al.* (1987), a decrease in ADF was obtained for wheat straw treated with an alkaline solution. A significant increase ($P < 0.05$) of ADL was observed with the 6% and 8% NaOH treatments compared with the control. Lignin content is not normally altered by chemical treatment, but this treatment led to increases in the rate of digestion of cellulose and hemicellulose by breaking links between these fractions (Feist *et al.*, 1970; Klopfenstein, 1980). The effect on EE of NaOH treatment of *A. donax* was not easily explained as there were no previously published studies on which to rely. The mineral residue from NaOH resulted in the expected increase in ash content with the increasing levels of NaOH. This increase was seen earlier by Moss *et al.* (1993).

Unlike in the present study, Pereira Filho *et al.* (2003), observed a significant increase ($P < 0.05$) in the DMD of black Jurema hay as the level of NaOH with which it was treated increased. Chaudhry (1997) pointed out that normally DMD of forages treated with an alkaline solution had a positive linear relationship when increasing levels of NaOH up to 8%. NaOH has been used frequently with other alkaline compounds and physical treatment because it was believed to be one of the most efficient chemical products for delignification of cell wall compounds in plant cells. The increase in the DMD of forage treated with NaOH

occurred because of the greater exposure of the cell wall components, increasing the susceptibility of structural carbohydrates to digestibility (Chaudhry, 1998).

Cumulative gas production as affected by treatment of *A. donax* forage with various levels of NaOH is shown in Table 2 and Figure 1. The *in vitro* GP technique simulates the rumen fermentation process and has been used frequently to evaluate the potential of food to produce gas. When the authors observed the lag time, they could see that *A. donax* supplemented with 2% NaOH began to ferment after 1.5 hours, which was before any of the other samples. The treatment with NaOH triggered a lower gas production, especially in the treatment with 8%, which could be a positive aspect in mitigating enteric gas production and consequently combating climate change.

Table 2 Effect of sodium hydroxide treatment on *Arundo donax* on cumulative gas production (ml 200 mg⁻¹ DM) and gas kinetics

Incubation time, hours	Treatment				
	Control	2% NaOH	4% NaOH	6% NaOH	8% NaOH
4	1.18	1.45	1.53	1.28	0.94
8	3.54	3.64	4.08	3.41	2.84
12	5.74	5.66	6.40	5.39	4.72
24	11.40	10.87	12.20	10.49	9.49
48	19.54	18.29	19.86	17.82	16.52
72	24.73	24.20	24.22	22.49	21.18
96	28.04	26.69	26.69	25.74	24.26
Kinetic parameter estimates					
a	-1.36	0.91	-1.28	-1.01	-1.15
b	35.21	31.84	31.24	31.70	31.43
c	0.0188	0.0192	0.0235	0.0188	0.0172
Lag time	2.10	1.50	1.80	1.70	2.20

a: gas production from the immediately soluble fraction (ml 200 mg⁻¹ DM), b: gas production from the insoluble fraction (ml 200 mg⁻¹ DM), c: gas production rate constant for the insoluble fraction (ml h⁻¹); NaOH: sodium hydroxide

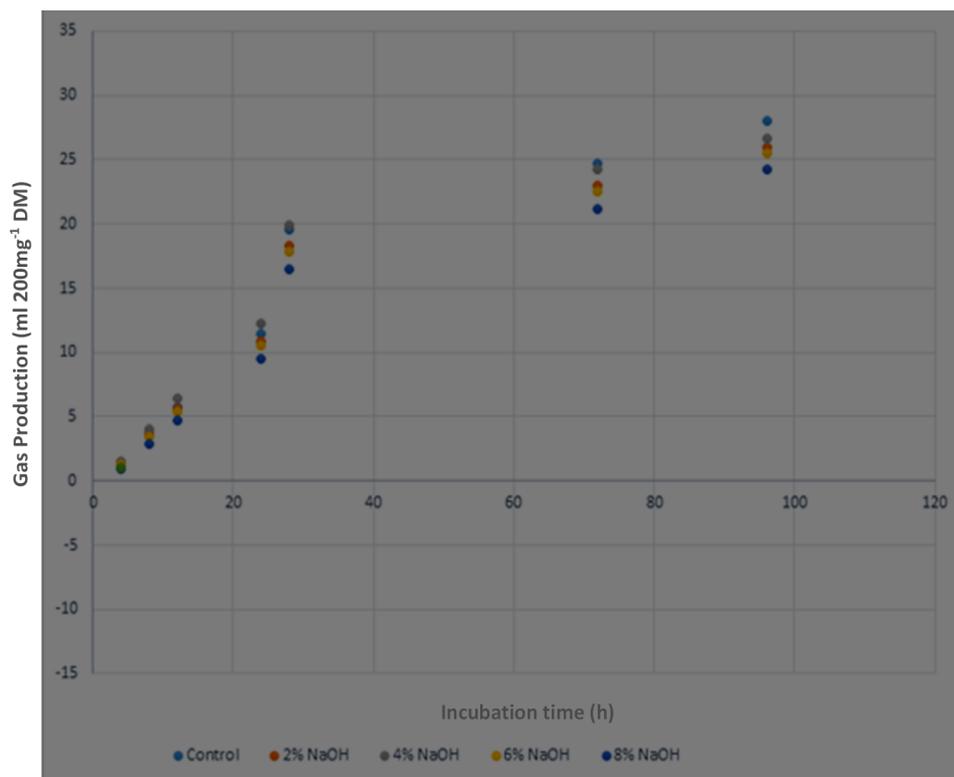


Figure 1 Pattern of in vitro gas production (fitted with exponential model) on incubation of *Arundo donax* treated with sodium hydroxide (NaOH) in buffered rumen fluid

Conclusions

Treatment of *A. donax* forage with 8% NaOH optimized its value because it decreased NDF and total gas production compared to the other treatments. Thus, treatment with NaOH improves *A. donax* as a feed for animals.

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Authors' Contributions

SMPT collected the data, performed the subsequent laboratory analysis and prepared the manuscript. CSAMMD and SMPT collaborated in the experimental design. CFMV and JSM conducted the statistical analysis. AESB supervised the work and reviewed the manuscript.

Conflict of Interest Declaration

None of the authors has any conflict of interest.

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