

# Optimizing nutritional value and biological profile of non-conventional forages for animal feeding by utilizing sodium hydroxide



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**Abstract** Increasing quality and decreasing quantity represent global trends in the forage processing for animal feed. Consequently, various techniques are applied to achieve these objectives. This study aims to evaluate the internal quality and biological attributes of leaves in animal reproduction by applying sodium hydroxide (NaOH) concentrations (control, 2, 4, 6, and 8%) and utilizing leaves from four non-conventional plants, namely *Hedychium gardnerianum* (HG), *Pittosporum undulatum* (PU), *Arundo donax* (AD), and *Acacia melanoxylon* (AM), while also examining their interactions simultaneously. NaOH doses did not affect the organic matter digestibility. A 2% NaOH concentration positively affected both acid detergent fibre and neutral detergent fibre. Notably, 6% NaOH significantly impacted dry matter, while ash content and dry matter digestibility peaked under treatment with 8% NaOH. The *Arundo donax* variety recorded the best values in ash and organic matter digestibility, while the leaves of *Acacia melanoxylon* significantly ( $P \leq 0.05$ ) influenced dry matter, acid detergent fibre, acid detergent lignin, and crude protein. The co-application of 2% NaOH with plant varieties demonstrated the most significant effectiveness on the observed characteristics, followed by 4% NaOH showing a subsequent impact. The findings of this study propose that utilizing NaOH concentrations with these plants represents a promising approach for including these forages in animal feeds.

**Keywords:** in vitro digestibility, ruminant nutrition, animal feeding, alternative forages, NaOH

## 1. Introduction

The primary global economic sector revolves around animal production. Nutrition stands out as a paramount factor significantly influencing animal production, often constituting over 50% of production costs. Effectively addressing the escalating challenges in competitiveness requires a focus on enhancing the quality, quantity, and availability of nutritional resources (Dias et al., 2023). The prevailing production system heavily relies on grazing but often faces challenges during periods of fibre scarcity (Giridhar and Samireddypalle, 2015). This fiber deficiency can lead to a decrease in food intake, affecting the growth, milk production and reproduction of animals. Such shortages are typically driven by fluctuations in pasture production or variations in climate that influence grass yields. The repercussions of climate change and biodiversity loss have altered the availability of feedstuffs, consequently, the dry season imposes nutritional stress, leading to a decline in animal productivity (Nunes et al., 2023). To address forage deficiencies, there is a necessity to import feedstuffs. Nonetheless, augmenting diets with concentrates during the dry season might not be a cost-effective practice due to elevated expenses. An alternative approach to enhance the quality and accessibility of food for ruminants during this period could involve incorporating unconventional sources and forages into their feed (Maleko et al., 2018). Shrubs and unconventional forages are characterized by their low-quality fibrous composition, with cellulose, hemicellulose, and lignin constituting approximately 70% of their content. Additionally, they exhibit low concentrations of soluble carbohydrates, protein, minerals, and vitamins. These characteristics contribute to a limited stimulation of the cellulolytic flora in the reticulum-rumen, rendering their fibrous structure inadequately degradable. Due to their low nutritional value, poor digestibility, and reduced voluntary intake, these forages are insufficient to meet the maintenance needs of ruminants (Borba, 2015; Rocha, 2017). Given these factors, reducing reliance on concentrated feeds, and optimizing forage utilization have emerged as critical factors in formulating diets for ruminant animals (Nunes et al., 2022). Therefore, the diminished digestibility of forages compared to concentrates can compromise challenges to animal production (Wales and Kolver, 2017). To fill this gap in knowledge regarding the quality and digestibility of unconventional forages, as well as best practices for their use in animal feed were implemented strategies to

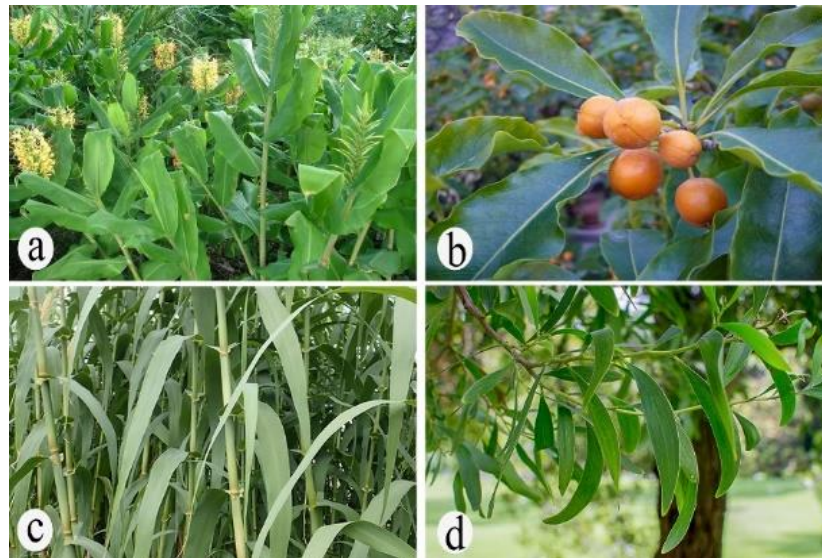


decrease quantity and enhance quality represents a profitable approach. Consequently, the treatment of forage leaves has a global history. In this regard, the initial reports on employing chemical treatments to enhance the digestibility of fibrous feeds trace back to 1881, with subsequent testing of various products (Borba et al., 2015; Mohamed et al., 2023). Chemical treatments, such as alkaline compounds, function by breaking down lignocellulosic complexes, leading to the solubilization of hemicellulose. This process increases cellulose digestibility by expanding the fibrous structure (Abe et al., 2021). Also, non-conventional plants play a significant role in shaping the internal quality of animal nutrition, as evidenced by studies on species such as *Pittosporum undulatum* and *Hedychium gardnerianum* (Nunes et al., 2017), *Arundo donax* (Al-Snafi, 2015) and *Acacia melanoxylon* have been recognized for their impact on animal nutrition (Maduro Dias et al., 2023). The objective of this investigation is to assess the internal quality of non-conventional forage for animal nutrition using different concentrations of sodium hydroxide (NaOH).

## 2. Materials and Methods

### 2.1. Experimental Design

In the current study, we collect leaves from non-conventional forages previously studied for animal nutrition. These included specimens such as *Hedychium gardnerianum* (HG), *Pittosporum undulatum* (PU), *Arundo donax* (AD), and *Acacia melanoxylon* (AM) in Figure 1. The plants were collected in Terceira Island, Azores, Portugal. The research involved the application of sodium hydroxide (NaOH) solutions at concentrations of 0 (control), 2, 4, 6, and 8% to treat leaves from four different species. The treatment included a ratio of 1 L of solution per 1 kg of dry leaves. Each treatment was made in triplicate. After seven days, samples from each chemical treatment were collected for laboratory analyses. The experimental design employed for this study was a randomized by Completely Randomized Design (RCD).



**Figure 1** The foliage of varieties was used for foraging processes such as. (a) *Hedychium gardnerianum* (HG), (b) *Pittosporum undulatum* (PU), (c) *Arundo donax* (AD), and (d) *Acacia melanoxylon* (AM).

### 2.2. Samples preparation

During this study, several parameters were analyzed to determine internal quality. Leaf dry matter content (DM%) was determined by taking the fresh weight of the leaf ( $W_b$ ) and drying it at 65°C in a forced-air oven for 72 hours until the weight was stable. Subsequently, it was weighed again ( $W_a$ ), and the dry matter percentage was calculated using the following equation (1):

$$DM\% = \frac{W_a}{W_b} \times 100 \quad (1)$$

The samples were ground using a Retsch mill (1 mm).

### 2.3. Chemical Analysis

The chemical characterisation of the plants was carried out using the Weende system. The adjusted dry matter (DM, method 930.15), crude protein (CP, method 954.01), ether extract (EE, method 920.39), and total ash (method 942.05) were determined according to the standard methods of A.O.A.C. Total ash content was assessed by igniting samples in a muffle furnace at 500°C for 12 hours. Crude protein was determined using the Kjeldahl method. The ether extract was measured by

refluxing forage samples with petroleum ether in a Soxhlet system. Acid detergent fibre (ADF), neutral detergent fibre (NDF), and acid detergent lignin (ADL) were analyzed following the procedures outlined by Goering and Van Soest (1970). Both NDF and ADF were expressed without residual ash.

In vitro, dry matter digestibility and organic matter digestibility were determined following the method of Tilley and Terry (1963), with modifications introduced by Alexander and McGowan (1966). The rumen fluid used to determine digestibility was collected at the local abattoir from 5 healthy dairy cattle fed ryegrass (*Lolium multiflorum*) and maize silage. The ruminal fluid was collected 10 minutes after slaughter, filtered through gauze, stored at 39° C under anaerobic conditions, and transported to the animal nutrition laboratory 30 minutes after collection [Borba et al., 2001].

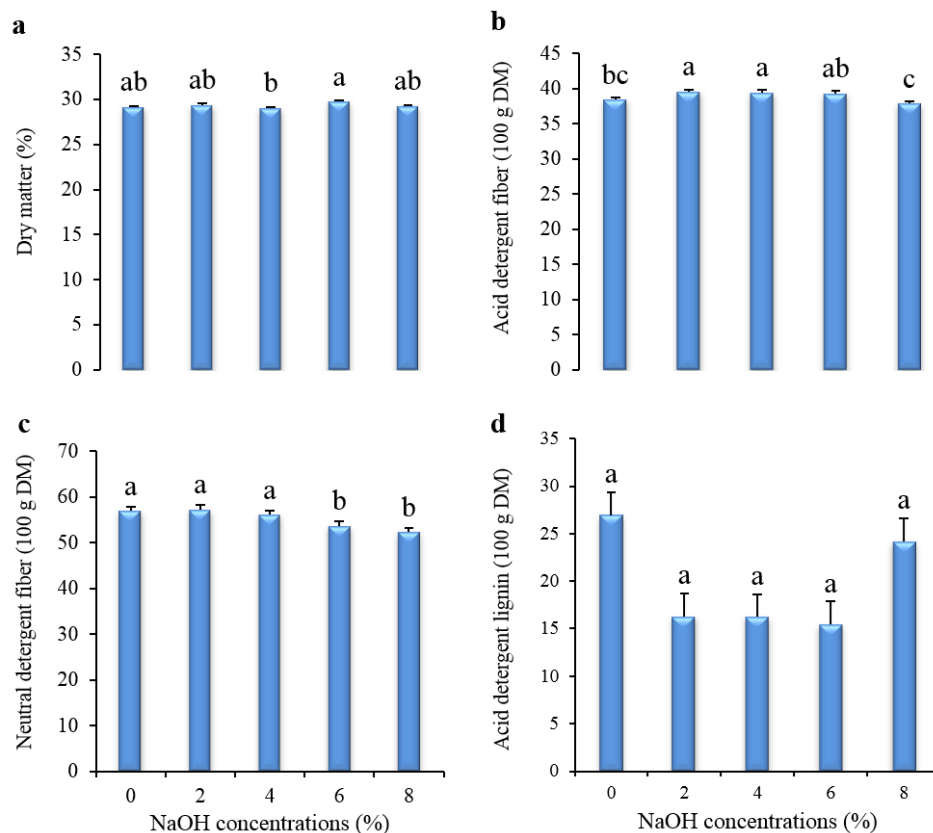
#### 2.4. Statistical analysis

A Completely Randomized Design (CRD) with three replications was followed in this study. The analysis of variance (One-way ANOVA), Duncan's new multiple range test at  $P \leq 0.05$ , the multiple correlation test, and principal component analysis (PCA) were implemented using XLSTAT software (version 2019.2.2).

### 3. Results

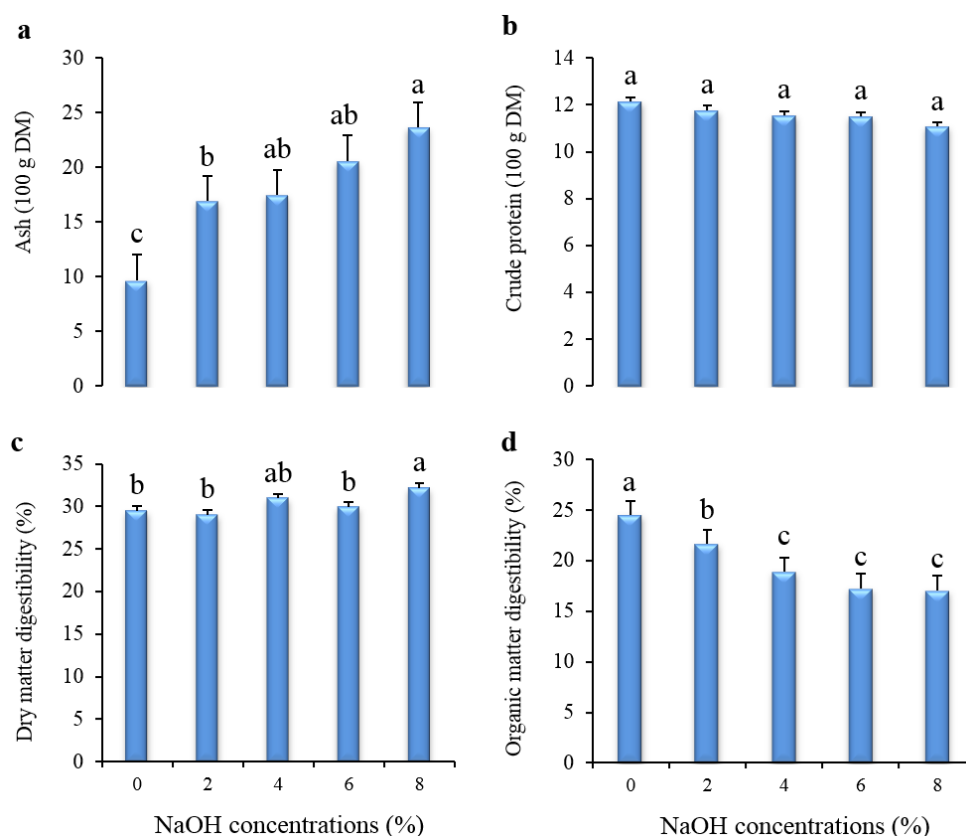
#### 3.1. Effect of NaOH concentrations on the studied variables

The present investigation evaluated the impact of optimal NaOH concentrations on the variables under consideration. The statistical analysis, specifically Analysis of Variance (ANOVA), conducted on the measurements revealed an impact of the treatments on the leaves used for the forage process, as shown in Figure 2. Regarding the percentage of dry matter, it was observed that leaves treated with 6% NaOH exhibited the highest values at 29.77%. In contrast, the characteristic recorded the lowest values at 29.05% for plant leaves under the 4% NaOH condition, as depicted in Figure 2a. Figure 2b indicates the optimal value for acid detergent fiber (39.53 g/100 g DM), achieved under the 2% NaOH, with a similar trend observed for the 4% NaOH. Conversely, the lowest value (37.86 g/100 g DM) was recorded with 8% NaOH. A 2% concentration of NaOH resulted in the highest production of neutral detergent fiber (57.27 g/100 g DM) in the leaves, followed by the control and 6% NaOH treatments. Meanwhile, the 8% NaOH treatment recorded the minimum production (52.28 g/100 g DM), although it did not show a statistically significant difference from the 6% NaOH in Figure 2c. Moreover, applying NaOH at all concentration levels did not significantly influence the leaves for acid detergent lignin (Figure 2d).



**Figure 2** Effect of various doses of NaOH on (variables of leaf plant. According to Duncan's test, each bar represents a mean  $\pm$  SE (n=3), and different letters denote significant statistical differences within a range of  $P \leq 0.05$ .

As shown in Figure 3, applying different NaOH doses substantially impacts biological attributes in the plant leaves for forage purposes. According to Figure 3a, increasing NaOH doses led to a significant elevation in ash measurements. The highest value (23.63 g/100 g DM) was recorded at 8% NaOH. Statistically, untreated leaves exhibited significantly lower ash values (9.71 g/100 g DM) than the other treatments. In Figure 3b, no significant differences were observed in crude protein content between the NaOH and control treatments. For dry matter digestibility, the percentage positively responded to varying levels of NaOH. The best rate (32.18%) was attained by applying 8% NaOH, while the minimum (29.05%) was registered with 2% NaOH, as stated in Figure 3c. The organic matter digestibility percentages of the treated plant leaves differed from those of the others (Figure 3d). The treatments that maximized digestibility (24.49%) were observed in leaves under the control condition. In contrast, with increasing NaOH doses, a decreasing trend in percentage was noted, reaching 17.09% with 8% NaOH concentration, with significant differences ( $P \leq 0.05$ ).

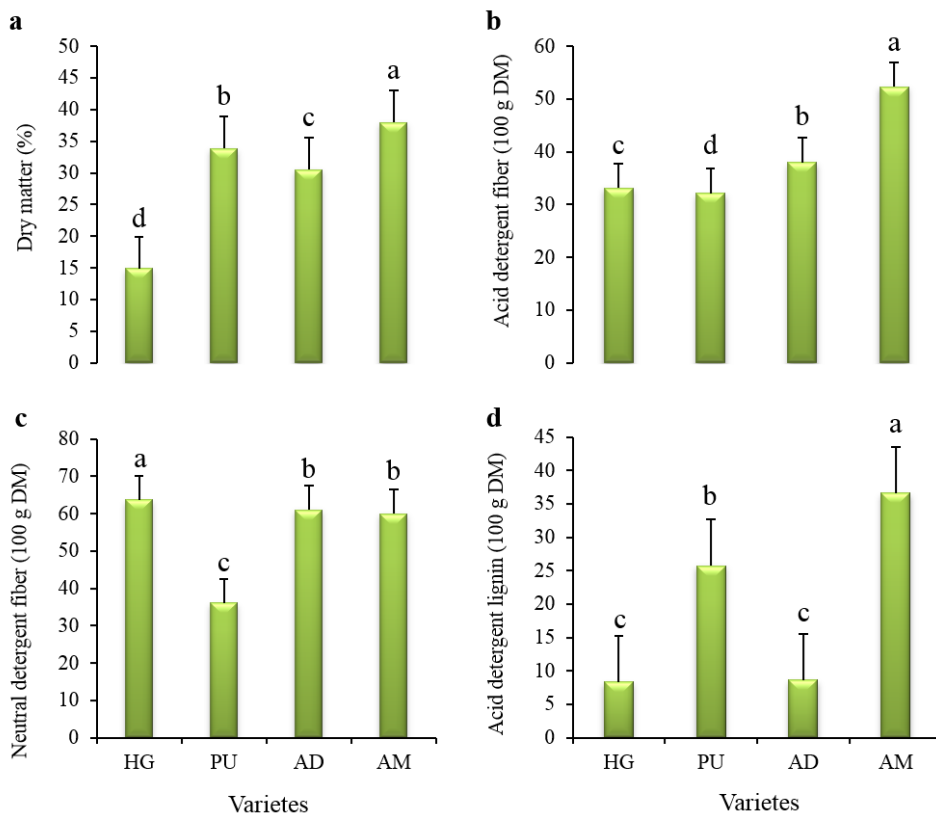


**Figure 3** Effect of various doses of NaOH on variables of leaf plant. According to Duncan's test, each bar represents a mean  $\pm$  SE ( $n=3$ ), and different letters denote significant statistical differences within a range of  $P \leq 0.05$ .

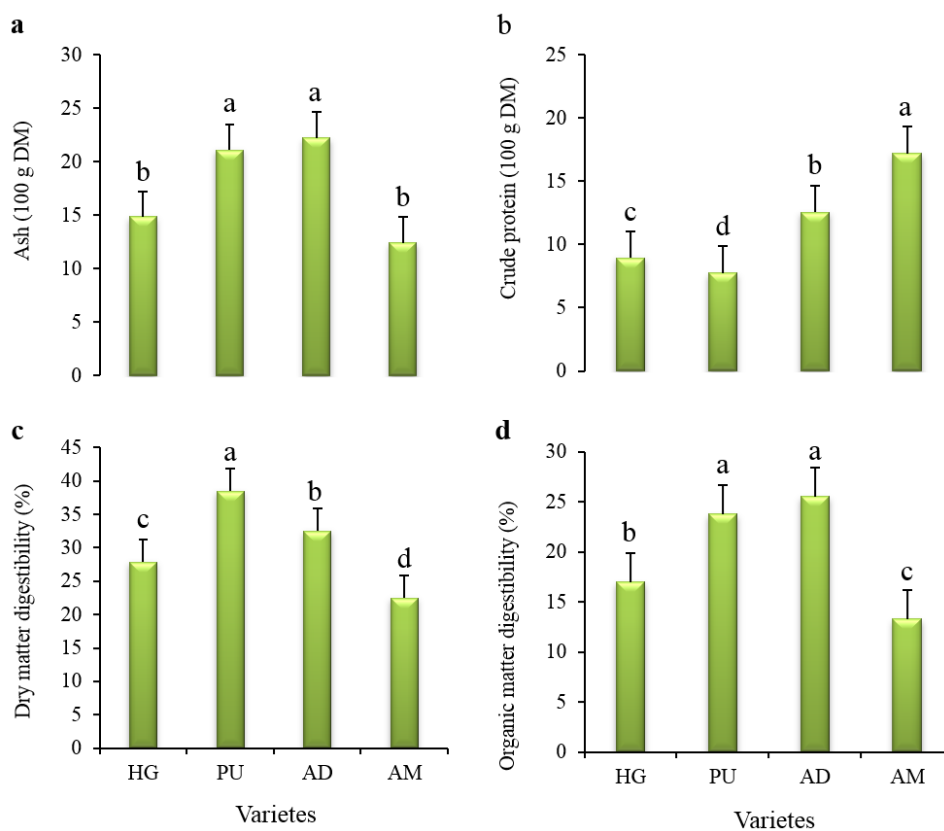
### 3.2. Effect of varieties on the studied variables

The findings in Figure 4 suggest an influence on the nutritional values of leaves, contributing significantly ( $P < 0.05$ ) to the nutritional content available for animal forage. Figures 4a, b, and d showed the concentration of dry matter, acid detergent fiber, and lignin content exhibited substantial variations among different leaf varieties. Triple contents were observed in the AM variety, with values of 38.01%, 52.34, and 36.58 100 g DM, respectively. These values surpassed those of HG for dry matter (14.93%), lignin 8.32 100 g DM, and PU for fiber content (32.18 100 g DM). In addition, regarding neutral detergent fiber, the HG leaf variety displayed the highest measurement for this trait, registering at 63.78 100 g DM. In contrast, PU recorded the lowest fiber content value, with a 36.06 100 g DM measurement in Figure 4c.

The analysis of variance for some biological traits in four leaf non-conventional plants indicated significant variations in nutritional values, as shown in Figure 5. According to the data presented in Figures 5a and d, it was evident that AD plant leaves resulted in the highest increases in ash (22.24 100 g DM) and organic matter digestibility (25.51%), with PU leaves following closely. In contrast, both characteristics reached their lowest levels under the AM variety, with 12.44 100 g DM values for ash and 13.30% for organic matter digestibility. Moreover, mean different leaves of varieties significantly influenced crude protein. The highest protein level, at 17.21 100 g DM, was detected in the AM plant. PU leaves produced a minimal level at 7.75 100 g DM (Figure 5b). Regarding dry matter digestibility, the highest measurement was observed in the leaves of the PU variety, with a value of 38.49%, while the AM plant had the lowest digestibility at 22.47% (Figure 5c).



**Figure 4** Effect of different varieties of leaves on variables of leaf plant. According to Duncan's test, each bar represents a mean  $\pm$  SE (n=3), and different letters denote significant statistical differences within a range of  $P \leq 0.05$ .



**Figure 5** Effect of different varieties of leaves on variables of leaf plant. According to Duncan's test, each bar represents a mean  $\pm$  SE (n=3), and different letters denote significant statistical differences within a range of  $P \leq 0.05$ .



### 3.3. Effect of interaction between varieties and NaOH concentrations on the studied variables

Concerning the dry matter percentage and acid detergent fiber in the leaves, the AM variety exhibited the highest percentage (38.27%) and fiber content (54.63 g/100 g DM) when the leaves were treated with a 6% NaOH concentration compared to other varieties and NaOH doses. The HG plant, when treated with 6% NaOH, displayed a significantly lower dry matter percentage of 15.69%, and similarly, PU under 9% NaOH exhibited a decreased value of 29.66 g/100 g DM. The neutral detergent fiber reached its peak value (68.73 g/ 100 g DM) when the HG plant and 2% NaOH were combined. Meanwhile, PU leaves under control conditions had minimal fiber content, measuring 33.26 g/100 g DM. Furthermore, treatments involving the PU variety and NaOH doses at 8% resulted in the most substantial increases in acid detergent lignin, reaching 50.90 g/100 g DM. In contrast, AD leaves treated with NaOH under 6% achieved the lowest rate, at 5.68 g/100 g DM.

**Table 1** Effect of interaction between dry matter, fibers, and NaOH concentrations on the studied characteristics.

Treatments	Dry matter (%)	g/100 g DM			
		Acid detergent fibre	Neutral detergent fibre	Acid detergent lignin	
HG	0% NaOH	15.71 ± 0.69 <sup>e</sup>	32.78 ± 2.10 <sup>hi</sup>	67.06 ± 1.41 <sup>a</sup>	13.79 ± 4.80 <sup>bcde</sup>
	2% NaOH	14.83 ± 0.37 <sup>ef</sup>	31.48 ± 0.32 <sup>ij</sup>	68.73 ± 1.46 <sup>a</sup>	7.81 ± 0.70 <sup>cde</sup>
	4% NaOH	13.83 ± 0.44 <sup>f</sup>	32.10 ± 0.41 <sup>hi</sup>	62.70 ± 1.38 <sup>b</sup>	6.88 ± 0.38 <sup>cde</sup>
	6% NaOH	15.69 ± 0.56 <sup>e</sup>	35.59 ± 0.95 <sup>fg</sup>	60.72 ± 2.26 <sup>bcd</sup>	6.57 ± 0.37 <sup>cde</sup>
	8% NaOH	14.61 ± 0.56 <sup>ef</sup>	33.85 ± 1.14 <sup>gh</sup>	59.69 ± 1.58 <sup>bcd</sup>	6.55 ± 1.36 <sup>cde</sup>
PU	0% NaOH	33.06 ± 0.35 <sup>b</sup>	31.96 ± 0.67 <sup>hi</sup>	33.26 ± 0.98 <sup>g</sup>	29.46 ± 0.63 <sup>abcde</sup>
	2% NaOH	34.38 ± 0.90 <sup>b</sup>	33.87 ± 0.61 <sup>gh</sup>	37.91 ± 0.31 <sup>f</sup>	16.64 ± 1.00 <sup>bcde</sup>
	4% NaOH	33.49 ± 0.71 <sup>b</sup>	32.43 ± 0.80 <sup>hi</sup>	36.88 ± 0.70 <sup>f</sup>	15.74 ± 1.19 <sup>bcde</sup>
	6% NaOH	33.97 ± 1.02 <sup>b</sup>	32.97 ± 1.15 <sup>hi</sup>	36.49 ± 1.41 <sup>f</sup>	15.81 ± 0.69 <sup>bcde</sup>
	8% NaOH	34.25 ± 0.57 <sup>b</sup>	29.66 ± 0.44 <sup>j</sup>	35.78 ± 0.86 <sup>fg</sup>	50.90 ± 51.22 <sup>a</sup>
AD	0% NaOH	29.74 ± 0.45 <sup>d</sup>	38.54 ± 1.21 <sup>de</sup>	66.22 ± 0.38 <sup>a</sup>	18.60 ± 2.23 <sup>bcde</sup>
	2% NaOH	30.51 ± 0.58 <sup>cd</sup>	39.54 ± 0.82 <sup>d</sup>	61.91 ± 2.70 <sup>bc</sup>	6.62 ± 0.27 <sup>cde</sup>
	4% NaOH	30.74 ± 0.61 <sup>cd</sup>	38.70 ± 1.34 <sup>de</sup>	62.61 ± 0.57 <sup>b</sup>	6.49 ± 0.39 <sup>cde</sup>
	6% NaOH	31.16 ± 0.75 <sup>c</sup>	37.04 ± 0.89 <sup>ef</sup>	58.92 ± 2.13 <sup>cde</sup>	5.68 ± 0.28 <sup>e</sup>
	8% NaOH	30.48 ± 0.65 <sup>cd</sup>	36.10 ± 0.29 <sup>f</sup>	55.68 ± 0.45 <sup>e</sup>	5.98 ± 0.12 <sup>de</sup>
AM	0% NaOH	38.02 ± 0.08 <sup>a</sup>	50.34 ± 0.38 <sup>c</sup>	61.07 ± 0.56 <sup>bcd</sup>	45.88 ± 0.70 <sup>a</sup>
	2% NaOH	37.89 ± 0.50 <sup>a</sup>	53.24 ± 0.39 <sup>ab</sup>	60.51 ± 1.24 <sup>bcd</sup>	34.03 ± 0.17 <sup>abc</sup>
	4% NaOH	38.14 ± 0.44 <sup>a</sup>	54.63 ± 2.02 <sup>a</sup>	62.06 ± 0.71 <sup>bc</sup>	35.83 ± 0.67 <sup>ab</sup>
	6% NaOH	38.27 ± 0.18 <sup>a</sup>	51.66 ± 0.57 <sup>bc</sup>	58.63 ± 1.91 <sup>cde</sup>	33.77 ± 0.44 <sup>abcd</sup>
	8% NaOH	37.73 ± 0.74 <sup>a</sup>	51.84 ± 0.62 <sup>bc</sup>	57.96 ± 2.53 <sup>de</sup>	33.38 ± 0.22 <sup>abcde</sup>

\*HG: *Hedychium gardnerianum*, PU: *Pittosporum undulatum*, AD: *Arundo donax*, and AM: *Acacia melanoxylon*.

The data presented in Table 2 reveal that non-conventional varieties and different levels of NaOH significantly influenced the success of additional biological attributes related to nutritional values in the leaves used for forage. Regarding ash content, the optimal value (36.11 g/100 g DM) was observed in AD plants treated with a 2% NaOH concentration. Conversely, the untreated AM leaves exhibited significantly lowest levels (5.47 g/ 100 g DM) of this characteristic compared to plants subjected to other treatments. The combination of the AM plant and 2% NaOH significantly affected the crude protein, as evidenced by the highest content of 19.02 g/100 g DM. The leaves of plants treated with NaOH, precisely the PU variety at 8% doses, showed a decreasing trend in protein value with increasing doses and exhibited a minimal available content, measuring 7.39 g/ 100 g DM. Moreover, variations in dry matter digestibility were observed among different varieties and treatments, especially with 2% and 8% NaOH. PU plants with NaOH at 4% treated leaves for forage contributed to the highest percentage (42%), contrasting with the reduced percentage observed in the combined AM leaves treated with 2% NaOH (21.30%). Organic matter digestibility explained that the PU variety and untreated leaves exhibited the maximum percentage digestibility (35.04%). Nevertheless, incorporating AM plants with 6% NaOH resulted in a significant decrease ( $P \leq 0.05$ ) in the percentage, reaching the lowest value at 8.21%.

### 3.4. PCA NaOH concentrations

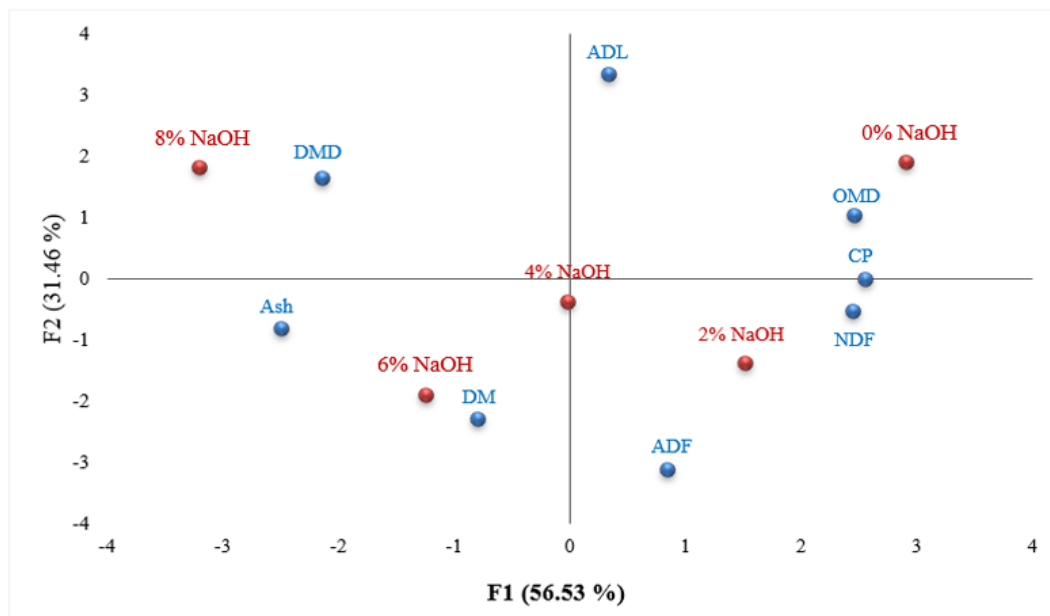
The Principal Component Analysis (PCA) was utilized to evaluate the relationships between the variables. The initial primary components, F1 and F2, collectively accounted for 87.99% of the observed variability. Consequently, these components were visualized in a two-dimensional representation, as illustrated in Figure 6. F1 explained most of the variance on the horizontal axis, accounting for 56.53%. Simultaneously, F2, depicted on the vertical axis, contributed 36.46% to the overall variation. The outcomes of the PCA revealed a notable influence of control conditions (0% NaOH) on the organic matter

digestibility (OMD) of leaves. A strong correlation was observed between ADF and NDF with 2% NaOH. Applying 6%, NaOH significantly impacted DM, whereas NaOH at 8% strongly influenced ash content and DMD. Interestingly, according to the PCA results, no discernible correlation was found between the variables and applying 4% NaOH.

**Table 2** Effect of interaction between varieties and NaOH concentrations on the studied characteristics.

Treatments	g/100 g DM			%	
	Ash	Crude protein	Dry matter digestibility	Organic matter digestibility	
HG	0% NaOH	12.97 ± 0.02 <sup>defgh</sup>	9.26 ± 0.38 <sup>e</sup>	26.62 ± 0.72 <sup>efgh</sup>	16.86 ± 0.97 <sup>efg</sup>
	2% NaOH	11.25 ± 0.61 <sup>efgh</sup>	9.01 ± 0.49 <sup>e</sup>	25.59 ± 0.68 <sup>fghi</sup>	17.92 ± 1.15 <sup>efg</sup>
	4% NaOH	14.75 ± 0.20 <sup>cdefgh</sup>	9.34 ± 0.71 <sup>e</sup>	27.48 ± 0.68 <sup>efg</sup>	16.81 ± 0.28 <sup>efg</sup>
	6% NaOH	16.41 ± 1.79 <sup>bcdefgh</sup>	9.19 ± 0.27 <sup>e</sup>	29.95 ± 1.79 <sup>def</sup>	18.03 ± 0.67 <sup>efg</sup>
	8% NaOH	18.87 ± 1.35 <sup>bcdefgh</sup>	7.85 ± 0.71 <sup>e</sup>	29.50 ± 1.20 <sup>def</sup>	15.18 ± 2.64 <sup>fgh</sup>
PU	0% NaOH	6.76 ± 0.26 <sup>gh</sup>	7.99 ± 0.40 <sup>e</sup>	36.72 ± 3.57 <sup>bc</sup>	35.04 ± 0.64 <sup>a</sup>
	2% NaOH	12.52 ± 1.00 <sup>defgh</sup>	7.42 ± 0.42 <sup>e</sup>	39.52 ± 3.01 <sup>ab</sup>	31.15 ± 2.69 <sup>ab</sup>
	4% NaOH	27.06 ± 0.76 <sup>abcd</sup>	7.54 ± 0.09 <sup>e</sup>	42.00 ± 3.82 <sup>a</sup>	20.74 ± 4.18 <sup>de</sup>
	6% NaOH	28.89 ± 1.26 <sup>abc</sup>	8.41 ± 1.85 <sup>e</sup>	33.66 ± 3.34 <sup>cd</sup>	17.12 ± 1.69 <sup>efg</sup>
	8% NaOH	30.24 ± 1.33 <sup>ab</sup>	7.39 ± 0.75 <sup>e</sup>	40.54 ± 2.29 <sup>ab</sup>	14.96 ± 2.41 <sup>fgh</sup>
AD	0% NaOH	13.62 ± 1.37 <sup>defgh</sup>	14.27 ± 1.18 <sup>c</sup>	31.46 ± 1.81 <sup>de</sup>	26.96 ± 2.26 <sup>c</sup>
	2% NaOH	36.11 ± 25.68 <sup>a</sup>	11.66 ± 1.45 <sup>d</sup>	29.79 ± 1.22 <sup>def</sup>	23.61 ± 1.27 <sup>cd</sup>
	4% NaOH	17.85 ± 0.84 <sup>bcdefgh</sup>	12.31 ± 1.15 <sup>cd</sup>	31.40 ± 1.93 <sup>de</sup>	23.83 ± 1.60 <sup>cd</sup>
	6% NaOH	21.21 ± 0.68 <sup>bcdefg</sup>	12.11 ± 0.84 <sup>cd</sup>	33.26 ± 1.27 <sup>cd</sup>	25.82 ± 2.91 <sup>c</sup>
	8% NaOH	22.42 ± 1.02 <sup>abcdef</sup>	12.40 ± 1.35 <sup>cd</sup>	36.58 ± 1.53 <sup>bc</sup>	27.33 ± 1.32 <sup>bc</sup>
AM	0% NaOH	5.47 ± 0.03 <sup>h</sup>	17.04 ± 0.25 <sup>ab</sup>	23.27 ± 1.63 <sup>ghi</sup>	19.11 ± 1.64 <sup>ef</sup>
	2% NaOH	7.79 ± 0.27 <sup>fgh</sup>	19.02 ± 2.51 <sup>a</sup>	21.30 ± 1.45 <sup>i</sup>	13.99 ± 0.46 <sup>gh</sup>
	4% NaOH	10.11 ± 0.05 <sup>efgh</sup>	17.01 ± 0.13 <sup>ab</sup>	22.91 ± 1.47 <sup>ghi</sup>	14.31 ± 1.85 <sup>gh</sup>
	6% NaOH	15.82 ± 3.66 <sup>bcdefgh</sup>	16.37 ± 0.21 <sup>b</sup>	22.79 ± 3.11 <sup>ghi</sup>	8.21 ± 0.50 <sup>i</sup>
	8% NaOH	22.99 ± 7.93 <sup>abcde</sup>	16.61 ± 1.07 <sup>b</sup>	22.10 ± 1.16 <sup>hi</sup>	10.90 ± 1.85 <sup>hi</sup>

\*HG: *Hedychium gardnerianum*, PU: *Pittosporum undulatum*, AD: *Arundo donax*, and AM: *Acacia melanoxylon*



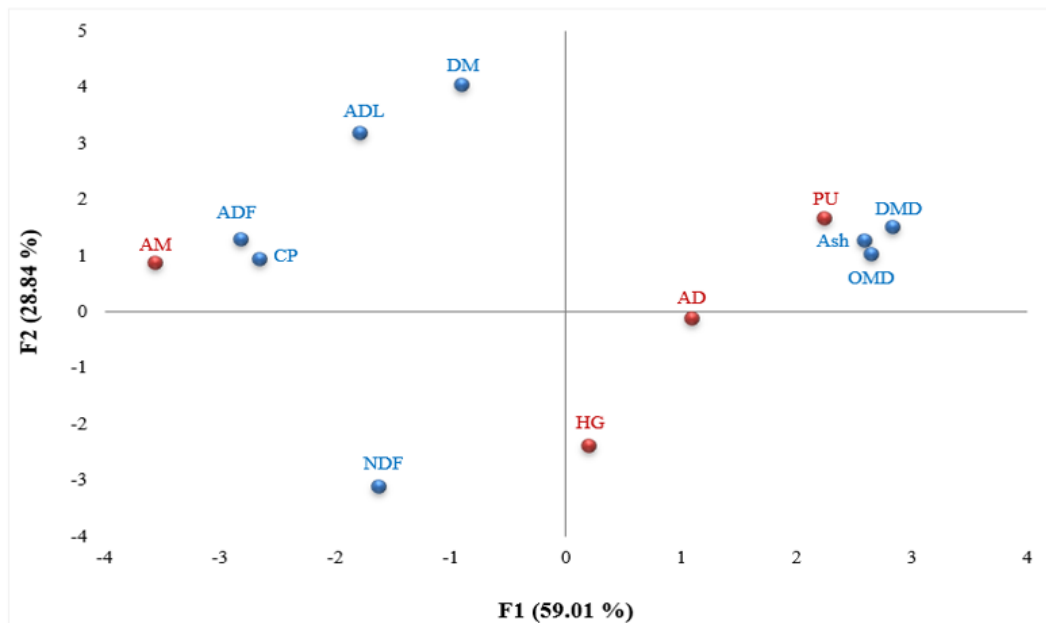
**Figure 6** A PCA biplot was employed to visually illustrate the patterns of variation in NaOH concentrations concerning the investigated variables related to the nutritional value of leaves for forage purposes.

### 3.5. PCA varieties

The PCA was employed to explore the relationships among various variables related to the biological compositions of leaves intended for the forage process for different varieties, such as HG, PU, AD, and AM. The initial principal components, labeled as F1 and F2, accounted for 87.85% of the overall observed variation and were visually depicted in a two-dimensional format, as illustrated in Figure 7. F1, positioned along the horizontal axis, explained most of the variance at 59.01%, while F2,



situated along the vertical axis, contributed an additional 28.84% to the total variation. The PCA results revealed an influence of different varieties on the investigated variables. Specifically, the HG variety significantly affected NDF, while the PU variety had a notable impact on DMD. The AD variety exhibited a strong correlation with ash and OMD. Additionally, the AM variety demonstrated significant associations with DM, ADF, ADL, and CP.



**Figure 7** A PCA biplot was employed to visually represent the patterns of variation among different varieties concerning the investigated variables associated with the nutritional value of leaves for the foraging process.

### 3.6. Multivariate Analysis

A Pearson correlation analysis was conducted to explore the relationships among the variables in leaf parameters for forage animal feed depicted in Figure 8. The results revealed a strong correlation between DM: dry matter with ADF ( $p = 0.01$ ), ADL ( $p = 0.00$ ), and CP ( $p = 0.01$ ). Furthermore, ADF demonstrated a positive association with ADL ( $p = 0.02$ ) and CP ( $p < 0.0001$ ) while exhibiting a negative correlation with DMD ( $p = 0.00$ ) and OMD ( $p = 0.04$ ). A statistically significant positive correlation was observed between NDF and CP ( $p = 0.02$ ), whereas NDF showed a negative correlation with DMD ( $p = 0.00$ ). CP displayed a negative correlation with DMD ( $p = 0.00$ ). A strong positive association was found between DMD with OMD ( $p = 0.00$ ).

ADF	<b>0.59</b>							<b>1</b>
NDF	-0.40	0.38						0.5
ADL	<b>0.65</b>	<b>0.52</b>	-0.28					0.0
Ash	0.06	-0.31	-0.29	-0.23				-0.5
CP	<b>0.54</b>	<b>0.95</b>	<b>0.50</b>	0.44	-0.33			-1
DMD	0.04	-0.73	-0.74	-0.20	0.43	-0.73		
OMD	0.03	-0.46	-0.32	-0.34	-0.09	-0.36	<b>0.62</b>	
	DM	ADF	NDF	ADL	Ash	CP	DMD	

**Figure 8** Pearson's correlation analysis (with a significance threshold of  $P \leq 0.05$ ) was conducted for all investigated variables, offering insights into the direction and significance of the correlations. PCA biplot was employed to visually represent the patterns of variation among different varieties concerning the investigated variables associated with the nutritional value of leaves for the foraging process.

### 4. Discussion

We assessed different concentrations of NaOH and varieties to determine the internal quality of leaves for forage in animal production. A key challenge in the contemporary ruminant animal industry revolves around optimizing the utilization of feed resources by the most successful players. This strategic use of feed resources is crucial for maximizing efficiency in feed utilization, as it directly impacts the industry's profitability and long-term sustainability. The predominant portion of overall input expenses is generally attributable to the costs associated with feeding (Connor, 2015; Moore and Jung, 2001). Forages play an important role in supplying the necessary energy and nutrients vital for the growth and productivity of ruminants,

rendering them a significant feed source in ruminant production. Despite their importance, the relatively elevated levels of lignin and fiber in forages constrain their digestibility in ruminants (Al-Khateeb, 2015). Consequently, various approaches to enhance the digestibility of forages have been investigated.

Based on the information presented in Figures 2 and 3, it is evident that the presence of NaOH impacts improving the quality of leaves for forage purposes. In relation to the cellular structure, an impact resulting from the application of NaOH was observed in diminishing the NDF content, with a more pronounced effect noticed at the highest NaOH concentration dose. The ADF showed a significant decrease with escalating NaOH treatment, particularly affecting hemicellulose more than cellulose. Despite the proportional reduction in ADL levels with increasing NaOH concentration, it is noteworthy that Maduro Dias et al. (2023). The application of NaOH enhanced cellulose and hemicellulose hydrolysis in sorghum and wheat straws (Mancini et al., 2018; Rollini and Manzoni, 2012). The alkaline treatment facilitated the solubilization of hemicellulose and lignin without adversely affecting cellulose. In a comparative study evaluating various treatments (anhydrous ammonia, urea, sodium hydroxide, and calcium hydroxide) on the nutritional value of roughage, this observation highlights the specific effectiveness of the alkaline treatment in selectively targeting non-cellulosic components while preserving cellulose integrity (de Carvalho et al., 2016).

The ash content demonstrates an increase ( $P \leq 0.05$ ) in correlation with the NaOH concentration in Figure 3a, a finding consistent with observations reported by Maduro Dias et al. (2023). The NaOH treatment did not significantly affect the CP content, consistent with expectations. This outcome aligns with the anticipated result, considering that the action of NaOH is primarily on the cell wall; therefore, it should not influence the concentration of CP in Figure 3b; it was in agreement with Pereira Filho et al. (2003). The DMD of the leaves exhibited a positive correlation with the escalating NaOH concentration employed in the chemical treatment, indicating that higher NaOH concentrations were associated with increased DMD (Figure 3c). This observation is consistent with the findings reported by Granzin and Dryden (2003). The rise in DMD with NaOH is attributed to the heightened exposure of cell wall components to this compound. This increased exposure enhances the susceptibility of structural carbohydrates to digestion, contributing to the observed increase in DMD (Chaudhry, 1998; Liu, 2011). The study conducted by Jami et al. (2014) observed that the semi-dry treatment of corn straw with 5% NaOH led to a notable reduction in NDF contents by 14%. Simultaneously, substantial solubilization occurred, including 35% of hemicellulose, 8.7% of cellulose, and 11.3% of lignin fractions. These alterations contributed to a significant 9.1% increase in vitro dry matter digestibility of corn straw. OMD exhibited a similar trend to DMD in response to the treatment. Pires et al. (2003) observed that the NaOH acts on the cell wall components by inducing alkaline hydrolysis of ester-type covalent bonds, especially those between lignin and structural carbohydrates. This process results in the solubilization of hemicellulose and phenolic compounds, decreasing NDF values. Consequently, this alkaline treatment facilitates the decomposition of cellulose and hemicellulose by the rumen. Pedro et al. (2023) stated that the ADL determination is conducted to extract all cellulose from the samples, effectively remove digestible components, and retain only indigestible constituents.

The leaves of various plant varieties, including *Hedychium gardnerianum* (HG), *Pitosporum undulatum* (PU), *Arundo donax* (AD), and *Acacia melanoxylon* (AM), were found to impact both secondary metabolites and nutritional value significantly. These alterations could potentially influence animal reproduction at a physiological level in Figures 4 and 5. In the current study, the AM plant recorded the highest values as dry matter, acid detergent fiber, acid detergent lignin, and crude protein. Also, AD has the best value for ash and organic matter digestibility. Thus, farmers need to exercise caution when using these plants as the foundation of ruminant nutrition. Despite their potential as a feed source, it's crucial to note that these plants exhibit low nutritional value. Additionally, the presence of secondary compounds in these plants raises concerns, as these compounds may potentially influence the physiological mechanisms related to animal reproduction. Therefore, careful attention and monitoring are essential to ensure optimal ruminant nutrition and reproductive outcomes (Nunes, 2017). In numerous regions globally, forages serve as the primary feed source for ruminants, primarily due to their widespread availability and cost-effectiveness. Carbohydrates constitute an important portion of ruminant diets, and forages play a crucial role as a spirited source of energy (Wang et al., 2002). According to Al-Khateeb (2015), forage-based diets are paramount in ruminant nutrition, constituting the most crucial component and providing between 40-70% of the net energy required for milk production in dairy animals.

## 5. Conclusions

Various factors contribute to the nutrient values of feed for forage leaves. The use of NaOH, the selection of appropriate plant varieties, and their interaction are among the factors that directly influence various physiochemical and biological properties. These considerations highlight the importance of chemical treatments and various varieties in shaping the nutrient profile of feed resources for animal production. The concentrations of NaOH and the choice of different plant varieties, including HG, PU, AD, and AM, significantly impact the leaves' characteristics during the forage quality determination process. These factors play a crucial role in influencing and modifying the nutritional and chemical properties of the leaves, ultimately affecting the overall forage quality. Indeed, this research provides valuable insights that can assist farmers in making informed decisions. Farmers can effectively enhance the quality of their feed production by identifying optimal NaOH concentrations

and suitable plant varieties. Understanding the ideal chemical doses and plant varieties contributes to developing strategies for producing high-quality feed, finally benefiting livestock nutrition and farm productivity.

### Ethical considerations

Not applicable.

### Conflict of Interest

The authors declare no conflicts of interest.

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