fermentation

Article

Effect of Sodium Hydroxide Treatment on Chemical Composition and Feed Value of Common Reed (*Phragmites australis*) Straw

Ahmet Uzatici [1], Onder Canbolat [2] and Adem Kamalak [3,*]

[1] Biga Vocational College, Canakkale Onsekiz Mart University, 1720 Canakkale, Turkey
[2] Department of Animal Science, Faculty of Agriculture, Bursa Uludag University, 16059 Bursa, Turkey
[3] Department of Animal Science, Faculty of Agriculture, Kahramanmaras Sutcu University, 46100 Kahramanmaras, Turkey
* Correspondence: akamalak@ksu.edu.tr

Abstract: This research was undertaken with the aim of determining the effect of sodium hydroxide (NaOH) treatment on the chemical composition, in vitro gas production, neutral detergent fiber digestibility (NDFD) and true dry matter digestibility (TDMD), dry matter intake (DMI), and relative feed value (RFV) of common reed (*Phragmites australis*) straw. Reed straw was treated with 0% (control), 1%, 2%, and 3% NaOH and stored in 1.5-L glass jars in triplicate for 21 days. NaOH treatment had a significant effect on the chemical composition, in vitro gas production, NDFD and TDMD, DMI, and RFV of the reed straw. While it decreased the cell wall content of the reed straw, it significantly increased the NDFD, TDMD, DMI, and RFV. The neutral detergent fiber content of the reed straw decreased with NaOH treatment in a dose-dependent manner and ranged from 56.03% to 65.05%, whereas the NDFD increased and ranged from 53.10% to 59.99%. Metabolizable energy, organic matter digestibility, and TDMD values were improved, ranging from 9.15 to 10.19 MJ/kg DM, 58.46% to 65.05%, and 55.29% to 62.33%, respectively. The estimated RFV and DMI also improved, ranging from 84.70% to 95.58% and from 1.87% to 2.14% of body weight, respectively. The most effective treatment dose of NaOH was 3%. Therefore, it can be suggested that NaOH treatment has the potential to improve the nutritive value of reed straw. However, before large applications, further in vivo investigations are required to determine the effects of NaOH treatment on the feed intake and production of ruminant animals.

Keywords: alkali treatment; chemical composition; digestibility; in vitro gas production; reed straw

check for updates

Citation: Uzatici, A.; Canbolat, O.; Kamalak, A. Effect of Sodium Hydroxide Treatment on Chemical Composition and Feed Value of Common Reed (*Phragmites australis*) Straw. *Fermentation* **2022**, *8*, 749. https://doi.org/10.3390/fermentation8120749

Academic Editor: Mengzhi Wang

Received: 11 November 2022
Accepted: 14 December 2022
Published: 16 December 2022

1. Introduction

Forage is one of the important components of ruminant diets, and forage shortages have caused many problems in ruminant nutrition. Therefore, considerable efforts have been devoted to finding alternative forage sources to meet ruminant requirements [1]. In this context, common reed (*Phragmites australis*), which grows naturally in swampy areas, has gained importance in recent years [2–4]. This species grows widely in alkaline and salty soils [5] and reproduces by seeds or rhizomes [6,7]. It generally has a strong stem structure, grows to 2–4 m in height [2,8,9], is cultivated around the world, and is harvested once a year by collecting the upper parts of the plants. The average annual growth rate depends on fertilization. These plants are reported to yield 9–63 tons/ha of dry matter [6,8,10]. Common reed is consumed by many animals, especially farm animals living near the water, such as buffalo, cattle, and sheep [9,11]. However, the protein and energy contents of common reed are low, and the levels of cellulose and cell wall components are very high [3,12]. Acids and alkalis that do not pose a risk to animal health can be used to increase the nutritional value of feeds that contain high levels of ligno-cellulosic substances and have low digestibility [4,13–16]. It has been reported that forages treated with alkaline sodium hydroxide (NaOH) are broken down due to saponification effects in the ester bonds between the lignin and compounds such as acetic acid, phenolic acids, cellulose, and hemicellulose in cell walls [4,16–18]. Thus, polysaccharides such as cellulose and hemicellulose in the cell walls of the plant become more open to enzymes that will hydrolyze them [4,13,17–19].

Chemical combinations with in vitro gas production techniques are widely used to determine the metabolizable energy (ME) and organic matter digestibility (OMD) of feedstuffs for ruminant animals [20]. In this study, we aimed to determine the effects of NaOH treatment on the chemical composition, in vitro gas production, neutral detergent fiber digestibility (NDFD) and true dry matter digestibility (TDMD), dry matter intake (DMI), and relative feed value (RFV) of reed straw.

2. Materials and Methods

The feed material was based on common reed (*Phragmites australis*) growing in the marsh area of Uluabat Lake in Bursa, Türkiye. Reed plants were harvested in August, dried, and shredded with a straw machine to be used in the form of straw in the present study. The obtained reed straw was divided into 4 equal parts of 5 kg and 0% (control: water), 1%, 2%, and 3% NaOH treatments were administered to dilute the dry matter (DM) to 50%. Straw was then stored in 1.5-L glass jars in triplicate for 21 days. Jars were opened on the 21st day and the straw was dried at 65 °C, ground to a particle diameter of 1.0 mm, and used in the analysis.

Gas production, OMD, and ME values of the reed straw samples were determined by the in vitro gas production technique reported by Menke and Steingass [20]. In this process, samples weighed to approximately 200 ± 15 mg DM were placed in special glass tubes (Model Fortuna, Häberle Labortechnik, Lonsee-Ettlenschieß, Germany) and 30 mL of rumen fluid/buffer solution was added [21]. The glass tubes were then incubated in a water bath at 39 °C. In vitro gas production was measured at 3, 6, 12, 24, 48, 72, and 96 h during the incubation period. The ME and OMD of the samples were calculated with the following equations [20]:

$$\text{ME, MJ/kg DM} = 2.20 + 0.1357 \times \text{GP} + 0.0057 \times \text{CP} + 0.0002859 \times \text{CF}^2 \quad (1)$$

$$\text{OMD, \%} = 15.38 + 0.8453 \times \text{GP} + 0.0595 \times \text{CP} + 0.0675 \times \text{CA} \quad (2)$$

Here, GP is the gas produced by 200 mg of feed sample in 24 h while CP is crude protein, CF is crude fat, and CA is crude ash (g/kg DM).

The TDMD and NDFD of the straw were determined using the Ankom Daisy^{II} incubator technique (Ankom Technology Corp., Fairport, NY, USA). In this process, approximately 0.5 g of reed straw was weighed into technique-specific bags (F57) and the mouths of the bags were sealed. Subsequently, 2 L of incubation fluid specific to the technique (1600 mL of buffer solution + 400 mL of rumen fluid) was added to the technique-specific glass jars in the presence of CO_2 gas. Glass jars prepared in this way were incubated for 48 h at 39 °C in a Daisy^{II} incubator device. At the end of the incubation period, all bags were removed from the jars and held under tap water until the water flowed clear. They were then dried in an oven at 105 °C for 4 h and TDMD and NDFD were determined [22]. The relative feed value (RFV) of the straw was calculated based on the following equations [23]:

$$\text{DMD} = \text{Dry matter digestibility (\%)} = 88.9 - (0.779 \times \text{\%ADF}) \quad (3)$$

$$\text{DMI} = \text{Dry matter intake (\% of body weight)} = 120 / (\text{\%NDF}) \quad (4)$$

$$\text{RFV} = \text{Relative feed value} = (\text{DMD} \times \text{DMI}) / 1.29 \quad (5)$$

RFVs of greater than 100 are taken as indicating high-quality feed, while lower values indicate lower quality [23]. The dry matter (DM), crude ash (CA), crude protein (CP), crude cellulose (CC), and ether extract (EE) contents of the samples were analyzed according to

AOAC methods [24]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents, which constitute the cell wall components of reed straw, were determined according to the methods reported by Van Soest and Robertson [22].

One-way analysis of variance (ANOVA) was used to determine the effect of NaOH treatment on the chemical composition, in vitro gas production, digestibility, and RFV of the reed straw. Significant differences ($p < 0.05$) among the mean values of the samples were determined with Tukey's multiple range test.

3. Results and Discussion

The effects of NaOH treatment on the chemical composition of reed straw are given in Table 1. The NaOH treatment had significant effects on the chemical composition of the straw. It significantly increased the CA content of the samples and decreased the OM content ($p < 0.05$). The highest level of CA (8.17%) was determined in the straw treated with 3% NaOH. However, treatment with NaOH did not significantly affect the CP or CF contents of the reed straw ($p > 0.05$).

Table 1. Effects of NaOH treatment on the chemical composition of reed straw.

Chemical Composition, %	NaOH Treatment				SEM
	0%	1%	2%	3%	
Organic matter (OM), %	92.68 [a]	92.42 [b]	92.41 [b]	91.82 [c]	0.038
Crude ash (CA), %	7.31 [d]	7.58 [c]	7.99 [b]	8.17 [a]	0.036
Crude protein (CP), %	9.58 [a]	9.57 [a]	9.59 [a]	9.62 [a]	0.029
Crude fat (CF), %	2.66 [a]	2.64 [a]	2.62 [a]	2.60 [a]	0.034
Crude cellulose (CC), %	47.91 [a]	45.89 [b]	43.76 [c]	42.41 [d]	0.659
Neutral detergent fiber (NDF), %	64.05 [a]	61.44 [b]	58.99 [c]	56.03 [d]	0.587
Acid detergent fiber (ADF), %	39.36 [a]	38.07 [b]	36.31 [c]	35.51 [d]	0.540

[a, b, c, d]: Differences between means indicated by different letters in the same row are significant ($p < 0.05$); SEM: standard error of the mean; NaOH: sodium hydroxide.

Although the CF levels of the straw samples were similar to the values reported by Canbolat [4], the CP levels were lower than those reported by Canbolat [4]. Treatment with NaOH significantly decreased the CS, NDF, and ADF contents of the straw ($p < 0.05$). The NDF and ADF contents ranged from 56.03% to 65.05% and from 35.51% to 39.36%, respectively. The lowest levels of CC, NDF, and ADF were obtained for the reed straw treated with 3% NaOH with values of 42.41%, 56.03%, and 35.51%, respectively. It can be said that the cell wall components decreased as a result of NaOH breaking down the ester bonds between compounds such as acetic acid, phenolic acids, cellulose, hemicellulose, and lignin in the cell walls of the straw with the effects of saponification [4,16–18]. The chemical composition of the reed straw was found to be similar to the values reported by Baran et al. [2], El-Talty et al. [11], Büyükkilic and Sirakaya [3], and Canbolat [4].

The effects of NaOH treatment on the gas production of reed straw are illustrated in Figure 1. Treatment with NaOH significantly affected the gas production of the straw over the course of the incubation period ($p < 0.05$). In vitro gas production increased significantly ($p < 0.05$) with NaOH treatment in a dose-dependent manner. The gas production of the straw samples at 96 h of incubation ranged from 61.67 to 69.41 mL/200 mg DM. The highest level of gas production was observed for the straw treated with 3% NaOH and the lowest for the control group. These findings can be attributed to the fact that NaOH breaks down the ester bonds between compounds such as cellulose and hemicellulose in cell walls and lignin [4,13,18,25]. This process makes nutrients more accessible to microorganisms [16,19]. Several previous studies have similarly showed that the treatment of wheat straw, reed grass, corn straw, and rice straw with NaOH increased gas production in a dose-dependent manner [4,16,18,26].

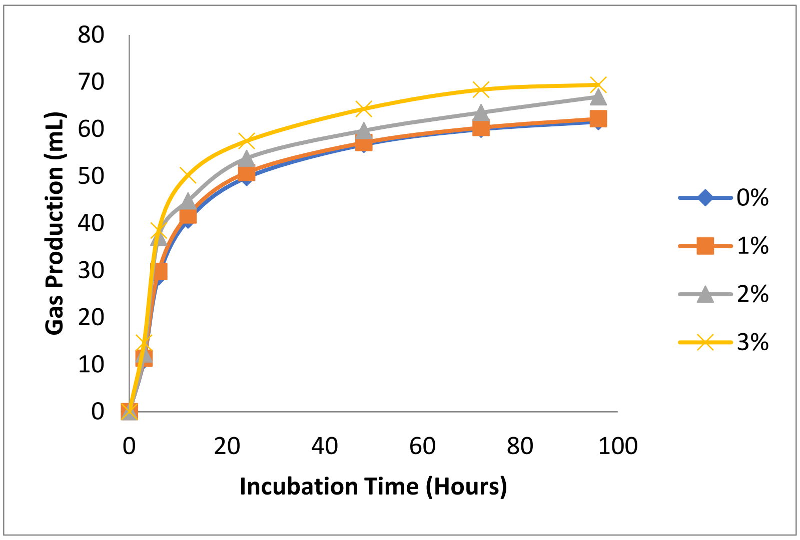

Figure 1. Effects of NaOH treatment on the in vitro gas production of reed straw.

The effects of NaOH treatment on ME and digestibility of the straw samples are shown in Table 2. Treatment with NaOH significantly affected the ME content of the straw ($p < 0.05$). The ME values increased in a dose-dependent manner with NaOH treatment, ranging from 9.15 to 10.19 MJ/kg DM. The highest level of ME (10.19 MJ/kg DM) was determined for the straw treated with 3% NaOH. The ME values of reed straw obtained in the present study were higher than those reported by Mokhtarpour and Jahantigh [9] and Büyükkilic and Sirakaya [3]. On the other hand, the ME values were similar to those obtained by Canbolat [4].

Table 2. Effects of NaOH treatment on the metabolizable energy and digestibility of reed straws.

Parameters	NaOH Treatment				SEM
	0%	1%	2%	3%	
ME, MJ/kg DM	9.15 ^{c}	9.29 ^{c}	9.70 ^{b}	10.19 ^{a}	0.102
OMD, %	58.46 ^{c}	59.37 ^{c}	61.93 ^{b}	65.05 ^{a}	0.641
TDMD, %	55.29 ^{d}	56.45 ^{c}	58.79 ^{b}	62.33 ^{a}	0.463
NDFD, %	53.10 ^{c}	54.03 ^{c}	55.80 ^{b}	59.99 ^{a}	0.523

$^{a–d}$: Differences between means indicated by different letters in the same row are significant ($p < 0.05$); SEM: standard error of the mean; NaOH: sodium hydroxide; ME: metabolizable energy; OMD: organic matter digestibility; TDMD: true dry matter digestibility; NDFD: neutral detergent fiber digestibility.

Treatment with NaOH significantly affected the OMD, TDMD, and NDFD levels of the reed straw ($p < 0.05$). The OMD, TDMD, and NDFD contents of the samples ranged from 58.46% to 65.05%, 55.29% to 62.33%, and 53.10% to 59.99%, respectively. The most pronounced effects on the digestibility of the reed straw were obtained with 3% NaOH. As described above, the breakages that occur with NaOH treatment make nutrients more available to microorganisms and more digestible compared with untreated reed straw. These results are in agreement with the findings of Canbolat [4], Mokhtarpour and Jahantigh, [9], Harada et al. [17], Liu et al. [26], and Canbolat et al. [18]. The TDMD and OMD values of the reed straw were similar to those reported by Büyükkilic and Sirakaya [3] and Canbolat [4].

The effects of NaOH treatment on the DMD, DMI, and RFV of the straw samples are shown in Table 3. Treatment of reed straw with NaOH significantly affected DMD, DMI, and RFV in a dose-dependent manner ($p < 0.05$) and the most effective dose of NaOH was 3%. When the straw samples were evaluated in terms of RFV, they were found to be of satisfactory quality, with a value of 104.27 obtained for the straw treated with 3% NaOH. Treatment of reed straw with NaOH breaks the ligno-cellulosic bonds in the cell walls [4,9,16,17,19,25] as a result of decreases in NDF and ADF levels. The values of DMD, DMI, and RFV obtained in the present study are similar to those reported by Büyükkilic and Sirakaya [3], who worked with sedge plants harvested in different time periods, although the DMI and RFV were higher. In addition, among the results reported by Canbolat [4], who worked with sedge grass, the obtained levels of DMD and DMI were similar, while the RFV was lower. These differences are likely due to the harvesting time, variety, method of chemical processing, and climate differences.

Table 3. Effects of NaOH treatment on digestibility, dry matter intake, and relative feed values of reed straws.

Parameters	NaOH Treatment				SEM
	0%	1%	2%	3%	
Dry matter digestion (DMD), %	58.31 [d]	59.24 [b]	60.61 [c]	62.79 [a]	0.420
Dry matter intake (DMI), BW, %	1.87 [d]	1.95 [c]	2.03 [b]	2.14 [a]	0.021
Relative feed value (RFV)	84.70 [d]	89.68 [c]	95.58 [b]	104.27 [a]	1.073

[a–d]: Differences between means shown with different letters in the same row are significant ($p < 0.05$); SEM: standard error of the mean; NaOH: sodium hydroxide. BW: body weight.

The increased DMI and DMD values obtained in the present study may be associated with decreases in the cell wall content of the straw samples due to NaOH treatment. These increased DMI and DMD values are consistent with the findings of Nocek and Russel [27] and Oba and Allen [28].

The NDF content of forages are routinely analyzed due to their nutritional importance and possible variations. NDF digestibility is one of the important parameters affecting forage quality and animal performance [27]. Oba and Allen [28] suggested that the digestibility of NDF as determined in vitro or in situ is a better indicator of the potential of the forage to increase DMI than NDF digestibility as determined in vivo [28]. Therefore, NDF digestibility has become an important variable in estimations of the total digestibility of forage as lactating cows consume more dry matter and produce more milk when fed forages that have higher NDF digestibility. Although dairy cows require NDF in their diets for maximum productivity, excess dietary NDF often limits voluntary feed intake because of the physical filling of the rumen. It is difficult to isolate the effect of NDF digestibility on animal performance due to other confounding factors, but it was previously suggested that forages with higher levels of NDF digestibility might have shorter rumen retention times, allowing for greater DMI. A one-unit increase in NDF digestibility in vitro was found to result in a 0.17 kg increase in DMI and a 0.25 kg increase in 4% fat-corrected milk [28]. Therefore, the increase in NDF digestibility of reed straw obtained in the present study with NaOH treatment is likely to increase DMI and milk production if NaOH-treated reed straw is included in ruminant diets. However, further studies are required to test the effects of NaOH-treated reed straw on DMI and animal performance.

The CP content of the straw samples ranged from 9.58% to 9.62%, which is higher than the minimum level of 7–8% DM for optimum rumen function and feed intake in ruminant animals. However, the high NDF content of reed straws should be taken into consideration when reed straw is included in ruminant diets. It is well-known that the NDF content of forages are among the most important factors affecting potential nutritive values for ruminant animals. Therefore, the high cell contents and especially the NDF content of reed straw are likely to be the most limiting factors for the use of this straw in ruminant diets. However, in the present study, treatment of reed straw with NaOH decreased the NDF content and subsequently improved the NDFD and TDMD of the straw. NaOH-treated reed straw warrants further in vivo investigations to determine the proper inclusion rate in ruminant diets and the effects on feed intake and production.

There is limited information to date regarding the chemical composition and nutritive value of NaOH-treated reed straw. Therefore, the results presented here provide invaluable information about the potential nutritive value of NaOH-treated reed straws. The in vitro gas production technique and Ankom Dacron bags utilized in this study successfully discriminated the experimental samples treated with NaOH and provided invaluable information about the digestibility and ME of reed straw.

4. Conclusions

In the present study, NaOH treatment was shown to have significant effects on the chemical composition, in vitro gas production, NDFD and TDMD, DMI, and RFV of reed straw (*Phragmites australis*). The treatment of reed straw with NaOH significantly decreased the NDF content and improved the ME, NDFD, TDMD, and RFV in a dose-dependent manner. The most effective treatment dose of NaOH was 3%. However, before large applications, further in vivo investigations are required to determine the effects of NaOH treatment on the feed intake and production of ruminant animals. NaOH-treated reed straw warrants further in vivo investigations to determine the proper rate of inclusion in ruminant diets and the effects on feed intake and production.

Author Contributions: Conceptualization, A.U. and O.C.; methodology, O.C. and A.K.; software, A.U. and O.C.; validation and formal analysis, A.U. and O.C.; investigation, A.U. and O.C.; data curation, A.U. and O.C., and A.U.; O.C. and A.K.; writing—original draft preparation, A.U., O.C. and A.K.; writing—review and editing, A.U., O.C. and A.K.; project administration, A.U., O.C. and A.K.; funding acquisition, A.U., O.C. and A.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study does not contain any work with human or animal subjects performed by any of the authors.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mohamoud Abdi, A.; Kilic, U. Effect of lignin peroxidase enzyme on feed values of different straws. *KSU J. Agric. Nat.* **2018**, *21*, 374–384. [CrossRef]
2. Baran, M.; Varadyova, Z.; Kracmar, S.; Hedvabny, J. The common reed (*Phragmites australis*) as a source of roughage in ruminant nutrition. *Acta Vet. Brno* **2002**, *71*, 445–449. [CrossRef]
3. Büyükkilic, B.S.; Sirakaya, S. Determination of feed value of common reed (*Phragmites Australis*) in different sowing periods. *KSU J. Agric. Nat.* **2019**, *22*, 487–491. [CrossRef]
4. Canbolat, O. The effect of different chemical processes on the nutrient composition and feed value of the reed plant. In Proceedings of the 4th International Congress on Agriculture, Animal Science and Rural Development, Ankara, Turkey, 12–14 June 2020; pp. 144–158.
5. Haslam, S.M. Biological Flora of the British Isles: *Phragmites australis*. *J. Ecol.* **1972**, *60*, 585–610. [CrossRef]
6. Lewandowski, I.; Scurlock, J.M.O.; Lindvall, E.; Christou, M. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass Bioenergy* **2003**, *25*, 335–361. [CrossRef]
7. Scragg, A.H. *Biofuels: Production, Application and Development*, 1st ed.; CABI: Wallingford, UK, 2010; 256p, ISBN -13.
8. Köbbing, J.F.; Thevs, N.; Zerbe, S. The utilisation of reed (*Phragmites australis*): A review. *Mires Peat* **2013**, *13*, 1–14.
9. Mokhtarpour, A.; Jahantigh, M. Effect of supplementing common reed (*Phragmites australis*) with urea on intake, apparent digestibility and blood metabolites of Baluchi sheep. *Iran. J. Appl. Anim. Sci.* **2018**, *8*, 623–628. Available online: https://ijas.rasht.iau.ir/article_544731_b9c8df09d507b43446d657e9b713d3bd.pdf (accessed on 10 November 2022).
10. Hidalgo, M.; Fernandez, J. Biomass Production of Ten Populations of Giant Reed (*Arundo donax* L.) Under the Environmental Conditions of Madrid (Spain). In *Biomass for Energy and Industry: Proceeding of the First World Conference, Sevilla, Spain, 5–9 June 2000*; James & James (Science Publishers) Ltd.: London, UK, 2000; pp. 1881–1884.
11. El-Talty, Y.I.; Abdel-Gwad, M.H.; Mahmoud, A.E.M. Effect of common reed (*Phragmites australis*) silage on performance of growing lambs. *Asian J. Anim. Sci.* **2015**, *9*, 1–12. [CrossRef]
12. Mashayekh, M.R.; Ghorbani, G.R. Variation of chemical composition and digestibility of common reed forage during growth stage and characteristics of reed forage ensilage. *Anim. Sci. J.* **2004**, *68*, 93–98.
13. Harada, C.; Nakamura, Y.; Minato, H. Effect of sodium hydroxide treatment of rice straw on cell wall composition and digestibility of dry matter. *Anim. Sci. J.* **1999**, *70*, 61–66. [CrossRef]

14. Nair, V.P.; Verma, A.K.; Dass, R.S.; Mehra, U.R. Growth and nutrient utilization in buffalo calves fed urea-ammoniated wheat straw and hydrochloric acid plus urea treated wheat straw. *Asian-Australas. J. Anim. Sci.* **2002**, *15*, 682–686. [CrossRef]
15. Gunun, P.; Wanapat, M.; Anantasook, N. Effects of physical form and urea treatment of rice straw on rumen fermentation, microbial protein synthesis and nutrient digestibility in dairy steers. *Asian-Australas. J. Anim. Sci.* **2013**, *26*, 1689–1697. [CrossRef]
16. Canbolat, O. Effect of alkali treatment on nutrient composition, in vitro gas production and feed value of corn straw. *J Food Sci. Technol.* **2022**, 61–67.
17. Harada, C.; Saito, Y.; Nakamura, Y.; Minato, H. The Effect of sodium hydroxide treatment of rice straw on in situ disappearance of hemicellulose and lignin in its cell wall. *Anim. Sci. J.* **2001**, *72*, 19–25. Available online: https://www.jstage.jst.go.jp/article/chikusan1924/72/1/72_1_19/_pdf (accessed on 10 November 2022). [CrossRef]
18. Canbolat, O.; Ozgur, C.O.; Kamalak, A. Effects of NaOH treatment on condensed tannin contents and gas production kinetics of tree leaves. *Anim Feed Sci. Technol.* **2007**, *138*, 189–194. [CrossRef]
19. Arisoy, M. The Effect of sodium hydroxide treatment on chemical composition and digestibility of straw. *Turk. J. Vet. Anim. Sci.* **1998**, *22*, 165–170. Available online: https://journals.tubitak.gov.tr/veterinary/vol22/iss2/11 (accessed on 10 November 2022).
20. Menke, K.H.; Steingass, H. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim. Res. Dev.* **1988**, *28*, 7–55.
21. Menke, K.H.; Raab, L.; Salewski, A.; Steingass, H.; Fritz, D.; Schneider, W. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor in vitro. *J. Agric. Sci.* **1979**, *93*, 217–222. [CrossRef]
22. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef]
23. Rohweder, D.A.; Barnes, R.F.; Jorgensen, N. Proposed hay grading standards based on laboratory analyses for evaluating quality. *J. Anim. Sci.* **1978**, *47*, 747–759. [CrossRef]
24. AOAC. *Official Methods of Analysis, 17th ed*; AOAC International: Gaithersburg, MD, USA, 2000.
25. Chaudhry, A.S. Rumen degradation in sacco in sheep of wheat straw treated with calcium oxide, sodium hydroxide and sodium hydroxide plus hydrogen peroxide. *Anim. Feed Sci. Technol.* **2000**, *83*, 313–323. [CrossRef]
26. Liu, J.X.; Susenbeth, A.; Südekum, K.H. In vitro gas production measurements to evaluate interaction between untreated and chemically treated rice straws, grass hay, and mulberry leaves. *J. Anim. Sci.* **2002**, *80*, 517–524. [CrossRef] [PubMed]
27. Nocek, J.; Russell, J. Protein and Energy as an Integrated System. Relationship of Ruminal Protein and Carbohydrate Availability to Microbial Synthesis and Milk Production. *J. Dairy Sci.* **1988**, *71*, 2070–2107. [CrossRef]
28. Oba, M.; Allen, M.S. Evaluation of the Importance of the Digestibility of Neutral Detergent Fiber from Forage: Effects on Dry Matter Intake and Milk Yield of Dairy Cows. *J. Dairy Sci.* **1999**, *82*, 589–596. [CrossRef]