International Journal of Veterinary Sciences and Animal Husbandry 2023; 8(5): 271-274

ISSN: 2456-2912
VET 2023; 8(5): 271-274

www.veterinarypaper.com
Received: 08-07-2023
Accepted: 12-08-2023

MA Ouma
Egerton University, Faculty of Agriculture, Department of Animal Science; P.O. Box 536-20115, Egerton, Kenya

JO Ondiek
Egerton University, Faculty of Agriculture, Department of Animal Science; P.O. Box 536-20115, Egerton, Kenya

PK Migwi
Egerton University, Faculty of Agriculture, Department of Animal Science; P.O. Box 536-20115, Egerton, Kenya

Corresponding Author:
MA Ouma
Egerton University, Faculty of Agriculture, Department of Animal Science; P.O. Box 536-20115, Egerton, Kenya

The impact of ratio and treatment with bentonite and wood ash on proximate composition and phenolic compounds in mesquite (*Prosopis juliflora*) leaves and pods

MA Ouma, JO Ondiek and PK Migwi

Abstract
Prosopis juliflora is a good alternative protein source and fodder for all livestock at any time of the year. However, it is high in polyphenolic compounds which hinder its palatability and digestibility. This study aimed at investigating the effect its leaves and pods combined at different ratios and on treatment have on their nutritive value and chemical composition. As a result, proximate analysis, and determination of polyphenols was carried out. The analyses were carried out on leaves and pods at ratios of 0:100, 27:75, 50:50, 75:25, and 100:0 to determine the effect of ratio and treatment. Results showed that ratio and treatment affected chemical and nutritive composition of *P. juliflora* leaves and pods (LP). The crude protein (CP) availability and ash contents were significantly increased while the crude fibre (CF), ether extracts (EE), and polyphenols were significantly decreased in leaves while performance differed in pods when the two were mixed at the different ratios. Treatment with wood ash and bentonite significantly increased availability of CP in *P. juliflora* LP and decreased CF, EE, and polyphenols of both of them significantly. Total extractable phenolics (TEPH) were significantly reduced in all the ratios while the performance differed with the other factors and depending on the binder used.

Keywords: Condensed tannins, metabolizable energy, organic matter digestibility, polyphenols, total extractable phenolics, total extractable tannins

1. Introduction

Leguminous tree forages are good alternative protein sources for ruminants to replace the poor quality and seasonal crop residues and grasses [18]. They are not affected by the ever changing climatic conditions and are evergreen throughout the year. Moreover, they are nutritionally rich with high crude protein content, metabolizable energy, minerals and organic matter [17].

Prosopis juliflora, which is deemed by many a bane due to its invasiveness has been found to have all these qualities. It is of high nutritional value, suppling protein of high quality, and a good source of fodder palatable to all livestock [9]. However, it contains polyphenolic compounds which bind to its protein component making it unavailable. These compounds also affect its nutritive value, palatability, digestibility and intake [22].

Treatment with various types of tannin binders has been shown to reduce the effect of these compounds and enhance its digestibility and nutritive value. Most researchers have focused on the use of synthetic binders like polyphenol glycol (PEG) which are expensive, difficult to access and their effect on the environment, for example, PEG-tannin complex, is vaguely known. Due to these limitations, some researchers have proposed that further research be done to explore on the use of natural and locally available binders such as wood ash and bentonite which have been proven to be good binders [4, 8]. However, their effect on the nutritional composition of *P. juliflora* is still unknown.

Bentonite and wood ash were used in this research due to their availability and effectiveness. More than 90% of Kenyans use either wood or charcoal for cooking and almost 10% of this is converted to wood ash [1]. Wood ash is rich in calcium and is mostly used as a calcium supplement for livestock.

However, its extensive used is discouraged due to variations in its minerals depending on the source hence, there might be need for supplementation, especially of phosphorus that becomes deficient with high calcium in the diet [23]. Wood ash is a non-corrosive alkaline that has long been used in roughages for digestibility improvement and tannin reduction in place of hazardous ones such as Sodium hydroxide. Its use is highly applicable in farms with limited resources.

On the other hand, bentonite occur naturally as a result of siltation in many riverbeds. It is also as a result of volcanic eruptions with United States being the leading producer in the world. This clay is of high cohesiveness and a strong binder that is used in softening vegetables, as a face mask, for wine refining, among others [11], It is also as good as PEG in tannin binding, yet PEG is harmful to the environment, and the effect of PEG-tannin complex on animals is still unknown. According to [5], use of bentonite in ruminant diets does not result in any adverse effects on rumen fermentation but can slightly alter the rumen's metabolome and concentration of some minerals.

Chemical analyses were therefore carried out on *P. juliflora* leaves and pods at different ratios to determine the effect of ratio on its nutritive composition and the effect of wood ash and bentonite on its nutritive composition.

2. Materials and Methods

2.1 Study site: The study was conducted were carried out in Egerton University main campus, Njoro sub-county, Nakuru county, Kenya in the Animal Science laboratory. The University situated at 0° 23 S, 35° 55 N with an altitude of 2,238 m above sea level, and latitudes and longitudes of 0.369734°S and 35.932779°E respectively. The area receives an average annual rainfall of 900-1,200 mm and the average daily temperatures ranges from 17 °C-22 °C [7].

2.2 Source of materials: Mature leaves and pods that were used in this study were sourced from Marigat Sub-County, Baringo county, which is 0° 20'N and 35° 37'E. The collection was carried out during the dry months of December and January. Marigat is approximately 20 Km from both Lake Baringo and Bogoria Kenya, and lies 1,080 m above sea level. It receives rainfall of 700-950 mm per year with peaks in the April/May and July/August but usually erratic in nature [3].

2.3 Preparation of samples for analysis: The collected leaves and pods for this study were transported to Egerton University where they were assorted by removing green pods and spoilt and mouldy leaves and pods. They were spread out for efficient drying then kept in the oven set at 60 °C for 6 hr for complete drying. Later, they were ground to pass through 1mm sieve before they were placed in airtight containers after weighing and mixing at ratios of 0:100, 25:75, 50:50, 75:25 and 100:0. Bentonite was used at the rate of 20 g/kg and wood ash collected was mixed thoroughly, sieved to remove foreign particles and used at 400g/kg in the analysis. Wood ash tainted with kerosene was not used. The animal handling procedures were approved and certified by the Ethical Clearance Committee, Egerton University, and permitted by the National Commission for Science and Innovation (NACOSTI), Kenya.

2.4 Proximate analysis: Moisture content, Ether extract, Crude fibre and ash contents were determined according to the procedures outlined in (AOAC, 2006) methods 934.01, 920.39, 962.09 and 942.05 respectively. Nitrogen content was determined using Kjeldahl method stipulated in AOAC method 984.13 and crude protein calculated using the formula; N×6.25.

2.5 Determination of Total Phenolics, Total Tannins and Condensed Tannins: Total Phenolics were extracted using 70% aqueous whereas total extractable tannins were determined through Folin-ciocalteu procedures acetone as described by [13]. In this procedure, regression equation of tannic acid standard is used the calculation. Condensed tannins were determined as per the method described by [19]. Polyvinypolypyrrolidone (PVPP) was bound to determine tannin content by initially determining total phenolics content then precipitating the tannins together with PVPP after which the results were subtracted from the phenols to determine the condensed tannins in dry matter as a percentage of tannic acid equivalent.

3. Results

Chemical composition of *P. juliflora* leaves and pods was significantly affected by ratio (Table 1). The leaves were more affected than the pods. Ash content of the leaves was significantly increased while the availability of CP, and presence of EE, CF, TEPH, TET and CT were significantly decreased. The effects on pods varied as shown in Table 1.

Table 1: Effects of ratio on chemical composition of *P. juliflora* leaves and pods

Parameter	ASH	EE	CP	CF	TEPH	TET	CT
PL100	8.55d	2.97a	23.04a	26.92a	7.69a	6.05a	0.86d
PP100	10.38c	2.22bc	13.09d	16.85c	5.51c	3.30cd	2.43a
PPL50:50	10.70b	1.93cd	14.141c	18.50c	5.35c	3.80c	1.34c
PPL75:25	7.09e	2.45b	13.99c	23.17b	5.21c	3.04d	1.55b
PPL25:75	12.85a	1.80d	20.22b	22.18b	6.72b	4.89b	0.912d
SEM	0.06	0.08	0.19	0.38	0.09	0.13	0.02

EE = Ether Extracts (%), CP = Crude Protein (%), CF = Crude Fibre (%), TEPH=Total Extractable Phenolics (g/kg DM), TET=Total Extractable Tannins (Tannin Acid Equivalent in g/kg DM,), CT = Condensed Tannins (g/kg DM), abcd Means with different superscripts in the same column are significantly different at $p<0.05$; SEM=Standard error of the mean.

Chemical composition of *P. juliflora* leaves was significantly affected by treatment (Table 2). The availability of CP was significantly increased by both binders while ash content was significantly increased by wood ash. EE, CF, TEPH, TET and CT content were significantly reduced by the binders. Pods were also affected, with EE, CF, TEPH, TET and CT being significantly reduced, while CP availability and ash content were only significantly increased by bentonite.

Table 2: Effects of treatment with bentonite and wood ash on the chemical composition of P. juliflora leaves and pods

Parameter	ASH	EE	CP	CF	TEPH	TET	CT
100% *P. juliflora* leaves							
Untreated	8.55^{b}	2.97^{a}	23.04^{b}	26.92^{a}	7.69^{a}	6.05^{a}	1.06^{a}
Wood ash treated	11.98^{a}	1.32^{c}	23.93^{a}	23.08^{b}	5.60^{b}	4.51^{b}	0.86^{c}
Bentonite treated	8.29^{c}	1.89^{b}	23.67^{ab}	23.08^{b}	5.39^{b}	4.05^{c}	0.92^{b}
SEM	0.05	0.11	0.15	0.5	0.08	0.1	0.01
100% *P. juliflora* pods							
Untreated	10.38^{b}	2.22^{a}	13.09^{b}	16.85^{b}	5.51^{a}	3.30^{a}	2.43^{a}
Wood ash treated	8.72^{c}	1.51^{b}	12.51^{c}	17.92^{a}	2.83^{c}	1.55^{b}	2.10^{c}
Bentonite treated	13.81^{a}	2.12^{a}	13.90^{a}	17.92^{a}	3.56^{b}	2.31^{ab}	2.18^{b}
SEM	0.07	0.07	0.1	0.08	0.11	0.23	0.01

EE=Ether Extracts (%), CP=Crude Protein (%), CF=Crude Fibre (%), TEPH=Total Extractable Phenolics (g/kg DM), TET=Total Extractable Tannins (Tannin Acid Equivalent in g/kg DM,), CT=Condensed Tannins (g/kg DM), abc Means in the same column with different superscripts differ significantly at $P<0.05$; SEM=Standard error of the mean

At PPL50:50, ash, EE, TEPH and CT were significantly decreased, CP availability and CF were significantly increased while TET was not significantly affected (Table 3). With the remaining two ratios (PPL75:25 and PPL25:75), CF, TEPH, and CT were significantly reduced while availability of CP was significantly increased. TET was insignificantly affected at PPL75:25 and significantly affected with wood ash at PPL25:75. Ash and EE were significantly increased at PPL75:25 and significantly decreased at PPL25:75.

Table 3: Effects of treatment with bentonite and wood ash on the chemical composition of *Prosopis juliflora* leaves and pods at 50:50, 25:75 and 75:75 ratios

Parameter	ASH	EE	CP	CF	TEPH	TET	CT
50% leaves and 50% pods of *Prosopis juliflora*							
Untreated	10.70^{a}	1.94^{a}	14.14^{c}	18.50^{b}	5.35^{a}	3.80^{a}	1.44^{a}
Wood ash treated	1.52^{c}	1.58^{b}	23.17^{a}	20.78^{a}	4.17^{c}	3.05^{b}	1.27^{b}
Bentonite treated	6.62^{b}	1.29^{c}	18.72^{b}	20.78^{a}	4.96^{b}	3.66^{a}	1.34^{b}
SEM	0.05	0.06	0.2	0.04	0.03	0.11	0.02
25% leaves and 75% pods of *Prosopis juliflora*							
Untreated	7.09^{c}	2.45^{a}	13.99^{c}	23.17^{a}	5.21^{a}	3.04^{a}	1.91^{a}
Wood ash treated	9.60^{b}	1.65^{b}	15.82^{a}	21.51^{b}	3.38^{c}	2.25^{a}	1.62^{b}
Bentonite treated	12.43^{a}	2.32^{a}	14.98^{b}	21.51^{b}	4.03^{b}	2.72^{a}	1.55^{b}
SEM	0.05	0.04	0.15	0.06	0.03	0.31	0.02
75% leaves and 25% pods of *Prosopis juliflora*							
Untreated	12.84^{a}	1.80^{a}	20.22^{b}	22.18^{a}	6.72^{a}	4.89^{a}	1.14^{a}
Wood ash treated	11.01^{b}	1.49^{b}	22.19^{a}	21.32^{b}	4.65^{c}	3.41^{b}	0.97^{b}
Bentonite treated	9.69^{c}	1.80^{a}	21.61^{ab}	21.32^{b}	6.02^{b}	4.66^{a}	0.91^{b}
SEM	0.03	0.03	0.33	0.08	0.08	0.07	0.01

EE=Ether Extracts (%), CP=Crude Protein (%), CF=Crude Fibre (%), TEPH=Total Extractable Phenolics (g/kg DM), TET=Total Extractable Tannins (Tannin Acid Equivalent in g/kg DM), CT=Condensed Tannins (g/kg DM), $^{a, b, c}$ Means in the same column with different superscripts differ significantly at $p<0.05$.; SEM=Standard error of the mean.

4. Discussion

The nutritive value of *P. juliflora* leaves and pods was significantly affected by both ratio and binders. The leaves were more affected by ratio compared to pods. Leaves have lower ash content (8.55%) but when combined with pods (10.38%), this is significantly increased at all other ratios except PPL75:25 (7.05%). The same applies to EE, CF, TEPH, TET, and availability of CP which were significantly reduced at all the ratios.

This shows that when pods are combined with leaves at different ratios, the effect of polyphenolics present in leaves is significantly reduced and CT in pods is significantly reduced. This is in line with the findings of [20] who investigated the composition and degradability of different fractions of Calliandra leaves, pods and seeds and found out that when leaves are mixed with either seeds or pods, its degradability and composition was affected. It is also in line with the findings and conclusions of [2]. Wood ash was found to be more effective with leaves compared to bentonite. It significantly increased the availability of CP and ash content, and reduced its EE, CF, TEPH, TET, and CT. Wood ash was also more effective in reducing the polyphenols in pods compared to bentonite which was more effective with ash and CP significantly increasing their availability. This confirm the findings of [4] that wood ash solution has a potential of minimizing the concentration of tannins by up to 70%.

[16], when investigating the effect of wood ash in tannin reduction found out that wood ash reduced 75% and 96% of tannins in *A. nilotica* and *D. cineraria* fruits respectively. [6], after carrying out a research on the effect of bentonite on alfalfa hay found out that it was very effective in increasing or maintaining the concentration of crude protein and ash content. At 50:50 ratios, the effects of wood ash and bentonite varied with wood ash more significantly increasing ash content and CP availability while reducing TEPH content compared to bentonite which only significantly reduced EE than wood ash. In the remaining parameters, the effect of wood ash and bentonite were not significantly different.

At 25% leaves and 75% pods, bentonite performed better in increasing ash content, wood ash was however, better in EE and TEPH reduction and CP availability increment. Their performances were not significantly different in the other parameters. Wood ash was the most effective binder at 75% leaves and 25% pods significantly increasing ash and protein content while decreasing EE, TEPH and TET content. The ratios depict the effect of ratios on the binders with wood ash being more effective than bentonite in these ratios.

Polyphenols that are a hindrance to effective usage of *P. juliflora* leaves were found to be significantly decreased on treatment with wood ash and bentonite. This confirms the findings of [10] that bentonite is as effective as PEG in tannin binding. This is also in agreement with the study done by [21] that wood ash improves digestibility and performance of Bonga lambs in Ethiopia when it was included in a high tannin diet. However, it is worth noting that wood ash varies in strength and effectiveness depending on the tree of origin [12, 15].

5. Conclusion

It was concluded that ratio affects the nutritive value and chemical composition of *P. juliflora* leaves and pods ration. Treatment with wood ash and bentonite also significantly affected the nutritive value of *P. juliflora* leaves and pods. Therefore, it is safe to use ratio and treatment using wood ash and bentonite as methods of enhancing nutritive value and chemical composition of *P. juliflora* leaves and pods.

6. Acknowledgement

Egerton University together with the Animal Science Department are highly appreciated for making this research possible. TAGDev and RUFORUM are also acknowledged for funding this research.

7. Conflict of Interest

The authors declare no conflict of interest and agree to publish the paper.

8. References

1. AL-Kharabsheh BN, Arbili MM, Majdi A, Ahmad J, Deifalla AF, Hakamy A. A review on strength and durability properties of wooden ash based concrete. Materials. 2022;15:7282. https://doi.org/10.3390/ma15207282
2. Askari F, Fazaeli H. Chemical composition and digestibility of Prosopis juliflora pods and leaves and effect of pods in ration on the performance of fattening Tali kids. Journal of Ruminant Research. 2019;7(2):1-16. https://doi.org/10.22069/ejrr.2019.15817.1661
3. Baringo County Government. County overview (Who we are); c2022. https://www.baringo.go.ke
4. Salem BH, Nefzaoui A, Makkar HPS, Hochlef H, Ben Salem I, Ben Salem L. Effect of early experience and adaptation period on voluntary intake, digestion, and growth in Barbarine lambs given tannin-containing (*Acacia cyanophylla* Lindl. foliage) or tannin-free (oaten

hay) diets. Animal Feed Science and Technology. 2005;122(1-2):59-77. https://doi.org/10.1016/j.anifeedsci.2005.04.009

5. Damato A, Vanzani P, Giannuzzi D, Giaretta E, Novelli E, Vianello F, *et al*. Bentonite does not affect in-vitro ruminal gross fermentations but could modify ruminal metabolome and mineral content. Research in Veterinary Science. 2022;144:78-81. https://doi.org/10.1016/j.rvsc.2022.01.012
6. Ding W, Yang F, Guo X. Screening of compound additive formula for improving quality of high moisture alfalfa hay. Transactions of the Chinese Society of Agricultural Engineering. 2013;29(4):285-292. https://www.ingentaconnect.com/2013/00000029/00000004/
7. Engineering Meteorological Station. Rainfall and temperature data for Egerton University Tatton farm; c2009.
8. Hasnain MS, Nayak AK. Alginate-inorganic composite particles as sustained drug delivery matrices. Applications of Nano composite materials in drug delivery. Wood head publishing; c2018. p. 39-74. https://doi.org/10.1016/B978-0-12-813741-3.00003-0
9. Jaimes-Morales J, Marrugo-Ligardo YA, Acevedo-Correa D. Analysis of mesquite (*Prosopis juliflora*) protein concentrate for possible use as supplementary protein. International Journal of Food Science; c2022. https://doi.org/10.1155/2022/7621818
10. Kemboi F, Ondiek JO, King'ori A, Onjoro PA, Museti JLK. Effects of Polyethylene Glycol (PEG 6000) and bentonite clay incorporation in selected local browse-based diets on the performance of small East African Goats. International Journal of Veterinary Sciences and Animal Husbandry. 2021-2022;6(5):43-47. https://orcid.org/0000-0001-9544-0778
11. Lukić I, Horvat I. Moment of bentonite addition, co-addition of tannins, and bentonite type affect the differential affinity of pathogenesis-related grape proteins towards bentonite during fermentation. Foods. 2020;9(11):1534. https://www.mdpi.com/2304-8158/9/11/1534
12. Makkar HPS, Singh B. Effect of wood ash on tannin content of oak (*Quercus incana*) leaves. Bioresource technology. 1992;41(1):85-86. https://doi.org/10.1016/0960-8524(92)90103-5
13. Makkar HP, Makkar HP. Measurement of total phenolics and tannins using Folin-Ciocalteu method. Quantification of tannins in tree and shrub foliage: A Laboratory Manual; c2003. p. 49-51. https://doi.org/10.1007/978-94-017-0273-7_3
14. Menke KH, Steingass H. Estimation of the energetic feed value obtained from chemical analysis and in-vitro gas production using rumen fluid. Animal Research and Development. 1988;28:7-55. https://cir.nii.ac.jp/crid/1573668925633603328
15. Misra MK, Ragland KW, Baker AJ. Wood ash composition as a function of furnace temperature. Biomass and Bioenergy. 1993;4(2):103-116. https://doi.org/10.1016/0961-9534(93)90032-Y
16. Mlambo V, Sikosana JLN, Smith T, Owen E, Mould FL, Mueller-Harvey I. An evaluation of NaOH and wood ash for the inactivation of tannins in *Acacia nilotica* and *Dichrostachys cinerea* fruits using an in-vitro rumen fermentation technique. Trop. Agric. (Trinidad). 2011;88:44-54. https://d1wqtxts1xzle7.cloudfront.net/66888716
17. Ondiek JO, Ogore PB, Shakala EK, Kaburu GM. Feed intake, digestibility and performance of growing small East African goats offered maize (*Zea mays*) stover supplemented with *Balanites aegyptiaca* and *Acacia tortilis* leaf forages. Basic Research Journal of Agricultural Science and Review. 2013;2(1):21-26.
18. Osuga IM, Wambui CC, Abdulrazak SA, Ichinohe T, Fujihara T. Evaluation of nutritive value and palatability by goats and sheep of selected browse foliages from semiarid area of Kenya. Animal Science Journal. 2008;79(5):582-589. https://doi.org/10.1111/j.1740-0929.2008.00567.x
19. Porter LJ. Number-and weight-average molecular weights for some proanthocyanidin polymers (condensed tannins). Australian Journal of Chemistry. 1986;39(4):557-562. https://doi.org/10.1071/CH9860557
20. Salawu MB, Acamovic T, Stewart CS, Roothaert RL. Composition and degradability of different fractions of Calliandra leaves, pods and seeds. Animal Feed Science and Technology. 1999;77(3-4):181-199. https://doi.org/10.1016/S0377-8401(98)00259-4
21. Tadesse W, Kechero Y, Tolemariam T. Comparison of polyethylene glycol and wood ash extract on feeding value and economic efficiency of mixes of high-tannin feed sources in growing Ethiopian Bonga lambs. Tropical Animal Health and Production. 2018;50:161-167. https://doi.org/10.1007/s11250-017-1417-2
22. Taiz L, Zeiger E, Møller IM, Murphy A. Plant physiology and development. Sinauer Associates Sunderland; c2015. https://www.cabdirect.org/cabdirect/abstract/20173165866
23. Ryssen VJBJ. Wood ash in livestock nutrition: 2. Different uses of wood ash in animal nutrition. Applied Animal Husbandry and Rural Development. 2018;11:62-67. https://www.sasas.co.za/aahrd