THE IMPACT OF RATIO COMBINATION AND TREATMENT OF MATHENGE (*Prosopis juliflora*) ON THE PERFORMANCE OF DAIRY GOATS

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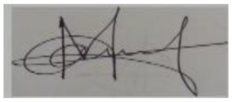
DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted or presented for examination in any institution for the award of a degree.

Signature:  Date…02/07/2024

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Recommendation

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DEDICATION

This work is dedicated to my immediate family who have supported me unconditionally throughout my study and research period. I also dedicate it to my siblings; Seline, Florence, Collins, Hellen and Ben who always have me in their prayers. Thank you all so much.

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ABSTRACT

Prosopis juliflora is a drought-resistant, locally available leguminous tree forage that can be used in place of expensive, seasonal and less nutritious feed supplements. The leaves and pods contain crude protein content of 21% and 14% respectively that can meet the demand of a lactating dairy goat of 17%. However, its efficient utilization in animals is hindered by anti-nutritional factors. Treatment of these factors using locally available, relatively cheap and easy to apply methods will enhance efficient utilization of this forage. This study aimed at determining the most digestible ratio combination of *P. juliflora* leaves and pods (LP) with or without treatment with wood ash or bentonite, and the impact of these binders on its chemical composition. The leaves and pods were combined at ratios of 0:100, 25:75, 50:50, 75:25 and 100:0 treated or untreated. A feeding trial was also undertaken to determine the effect on feed intake, milk production, composition, and mineral content. The results were subjected to a one way ANOVA using the generalized linear model of SAS version 9.4. The most digestible ratio of leaves and pods which was found to be at 75% leaves and 25% pods treated or untreated with wood ash and bentonite was used for the feeding trial. *Prosopis juliflora* treated with wood ash was more digestible compared to bentonite treated. Wood ash was especially effective on pods producing 32.95ml and 38.61ml of gas at 24 hr and 48 hr respectively which was significantly different to that produced by bentonite which was 30.42 ml and 33.91 ml at 24 hr and 48 hr respectively. Overall leaves (100%) were significantly more digestible than pods (100%) without treatment with 25.15 ml and 24.51 ml gas production respectively at 48 hr. Treatment with wood ash or bentonite had no adverse effects on the proximate composition of *P. juliflora* LP. Moreover, they were effective in minimizing phenolic compounds. For instance, total extractable phenolics were reduced significantly in leaves from 7.69 g/kg to 5.60 g/kg and 5.39 g/kg with wood ash and bentonite treatment respectively. In pods, they were minimized significantly from 5.51 g/kg to 2.83 g/k and 3.56 g/kg with wood ash and bentonite treatment respectively. On the feeding trial, treated diets had significantly higher intake compared to the untreated (control diets) with 1.90 kg dry matter intake for control, 2.01 kg and 1.96 kg for wood ash and bentonite treated respectively. Milk production, composition and mineral content were also significantly higher in the treated than untreated diets. It was concluded that the use of these natural binders was efficient and was highly recommended as they will enhance usage of leguminous tree forages like *P. juliflora* that are relatively nutritious aand available at any time of the year.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BW	Body weight
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
EFSA	European Food Safety Authority
FAO	Food and Agricultural Organization of the United Nations
GDP	Gross Domestic Product
GE	Gross Energy
GLM	Generalized Linear Model
GM	Gross Margin
In-vitro	Test done on artificial environment in the laboratory
IUCN	International Union for Conservation of Nature
KDB	Kenya Dairy Board
KNBS	Kenya National Bureau of Statistics
NDF	Neutral detergent fiber
PEG	Polyethylene glycol
PVP	Polyvinylpyrrolidone
RCBD	Randomized Complete Block Design
SHP	Smallholder high-potential system
SLP	Smallholder low potential system
SMP	Smallholder medium-potential system
TDN	Total digestible nutrients
USAID	United States Agency for International Development

CHAPTER ONE
INTRODUCTION

1.1 Background Information

The Kenyan population is approximately 47.5 million people (KNBS, 2019). This is expected to double to 96 million by 2050 as that of urban areas also increases from the current 27 to 50% by the same year (FAO, 2017). This rapid growth in population will result in further land subdivision and therefore reduction in arable land which will negatively affect livestock production. According to FAO (2018), the consumption of meat and milk is likely to increase by over 170%, from 9,074 tonnes and 22,873 tonnes respectively in 2015 to 17,405 tonnes and 34,445 tonnes by 2050 in rural areas. At the same time, that of urban areas will rise from 11,747 tonnes and 28,075 tonnes respectively to 39,596 tonnes and 73,393 tonnes. The increase in demand for livestock and their products is chiefly driven by the growth in per capita GDP which is anticipated to increase by 140% by 2050 (FAO, 2017), rapidly growing human population, and urbanization (Bett *et al.,* 2012). Behnke and Muthami (2011) also stated that, 11.4% of the household money meant for food is spent on food products derived from livestock at 13.1% in rural and 9.7% in urban areas of Kenya.

Goats are found in many households where they are kept for a variety of reasons including food security, as a hobby or as a source of income. Mostly, they are used as a source of milk and have been used as a source of milk long before the cow (Lad *et al.*, 2017). Goat milk is generally more preferred than cows' because of its superior nutritional value and has no allergens. People allergic to cow milk protein can safely consume goat milk without any negative effect (Getaneh *et al.*, 2016). The current goat population in Kenya is slightly over 15 million, with about 400,000 being dairy goats (KNBS, 2019). Most of the dairy goats in Kenya are found in the highlands accounting for 80% of their population (Mbindyo *et al.*, 2018). However, dairy goat farmers in Kenya encounter numerous of challenges that are yet to be fully addressed. These include; poor market structure for the dairy goats and their products, diseases, costly concentrates, lack of year-round supply of good quality feeds, breeding problems such as unreliable buck rotation programme, insecurity, climate change and parasites (Kahi & Wasike, 2019; Mbindyo *et al.*, 2018).

The inadequate and poor quality protein and energy feed resources and which are in most cases expensive, coupled with the changing climatic conditions are the most significant limitations to small ruminant production (Olafadehan & Okunade, 2018). There is therefore a need to explore locally available and inexpensive alternative feed resources for goat production. Leguminous tree forages and browses can be used as alternative feed resources. They are not only rich in crude protein, but also mineral and organic matter content thus efficient for use as replacement and supplements for the poor quality and expensive feeds (Ondiek *et al.,* 2013).

Prosopis juliflora has been identified as one of the tree browses that can be exploited as a feed resource since it is drought resistant and nutritious. However, efficient utilization of this shrub as a protein source is hindered by its anti-nutritional factors more so in the leaves, and the fact that it is invasive. These polyphenolic compounds interfere with the digestibility and availability of nutrients such as protein to the animal (Taiz *et al.,* 2015). Ameliorating the effect of these anti-nutrients is key to unlocking the full potential of *P. juliflora* as a feed resource for small ruminants in arid and semi-arid areas. The objective of this study is to evaluate the use natural, local, affordable and readily available tannin binders to minimize polyphenols in this forage and enhance its usage by small ruminants as a protein source in place of the expensive feed supplements. Consequently, wood ash and bentonite were used in this research in *P. juliflora* leaves and pods to determine their effects when fed to dairy goats.

1.2 Statement of the Problem

Poor quality, scarce, seasonal and expensive protein sources is the major hindrance to small ruminants' production. However, leguminous forages like *P. juliflora* can be efficiently used in Arid and Semi-Arid Lands (ASALs) as protein sources but they contain polyphenolic compounds that bind to proteins interfering with their availability, nutritive value, palatability, digestibility and intake. As such, there is need to counteract these anti-nutrients using natural, relatively cheap and locally available binders which literature has recommended further research on. This study focused on bentonite and wood ash which have for a long time been used as tannin binders due to their high efficiency and effectiveness. However, there is scarce information on their effect on *P. juliflora* leaves and pods fed to lactating dairy goats.

1.3 Objectives of the study

1.3.1 General Objective

To contribute to enhancement of food security through promotion of climate smart agriculture by sustainable dairy goat production.

1.3.2 Specific Objectives

i. To determine the effect of combining different ratios of leaves and pods and/or binders on the digestibility of mesquite (*Prosopis Juliflora)*

ii. To determine the effect of ratio combination and treatment using wood ash and bentonite on proximate composition and phenolic compounds in *P. juliflora* leaves and pods.

iii. To determine the effect of treatment of *P. juliflora* leaves and pods with bentonite and wood ash on feed intake, milk production, composition, and mineral content in dairy goats.

1.4 Hypotheses

The following Null hypotheses were postulated for this study: -

i. That combining different ratios of leaves and pods and/or binders does not significantly affect the digestibility of mesquite (*Prosopis Juliflora)*

ii. That proximate composition and phenolic compounds in *P. juliflora* leaves and pods are neither significantly affected by treatment with wood ash or bentonite nor ratio combination.

iii. That treatment of *P. juliflora* leaves and pods with bentonite or wood ash does not significantly affect feed intake, milk production, composition and mineral content in dairy goats.

1.5 Justification of the study

Approximately 83% of Kenya's land mass is either arid or semi-arid and this continually increases due to climate change. These areas receive rainfall ranging from 150mm to 550mm in arid areas and between 550mm to 850mm in semi-arid areas (EAC, 2019). This amount of rainfall can comfortably support leguminous shrubs such as *P. juliflora* which can sprout spontaneously in arid tropical zones with utmost rainfall stress of as low as below 100mm per annum (Haregeweyn *et al.,* 2013). It is of high nutritional value, producing proteins of high quality, and a good source of fodder palatable to all livestock. However, *P. juliflora* has high tannin content which hinder its palatability and digestibility, hence, efficient utilization in animal's diet.

Treatment with bentonite and wood ash can promote availability of protein by neutralizing the tannins, hence, enhancing palatability and digestibility of the leaves and pods. Bentonite and wood ash are natural tannin binders that are locally and readily available, and affordable compared to synthetic binders such as Polyethylene glycol (PEG) which is well documented. More than 90% of Kenyans use either wood or charcoal for cooking, and 6-10% of this is converted to wood ash (AL-Kharabsheh *et al.*, 2022; Bailis *et al.*, 2020). Potassium Carbonate is the major component in wood ash and forms a strong alkaline with high adsorption capacity, called lye when mixed with water. It is this solution that when soaked with feed binds the tannins. Wood ash has long been used for softening vegetables, as a source of alkaline for crops, for smearing houses, pests repellent, insecticide among others. Hence it is very valuable. On the other hand, Bentonite has been reported to be as good in tannin binding as PEG. It is a clay of high cohesiveness that has been used in softening vegetables, fining of wine, removal of toxins from the body, and as the most effective *in-vivo* aflatoxin binder among other uses (Lukić & Horvat, 2020). This study utilized all the above facts on wood ash and bentonite to make efficient use of *P. juliflora* in animals' diet. The study will also assist controlling the menace of *P. juliflora* by utilization, and contributes to climate smart agriculture and food security as stipulated in the Sustainable Development Goals numbers two and thirteen.

1.6 Limitations of the study

i. Quality of wood ash varies depending on the chemical composition of the tree which are also affected by climatic conditions
ii. Kerosene interferes with the chemical composition of wood ash hence kerosene tainted wood ash should be avoided
iii. The locally available bentonite clay might be having impurities hence amount required might be higher compared to the purified one that was used in this research

1.7 Definition of terms

i. Tannin s are yellowish or brownish bitter-tasting organic substances present in some galls, barks, leaves and other plant tissues and it consists of derivatives of gallic acid (Thakker & Sun, 2021)
ii. Polyphenols/ phenolic compounds are secondary metabolites synthesized exclusively in plants and found naturally in plant like fruits, vegetables, herbs among others and are

responsible for their bitterness, flavour and colour, they include flavonoids, phenolic acids, stilbenes and lignins (De la Rosa *et al.*, 2019)

iii. Anti-nutritional factors/antinutrients are compounds in plants which tend to inhibit their intake and nutrient utilization. They include saponins, tannins, protease inhibitors, glucosinolates, phytic acid among others (Vikram *et al.*, 2020)

CHAPTER TWO
LITERATURE REVIEW

2.1 Climate smart agriculture

Climate smart agriculture (CSA) sprung up in 2009 as a methodology to guide management of agriculture to overcome climate change (FAO, 2018). Climate change has exacerbated uncertainties and risks among farmers and policy makers over the years (Singh *et al.,* 2020). This poses a challenge to food security given the constantly increasing population, urbanization and natural disasters such as drought, famine, floods and extreme temperatures (Duchenne-Moutien & Neetoo, 2021). It is estimated that the living conditions of as many as 9 billion people will continue worsening by 2050 due to these factors, increasing instances of hunger and poverty (Dániel, 2020). As a result, several international organizations in collaboration with the World bank and Food and Agriculture Organization of the United Nations have been working together to devise agricultural systems that will help curb this menace and CSA was one of these mechanisms that several researchers, policy makers, investors and experts applauded hence its implementation (FAO, 2013).

Climate Smart Agriculture is a sustainable and transformation oriented form of agriculture with improvement in food security and productivity as its core mandate (Matteoli *et al.,* 2020). It puts into consideration the key pillars of climate change such as resilience, adaptation and mitigation as well as innovative and smart technological know-how thus enhance profit and minimize susceptibility by reduction in greenhouse gas emissions (Adesipo *et al.,* 2020). According to FAO (2019), CSA has three pillars, the first one being to sustainably enhance the productivity of agriculture and income, secondly, to promote resilience and adaptation of people and food systems to climate change and thirdly to minimize or eradicate greenhouse gas emissions. Goats are hardy and can survive in extreme temperatures compared to other livestock, they are also cheaper to maintain and produce less greenhouse gases compared to cattle (Sejian *et al.,* 2021). Moreover, *P. juliflora* is drought resistant and available locally (Shiferaw & Demissew, 2022). On the other hand, natural binders such as wood ash and bentonite are neither harmful to the animal nor to the environment (Ghahri *et al.,* 2021). Considering the pillars of CSA, this research is qualified to be promoting climate smart agriculture.

2.2 Production of Dairy Goats in Kenya

In the recent years, popularity of goat farming has greatly improved among smallholder farmers both in rural and urban areas (Miller & Lu, 2019). This is because goats are deemed hardy in that they can overcome difficult conditions, endure and maintain their productivity given the changing environmental conditions that have continually imparted stress on animals in the past few years (Dwyer, 2022). Moreover, with the continually growing human population and land fragmentation, goats are the best animals to keep as they require less space, feed, capital, and more resistant to diseases compared to cattle and poultry (Kahi & Wasike, 2019). Moreover, they are good source of income given the higher demand for their products. They also require simple structures, can easily be sold when need arises, and their small size allows them to be easily and quickly slaughtered to satisfy family's food needs with less wastage (Dhakal *et al.*, 2021).

According to Lad *et al*. (2017), goats are found in many households in Africa, and Kenya is not an exception with goats found in virtually all counties in the country. Kenya's goat production has increased significantly over the years. According to statistics, there were 15 million goats in Kenya in 2019 with approximately 400,000 being dairy goats compared to just 200,000 dairy goats as reported in 2011 (KNBS, 2019). Dairy goat sector has been increasing exponentially over the past decades through production and sale of meat and milk such that it contributed 4.2% of the total milk produced in 2016 (KDB, 2016). The common dairy goat breeds in Kenya are the Saanen, Toggenburg, Boer, Alpines, and Anglo-Nubian together with crosses with indigenous goats (Waineina *et al.,* 2021). These exotic breeds were introduced in the Kenyan highlands during the pre-colonial periods by various development operations with the aim of enhancing food security and alleviating poverty (Mutunga *et al.,* 2023). However, in recent times, especially from 1990s, various development partners such as Farm Africa and Britain's agency for Overseas development (DFID) have played a key role in introducing more dairy goats in Kenya.

Goat production comes with a lot of advantages despite the many challenges that the sector faces. Goats can be reared in any climatic conditions and with low starting capital, they have better thermal and drought tolerance, better disease resistance and relatively high feed conversion efficiency compared to other ruminants (Sejian *et al.,* 2012). Furthermore, they can also survive on less pasture and produce less methane thus environment friendly (Nair *et al.,*2021). The poorly structured market of this sector has not hindered consumer's preference of goat milk and products due to its health and nutritional benefits. Goat milk has highly digestible and easily metabolized, and tastier than cow milk (Haenlein, 2004). It also resembles human milk more and lack allergens. It is thus preferred for infants than cow milk, having been used to make infant formula over the last 30 years (Tarola *et al.*, 2019).

2.3 Dairy Goat Production Systems in Kenya

The popularity of dairy goat farming is growing rapidly among smallholder farmers due to numerous factors. This include land subdivision which has resulted in small land holdings that cannot sustainably keep larger livestock such as dairy cattle (Mburu *et al.*, 2014). However, these diminished land sizes can be used to produce dairy goats and dairy goat products like milk, meat, hair and skin at an economic level and at an attractive price (Miller & Lu, 2019). Nevertheless, the issue of small land holdings is also a major challenge for dairy goat farmers, especially in the provision of year-round supply of good quality forage. In an attempt to address the challenges facing the dairy goat sub sector, non-governmental organizations, the Kenyan Government together with other bilateral and multilateral development agencies organizations such as USAID and Farm-Africa among others came together (Ojango *et al.*, 2010). They initiated a dairy goat production improvement programme focusing on key constraint such as nutrition, breeding and production system. According to Agossou *et al.* (2017), dairy goat production is majorly under the smallholder, crop-livestock mixed production systems. However, the systems vary from smallholder low potential (SLP), smallholder medium-potential (SMP) to smallholder high-potential (SHP) systems of production depending on the level of use of inputs (Shivairo *et al.*, 2013).

In SLP system of production, goats are grazed in the open field, while in SMP apart from being grazed in open fields they are supplemented with cut and carry fodder in the evening when they are under an enclosure/stall (Mburu *et al.*, 2014). For the SHP, they are restricted in stalls where they are fed with cut and carry fodder and supplemented with commercial feeds. Most dairy goat production systems entail the low input systems characterized by spontaneous feeding and housing (Dubeuf & Boyazoglu, 2009; Nampanzira *et al.*, 2015). For housing, most farmers use raised slated floor, some use earthen floor and others prefer concrete floor with wooden walls (Kaberia *et al.*, 2003). There are hardly any commercial nor large scale dairy goat farms in the peri-urban areas of Kenya. The few that exist have a ready market in the urban areas for their dairy goats and products (Peacock, 2005).

2.4 Dairy Goat Nutrition

Feeding and nutrition is one of the chief challenges facing dairy goat farmers besides diseases and parasites, climate change, breeding and reproduction, and underdevelopment in the dairy goat products market (Bosman *et al.*, 2015). Feeding is the most expensive factor in any animal production and intensive dairy goat production is not an exception. To maintain good health and better expression of genetic potential for milk production, goats ought to be fed a balanced diet constituting carbohydrates, proteins, vitamins, and minerals containing the key macro elements like calcium, phosphorus and trace minerals (Amole & Ayantunde, 2016). Feeding of dairy goats among small holder dairy goat farmers relies on readily available forages which are used in making homemade rations (Roy & Rana, 2024).

Generally, goats are great browsers and are known to have a greater ability to select high quality plants and plant parts that are more palatable and nutritive and with good botanical composition depending on the plant species available (Siemens, 2019). In most dairy goat production systems, they are fed on forages such as hay, silage and pasture, and fresh grass fodder based in stalls (Amole & Ayantunde, 2016). However, fresh pastures and fodders are seasonal and are only available during the rainy season. During dry spell, dairy goats are majorly fed on hay and crop residues and which are generally considered to be of low quality (Recha *et al.*, 2013). These crop residues also vary depending on crops grown in a particular area and are usually treated with urea and fortified molasses before feeding. When being grazed, dairy goats are often grazed together with other animals like sheep or larger livestock or tethered in some systems (Partey *et al.*, 2019).

Farmers mainly supplement lactating goats with agro-industrial by-products like oil seed cakes from cotton, sunflower and groundnut, maize bran and wheat bran (Shamsi *et al.*, 2012). Exotic dairy goat breeds are the majorly supplemented with vitamin in the form of vitamin premixes and minerals in the form of mineral blocks. The local breeds get most of the minerals and vitamins they require from forages when browsing (Roy & Rana, 2024). Various grasses and legumes are also used as fodder supplements for the goats.

2.4.1. Nutrient Requirement of Dairy goats

Goats require dry matter intake (DMI) of 4-5% of their live weight daily (Singh, 2018). DMI is affected by body weight, percentage dry matter (86-92% in hay and concentrates, and 12-35% in forages), palatability, stage of growth, pregnancy or stage of lactation (Oliveira *et al.*, 2014). The nutrient requirements by dairy goats for maintenance, during early and late gestation and during lactation for a mature doe are shown below (**Table 2.1**). Half of goat's ration should constitute of forages made up of either hay or pasture, that is 12% crude fiber, to avoid high energy related problems that come about mainly when fed with too much grains (Mkutche, 2020). These problems include; urinary calculi in castrated males, lactic acidosis, enterotoxaemia, and polio-encephalomalacia. Demand for energy varies depending on the psychological state of an animal, whether for growth or lactation, maintenance or production. Lactating does have the highest energy demand and therefore, it is cru cial to feed them with a ration that is high in energy during breeding, late gestation and lactation (Shaw & Phillips, 2023). Dairy goat kids require 21% higher energy than the average.

Table 2.1: Nutrients required by does

Stage		**Nutrients required on DM basis**	
DMI based on BW(%)		**%CP**	**%TDN**
Maintenance	1.8-2.4	7.0	53.0
Early lactation	2.4-3.0	9.0-10.0	53.0
Late gestation	2.4-3.0	13.0-14.0	53.0
Lactation	2.8-4.6	12.0-17.0	53.0-66.0

Source: NRC (2007)

Protein are the most expensive feed ingredients (Parisi *et al.,* 2020). Its requirements are higher during fast rate of growth, lactation and growth of mohair. During certain times of the year like winter, there is also need for protein supplementation. However, there is no need for supplementation when hay being fed is of a high quality (12-15% of protein).

2.5 Overview of Prosopis juliflora

Prosopis juliflora, also known locally in Kenya as '*Mathenge*', and *meskit or mesquite* in the US. It is an evergreen forage tree native to Central and North America, the Caribbean, and some parts of Asia. It is one of the 44 species of Prosopis genus and grows up to 10-15 m high (Awale, 2021). In Africa, the tree was first introduced in Senegal in the early 1800's and later in South Africa and Egypt in the late 1800 and 1900 respectively. It was introduced in Kenya much later in 1973 from Brazil and Hawaii to reclaim the limestone quarries of Mombasa. It was later introduced in the ASALs of Baringo, Tana River, Mandera, and Garissa counties in 1980 as a cover tree to control extensive soil erosion in those areas (Rashed *et al.,* 2022). The tree is also a source of food for humans, fodder for livestock, timber, firewood, charcoal, honey production, provide shade, contribute to soil amelioration, and generally add aesthetic value to the land (Choge *et al.,* 2022).

Since its introduction, this tree has spread extensively and very aggressively to various parts around the country and there are fears that it might spread beyond control with serious environmental consequences to the ecosystem. According to Tabe-Ojong *et al.* (2021), this tree is a potential threat to Kenya's ASAL rangelands since it spreads rapidly in hot and dry lowland areas. Currently, the tree has spread to almost all ASALs of the tropics and sub-tropics (Sawal *et al.,* 2004). In 2004, this *P. juliflora* was among the 100 most invasive weed species in the world as listed by the International Union for Conservation of Nature (IUCN) (Mwangi & Swallow, 2005). However, the pods and leaves have been found to be very nutritive and with a capability of providing good forage quality for livestock (Haregeweyn *et al.,* 2013). Furthermore, the shrub can grow in harsh environmental conditions where most plants cannot survive and still remain evergreen all year round unlike other plants that either shed their leaves, pods or flowers during the dry season or drought.

This tree/shrub can survive to more than 25 years and hits its maximum fruit production at 15-20 years (Jaimes-Morales *et al.,* 2022). It can grow in all climatic conditions, in both fertile and unfertile soils as well as saline soils. It can do well in areas receiving rainfall as low as 50mm per annum (Haregeweyn *et al.*, 2013). It becomes a full tree within three years when it starts producing fruits hence becomes a good source of both fruits and foliage. *P. juliflora* is a potential and excellent source of fodder at any time of the year. However, complaints have been received that the leaves and more so, the pods cause discouloration, cracking and subsequent breakage of livestock's teeth and bones, and loss of body condition since they could not browse efficiently (Sharma *et al.,* 2021). This was confirmed by Mutavi (2020) when he subjected teeth and bones of slaughtered goats in aqueous solution of leaves and pods and found that that of the pods was more acidic and had adverse effects than leaves. However, feeding well dried and ground *P. juliflora* not only controls its invasiveness but also the effect on bones as the ground ones are less acidic (Shitanda *et al.,* 2013). Drying and grinding also minimizes tannin content and increases digestibility in both pods and leaves.

2.5.1 *Prosopis juliflora* Pods

Prosopis juliflora pods are straight with an incurved apex, and a length of 8-29 cm, thickness of 4-8 mm, and breadth of 9-17mm. The pods are usually seen to be constricted between the seeds and can contain between 10-30 seeds. Mature and dry ones are either yellow or brown but they are green when young and unripe. The seeds are broad and ovoid measuring about 6 mm by 4 mm, are brownish in colour and contained in a whitish sweet pulp (Azani *et al.,* 2017). A mature *P. juliflora* tree of about 8.5m tall can produce from 7.2-90 kg of pods per year. At different seasons of the year, the chemical composition of the pods varies minimally. Pods have been reported to contain 10-15% CP and 16-41% soluble sugars with a digestibility of 68-75% (Azani *et al.,* 2017). They are also endowed with saccharose at a dry matter content of 20-25%. At 70% dry matter, sucrose is the main sugar in the pods making them sweet to taste hence highly palatable (Sawal *et al.,* 2004). The pods have been used to feed goats, sheep, cattle, poultry and camels either as whole or milled form. The milled form is more preferred as it enhances digestibility and minimizes chances of seed dispersal which is undesired (Peña-Avelino *et al.,* 2014).

However, the pods are rapidly becoming commercial with companies in South Africa currently using more than 200 tons of the pods per year to produce food supplements more so for the management of diabetes (Shitanda *et al.,* 2013). In areas like Baringo, Kenya, it is commercially sold on the roadsides in bulks for passers-by and to feed millers for dairy meal formation. The United States, Latin America and India use the pods in large scale in ranches to feed their livestock while in Peru, they are used to make various foods and a local brew known as Algarobina. It is also used as a coffee substitute and the endocarp as a source of fuel. In the near future, the pods will be commercial feeds like any other feeds sold out there and the farmers will have to pay more to purchase them. The competition between man and animals for the pods is also intensifying as shown above. Apart from that, when grazed upon, seeds are an excellent medium of dispersal when not chewed and bypass rumen digestion. Furthermore, when passing through the animal's digestive system they tend to undergo treatment that enhances their germination once excreted. The excreta also act as fertilizer during the early stages of germination and therefore ensuring establishment of the seedlings (Alvarez *et al.,* 2017).

2.5.2 *Prosopis juliflora* Leaves

The leaves are bi-pinnate, 3-11 cm long and the leaflets can be 11-15 pairs. They are very nutritive, yet less digestible and palatable compared to the pods as shown in **Table 2.2**. They have a more bitter taste than the pods but are more abundant (Azani *et al.,* 2017). They are however, consumed by animals when nothing else is available.

Table 2.2: Chemical composition of P. juliflora leaves, pods and their mixture (%DM)

Plant part	DM	CP	NDF	ADF	ADL	Ash
Pods	89.7	14.9	28.8	17.04	4.2	5.3
Leaves	92.3	21.6	27.1	18.2	3.2	8.7
Pods and leaves	91.4	18.3	28.3	17.5	3.6	7.1

Source:Ali *et al.* (2012)

Browsers are also known to feed on both leaves and pods (Kandie, 2022). This is due to the diversity in terms of nutritive value and mineral content of both leaves and pods (**Table 2.3**). Goats require crude protein content of 12-17% during lactation and this can be met by both leaves and pods without supplementation.

Table 2.3: Mineral composition of P. juliflora leaves and pods

Elements (%)	Leaves	Pods
Sodium	0.04±0.003	0.02±0.002
Phosphorus	0.21±0.018	0.23±0.066
Magnesium	2.83±0.024	1.52±0.065
Potassium	1.50±0.007	2.15±0.055
Calcium	6.38±0.248	3.46±0.302
Zinc	2.09±0.072	18.86±1.038

Source: Shitanda *et al.* (2013)

Choge *et al.* (2022), reported that the leaves are more efficiently used by goats compared to sheep, and hardly palatable to camels, while unpalatable to horses. However, when the leaf debris are cut off from the tree and dried, they become more palatable since the toxic substances like tannins clear off a few days later due to wilting effect.

2.6 Tannins on Ruminants

Tannins are described as compounds which result from phenolic acids. They can be distinguished from other phenolic compounds with their capacity to bind with proteins and form complexes with them making them difficult to decompose (Habtamu & Negussie, 2014). These phenolic compounds are widely spread in many plant species, more so leguminous forages with their seeds and pods, globally and in all climatic conditions. They are the most plentiful metabolites synthesised by plants accounting for about 5-10% dry weight of the leaves (Gourlay *et al.*, 2020). Tannins are grouped into hydrolysable tannins and condensed tannins which are found in most legumes, shrubs and leaves of trees and pseudo-tannins. Generally, tannins are more widely found in leguminous trees, plants, fruits, flowers, forages, and grasses (Blackmon *et al.*, 2016).

Tannins were for a long time thought to be detrimental to all animals (Tong *et al.*, 2022). However, with more research, they have been found to be beneficial as well. Their harmfulness is subject to the quantity consumed, the chemical structure, molecular weight and the type of animal that consumes them (Dey & De, 2014). Goats can utilize tannins more efficiently than other ruminants such as cattle and sheep (Schmitt *et al.*, 2020). It is said that this ability is due to proline found in their saliva which act as the initial defence mechanism against tannins once consumed. The goats are compared to the rats, deer, mouse and mules which contain this substance (Niezen *et al.*, 1995).

2.6.1 Positive Effects of Tannins on Ruminants

Tannins consumed in moderate quantity, and with the right structure, molecular weight and ingested by the right animal are very critical in enhancing efficiency in digestion more so in ruminants (Dey & De, 2014). They enhance feed protein and microbial protein by-pass by about 60% to the abomasum where they undergo enzymatic digestion rather than undergo microbial degradation in the rumen which usually result in wastage (Jonker & Yu, 2017). The wasteful ruminal degradation of protein is reduced when the tannins form a tannin-protein complex which only dissociates at low pH, past the rumen hence become available for enzymatic digestion. At the same time, they enhance flow of amino acids into the small intestine where they are absorbed into the blood stream thus increase supply of non-ammonia nitrogen which is critical for production purposes (Besharati *et al.*, 2022). Tannins being bulky, reduce the rate of fermentation thus reduce methane and nitrogen gas production and introduction of these gases to the environment. This act also reduces formation of frothy bloat in the rumen (Blackmon *et al.*, 2016).

Tannins have also been found to have an anthelmintic effect and therefore suppressing proliferation of gastrointestinal parasites which is likely to confer beneficial healthy effect on the animal. When being passed out, they come out with both larval and adult worms thus keeping them in check (Gunun *et al.,* 2017). At the same time, when they bind with the available proteins, they limit nutrients available for these worms leading to their starvation and death. Worms are deleterious to an animal's health as they cause diarrhoea and loss in body condition (Dauda & Kuka, 2021). Furthermore, they compete for scarce dietary nutrients such as trace minerals and vitamins thus compromising the nutritional status of the animal.

2.6.2 Negative Effects of Tannins on Ruminants

Leguminous forages are increasingly being utilized in small ruminant's nutrition as feed supplements to replace cereal concentrates which are costly. This minimizes the cost of feeding hence cost of production and enhance the quality of these forages (Vasta & Luciano, 2011). However, they have high tannin content and when included in the diet at more than 1.5% of the dry matter, they significantly interfere with alpha–amylase, trypsin, general proteinase activity, and lessen the ability of the tract to absorb nutrients thus result in depressed performance of animals (Huang *et al.,* 2018). They also decrease feed intake, availability of proteins to the animal, nutrient digestibility and production. Tannins higher than 5% greatly reduce feed intake and when more than 9% can result in mortality (Mustabi & Prahesti, 2019). They interfere with the fermentation process by limiting microbial activities, protein break down hence high protein losses in feaces and urine, increase methane production and reduce the ability of an animal to get rid of micro-organisms and toxins in feed (Addisu, 2016).

The parts of plants that are high in tannins tend to have a severely bitter taste which protect them from being browsed on but when this occurs, tannins decrease the value of the feed by binding and precipitating proteins, amino acids and alkaloids (Habtamu & Negussie, 2014). When the tannin content is too high, the complexes they form with proteins fail to be broken post-rumen hence the protein is removed as waste through feaces and urine, thus become unavailable to the animal. Intestinal permeability to tannins and eventual introduction into the blood stream may also be enhanced (Huang *et al.,* 2018). This can be as a result of consumption of an intestinal irritant, intake of condensed tannins in high quantity or together with saponins. This may cause damage to vital organs such as kidney, liver and spleen just like the hydrolysable tannins.

According to EFSA (2014), when poultry consume rations with tannin content above 40,000 mg tannic acid/kg DM, it causes decrease in feed intake and egg production. When pigs are given above 1000 mg tannic acid/kg DM, it results in increased feed conversion ratio, decreased daily weight gain and lower blood cell counts. Similarly, calves experience diarrhoea which might result in mortality, renal failure and meta-haemoglobinaemia when taken above 4400 mg/kg body weight.

2.7 Tannin Binding

Tannins are important in ruminants, however, when taken in high quantities, they cause adverse effects that may be detrimental to an animal. They may interfere with productivity and lead to death of the animal. Therefore, there is need to minimize tannin content in browses so that ruminants can use them more efficiently and realize high production and good health. There are many methods that have been used since time immemorial to reduce tannin in forages, more so leaves to desirable quantities. According to Brown *et al.* (2016), drying is an effective method of tannin reduction in high moisture content multipurpose trees. When dried at 60^{o}C for 48 hr, sun-dried for 24 hr to 48 hr, or shade-dried for up to 72 hr, tannin present in leucaena and cassava are reduced significantly.

The same authors also recognized wood ash as an economical source of alkali that can effectively minimize tannin content in feedstuffs, it achieves this by oxidizing the tannin compounds. When feeds are soaked with this alkaline solution, the toxic compounds (tannins) are separated from the nutritious parts. It has been traditionally used to reduce tannin content in feeds like sorghum, millet and vegetables for human consumption. The more the content of wood ash, in the solution, the better the results. However, 180 to 200 g per litre of water has been recommended by several authors. Ben Salem *et al*. (2005) experimented on green and dried *Acacia cyanophylla* leaves which were soaked for 6 hr in wood ash solution, and observed that the concentration of condensed tannins was reduced in each of them by 44.8 and 58.2% respectively.

According to these authors, the wood ash solution can minimize tannin concentration by up to 70%, improve fibre and protein digestibility by 14% and 8% respectively, nitrogen retention from -0.4 to +2.4 g/day and increase supply of microbial protein by 15%. Wood ashes from diverse woods perform differently in tannin reduction, and this can be attributed to the concentration of Calcium carbonate in the diverse tree species. Although conclusive research has not been conducted in this area, Ben Salem *et al.* (2005) compared the performance of Aleppo pine and acacia wood ashes in tannin reduction and found Aleppo pine wood ash more effective. Calcium Carbonate, the major mineral in wood ash, forms a strong alkaline with high adsorption capacity, called lye when mixed with water. It is this solution that when soaked with feed binds the tannins which are then excreted via urine and feaces.

Brown *et al.* (2016) also recommend cooking at 100°C for 30 minutes that it decreases tannin content by 71.91%. Autoclaving or steaming was also found to be very effective in ground oak leaves by elevating the digestibility of dry matter from 24% to 27%. When steaming is carried out for 10 minutes, protein precipitation is increased to 53%. Magadi soda has also been used to this effect and found to decrease tannin content in sorghum by 40 to 50% (Ben Salem *et al.,* 2005). Chopping of feeds and then adding urea, and thereafter storing them for 5-10 days rather than feeding on the same day can help in tannin reduction. Urea releases ammonia which inactivates tannins (Ben Salem *et al.*, 2005).

Makkar (2003), did a study on solid-state fermentation as a method of tannin biodegradation using white-rot fungi on oak leaves and found out that, the use of *Sporotrichum pulverulentum* was very effective in tannins and total phenol's reduction at 66% and 58% respectively in just ten days. Others like *Ceriporiopsis subvermispora* and *Cyathus stercoreus* took longer (3 weeks) and reduced tannin content by 56-65%. Use of chemicals including organic solvents such as 50% methanol, 30% acetone and 40% ethanol can minimize up to 70% of tannins present in leaves (Cuong *et al.,* 2019). Others such as alkalis like Sodium hydroxide (0.05 M) oxidize Phenolics, and Hydrogen peroxide decrease tannin content by 99% (Brown *et al.*, 2016).

Polyethylene glycol (PEG) releases proteins from the tannin-protein complex and binds with the tannins. It is mixed with water at 0.5 g PEG/ml then sprayed on the feeds and left overnight before feeding (Putri *et al.,* 2021). It is mainly effective on feed containing 5-10% condensed tannins. However, it is considered costly and difficult to acquire. Moreover, the effect of PEG-tannin complex on the environment is still unknown, and given that it is synthetic, might be detrimental even to the animals themselves. Polyvinylpyrrolidone (PVP) is another commonly used synthetic tannin binder and its effects in animals and the environment is equally unknown. When used in high concentrations, both PEG and PVP are highly toxic (Kizhakkumpat *et al.,* 2021).

Bentonites can be used up to 20 g/kg of feed (EFSA, 2010). Kemboi *et al.* (2022) found out that bentonite can successfully bind with tannins in *Acacia brevispica* and *Berchemia discolor* resulting in significantly higher total and daily dry matter intake and weight gain in Small East African goat. The study also recommended its use among the pastoral communities in Baringo due to its effectiveness and low cost. In Kenya, bentonite is naturally found in the Western region, Baringo county, and around Mt. Kenya region hence easily accessible to the inhabitants in those areas. It is also sold locally at very affordable prices. The active ingredient in bentonite that makes it effective in tannin binding is Montmorillonite which is made up of alumino silicate layers giving it a higher cation exchange capacity thus making it a good adsorbent (Hasnain & Nayak, 2018).

In this study, wood ash and bentonite were used due to their high effectiveness, availability, low cost, and the fact that they are natural hence have no negative effects on animals unlike the synthetic ones. Moreover, authors like Ben Salem *et al*. (2005) encouraged further research on natural and local binders like wood ash and bentonite as tannin binders. They also suggested that using them in combination might be more effective than when used singly. Therefore, this study was also carried out to confirm or dispute the recommendations by these authors. The effectiveness of these binders have not been determined when used to treat *Prosopis juliflora* leaves and pods. Moreover, the effect of ratio combination of leaves and pods on their digestibility have not been determined before. *Prosopis juliflora* leaves and pods combined at different ratios and treated with wood ash and bentonite clay have also not been tried on dairy goats.

CHAPTER THREE

THE EFFECT OF COMBINING DIFFERENT RATIOS OF LEAVES AND PODS AND/OR BINDERS ON THE DIGESTIBILITY OF MESQUITE (*Prosopis Juliflora)*

Abstract

Prosopis juliflora has long been used as a leguminous tree forage. However, the availability of high concentrations of tannins has hindered its usage. The study aimed at determining the most digestible leaves and pods (LP) ratio of *P. juliflora*) and the effect of wood ash and bentonite on their digestibility. Therefore, *in-vitro* digestibility (IVD) trial was conducted on *P. juliflora* LP at ratios of 0:100, 25:75, 50:50, 75:25, and 100:0 for 96 hr with and without treatment. Gas produced was recorded at intervals of 0, 3, 6, 9, 12, 24, 48, 72, and 96 hr. The results showed that ratio combination significantly affect the digestibility of *P. juliflora* LP. At the ratio of 75:25 leaves: pods, the gas production was 26.16 ml at 24 hr and 31.05 ml at 48 hr which was significantly higher than that of leaves which was 22.74 ml at 24 hr and 25.15 ml 48 hr and pods which was 23.79 ml at 24 hrs and 24.51 ml at 48 hrs. It also showed that wood ash and bentonite significantly ($P<0.05$) affected the digestibility of *P. juliflora* LP at different ratios. Treated samples showed better digestibility than the untreated ones. Wood ash treated pods were the most digestible with gas production of 32.95 ml at 24 hr and 38.61 ml at 48 hr. It was concluded that the use of binders enhanced the digestibility of *P. juliflora* LP. However, they significantly positively affect pods compared to leaves.

3.1 Introduction

The inadequate, costly, seasonal, and poor-quality protein sources coupled with the changing climatic conditions are the major hindrances to small ruminants' production (Olafadehan *et al*., 2018). Use of leguminous tree forages can be a solution to this challenge among livestock farmers and, those keeping dairy goats. They have relatively high content of minerals, organic matter, and crude protein. Moreover, they are also drought resistant due to their ability to access subterranean water due to their deep root system. They can therefore be used to replace the seasonal and low quality forages (Ondiek *et al.*, 2013). *Prosopis juliflora* which is one of the tree forages is an evergreen, available throughout the year and is generally nutritious. However, the high tannin content, more so in the leaves has hindered efficient utilization of this forage as a protein source. The tannins bind to proteins forming complexes that interfere with their availability to the animal (Taiz *et al.,* 2015). Natural, local, affordable, and readily available tannin binders can be used to minimize the effect of tannins in this forage and replace the expensive synthetic binders. Makkar *et al.* (2003), recommend further research on these natural tannin binders as a replacement for synthetic binders which are also detrimental to the environment. This will enhance the adoption and usage of these leguminous forages.

Wood ash and bentonite were used as tannin binders in this research because wood ash is readily available due to the fact that more than 90% of Kenyans depend on either charcoal or firewood as source of energy for cooking (Bailis *et al.,* 2020). Furthermore, 6-10% of wood is converted to wood ash (AL-Kharabsheh *et al.,* 2022). Potassium Carbonate, a major component of wood ash, forms a strong alkaline solution with high adsorption capacity, called lye when mixed with water. This solution binds the tannins when soaked with feeds. Bentonite has been reported to be as effective as polyethylene glycol (PEG) in binding tannin (Kemboi *et al.,* 2022). It is a clay of high cohesiveness that has been used in softening vegetables, and wine fining, as a face mask for removing toxins from the body, and as the most effective *in-vivo* aflatoxin binder (Lukić & Horvat, 2020).

In-vitro digestibility trials were conducted in the laboratory to determine the most digestible ratio of *P. juliflora* Leaves and pods, and the effect of bentonite and wood ash on the digestibility of these ratios. The trials were carried out for 96 hr and gas produced was recorded at intervals of 0, 3, 6, 9, 12, 24, 48, 72, and 96 hr. The calculations were carried out using Boisen and Fernandez (1997) formulae and fitted into the model of Ørskov and McDonald (1979).

3.2 Materials and Methods

3.2.1 Study site

The experiments were undertaken at Egerton University, main campus, in the Animal Science laboratories. It is located in the sub-county of Njoro, the County of Nakuru, Kenya. It is approximately 25 kilometres to the southwest of Nakuru town and 5 kilometres from Njoro centre. The GPS coordinates are 0°22'11.0"S and 35°55'58.0" E, and the latitude and longitudes are 0.369734°S, and 35.932779°E respectively. The altitude above sea level is approximately 1800 metres, and receives averagely 900-1,200 mm of rainfall annually, with average daily temperatures ranging between 17°C-22°C (Egerton University Meteorological Station, 2019).

3.2.2 Source of materials

Mature pods and leaves were collected from Marigat Sub-County, Baringo County, Kenya, and transported to Egerton University. Marigat Sub-County is located at 0° 20'N and 35° 37'E and approximately 20 km from both Lake Baringo and Bogoria. It lies at 1080 m above sea level and receives rainfall of 700 – 950 mm per year with peaks in April/May and July/August but generally erratic. (Baringo County Government, 2022).

3.2.3 Preparation of samples for analysis

P. juliflora leaves and pods samples were sourced from Marigat sub-county in Baringo County by either stripping off or shaking *P. juliflora* trees and shrubs in homesteads, and grazing areas. This was undertaken in the dry months of December to January. The samples were spread out to dry under the shade, sorted out, and packed in sacks. Afterward, they were transported to the Animal Nutrition laboratory of Egerton University for analysis. They were ground to pass through 1 mm sieve, weighed, mixed at ratios of 0:100, 25:75, 50:50, 75:25 and 100:0 then packed in airtight containers. Bentonite was mixed with the samples at the rate of 20g/kg. The samples were made wet with distilled water then mixed with bentonite and left overnight. Wood ash was soaked in water at the rate of 400g/l and left for two hours then decantation was carried out to remove the liquid part. This was mixed with the samples at 400 g/kg and left overnight as well.

3.2.4 Data collection

Data collection was carried out to determine the most digestible ratio of *P. juliflora* leaves and pods, and the most effective binder on their digestibility. Gas produced was recorded at intervals of 0, 3, 6, 9, 12, 24, 48, 72, and 96 hr in ml. The cumulative gas production technique model as developed by Ørskov and McDonald (1979) was used to determine the best ratio after calculation using Boisen and Fernandez (1997) formulae. The ratios used were 0:100, 25:75, 50:50, 75:25, and 100:0 for *P. juliflora* LP, wood ash, and bentonite.

3.2.5 *In-vitro* gas production

The set up for the study began a day earlier. The required instruments were put together, and 200 mg of the samples were weighed in duplicate into clearly marked 100 ml glass syringes. The following morning, the water bath was refilled to the required level, the thermostat heater was switched on, then allowed to heat to 39°C. The thermo-flask was then filled with warm water to keep warm. Approximately 500 ml of rumen fluid was collected from two donor goats before feeding and 15 minutes before the digestibility trial began. The collection of rumen fluid was carried out using a vacuum pump and a stomach tube. They were inserted in the rumen as described by Muizelaar *et al.* (2020), then kept in the warm thermos flask.

The extracted rumen fluid was filtered using two-layered cheesecloth to get particle-free rumen fluid that was kept in the warm thermo-flask and continually pumped with CO_2 to maintain an anaerobic environment. A colourless buffer mineral medium continuously flushed with CO_2 was added into the fluid to emulate the action of saliva at 1:2 (v/v) ratio. The syringes containing samples, and the blank ones were filled with approximately 30 ml of the buffer medium containing rumen fluid and swirled gently to expel bubbles. Thereafter, the clips were tightly closed before the solution was well mixed with the feed samples (200 mg). More air bubbles were expelled from the syringe and the exercise stopped before the solution went into the inlet which was tightly closed using rubber bands.

The syringes were then inverted, and the initial reading was recorded at time = 0 before they were placed into the water bath. Gas production readings were recorded at specific intervals of 0, 3, 6, 9, 12, 18, 36, 48, 72, and 96 hr. After each reading, the syringes were carefully swirled to efficiently mix all floating samples with the solution. When gas produced went past 70ml, it was evacuated by pushing the piston inwards until the red marker on the piston went to 40ml or below and recorded to enhance efficient gas production. The clips were then closed and the syringes placed back in the water bath. The entire digestibility procedure followed the Menke technique developed by Menke and Steingass (1988).

After the data collection exercise, net gas produced was arrived at by deducting mean blank value from gas produced by all the samples from the total increase in volume. To determine the degradability of *P. juliflora* leaves and pods at different ratios and when binders are added, Ørskov and McDonald (1979) model was then applied and the values fit in the formula:

$$Y= a + b(1-e^{-ct})$$

where: -

Y = volume of gas produced with time (t)

a = initial gas produced

b = gas produced at incubation at time t

c = rate of gas production (hour)

(a+b) = potential extent of the gas production.

e = standard error

t = incubation time

3.2.6 Determination of Organic Matter Digestibility, Metabolizable Energy and Short Chain Fatty Acids

Organic matter digestibility, Metabolizable Energy, and Short Chain Fatty Acids contents were approximated after undertaking an *in-vitro* digestibility trial of the samples using the formulas for roughages described by Menke and Steingass (1988).

SCFA (m mol/200 mg DM) =0.0222 GP-0.00425,

OMD%=18.53+0.9239*(gas production at 48 hr) +0.0540*CP,

ME (MJ/kg DM) = 2.2 + 0.1357 × GP + 0.0057 × CP (g/kg DM) + 0.0002859 × EE^2 (g/kg DM)

Where GP is 24 hr net gas production (ML/200 mg DM).

3.2.7 Statistical model and analysis

$$\mathbf{Y_{ij} = \mu + \tau_i + \varepsilon_{ij}}$$

where:

$\mathbf{Y_{ij}}$= dependent variable (gas production)

$\boldsymbol{\mu}$= overall mean,

$\boldsymbol{\tau_i}$ = effect due to binders

$\boldsymbol{\varepsilon_{ij}}$=random error factor effect

The outcome of the digestibility trial was subjected to a one-way analysis of variance using generalized linear model (GLM) of SAS version 9.4. The means were separated using Tukey's honest significance difference (HSD) test at P<0.05 level of significance.

3.3 Results

The *in-vitro* digestibility (IVD) of *P. juliflora* leaves and pods at ratios of 100:0, 75:25, 50:50, 25:75, and 0:100 varied greatly (**Table 3.1** and **Figure 3.1**). Gas production at 24 hr and 48 hr in ml/200mgDM clearly showed these variations. *Prosopis juliflora* leaves and pods at 75% and 25% respectively (26.16 ml at 24 hr and 31.05 ml at 48 hr) has a higher digestibility, and PPL 75:25 (21.32 ml at 24 hr and 22.72 ml at 48 hr was the least digestible.

Table 3.1: *In-vitro* digestibility of P. juliflora leaves and pods at different ratios

Parameter	**24 hr**	**48 hr**	**a**	**b**	**c**	**a+b**	**RSD**	**SCFA**	**OMD%**	**ME**
PL100	22.74***	25.15**	6.2***	5.81***	0.05^{ns}	12.01***	2.05***	0.22***	22.00***	4.56***
PP100	23.79**	24.51**	4.07***	65.07***	0.00^{ns}	69.04***	2.50***	0.08**	19.90***	3.62***
PPL50:50	21.39***	24.20**	3.89***	31.73***	0.10*	36.39***	1.98***	0.06*	21.89***	3.53***
PPL75:25	21.32***	22.72*	3.71***	26.07***	0.00^{ns}	29.86***	1.57***	0.08**	20.58***	3.72***
PPL25:75	26.16***	31.05***	4.64***	2.25***	0.01^{ns}	7.15***	2.49***	0.10**	23.80***	4.11***
SEM	0.48	0.35	0.29	0.12	0.05	0.44	0.24	0.02	0.49	0.20

*Significant at P<0.05, **Significant at P<0.001, ***Significant at P<0.0001, ^{ns} not significant at P<0.05; a, b, c refer to constants described by Ørskov and McDonald (1979), RSD=Relative standard deviation; SCFA=Short chain fatty acids in m mol/200 mg DM, OMD=Organic matter digestibility, ME=Metabolizable energy in MJ/Kg DM; PL 100=100% leaves; PP 100=100% pods; PPL 50:50=pods and leaves at 50% each; PPL 75:25=pods at 75% and leaves at 25%; PPL 25:75=pods at 25% and leaves at 75%; SEM=Standard error of the mean.

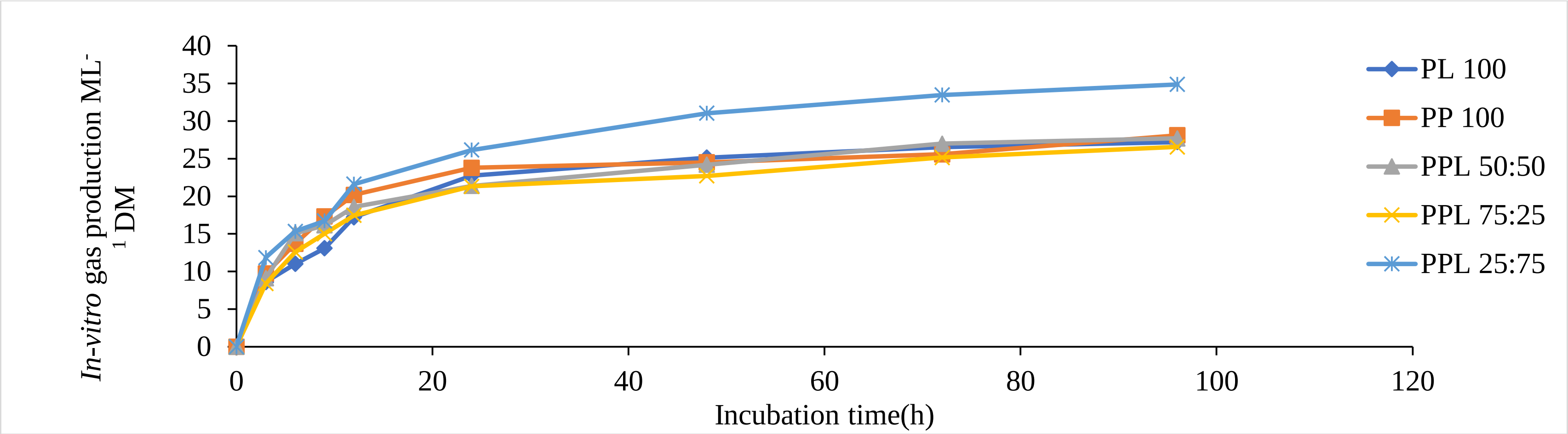

Figure 3.1: Graphical representation of the effect of ratio on the IVD of Prosopis juliflora leaves and pods in an interval of 96 hr

P. juliflora leaves were more digestible when untreated than when treated (**Table 3.2** and **Figure. 3.2).** Untreated leaves had the highest digestibility at 24 hr (22.74 ml) unlike wood ash treated (21.07 ml) and bentonite treated (18.79). Treated pods had higher digestibility than untreated pods (**Table 3.2** and **Figure. 3.2**). Wood ash performed better in enhancing the digestibility of pods with 32.95 ml and 38.61 ml gas production at 24 hr and 48 hr respectively compared to bentonite which produced 30.42 ml and 33.91 ml of gas at 24 and 48 hr respectively.

Table 3.2: Effects of treatment with bentonite and wood ash on the In-vitro digestibility of P. juliflora leaves and pods

Parameter	24 hr	48 hr	a	b	c	a+b	RSD	SCFA	OMD%	ME
100% *Prosopis juliflora* leaves										
Untreated	22.74***	25.15**	6.2***	5.81***	0.05*	12.01***	2.05***	0.22***	22.00***	4.56***
Wood ash treated	21.07***	24.42**	3.11^{ns}	0.03^{ns}	0.05*	3.41***	2.12***	0.15***	22.88***	3.50***
Bentonite treated	18.79***	22.14**	2.50**	1.67***	0.02^{ns}	4.17***	2.32***	0.15***	22.14***	4.73***
SEM	0.58	0.6	0.33	0.06	0.02	0.3	0.18	0.01	0.5	0.22
100% *Prosopis juliflora* pods										
Untreated	23.79***	24.51*	4.07***	6.07***	0.00ns	6.04***	2.50***	0.08*	19.90***	3.62***
Wood ash treated	32.95***	38.61***	1.05***	3.14***	8.50***	4.72***	2.89***	0.21***	24.43***	3.72***
Bentonite treated	30.42***	33.91***	0.00***	5.87***	5.10***	5.87***	3.04***	0.12**	21.66***	3.75***
SEM	0.1	0.32	0.11	0.09	0.09	0.12	0.18	0.02	0.3	0.13

*Significant at P<0.05, **Significant at P<0.001, ***Significant at P<0.0001, ^{ns} not significant at P<0.05; a, b, c are constants described by Ørskov and McDonald (1979), RSD=Relative standard deviation; SCFA=Short chain fatty acids in m mol/200mg DM, OMD=Organic matter digestibility, ME=Metabolizable energy in MJ/Kg DM; SEM=Standard error of the mean.

At a 50:50 ratio combination of *P. juliflora* LP, wood ash had a significantly higher effect on digestibility (**Table 3.3** and **Figure3.2**). Untreated *P. juliflora* LP produced 21.39 ml and 24.20 ml of gas at 24 hr and 48 hr respectively. When treated with wood ash, this increased to 23.58 ml and 30.56 ml at 24 hr and 48 hr respectively. Bentonite reduced the digestibility at 24 hr (20.27ml) but increased at 48 hr (26.11 ml).

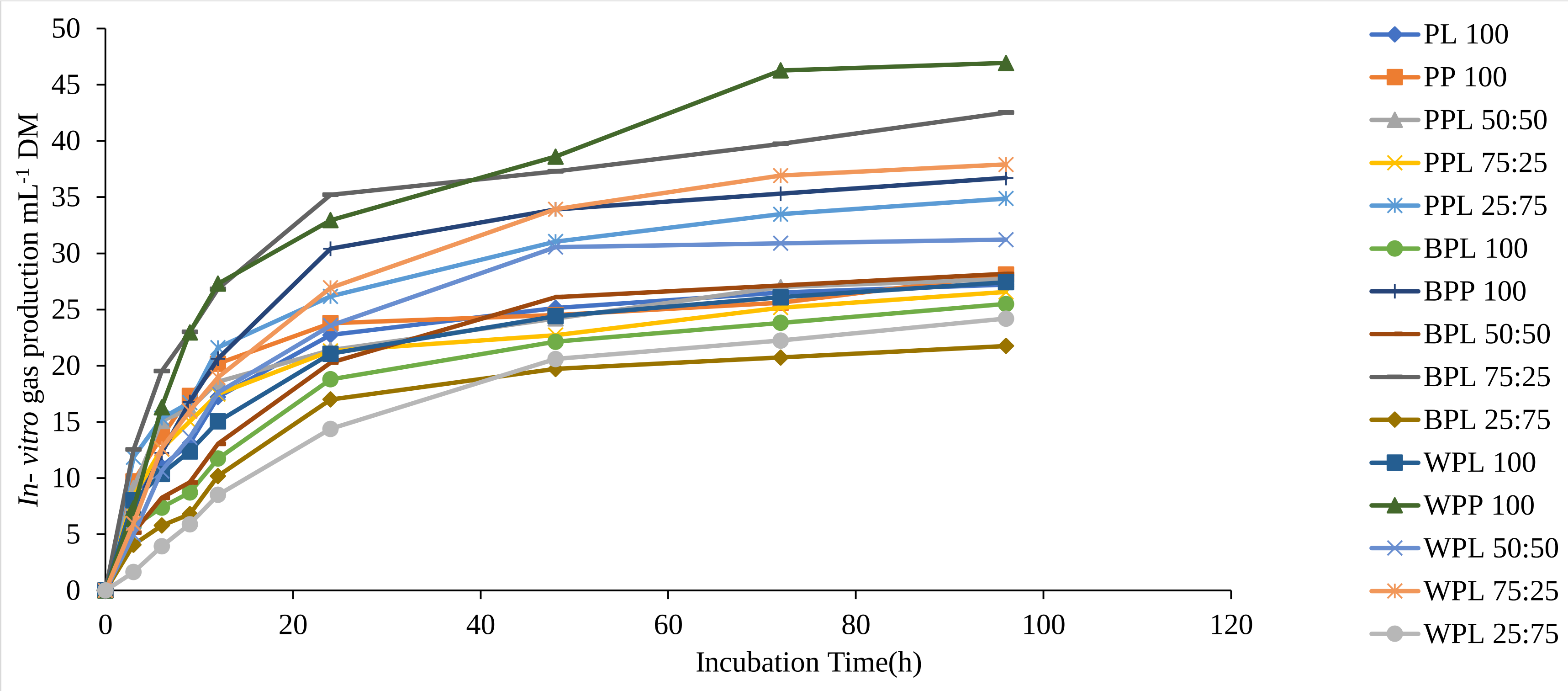

Figure3.2: Graphical representation of the effect of binders (wood ash and bentonite) on the In-vitro digestibility of Prosopis juliflora leaves and pods in an interval of 96 hr

At an inclusion level of 75% pods and 25% leaves, bentonite had a significantly greater effect on *P. juliflora* LP than wood ash while untreated *P. juliflora* LP was the least digestible (**Table 3.3** and **Figure. 3.2**). The rate of gas production at 24 and 48 hr were 35.21 ml and 37.30 ml for BPL75:25, 26.94 ml and 33.92 ml for WPL75:25 and 21.32 ml and 22.72 ml for PPL75:25.

Table 3.3: Effects of treatment with bentonite and wood ash on the digestibility of P. juliflora leaves and pods at ratios of 50:50, 25:75 and 75:25

Parameter	24 hr	48 hr	a	b	c	a+b	RSD	SCFA	OMD%	ME
				50% leaves and 50% pods of *Prosopis juliflora*						
Untreated	21.39***	24.20***	3.89***	31.73***	0.10^{ns}	36.39***	1.98***	0.06**	21.89***	3.53***
Wood ash treated	23.58***	30.56***	1.03***	2.37***	4.88***	3.39***	2.48***	0.16***	26.23***	4.29***
Bentonite treated	20.27***	26.11***	0.00***	3.90***	4.75***	3.90***	2.73***	0.13***	25.18***	4.41***
SEM	0.18	0.28	0.37	0.39	0.12	0.49	0.22	0.01	0.26	0.08
				25% leaves and 75% pods of *Prosopis juliflora*						
Untreated	21.32***	22.72*	3.71***	26.07***	0.00^{ns}	29.86***	1.57***	0.08***	20.58***	3.72***
Wood ash treated	26.94***	33.92**	3.54***	2.30***	0.03^{ns}	5.64***	2.56***	0.18***	21.23***	3.62***
Bentonite treated	35.21***	37.30***	0.00***	4.70***	11.38***	4.70***	2.71***	0.17***	25.84***	4.27***
SEM	0.4	0.4	0.08	0.08	0.05	0.14	0.23	0.01	0.37	0.12
				75% leaves and 25% pods of *Prosopis juliflora*						
Untreated	26.16***	31.05***	4.64***	2.25***	0.01^{ns}	7.15***	2.49***	0.10**	23.80***	4.11***
Wood ash treated	14.38***	20.60***	2.76***	0.05^{ns}	0.04^{ns}	2.89***	2.12***	0.15***	22.18***	4.11***
Bentonite treated	16.99***	19.71***	0.00***	3.02***	7.46***	3.15***	2.12***	0.13***	25.10***	4.33***
SEM	0.3	0.18	0.18	0.08	0.08	0.08	0.17	0.02	0.6	0.14

*Significant at P<0.05, **Significant at P<0.001, ***Significant at P<0.0001, ^{ns} not significant at P<0.05; a, b, c are constants described by Ørskov and McDonald (1979), RSD=Relative standard deviation; SCFA=Short chain fatty acids in m mol/200mg DM, OMD=Organic matter digestibility, ME=Metabolizable energy in MJ/Kg DM; SEM=Standard error of the mean.

At an inclusion level of 25% pods and 75% leaves, untreated *P. juliflora* LP has a higher digestibility compared to wood ash and bentonite treated samples at 24 and 48 hr (**Table 3.3** and **Figure. 3.2**). *P. juliflora* leaves and pods at 75% and 25% respectively has a gas production level of 26.16 ml at 24 hr and 31.05 ml at 48 hr and WPL25:75 had the lowest digestibility rate producing 14.38 ml and 20.60 ml of gas at 24 and 48 hr respectively.

3.4 Discussion

The IVD of *P. juliflora* LP was positively and negatively affected by the different ratio combinations of PL100, PP100, PPL50:50 PPL75:25, and PPL25:75. The rate of digestibility was significantly higher when *P. juliflora* LP was combined at the rate of 25% pods and 75% leaves (PPL25:75) than when used individually (PL100 and PP100). However, the other two ratio combinations (PPL50:50 and PPL75:25) negatively affected the digestibility of *P. juliflora* LP, significantly reducing the performance. As expected, the digestibility of leaves (PL100) was also found to be significantly higher than that of pods (PP100).

The good performance of PPL25:75 can be attributed to the higher OMD (23.80%) compared to the other ratios. *P. juliflora* leaves (100%) follows closely in terms of OMD at 22% making it the second most digestible sample. *P. juliflora* leaves (100%) is also higher in ME and SCFA at 4.56 MJ/Kg DM and 0.22 m mol/200 mg DM respectively compared to PP100 which has a lower OMD (19.9%), ME (3.62 MJ/Kg DM) and SCFA (0.08 m mol/200 mg DM). The higher digestibility of leaves to pods can be attributed to the higher crude protein content in leaves (21.6%) compared to the pods (11.4%) (Ali *et al*., 2012; Odero-Waitituh *et al.*, 2016). According to Putri *et al.* (2021), the higher the protein content in a feed, the better the digestibility in ruminant diets. This is because proteins act as a source of rumen degradable N and therefore ammonia–N for the rumen microbes. This is despite the high condensed tannins and crude fibre in leaves as described by Abdulrazak *et al.* (1999).

When binders are introduced on *P. juliflora* leaves, the untreated leaves still stand out having a higher digestibility (22.74 ml at 24 hr and 25.15 ml at 48 hr) compared to the treated leaves (WPL= 21.07 ml at 24 hr and 24.42 ml at 48 hr; BPL=18.79 ml at 24 hr and 22.14 ml at 48 hr). PL100 has higher SCFA indicating that the binders interfere with SCFA content in *P. juliflora* leaves. According to Shen *et al.* (2019), diet-SCFA are very essential to the rumen microbiota, they uphold the reliability of the rumen epithelium, and maintain the microbial rumen's homeostasis. This can explain the higher digestibility of untreated leaves compared to treated ones.

The digestibility of *P. juliflora* pods is significantly enhanced on treatment with the tannin binders. The digestibility of the pods is much better with wood ash than with bentonite treatment. Figure. 3.2 shows that wood ash-treated pods perform significantly better than all the other samples and pods perform much better on treatment than leaves. The results also shows bentonite-treated pods and leaves at 75% and 25% respectively coming second in terms of digestibility. This shows that leaves perform better when untreated, but pods perform much better when treated. This can be because the inhibition in pods is much higher, hence treatment becomes more effective and with better results. The significant effect of wood ash on the digestibility of pods concurs with the findings and conclusions of Mlambo *et al.* (2004) that wood ash is very effective in minimizing the detrimental effects of tannins and the higher the concentration the more the effectiveness.

At 50:50 *P. juliflora* LP concentration, wood ash still performs significantly much better (23.58 ml at 24 hr and 30.56 ml at 48 hr) than untreated (21.39 ml at 24 hr and 24.20 ml at 48 hr) and bentonite treated (20.27 ml at 24 hr and 26.11 ml at 48 hr) samples. This can be attributed to its higher OMD (26.23%), SCFA (0.16 m mol/200mg DM), and ME (4.29 MJ/Kg DM) concentrations than PPL50:50 and BPL50:50. It is worth noting that it is at this combination of *P. juliflora* LP that OMD of wood ash treated samples is highest.

When *P. juliflora* LP are combined at a ratio of 75% pods and 25% leaves, bentonite-treated samples have a significantly higher digestibility (35.21 ml at 24 hr and 37.3 ml at 48 hr) than wood ash treated (26.94 ml at 24 hr and 33.92 ml at 48 hr) and untreated (21.32 ml at 24 hr and 22.72 ml at 48 hr) samples. Bentonite-treated *P. juliflora* LP also has the highest OMD of 25.84% which is the highest for all bentonite-treated samples, and ME of 4.27 MJ/Kg DM at this ratio combination. At 25% pods and 75% leaves, the untreated sample is still more digestible (26.16 ml at 24 hr and 31.05 ml at 48 hr) than treated samples (WPL25:75=14.38 ml at 24 hr and 20.60 ml at 48 hr; BPL25:75=16.99 ml at 24 hr and 19.71 ml at 48 hr).

Bentonite was found to be as effective as PEG by Kemboi *et al.* (2022). Ben Salem *et al*. (2005), when experimenting on green and dried *Acacia cyanophylla* leaves found that on soaking them for 6 hr in wood ash solution (180 to 200 g per litre of water), the concentration of condensed tannins was minimized by 44.8% and 58.2% respectively. In this trial, 400 g/l of wood ash was used and the result showed that, when it is combined with pods at this rate, the performance is much better than in all the other samples. This finding is in agreement with that of Mlambo *et al.*

(2004) that wood ash oxidizes even phenolics of lower molecular weight. It might also be that the pods have fewer low molecular weight phenolics than the leaves.

3.5 Conclusion

It was concluded that *in-vitro* digestibility of *P. juliflora* leaves and pods is affected by ratio combination and treatment with wood ash and bentonite. Bentonite and wood ash are less effective on *P. juliflora* leaves but more effective with the pods.

CHAPTER FOUR

THE IMPACT OF RATIO AND TREATMENT WITH BENTONITE AND WOOD ASH ON THE PROXIMATE COMPOSITION AND PHENOLIC COMPOUNDS IN MESQUITE (*Prosopis juliflora)* LEAVES AND PODS

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. An experiment was carried out on *P. juliflora* LP treated or untreated with wood ash and bentonite at different ratios to determine the effect of treatment and ratio combination on proximate composition and phenols in *P. juliflora* LP. The analyses were carried out on leaves and pods at ratios of 0:100, 27:75, 50:50, 75:25, and 100:0 to. Results showed that ratio and treatment affected proximate composition and phenolic compounds in P. *juliflora* LP. The Crude protein (CP) content of leaves (23.04%) is significantly reduced by ratio combination while that of pods (13.09%) is positively affected. The ratios of PPL 50:50, PPL 75:25, PPL25:75 had CP contents of 14.14%, 13.99%, and 20.22% respectively. Pods had significantly lower contents of total extractable phenolics (TEPH) and total extractable tannins (TET) of 5.51% and 3.30% respectively while leaves had 7.69% and 6.05% respectively. Total extractable tannins were lowest in PPL 75:25 at 3.04%. Treatment with bentonite most significantly affected TET content in leaves to 4.05%. It was concluded that both treatment and ratio combination had significant effect on phenolic compounds without negatively significantly affecting proximate composition of P. *juliflora* leaves and pods.

4.1 Introduction

Leguminous tree forages are good alternative protein sources for ruminants to replace the poor quality and seasonal crop residues and grasses (Osuga *et al.,* 2008). 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 (Ondiek *et al.,* 2013).

P. 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, supplying protein of high quality, and a good source of fodder palatable to all livestock (Jaimes-Morales *et al.,* 2022). 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 (Taiz *et al.,* 2015).

Treatment with various types of natural 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 polyethylene glycol (PEG) which are expensive, difficult to access and their effect on the environment, for example, PEG-tannin complex, is vaguely known (Kemboi *et al.,* 2021). 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 (Ben Salem *et al.,* 2005; Hasnain & Nayak, 2018). 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 (Nweke, 2019). Wood ash is rich in calcium and is mostly used as a calcium supplement for livestock (Ohanaka *et al.,* 2022). 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 (Van Ryssen, 2018). 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 (Chakraborty, 2021). 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 (Lukić & Horvat, 2020), 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 Damato *et al.* (2022), 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.

4.2 Materials and Methods

4.2.1 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 17-22°C (Egerton University Meteorological Station, 2019).

4.2.2 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. (Baringo County Government, 2022).

4.2.3 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 20g/kg and wood ash collected was mixed thoroughly, sieved to remove foreign particles and used at 400g/kg in the analysis.

4.2.4 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.

4.2.5 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 Makkar and Makkar

(2003). In this procedure, regression equation of tannic acid standard is used the calculation. Condensed tannins were determined as per the method described by Porter *et al* (1986). 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.

4.2.6 Statistical model and analysis

$$\mathbf{Y_{ij} = \mu + \tau_i + \varepsilon_{ij}}$$

where:

$\mathbf{Y_{ij}}$= dependent variable (chemical composition)

$\boldsymbol{\mu}$= overall mean,

$\boldsymbol{\tau_i}$ = effect due to binders

$\boldsymbol{\varepsilon_{ij}}$=random error factor effect

Results of the chemical analyses were taken through a one-way analysis of variance by means of a generalized linear model (GLM) of SAS version 9.4. The means were separated using Tukey's honest significance difference (HSD) test at P<0.05 level of significance.

4.3 Results

Table 4.1: Effects of ratio on chemical composition of *P. juliflora* leaves and pods

Parameter	ASH	EE	CP	CF	TEPH	TET	CT
PL100	8.55^{d}	2.97^{a}	23.04^{a}	26.92^{a}	7.69^{a}	6.05^{a}	0.86^{d}
PP100	10.38^{c}	2.22^{bc}	13.09^{d}	16.85^{c}	5.51^{c}	3.30^{cd}	2.43^{a}
PPL50:50	10.70^{b}	1.93^{cd}	14.141^{c}	18.50^{c}	5.35^{c}	3.80^{c}	1.34^{c}
PPL75:25	7.09^{e}	2.45^{b}	13.99^{c}	23.17^{b}	5.21^{c}	3.04^{d}	1.55^{b}
PPL25:75	12.85^{a}	1.80^{d}	20.22^{b}	22.18^{b}	6.72^{b}	4.89^{b}	0.912^{d}
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 (% DM), TET=Total Extractable Tannins (Tannin Acid Equivalent in % DM,), CT=Condensed Tannins (% DM), PL 100=100% leaves; PP 100=100% pods; PPL 50:50=pods and leaves at 50% each; PPL 75:25=pods at 75% and leaves at 25%; PPL 25:75=pods at 25% and leaves at 75%; ^{abcM}*eans 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 and pods was significantly affected by ratio (**Table 4.1**). Ash content of the leaves was significantly increased while the contents of CP, EE, CF, TEPH, TET and CT were significantly decreased. The effects on pods varied as shown in **Table 4.1**.

Chemical composition of *P. juliflora* leaves was significantly affected by treatment (**Table 4.2**). The content of CP was significantly increased by both binders while ash content was significantly increased by wood ash and reduced by bentonite. EE, CF, TEPH, TET and CT content were significantly reduced by the binders. There was no significant difference between wood ash and bentonite in TEPH reduction. However, on TET reduction, bentonite performed significantly better while wood ash significantly performed better than bentonite in CT reduction.

Table 4.2: Effects of treatment with bentonite and wood ash on the chemical composition of *Prosopis 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 (% DM), TET=Total Extractable Tannins (Tannin Acid Equivalent in % DM,), CT=Condensed Tannins (% DM), ^{abc}*Means in the same column in the same group with different superscripts differ significantly at P<0.05*; SEM=Standard error of the mean.

Pods were also affected, with ash, CP, EE, TEPH, TET and CT being significantly reduced by wood ash compared to bentonite. Crude protein and ash content were significantly increased by bentonite while there was no significant difference in EE and TET content of untreated and bentonite treated *P. juliflora* pods.

At the ratio of 50% leaves and 50% pods, wood ash treatment significantly reduced ash, TEPH and TET contents and increased CP content compared to bentonite. The effects of wood ash and bentonite are not significantly different on CF and CT contents. At the ratio of 25% leaves and 75% pods, wood ash significantly decreased EE and TEPH contents and significantly increased CP contents while bentonite significantly increased ash contents. There was no significant difference in CF, TET and CT reduction using wood ash or bentonite.

Table 4.3: 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 (% DM), TET=Total Extractable Tannins (Tannin Acid Equivalent in % DM), CT=Condensed Tannins (% DM), ^{abc}*Means in the same column in the same group with different superscripts differ significantly at $P<0.05$.*; SEM=Standard error of the mean.

At the ratio of 75% leaves and 25% pods Untreated samples had significantly higher contents of ash, CF, TEPH, TET and CT compared to treated samples. Wood ash treated sample had significantly higher CP Content, and lowest TEPH and TET contents compared to other samples. On the other hand, bentonite had significantly higher contents of ash while there was no significant difference in CP, CF and CT contents in wood ash and bentonite treated samples.

4.4 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 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 Salawu *et al.* (1999) 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 Askari and Fazaeli (2019). Wood ash was found to be more effective with leaves compared to bentonite. It significantly increased the CP and ash contents, 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 Ben Salem *et al.* (2005) that wood ash solution has a potential of minimizing the concentration of tannins by up to 70%.

Mlambo *et al*. (2011), 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. Ding *et al.* (2013), 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 and CP content 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 content 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 Kemboi *et al.* (2022) that bentonite is as effective as PEG in tannin binding. This is also in agreement with the study done by Tadesse *et al.* (2018) 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 (Makkar & Singh, 1992; Misra *et al.*, 1993).

4.5 Conclusion

It was concluded that ratio combination affect proximate and phenolic compounds in *P. juliflora* leaves and pods hence the ratio to use should be decided upon depending on the desired parameters of proximate composition and polyphenols. Treatment with wood ash and bentonite also significantly affected the proximate and phenolic compounds in *P. juliflora* leaves and pods at the different ratios hence the binder to use should be decided upon depending on the desired parameters and the availability and cost of the binder.

CHAPTER FIVE

THE EFFECTS OF TREATMENT OF MATHENGE (*Prosopis juliflora)* LEAVES AND PODS WITH BENTONITE AND WOOD ASH ON FEED INTAKE, MILK PRODUCTION AND COMPOSITION IN DAIRY GOATS

Abstract

The high tannin content in *Prosopis juliflora* leaves and pods (LP) has hindered its efficient usage as a leguminous tree forage despite its high nutritive value. Goats are capable of using it but in a limited quantity despite being capable of meeting their nutritional requirements. An experiment was conducted to determine the impact of natural binders on intake, milk production, composition, and mineral content of dairy goats when used to treat *P. juliflora* LP. Twelve lactating dairy goats of the Toggenburg breed and their crosses weighing 35±2kg were used in the experiment. They were placed in four treatments in a randomized completely block design (RCBD) with the breed being the blocking factor (there were two pure breeds and one cross in each treatment). They were housed individually with three goats in each treatment. A digestibility trial was initially conducted to decide on the ratio of *P. juliflora* (LP) that was more digestible for the goats and 25% pods with 75% leaves (PPL 25:75) was found more digestible compared to the other ratios. There were four treatments; untreated PPL 25:75, bentonite treated PPL 25:75, wood ash treated PPL 25:75 and wood ash (50%) and bentonite (50%) treated PPL 25:75. The feeding trial took nine weeks with fourteen days' adaptation period. Feed leftovers were collected every morning to determine intake, and were also milked in the morning and evening to determine milk production. At the end of the experiment, milk samples from every treatment were analyzed to determine milk composition and mineral content. Data was analyzed using the GLM of SAS and the results showed that treatment improved intake with wood ash treated diet having significantly the highest intake of 2.01kg DMI compared to untreated diet (1.9kg DMI). There was no significant difference in milk production in all the treated diets and they were significantly higher than untreated diet. Treated diets also had better milk composition and mineral content compared to the untreated diet.

5.1 Introduction

Goats are found in many households. The poor man's cow has been credited for its ability to survive in almost all climatic conditions, better resist diseases, and its high feed conversion efficiency (Nair *et al.,* 2021). Their high feed conversion capacity coupled with presence of proline in their saliva that act as an initial defence against tannins on consumption, enables them to utilize tannins better than cattle and sheep (Niezen *et al.,* 1995). *Prosopis juliflora*, a leguminous tree forage that does excellently in arid and semi-arid areas including those receiving rainfall as low as 100mm per annum can be utilized by goats (Haregeweyn *et al.,* 2013). However, their inclusion in the diet is limited to 30% due to its high antinutritional factors and effect on teeth (Mutavi, 2020).

This study focused on contributing to enhanced usage of the forage by reduction of the ant nutritional factors, particularly tannins using wood ash and bentonite as binders. These binders are highly effective in tannin reduction and enhancement of feed intake and digestibility (Ben Salem *et al.,* 2005). Apart from that, they have no adverse effect on the environment unlike synthetic binders like polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP) whose intensity in affecting the environment is barely known. Moreover, they are locally available and affordable, and has been used since time immemorial in softening vegetables. Bentonite has been reported to be as effective as PEG, and it is also applied at a lower quantity of 20 g/kg (Kemboi *et al*., 2022). More than 90% of Kenyans rely on either charcoal or wood for cooking, and 6-10% of this is converted to wood ash (AL-Kharabsheh *et al.,* 2022; Bailis *et al.,* 2020). According to Mlambo *et al.* (2011), wood ash can reduce 75% and 96% of tannins in *A. nilotica* and *D. cineraria* fruits respectively.

An experiment was carried out to determine the effect of the treatment of *P. juliflora* LP with wood ash, bentonite, and synergistically on feed intake, milk production, and milk quality of the Toggenburg dairy goats. It aimed at enhancing the usage, adoption, and appreciation of *P. juliflora* as an alternative protein source. According to Azani *et al.* (2017), *P. juliflora* can meet the nutritional requirements of lactating dairy goats of 17% crude protein.

5.2 Materials and Methods

5.2.1 Study site

The study was carried out at Tatton Agriculture Park (TAP) of Egerton University's main campus which is situated in Njoro sub-county, Nakuru county, Kenya. It is at 0° 23 S, 35° 55 N with an altitude of 2,238 m above sea level. The latitudes and longitudes are 0.369734°S and 35.932779°E respectively. The average annual rainfall ranges from 900-1,200 mm with average daily temperatures of 17-22°C (Egerton University Meteorological Station, 2019).

5.2.2 Collection and Preparation of feeding materials

Mature leaves and pods 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 April/May and July/August but usually erratic. (Baringo County Government, 2022). The collected leaves and pods were transported to Egerton University where they were assorted by removing green pods and spoilt and mouldy leaves and pods. They were then spread out for efficient drying. Bentonite was mixed with the feed at the rate of 20g/kg, and wood ash collected mixed thoroughly, sieved to remove foreign particles, and used at 400g/L of water while avoiding kerosene-tainted wood ash.

5.2.3 Experimental animals and design

Toggenburg dairy goat breeds and their crosses used in this study were sourced from TAP. Twelve lactating and healthy ones weighing 35±2 kg were used. There were eight pure Toggenburg breeds and four crosses. The goats were arranged in a randomized completely block design(RCBD) with a cross in each treatment. They were housed individually with feed troughs, watering cans, and salt licks. They were given fourteen days' adaptation period before data collection commenced.

5.2.4 Experimental diets and feeding

Rhodes grass hay sourced from a local supplier was used as the basal diet. They were ground to pass through a 4 mm sieve. The goats were also provided with a mineral supplement in form of a salt lick and water *ad-libitum.* The four diets were decided upon after an *in-vitro* digestibility trial that was carried out earlier on *P. juliflora* leaves and pods, where 75% leaves mixed with 25% pods were found to be the most digestible. This was used as the supplement at 30% of the diet in all the treatments. The dietary treatments were as follows: -

T1) - Rhodes grass hay (*ad-libitum*) plus 75:25 ratios of *P. juliflora* LP (control)

T2- Rhodes grass hay (*ad-libitum*) plus wood ash treated 75:25 ratios of *P. juliflora* LP

T3- Rhodes grass hay (*ad-libitum*) plus bentonite treated 75:25 ratios of *P. juliflora* LP

T4- Rhodes grass hay (*ad-libitum*) plus 75:25 ratios of *P. juliflora* LP treated with a 50:50 ratio combination of wood ash and bentonite

5.2.5 Data collection

Data collection was carried out for nine weeks with a 14-days' adaptation period. The supplements and the treatments were mixed thoroughly and left overnight before being fed in the morning just after milking. They were given two hours with the supplement before the basal diet, given *ad-libitum*, was introduced. The goats were milked daily in the morning at 07:00 hr and in the evening 16:00 hr, and the milk produced recorded. Feed refusals were collected just before milking and introduction of fresh feeds, then weighed and recorded.

5.2.6 Milk analysis

During the last week of data collection, milk samples were retrieved from goats in all treatments and analyzed for fats, solids, proteins, lactose, salts, freezing point, and density using an Ultra scan milk analyzer.

5.2.7 Determination of minerals

Calcium, zinc, and iron content in milk of the four treatments were determined using the Atomic Absorption Spectrometer 3300 as was used by Perkin-Elmer, Massachusetts, USA. The ascorbic acids method was used to determine phosphorus content in the milk samples. The milk samples were prepared according to Brooks *et al.* (1970) by precipitation of casein using trichloroacetic acid (TCA).

5.2.8 Statistical model and analysis

$$\mathbf{Y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk}}$$

Where;

$\mathbf{Y_{ijk}}$ = observation on kth goat of the ith treatment in the jth block

$\boldsymbol{\mu}$ = overall population mean

$\boldsymbol{\alpha_i}$ = effect due to the ith treatment

$\boldsymbol{\beta_j}$ = effect due to the jth block

$\mathbf{e_{ijk}}$ = random error associated with Y_{ijk}

Results of the feeding trial were taken through a one-way analysis of variance using the generalized linear model (GLM) of SAS version 9.4. The means were separated using Tukey's honest significance difference (HSD) test at $P<0.05$ level of significance.

5.3 Results

Dairy goats fed treated diets of *P. juliflora* leaves and pods had higher dry matter intake, milk production and better milk composition and mineral contents compared to those fed untreated diets of *P. juliflora* leaves and pods (**Table 5.1**). In terms of dry matter intake, there was no significant difference (P>0.05) between T1, T3, and T4, while there was no difference between T4, T3, and T2. However, T1 and T2 were different. There was no difference in milk production in all the treatments except for the control.

Table 5.1: Average daily dry matter intake and milk production of dairy goats fed Rhodes grass hay and supplemented with treated and untreated *Prosopis juliflora* leaves and pods

Treatments	**Total DMI (kg)**	**Milk Production (ml)**
Control (T1)	1.90^{b}	108.02^{b}
Wood ash treated (T2)	2.01^{a}	137.67^{a}
Bentonite treated (T3)	1.96^{ab}	124.26^{a}
Wood ash and Bentonite treated (T4)	1.94^{ab}	123.89^{a}
SEM	0.01	2.07
p-value	0.0021	< .0001

^{abc}*Means within a column with different superscripts differ significantly at P<0.05., DMI=dry matter intake:* SEM=Standard error of the mean; DMI=Dry matter intake.

Milk sourced from dairy goats fed diets with untreated *P. juliflora* leaves and pods (T1) was low in all parameters (**Table 5.2**). Wood ash (T2) and bentonite treated (T3) had similar fat contents, solids, density, and salts while solids, freezing points, proteins, and lactose contents were similar in all treatments except T1. Treatment1 and 50:50 wood ash and bentonite (T4) had similar amounts of salts.

Table 5.2: Comparison of various parameters of milk composition of the treatment diets

Parameters	**T1**	**T2**	**T3**	**T4**	**SEM**	***p*-value**
Fats (%)	2.30^{c}	5.20^{b}	6.15^{b}	8.70^{a}	0.33	0.0001
SNF (%)	6.45^{b}	9.55^{a}	9.60^{a}	8.35^{a}	0.37	0.0009
Density (g/l)	1021.98^{c}	1032.02^{a}	1032.55^{a}	1026.65^{b}	0.4	0.0001
Freezing point (°C)	0.41^{b}	0.65^{a}	0.66^{a}	0.59^{a}	0.03	0.0007
Protein (%)	2.40^{b}	3.60^{a}	3.65^{a}	3.30^{a}	0.14	0.0009
Lactose (%)	3.60^{b}	5.40^{a}	5.45^{a}	4.70^{a}	0.21	0.0009

Salts (%)	0.45^{b}	0.75^{a}	0.70^{a}	0.48^{b}	0.03	0.0002

^{abc}*Means within a row with different superscripts differ significantly at P<0.05;* SNF=Solids-Not-Fat SEM=Standard error of the mean. T1=control, T2=wood ash treated, T3=bentonite treated, T4=wood ash and bentonite treated.

Treatment 3 had the highest amounts of iron, while other treatments had equal amounts (**Table 5.3**). Treatment 3 and T4 had similar and highest amounts of calcium while T1 had the lowest. Treatment2 had the highest amount of zinc whereas the rest of the treatments had comparable amounts. Treatment2 also had the highest similar amounts of phosphorus with T1 while T3 and T4 had equal amounts.

Table 5.3: Sampled major and minor minerals (%) of milk sourced from the four treatments

Parameters	**T1**	**T2**	**T3**	**T4**	**SEM**	***p*-value**
Iron	13.85^{b}	18.08^{a}	12.12^{b}	11.15^{b}	0.7	0.0005
Calcium	0.32^{c}	2.30^{a}	1.98^{b}	2.16^{ab}	0.05	0.0001
Zinc	0.08^{b}	0.08^{b}	0.15^{a}	0.09^{b}	0.01	0.0015
Phosphorus	2.60^{ab}	2.45^{b}	2.86^{a}	2.38^{b}	0.07	0.0055

$^{a,\ b}$ *Means within a row with different superscripts differ significantly at P<0.05;* SEM=Standard error of the mean.

5.4 Discussion

The results indicate that treatment with tannin binders enhanced feed intake. As much as there was no difference between T3 and T4 with the control, there was a difference between T2 and the control, and no difference between T2, T3, and T4. This makes wood ash more effective in enhancing feed intake as compared to other forms of treatment when used at 400g/l. This is in agreement with the findings of Brown *et al.* (2016) that the more the amount of wood ash used, the better the results. This can be attributed to the fact that wood ash is alkaline and can be used to inhibit ant nutritional factors such as toxins in tannins in diets (Van Ryssen, 2018).

Tannins not only interfere with the digestibility of feeds but also limit their palatability hence intake. Wood ash has been used in the treatment of sorghum grain, millet, vegetables, and the leaves of leguminous tree forages like *Acacia cyanophylla*. Treatment1 might have performed the same as T3 and T4 because the ratio of leaves and pods in the diet can also be used to minimize the effect of tannins. According to Mueller-Harvey (2006), mixtures can be applied to thwart the harmful effects of tannins. Salawu *et al.* (1999) also discovered that leaves of calliandra were affected when mixed with its leaves or pods. An *in-vitro* digestibility study done earlier prior to this experiment also showed that wood ash at 400 g/l of water was more digestible than bentonite, and the ratio combination of leaves and pods also affected their digestibility.

Milk production was higher in all the treatments except the control. This could be because the ratio combination does not have as much effect on tannins as treatment with either wood ash, bentonite, or wood ash and bentonite at a 50:50 ratio combination. Henke *et al*. (2017) carried out research to determine the effect of quebracho tannin extract, a source of condensed tannins, on milk production in dairy cows among other factors, and found out that at high inclusion level, it had detrimental effects on not only milk production, but also diet digestibility, and general performance of the animals. This study shows the effects tannins have on milk production, with the treated diets generally performing better than the untreated control. In addition to that, with high tannin diets, there is reduced voluntary intake and animals tend to spend more time and energy ruminating due to the astringent factor that is formed as a result of the complexes formed with tannins and salivary proteins (Muir, 2011).

The results also show that the composition of milk sourced from goats in the treated diets was higher in all the parameters that were determined (fats, solids, density, freezing point, proteins, lactose, and salts) than in the control. According to Nascimento *et al.* (2021), high tannin content in the diet reduce the productive efficiency of goats. In their study, high tannin content decreased the concentration of milk fat. This is also in agreement with the findings of Wang *et al.* (1996) that investigated the effect of condensed tannins present in *Lotus corniculatus* on the lactation performance of ewes.

Min *et al.* (2005) investigated the impact of condensed tannins on milk composition in angora goats and found out that they not only interfere with lactose content, lowering them, but also decrease the level of solids, solid-not fats and somatic cell count in milk. Tannins generally interfere with protein degradation in the rumen and increase rumen and intestinal protein by-pass by forming tannin-protein complexes which make the protein component of the feed unavailable to the animal (Mueller-Harvey, 2006). This can explain the lower protein component in milk. It can also explain the low freezing point, density, salts, and mineral contents (iron, calcium, zinc, and phosphorus) in the control. The density of the milk derived from the control diet was below the minimum and average in goats of 27 and 30 respectively. The high calcium content in a wood ash-treated diet can be attributed to the fact that calcium is the major mineral component in wood ash. According to Van Ryssen (2018), the higher the calcium content in the diet, the lower the phosphorus content hence phosphorus deficiency in livestock. This can explain the lower phosphorus content in milk derived from goats-fed wood ash treated diets compared to bentonite treated.

5.5 Conclusion

It was concluded that lactating dairy goats fed diets supplemented with treated *P. juliflora* leaves and pods (75:25) performed better in terms of dry matter intake, milk production, as well as milk composition.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS, RECOMMENDATIONS AND FURTHER RESEARCH

6.1 General discussion

The most digestible ratio combination was found to be PPL25:75. This can be attributed to the higher OMD (23.80%) compared to the other ratios. *P. juliflora leaves* 100 follows closely in terms of OMD at 22%, and was the second most digestible sample. *P. juliflora leaves* 100 was also higher in ME and SCFA at 4.56 MJ/Kg DM and 0.22 m mol/200 mg DM respectively compared to PP100 which has a lower OMD (19.9%), ME (3.62 MJ/Kg DM) and SCFA (0.08 m mol/200 mg DM). The higher digestibility of leaves to pods can be attributed to the higher crude protein content in leaves (21.6%) compared to the pods (11.4%) (Ali *et al.*, 2012; Odero-Waitituh *et al.,* 2016). Proteins are an energy source for microorganisms in the rumen thus enhance fermentation. This is despite the high condensed tannins and crude fibre in leaves as described by Abdulrazak *et al.* (1999).

The use of binders hardly affected the performance of leaves in that the untreated performed much better (22.74 ml at 24 hr and 25.15 ml at 48 hr) compared to the treated (WPL= 21.07 ml at 24 hr and 24.42 ml at 48 hr; BPL=18.79 ml at 24 hr and 22.14 ml at 48 hr). Untreated. *P juliflora* leaves had higher SCFA than the treated indicating that the binders interfere with their SCFA content. According to Shen *et al.* (2019), diet-SCFA are very essential to the rumen microbiota, they uphold the reliability of the rumen epithelium, and maintain the microbial rumen’s homeostasis. The digestibility of *P. juliflora* pods is greatly enhanced on treatment with wood ash and bentonite at 75% pods and 25% leaves. The significant effect of wood ash on the digestibility of pods concurs with the findings and conclusions of Mlambo *et al.* (2004) that wood ash is very convenient in minimizing the detrimental effects of tannins and the higher the concentration the more the effectiveness.

At 50:50 *P. juliflora* LP concentration, wood ash performs better (23.58 ml at 24 hr and 30.56 ml at 48 hr) than the untreated (21.39 ml at 24 hr and 24.20 ml at 48 hr and bentonite treated (20.27 ml at 24 hr and 26.11 ml at 48 hr) samples. This can be due to the higher OMD (26.23%), SCFA (0.16 m mol/200mg DM), and ME (4.29 MJ/Kg DM) concentrations compared to other samples. At 75:25 LP, bentonite-treated samples have the highest digestibility (35.21 ml at 24 hr and 37.3 ml at 48 hr), OMD (25.84%), and ME (4.27 MJ/Kg DM). At 25% pods and 75% leaves, the untreated sample is still more digestible (26.16 ml at 24 hr and 31.05 ml at 48 hr) than treated.

The proximate and phenolic compounds of *P. juliflora* leaves and pods were significantly affected by both ratio combination 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, CP, CF, TEPH, and TET which were decreased at all the ratios. This is in line with the findings of Salawu *et al.* (1999) 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 Askari and Fazaeli (2019). Wood ash was found to be more effective with leaves compared to bentonite. It significantly increased its ash and CP availability, 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 just more effective on ash and CP availability significantly increasing them. This confirm the findings of Ben Salem *et al.* (2005), that wood ash solution has a potential of minimizing the concentration of tannins by up to 70%.

Ding *et al.* (2013), when investigating the effect of sodium 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 and 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 increment of CP availability. 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. Tadesse *et al*. (2018) found out that wood ash improves digestibility and performance of Bonga lambs in Ethiopia when it was included in a high tannin diet.

During the feeding trial, treatment enhanced feed intake, milk production, composition and mineral content. Wood ash treated diet performed better (T2), though not different from bentonite (T3) and wood ash and bentonite (50:50) treated (T4). This conquers with the findings of Brown *et al.* (2016) that the more the amount of wood ash used, the better the results. This can be attributed to the fact that wood ash is alkaline and can be used to inhibit antinutritional factors and the effects of toxins in tannins (Van Ryssen, 2018). In this trial 400g/l of wood ash was used. The control (T1) might have performed the same as T3 and T4 because the ratio of leaves and pods in the diet can also be used to minimize the effect of tannins. According to Mueller-Harvey (2006), mixtures can be applied to thwart the harmful effects of tannins. Milk production was significantly higher in all the treatments except the control. Henke *et al*. (2017) carried out research to determine the effect of quebracho tannin extract, a source of condensed tannins, on milk production in dairy cows among other factors, and found out that at high inclusion level, it had detrimental effects on not only milk production, but also diet digestibility, and general performance of the animals. In addition to that, with high tannin diets, there is reduced voluntary intake and animals tend to spend more time and energy ruminating due to the astringent factor that is formed as a result of the complexes formed with tannins and salivary proteins (Muir, 2011).

The composition of milk sourced from goats in the treated diets was higher in all parameters (fats, solids, density, freezing point, proteins, lactose, and salts) compared to the control. According to Nascimento *et al.* (2021), high tannin content in the diet reduce the productive efficiency of goats. This is also parallel to the findings of Wang *et al.* (1996). Min *et al.* (2005) investigated the impact of condensed tannins on milk composition in angora goats and found out that they not only interfere with lactose content, lowering them, but also decrease the level of solids, solid-not fats and somatic cell count in milk. Tannins generally interfere with protein degradation in the rumen and increase rumen and intestinal protein by-pass by forming tannin-protein complexes which make the protein component of the feed unavailable to the animal (Mueller-Harvey, 2006). This can explain the lower protein component in milk from the untreated diet. It can also explain the low freezing point, density, salts, and mineral contents (iron, calcium, zinc, and phosphorus) in the control. The high calcium content in a wood ash-treated diet can be attributed to the fact that calcium is the major mineral component in wood ash. According to Van

Ryssen (2018) high calcium in the diet can lead to phosphorus deficiency in livestock hence the lower phosphorus content in wood ash treated compared to bentonite treated diets.

6.2 Conclusions

i. Ratio combination as well as treatment with wood ash and bentonite affected the digestibility of *P. juliflora* leaves and pods. Leaves are more digestible when untreated than when treated with either wood ash or bentonite, while pods are much more digestible when treated with wood ash than when untreated or bentonite treated.

ii. Mixing ratio and treatment significantly affected proximate composition and phenolic compounds in*. juliflora* leaves and pods.

iii. Lactating dairy goats fed diets supplemented with treated *P. juliflora* leaves and pods (75:25) treated with wood ash and bentonite performed better in terms of dry matter intake, milk production, composition and mineral content.

6.3 Recommendations

i. Ratio combination of leaves and pods and natural binders like wood ash and bentonite can be used to enhance digestibility of fibrous leguminous forages like *Prosopis juliflora*.

ii. It is safe to use ratio and treatment with wood ash and bentonite as methods of tannin binding since they are effective on the phenolic compounds and have no effect on the proximate composition of *P. juliflora* leaves and pods.

iii. Treatment of *P. juliflora* leaves and pods with natural binders (wood ash and bentonite) can be used to enhance the performance of lactating dairy goats.

6.4 Further Research

i. Conclusive research to determine trees with the best source of wood ash for tannin binding in Kenya and the maximum amount of wood ash that can be used in tannin binding in various leguminous tree forages

ii. Natural binders that are more effective on the digestibility of *P. juliflora* leaves and the effect of ratio combination of pods and leaves and treatment with wood ash and bentonite in other high tannin leguminous tree forages.

iii. Effect of the other ratios of *P. juliflora* leaves and pods treated with wood ash and bentonite on feed intake, milk production and quality in dairy goats

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APPENDICES

Appendix A: Research Permit and Ethical Clearance

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: **289113** Date of Issue: **31/May/2023**

RESEARCH LICENSE

This is to Certify that Miss... MARY ATIENO OUMA of Egerton University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nakuru on the topic: PERFORMANCE OF DAIRY GOATS SUPPLEMENTED WITH Prosopis juliflora TREATED WITHWOODASH AND BENTONITE CLAY for the period ending: 31/May/2024.

License No: **NACOSTI/P/23/26222**

289113

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Verification QR Code

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See overleaf for conditions

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EGERTON

EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW COMMITTEE

EU/RE/DIR/009

Approval No. EUISERC/APP/239/2023 ***4th May*** **2023**

Mary Atieno Ouma
Department of Animal Sciences
Egerton university
P. O. Box 536– 20115,
Egerton – Kenya.
Telephone: 0712148324
E-mail: Oumamary08@gmail.com

Dear Mary,

RE: ETHICAL APPROVAL: PERFORMANCE OF DAIRY GOATS FED DIETS WITH *PROSOPIS JULIFLORA* LEAVES AND PODS DE-TANNED WITH WOODASH AND BENTONITE CLAY

This is to inform you that ***Egerton University Institutional Scientific and Ethics Review Committee*** has reviewed and approved your above research proposal. Your application approval number is ***EUISERC/APP/239/2023.*** The approval period is ***4th May, 2023 –5th May, 2024***
This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.

ii. You are required to adhere Institutional Experimental Animals Use and Care policy.

iii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***Egerton University Institutional Scientific and Ethics Review Committee***.

iv. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***Egerton University Institutional Scientific and Ethics Review Committee*** within 72 hours of notification

v. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to ***Egerton University Institutional Scientific and Ethics Review Committee*** within 72 hours.

"Transforming Lives through Quality Education"

vi. Clearance for Material Transfer of biological specimens must be obtained from relevant institutions.

vii. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal. viii. Submission of an executive summary report within 90 days upon completion of the study to ***Egerton University Institutional Scientific and Ethics Review Committee***.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

EGERTON UNIVERSITY Date. 04/05/2023 RESEARCH ETHICS

Yours sincerely.

Prof. Raphael M. Ngure

CHAIRMAN, EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW CTTEE

RMN/BK/

Appendix B: Analysis of variance

Digestibility of *Prosopis juliflora* at 24 hr

Dependent variable: Leaves(PL100)

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Mode	l2	24.41449267	12.20724633	1413.11	<.0001
Error	6	0.05183133	0.00863856		
Corrected Total	8	24.46632400			

R-Square	Coeff Var	Root MSE	Leaves Mean
0.997882	0.445354	0.092944	20.86967

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Leaves

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.008639
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	0.2328

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	22.73967	3	24HRS
B	21.13733	3	W24HRS
C	18.73200	3	B24HRS

Dependent variable: Pods (PP100)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	133.1330276	66.5665138	6431.75	<.0001
Error	6	0.0620980	0.0103497		
Corrected Total	8	133.1951256			

R-Square	Coeff Var	Root MSE	Pods Mean
0.999534	0.350620	0.101733	29.01522

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Pods

Alpha	0.05
Error Degrees of Freedom	6

Error Mean Square 0.01035
Critical Value of Studentized Range 4.33902
Minimum Significant Difference 0.2549

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	32.86767	3	W24HRS
B	30.41433	3	B24HRS
C	23.76367	3	24HRS

Dependent variable: FiftyPods(PPL50:50)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	17.18008467	8.59004233	2354.58	<.0001
Error	6	0.02188933	0.00364822		
Corrected Total	8	17.20197400			

R-Square	Coeff Var	Root MSE	FiftyPods Mean
0.998728	0.278040	0.060401	21.72367

The GLM Procedure

Tukey's Studentized Range (HSD) Test for FiftyPods

Alpha 0.05
Error Degrees of Freedom 6
Error Mean Square 0.003648
Critical Value of Studentized Range 4.33902
Minimum Significant Difference 0.1513

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	23.57933	3	W24HRS
B	21.32567	3	24HRS
C	20.26600	3	B24HRS

Dependent variable: SeventyfivePods(PPL75:25)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	290.2163127	145.1081563	25731.9	<.0001
Error	6	0.0338353	0.0056392		
Corrected Total	8	290.2501480			

R-Square	Coeff Var	Root MSE	SeventyfivePods Mean
0.999883	0.269659	0.075095	27.84800

The GLM Procedure

Tukey's Studentized Range (HSD) Test for SeventyfivePods

Alpha 0.05
Error Degrees of Freedom 6
Error Mean Square 0.005639
Critical Value of Studentized Range 4.33902
Minimum Significant Difference 0.1881

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	35.20133	3	B24HRS
B	26.96700	3	W24HRS
C	21.37567	3	24HRS

Dependent variable: TwentyfivePods(PPL25:75)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	230.4080082	115.2040041	12144.9	<.0001
Error	6	0.0569147	0.0094858		
Corrected Total	8	230.4649229			

R-Square	Coeff Var	Root MSE	TwentyfivePods Mean
0.999753	0.507877	0.097395	19.17689

The GLM Procedure

Tukey's Studentized Range (HSD) Test for TwentyfivePods

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.009486
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	0.244

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	26.16467	3	24HRS
B	17.01700	3	B24HRS
C	14.34900	3	W24HRS

Digestibility of *Prosopis juliflora* at 48 hr
Dependent variable: Leaves(PL100)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	15.06112156	7.53056078	2226.59	<.0001
Error	6	0.02029267	0.00338211		
Corrected Total	8	15.08141422			

R-Square	Coeff Var	Root MSE	Leaves Mean
0.998654	0.243224	0.058156	23.91044

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Leaves

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.003382
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	0.1457

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	25.16600	3	48HRS
B	24.43500	3	W48HRS
C	22.13033	3	B48HRS

Dependent variable: Pods(PP100)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	311.2493416	155.6246708	7585.77	<.0001
Error	6	0.1230920	0.0205153		
Corrected Total	8	311.3724336			

R-Square	Coeff Var	Root MSE	Pods Mean
0.999605	0.442330	0.143232	32.38122

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Pods

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.020515
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	0.3588

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	38.6917	3	W48HRS
B	33.9173	3	B48HRS
C	24.5347	3	48HRS

Dependent variable: FiftyPods(PPL50:50)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	63.90269489	31.95134744	6464.39	<.0001
Error	6	0.02965600	0.00494267		
Corrected Total	8	63.93235089			

R-Square	Coeff Var	Root MSE	FiftyPods Mean
0.999536	0.260819	0.070304	26.95511

The GLM Procedure

Tukey's Studentized Range (HSD) Test for FiftyPods

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.004943
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	0.1761

Means with the same letter are not significantly different.

Tukey Grouping Mean N TRT

Tukey Grouping	Mean	N	TRT
A	30.54633	3	W48HRS
B	26.14833	3	B48HRS
C	24.17067	3	48HRS

Dependent variable: SeventyfivePods(PPL75:25)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	346.8873449	173.4436724	26612.2	<.0001
Error	6	0.0391047	0.0065174		
Corrected Total	8	346.9264496			

R-Square	Coeff Var	Root MSE	SeventyfivePods Mean
0.999887	0.257886	0.080731	31.30478

The GLM Procedure

Tukey's Studentized Range (HSD) Test for SeventyfivePods

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.006517
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	0.2022

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	37.26100	3	B48HRS
B	33.91300	3	W48HRS
C	22.74033	3	48HRS

Dependent variable: TwentyfivePods(PPL25:75)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	238.7375362	119.3687681	24485.3	<.0001
Error	6	0.0292507	0.0048751		
Corrected Total	8	238.7667869			

R-Square	Coeff Var	Root MSE	TwentyfivePods Mean
0.999877	0.293319	0.069822	23.80411

The GLM Procedure

Tukey's Studentized Range (HSD) Test for TwentyfivePods

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.004875
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	0.1749

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	31.07133	3	48HRS

B	20.59500	3	W48HRS
C	19.74600	3	B48HRS

Dependent variable: CONDENSEDTANNINS (Untreated samples)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	4.81729333	1.20432333	1204.32	<.0001
Error	10	0.01000000	0.00100000		
Corrected Total	14	4.82729333			

R-Square	Coeff Var	Root MSE	CONDENSEDTANNINS Mean
0.997928	2.231146	0.031623	1.417333

The GLM Procedure

Tukey's Studentized Range (HSD) Test for CONDENSEDTANNINS

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.001
Critical Value of Studentized Range	4.65429
Minimum Significant Difference	0.085

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	2.42667	3	PP100
B	1.54667	3	PPL75:25
C	1.34000	3	PPL50:50
D	0.91000	3	PPL25:75
D			
D	0.86333	3	PL100

CONDENSEDTANNINS (Treated samples)
Dependent variable: Leaves

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.06206667	0.03103333	73.50	<.0001
Error	6	0.00253333	0.00042222		
Corrected Total	8	0.06460000			

R-Square	Coeff Var	Root MSE	Leaves Mean
0.960784	2.170568	0.020548	0.946667

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Leaves

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.000422
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	0.0515

Means with the same letter are not significantly different.

Tukey Grouping Mean N TRT

A	1.06000	3	WCtannin
B	0.91667	3	BCtannin
C	0.86333	3	Ctannins

Dependent variable: Pods

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.17126667	0.08563333	220.20	<.0001
Error	6	0.00233333	0.00038889		
Corrected Total	8	0.17360000			

R-Square	Coeff Var	Root MSE	Pods Mean
0.986559	0.881681	0.019720	2.236667

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Pods

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.000389
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	0.0494

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	2.42667	3	Ctannins
B	2.18000	3	BCtannin
C	2.10333	3	WCtannin

Dependent variable: FiftyPods

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.04402222	0.02201111	30.48	0.0007
Error	6	0.00433333	0.00072222		
Corrected Total	8	0.04835556			

R-Square	Coeff Var	Root MSE	FiftyPods Mean
0.910386	1.987409	0.026874	1.352222

The GLM Procedure

Tukey's Studentized Range (HSD) Test for FiftyPods

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.000722
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	0.0673

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1.44333	3	BCtannin

Tukey Grouping	Mean	N	TRT
B	1.34000	3	Ctannins
B			
B	1.27333	3	WCtannin

Dependent variable: SeventyfivePods

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.22006667	0.11003333	79.22	<.0001
Error	6	0.00833333	0.00138889		
Corrected Total	8	0.22840000			

R-Square	Coeff Var	Root MSE	SeventyfivePods Mean
0.963514	2.200854	0.037268	1.693333

The GLM Procedure

Tukey's Studentized Range (HSD) Test for SeventyfivePods

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.001389
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	0.0934

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1.91000	3	BCtannin
B	1.62333	3	WCtannin
B			
B	1.54667	3	Ctannins

Dependent variable: TwentyfivePods

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.08540000	0.04270000	64.05	<.0001
Error	6	0.00400000	0.00066667		
Corrected Total	8	0.08940000			

R-Square	Coeff Var	Root MSE	TwentyfivePods Mean
0.955257	2.564890	0.025820	1.006667

The GLM Procedure

Tukey's Studentized Range (HSD) Test for TwentyfivePods

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.000667
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	0.0647

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1.14000	3	BCtannin
B	0.97000	3	WCtannin

```
B
B      0.91000     3    Ctannins
```

Dependent variable: TwentyfivePods

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	52	11.87706599	0.22840512	3.62	<.0001
Error	535	33.73072177	0.06304808		
Corrected Total	587	45.60778776			

R-Square	Coeff Var	Root MSE	Intake Mean
0.260417	12.86987	0.251094	1.951020

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Intake

Alpha	0.05
Error Degrees of Freedom	535
Error Mean Square	0.063048
Critical Value of Studentized Range	3.64466
Minimum Significant Difference	0.0755

Means with the same letter are not significantly different.

```
Tukey Grouping         Mean      N    TRT

          A      2.00796    147    T2
          A
      B   A      1.96000    147    T3
      B   A
      B   A      1.94027    147    T4
      B
      B          1.89585    147    T1
```

Appendix C: Publications from this thesis

International Journal of Veterinary Sciences and Animal Husbandry 2023; 8(4): 158-163

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The effect of ratio combination and binders on the digestibility of mesquit (*Prosopis Juliflora)* leaves and pods

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Abstract
Prosopis juliflora has long been used as a leguminous tree forage. However, the availability of high concentrations of tannins has hindered its usage. The study aimed at determining the most digestible ratio combination of *P. Juliflora* (PJ) leaves and pods and the effect of wood ash and bentonite on their digestibility. Therefore, *in-vitro* digestibility (IVD) trials were conducted on PJ leaves and pods (PJLP) at ratios of 0:100, 25:75, 50:50, 75:25, and 100:0 for 96 h with and without treatment. Gas produced was recorded at intervals of 0, 3, 6, 9, 12, 24, 48, 72, and 96 h. The results showed that the ratio combination significantly affects the digestibility of PJLP. It also showed that wood ash and bentonite affect the digestibility of PJLP at different ratios. It was concluded that the use of binders enhanced the digestibility of PJLP. However, they significantly positively affect pods compared to leaves.

Keywords: Bentonite, *in-vitro* digestibility, leguminous tree forages, natural binders, tannins, wood ash

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

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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

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The effects of treatment of *Prosopis juliflora* leaves and pods with bentonite and wood ash on feed intake, milk production and composition in dairy goats

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Abstract
The high tannin content in *Prosopis juliflora* leaves and pods (LP) has hindered its efficient usage as a leguminous tree forage despite its high nutritive value. Goats are capable of using it but in a limited quantity despite being capable of meeting their nutritional requirements. An experiment was conducted to determine the impact of natural binders on intake, milk production, composition, and mineral content of dairy goats when used to treat *P. juliflora* LP. Twelve lactating dairy goats of the Toggenburg breed and their crosses weighing 35±2 kg were used in the experiment. They were placed in four treatments in a randomized completely block design (RCBD) with the breed being the blocking factor. They were housed individually with three goats in each treatment. A digestibility trial was initially conducted to decide on the ratio of *P. juliflora* (LP) that was more digestible for the goats. Feed leftovers were collected every morning to determine intake, and were also milked in the morning and evening to determine milk production. At the end of the experiment, milk samples from every treatment were analyzed to determine milk composition and mineral content. The results showed that treatment not only improved intake but also milk production, composition, and mineral content.

Keywords: Bentonite, *in-vitro* digestibility, natural binders, tannins, wood ash