Nutritive Value, Tannin Bioassay and Processing Effects of *Acacia brevispica, A. mellifera* and *A. tortilis* Pods as Potential Supplements for Growing Small East African Goats (SEAGs) in Baringo County-Kenya

[1*]Mutai A. Paul, [1]Nandwa Anastacia., [1]Ronoh Salina., [1]Sergon Philomena, [2]Oliech O. George, [2]Yator Monica, [3]Meso N. Doreen and [1]Koech K. Julius

[1]School of Science, Department of Biological Sciences, University of Eldoret, P.o. Box 1125, Eldoret, Kenya

[2]School of Agriculture and Biotechnology, Department of Animal science, University of Eldoret, P.o. Box 1125, Eldoret, Kenya

[3]School of environmental science, Department of Biology and Health, University of Eldoret, P.o. Box 1125, Eldoret, Kenya

***Corresponding author's email address:** mutaipaul52@gmail.com

Abstract

This study was conducted in Radat sub-location, Mogotio sub-county, Baringo county. The objective of the study was to evaluate nutritive value, tannin bio assay and processing effects of mature green Acacia species pods as potential supplements for growing goats in Baringo. Acacia pods were randomly collected, and prossed as: T1-control (untreated), T2- shade dried, T3-sun-dried, and T4-pods soaked in wood ash (alkali) mixed at 200gm per/liter of water for 48 hrs. respectively. They were oven- dried at 500^{0C} for 24hrs, then ground to be able to pass through 1mm sieve, packed and transported for analysis. Experimental Design was Randomized complete block design (RCBD). Nutrient composition by proximate and Van-Soest procedures (AOAC-1995), tannin bioassay by (Makkar,2003) was used. Total extractable tannins (TET) were determined indirectly after being absorbed by the insoluble tannin–binding compound polyvinylpolypyrrolidone (PVP)and TET concentration by subtracting TET remaining after treatment by use of PVP. Concentrations of Total phenolics and Total Tannins were calculated as tannic acid equivalents (eq) expressed as g/kg DM. Total extractable condensed tannins was assayed by addition of butanol HCl (normal concentration) Fe^{3+} assay, which hydrolyses the Hydrolizable tannins. Data analysis was done using ANOVA and least significant difference (LSD). There was significant difference ($p \leq 0.005$) in all the parameters tested for all the 3 species of acacia except in the total condensed tannins. It was concluded that Acacia tortilis with a crude protein of 11.890(g Kg^{-1} DM), and having the least tannin content of 0.00020 mg/g DM after processing can be used as a non-conventional plant protein supplement for ruminants, and that alkali processing method can be used to reduce tannins.

Keywords: Acacia species, nutritive value, tannin bioassay, processing effects, goats.

INTRODUCTION

Livestock sector is an important global player with enormous economic, social and environmental impacts (Steinfeld et al., 2006). Worldwide, livestock production contributes 40% of global agricultural GDP, employs 1.3 billion people, providing livelihoods for 1 billion of the world's poor people (Blümmel *et al.*, 2015). The impact of the livestock subsector are being increasingly felt due to demographic increase and income growth

(Thornton, 2010). Global meat demand is projected to more than double from 229 million tons in 1999-2001 to 465 million tons in 2050, and the minimum per capita protein intake recommended for maximum human physical and mental development is about 56gms per day, (Higgin *et al.*, 2011). In Kenya, the livestock subsector contributes about 12% of GDP ($4.5 billion per year) which is 40% of agricultural GDP, employing over 50% of agricultural labor force and the beef sector is ranked as the fastest rising economic sector with the per capita meat consumption being 10.8kg in the year 2003. Meat consumption has increased by about 10% in the last six years and production from 287,000 metric tons in 2001 per year to almost 300,000 MT in the year 2008 (Senerwa *et al.*, 2016; Steinfeld *et al., 2007).* There is an escalating demand not only for a bigger scope of quality attributes from livestock but also of the practices used to produce them, (Makkar 2003). There are 9 million goats in Kenya (Government of Kenya livestock census 2010). It is therefore imperative to research on acacia tree legumes nutritional potential to mitigate the effects of livestock malnutrition

Kenya is divided into seven agro-ecological zones and each zone has its unique physiognomic characteristics. There are over 1,342 acacia species distributed throughout the world and Kenya has 52 acacia species widely distributed across the varied agro- ecological zones, (Najma Dharani, 2006). The pastoralists depend solely on or partially on livestock and droughts usually result in high livestock mortality rate rendering these pastoralists among the most economically vulnerable communities in Kenya, (United Nations World Food Proggramme, Regional Bureau for East and Central Africa, Nairobi Kenya (Opiyo *et al.*, 2013).

The usually mixed grasses and browse vegetation in the field is low in nutritional value and hardly support fast growth rate and early maturity. Usually at the onset of the dry season, the quality of feed worsens quickly followed by reduced quantity due to increased grazing pressure and senescence of forages (Landers, 2007). In Kenya, artificially established field legume pastures like Lucerne, Desmodium, Vetch and Beans have high protein content of over 20% (CP.) (Mutai, 2017). The acacia tree legumes being members of the same family (*leguminiceae)*, are expected to contain reasonably good proportions of plant protein and other nutrients for maintenance and production to support livestock growth and development.

Among the acacia species in Kenya are *Acacia brevispica, Acacia. mellifera* and *Acacia tortilis* and are the subject of this study since they are the most occurring and preferred by goats in the Arid and Semi-Arid Lands (ASALs) of Kenya, which constitutes 80% of Kenya’s land mass (Kandie *et al.,* 2020). These tree-legumes also have the ability to fix atmospheric nitrogen and are good for silvo-pasture systems. Some field legumes in Kenya like Beans, Vetch, Lucerne and Desmodium (in their decreasing order) possess Anti-Nutritional Factors (ANFs) which cause poor nutrient utilization and growth retardation hence poor animal productivity (Mutai, 2017).

Most acacia species possess Anti-nutritional factors called tannins which protect them from excessive feeding by herbivores and the tannins protect dietary plant proteins from ruminal microbial digestion by forming tannin- protein complexes hence making them unavailable for degradation and consequently increasing their output in fasces (Gxasheka *et al.,* 2015).

In plant cells, Hydrolysable tannins (HT) and Condensed tannin (CDs) molecules are situated in the vacuoles of plants and are said to be discharged to the cytoplasm during cell damage such as during mastication by ruminants, and they have an affinity to bind chemically not only with proteins but also with polysaccharides, nucleic acids, alkaloids, saponins and steroids (de Sousa Lamy, 2008). This study aimed at determining the nutritional value, tannin bioassay and processing effects of mature green *Acacia- brevispica,* *A. mellifera* and *A. tortilis* pods processed differently as supplements to growing Small East Africa Goats (SEAG) in Baringo County-Kenya.

MATERIALS AND METHODS

Experimental Site and Characteristics

The research was done in Baringo county, Mogotio Sub- County, Kimose sub-location, Radat location, Kenya. This study area was selected since it falls within Kenya's agro-ecological transitional zone iv which forms part of Kenya's ASAL regions and is the habitat of most of the acacia species and frequently facing severe draught seasons (Wetang'ula *et al.,* 2007). The station is located at latitude-0.4N of Equator, Longitude 35.8 E of GM. with an average unimodal rainfall pattern of 400-700 mm per year which usually starts from April to June and has day temperatures ranging from 29-32 ^{0C}. It has a mid-altitude of 1000-1500 m.a.s.l and known for high goat population, practiced by the Capro-agro-pastoralist communities, (MOAlF, 2015).

Nutritive value

One kg of mature green pods was randomly collected from at least 7-10 trees from each of the three acacia species and placed in bags made of cloth after being washed and rinsed to get rid of dust and oven-dried at 105^{0c} for 12 hrs., to reduce moisture content to a constant weight. The samples were then ground using a laboratory grinder (Ten Can make) to be able to pass 1mm sieve and then packaged into air-tight polythene bags and clearly labeled. 200 gm sub samples in triplicates from each acacia species. were taken and subjected to proximate and Van Soest nutrient analysis (as per AOAC procedures-1995), for the four treatments i.e., T1, unprocessed pods, T2, shade-dried pods, T3 sun dried pods and T4, pods soaked in wood ash (alkali treatment) solution. for 48 hrs. This provided results for % Crude Protein (CP)), Dry Matter (DM), Crude fiber (CF), Neutral Detergent Fiber (NDF), Ether Extract (EE), Ash (minerals), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) respectively. Moisture content (MO)-was determined by oven-drying procedure (Association of analytical chemist (AOAC) International,1995). CP-The micro-Kjeldahl method (AOACInternational,1995) was used to determine the crude protein content. The % N in the sample was obtained by multiplying the %nitrogen by a factor (6.25). ASH (MINERALS) was determined using (AOACInternational,1995) Method923.03. Total extractable tannins and condensed tannins Extraction. A 200 gm (on DM basis) sub- sample was ground to be able to pass 1.0 mm sieve and used for tannin bioassay experiments where total extractable phenolics (TEPH) was extracted by use of 70% aqueous acetone (Makkar2003).

Determination of total extractable tannins (TET)

The tannins were measured indirectly after being precipitated by Polyvinylpolypyrrolidone (PVPP). TET (mg/g DM) was calculated as: Conc. Of total extractable phenolics (TEPH) remaining after Polyvinylpolypyrrolidone (PVP) treatment. Calibration curve:10ml aliquots of the standard tannic acid was pipetted into 100ml volumetric flask containing 75ml water and 5ml folin Denis's reagent was added and 10ml sodium carbonate solution added and diluted to the mark with water, then mixed well and absorbance was determined and plotted against concentration of tannic acid. equation was used to calculate the concentrations of the samples.

Determination of condensed tannins (Proanthocyanicidins)

Reagent

Butanol -HCL (Butanol - HCL 95:5 v/v), Ferric reagent (2% ferric ammonium sulphate in 2N HCL) was calculated as equivalent using the formulae: A_{550nm} x 78.26 x dilution factor $^{++}$)/ (% DM of the sample) (Porter et al., 2006). The regression curve showed a very high

Regression coefficient, y=0.0328+1.9715, (R^2=0.9544), between height, concentration(ppm) and Retention time from the chromatography performed.

Tannins bioassay

This was performed as per the procedures of (Makkar, 2003). Incubation was done on 500mgDM of acacia species samples, with or without 1.0 polyethylene glycol (PEG) molecular weight of 6000. Syringes were first warmed at 390C before addition of 40+-0.5ml of rumen liquor at a buffer mixture of 1:3, then incubated in triplicate in a
water bath maintained at 390C.

RESULTS AND DISCUSSIONS

Nutrient Composition

The results in Table 1 give the means for nutrient composition of A. *brevispica*, *A. tortilis* and *A. mellifera*, pods and LSD. The LSD test show variation in the means for pre-processed A. *brevispica*, *A. tortilis* and *A. mellifera* pods for observed nutritive parameters except for the CDs where there is little variation.

Table 1: Least significant difference (LSD)for chemical composition of acacia species pods.

	%DM	%MO	% CP	% CF	% OM
A. *brevispica*	96.715[a]	3.284[b]	9.359[b]	28.880[c]	92.582[a]
A. tortilis	96.141[a]	4.532[b]	9.877[b]	23.779[b]	91.406[a]
A. mellifera	90.965[b]	9.034[a]	20.707[a]	30.084[a]	84.139[b]

* *0.05 Significance levels*

[a, b, c] *Means with different superscripts in a column differ significantly (P<0.05).*

Table 2: LSD for Total Tannins (mg/g DM) for the treatments(processed)

Treatments	Total Tannins (mg/g DM)
A. tortilis, -T4 (AK)	11.025[b]
A. totilis, -T3 (Sun)	11.051[b]
A. totilis, -T2 (Sd)	11.513[b]
A. mellifera -T4 (AK)	8.218[d]
A. mellifera –T3 (Sun)	7.718[d]
A. mellifera –T2 (Sd)	5.551[e]
A. brevispica T3(Sun)	16.474[a]
A. brevispica –T2(Sd)	9.538[c]
A. brevispica -T4(Ak)	9.589[c]
T1A. brevispica-OE	8.320[d]
T1A. totilis -OE	11.243[b]
T1A. mellifera -OE	10.871[b]

All the three acacia species differed significantly ($p \leq 0.005$), in all the chemical components tested except in the tannins concentration where they depicted close similarity. This can be explained by the fact that tannins concentration in forage species can be influenced by the ecological zone, soil (edaphic factors) and genetic factors amongst others (Kirti 2008). Dry matter (DM). There was significant difference ($p \leq 0.005$) in dry matter content, (g Kg^{-1} DM) of acacia species pods processed differently among the acacia species i.e.,483.58, 480.71, and 454.83, for *A. brevispica, A. tortilis*, and *A. mellifera*, respectively. These findings differs with another study where the DM was 956.20, 970.07 and 958.07 (g Kg^{-1} DM), for A. brevispica; A. tortilis and A. mellifera, respectively (Kandie et al., 2022).

The crude protein content (g Kg^{-1} DM) differed significantly ($p \leq 0.005$) and were: 46.80, 49.39 and 103.54, for A. *brevispica*, *A. tortilis* and A. *mellifera*, respectively, with *A. mellifera* having the highest CP value of 103.54 (g Kg^{-1} DM). In this study, the levels of CP for the three acacia species i.e. *A. brevispica, A. tortilis* and *A. mellifera* was above the requisite amount of 7-8% needed for optimal rumen microbiota functioning (VanSoet,1994) and in agreement with the findings of Abdulrazak *et al*., 2020) and (Ngambi *et al.*, 2009) whose findings indicated a CP range of 12-21.3% depending on the ecological zone of the area and the dryness.

Neutral Detergent fiber (NDF)comprises the plant cell-wall polysaccharides and the more the NDF in the acacia species pods, the better the nutritive value of the particular acacia species. In this study the acacia species gave the following results with significant difference ($p \leq 0.005$):284.98, 237.86 and 222.50 (g Kg^{-1} DM) for *Acacia brevispica*, *A. mellifera* and *A. tortilis* pods, respectively. This finding differs with those of Isaac M Osuga *et al.* (2007**)** who reported NDF values of 382.5and 417.6 (g Kg^{-1} DM) for *A. brevispica* and *A. mellifera* respectively (Kassi et al., 2000).

Acid Detergent Fiber (ADF) And Acid Detergent Lignin (ADL)

These feed components varied significantly ($p \leq 0.005$) ranging from (g Kg^{-1} DM) 34.658, 35.500 and 39.670 for (ADF), and 32.403, 29.808 and 28.629 for (ADL) respectively. This implies that, the pre-processing like the sun drying and shade drying of the pods for all the acacia species seem to have had effect on the composition of CF%, NDF%, and ADL%. The results in this study suggests that *A. tortilis* with ADF and ADL of 35.500 and 29.808 (g Kg^{-1} DM), respectively have better nutritive value than *A. brevispica* and *A. mellifera*. According to this study, the best time for pods collection for processing and storage is at early maturity stage when ADF and ADL concentrations are still low and also when the tannins concentration is still low.

Ether extract (EE)

This is the fat component and a good energy source to the goats in the acacia species and there was significant difference($p \leq 0.005$) among the acacia species, where *A. brevispica, A. tortilis* and *A. mellifera* had 1.88, 2.15 and 1.66 % EE, respectively. This finding differs with those of Kandie *et al*. (2020) whose findings for the unprocessed acacia pods were: 1.66%,1.19% and 1.88%, respectively. The minerals (Ash components) of the processed acacia species were % - 4.133, 4.734, and 6.826 for, A. *brevispica, A. tortilis and A. mellifera*, respectively. Tannin bioassays: ANOVA results are as presented in tables-1.0a and 1.0b: It Indicates that there was significant difference ($P<0.05$) in the means between and within the groups for all the parameters indicating that the pre-processed acacia species pods were different, except for the total condensed tannins (CDs. The %CT (mg/g DM) was: 0.00138, 0.00020 and 0.01070, for A. *brevispica, A. tortilis* and *A. mellifera* respectively, with *A.tortilis* having the least amount and *A.mellifera* with the highest amount **,**Table 1.2.These findings differ with the findings of Isaac M Osuga *et al;* (2007**)** who recorded a Total Extractable Phenolics (TEPH) of 18.4Kg-1DM for *A. mellifera* and (7.1gkg[1] DM) for Total Extractable Tannins (TET) in unprocessed *Acacia mellifera* forage, unlike in this current study where the *Acacia* pods were processed differently.Table1 LSD for Total Tannins (mg/g DM) and Hydrolysable tannins(HTs) for the treatments gave the means for Total Tannins composition of the selected acacia species for all the treatment .There was no significant difference ($p \geq 0.005$) between the means for treated *A. tortilis* and the control(OE) for Total Tannins (TT) which was *A. tortilis,* -T4 (AK) 11.025 mg/g DM, *A. tortilis,* -T3 (Sun) 11.051 mg/g DM, *A. tortilis,* -T2 (Sd), 11.243 mg/g DM and 11.513 mg/g DM for *A. tortilis* –OE(control) respectively. This occurrence in *A. tortilis* may be associated with the different types of condensed tannins (*proanthocyanidines (*PA)-monomers), their degree of polymerization, molecular weights and stereochemistry of their carbon atoms and the types of hydrolysable tannins (*anthocyanidines* (AC)), (Tharayi *et al.*, 2011). Some studies have reported that condensed tannins having a high molecular weight have stronger reactivity due to their molecular weights and degree of polymerization with enzymes and some proteins than condensed tannins having reduced molecular weight, and that the interaction of CT increases with raising prodelphinidin content, (Isaac M Osuga *et al;* 2007). These characteristics may also have affected the capacity of wood ash (potassium hydroxide) to precipitate the tannins in *A. tortilis,* as was suggested in a study by (Florian Weber *et al*;2020). There was a strong Regression coefficient (R^2=0.944), between Height, Concentration and Parts per million(pmm).

CONCLUSION AND RECOMMENDATIONS

It was concluded that mature green pods of *acacia tortilis* have the requisite nutritional composition to be used for both maintenance and production of goats and other ruminants. It has also the lowest tannin levels as compared to the other acacia species in this study hence has the capacity to improve organic matter digestibility and improve feed intake, growth performance and carcass yield of ruminants. This is because it gave the overall best results in the parameters tested and therefore is recommended for use as a supplement for growing goats (and other ruminants) in Baringo county and other parts of the ASALs.

REFERENCE

Aganga, A. A., Omphile, U. J., & Baleseng, L. (2002). Performance of tswana goats fed Acacia mellifera, Euclea undulata, and Peltophorum africanum as a supplement to buffel grass. *Archivos de zootecnia*, *50*(191), 10.

Blümmel, M., & Becker, K. (1997). The degradability characteristics of fifty-four roughages and roughage neutral-detergent fibres as described by in vitro gas production and their relationship to voluntary feed intake. *British Journal of Nutrition*, *77*(5), 757-768.

Blümmel, M., Hailedlassie, A., Herrero, M., Beveridge, M., Phillips, M., & Havlik, P. (2015). Feed resources vis-á-vis livestock and fish productivity in a changing climate. *Livestock production and climate change*, *6*, 8.

de Sousa Lamy, E. C. C. (2008). *Salivary Proteomics as a Tool to Understand Ingestive Behavior: An Experimental Study in Sheep (Ovis aries), Goat (Capra hircus) and Mice (Mus musculus)* (Doctoral dissertation, Universidade de Evora (Portugal)).

Gxasheka, M., Tyasi, T. L., Qin, N., & Lyu, Z. C. (2015). An overview of tannins rich plants as alternative supplementation on ruminant animals: a review. *Int. J. Agric. Res. Rev*, *3*(6), 343-349.

Higgin, M., Evans, A., & Miele, M. (2011). A good kill: socio-technical organizations of farm animal slaughter. In *Human and Other Animals* (pp. 173-194). Palgrave Macmillan, London.

Kassi, A. L., Newbold, C. J., & Wallace, R. J. (2000). Chemical composition and degradation characteristics of foliage of some African multipurpose trees. *Animal feed science and technology*, *86*(1-2), 27-37.

Landers, J. N. (2007). *Tropical crop-livestock systems in conservation agriculture: the Brazilian experience*. Food & Agriculture Org.

Makkar, H. P. S. (2003). Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small ruminant research*, *49*(3), 241-256.

MoALF (2015). Economic Review of Agriculture 2015

Mutai, P. A. (2017). *Evaluation Of Nutritional Value of Field Legumes Using Rabbit (Oryctalogus Cunniculus) As The Animal For Bioassay* (Doctoral Dissertation, University Of Eldoret).

Opiyo, F., Wasonga, O., Nyangito, M., Schilling, J., & Munang, R. (2015). Drought adaptation and coping strategies among the Turkana pastoralists of northern Kenya. *International Journal of Disaster Risk Science*, *6*(3), 295-309.

Senerwa, D. M., Sirma, A. J., Mtimet, N., Kang'ethe, E. K., Grace, D., & Lindahl, J. F. (2016). Prevalence of aflatoxin in feeds and cow milk from five counties in Kenya. *African Journal of Food, Agriculture, Nutrition and Development*, *16*(3), 11004-11021.

Steinfeld, H., Gerber, P., Wassenaar, T. D., Castel, V., Rosales, M., Rosales, M., & de Haan, C. (2006). *Livestock's long shadow: environmental issues and options*. Food & Agriculture Org.

Thornton, P. K. (2010). Livestock production: recent trends, future prospects. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1554), 2853-2867.

Wassenaar, T., Gerber, P., Verburg, P. H., Rosales, M., Ibrahim, M., & Steinfeld, H. (2007). Projecting land use changes in the Neotropics: The geography of pasture expansion into forest. *Global Environmental Change*, *17*(1), 86-104.

Wetang'ula, G. N., Kubo, B. M., & Were, J. O. (2007). Environmental baseline study for geothermal developments: case study Arus-Bogoria geothermal prospects, Kenya. *Proceedings of the Short Course II on Surface Exploration for Geothermal Resources, Lake Naivasha, Kenya,* 2-17.