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Nutritive and Anti-Nutritive Evaluation of *Kleinhovia hospita, Leucaena leucocephala* and *Gliricidia sepium* with Respect to Their Effects on *in Vitro* Rumen Fermentation and Gas Production

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ABSTRACT

The nutritive and tannin content of tree forages (*Kleinhovia hospita***,** *Leucaena leucocephala,* **and** *Gliricidia sepium* **and their effects on** *in vitro* **rumen fermentation, digestibility and gas production were examined. Rumen fluid was obtained from three fistulated Boer goats with an average weight range of 31-32 kg fed forages. The fluid was incubated with 0.2 g of each forage at 39 °C for 48 h to determine the** *in vitro* **gas production, DM digestibility, metabolizable energy and volatile fatty acid. The proximate composition and the polyphenol composition of the forage were also evaluated. The experimental design was a completely randomized design and the treatments were** *K. hospita***,** *L. leucocephala* **and** *G. sepium***. The chemical composition, percentage of total polyphenol, non-tannin polyphenol, condensed tannin and hydrolysable tannin differed (P<0.05) among the forages. The** *K. hospita* **had higher (P<0.05) net gas production and** *in vitro* **dry matter digestibility compared with other forages. Similarly,** *K. hospita* **had higher (P<0.05) concentration of total volatile fatty acid and propionic acid followed by** *L. leucocephala* **and** *G. sepium***. The molar proportion of acetic and butyric acid did not differ among the forages. The outcome of this study present** *K. hospita* **as a good potential forage to be used in ruminant diet as a result of better nutrient composition, moderate anti-nutritive value and best ivDMD in comparison with** *L. leucocephala* **and** *G. sepium***.**

Keywords: Kleinhovia hospital, Leucaena leucocephala, Gliricidia sepium, nutritive value, tannin

INTRODUCTION

In the tropical and sub-tropical developing countries of the world, there is a wide and distinct difference between obtainable and needed livestock feeds. Ruminants in these areas are frequently fed highly lignified roughages and crop residues, which are low in available energy, nitrogen and minerals (Lunagariya *et al.,* 2017). A large proportion of these plants compose of structural carbohydrate shielded by lignin, which is not easily degraded by rumen microbes (Sallam *et al.,* 2007). Some of the arable crops used for ruminant are too expensive to be afforded by the small holders. Some are also limited in terms of quantity, undependable owing to the depletion of land by erosion or cyclone, climatic instability, encroachment of lands due to increase in human population. The aforementioned necessitate the need to diversify and search for alternative feed sources, which are cheap, in close proximity, available, and are not competed for by human and livestock (Chanjula *et al.,* 2011).

Feed quantity and quality are frequently the most challenging factors affecting the success of global livestock industries. The increase in human population has created an impetus for increased demand for animal protein for the sustenance of the populace protein requirement (Thornton, 2010). The need to intervene to rescue the livestock production from the usage of poor roughages through either supplementation or partial replacement by forage is very essential (Hills *et al.,* 2015). Tree forages which often play a central role in livestock feed, are rapidly gaining interest to be used as a result of the high content of rapidly degradable fiber as supplements to ruminants consuming poor quality roughage diets. Tree forages have successfully been used as supplements in sheep and goats (Mahgoub *et al.,* 2007).

In addition, the utilization of various tree forages are mostly employed in animal feeding on account of high protein content. *Kleinhovia hospita* has been reported by Ahmed *et al.* (2017) to improve the rumen fermentation profile in goat. The supplementation of *Leucaena leucocephala* in livestock feed bring about an increase in the milk yield, meat quality and live weight

gain of cattle, water buffalo and goats (Thornton & Herrero, 2010). In poultry, the extract from *L. leucocephala* (Xanthophylls) increase the quality of yolk color while the feed efficiency and feed intake in rabbit fed *L. leucocephala* leaf meal was ameliorated (Zongo *et a*l., 1997). The inclusion of *Gliricidia sepium* in sheep diet was found to increase the average daily body weight, total crude protein intake, total DM digestibility, digestibility coefficient and retained nitrogen has reported by Mpairwe *et al.* (1998). On the other hand, there was no significant difference among the forages (lablab, clitoria, mucuna and gliricidia) fed to dairy cattle in total dry matter intake and milk yield (Muinga *et al.,* 2000).

Nonetheless, the quantity of tannin and other phenolic components present in tree forages could hinder their utilization as feed for animals (Nahand *et al.,* 2011). Nutrient digestibility, voluntary feed intake and nitrogen (N) retention could be retarded because of high level of tannins in the leaves (Waghorn, 2008). Moreover, tannin concentration below 5% in the leaves may not be deleterious to the animal but could increase the protein by pass to the intestine boosting protein efficiency and productivity of ruminants fed with forage diet (Barry & McNabb, 1999).

Tannins are polyphenolic substances with different molecular weight of varied complexity. These groups of substances are chemically undefined with the quality to cling protein in aqueous solution (Waghorn, 2008). The multiple phenolic hydroxyl groups lead to the formation of complexes majorly with proteins and to a lesser extent with metal ions, amino acids, and polysaccharides (Piluzza *et al.,* 2012; Valiollah & Peyman, 2013). Due to the quest for tree forages for animal feed, assessing the nutritional value and biological effect of tree forages are highly essential (Azevedo Junior *et al.,* 2012).

Chemical composition alone could not ascertain the genuine nutritional potential, anti-nutritional factors and biological consequences of tannin in forage trees except with the combination of *in vitro* technique, which determines the *in vitro* digestibility as well as metabolizable energy (ME) content (Ammar *et al.,* 2005). A plethora of studies has utilized *in vitro* technique, which has proven to be efficient in the determination of the nutritive value of feeds containing anti-nutritive factors (Fievez *et al.,* 2005; Zmora *et al.,* 2012).

Nonetheless, there is dearth of information on the nutritional and anti-nutritional factors and *in vitro* fermentation of *K. hospita*, *L. leucocephala* and *G. sepium*. Thus, the objective of this present study was to evaluate the nutritive and phenolic contents of *K. hospita*, *L. leucocephala* and *G. sepium*) and their effects on *in vitro* rumen fermentation and gas production.

MATERIALS AND METHODS

Forage Samples and Collection

The forages (*K. hospita, L. leucocephala,* and *G. sepium*) were randomly harvested from four different parts of Ladang 2 (longitude 101°42′09.4″E and latitude 3°00′27.7″N) Ruminant Farm, Department of Animal Science, Plant unit of Animal Science Department, Faculty of Agriculture, Universiti Putra Malaysia.

Chemical Analyses

The forages were oven-dried at 100 °C until a stable weight was achieved to determine the dry matter content. The tree forages were ground into powder, sieved (mesh size of 2 mm), packed in air-free polyethylene bags and stored in a cool, dry place until further analysis. The chemical composition of the forages were analyzed in accordance with the procedure of AOAC (2007). The Van Soest *et al.* (1991) procedure was used to determine neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL).

Phytochemical Analysis

Sample preparation and extraction of tannin. The plants were harvested and freeze-dried as recommended by (Orians, 1995). Each of the plants was grounded to pass through 1 mm sieve. The samples were prepared for analysis according to the procedure of (Barman, 2004). About 400 mg of ground sample was placed in a test tube with a mixture of 40 mL diethyl ether with one % acetic acid (v/v) added and shaken in order to get rid of the pigment contained in the plant material. Five minutes later, the supernatant was discarded and 20 mL of aqueous acetone (70%) was included before the test tube was covered. For optimum extraction, the covered tubes were transferred into an orbital electrical shaker for 2 h. The samples were filtered through Whatman No. 1 filter paper and kept in a refrigerator (4 °C) for subsequent analysis.

Estimation of total phenol and tannins. Estimation of total polyphenol and tannins were done following the procedure of (Makkar *et al.,* 1993). About 50 µL of tannins extract was transferred into a separate test tube and distilled water (DH_2O) was added which gave a final volume of 1.0 mL. Thereafter, 0.5 mL of the prepared Folin Ciocalteu reagent was included and regularly agitated using an electrical orbital shaker. Then 2.5 ml of 20% (w/v) solution of sodium carbonate was added, mixed and kept for 40 min at 25 °C room temperature. The absorbance was measured at a wavelength of 725 nm using a spectrophotometer (*Genesys 20 Spectrophotometer with US plug*) before determining the concentration based on a standard curve. Total phenol was estimated as tannic acid equivalent and expressed on a DM basis.

Estimation of non-tannin phenol. The precipitation of tannins with polyvinyl polypyrrolidone (PVPP) as tannin binder is one of the way to determine non-tannin phenol. Approximately 200 mg of PVPP was measured into each test tube, 2 mL of DH_2O and 2 mL of tannin extract were added. The mixture were convoluted and kept at 4 °C within 15 min. The mixture was vortex and strained through Whatman No. 1 filter paper. Determination of non-tannin phenol was done using the filtrate. Similar to the total phenol estimation, non-tannin phenol content

was estimated by measuring 150 µL of filtrate into another test tube followed by addition of DH_2O to make up 1 mL by volume. The tannic acid standard curve obtained based on DM was used to estimate the non-tannin phenol concentration.

The estimation of total tannins was done by subtracting the non-tannin phenol from the total phenol. A standard solution from the stock solution of tannic acid (0.5 mg/mL) was prepared using 0, 10, 20, 30, 40 and 50 µL in test tubes. In each of the test tube, the standard prepared was made up to a volume of 1000 µL. This resulted into 0, 5, 10, 15, 20 and 25 µg of concentration of tannic acid respectively. Then 0.5mL of Folin reagent and 2.5 mL of 20 % sodium carbonate were added. The whole content was mixed thoroughly and after 40 min, measurement was carried out at a wavelength of 725 nm using a spectrophotometer (*Genesys 20 Spectrophotometer with US plug*).

Determination of condensed tannin. The concentration of condensed tannin (CT) was determined following the protocol of Porter *et al.* (1986). Approximately 0.5 mL of the tannin extract was pipetted into test tubes in triplicate and mixed with 3.0 mL of prepared butanol- HCl reagent and 0.1 mL of ferric reagent. The mouth of the tube was covered with glass marble and then boiled for 60 min in a water bath. Similarly, a blank was also prepared for each sample but the reagent was not subjected to heating. The tube was allowed to cool to room temperature before readings were taken at 550 nm using a spectrophotometer (Labomed Inc., Culver City, CA). Condensed tannin as leucocyanidin equivalent was calculated as follows:

Condensed tannin (%)= (A550 nm x 78.26 x dilution factor) / (% DM)

Estimation of hydrolysable tannin. Hydrolysable tannin was estimated by subtracting the condensed tannins from the total tannin phenol.

In Vitro Fermentation Characteristics

Collection of rumen fluid and *in vitro* incubation. The rumen fluid was obtained from three fistulated Boer goats fed with concentrate and forage and were within the weight range of 31-32 kg. The rumen fluid was obtained prior to morning feeding into a pre-warmed thermo flask already flushed with CO_2. The rumen fluid was pooled and squeezed through four layers of cheese cloth. The *in vitro* incubation was conducted following the protocol of Menke & Steingass (1988). Subsequently, the filtrate was mixed with phosphate and bicarbonate buffer at a ratio of 1:2 under continuous flushing of carbon dioxide (CO_2) to make a rumen buffer medium. The medium was made up of 400 mL distilled water, 200 mL of buffer solution, 200 mL macro mineral, 0.1 mL micro mineral, 1 mL of resazurin and 40 mL of freshly prepared reducing solution (4 mL of 1 N NaOH, 0.625 g of $NaS.9H_2O$ and 95 mL distilled water) which were thoroughly mixed together under a continuous CO_2 flushing.

About 0.2 g of each substrate was incubated with a 30 mL of the buffer medium in syringe at 39 °C for 48 h. Three syringes containing only rumen liquor-buffer medium were incubated as blanks while the other samples were replicated thrice in three different incubation runs. The gas production was measured at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 36 and 48 h. After 48 h of incubation, the net gas production of the samples were determined by subtracting the average volume of gas produced from blank from the average volume of gas produced by the sample.

The rumen fermentation kinetic data was fitted into the model of Ørskov & McDonald model (1979) by NEWAY software program. The equation is as follows:

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

Where a= gas production from the immediately soluble fraction (mL), b= gas production from the insoluble fraction (mL), c= gas production rate constant (mL/h), a + b = the potential gas production (mL), t= the incubation time (h), y= the gas produced in time t

The metabolizable energy (ME) was also determined by using the equation in accordance to Menke & Steingass (1988) as follows:

ME (MJ/kg DM)= 2.20 + 0.136 GP + 0.057 CP + 0.0029 CF

Where: ME= metabolizable energy, GP= net gas production (ml/200 mg), CP= crude protein, CF= crude fiber

The methane (CH_4) concentration was calculated using the formula proposed by Moss *et al.* (2000), as follows;

CH_4= 0.5 acetate – 0.25 propionate + 0.5 butyrate.

***In vitro* dry matter digestibility (%ivDMD).** The *in vitro* dry matter digestibility (*iv*DMD) was estimated at the end of the incubation. The content of the syringe for each sample and blank was emptied into dried and preweighed beaker. The dry weight of each beaker was recorded and labeled accordingly. Distilled water (DH_2O) was used to rinse the interior and the plunger of the syringes to reduce chances of under or over estimation of (*iv*DMD). The beakers were then oven dried at 105 °C until its content dried up and weight become stable prior to weight determination.

Determination of volatile fatty acids in rumen liquor. The concentration of VFA quantified by gas chromatography (Agilent 69890N Series) fitted with a flame ionization detector. About 3 to 5 drops of 10% H_2SO_4 was added to the rumen liquor to stop fermentation, then kept in -20 °C and later analyzed for VFA. At the onset of the analysis, the rumen fluid was allowed to thaw. Approximately 1.5 mL of rumen fluid was pipetted into a micro centrifuge tube and 0.3 mL of 3:1 (v/v) metaphosphoric acid (25%) and formic acid solution was added to the rumen fluid inside the micro centrifuge tube. The mixture was centrifuged at 3000 x g for 10 min and 0.5 mL of the supernatant was dispensed inside Agilent GC vial and 0.5 mL 20 mmol/L 4 methyl-n-valeric acid (internal standard) was added.

Statistical Analyses

All variables were analyzed as a completely randomized design (CRD) using the general linear model

(GLM) procedure of Statistical Analysis System package (SAS) Version 9.4 (2012). Means were separated by Duncan multiple range test at significance level of $P< 0.05$. The following statistical model was used;

$Yij = \mu + \alpha i + \varepsilon ij$

Where: Yij= dependent variables; μ= overall mean of the observations; αi = effect of forage types (i = 1, 2, 3) in the rumen fermentation of fistulated Boer goat and εij = difference within the forage types (error term). Also for the nutrient composition and phenol substance, SAS was used for the analysis.

RESULTS

Chemical Composition of the Selected Tree Forages

The nutrient composition of the selected tree forages in the current studies is presented in Table 1. The dry matter analysis among the forages in the present student was not significantly different whereas the ash content varied among the forages with *G. sepium* being the highest followed by *K. hospita* and *L. leucocephala* respectively. Also, the organic matter between *K. hospita* and *L. leucocephala* were not significant but both were significantly different from *G. sepium*. Furthermore, the crude proteins among the forages were significantly different (P<0.05) with *L. leucocephala* having highest crude protein compared to *K. hospita* and *G. sepium* which had similar crude protein value. The crude fibre (CF), NDF and ADF also differed (P<0.05) among the forages. *K. hospita* had the lowest values of fibrous components among the selected tree forages while *L. leucocephala* and *G. sepium* fibrous component increased but the highest fibrous components was recorded in *G. sepium*. In addition to the nutrient composition of the present studies, the lignin content of the forages varied (P<0.05). The lowest lignin value was recorded in *K. hospita* and the highest value was found in *L. leucocephala* with *G. sepium* having an intermediate lignin value. There was significant difference (P<0.05) among the forages in the gross energy content with *K. hospita* having the highest value of gross energy content followed by *G. sepium* and *L. leucocephala* had an intermediate values.

Polyphenol of Selected Tree Forages

The polyphenolic contents in *K. hospita*, *L. leucocephala* and *G. sepium* are presented in Figure 1. The polyphenolic contents differed (P<0.05) among the tree forages. The *K. hospita* leaves had higher (P<0.05) total polyphenols (3.77%), tannin polyphenols (2.56%), hydrolysable tannin (1.667%), condensed tannin (0.897%) and non-tannic polyphenols (1.248%) when compared to *L. leucocephala* and *G. sepium*.

In Vitro Fermentation Kinetics

The *in vitro* fermentation kinetics differed among the forages (P<0.05) and the data are presented in Table 2. The gas produced from soluble fraction "a" in *K. hospita* was observed to be the highest followed by *L. leucocephala* and *G. sepium* respectively. Also, from the result obtained from the insoluble fraction "b", it was found that *K. hospita* was significantly different (P<0.05) with the highest value when compared with *L. leucocephala* and *G. sepium* while *L. leucocephala* and *G. sepium* had similar value. For the potential gas production "(a+b)", ME and *iv*DMD, a significant difference (P<0.05) among the forages was observed. The result for *K. hospita* was the highest in comparison with *L. leucocephala* and *G. sepium* which had similar results. The net gas production (NGP) among the forages differed (P<0.05) and the highest result was obtained from *K. hospita* followed by *G. sepium* while the lowest was *L. leucocephala*. There was no statistical difference among the forages pH values.

In Vitro Volatile Fatty Acids

Based on the *in vitro* fermentation profile result, there was significant difference (P<0.05) in the total VFA production among the forages and has evinced in Table 3. *K. hospita* had higher total VFA result (P<0.05) when compared with *G. sepium* while *K. hospita* and *L. leucocephala* as well as *L. leucocephala* and *G. sepium* were similar. In the acetic, butyric, valeric and iso-valeric acid there was no significant difference among the forages. Furthermore, in the propionic acid, the result varied

Table 1. Chemical composition of selected tree forages

Variables (%DM)	Forages			SEM	P-value
	K. hospita	*L. leucocephala*	*G. sepium*		
Dry matter	94.57	94.08	94.33	0.245	0.385
Ash	7.54b	7.30^{c}	9.51^{a}	1.213	<.0001
Organic matter	92.44^{a}	92.68^{a}	90.47^{b}	1.213	0.003
Crude protein	18.99^{b}	23.30^{a}	20.88^{b}	2.16	0.006
Ether extract	2.60^{c}	3.98^{b}	4.71^{a}	1.072	<.0001
Crude fibre	13.39^{c}	16.10^{b}	17.04^{a}	1.895	<.0001
Neutral detergent fibre	45.86^{c}	52.81^{b}	59.21^{a}	6.677	<.0001
Acid detergent fibre	22.89^{c}	28.38^{b}	29.49^{a}	3.534	<.0001
Lignin	8.85^{c}	14.86^{a}	12.19^{b}	3.011	<.0001
Gross energy	18.64^{a}	18.10^{ab}	17.18^{b}	0.738	0.031

Note: Means in the same row with different superscripts differ significantly (P<0.05). SEM: standard error mean.

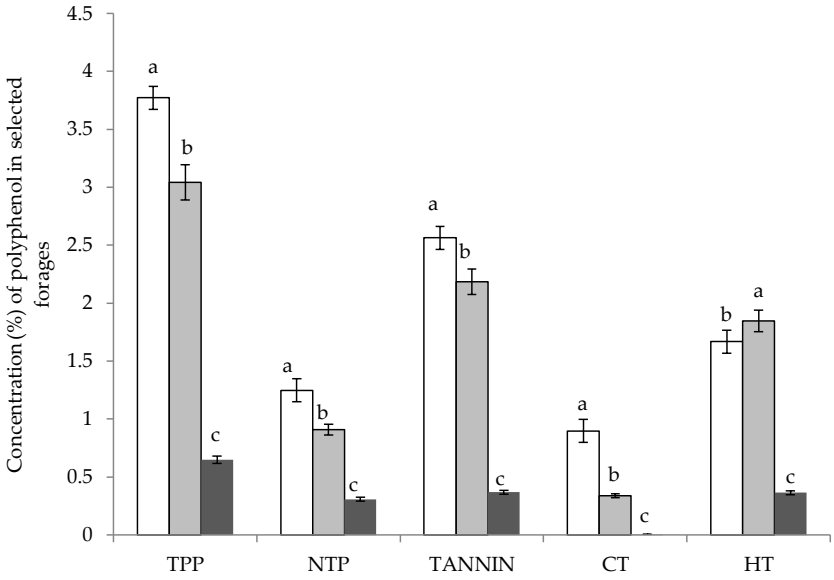

Figure 1. Concentrations of polyphenols in selected tree forages. [a,b,c] Bars with distinct superscript are significantly different (P<0.05); KH= *Kleinhovia hospital* (□); LL= *Leucaena leucocephala* (■); GS= *Gliricidia sepium* (■); TPP= total polyphenols; NTP= non tannin polyphenols; CT= condensed tannin; HT= hydrolysable tannin.

Table 2. *In vitro* fermentation kinetics, gas production, dry matter digestibility of selected forages

Variables	Treatments			SEM	P-value
	K. hospita	*L. leucocephala*	*G. sepium*		
a (mL)	1.28[a]	0.67[b]	0.33[c]	0.479	<.0001
b (mL)	31.80[a]	25.73[b]	26.88[b]	3.220	0.001
c (mL/h)	0.043[ab]	0.04[b]	0.045[a]	0.003	0.033
a+b (mL)	33.07[a]	26.40[b]	27.44[b]	3.588	0.000
NGP (mL)	28.17[a]	22.99[c]	26.19[b]	2.615	0.003
ME (MJ/KG/DM	7.05[a]	6.46[b]	6.62[b]	0.307	0.001
ivDMD (%)	62.83[a]	61.217[b]	61.50[b]	0.863	0.002
pH	6.71	6.79	6.85	0.104	0.375

Note: Means in the same row with different superscripts differ significantly (P<0.05); SEM: standard error mean; a= quantity of gas produced from soluble fraction (mL/200mg); b= quantity of gas produced from insoluble fraction (mL/200mg); (a+b)= potential gas produced (mL/200mg); c= rate of gas production from the insoluble fraction; ivDMD= *in vitro* dry matter digestibility; ME= metabolizable energy; NGP= net gas production; pH= ruminal pH.

Table 3. *In vitro* fermentation profiles of selected forages

Variables (mol/100mol)	Treatments			SEM	P-value
	K. hospita	*L. leucocephala*	*G. sepium*		
Total VFA Mm	59.405[a]	57.775[ab]	56.875[b]	1.282	0.054
Aetic acid (C2)	37.800	38.940	37.046	0.551	0.144
Propionic acid (C3)	18.745[a]	17.820[b]	16.790[c]	0.978	<.0001
Butyric acid (C4)	9.013	9.167	10.527	0.833	0.082
Iso-butyric acid	1.495[c]	2.055[b]	2.532[a]	0.519	0.002
Valeric acid	2.535	2.445	2.792	0.179	0.160
Iso-valeric acid	2.003	1.830	1.798	0.115	0.370
C2:C3	2.018[b]	2.188[a]	2.209[a]	0.105	0.022
CH_4	18.720[b]	19.599[a]	19.589[a]	0.505	<.0001
pH	6.723	6.783	6.837	0.057	0.378

Note: Means in the same row with different superscripts differ significantly (P<0.05). SEM: standard error mean.

among the forages with *K. hospita* having the highest value of propionic acid followed by *L. leucocephala* and *G. sepium* respectively. The proportion of acetic and propionic acids as well as CH_4 gas production were noticed to differ among the forages with *K. hospita* being the lowest in comparison with *L. leucocephala* and *G. sepium* which were similar. Differences were also noted in the iso-butyric acid among the forages. However, there was no significant difference in the pH among the forages.

DISCUSSION

The nutritional composition of every component of animal feeds perhaps forages are very essential which cannot be under estimated owing to its feed quality determination and contribution on animal performance. The dry matter of most feed ingredient fed to animals is an indicator of the quantity of nutrients that are available to animal in a particular feed. In the present finding, the dry matters of all the forages (*K. hospita*, *L. leucocephala*, and *G. sepium*) were not significantly different which suggest high chances of the forage nutrient component to be mostly alike. The high ash content in *L. leucocephala* compared to other forages (*K. hospita* and *L. leucocephala*) could be due to the high mineral element present which could impact changes on the physicochemical as well as the nutrition (essential and toxic mineral elements) of the forage (Mbatchou & Dawda, 2013). The similarity in the organic matter content observed in *K. hospita* and *L. leucocephala* could be due to the quantity of available energy present in the forage which organic matter served as an indicator. The organic matter of *G. sepium* was lower than *K. hospita* and *L. leucocephala* due to reduced available energy has validated in the gross energy determination in the present study.

Furthermore, the crude protein content obtained in *L. leucocephala* was significantly higher than *G. sepium* and *K. hospita* which had similar value. Although, the protein contents among the forage in the present study exceeded 12% DM which is the protein requirement for ruminants (Njidda & Ikhimioya, 2010). This implies that the protein content in the tree leaves in this study could be optimized when supplemented in ruminants fed poor roughages. The influence of fibrous component of forages with regards to digestibility and intake was reported by Ball *et al.* (2001) who stated that increase in the concentration of NDF and ADF reduced the potential voluntary intake of the forage and digestibility respectively owing to the high fibre contents. In the present study, low CF, NDF and ADF recorded in *K. hospita* compared with *L. leucocephala* and *G. sepium*. This suggests that *K. hospita* possess less of indigestible fibrous component in the forage and this could enhance digestibility, voluntary intake and utilization. This was in agreement with what was reported by (Ball *et al.*, 2001). Lignin composition of most forage has been established as one of the major militating component affecting digestibility of cell wall material by rumen microbes and increase in the lignin content could hindered the optimization of such forage (Moore & Jung, 2001). *K. hospita* had the lowest lignin component followed by *G. sepium* and *L. leucocephala* respectively in the present study. The highest gross energy obtained in *K. hospita* evinced the energy composition of the forage compared to other forages (*L. leucocephala* and *G. sepium*).

Polyphenols are large group of phytochemicals obtained in ample natural plant with bioactive content which are beneficial to animal and human depending on the composition. Tannin has been reported by Naumann *et al.* (2017) to be the most profuse compound made up of condensed and hydrolyzed tannin. These have been found to reduce ruminal degradation of plant protein and increase crude protein flow to the intestine for absorption by the animal and reducing CH_4 emission. In addition, the morphology and species of the forages has reported by Min *et al.* (2002) to initiate the variation in the composition of phenolic compound. The highest total polyphenol, non-tannin phenol and tannin obtained in *K. hospita* compared to other forages (*L. leucocephala* and *G. sepium*) in the present study indicate the huge potential of bioactive compound of *K. hospita*. Moreover, the highest tannin content noted in the current study is below the 5% which has been stated as the maximum level of tannin that could be tolerated by animals (Silanikove *et al.*, 1997; Barry & McNabb, 1999).Higher concentration of tannin in the diet beyond the tolerated level could be detrimental.

The gas produced from soluble fraction "a" during the *in vitro* fermentation kinetics was reported by Cone & Van Gelder, (1999) to be an indication of presence of high soluble nutrient in the forage, which can easily be fermented by the rumen microbes. The highest "a" in the present study was recorded in *K. hospita* followed by *L. leucocephala* and *G. sepium* respectively. This was in line with the aforementioned finding as well as Valiollah & Peyman, (2013) and it is an obvious affirmation of the good potentiality of *K. hospita* to be selected as forage. Also, the *in vitro* gas produced from insoluble fraction "b" as well as potential gas production "(a+b)" in *K. hospita* was significantly the highest when compared with *L. leucocephala* and *G. sepium*. This could be as a result of high degradable content in the former (*K. hospita*) than the latter (*L. leucocephala* and *G. sepium*).

Furthermore, the cumulative net gas production, ME and *iv*DMD differed among the forages. In the present study, *K. hospita* had highest cumulative net gas production, ME and *iv*DMD in comparison with *L. leucocephala* and *G. sepium*. The increase in *iv*DMD brought about an increase in cumulative gas production. This observation was similar to the findings obtained from Banik *et al.* (2013); Njidda & Nasiru (2010). The relationship between (cumulative net gas production and *iv*DMD) noted in the present study is a reflection of feed digestibility and this was in line with the view of Holtshausen *et al.* (2009). The feed digestibility reflection could be employed in the present study to examine the nutritive value in order to justify the potential digestibility and energy contents of forages has reported by (Sallam, 2005). In addition, the NDF and ADF concentration of forages also contribute to the fermentation of forages has stated by (Wilson & Mertens, 1995). Hence,

the relationship between NDF and ADF with gas production exist in the recent finding. This implies that increase in the former (NDF and ADF) led to decrease in the latter (gas production) during the incubation has noticed by Menke & Steingass, (1988); Getachew *et al.* (2004); Maheri-Sis *et al.* (2008). The low concentration of NDF and ADF in *K. hospita* could be responsible for its high gas production compared with *L. leucocephala* and *G. sepium* in the present study.

Nevertheless, the *in vitro* total VFA production obtained in *K. hospita* was more than other forage types (*L. leucocephala* and *K. hospita*) and this was similar to what was noticed in the cumulative gas production and *iv*DMD. These observations were in tandem with the findings of (Elmenofy *et al.* (2012); Zmora *et al.* (2012) who reported that increase in gas production in the sample suggest an increase in fermentation activity by rumen microorganisms. An increase in the molar proportion of propionic acid in *K. hospita* brought about a decrease in acetic:propionic acid ratio compared to other forages (*L. leucocephala* and *G. sepium*). Reduction in acetic:propionic acid ratio could also impede hydrogen transfer and decrease the rate of methanogenesis thereby reducing CH_4 emission has observed in the present study and enhance energy utilization which were in agreement with the findings of (Naumann *et al.*, 2017). The result of the current study concurs with those of Garcia-Gonzales *et al.* (2010); Szumacher-Strabel & Cieślak, (2010) who observed that rhubarb decreased CH_4 emission with little changes in the VFA profile devoid of deleterious effects on DM digestibility. The differences noted among the forages in iso-butyrate could be due to differences in protein degradation since the branched-chain VFAs are primarily derived from dietary proteins (Berthiaume *et al.*, 2010). The pH between the forages in the present study was similar and is within the range of normal pH for rumen microbial activities (Dehority, 2003).

CONCLUSION

The results of the present study indicate that *K. hospita* nutritive composition is worthwhile and comparable with the other forages (*L. leucocephala* and *G. sepium*). The anti-nutritive component is within the tolerated level without any deleterious effect. Also, in the *in vitro* fermentation kinetics, it shows a positive result and it stands a great chance to be exploited in ruminant feed.

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