Chemical composition of *Lablab purpureus* and *Vigna unguiculata* and their subsequent effects on methane production in Xhosa lop-eared goats

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Abstract

The objective of this study was to evaluate the nutritive value and anti-nutrient contents of *Lablab purpureus* (*Lablab*) and *Vigna unguiculata* (cowpea) and their effects on methane production in goats. Legume forages were grown and harvested at three stages of growth of pre-anthesis, anthesis, and post anthesis. Samples were collected at each stage and examined for proximate composition, total phenolics, condensed tannins, and saponins using standard methods. Hay was harvested at the anthesis stage and used in a growth study to evaluate the effects of forage legumes on methane production. Eighteen one-year-old goats, nine males and nine females, were used in the feeding trial. The goats were subjected to three treatment diets with six goats in each treatment, representing both sexes equally, for 60 days in a complete randomized design. Methane was measured with a laser methane detector (LMD). Cowpea showed higher ash (13.11%), acid detergent fibre (ADF) (38.42%), and crude protein (CP) (20.23%) than Lablab, which had values of 11.45 %, 36.17%, and 19%, for ash, ADF, and CP, respectively. Lablab had significantly higher fat content (2.41%), neutral detergent fibre (NDF) (49.27%), and hemicellulose (13.07%) than cowpea (2.1%, 46.91%, and 8.48%, respectively). The tannin, phenolic, and saponin content were influenced significantly by forage species and stage of growth. The diet and sex of the animal affected enteric methane production significantly. Forage legumes met animal requirements for fat, ADF, NDF, and CP. The energy and tannin levels of forage legumes were shown to reduce enteric methane production in goats.

Introduction

Livestock production is a critical and perhaps the most successful business enterprise for underprivileged small-scale farmers that live in marginalized drought-prone areas. Nevertheless, this lucrative business is characterized by severe animal feed shortages, particularly during the dry season. Furthermore, changes in the climate of the southern hemisphere are likely to influence feed availability (Scholtz *et al.*, 2013; Meissner *et al.*, 2013). Many studies have focused on strategies to improve animal nutrition during dry periods. One such proposal was the use of improved pasture grasses and forage legumes (Mapiye *et al.*, 2007). Improved pasture grasses provide sufficient metabolizable energy (ME), averaging between 8 ME MJ/kg dry matter (DM) and 12 ME MJ/kg DM (McDonald *et al.*, 2011). However, they tend to have inadequate protein, especially in the dry season. Conversely, forage legumes provide enough protein, ranging from 12% to as high as 25% (McDonald *et al.*, 2011) for maintenance and production, depending on the species. The use of legumes has also been limited by the availability of fermentable fibre (Mupangwa, 2000). Although legumes provide enough proteins, the amount of their fermentable carbohydrates tends to be limiting. Therefore, in most intensive systems, their use is usually of a supplemental nature to pasture grasses (Jingura *et al.*, 2001). The amount of biomass is crucially important, although the nutritive value of the forages is of concern to livestock farmers. For pasture legumes and grass

forages, biomass and age are generally negatively correlated with forage quality. Nutritive value has been linked to a number of factors, ranging from species to harvesting and curing methods. Furthermore, as reported by Mupangwa (2000), the stage of harvesting of most legume forages influences their nutritive value significantly. A similar conclusion was reached by Jingura *et al.* (2001). In addition, legume forages are known to contain high levels of anti-nutrients that form complexes with proteins and carbohydrates, rendering them unavailable. As a result of these limiting factors, it is necessary to evaluate the amount and extent of these complexities to validate the available protein. The presence of tannins and low fibre content can be advantageous in mitigating enteric methane (CH_4) production. Valenciaga *et al.* (2009) reported that forage legumes possess lower levels of soluble carbohydrate content compared to grasses, which can influence methane production. Soluble carbohydrates, such as starch, are easily fermented, resulting in high levels of free hydrogen (H_2), which promotes methanogenesis. On the other hand, tannins have been reported to decrease the number of cellulolytic bacteria (McSweeny *et al.*, 2001), shift short-chain fatty acid (SCFA) production, and reduce DM and organic matter digestibility (OMD) (Hess *et al.*, 2006; Abdalla *et al.*, 2007; Animut *et al.*, 2008; Tiemann *et al.*, 2008), all of which stimulate the release of high H_2.

Enteric CH_4 production in ruminants accounts for about 11–17% of global methane (Storm *et al.*, 2012). Methane arises from the activity of bacterial agents, called methanogens, in the rumen. These organisms use H_2 to reduce carbon dioxide (CO_2). By so doing, they prevent the accumulation of reducing equivalents, which are known to impede ruminal fermentation. Goats are ruminant animals and produce enteric CH_4. As reported by Du Toit *et al.* (2013), South African goats produce in the range of 15 to 17 g/kg DM methane per day. Methane as a greenhouse gas (GHG) is a cause for concern in global warming (Storm *et al.*, 2012). This has resulted in enormous research work that sought to elucidate and quantify the amount of gas produced by ruminant animals. Detailed reviews were done by Storm *et al.* (2012) and Moss *et al.* (2000), which highlighted the methods by which enteric CH_4 from ruminant animals can be quantified. Regardless of the effectiveness of any method, the greatest challenge has been practical applicability of these enteric measurement methods. In these reviews, it is clear that ruminant animals are contributing to GHGs chiefly in the form of CH_4. Therefore, the objective of this study was to evaluate the nutritive value, anti-nutrient content, and effects of *Lablab purpureus* and *Vigna unguiculata* on enteric CH_4 production in goats.

Materials and Methods

The research was conducted at the University of Fort Hare Research Farm, Eastern Cape, South Africa, during the summer season in November 2014. The farm is located at a latitude of 32°46' S and longitude 26°50' E, at an altitude of 535 metres above sea level. It has a warm temperate climate with an average annual rainfall of about 575 mm, which is received mainly during the summer months of November to March. The maximum temperature is 24.6 °C, minimum temperature is 11.1 °C, and average temperature is 17.8 °C. The soils are deep and alluvial, of the Oakleaf form (Oa), belonging to the Ritchie family, according to the South African system of soil classification (Soil Classification Working Group (SCWG), 1991). According to the soil map of the world, the soils are Eutric Fluvisols (Fle). The vegetation is dominated by grasses such as *Themeda triandra* and *Cympogon plurinodis* with woody plants such as *Acacia karro* and shrubs encroaching the grazing lands (Mucina & Rutherford, 2006).

The forage legumes *L purpureus and V. unguiculata* were each grown in the 2014/15 season. Each legume was established in rows 0.60 m by 0.30 m in plots, two for each forage legume, measuring 16 x 32 m, at Fort Hare Research Farm. A basal fertilizer of single superphosphate at 300 kg/ha was applied on the day of planting. Legumes were grown under dry land conditions with no irrigation. The experiment was a 2 x 3 factorial experiment in a completely randomized design to examine the effects of forage legumes (Lablab and cowpea) and stage of growth (pre-anthesis, anthesis and post-anthesis) on nutrient, tannin, saponin and phenolic content. Three legume samples were cut randomly using a 0.5 m^2 quadrant from each block to a 10-cm stubble height. These samples were harvested from each block, making a total of 12 samples (six for each forage species). Twenty percent of the samples were oven dried at 60 °C for 48 hours and stored at room temperature for further analysis**.** The dried legume samples were milled through a 1-mm screen. Triplicate samples of each legume were analysed for crude protein (CP) using Kjeldahl's procedure (AOAC, 2005), while neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed by methods described by Goering & Van Soest (1970). Total ash was obtained by igniting a dried sample in a muffle furnace at 500 °C for 24 hours and cooled to room temperature before determining ash content by difference. Pulverised samples with an average weight of 2 g were soaked in separate conical flasks with 50 ml organic solvents, which included acetone, methanol, ethanol and water and shaken in an orbital shaker (Gallenkamp 202 Ilanga Trading cc.) for 24 hours. The crude extracts were filtered using a Buchner funnel and Whatman No. 1 filter paper.

Phenol determination was estimated spectrophotometrically using the Folin-Ciocalteu method as described by Samatha *et al.* (2012) with some modifications. The amounts of 0.5 mL of the plant extracts (1 mg/ml) and standard gallic acid with levels ranging from 0.02 mg/ ml to 0.1 mg/ml were pipetted into different test tubes. To this, 2.5 ml 10% (v/v) Folin-Ciocalteu's reagent, prepared in distilled water, was added and the mixture was vortexed. The reaction was allowed to stand at room temperature for about 5 mins. After 5 mins, 2 ml 7.5% (w/v) anhydrous sodium carbonate was added to the solution, vortexed and incubated at 40 °C for 30 mins. A control solution, which had neither extract nor gallic acid, was used as a blank. After incubation, the absorbance was measured at 765 nm using an AJI-C03 UV-Vis spectrophotometer (METTLER TOLEDO). The experiment was done in triplicate. The phenol content was extrapolated from the gallic acid standard/calibration graph equation: $y = 0.0091x - 0.0527$, R^2 0.9979, and was expressed as mg gallic acid equivalent (GAE)/g DM from the equation:

$$TP = CV/m$$

Where: TP is total phenolics
C is the concentration as derived from the calibration curve equation in mg/ml
V is the volume of the extract used in the assay in ml
m is the mass of the extract used in the assay in g

The total proanthocyanidin was determined using a procedure described by Sun *et al.* (1998). A mixture containing 3 ml vanillin-methanol (4% w/v), 1.5 ml hydrochloric acid, and standard catechin was added to 0.5 ml 1 mg/ml extract solution at various concentrations from 0.02 mg/mL to 1 mg/mL. The mixture was vortexed and allowed to stand for 15 min at room temperature. A blank control solution was used, which had neither extract nor catechin. The absorbance was measured at 500 nm using a UV-3000 PC spectrophotometer. Triplicate samples for each forage were used in this experiment. The proanthocyanidin content was evaluated using a calibration curve equation, namely $y = 0.0014x + 0.015$, R^2 0.9965, and was expressed as mg catechin equivalent per gDM using the formula, CV/m, as referred to above.

The saponin content in the plant extracts was determined using the method described by Omoruyi *et al.* (2012). The procedure included mixing 1 mg of the various solvent extracts with 50 ml 20% ethanol in a shaker for 30 min. This was then heated in a water bath at 55 °C for 4 hours with continuous stirring. After heating, the mixture was filtered and the residue was re-extracted with another 20 ml 20% ethanol. The combined extracts were reduced to 40 ml in a water bath at 90 °C. The concentrated solution was then transferred into a 250 ml separating funnel and extracted twice using 20 ml diethyl ether. The ether layer was discarded, while the aqueous layer was retained, and 60 ml *n*-butanol was added. The *n*-butanol extracts were washed twice with 10 ml 5% sodium chloride. The butanol layer was collected and evaporated in a water bath and later oven dried at 40 °C to a constant weight. This was done in triplicate and the percentage saponin content was calculated using the formula:

% saponin = final weight of sample / initial weight of sample ×100

Eighteen goats were used in this experiment. The average age of goats was 12 months and they had an average live weight of 14.2 ± 0.24 kg, with equal representation for sexes (nine castrated males and nine empty females). Goats were dewormed using niclosamide 20% (Lintex L), and dipping was done with a pour-on acaricide (Coopers Redline). They were subjected to three treatments: treatment 1 (T_1): 71% Vigna hay, 19% Katambora hay, salt (0.5%), molasses (3%), maize (5%), and mineral vitamin premix (1.5%); treatment 2 (T_2): 90% lamb and ewe pellet plus 10% Katambora grass hay; and treatment 3 (T_3): 72% Lablab hay 19% Katambora hay, salt (0.5%), molasses (2%) maize (5%), and mineral vitamin premix (1.5%). T2 was the positive control diet. All diets were formulated to contain CP and energy to meet the minimum recommendation for intensive feeding (i.e. 14% CP and 9 MJ ME/ kg DM), according to NRC (2001). Animals were injected with a mineral and vitamin complex (Cipla Agrimed, Pretoria, South Africa) prior to housing and after every 14 days. Animals were housed individually in metabolic pens measuring 1.5 x 1.0 m and acclimatized to the environment and experimental conditions for two weeks; this was followed by 40 days of growth/feeding trial and 6 days of digestibility trial, respectively. The experiment was arranged in a complete randomized design with 3 x 2 factorial arrangements (three diets and two sexes). Animals were housed according to sex. Animals were fed in two equal portions at 08:00 and 15:00 hour daily and the amount offered was adjusted based on bodyweight measured every 15 days. Clean water was available to animals *ad libitum*.

Methane was measured using an LMD (Crowcon Detection Instruments Ltd., Oxfordshire, United Kingdom), weekly from the adaptation period when animals were resting, feeding and ruminating, then daily

for the last seven days of the trial. The LMD equipment measures the concentration of CH_4 between the equipment and the target point. It is based on infrared absorption spectroscopy and measures CH_4 values as a plume. Hence the measurements are in parts per million-metre (ppm-m). The equipment operates normally in the temperature range between 0 and 40 °C, in the humidity range of 20–90%, with a reaction time of 0.1 seconds. The LMD can detect CH_4 concentrations between 1 and 50,000 ppm within a distance of up to 150 m. Gas column density was measured by directing the auxiliary LMD targeting (visible HeNe) laser beam at the nostrils of goats for a maximum of five minutes per animal at a distance of 1.5 m. This distance was considered safe enough not to disturb animal activity, as described by Chagunda *et al.* (2009). All measurements were taken at approximately the same time of day (morning and late afternoon). Three measurements were taken from each animal at each activity and the average value was calculated.

Methane eructed was determined per each activity using standard respiratory coefficients per activity, then translated to an equivalent emission per day. Methane production was also evaluated in relation to dry matter intake (DMI) consumed.

Methane eructed during activity

$$M_{TV} = M_{MD} \times T_{Vr} / 10^6 ml \text{ (Chagunda } et\, al.,\text{ 2009)}$$

Where: M_{TV} is the enteric methane in breath in ml during ruminating
M_{MD} is the enteric methane detected by LMD converted from ppm-m to ml
T_{Vr} is the tidal volume during different activities

Additionally, T_{Vr} feeding = 620 ml, T_{Vr} standing = 760 ml, T_{Vr} ruminating = 760 ml
Tidal volume (feeding) = 3100 ml, tidal volume (standing) = 3800 ml for dairy animals. These were then converted using livestock units to represent goats, where 0.5 LU cow is equivalent to 0.1 livestock unit (LU) goats (Chilonda & Otte, 2006) for sub-Saharan Africa.

Methane eructed per activity per day

$$M_{TTA} = M_{TV} \times R_{TA} \text{ (Chagunda } et\, al.,\text{ 2009)}$$

Where: M_{TTA} is the amount of enteric methane produced during an activity (rumination, feeding, just standing).

Methane eructed per day

$$M_D = MTTA \times (T_D \times R_{TA}) \text{ ml/day (Chagunda } et\, al.,\text{ 2009)}$$

Where: M_D is daily enteric methane
T_D is daytime in seconds
R_{TA} is total time spent on activity

R_{TA} standing = 1440, R_{TA} feeding = 2880, and R_{TA} ruminating = 7200

By substitution and use of specific density conversion factor, daily enteric methane in grams (MDG) is:

M_{DG} (g/day) = M_D x 0.00066715 (CH_4 density in g/ml) (Chagunda *et al.*, 2009)

Methane from dry matter intake

1. Methane (l/day) = 0.0305 DMI(g/day) – 4.441 (Shibata *et al.*, 1992)
2. M (kg/head/day)= DMI x 0.0188 + 0.00158 (Howden & Reyenga, 1987)

Analysis of variance was used to determine the effects of legume species and stage of growth and their interactions on biomass yield, CP, NDF, ADF, Ash, fat, and hemicellulose, using the general linear model's procedure of SAS (SAS, 2003). The analytical model was as follows:

$$Y_{ijk} = \mu + F_i + S_j + (FS)_{ij} + e_{ijk}$$

Where: Y_{ijkl} = biomaas, DM yield, CP, CF, etc

μ = overall mean

F_i = effect of type of forage (j = 1, 2 Vigna, Lablab)

S_j = effect of stage of growth (i = pre-anthesis, anthesis, post-anthesis)

$(SF)_{ij}$ = effect of interaction between stage of growth and forage type

e_{ijk} = error term

Means were separated using Tukeys' studentized range test.

The effect of diet, sex and their interactions on methane production were analysed using the general linear model (PROC GLM) procedure of SAS (2003) and initial bodyweight was regarded as a covariate Methane was also measured over time, and data were analyzed using repeated measures of SPSS version 17. Tukeys' studentized range test was used to test the significant differences between means when F-test was found to be significant ($P <0.05$). The statistical model used was:

$$Y_{ijkl} = \mu + S_i + T_j + D_k + (ST)_{ij} + (SD)_{ik} + (TD)_{jk} + (STD)_{ijk} + e_{ijkl}$$

Where: Y_{ijk} is the dependent variable (methane emission)

μ is the overall mean

S_i is the effect of sex of animal (i = 1,2)

T_js the effect treatment (j = 1,2,3)

D_k is the effect of time in weeks (k,= 1,2,3,4,5,6)

$(ST)_{ij}$is the interaction effect between sex treatment

$(SD)_{ik}$ is the interaction effect between sex and time

$(TD)_{jk}$ is the interaction effect between treatment and time

$(STD)_{ijk}$ is three-way inteaction amoung sex treatment and time

e_{ijkl} is the error term

Results

The proximate value of Lablab and Cowpea were evaluated, and the results are shown in Table 1.

Table 1 Proximate composition of Lablab and cowpea used in the study

Forage species (F)	Stage of Growth (S)	Moisture %	Ash %	CP %	Fat %	ADF %	NDF %	Hemi-celluloses %
Lablab	Pre-anthesis	7.58 ± 1.65	12.67[a] ± 0.22	21.14[a] ± 0.23	3.00[a] ± 0.12	35.13[c] ± 5.02	48.7[bc] ± 7.76	13.61[a] ± 1.78
	Anthesis	7.47 ± 1.65	11.00[c] ± 0.22	17.73b ± 0.23	2.37[b] ± 0.12	33.65[c] ± 5.02	46.51[c] ± 7.76	12.86[a] ± 1.78
	Post anthesis	7.44 ± 1.65	10.67[c] ± 0.22	17.51[b] ± 0.23	1.87[c] ± 0.12	39.87[b] ± 5.02	52.61[b] ± 7.76	12.74[ab] ± 1.78
Vigna	Pre-anthesis	7.56 ± 1.54	17.33[b] ± 0.12	24.88[c] ± 0.23	2.90[b] ± 0.12	38.36[b] ± 5.02	45.53[c] ± 7.76	7.16[b] ± 1.78
	Anthesis	7.89 ± 1.54	11.00[c] ± 0.12	19.10[d] ± 0.23	2.03[b] ± 0.12	30.4[d] ± 5.02	37.58[d] ± 7.76	7.18[b] ± 1.78
	Post anthesis	7.70 ± 1.54	11.00[c] ± 0.12	16.71[b] ± 0.23	1.33[c] ± 0.12	46.5[a] ± 5.02	57.63[a] ± 7.76	11.10[ab] ± 1.78
Significance	F	0.4337	0.001	0.001	0.002	0.002	0.027	0.022
	S	0.4337	0.001	0.001	0.021	0.020	0.023	0.031
	F x S	0.323	0.153	0.398	0.236	0.001	0.024	0.661

[abcd] Column means with different superscripts differ significantly at $P <0.05$, CP: crude protein, ADF: acid detergent fibre, NDF: neutral detergent fibre

The average moisture contents for cowpea and Lablab (7.72% vs 7.50%) were similar ($P >0.05$), irrespective of the stage of growth. Cowpea exhibited higher ($P <0.05$) ash (13.11%), ADF (38.42%), and CP (20.23%) than Lablab (11.45%, 36.22%, and 19%, respectively). Lablab recorded significantly higher fat content (2.41%) than cowpea (2.09%). On average, cowpea recorded lower ($P <0.05$) NDF values compared

with Lablab (46.9% vs 48. 78%).There was a significant interaction between stage of growth and forage species for ADF, NDF, and hemicellulose content. Lablab showed higher (*P* <0.05) NDF (48.78%) and hemicelluloses (13.07%) values than cowpea. Lablab showed higher (*P* <0.05) ADF and NDF values post anthesis, a trend that was similar with cowpea. Furthermore, the NDF content increased (*P* <0.05) post anthesis for both forages. Cowpea recorded a 6.33%, 8.17%, and 8.14% decline in ash, CP, and ADF, respectively, from pre-anthesis to post anthesis. These percentages were significantly higher than those exhibited by Lablab of 2%, 3.63%, 4.74% for ash, CP and ADF, respectively. Three ANFs were evaluated for Lablab and cowpea, and the results are shown in Table 2.

Table 2 Anti-nutritional factors in Lablab and cowpea forage legumes used in the study

Forage species (F)	Stage of growth (S)	Tannins (mgCAE/gDM)	Phenolic (mgGAE/gDM	Saponins %
Vigna	Pre-anthesis	$2.69^c \pm 0.047$	$11.65^d \pm 0.06$	$0.50^b \pm 0.73$
	Anthesis	$1.24^e \pm 0.047$	$12.04^c \pm 0.06$	$0.55^b \pm 0.73$
	Post-anthesis	$0.56^f \pm 0.047$	$4.52^e \pm 0.06$	$1.22^b \pm 0.73$
Lablab	Pre-anthesis	$3.31^b \pm 0.047$	$15.41^a \pm 0.06$	$2.53^a \pm 0.73$
	Anthesis	$1.59^d \pm 0.047$	$11.88^{cd} \pm 0.06$	$0.97^b \pm 0.73$
	Post-anthesis	$5.14^a \pm 0.047$	$13.14^b \pm 0.06$	$1.16^b \pm 0.73$
Significance	F	0.001	0.021	0.001
	S	0.003	0.001	0.023
	F x S	0.033	0.022	0.041

[abcdef] Means in a column with different superscripts are significant at *P* <0.05

The legume forage species and stage of growth influenced CT, phenolic and saponin levels significantly. Lablab exhibited a higher (*P* <0.05) average tannin content compared with cowpea (3.345 ± 0.047 mg CAE/gDM vs. 1.494 ± 0.047 mg CAE/gDM). Lablab showed significantly higher average phenolic content (13.47 ± 0.0693 mg GAE/gDM) than cowpea (9.402 ± 0.0693 mg GAE/gDM). The tannin content of cowpea shows a general decline with stage of growth, while for Lablab there is a significant increase of 1.828 ± 0.0693 mg CAE/gDM from pre-anthesis to post anthesis. Both forages showed a general decline (*P* <0.05) in phenolic content with advancing stage of growth. Lablab exhibited the highest phenolic content in early stages of growth. The average saponin content was higher (*P* <0.05) for Lablab than for cowpea. Lablab recorded the highest (*P* <0.05) saponin content pre-anthesis, while cowpea had higher saponin levels post anthesis. There was a general increase with stage of growth for saponins in cowpea, yet Lablab showed a decline with advancing stage of growth (*P* <0.05).

Chemical compositions of the three diets given to growing goats over a period of sixty days and the dietary inclusions are listed in Table 3. The three diets were formulated to provide the same amount of protein (iso-nitrogenous) and energy (iso-energetic). The control was a pellet diet, which had a CP level of 14% and ME of 8.87 Mj/kg. The CP level was above the minimum requirement, as indicated by NRC (2007) of 12.6%. The acid detergent lignin (ADL), acid detergent insoluble nitrogen (ADIN), and neutral detergent insoluble nitrogen (NDIN) compositions of treatment diets were also evaluated and the results are shown in Figure 1. Cowpea exhibited significantly higher ADL and NDIN contents compared with Lablab, which showed a significantly higher ADIN content. Enteric CH_4 production figures are shown in Table 4. Treatment diets had a significant effect on enteric CH_4 when animals were ruminating (*P* <0.05), but had no effect (*P* >0.05) when animals were standing or feeding. The control diet exhibited higher (*P* <0.05) CH_4 emissions when animals were standing (18.63 ± 1.38 ppm-m) and ruminating (48.10 ± 2.055 ppm-m), while T_1 showed the lowest CH_4 emissions when animals were standing (15.92 ± 1.38 ppm-m) and highest emissions when ruminating (46.86 ± 3.760ppm-m).

Table 3 Chemical composition (% for DM and % DM for others) of Katambora hay supplemented with either Lablab or Cowpea and ewe pellets

Nutrient	Diet 1	Diet 2	Diet 3
	Katambora/Vigna (1:4)	Pellets	Katambora/Lablab (1:4)
DM%	92.3	89.92	92.72
CP%	13.93	13.49	13.77
CF%	-	23.51	-
Fat%	2.17	5.92	1.97
Me Mj/kg	8.60	8.87	8.90
NE Mcal/kg	-	0.78	-
NDF%	35.41	32.39	37.51

DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; CF: crude fibre; Me: metabolizable energy; NE: net energy.
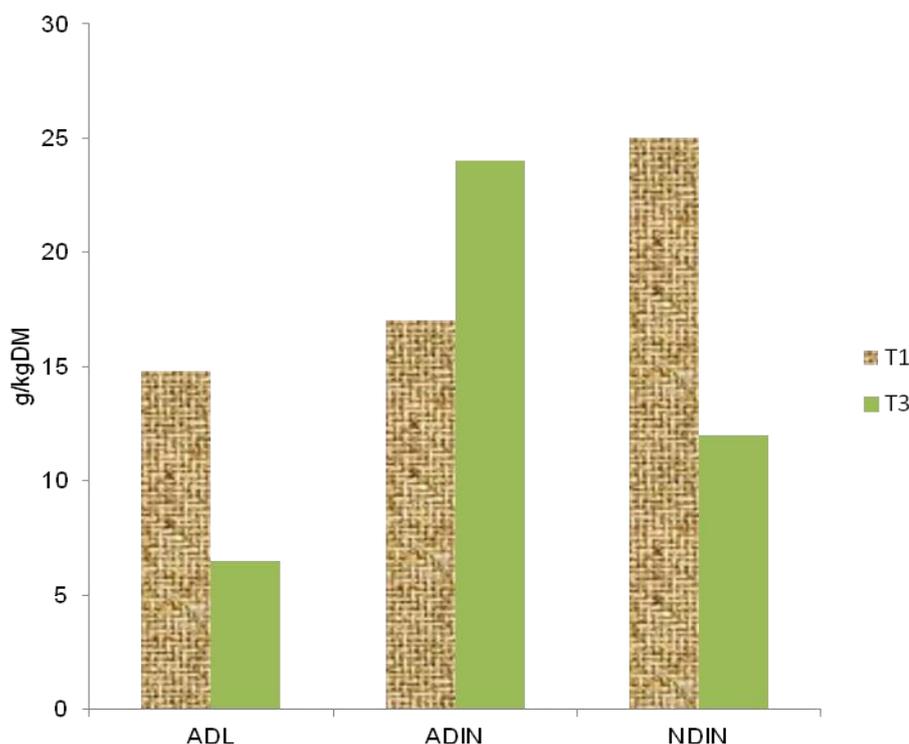
Key: ADL= acid detergent lignin; ADIN= acid detergent insoluble nitrogen; NDIN neutral detergent insoluble nitrogen

Figure 1 Chemical composition of acid detergent lignin, acid detergent insoluble nitrogen and neutral detergent insoluble nitrogen for Katambora hay supplemented with either Lablab or Cowpea and ewe pellets.

Treatment three (T_3) had significantly lower emissions when animals were ruminating (35.28 ± 3.64 ppm-m) compared with T_1 (46.86 ppm-m) and T_2 (48.10 ± 2.97 ppm-m). On average, animals in the control diet exhibited higher ($P < 0.05$) CH_4 emissions than in the other treatment diets: 35.62 ± 0.0032 ppm-m, 34.89 ± 0.0032 ppm-m, and 31.04 ± 0.0032 ppm-m for T_2, T_1, and T_3, respectively. The sex of animal and number of days (time in weeks) significantly affected ($P < 0.05$) enteric CH_4 emissions. Male animals (48.16 ± 1.218 ppm-m) produced higher methane emission than female animals (33.57 ± 1.218 ppm-m). There was a wide

significant difference in CH_4 emissions between male and female animals in the control diet of 36.46 ± 2.66ppm-m. This variation was significantly higher than other treatments, namely 17.5 ± 2.66ppm-m and 0.48 ± 2.66ppm-m for T_1 and T_3, respectively

**Table 4** Least square means (ppm–m) of enteric methane emission from goats

Activity	Treatment (T)			Sex (S)		P values		
	T_1	T_2	T_3	M	F	T	S	T x S
Standing	15.92 ± 1.38	18.63 ± 1.38	17.26 ± 1.38	17.90 ± 1.21	16.64 ± 1.21	0.47	0.001	0.300
Feeding	41.89 ± 2.66	40.13 ± 2.66	40.59 ± 2.66	48.16[a] ± 1.21	33.57[b] ± 1.21	0.903	0.001	0.14
Ruminating	46.86[ab] ± 3.64	48.10[a] ± 3.64	35.28[b] ± 3.64	46.48 ± 1.21	40.34 ± 1.21	0.028	0.148	0.001

[ab] Row means with different superscript within treatments and sex, differ significantly at *P* <0.05
T_1: 71% Vigna hay, 19% Katambora hay, salt (0.5%), molasses (3%), maize (5%) and mineral vitamin premix (1.5%); T_2 =90% lamb and ewe pellet plus 10% Katambora grass hay
T_3: 72% Lablab hay 19% Katambora hay, salt (0.5%), molasses (2%) maize (5%) and mineral vitamin premix (1.5%.

Methane was also measured over time. The results are shown in Figure 2.
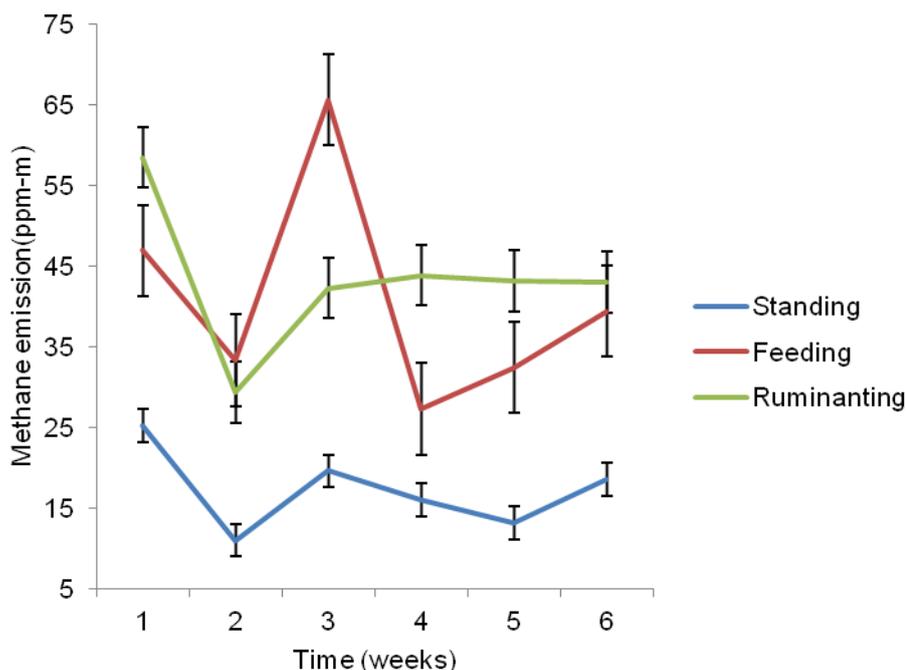
Figure 2 Methane emission of goats fed Lablab, Cowpea and ewe pellets

Methane production was not consistent (*P* <0.05) with time, although there was a general decline for the measured activities: 6.66 ± 1.38 ppm-m, 7.52 ± 2.97 ppm-m, and 15.42 ± 3.64 ppm-m for standing, feeding, and ruminating, respectively, from week 1 to week 6. Animals produced more CH_4 with time during ruminating (43.39 ± 3.769 ppm-m) than feeding (40.86 ± 5.673 ppm-m) and standing (17.27 ± 2.055 ppm-m). Generally, male animals emitted more CH_4 than females, except for T_1, in which females emitted more than males by 17.5 ± 3.211ppm-m. Since LMD measures CH_4 only in ppm-m, which are not equivalent to grams per kg or per day, CH_4 can also be determined on a DMI basis to give an indication as to how much CH_4 is produced by animals in friendly measurements (Table 5). These results show CH_4 measurements within the last six days of the trial measured consecutively. Dry matter intake was positively correlated with CH_4

production. Animals that consumed more feed produced significantly more CH_4, as shown in Table 5. Treatment diets and sex affected methane production significantly. T_2 (control) had higher (P <0.05) CH_4 emissions than T_1 and T_3. For all treatments animals produced significantly more gas when ruminating than feeding or just standing. T_1 exhibited significantly more gas than T_2 and T_3, namely 0.206 ± 0.006 g/day, 0.2007 ± 0.006 g/day, and 0.1685 ± 0.006 g/day for T_1, T_2, and T_3, respectively (P <0.05). On average, 4.602 ± 0.400 kg CH_4, 4.767 kg ± 0.400 CH_4 and 4.719k ± 0.400g CH_4 is produced each year for T_1, T_2, and T_3, respectively. The control exhibited higher (P <0.05) CH_4 emissions per kg of DMI. Sex significantly influenced the amount of gas produced, with male animals (17.400 ± 008 L/day; 12.46 ± 0.008 g/kg DMI; 0.126 ± 0.008 g/day) producing more (P <0.05) gas than females (15.47L ± 0.002 L/day; 12.28 ± 0.002 g/kg DMI; 0.0109 ± 0.002 g/day). Sex and treatment diets both contributed significantly to CH_4 emissions individually and interactively (P <0.05).

Table 5 Least square means of methane emissions (Litres/day); (grams /kgDMI) and (grams /day)

Activity	Treatment (T)			Sex (S)		P values		
	T_1	T_2	T_3	M	F	T	S	T x S
DMI (g)	670.8[c] ± 4.61	694.8[a] ± 4.61	688.1[b] ± 4.61	716.1[a] ± 3.76	653[b].03 ± 3.76	0.001	0.001	0.0266
[1]Methane (l/day)	16.01[c] ± 0.006	16.75[a] ± 0.006	16.54[b] ± 0.006	17.40[a] ± 0.008	15.47[b] ± 0.02	0.001	0.001	0.0001
[2]Methane (g/kg DMI)	12.61[c] ± 0.40	13.06[a] ± 0.40	12.93[b] ± 0.04	12.46[a] ± 0.008	12.28[b] ± 0.02	0.001	0.001	0.0001
[3]Methane (g/day)								
Standing	0.004[a] ± 0.48	0.0037[b] ± 0.48	0.0035[ab] ± 0.48	0.595 ± 0.28	0.62 ± 0.28	0.031	0.720	0.1640
Feeding	0.082[a] ± 0.005	0.066[a] ± 0.005	0.065[a] ± 0.005	0083[a] ± 0.06	0.059[b] ± 0.06	0.051	0.002	0.0001
Ruminating	0.120[a] ± 0.008	0.131[b] ± 0.008	0.100[a] ± 0.008	0.126 ± 0.006	0.0109 ± 0.006	0.007	0.089	0.0001

[abc] Row means with different superscript letters within treatments and sex differ significantly at P <0.05
T_1: 71% Vigna hay, 19% Katambora hay, salt (0.5%), molasses (3%), maize (5%) and mineral-vitamin premix (1.5%); T_2: 90% lamb and ewe pellet plus 10% Katambora grass hay; T_3: 72% Lablab hay 19% Katambora hay, salt (0.5%), molasses (2%) maize (5%) and mineral-vitamin premix (1.5%)
[1]Methane calculated according to Shibata *et al.* (1992)
[2]Methane calculated according to Howden & Reyenga (1987)
[3]Methane calculated according to Chagunda *et al.* (2009)

Discussion

The voluntary intake of feed and the extent to which the quantity of DM consumed supplies energy, proteins, minerals and vitamins to the animal determines its nutritive value. The DM contents of cowpea and Lablab were similar, and the current results were similar to those obtained by Ayan *et al.* (2012) and Ayana *et al.* (2013). Forage legumes generally have a CP range of 14–20%, as indicated by Norton & Poppy (1995), Kalamani & Gomez (2001), and Mahala *et al.* (2012). The results from this study confirm earlier observations for Lablab and cowpea. However, cowpea had higher ash, CP, and ADF content than Lablab. This was expected because cowpea exhibited indeterminate (continuous growth after florescence) nature of growth. On the other hand, Lablab had higher fat and NDF content and these are known to reduce feed intake. However, a high NDF content is indicative of higher forage degradability (Mahala *et al.*, 2012; Waters *et al.*, 2013). The high ADF values for cowpea indicate that it is not easily digestible and or degradable. This is because ADF is composed principally of cellulose and lignin, which are not easily degradable. A report by Meale *et al.* (2012) showed that the higher the fibre content of a diet, the lower the DMI and fermentability, and the higher the residence time, and gas production.

The results show a general increase in NDF and ADF with advancing stage of maturity. Jingura *et al.* (2001) and Mahala *et al.* (2012) also confirmed these findings. Legume forages accumulate carbohydrates as the plant matures. This is in line with what was concluded earlier by Mupangwa (2000). The effects of this silication and lignification translate into low degradability values within the rumen. This can be preferred if the animal has enough rumen degradable protein in the diet. The NDF content of legume forages has been the major drawback to their full utilization in animal nutrition. Legumes lack enough fibre to optimize rumen functionality, hence the requirement that they should be used as supplements to grass forages (Jingura *et*

al., 2001; Tibayungwa *et al.*, 2010). The low values of ADF recorded with Lablab makes it a legume of choice since high ADF values limit rumen microbial protein synthesis (Ayan *et al.*, 2012). Although tropical browse species and forage legumes are used as animal feed, they contain substantial amounts of anti-nutrients, including phenolic compounds, tannins, saponins and other secondary compounds (Makkar, 2003). Phenols are the largest category of phytochemicals and are widely distributed in the plant kingdom. These are generally divided into three important groups: flavonoids, phenolic acids, and polyphenols. They show a diversity of biological activities (Silva *et al.*, 2007), thereby influencing digestibility of forages. The results from the current study indicated that total phenols fall within a range that does not influence digestibility (Makkar, 2003; Abarghuei *et al.*, 2014).

There are two forms of tannins in the plant kingdom: hydrolysable tannins (HTs) and condensed tannins (CTs). The existence of tannin in plants has the potential to reduce the nutritional value of forages. Tannins, in particular, are known to bind feed proteins, making them unavailable for rumen microbial degradation (Mueller-Harvey, 2005), thereby limiting rumen microbial protein synthesis, which is important in ruminant digestive physiology. A report by Mueller-Harvey (2005) indicated that HTs are generally harmful, in comparison with CTs, which are considered safe if they account for less than 5% of DM in the feed. Min *et al.* (2003) also reported that forages containing more than 50 g CT/kg DM have limited palatability, voluntary intake, digestibility and N retention. However, Goel & Makkar, (2012) reported that low dietary tannin levels improve nitrogen utilization by ruminants. This is possible because tannins have the capability to alter the site of protein digestion from the rumen to the intestines, hence improving amino acid absorption. In the language of ruminant digestive physiology, this is referred to as rumen escape protein and is purported to lead to higher growth rates, milk yield, and fertility (Mueller-Harvey, 2005). Tannins have also been shown to reduce enteric CH_4 production in ruminants (Makkar 2003; Tavendale *et al.*, 2005; Carulla *et al.*, 2005 Hess *et al.*, 2011), promote high digestibility of nutrients (Yisehak *et al.*, 2014), and decrease the number of cellulolytic bacteria (McSweeny *et al.*, 2001). In addition, they are resistant to enzymatic digestion (Goel & Makkar, 2012), shift short-chain fatty acid (SCFA) production, and reduce DMD and OMD (Hess *et al.*, 2006; Abdalla *et al.*, 2007; Animut *et al.*, 2008; Tiemann *et al.,* 2008). In the current study, Lablab showed higher CTs levels than cowpea. However, these results still fall below 50 gCT/kgDM, as reported to have negative effects on ruminants (Makkar, 2003). Thus, tannin levels influenced DM and OMD in the current study.

Saponins are natural detergents, high molecular weight glycosides in which sugars are linked to a triterpene or steroidal aglycone moiety (Goel & Makkar, 2012). Plants rich in saponins enhance the flow of microbial protein from the rumen, as reported by Das *et al.* (2012), increase the efficiency of feed utilization (Jayanegara *et al.*, 2012), decrease protozoal populations (Das *et al.,* 2012), and consequently reduce CH_4 production (Goel & Makkar, 2012). Reduction in CH_4 production is achieved by increasing propionate production and decreasing protozoal numbers (Hess *et al.*, 2006). Higher DM and OM digestibility co-efficiencies have been reported in sheep supplemented with a saponin content of between 2% and 4 % (Das *et al.*, 2012; Jayanegara *et al.*, 2012; Lu & Jorgensen, 1987). However, Azami *et al.* (2013) reported no changes in OM or DM digestibility at the same level of saponin inclusions. In the present study, the saponin content differs with the stage of growth and generally fall below the 2% minimum level expected to influence digestibility. Tannins and saponins have been reported to lower digestibility of nutrients. However, the level at which they achieve this is still unknown (Norrapoke *et al.*, 2012; Wanapat *et al.*, 2015). Coulman *et al.* (2000) suggested that forages have lower fibre content, higher DMI, and faster rate of passage through the rumen; hence, they have the capacity to reduce enteric CH_4 production. This was confirmed by Beauchemin *et al.* (2009) and Archime`de *et al.* (2011).

The contribution of ruminant livestock production systems to global anthropogenic CH_4 emissions has long been hypothesized. According to Steinfeld (2006), 50% or more of GHG emissions come from enteric fermentation. Nevertheless, Scholtz *et al.* (2013) dispute this and argue that only 5% of total global CH_4 is enteric. This enteric production is a loss in dietary energy, as has been extensively researched and reviewed by Eckard *et al.* (2010), Morgavi *et al.* (2010), and Cottle *et al.* (2011). Methane production in the tropics exhibits great variations and is governed by such animal factors as weight, age, species, and breed, together with feed characteristics (Brouček, 2014). According to Ramin & Huhtanen (2013), Lovett *et al.* (2005), and Du Toit *et al.* (2013), DMI is one of the main determinants of total CH_4 production. These authors also observed that gross energy intake is negatively related to CH_4 production, and positively correlated with diet digestibility. On the other hand, Scholtz *et al.* (2013) concluded that the production system is the major determinant factor in the total amount of CH_4 produced. They cited that animals on rangelands would produce more CH_4 gas than those on intensive systems, particularly feedlots. Results from this study confirmed that DMI has a significant bearing on CH_4 emissions. As animals consumed more, they produced significantly more gas compared with their contemporaries. In the current study, growing goats produced between 12 to 13 g/kg DM of CH_4. This is considered lower when compared with results obtained by Du Toit *et al.* (2013). This was the effect of forage legumes on CH_4 production. Woodward *et al.* (2004) indicated that

forage legumes can be used to influence rumen characteristics significantly to achieve reduced CH_4 emissions. According to Waghorn & Clark (2004) and Peters *et al.* (2013), tropical forage species have the capability of reducing ruminant CH_4 emissions per unit livestock product compared with lower-quality rangeland species. This is because they contain less structural carbohydrates and more CTs than grass. Results from this study also showed that goats produce between 16 and 17 litres CH_4 per day. These results are similar to those proposed by Du Toit *et al.* (2013) for animals in the Eastern Cape of South Africa.

From a practical point of view, animal enteric CH_4 production is relative to activities by the animal. Results show that ruminating animals produce more gas than feeding or idle animals. This was confirmed earlier by Chagunda *et al.* (2009). Although the animal appears quiet and relaxed during rumination, the activities and process in its digestive system dominate enteric CH_4 production compared with any other activities that a ruminant animal would perform per its daytime budget. This was reported earlier by Marik & Levin (1996). The ruminant digestive tract is a reservoir of microorganisms that are beneficial to the host animal. Among these microorganisms, found is a group called methanogens. These methanogens produce CH_4 by a process called methanogenesis or biomethanation as a by-product of anaerobic fermentation. This process represents an inefficient utilization of feed (Chagunda *et al.*, 2009). The process of methanogenesis is a two-stage process. First, glucose equivalents are hydrolysed to yield pyruvate. This hydrolysis is achieved by extracellular microbial enzymes in the presence of protozoa and fungi. Also, pyruvate undergoes oxidation reactions under anaerobic conditions to produce reduced co-factors like nicotinamide adenine dinucleotide hydrogen (NADH). This reduced co-factor, for example, is then re-oxidized to nicotinamide adenine dinucleotide (NAD) to complete the synthesis of VFAs. In essence, these two processes are crucial to VFA syntheses and therefore are inevitable for ruminants. Ruminants use VFAs as a source of energy since all the dietary glucose is quickly used up by symbiotic microorganisms for their own microbial protein synthesis. The production of VFAs from pyruvate releases free hydrogen (H_2), which, if left unchecked, results in lower pH, which might culminate in metabolic conditions such as acidosis or rumen stasis. The ruminant system has a way of dealing with excess H_2. This is where methanogens become handy to achieve homeostasis. Methanogens eliminate the available H_2 by using CO_2 to produce CH_4 (Kebreab *et al.*, 2006a).

Bell & Eckard (2012), however, observed that elimination of enteric CH_4 has consequences as it could result in a reduction in rumen fermentation rate or in a shift in VFA production. They also observed that there is an inverse relationship between CH_4 production and the presence of propionate (Bell & Eckard, 2012). In its simplest form, propionate is deemed a hydrogen sink. This means it reduces the amount of H_2 available for methanogens, hence reduces the amount of CH_4 produced. The three main VFAs are generally produced in relation to each other at a ratio of 70: 20: 10 for acetate: propionate: butyrate. However, these proportions can be manipulated by dietary intervention to reduce the ratio of acetate to propionate in particular to less than 0.5 (Bell & Eckard, 2012). Any ratios above this margin result in excess H_2, which becomes available to form CH_4 (a function of methanogens). However, if the H_2 produced is not used correctly, ethanol or lactate can form. These inhibit microbial growth, forage digestion, and any further production of VFAs. Enteric CH_4 production is reduced if the flow of H_2 shifts towards alternative electron acceptors. Unfortunately, many of the alternative acceptors are less thermodynamically favourable; therefore, CO_2 becomes reduced to CH_4. From a dietary perspective, the CH_4 reduction is achieved by diluting starch (a major source of glucose) with a non-forage carbohydrate source that is less rapidly fermented, produces more propionate, without reducing ruminal pH. Forage legumes are known to possess such characteristics hence have been proposed to reduce CH_4 production (Coulman *et al.*, 2000; Peters *et al.*, 2013). However, Grainger & Beauchemin (2011) suggested that forage-based diets generally result in higher CH_4 emissions than mixed or concentrate-based diets. This was also alluded to by Scholtz *et al.* (2013). This is contrary to results from this study, which have shown that legume forage-based diets reduce CH_4 production significantly compared with a pellet-based diet. The reason is that in this study CH_4 was not measured per kilogram of weight gain of goats. Sanchis (2015) observed CH_4 emissions ranging between 19.6 to 29.7 g/day with Murciano-Granadina goats. The result from this study shows an even lower range of CH_4 emissions. This confirms the earlier proposals by Moss *et al.* (2000), Steinfeld *et al.* (2006) and Storm *et al.* (2012) that dietary manipulations subdue methanogenesis.

Conclusion

Legume forages (*Vigna unguiculata* and *Lablab purpureus*) reduced enteric methane production significantly from goats. This was achieved by the level of tannins in these forages and lower fibre content. Methane production is relative to animal activity with ruminating animals producing more gas compared with feeding or standing. The sex of the animal also affected methane production significantly, with male animals producing more gas than females. Enteric methane production is positively related to DMI.

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Authors' Contributions
SW worked on the original report and data analysis, JM conceptualised the paper and VM worked on the study design execution and manuscript preparation.

Conflict of interest Declaration
There is no conflict of interest associated with this manuscript.

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