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Unlocking the Potential of Indian Pasture Legumes: Nutrition, Mineral, Gas and Methane Production Insights for Use in Semiarid Regions

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Article

Unlocking the Potential of Indian Pasture Legumes: Nutrition, Mineral, Gas and Methane Production Insights for Use in Semiarid Regions

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Abstract: This study evaluated five annual and eleven perennial Indian pasture legumes species for their nutritive value, dry matter, mineral contents and *in vitro* fermentation parameters, aiming to boost their potential as animal feed. Legume species significantly differed ($p<0.05$) in organic matter, crud protein (CP), ether extracts, fibres, and protein fractions. *Clitoria ternatea* (CT) a perennial had higher ($p<0.05$) buffer soluble protein (477), while neutral detergent soluble protein was highest in annually grown *Lablab purpureus* (420 g/kg CP). *Atylosia scarabaeoides* (*AS*) had higher NSC (392 g/kg DM) than SC (367 g/kg DM). Rapidly degradable fraction (51.7 g/kg tCHO) was lower ($p<0.05$) than other fractions of carbohydrate. Total digestible nutrients, digestible energy and metabolisable energy varied and values were higher for *Desmenthus virgatus* and lowest for *Stylosanthas seabrana* (*SSe*). The NE values for lactation (NE_L), maintenance (NE_M) and gain (NE_G) were also higher for *DV* (6.6, 7.95 and 4.28) and lowest for *SSe* (3.54, 4.24 and 0.58 kJ/g DM), respectively. dry matter intake, digestible DM (DDM) and relative feed value of legumes differed ($p<0.05$) with mean values of 2.22% body weight, 592 g/kg DM and 102%, respectively. Annual grasses like *Dolichos biflorus, Macroptilium atropurpureum, Rhynchosia minima* (*RM*) were found better with micro minerals compared to other perennials. *In vitro* dry matter degradability, partition factor, short chain fatty acids and microbial protein production of legumes varied ($P<0.05$) and mean values were 578 g/kg DM, 5.61 mg DDM/mL, 2.42 mm/g and 352 mg/g, respectively. Gas and CH_4 production (mL/g and mL/g DDM) varied ($p<0.05$) being lowest gas from *AS* (55.5) and highest from *CT-blue* (141), while CH_4 production was lower ($p<0.05$) from *CT-white* and *AS* (8.24 and 9.14) and higher from *Arachis glabrata* (*AG*) and *Arachis hagenbackii* (*AH*) (15.2 and 15.1). Methane in total gas was low for *DV*, *RM* and *CT-w* (8.99, 9.72 and 9.51%) and loss of DE and ME as CH_4 varied ($P<0.05$) amongst the legumes with mean values of 4.62 and 7.86%, respectively. Based on these findings, each legume offers unique benefits, allowing for tailored combinations of annual and perennial legumes to optimize rumen feed efficiency.

Keywords: animal feed; annual legumes; perennial legumes; nutritional value; minerals; *in vitro* fermentation; methane production

1. Introduction

Inadequate availability feed and forage coupled with their low nutritive value (crop residues, grasses of pasture and grazing lands) are the prime factors for poor animal production in tropics and subtropics. For ages forage legumes had played an important role in dairy and meat production [1]. Forage legumes species usually had high feeding value as they are generally rich in protein, provides substantial amount of energy, mineral and vitamins with higher intake and digestibility [2, 3]. Even

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in grain-based feed lot livestock production, forage legumes are essential to maintain animal health [4]. In recent many researchers have reviewed the role of forage legumes, for sustenance of mixed crop-livestock production, sustenance of pasture and grazing lands along with ecosystem services [5-7]. Dietary supplementation of forage legumes not only increases livestock production primarily through higher intake, nutrient contents, digestibility than cereal crops-grasses, sorghum, maize etc. [8, 9] but also improves the rumen fermentation efficiency through enhanced metabolisable energy, protein ratio and ruminal bypass protein availability to animals [10], increased N retention [11] and reduced methane emission [8, 12, 13]. Further the introduction of forage legumes in grass production areas as grass-legume mixture could be one of the promising strategies to mitigate the GHG emissions from pasture-based livestock production [7].

As per Food and Agriculture Organization of the United Nations (FAO; www.feedipedia.org) lists around 169 legume species are being used as forage and about 20 Mha land area in under forage legume monoculture [14]. Species of *Aeschynomene*, *Arachis*, *Centrosema*, *Desmodium*, *Macroptilium*, and *Stylosanthes* offer promise for improved tropical pasture systems [15]. Alike the wider morphological genetic variability in legume species [16] there exists nutritional variability [17]. In addition to genetic differences the nutritional composition of legumes also influenced by the season and growing location. So, to have correct information on legumes level of supplementation and nutritive value, their evaluation for chemical composition has a substantial impact on the understanding of their nutritional value and also a great influence on animal nutrition [8, 18]. The chemical composition (CP, NDF, ADF and lignin) and total digestible nutrients TDN, in vitro digestibility has limited application for formulating precise diet specific to animal species and their physiological stages [19, 20]. To have comprehensive information on feeding value of each legume, the present study was planned to evaluate 16 annual and perennial different legume species from nine genera for protein, cell wall constituents, carbohydrate fractions, protein fractions, energy contents, minerals, intake, digestibility and *in vitro* fermentation pattern (gas and methane production) for their judicious use in ruminant diets.

2. Materials and methods

2.1. Experimental Sites

The study was carried out at Plant Animal relationship Division, ICAR-Indian Grassland and Fodder Research institute, Jhansi (India). Laboratory procedures and animal management for donor sheep were carried out as per Institute animal ethics committee guidelines.

2.2. Sample collection and processing of forage legumes

Annual species: Dolichos biflorus (DB), Lablab purpures (LLP), Macroptilium atropurpureum (MA), Rhynchosia minima (RM), Stylosanthas hamata (SH); Perennial species: Arachis glabrata (AG), Arachis hagenbackii (AH), Atylosia scarabaeoides (AS), Clitoria ternatea-white (CT-W), Clitoria ternatea-blue (CT-B), Centrosoma pubescene (CPb), Desmenthus virgatus (DV), Stylosanthas scabra (SSc), Stylosanthas scofield (SSco), Stylosanthas seabrana (SSe) and Stylosanthus viscosa (SV) were collected randomly from plots (30×10 m) maintained by Grassland and Silvipasture Management Division of Institute. The legumes samples were harvested after monsoon (rainy season) growth in first week of September 2016. Collected samples were dried initially under shade on cemented floor and then in hot air oven at 60°C for 2-3 consecutive days. The DM contents were 36.03, 29.26, 22.21, 17.98, 16.62, 31.14, 28.48, 32.23, 29.13, 35.57, 20.40, 30.38, 39.04, 29.77, 15.83 and 17.71% for AG, AH, CP, DB, CT-w, SSe, SH, SSc, SSco, MA, AS, DV, RM, CT-b and LLP, respectively.

2.3. Chemical analyses

Samples dry matter (DM), crude protein (CP), ether extract (EE) and ash were estimated as per methods of AOAC (1995). CP of samples was estimated as Kjeldahl N × 6.25 by digesting in sulfuric acid and digestion mixture (Consisting of sodium/ potassium sulphate and copper sulphate in 10:1 ratio) using semi auto analyser (Kel Plus Classic-DX, Pelican). The EE was determined by refluxing

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samples in petroleum ether using extraction apparatus. For ash estimation samples were put in tarred silica basins, desmoked and then basins were put into a muffle furnace at 600˚C temperature for 4h. Neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose and lignin (ADL) were estimated by sequential procedure modified by Van Soest *et al*. [21] using fiber tech (Fibra Plus FES 6, Pelican). Heat labile alpha amylase and sodium sulphite were not used in NDF solution. For lignin (ADL) estimation sample left after ADF estimation were treated with 72% H_2SO_4 followed by ashing in muffle furnace.

2.4. Carbohydrate and protein fractionation

Carbohydrate fractions were estimated according to the CNCPS [22]. This is broadly classified into 4 fractions as follows:

a) C_A: rapidly degradable CHO including sugars.
b) C_{B1}: intermediately degradable starch and pectin.
c) C_{B2}: slowly degradable cell wall.
d) C_C: unavailable/lignin bound cell wall.

Total carbohydrate (tCHO g/kg DM) was determined by subtracting CP, EE and ash contents from 1000. Structural carbohydrates (SC) were calculated as the difference between NDF and neutral detergent insoluble protein and non fiber carbohydrates were estimated as the difference between total CHO and SC [23]. Starch estimated by samples extraction with 80% ethyl alcohol to solubilize free sugars, lipids, pigments and waxes. Residue rich in starch was solubilized with perchloric acid and extract was treated with anthrone-sulphuric acid to determine glucose colorimetrically using standard glucose [24].

Protein fractions of samples were partitioned into five fractions according to Licitra et al. [25]. These are as follows:

P_A: non-protein nitrogen (NPN), the difference between total nitrogen and true CP nitrogen precipitated with sodium tungstate (0.30 *M*) and 0.5 *M* sulfuric acid.

P_{B1}: buffer soluble protein, the difference between true protein and buffer-insoluble protein, estimated with borate-phosphate buffer (pH 6.7 to 6.8) and freshly prepared 10% sodium azide solution.

P_{B2}: neutral detergent soluble protein (NDSP), buffer-insoluble protein minus ND insoluble protein.

P_{B3}: acid detergent soluble CP, the difference between ND insoluble protein and acid detergent insoluble CP.

P_C: indigestible.

All fractions, including CP, were analysed in triplicate and N content determined by Kjeldahl. Crude protein was determined as Kjeldahl N × 6.25 using semi auto analyzer (Kel Plus Classic-DX Pelican India).

2.5. Dry matter intake, digestibility and energy calculations

Legumes dry matter intake (DMI), digestible DM (DDM), relative feed value (RFV), total digestible nutrients (TDN) and net energy for different animal functions i.e. lactation (NEl), gain (NEg) and maintenance (NEm), were calculated using the following equations (DMI = 120/NDF; DDM =88.9-0.779×ADF; RFV =(DDM×DMI)×0.775; TDN = 104.97–(1.302×ADF); NEl =(TDN×0.0245)-0.012; NEg=TDN×0.029)-1.01; NEM = (TDN×0.029)-0.29) of Undersander *et al.* [26]. Digestible energy (DE, KJ/g DM; DE= TDN×0.04409) and metabolizable energy (ME, KJ/g DM) values were calculated using the equations of Fonnesbeck *et al.* [27] and Khalil *et al.* [28], respectively. Metabolizable energy was calculated as 0.821 × DE.

2.6. Estimation of minerals

For minerals estimation legumes samples were wet digested with 3:1 HNO_3: perchloric acid mixture, cooled and filtered through Whatman 42 filter paper. The aliquot was used for estimation

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of calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn) using an atomic absorption spectrophotometer (Varian AA 240) against their standards.

2.7. Donor animals and inoculum preparation

Four male adults of *Jalauni* sheep with mean body weight of 36.2± 0.217 kg were used as inoculums donors. These animals were maintained on sole berseen hay diet and had free access to clean drinking water. Rumen liquor was collected in a pre-warmed thermos from each animal before feeding using a perforated tube from stomach with the help of vacuum pressure pump. Collected rumen liquor was filtered through four layers of muslin cloth and thus strained rumen liquor obtained from different animals was mixed, kept at 39°C in water bath, gassed with CO_2 till used for mixing with incubating buffer media.

2.8. In vitro incubations

In vitro gas production was determined according to the pressure transducer technique of Theodorou *et al.* [29]. Ruminal fluid was collected in pre warmed thermos using a stomach tube before feeding from adult male *Jalauni* sheep maintained on berseem hay diet. Collected rumen fluid was filtered through a double layer of cheese cloth and bubbled with CO_2. The incubation medium (1L) was prepared by sequential mixing of buffer solution, macro-mineral solution, micro-mineral solution and resazurin solution (Menke and Steingass 1988). Incubation medium was fluxed with CO_2 till the pink colour turned colourless and then 250 mL rumen liquor was added to attain incubation medium: rumen liquor ratio of 80:20. Samples (0.5g) of air dry foliages were weighed into three 100 mL serum bottles. Three serum bottles without substrate/sample were used as blanks. Sample and control serum bottles were initially gassed briefly with CO_2 before adding 50 mL of inocuolum medium. Bottles were continuously fluxed with CO_2 and then sealed with aluminium crimps. Before incubation, the gas pressure transducer was used to adjust head space gas pressure in each bottle to adjust the zero reading on the LED display and then incubated at 39°C for 24 h to estimate the total gas production.

2.9. Methane measurements

Methane in total gas measured at 24h from three bottles incubated for each of the tree leaves was analysed by gas chromatography (Nucon 5765 Microprocessor controlled gas chromatograph, Okhla, New Delhi, India) equipped with a stainless-steel column packed with Porapak-Q and a Flame Ionization Detector. One ml gas sampled using a Hamilton syringe from total gas produced was injected manually (pull and push method of sample injection) into the GC, which was calibrated with standard CH_4 and CO_2. Methane was also measured from blank bottles incubated for 24h and used for correction of CH_4 produced from the inoculum. Methane measured was related to total gas to estimate its concentration [30] and converted to energy and mass values using 39.54 kJ/l CH_4 and 0.716 mg/mL CH_4 factors, respectively [31]. Short chain fatty acids (SCFA) were calculated using 24h gas production [32], while the partition factor (PF) and microbial mass (MBM) were estimated as described in previously described method [33].

2.10. Statistical analysis

The means of nutritional and gas fermentation parameters of legumes were compared using one way ANOVA of SPPS (version 16) with legumes as fixed factor and parameters as dependent variables. Post hoc multi comparison was performed using Duncan Multiple Range test to differentiate the means at $p<0.05$ level.

3. Results

3.1. Chemical composition

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Legumes differed (p<0.05) in OM, CP, EE, NDF, ADF, cellulose and lignin contents and their mean values were 906, 125, 33.4, 549, 382, 281 and 93.9 g/kg DM, respectively (Table 1). The protein contents were > 150 g/kg DM in *CP, CT-b* and *LLP* and lower < 100 g/kg DM in *DB, SSe, SH, SSc* and *SSco* (87.9, 80.4, 93.5, 92.2 and 94.8 g/kg DM). The EE contends were higher in *CT-w, CT-b* and *DV* (48.3, 46.6 and 63.8 g/kg DM), while lignin contents were lower in *LLP* (62.7) and *AH* (68.1) against highest in *SSe* (134) and *CT-w* (124 g/kg DM). *Stylosanthes* species had higher ADF (401 to 508) and cellulose (297 to 365 g/kg DM) contents except (*DB*) than other evaluated legumes.

Table 1. Chemical composition of range legumes (g/kg DM).

Legumes	OM	CP	EE	NDF	ADF	Cellulose	Lignin	Hemi cellulose
AG	873^{b}	123^{f}	20.2^{ab}	479^{b}	381^{cd}	2725^{e}	101^{ef}	976^{a}
AH	878^{c}	116^{e}	23.4^{abc}	478^{b}	342^{b}	273^{e}	68.1^{ab}	136^{bcd}
CP	905^{g}	172^{i}	27.5^{cd}	549^{cde}	380^{c}	266^{c}	103^{ef}	169^{d}
DB	897^{f}	88^{b}	28.1^{cd}	730^{h}	414^{ef}	325^{e}	82.4^{bcd}	316^{g}
CT-w	919^{i}	146^{h}	48.3^{f}	566^{f}	396^{cde}	270^{c}	124^{gh}	171^{d}
SSe	930^{L}	80^{a}	26.6^{cd}	604^{g}	501^{g}	365^{g}	134^{i}	103^{ab}
SH	923^{j}	93^{c}	27.0^{cd}	624^{g}	403^{e}	297^{d}	99.2^{e}	221^{e}
SSc	915^{h}	92^{c}	43.2^{ef}	548^{cde}	423^{f}	345^{f}	72.9^{abc}	124^{abc}
SSco	886^{d}	95^{c}	18.5^{a}	529^{cd}	401de	307de	83.8^{bc}	129^{abc}
SV	927^{k}	105^{d}	24.6^{bcd}	545^{cde}	407^{ef}	320^{g}	80.7^{bc}	139^{bcd}
MA	855^{a}	141^{g}	24.9^{bcd}	482^{b}	382^{cd}	271^{e}	105^{ef}	99.0^{a}
AS	894^{e}	105^{d}	29.7^{d}	439^{a}	342^{b}	213^{b}	115^{fg}	98.2^{a}
DV	928^{kl}	143^{gh}	63.8^{g}	519^{c}	266^{a}	177^{a}	73.5^{abc}	253^{ef}
RM	934^{m}	143^{gh}	41.5^{e}	595^{fg}	337^{b}	229^{b}	1027^{ef}	258^{f}
CT-b	937^{n}	183^{j}	46.6^{f}	544^{cde}	396^{cde}	296^{d}	94.6^{de}	147^{cd}
LLP	896^{e}	180^{j}	40.3^{e}	556de	335^{b}	267^{c}	62.7^{a}	220^{e}
Mean	906	12.54	33.4	549	382	28.09	93.9	168
SEM	2.12	0.283	0.421	-	1.51	6.93	1.69	2.87
Significance	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

AG, Arachis glabrata; AH, Arachis hagenbackii; AS, Atylosia scarabaeoides; CPb, Centrosoma pubescene; CT-b; Clitoria ternatea(blue) CT-w, Clitoria ternatea(white); DV, Desmenths virgatus; DB, Dolichos biflorus; LLP, Lablab purpures; MA, Macroptilium atropurpureum; RM, Rhynchosia minima; SSe, Stylosanthas seabrana; SH, Stylosanthas hamata, SSc, Stylosanthas scabra, SSco, Stylosanthas scofield; SV, Stylosanthus viscosa.ADF, acid detergent fibre expressed inclusive residual ash; CP, crude protein; EE, ether extract; Lignin(sa), lignin solubilized with sulphuric acid; NDF, neutral detergent fibre expressed inclusive residual ash; OM, organic matter; SEM, standard error of means.

3.2. Protein and carbohydrate fractions

Contents of tCHO, SC and NSC varied (p<0.05) in legumes and their mean values were 747, 484 and 263 g/kg DM, respectively. Amongst the evaluated legumes *AS* is the legume which had higher NSC than SC (392 vs 367 g/kg DM Table 3). SC contents were highest in *DB* and lowest in AS (684 vs 367 g/kg DM). Carbohydrate fractions C_A, C_{B1}, C_{B2} and C_C differed (p<0.05) in legumes and ranged between 143-589, 33.5-84.7, 4.30-551 and 221-428 g/kg tCHO, respectively. Rapidly degradable carbohydrate fraction C_{B1} (51.7) was lower (p<0.05) than C_A (405), C_{B2} (242) and C_C (302 g/kg tCHO), respectively.

Legumes protein fractions P_A, P_{B1}, P_{B2}, P_{B3} and P_C differed (p<0.05 Table 2) and had mean values were 226, 295, 209, 167 and 102 g/kg CP, respectively. Legumes had highest (295) accumulation of rapidly degradable protein fraction (P_{B1}) against lowest (1023 g/kg CP) of lignin bound protein fraction (Pc). *Clitoria* species (*CT-w* and *CT-b*) and *MA* had higher P_{B1} fraction (437, 477 and 430) and lowest P_C fraction (52.9, 71.6 and 75.2 g/kg CP).

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Table 2. Carbohydrates (g/kg DM), its fractions (g/kg tCHO) and protein fractions (g/kg CP) of range legumes.

Legumes	Carbohydrate and its fractions							Protein fractions			
	tCHO	SC	NSC	C_A	C_{B1}	C_{B2}	C_C	P_A	P_{B1}	P_{B2}	P_{B3}	P_C
AG	730^{e}	424^{bc}	306^{ghi}	470^{f}	71^{g}	127^{bc}	315^{gh}	223de	394^{fg}	161^{cd}	116^{cd}	98.8^{d}
AH	739^{f}	430^{bcd}	309^{ghi}	439^{ef}	57^{e}	282^{gh}	221^{a}	228de	374^{ef}	224^{e}	92.8^{bc}	81.5^{c}
CP	705^{c}	457^{cde}	248^{def}	459^{ef}	48^{cd}	153^{cd}	349^{def}	214^{cd}	185^{b}	305^{g}	202^{ef}	93.7^{d}
DB	781^{i}	683^{h}	976^{a}	143^{a}	52^{d}	551^{j}	253^{a}	324^{h}	123^{a}	300^{g}	118^{cd}	134^{fg}
CT-w	725de	500^{f}	225^{cd}	429^{ef}	37^{a}	121^{bc}	428^{g}	211^{c}	437^{h}	91.5^{b}	208f	52.9^{a}
SSe	823^{L}	561^{g}	261^{ef}	358^{c}	43^{b}	208^{def}	391^{fg}	184^{b}	352^{e}	172^{d}	178^{e}	113^{e}
SH	802^{k}	589^{g}	213^{bc}	278^{b}	61^{e}	365^{i}	297^{bc}	277^{g}	232^{c}	346^{h}	60.8^{a}	83.7^{c}
SSc	780^{j}	483^{ef}	296^{gh}	406de	37^{a}	332^{hi}	224^{a}	236^{ef}	182^{b}	292^{g}	139^{d}	150^{h}
SSco	773^{i}	454^{cde}	319^{hi}	431^{ef}	58^{e}	251^{efg}	260^{ab}	238^{ef}	194^{b}	179^{d}	246^{g}	142^{gh}
SV	797^{k}	464de	333^{i}	414de	65^{f}	277^{fgh}	243^{a}	214^{cd}	265^{d}	138^{c}	254^{g}	129^{f}
MA	689^{b}	415^{b}	274^{fg}	478^{f}	85^{h}	70^{b}	367^{ef}	165^{a}	430^{h}	252^{f}	78.3^{ab}	75.2^{cd}
AS	759^{h}	367^{a}	392^{i}	589^{g}	43^{b}	4.3^{a}	364^{def}	250^{f}	205^{bc}	99.1^{b}	330^{h}	116^{e}
DV	721^{d}	458^{cde}	263^{ef}	410de	49^{cd}	296^{gh}	245^{a}	215^{cd}	281^{d}	310^{g}	110^{cd}	82.9^{c}
RM	749^{g}	516^{f}	233^{cd}	375^{cd}	45^{bc}	254^{efg}	326^{cde}	210^{c}	418^{gh}	25.2^{a}	245^{g}	1015^{d}
CT-b	708^{c}	453^{cde}	254^{def}	450^{ef}	42^{b}	187^{cde}	321^{cd}	205^{c}	477^{i}	33.8^{a}	213^{f}	71.6^{b}
LLP	676^{a}	484^{ef}	191^{b}	356^{b}	33^{a}	388^{i}	223^{a}	215^{cd}	177^{b}	420^{i}	76.8^{ab}	111^{e}
Mean	747	484	263	405	52	242	302	226	295	209	167	102
SEM	0.343	2.74	2.77	14.03	1.96	19.5	9.50	5.30	16.4	16.4	11.3	3.90
Significance	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

NSC, non-structural carbohydrates; P_A, non-protein nitrogen; P_{B1}, buffer soluble protein; P_{B2}, neutral detergent soluble protein; P_{B3}, acid detergent soluble protein; P_C, indigestible protein; SEM, standard error of means; SC, structural carbohydrates; tCHO, total carbohydrates.

3.3. Energy, energy efficiency, intake, digestibility and relative feed value of legumes

The TDN, DE and ME contents of legumes varied (p<0.05) and their values were higher for *DV* (703, 12.9 and 10.6) and lowest for *SSe* (398, 7.28 and 5.99 kJ/g DM Table 3). Similarly, the NE values of legumes for lactation (NE_L), maintenance (NE_M) and gain (NE_G) were higher for *DV* (6.66, 7.94 and 4.28) and lowest for *SSe* (3.53, 4.24 and 0.582 kJ/g DM), respectively. DMI, DDM and RFV of evaluated legumes differed (p<0.05) with mean values of 2.22 % body weight, 592 g/kg DM and 102%, respectively.

Table 3. Energy value of range legumes.

Legumes	TDN	DE	ME	NE_L	NE_M	NE_G	DMI	DDM	RFV
AG	553de	10.1^{e}	8.32^{d}	5.16^{f}	6.16^{e}	2.45^{de}	2.51^{g}	592de	114.94^{ef}
AH	604^{f}	11.1^{f}	9.11^{e}	5.66^{g}	6.74^{f}	3.08^{f}	2.51^{g}	623^{f}	121.26^{fg}
CP	555^{e}	10.2^{e}	8.36^{d}	5.16^{ef}	6.16^{e}	2.50^{e}	2.19^{def}	593^{e}	100.45^{cd}
DB	511^{ab}	9.36^{bc}	9.69^{bc}	4.70^{be}	5.62^{bc}	1.96^{bc}	1.64^{a}	567^{bc}	72.20^{a}
CT-w	534^{cde}	9.82^{cde}	8.03^{cd}	4.95^{cdef}	5.91^{cde}	2.25^{cde}	2.12^{cd}	581^{cde}	95.48^{c}
SSe	398^{a}	7.28^{a}	5.99^{a}	3.54^{a}	4.24^{a}	0.58^{a}	1.99^{bc}	499^{a}	76.80^{a}
SH	525^{c}	9.65^{cd}	7.90^{c}	4.87^{cde}	5.78^{c}	2.12^{c}	1.93^{b}	575^{c}	86.16^{b}
SSc	498^{b}	9.150^{b}	7.48^{b}	4.58^{b}	5.49^{b}	1.83^{b}	2.20^{def}	559^{b}	95.65^{c}
SSco	528^{cd}	9.69^{cde}	7.94^{cd}	4.87^{cde}	5.82^{cd}	2.16^{cd}	2.27^{ef}	577^{cd}	101.46^{cd}
SV	520^{ab}	9.52^{bc}	7.82^{bc}	4.78^{f}	5.74^{c}	2.08^{bc}	2.20^{def}	572^{bc}	97.68^{c}
MA	552de	10.1^{de}	8.32^{d}	5.12^{bc}	6.12^{de}	2.45^{de}	2.50^{g}	591de	114.36^{e}
AS	605^{f}	11.1^{f}	9.11^{e}	5.66^{g}	6.78^{f}	3.08^{f}	2.73^{h}	623^{f}	131.73^{h}
DV	703^{g}	12.9^{g}	10.6^{f}	6.66^{h}	7.95^{g}	4.28^{g}	2.31^{f}	682^{g}	122.22^{g}
RM	611^{f}	11.2^{f}	9.19^{e}	5.74^{e}	6.82^{f}	3.16^{f}	2.02^{be}	627^{f}	97.92^{c}
CT-b	534^{cde}	9.77^{cde}	8.03^{cd}	4.95^{b}	5.91^{cde}	2.25^{cde}	2.21^{def}	580^{cde}	99.45^{cd}
LLP	613^{f}	11.2^{f}	9.23^{e}	5.74^{e}	6.86^{f}	3.20^{f}	2.16^{de}	627^{f}	104.94^{d}
Mean	553	10.15	8.40	5.12	6.12	2.47	2.22	592	102.04
SEM	2.04	0.029	0.029	0.021	0.025	0.025	0.011	1.22	0.548

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Significance	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

DM, dry matter; EE, ether extract; Lignin(sa), lignin solubilized with sulphuric acid; fibre expressed inclusive residual ash; NE_L, net energy for lactation; NE_M, net energy for maintenance; NE_G, net energy for growth; NSC, non-structural carbohydrates; OM, organic matter; SEM, standard error of means; TDN, total digestible nutrients.

3.4. Minerals

Micro minerals (Cu, Zn, Fe and Mn) of legumes varied (p<0.05) in range of 11.11-76.74, 20.77-65.85, 31.53-1288.04 and 18.61-70.51 ppm, respectively (Table 4). Ca and Mg varied from 0.55-2.77 and 0.25-0.88% with mean values of 1.26 and 0.42%, respectively.

Table 4. Macro (%) and micro minerals (ppm) of range legumes.

Legumes	Cu	Zn	Fe	Mn	Ca	Mg
AG	76.7^{h}	65.8^{g}	1150^{cde}	47.5^{c}	2.77^{f}	0.59^{j}
AH	60.7^{g}	61.1^{g}	737^{bc}	47.3^{c}	2.74^{f}	0.58^{j}
CP	28.7^{ef}	42.2^{e}	246^{ab}	31.9^{ab}	1.48^{e}	0.37^{e}
DB	35.7^{f}	31.9^{bc}	1271de	70.5^{ef}	1.37^{e}	0.41^{g}
CT-w	24.9^{cde}	41.7^{de}	311^{ab}	40.8^{bc}	0.60^{a}	0.37^{f}
SSe	14.9^{ab}	35.0	215^{ab}	20.3^{a}	0.94^{c}	0.24^{e}
SH	11.1^{a}	26.7^{ab}	259^{ab}	77.37^{f}	0.80^{b}	0.25^{ab}
SSc	13.6^{ab}	32.0^{bc}	174^{a}	22.1^{a}	1.34^{e}	0.28^{bc}
SSco	16.1^{abc}	48.7^{f}	1023^{cd}	54.5^{cd}	1.47^{e}	0.29^{cd}
SV	12.4^{ab}	20.8^{a}	137^{a}	27.2^{a}	1.44^{e}	0.46^{h}
MA	21.1^{bcde}	30.1^{bc}	1573^{e}	61.4^{e}	1.17^{d}	0.34^{ef}
AS	18.5^{abcd}	28.5^{bc}	1288de	72.3^{f}	0.93^{c}	0.36^{ef}
DV	27.3^{def}	27.1^{ab}	32.8^{a}	28.3^{ab}	0.93^{c}	0.88^{k}
RM	59. 6^{g}	25.5^{ab}	183^{a}	18.6^{a}	0.58^{a}	0.32^{de}
CT-b	72.0^{h}	35.3^{cd}	31.5^{a}	41.9^{bc}	0.55^{a}	0.53i
LLP	62.8^{g}	39.3^{de}	39.4^{a}	41.6^{bc}	0.98^{c}	0.44^{gh}
Mean	34.8	37.0	542	44.0	1.26	0.42
SEM	0.726	0.544	41.41	1.08	0.11	0.003
Significance	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

SEM, standard error of means.

3.5. Fermentation pattern

Fermentation parameters (DMD, ME, PF, SCFA and MBP) of the legumes incubated in sheep inoculums varied (p<0.05 Table 5) and their mean values were 578 g/kg DM, 5.88 kJ/g DM, 5.61mL/mg DM, 2.42 mm/g and 351.65 mg/g, respectively. Microbial protein production efficiency varied (p<0.05) from 0.32 for DV to 0.68 for *CT-w*.

Table 5. Partition factor (PF), short chain fatty acids (SCFA), degradable dry matter and microbial protein production from fermentation of range legumes in sheep inoculum.

Legumes	ME kJ/g	DMD	PF	SCFA mm/g	MBM mg/g	EMBP mg/g
AG	6.07^{cd}	649^{fg}	6.09^{cde}	2.44 ^{cd}	421^{efg}	0.63 ^{cd}
AH	6.04^{cd}	663^{g}	6.35^{cde}	2.41 ^{cd}	445^{g}	0.64 ^{cd}
CP	5.77^{bcd}	513^{b}	5.35^{bc}	2.20^{bcd}	306 ^{bc}	0.60 ^{bcd}

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DB	6.11^{cd}	577^{cd}	5.26^{bc}	2.54 ^{cd}	346 ^{cde}	0.56^{bcd}
CT-w	4.86^{a}b	559^{cd}	7.05^{de}	1.80^{ab}	392^{defg}	0.68^{d}
SSe	5.61^{bc}	578^{cd}	5.49^{bc}	2.41 ^{cd}	355 ^{cdef}	0.59 ^{bcd}
SH	5.82^{bcd}	641^{fg}	6.20^{cde}	2.37^{cd}	417 ^{defg}	0.63 ^{cd}
SSc	6.18^{cd}	631^{efg}	5.60^{bcde}	2.61^{de}	385^{defg}	0.59 ^{bcd}
SSco	5.78^{bcd}	646 ^{fg}	6.25^{cde}	2.42 ^{cd}	424 ^{efg}	0.63^{cd}
SV	5.84^{bcd}	580 ^{cd}	5.24^{bc}	2.54 ^{cd}	342 ^{cd}	0.57 ^{bcd}
MA	5.59^{bc}	594^{cde}	7.08^{e}	2.00^{abc}	407 ^{defg}	0.66 ^{d}
AS	4.49^{a}	394^{a}	5.53^{bc}	1.64^{a}	238^{b}	0.58 ^{bcd}
DV	6.53^{cd}	409^{a}	3.45^{a}	2.77^{de}	142^{a}	0.32^{a}
RM	6.25^{cd}	547^{bc}	4.82^{abc}	2.60 ^{cde}	302 ^{bc}	0.53^{bc}
CT-b	6.43^{cd}	602de	4.37^{ab}	3.13^{e}	301 ^{bc}	0.48^{b}
LLP	6.73^{d}	660^{g}	5.56^{bcd}	2.76^{de}	402 ^{defg}	0.58 ^{bcd}
Mean	5.88	578	5.61	2.42	352	0.58
SEM	0.080	10.4	0.151	0.059	11.00	0.013
Significance	0.001	<.0001	<.0001	<.0001	<.0001	<.0001

DMD, dry matter degradability; ME, metabolisable energy; MBM, microbial protein production; EMBP, efficient of microbial protein production; PF, partition factor (gas mL/mg DM); SFCA, short chain fatty acids; SEM, standard error of means.

3.6. Gas, methane and loss of energy as methane

In vitro gas and methane production (mL/g and mL/g DDM) of legumes varied (p<0.05 Table 6). Gas production (mL/g) was lowest from *AS* (55.5) and highest from *CT-b* (141), while CH_4 production was lower (p<0.05) from *CT-w* and *AS* (8.24 and 9.14) and higher from *AG* and *AH* (15.2 and 15.1). Methane in total gas varied (p<0.05) and was low for *DV, RM* and *CT-w* (8.99, 9.72 and 9.51%) and higher for *MA, AG* and *AH* (14.1, 14.0 and 13.9%), respectively. The loss of DE and ME as CH_4 varied (p<0.05) amongst the legumes with mean values of 4.62 and 7.86%, respectively.

Table 6. Gas production, methane production and loss of energy as methane from in vitro fermentation of range legumes in sheep inoculum.

Legumes	Gas mL/g	CH_4 mL/g	Gas mL/g DDM	CH_4 mL/g DDM	CH_4% Gas	CH_4%DE	CH_4% ME
AG	110^{cde}	15.2^{d}	170^{ab}	23.5^{b}	13.9 ^{bc}	5.77^{ef}	7.03 ^{ef}
AH	109^{cde}	15.1^{d}	164 ^{ab}	22.7 ^{b}	13.9 ^{bc}	5.23 ^{cdef}	6.37 ^{cdef}
CP	99.1^{bcd}	11.0^{bc}	192 ^{abc}	21.4 ^{b}	11.1 ^{abc}	4.14 ^{abc}	5.04 ^{abc}
DB	114de	13.0^{cd}	199 ^{abc}	22.6 ^{b}	11.5 ^{abc}	5.31 ^{def}	6.47^{def}
CT-w	81.3^{b}	8.24^{a}	145 ^{a}	14.7 ^{a}	10.1 ^{ab}	3.23 ^{a}	3.94^{a}
SSe	108^{cde}	11.5^{bc}	188 ^{abc}	20.1 ^{ab}	10.8 ^{abc}	6.10^{f}	7.43 ^{f}
SH	107^{cde}	11.5^{bc}	166 ^{ab}	18.1^{ab}	11.2 ^{abc}	4.61 ^{bcd}	5.61 ^{bcd}
SSc	117de	12.8^{cd}	186 ^{abc}	20.4 ^{b}	11.3 ^{abc}	5.37 ^{def}	6.54 ^{def}
SSco	109^{cde}	11.9^{bc}	169 ^{ab}	18.6^{ab}	11.4 ^{abc}	4.75 ^{bcde}	5.79 ^{bcde}
SV	115de	11.4^{bc}	197 ^{abc}	19.7 ^{ab}	10.0 ^{a}	4.60 ^{bcd}	5.60 ^{bcd}
MA	90.4^{bc}	12.4^{cd}	153 ^{a}	21.0^{b}	14.1 ^{c}	4.72 ^{bcde}	5.75 ^{bcde}
AS	55.4^{a}	9.1^{ab}	188 ^{abc}	23.2^{b}	12.5 ^{abc}	3.17 ^{a}	3.86^{a}
DV	122^{def}	10.8 ^{bc}	309^{d}	26.4 ^{c}	8.99^{a}	3.24 ^{a}	3.94^{a}
RM	117de	11.4 ^{bc}	215^{bc}	21.0^{b}	9.72 ^{a}	3.92 ^{ab}	4.78 ^{ab}
CT-b	141^{f}	13.4^{cd}	235 ^{c}	22.3 ^{b}	9.51^{a}	5.27 ^{cdef}	6.42 ^{cdef}
LLP	124^{ef}	12.8 ^{cd}	190 ^{abc}	19.4 ^{ab}	10.4^{abc}	4.49 ^{bcd}	5.46 ^{bcd}

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Mean	107	12.0	192	21.0	11.3	4.62	5.63
SEM	1.80	0.219	5.86	0.495	0.315	0.134	0.163
Significance	<.0001	<.0001	<.0001	0.010	0.041	<.0001	<.0001

DDM, dry matter; ME, metabolisable energy; SEM, standard error of means.

4. Discussion

4.1. Chemical composition

Information on the chemical composition of forages has significant impact on the understanding of their nutritive value and animal production [8, 18]. Legumes evaluated in present study except *DB* and *Stylosanthes* species had CP contents above 110 g/kg DM recommended to fulfil the protein requirement of growing cattle. Crude protein and NDF contents of *Arachis, Centro, Stylo* and *Siratro* legumes in range of 129-191 and 452-592 g/kg DM [34] were within range of our values baring *Stylosanthes* species and *AS*. The earlier reported CP contents of *C. pubescens* (221) and *S. guyyanensis* (179 g/kg DM) reported were higher than our values (172 and 80.4-105 g/kg DM), respectively [17]. The CP, EE, NDF, AFD, cellulose and lignin contents of *C. pubesecens* were found similar to earlier reports [17, 35]. Whereas *S*. gyuyanensis was in lower contents than *Stylosanthes* except lignin. Further the EE contents of *C. pubescens* and *S. guyanensis* (24.0 and 47.0 g/kg DM) of these workers were similar to values of *CPb* (25.0) and *SSc* (43.2 g/kg DM), respectively. The OM, NDF and ADF contents in *C. pubescens*, *S. hamata*, and *S. scabra* was recorded as same by Musco *et al.* [36]. However, the lignin contents of *C. pubescens* (167) and *S. scabra* (187) were higher than our values (103 and 72.9 g/kg DM), respectively. The NDF and ADF contents of *C. pubescens, Macroptelium bracteatum* and *M. gracile* was at par with previous study [37]. Similarly, the range of CP, EE, OM, NDF, ADF, cellulose and lignin contents in *Macroptilium* species, *Rhynchosia minima, S. humilis, Clitoria ternatea* were found in coherence with other studies [38, 39]. In contrast, significant differences in DM contents (154-253 g/kg DM) of 13 legumes were also recorded previously [40]. The CP content in 24 accessions of *Arachis* species was ranged from 14.7 to 22.55 [41] that was like our results whereas in a study it was recorded even at higher range (184-250 g/kg DM) [42]. Whereases OM and lignin contents (873-919) and 63-82 g/kg DM) were like values presented in present study. The CP (73-129), EE (15-36) and ADF (373-424) except NDF (416-510 g/kg DM) of ten *Stylosanthes guyanensis* varieties recorded by Li *et al.* [43] were within the range of our observed values of *Stylosantehs* species. Furthermore, the value chemical composition recorded in *Arachis hypogeal*, *C. pubescens, Clitoria ternatea*, *M. atropurpureus* and *S. guyanensis* were found similar of earlier studies [44, 45].

4.2. Protein and carbohydrate fractions

Feeds protein fractions composition reflect rumen degradation rates that estimate the dietary nitrogen efficiency. Thus, the utilization level of the nitrogenous fraction is important at the evaluation of feeds and at the specification of nutritional requirements of ruminants [46]. Protein fractions (P_{B1}, P_{B2}, P_{B3} and P_C) differed (p<0.05) across legumes may be attributed to differences in concentrations of CP and lignin. About 50–150 g/kg CP of total forage N is bound to lignin, or rather, is unavailable to ruminal microorganisms [47] and our legumes values for P_C lies within this range (52.9 to 150 g/kg CP). The P_C fraction of *Arachis pinto* ten accessions in range of 178-276 g/kg CP [42] (Ferreira *et al.* 2012) was higher than our *Arachis* species values. Due to good source of protein the annual *Lablab purpures* is predominantly cultivated in dry and semi-dry tropical regions [48].

Carbohydrates constitute the main energy source of plants (500-800 g/kg DM) and play an important role in animal nutrition as a prime source of energy for rumen microorganisms [47]. Carbohydrate accumulation in fodder crops is influenced by several factors like plant species, variety, growth stage and environmental conditions during growth (Buxton and Fales 1994). Mlay *et al.* [49] reported mean tCHO content of 746 g/kg DM for *Macropttlium atropurpurus* legume which was higher than our values (689 g/kg DM). The higher tCHO contents for blue (800) and white (830 g/kg DM) varieties of *Clitoria ternatea* than our *CT-w* (725) and *CT-b* (708 g/kg DM) values was reported earlier

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[38]. The tCHO (626-701 g/kg DM) for ten *Arachis pinto* accessions found marginally lower than our *AG* and *AH* values [42]. Higher SC in DB, SSe, SH and RM may be due to their higher NDF contents recorded in the present study. The legumes *AG, AH, CPb, MA, CT-b* and *AS* had higher contents of C_A fraction (> 44 g/kg tCHO) and feeds higher in this fraction are considered good energy sources to stimulate rumen microorganism growth [50] and the synchronism between the protein and carbohydrate digestion rates, having an important effect on the end products of fermentation and on animal production [51]. Low contents of unavailable carbohydrate fraction (C_C) in *AH, DB, SSe, DV, SV* and *LLP* may be due to their lower lignin contents. This indigestible fraction with C_{B2} usually affects animal intake by the rumen fill, which can reduce animal performance [52]. In our study we recorded that *DB, DV, RM, SH* and *LLP* legumes with higher hemicelluloses contents (315, 253, 258, 221 and 220 g/kg DM) had higher C_{B2} fraction contents (551, 296, 254, 365 and 388 g/kg tCHO) which is more slowly degraded in the rumen, impacting microbial synthesis and animal performance [53]. Higher hemicellulose concentrations result in higher concentrations of C_{B2} fraction. Carvalho et al. [50] reported that NDF concentration influences carbohydrate fraction C_{B2} and forages high in NDF concentration usually have higher values of C_{B2}. Our results partially agree with these observations that most of the legumes with higher NDF contents had higher C_{B2} fraction value. Additionally, annual forage *RM* rich in flavonoids and found suitable for long-term rangelands improvement [54, 55].

4.3. Energy, energy efficiency, intake, digestibility and relative feed value of legumes

Feed or fodder nutritive value is a function of its dry matter intake and its ability to provide the nutrients in the right proportion required by animals for different physiological functions [56]. Greater calculated DMI value for *AG, AH, MA* and *DV* (2.51, 2.51, 2.50 and 2.73%) than other legumes (1.64-2.31%) may be due to lower NDF contents. The NDF of forages has been negatively correlated with DMI which is always not consistent, although the NDF is positively related with resistance to comminution [57]. Lowest and highest DMD of *SSe* and *DV* legumes may be due to their highest (501) and lowest (266 g/kg DM) ADF contents, respectively.

Musco *et al.* [36] reported higher NE_L values for *C. pubescens, S. hamata* and *S. scabra* (9.53, 6.16 and 5.49 kJ/g DM) than our recorded values (5.16, 4.88 and 4.58 kJ/g DM), respectively. Similarly, ME values of *C. pubescens* were higher (6.53) than our values (5.78 kJ/g DM) [58]. The ME contents (9.44-10.36 kJ/g DM) of 13 legumes were higher than ME values observed in present study [40]. The TDN consists of digestible nutrients that are available for livestock and is primarily related to forage ADF contents. With increase in ADF there is decline in TDN resulting unavailability of forage nutrients to animals [59]. The relatively higher values for TDN (634 g/kg DM), GE (17.2), DE (11.5) and ME (9.44 kJ/g DM) were recorded than values of *MA* in present study [49].

The *IVDMD* of *C. pubescens* and *S. guyanensis* (530 and 570 g/kg DM) reported earlier was like our values of *CPb* (513) and *SSe* and *SV* (578 and 580 g/kg DM) but lower to *SH* (641, *SSc* (631) and *SSco* (646 g/kg DM), respectively [17]. The OMD of *C. pubescens* (478), *S. hamata* (609) and *S. scabra* (593) were relatively higher than our values (457, 447 and 471 g/kg DM), respectively. Tona *et al.* [35] reported higher ME and OMD for *C. pubescens* than our values. The DMD and OMD (614-712 and 586-688 g/kg DM) for five forage legumes [37] were more than range values (395-663 and 375-526 g/kg DM) of legumes evaluated in present study. Similarly, the mean OMD and DMD (656 and 684 g/kg DM) were higher than average OMD and DMD values of tested legumes. The higher values for OMD (642-739 g/kg DM) in legume forage than our values are mentioned earlier[40]. Fernandes *et al.* [60] recorded DMD of ten *Arachis* species over three years in range of 501-632 g/kg DM. The values of IVOMD between 600 to 740 g/kg DM for 24 accessions of *Arachis* pinto substantiate our DMD values of *Arachis* species [41]. Highest and lowest TDN values for *DV* and *SSe* in present study may be attributed to differences in their digestibility and lower and higher ADF contents as ADF increased TDN values decreases.

The NDF and ADF contents are negatively associated with OMD and ME values of legumes [40]. Intake an important part of forages nutritive value is partially related to cell wall content and bulkiness of forages. Lowest value of DMI for *DB* (1.64) versus highest of *AS* (2.73%) may be

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attributed to their maximum (730) and minimum NDF contents (440 g/kg DM) as the NDF contents are negatively associated with DMI [47].

The relative feed value (RFV) of ten varieties of *S. guyanensis* in range of 102.8-130.5% [43] were higher than values determined for *Stylosanthes* species (70.80-101.46%). The values of *IVDMD* and TDN of *A. hypogeaea* (78.86 and 71.44), *C. pubescens* (59.89 and 51.30), *Clitoria ternatea* (74.15 and 60.20) *M. atropurpureus* (66.51 and 56.15) and *S. guyanensis* (66.22% and 54.43%) which were higher than our values for these legumes [45]. The DMD of *Arachis species* (*AG* and *AH*) was higher (650 and 663 g/kg DM) except *LLP* than other legume species is similar to previous observations [61] where reported that DMD of *Arachis* species in general is greater than other tropical legumes.

4.4. Minerals

The Ca contents (0.50-0.80%) of legumes studied here were found relatively lower than earlier reported [17], while Mg contents (0.32-0.63%) were like our values of legumes. Similar trend of Ca and Mg contents were reported in other study [62]. Legumes Fe, Cu, Mn and Zn contents in range of 441-494, 2.42-7.14, 55.2-61.3 and 34.8-65.3 ppm [62] were more or less similar to our micro mineral values. In contrast, the relatively lower Ca (1.01-1.62%) and higher Mg (0.92-1.96%) of different *Arachis* pinto cultivars under humid and sub-humid environment were reported than our values [63]. Further the Cu, Fe, Zn and Mn reported for this legume were inconsistent to our values. Macro (Ca and Mg) and micro minerals (Cu. Zn, Fe and Mn) of white and blue varieties of *Clitoria ternatea* reported earlier [38] were more or less similar to our *CT-w* and *CT-b* values. Fernandes *et al.* [60] reported Ca and Mg values in range of 1.4-2.47% and 0.08-0.59% for ten *Arachis* species over three years were similar to our *Arachis* species values. Macro (Ca and Mg) and micro minerals (Cu, Fe, Zn and Mn) of four herbaceous legumes and two browse legumes reported by [63] were in partial agreement to our mineral values. The variation in mineral contents of legume species may be due to differences in soil mineral contents, growth stage, fertilizer application and environment conditions [64, 65].

4.5. Fermentation pattern

The 1.15 µmole SCFA content for *C. pubescens* legume at 24h of fermentation was reported earlier [35]. The partition factor in range of 3.07-4.94 mg/mL reported earlier [36] was lower than PF values (3.45-7.08) of legumes evaluated in present study. The reason for higher PF values for evaluated legumes may be ascribed to less gas production due to lower DMD.

Partition factor an indicator of fermentation efficiency of a feedstuff is expressed as volume of gas (mL) produced per mg of substrate degraded. Legumes mean PF values were higher (5.61) than the theoretically possible maximum value (4.14) [33]. Higher PF values for *CT-w* (7.05) could not translate to higher microbial mass probably due to their less gas production. Higher SCFA for *DV, LLP, SSc* and *RM* may be due to their higher gas production as reported. For *DV* legume microbial protein production was lowest (143 mg/g) and methane production was highest (26.45 mL/g DDM) which is consistent with previous observations [66]. Greater PF values for *CT-w* did not translate to greater microbial mass as noted in previous study [67]. We expected with higher PF to translate to greater microbial mass as PF is the measure of efficiency of microbial production. We can only speculate the reason for this observation, but higher SCFA value noted for *DV* consistent with previous report that microbial mass and SCFA are inversely related [68]. It may be that *DV* partitioned more energy into SCFA *versus* microbial mass production, while in case of *LLP* energy partition was well distributed between SCFA and microbial mass production.

4.6. Gas, methane production and loss of energy as methane

One of the options to improve the feed efficiency through efficient rumen fermentation is that dry matter conversion to methane is less and utilization by animal is more. Methane an end-product of rumen fermentation causes 2-12% loss of dietary energy [69]. This loss of feed energy as methane varies with its quality [70-73] and animal species. Methane % of gas between 15.9 to 18.4%) from 13

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legumes [40] was higher than our recorded values for 16 legumes (9.0 to 14.1%). Our values for proportion of methane in total gas except for *DV, RM* and *CT-b* were within the value (12.3-15.9%) reported earlier [62]. Lopez et al. [74] categorised methane reduction potential as low potential (% of CH_4 in gas between 11 and 14%). So, legumes evaluated in present study had low methane production potential. Furthermore, gas production was higher from *AG* and *AH* (110.25 and 108.92) than *MA* and *CPb* (90.46 and 99.14 ml/g) as recorded in present study [34]. A higher gas production for *S. hamata* and *S. scabra* (195 and 193) than *C. pubescens* (136 mL/g OM) were reported and these values were higher with similar gas production pattern [36]. Similarly, a report on higher gas production at 24h for *C. pubescens* (25.4 mL/200mg) than our values was published [58]. Like our observations [40] recorded significant difference in gas production (42.56 to 51.42 mL/200mg) from hays of 13 legumes and these values were higher than our values (55.46 to 141.26 mL/g) from 16 legumes and these differences may be due to our lower OMD values as low OM fermented for gas production. Further the methane production in range of 7.36-8.78 mL/200mg was higher than our methane production values. The differences in gas and methane production of evaluated pasture legume species may be ascribed to the variation in their chemical composition and degradation. Such variation in methane and gas production of legume species has been recorded earlier [40, 75-77].

The proportion of methane to total gas production is an important indicator of methane emission potential of feed/fodder degradation. This ratio of methane to gas for common feeds (hays, concentrate, mixture of hays and concentrate) vary between 16-20%. Methane production from *Desmodium intortum* (3.67), *Medicago sativa* (5.90) and *Vicia sativa* (5.73 mL/200mg DM) legumes observed by [62] was also higher than most of our legumes but similar to *Arachis* species. Maccarana *et al.* [78] reported gas production, methane production and CH_4% of total gas in range of 72-480 mL/g DM, 7.3-77.5 mL/g and 9.4-40.61% from 390 observations of 30 studies. Relatively lower values of gas production, methane production and CH_4% total gas in present study may be due to sheep rumen liquor as values of gas and methane production and CH_4% total gas were higher when bovine rumen liquor was used for incubations [79, 80]. Greater methane production may be attributed to their higher *IVDMD* values. In line of present findings, previous studies reported that feedstuffs with higher gas production and *IVDMD* tended to have higher methane production per gram DM incubated [81]. Methane emission differences within legumes may be attributed to the variability in their chemical constituents as reported earlier [82-84]. The differences in relative proportion of CH_4 to energy values (CH_4% of DE and % of ME) may be attributed to variations in legumes energy values, level of methane production, dry matter degradability. Variability in proportion of methane to energy of feeds and fodders has been reported earlier [62, 84].

5. Conclusions

Range legumes evaluated in present study had CP more than 100 g/kg DM except for *Stylosanthes* species. Legumes *AG, AH, DV* and *AS* had higher energy values and relative feed values along with lower accumulation of structural carbohydrates. The CH_4% of total gas and CH_4 (% of DE and ME) were lowest for *DV* and *RM* legumes.

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