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Nutritional values and in vitro fermentation parameters of some fodder species found in two rangeland areas in the Republic of Benin

Euloge OGOUKAYODE AKAMBİ OLOMONCHİ[1,*], Ali Vaiz GARİPOĞLU[1], Nuh OCAK[1], Adem KAMALAK[2]

[1]Department of Animal Science, Faculty of Agriculture, Ondokuz Mayıs University, Samsun, Turkey

[2]Department of Animal Science, Faculty of Agriculture, Kahramanmaras Sutçu Imam University, Kahramanmaras, Turkey

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Abstract: In the present study, the nutritional values and in vitro rumen parameters of three browse forages (*Leucaena leucocephala* (LL), *Cajanus cajan* (CC) and *Khaya senegalensis* (KS)) and five grasses (*Andropogon gayanus* (AG), *Panicum maximum* C_1 local (PMCL), *Panicum maximum* C_1 ameliorate (PMCA), *Pennisetum purpureum* (PP), and *Brachiaria ruziziensis* (BR)) grown in the Republic of Benin were investigated. With this aim, it was determined dry matter (DM), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), crude protein (CP), ether extract (EE), metabolizable energy (ME), dry matter intake (DMI), dry matter digestibility (DMD) and relative feed value (RFV) as nutritive values, gas production (GP), methane production (MP), organic matter digestibility (OMD) as in vitro rumen fermentation parameters. Strained and anaerobically prepared rumen fluid was used for determining GP and OMD. Additionally, the correlations between chemical composition and fermentation characteristics were studied. The samples were collected in two rangeland areas in the Republic of Benin. The lowest and highest ADF contents were determined for CC (30.85%) and PMCA (43.14%). The lowest NDF content was found for CC (48.62%). The highest GP (33.8%) and ME (6.85 MJ/kg DM) have been determined for BR. The CC had the highest DMD (64.9%) and RFV (124.1). The lowest MP (mL) was recorded in PMCA, whereas the highest MP was recorded in AG. The crude protein (CP) content of forages was positively correlated with OMD, DMD, DMI, and RFV, while NDF was negatively correlated with GP, MP, OMD, DMD, DMI, and RFV. However, ADF was negatively correlated with GP, DMD, DMI, and RFV, whereas positively with ME. These results show that all the plant materials used in the present study can be used as alternative forage sources in ruminant rations.

Key words: Browse forages, grasses, methane, gas production, organic matter digestibility

1. Introduction

Herbaceous and forbs species, as well as shrub and tree species, are important for livestock in the world, including arid and semi-arid regions [1,2]. In West African countries, especially in the Republic of Benin, feed scarcities are the principal problem in animal nutrition [3,4,5,6]. Therefore, there has been an increasing awareness to determine the chemical composition of the grass and forage legumes or tree leaves found in the Republic of Benin [5,7].

Some authors noted that the natural pastures and crop residues are the main sources of feed for animal nutrition in sub-Saharan Africa [3,4,5]. As such, there is a need for the determination of forage species with high yield and quality based upon their nutritional characteristics to enhance livestock productivity in grazing or cut-and-carry system [5,8]. Indeed, evaluating the nutritional and in vitro fermentation parameters of available fodder species found in West Africa, especially in the Republic of Benin, is important because they could make an important contribution to animal nutrition [7,9].

The nutritional characteristics of forage species can be analyzed approximately [10,11] and can be calculated using these analysis results, whereas fermentation characteristics can be studied by using techniques such as the in vitro gas production system [7,9]. Information on nutritive and energy values of forage species consumed by animals on native rangelands in the Republic of Benin and their contribution to MP are limited. In this study, the nutrient values and in vitro fermentation parameters of three browse forages (*Leucaena leucocephala*, *Cajanus cajan* and *Khaya senegalensis*) and five grasses (*Andropogon gayanus*, *Panicum maximum* C_1 local, *Panicum maximum* C_1 ameliorate, *Pennisetum purpureum*, and *Brachiaria ruziziensis*) grown in the Republic of Benin was determined. With this aim, DM, Ash, ADF, NDF, CP, EE, ME, DMI, DMD, and RFV as nutritive values, GP, MP, and OMD as in vitro fermentation parameters were

* Correspondence: olomonchi@yahoo.fr

determined. Also, it was studied correlations between chemical composition and fermentation characteristics.

2. Material and methods

2.1. Experimental location and fodder samples

Three browse forages (*Leucaena leucocephala*, *Cajanus cajan* and *Khaya senegalensis*) and five grasses (*Andropogon gayanus*, *Panicum maximum C_1* local, *Panicum maximum C_1* ameliorate, *Pennisetum purpureum*, and *Brachiaria ruziziensis*) used in this study were collected from the natural rangelands located in the middle region of Benin (40°50′ to 41°51′ N and 37°08′ to 34°25′ E at nearly 1200 m above from sea level). The study area is characterized by two climates (hot and humid), with the presence of four seasons (two rainy seasons and two dry seasons), and an average annual rainfall of 583.6 mm.

The first rainy season lasts four months (April to July), and the second rainy season runs three months (September to November). While the principal dry season lasts five months (December to April), the short dry season runs from July to September.

The area is characterized by two types of soil (sandy and ferrallitic). The vegetation of the study area is made up of shrubs, swampy grasslands, swamp forests, mangroves, and dense forests [7].

To collect samples of the species, two lines of approximately 1000 m in different areas of four rangeland communities, which are at least 2 km away from each other, were selected and three samples (500 g) for each forage were collected at three times by 15 days intervals from before-flowering stage to after-flowering stage. Once collected, the samples were grouped by forage types. The samples were dried in the sun for 72 h and then crushed and sieved (with a 1 mm sieve). The samples obtained were used to determine the chemical composition and in vitro gas production of forage types.

2.2. Chemical analyses

The dry matter (DM; method 2001.12), Ash (method 930.05), crude protein (CP; method 978.04), and ether extract (EE, method 920.39) of all samples were performed in triplicate in accordance with the approved methods [12]. Analysis results obtained from the Laboratory of Animal Nutrition, Department of Animal Science, Ondokuz Mayis University, Samsun were expressed at DM basis. The fiber contents (NDF, and ADF) were analyzed using the ANKOM A200/220 (ANKOM Technology Corp., Fairport, NY, USA) fiber analyzer with filter bag technique [13].

2.3. In vitro gas and methane production

The fermentation parameters such as in vitro GP and MP were studied by incubating all the forages at 39°C under anaerobic conditions with buffered rumen fluid obtained from sheep. Approximately 200 mg DM of each fodder was weighed and placed in syringes (100 mL calibrated syringes),and then they were subjected to fermentation in four replicate for 24 h in a water bath set at 39°C under anaerobic conditions according to the method described by Menke et al. [14].

Rumen fluid was taken from two fistulated sheep fed with a diet, which was composed of 60% of alfalfa and 40% of the concentrate. Our concentrate contained 74% of wheat, 24% of sunflower meal, 0.99% of calcium carbonate, 1% of salt, and 0.01% of complex vitamin-mineral. Four syringes each contained 30 mL of rumen fluid were incubated and considered as the control group. Total gas values were obtained using empty syringes placed in incubation (control groups). An infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) was used to determine methane (CH_4) proportion, percentage of total gas produced after 24h fermentation [15].

2.4. Calculation of nutritional and fermentation characteristics

MP (mL) was calculated using the equation given below.

MP (mL) = Total GP (mL) * Percentage of methane produced

The metabolizable energy, net energy for lactation, and organic matter digestibility of each forage species were determined according to the following equations [14].

$$ME\ (MJ/kg\ DM) = 2.2 + 0.1357GP + 0.0084CP + 0.0002859EE^2$$

$$OMD\ (\%) = 14.88 + 0.8893GP + 0.448CP + 0.651Ash$$

The dry matter digestibility (DMD), dry matter intake (DMI), and RFV were determined as described by Van Dyke and Maccarana et al. [16,17].

$$DMD = 88.9 - (0.779 * ADF\ (\%))$$

$$DMI\ (\%\ Body\ weight) = 120 / NDF\ (\%)$$

$$RFV = (DMD * DMI) / 1.29$$

2.5. Statistical analyses

Data obtained in the present study were subjected to one-way analysis of variance (ANOVA). The differences between averages were identified with Duncan's multi-range test. Mean differences were considered as significant at $p < 0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance.

3. Results and discussion

Leaves and other parts of different tropical plants are extensively used as forage sources in some African countries. The nutritive values of these tropical forages were reported by various researchers [11,18,19]. The chemical composition of tropical forage species used in the present study was shown in Table 1.

Ash ranged from 5.53 ± 0.19% to 9.43 ± 0.14%. The highest ash content was recorded in *Pennisetum purperium* (9.43 ± 0.14%) and the lowest in *Andropogon gayanus* (5.53

Table 1. Chemical composition of some fodder types grown in Benin, %

Types	DM	Ash	ADF	NDF	CP	EE
		% DM				
Leucaena leucocephala	94.0 ± 0.09[b]	7.57 ± 0.17[de]	39.99 ± 0.09[b]	62.93 ± 0.07[c]	19.78 ± 0.17[b]	2.95 ± 0.06[c]
Khaya senegalensis	91.0 ± 0.28[c]	7.72 ± 0.06[d]	38.74 ± 0.06[c]	58.85 ± 0.43[d]	12.95 ± 0.08[c]	3.163 ± 0.05[bc]
Pennisetum purperium	90.9 ± 0.43[c]	9.43 ± 0.14[a]	41.98 ± 0.91[a]	67.87 ± 0.40[b]	8.98 ± 0.09[d]	3.29 ± 0.16[bc]
Andropogon gayanus	90.0 ± 0.46[c]	5.53 ± 0.19[g]	38.416 ± 0.2[cd]	58.36 ± 0.27[d]	8.55 ± 0.18[d]	3.51 ± 0.15[b]
Panicum maximum C_1 local	90.3 ± 0.25[c]	6.17 ± 0.17[f]	37.19 ± 0.17[e]	67.84 ± 0.28[b]	10.23 ± 0.23[d]	2.48 ± 0.12[d]
Panicum maximum C_1 ameliorate	90.8 ± 0.33[c]	7.13 ± 0.15[e]	43.14 ± 0.38[a]	70.37 ± 0.31[a]	7.87 ± 0.16[e]	2.95 ± 0.16[c]
Brachiaria ruziziensis	88.5 ± 0.24[d]	8.89 ± 0.21[b]	42.93 ± 0.24[a]	62.85 ± 0.18[c]	8.83 ± 0.19[d]	3.13 ± 0.11[bc]
Cajanus cajan	96.2 ± 0.07[a]	8.21 ± 0.14[c]	30.85 ± 0.47[f]	48.62 ± 0.45[e]	25.10 ± 0.05[a]	5.03 ± 0.16[a]

DM: Dry matter; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; CP: Crude protein; EE: Ether extract.
[a, b, c,...] Means in a column with different superscripts are different ($p < 0.05$).

± 0.19%). NDF and ADF ranged, respectively, from 48.62 ± 0.45 to 70.37 ± 0.31%, and 30.85 ± 0.47 to 43.14 ± 0.38%. While the highest values for NDF (70.37 ± 0.31%) and ADF (43.14 ± 0.38%) were recorded in *Panicum maximum C_1* ameliorate, the lowest values of NDF (48.62 ± 0.45%) and ADF (30.85 ± 0.47%) were recorded in *Cajanus cajan*. CP ranged from 25.10 ± 0.05 to 7.87 ± 0.16%.

The highest and lowest values for CP were recorded, respectively, in *Cajanus cajan* (25.10 ± 0.05%) and *Panicum maximum C_1* ameliorate (7.87 ± 0.16%). The differences in the chemical composition of forage species found in present study can be explained by the fact that these forages belong to different plant families such as legumes and grasses. *Pennisetum purperium*, *Andropogon gayanus*, *Panicum maximum C_1* local, *Panicum maximum C_1* ameliorate, and *Brachiaria ruziziensis* are forage grass species [20]. Hence, low levels of crude protein were recorded for these species. The CP contents of *Panicum maximum C_1* ameliorate were also similar to those reported by Yousuf et al. [21]. In general, while the grasses are poor in CP, the legumes are rich in CP.

CP and ADF contents of *Khaya senegalensis* and *Andropogon gayanus* were similar to those reported by some authors [11,13]. CP, EE, ash, and NDF contents of *Leucaena leucocephala* correspond to those found in previous studies [10,21]. *Khaya senegalensis* and *Leucaena leucocephala* are fodder trees found in rangeland. These forage trees are used for ruminant feeding in tropical areas of Africa. The use of tree leaves as a supplement or as a single feed improves the productivity and health of ruminants [11,22,23,24,25].

The values for DM, CP, ash, and EE for *Cajanus cajan* determined in the present study were higher than those reported in the previous studies [11,23,26,27]. According to recent studies, *Cajanus cajan* can produce high-quality fodder and can be integrated with the culture and livestock system as a dietary supplement [11,22,28]. The CP content (10%–15%) reported by some authors [11,27,28] for *Cajanus cajan* leaves is lower compared to the present study findings. In contrast, the present findings are in accordance with some previous studies [11,27,29]. These discrepancies can be explained by the differences in species and harvest time.

The GP, MP, DMD, DMI, OMD, ME, and RFV values obtained from the current study of forage species in Table 2 and their correlation with nutritional values in Table 3 can be seen.

The in vitro gas production method is a suitable technique for evaluating the nutritional value of forages in underdeveloped and developing countries where financial resources are limited [17,27,29]. This method provides useful information about the degradability of both insoluble and soluble nutrients [3,6,9], fermentation kinetics, and final products (NH_3-N and VFA, etc.) [7,8,9].

Differences in GP, MP, DMD, DMI, OMD, ME, and RVF values of fodder may be due to the effect of fiber content, expressed as ADF and NDF on rumen fermentation, as well as CP content [6,7,10]. These researchers determined that the fiber content (NDF and ADF) influenced GP. These were consistent with our results.

The effect of forage types was significant on GP, MP, DMD, DMI, OMD, ME, and RFV. Gas production ranged from 20.41 ± 0.88 to 33.75 ± 1.15 mL, the lowest for *Leucaena leucocephala* and the highest for *Brachiaria ruziziensis*. In line with our expectations, the factor that influenced in vitro GP was the NDF content of different forages incubated. However, in the present study, GP and NDF values are negatively correlated. This can be

Table 2. In vitro fermentation characteristics and nutrient content of some fodder types found in Benin (June to December).

Forages	GP, ml	MP, ml	DMD, %	DMI, % of BW	OMD, %	ME, MJ/kg DM	RFV
Leucaena leucocephala	20.4 ± 0.88^{d}	7.4 ± 0.16^{cd}	57.7 ± 0.07^{d}	1.90 ± 0.02^{c}	46.8 ± 0.80^{bc}	5.13 ± 0.12^{e}	85.4 ± 0.15^{c}
Khaya senegalensis	25.1 ± 1.33^{c}	8.2 ± 0.14^{a}	58.7 ± 0.01^{c}	2.03 ± 0.01^{b}	48.0 ± 1.19^{bc}	5.71 ± 0.18^{cd}	92.8 ± 0.76^{b}
Pennisetum purperium	22.8 ± 1.52^{d}	7.6 ± 0.27^{bc}	56.8 ± 0.71^{e}	1.76 ± 0.01^{d}	45.3 ± 1.41^{bc}	5.36 ± 0.20^{e}	77.0 ± 1.38^{e}
Andropogon gayanus	29.8 ± 1.00^{b}	8.3 ± 0.26^{a}	59.0 ± 0.22^{bc}	2.05 ± 0.01^{b}	48.8 ± 0.92^{b}	6.31 ± 0.13^{b}	94.0 ± 0.79^{b}
Panicum maximum C_1 local	23.8 ± 2.08^{d}	7.8 ± 0.02^{abc}	59.9 ± 0.13^{b}	1.76 ± 0.01^{d}	44.6 ± 2.05^{c}	5.51 ± 0.28^{cde}	82.2 ± 0.51^{d}
Panicum maximum C_1 ameliorate	25.4 ± 0.88^{c}	6.3 ± 0.18^{e}	55.3 ± 0.29^{e}	1.70 ± 0.007^{e}	45.7 ± 0.94^{bc}	5.71 ± 0.12^{cd}	73.1 ± 0.71^{f}
Brachiaria ruziziensis	33.8 ± 1.15^{a}	8.1 ± 0.12^{ab}	55.6 ± 0.18^{e}	1.90 ± 0.01^{c}	54.6 ± 0.91^{a}	6.85 ± 0.15^{a}	82.1 ± 0.51^{d}
Cajanus cajan	26.8 ± 0.57^{bc}	6.9 ± 0.11^{c}	64.9 ± 0.37^{a}	2.46 ± 0.02^{a}	55.3 ± 0.54^{a}	6.04 ± 0.07^{bc}	124.1 ± 1.01^{a}

GP: Gas production; MP: Methane production; DMD: Dry matter digestibility; DMI: Dry matter intake; BW: Body weight; OMD: Organic matter digestibility; ME: Metabolizable energy; DM: Dry matter; RFV: Relative feed relative.
$^{a, b, c,...}$ Means in a column with different superscripts are different ($p < 0.05$).

Table 3. Correlations between some nutritional value and in vitro fermentation parameters of some forage species grown in the Republic of Benin.

	GP	MP	ME	OMD	DMD	DMI	RFV
CP	–0.267	–0.269	–0.184	0.419^{*}	0.741^{**}	0.753^{**}	0.783^{**}
EE	0.215	0.333	0.279	0.658^{**}	0.652^{**}	0.862^{**}	0.843^{**}
CA	0.027	-0.071	0.044	0.327	–0.207	0.035	–0.022
ADF	-0.07^{***}	–0.117	0.006^{***}	–0.361	-1.000^{*}	-0.830^{**}	-0.913^{**}
NDF	-0.244^{***}	-0.159^{***}	–0.313	-0.678^{**}	-0.797^{**}	-0.992^{**}	-0.965^{**}
DM	–0.396	–0.249	–0.320	0.257	0.673^{**}	0.636^{**}	0.685^{**}

DM: Dry matter; CA: Crude ash; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; CP: Crude protein; EE: Ether extract; GP: Gas production; MP: Methane production; ME: Metabolizable energy; OMD: Organic matter digestibility; DMD: Dry matter digestibility; Dry Matter intake; RFV: Relative feed value. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

explained by forage quality. In the current study, the reduced degradability of DM is closely related to high NDF content. This results in low gas production. This result is in accordance with findings of previous studies [11,17,30,31]. Carbohydrate availability for microorganisms is determined by the gases produced after fermentation [11,27,32,33]. Some researchers claimed that the amount of gas produced is related to the amount of fermentable carbohydrates [11,32,33].

The methane production varies between 6.3 ± 0.11 and 8.30 ± 0.26 mL among the species in the present study. The lowest methane productions were recorded in *Panicum maximum C1* ameliorate (6.3 ± 0.18 mL), *Cajanus cajan* (6.9 ± 0.11 mL), and *Leucaena leucocephala* (7.4 ± 0.16 mL) and highest in *Andropogon gayanus* (8.3±0.26 mL), *Khaya senegalensis* (8.2 ± 0.14 mL), and *Brachiaria ruziziensis* (8.1 ± 0.12 mL). In summary, the highest quantity of methane production was recorded in *Andropogon gayanus* (8.3±0.26) and lowest in *Panicum maximum C_1* ameliorate (6.29 ± 0.18).

In present study, ME content of forage species varied between 6.85 ± 0.15 MJ/kg DM (*Brachiaria ruziziensis*) and 5.13 ± 0.12 MJ/kg DM (*Leucaena leucocephala*). After 24 hours of fermentation, forage species with high gas production also had a high proportion of ME. Many researchers reported that the carbohydrate content of animal feed contributes to 40% of total gas production [11,33,34]. The structural carbohydrates found in plants influence methane production [27,33,35]. Ammonia produced in the rumen comes from the metabolism of CP. Ammonia is subsequently used for methane mitigation [11,27,36]. Due to the relationship between methanogenic and cellulolytic bacteria in the rumen, methane is produced following the digestion of fiber material (ADF and NDF) in the feed sources [11,37,38,39]. It can be said that the raw fiber richness of feeds can be the basis of methane production.

The OMD of forage species ranged from 44.60 ± 2.05 to 55.30 ± 0.54. The highest OMD was found for *Cajanus cajan* (55.3 ± 0.54) and the lowest for *Panicum maximum* C_1 local (44.6 ± 2.05). In present study, DMD, DMI and RFV varied, respectively, from 55.30 ± 0.29 to 64.90 ± 0.37; 1.70 ± 0.007 to 2.46 ± 0.02; 73.10 ± 0.71 to 124.10 ± 1.01. *Panicum maximum* C_1 ameliorate had the lowest values for the DMI, DMD, and RFV parameters. The highest DMD, DMI, and RFV were obtained for *Cajanus cajan*. It can easily be seen that fiber fractions of fodder species are negatively correlated with OMD, DMD, DMI, and RFV. These results are consistent with those of authors who reported a negative correlation between legume cell wall contents with OMD and ME content of legume hays [11,27,32].

Khaya senegalensis [38,39,40,41,42], *Leucaena leucocephala*, and *Cajanus cajan* [7] are tree species, and their leaves are used in animal feed. They contain a tannin [43] and certain polyphenolic compounds [44]. The differences found in terms of GP, MP, DMD, DMI, OMD, ME, and RFV between the browse forages (*Leucaena leucocephala*, *Cajanus cajan* and *Khaya senegalensis*) and grasses (*Pennisetum purperium, Andropogon gayanus*, *Panicum maximum* C_1 *local*, *Panicum maximum* C_1 *ameliorate, Brachiaria ruziziensis*). During the dry season period, available natural pastures have been reported to be low in protein, nitrogen, sulfur, vitamins, and other nutrients. However, they are rich in fiber with a dry matter content of more than 30% [45,46,47].

4. Conclusion

The different forage species used in present study had different chemical composition.

It was found that a decrease in DMD in our study was caused by an increase in NDF.

Negative correlation was found between fiber content (NDF and ADF) and parameters such as OMD, DMD, DMI and RFV. From this study, it appears that the forage species found at the natural pastures of Benin are rich in nutrients (fiber, protein and energy). In contrast, some forages used in this study has low protein content. The amount of NDF for cows was recommended to be 25% of dietary DM with a condition that 19% of dietary DM must be NDF from forage [11,38]. In conclusion, various forages species used in the present study were found promising to approach the goal of improved nutrition of ruminants in the tropical regions (especially in the Republic of Benin) at simultaneously limited methane emissions.

Conflicts of interest

No potential conflict of interest was reported by the author(s).

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