In vitro rumen fermentation characteristics, methane production and rumen microbial community of two major Acacia species used in Sahelian region of Burkina Faso

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In vitro RUMEN FERMENTATION CHARACTERISTICS, METHANE PRODUCTION AND RUMEN MICROBIAL COMMUNITY OF TWO MAJOR *Acacia* SPECIES USED IN SAHELIAN REGION OF BURKINA FASO[†]

[CARACTERÍSTICAS DE FERMENTACIÓN RUMINAL, PRODUCCIÓN DE METANO Y COMUNIDAD MICROBIANA RUMINAL *in vitro* CON DOS ESPECIES PRINCIPALES DE *Acacia* UTILIZADAS EN LA REGIÓN SAHELIANA DE BURKINA FASO]

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SUMMARY

Acacia nilotica var adansonii (Guill. et Perr.) O. Ktze and *Acacia raddiana* (Savi) species are local ressources important to feed animals in Sahelian area of Burkina Faso. Thus, this experiment was to investigate the *in vitro* effect of both *Acacias* on ruminal fermentation, CH_4 production and rumen microbial community using a semi-automatic system. In all experiments, 25 mL of the inoculum solution was incubated with 50 mL of buffered rumen fluid at 39 °C and gas production was measured at 2, 4, 8, 12 and 24 h. The results show that *A. nilotica* was rich in condensed tannins and fiber content compared to *A. raddiana* which was rich in total tannins. *A. raddiana* treatment presented greater degradability of organic matter and lower CH_4 production compared to *A. nilotica* and control group ($P<0.05$). In addition, methanogenic archaeal were significantly lower in *A. raddiana* associated with increased fungal communities abundance compared to *A. nilotica* ($P<0.05$), but no effects was observed on rumen bacteria *R. flavefaciens* and *F. succinogenes* ($P>0.05$). We conclude that *A. nilotica* leaves showed negative effect on ruminal fermentation due to high fiber and CT content, and *A. raddiana* could be an interesting plant to increase fiber digestion and reduce CH_4 production *in vitro.*

Key words: *In vitro* gas production; *Acacia raddiana; Acacia nilotica;* Ruminant.

RESUMEN

Acacia nilotica var adansonii (Guill. et Perr.) O. Ktze y *Acacia raddiana* (Savi) son recursos locales importantes para alimentar animales en la región saheliana de Burkina Faso. Por lo tanto, este experimento fue para investigar el efecto *in vitro* de Acacia en la fermentación ruminal, la producción de CH4 y la comunidad microbiana del rumen utilizando un sistema semi-automático. La técnica de producción de gas *in vitro* se utilizó para evaluar el efecto de las plantas ricas en taninos en la formación de CH_4 por las ovejas. En todos los experimentos, se incubaron 25 ml de la solución de inóculo con 50 ml de fluido ruminal tamponado a 39 °C y se midió la producción de gas a las 2, 4, 8, 12 y 24 h. Los resultados muestran que *A. nilotica* es rica en taninos condensados y contenido de fibra (NDF, ADF y

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ADL) en comparación con *A. raddiana* que es rica en taninos totales. El tratamiento con *A. raddiana* presentó mayor degradabilidad de la materia orgánica (DMO) y menor producción de CH_4 en comparación con *A. nilotica* y el grupo control ($P < 0.05$). Además, las archaeas metanogénicas fueron significativamente menores en *A. raddiana* lo que fue asociado con un aumento de la abundancia de las comunidades fúngicas en comparación con *A. nilotica* ($P < 0.05$), pero no se observaron efectos en las bacterias del rumen *R. flavefaciens* y *F. succinogenes*($P > 0.05$). Concluimos que las hojas de *A. nilotica* mostraron un efecto negativo sobre la fermentación ruminal debido al alto contenido de fibra y CT, y *A. raddiana* podría ser una planta interesante para aumentar la digestión de la fibra y reducir la producción de CH_4 *in vitro*.

Palabras clave: Producción de gas *in vitro*; *Acacia raddiana*; *Acacia nilotica*; Rumiantes.

INTRODUCTION

In arid and semi-arid regions of the world, small ruminants play an important socio-economic role in the daily lives of rural farmers in mixed systems of agriculture and livestock (Belem et al., 2000). However, this sector faces with nutritional and health constraints that limit the expression of animal performance on farms (Zabré et al., 2017). Thus, rural herders in arid regions have developed endogenous strategies based on the exploitation of plant resources to feed and fight against diseases (Zabré et al., 2017). The main threats on production are i) the low biomass availability to achieve the nutritional requirements of animals, and ii) the infection with gastro intestinal parasites. In addition, ruminant livestock depends predominantly on natural pastures but the omnipresent processes of desertification affect vast surfaces primarily confined in the arid, semi-arid and sub-humid dry areas (Boufennara et al., 2013) which caused problems of availability and quality of feed for ruminants.

Nutritional constraints for ruminant livestock are: i) highly lignified plants that caused low digestibility; ii) forages with low nitrogen (N) content (Abdalla et al., 2012). Also, the type and the concentration of tannins widely present in tropical plants may affect feed intake and ruminant digestion including methane (CH_4) emissions (Jayanegara et al., 2012). Tannins in the foliage can exhibit anti-nutritional effects or positive nutritional merits. When the concentrations are high (>55 g CT/kg DM) in the browse foliage, the tannins could reduce ruminal and post-ruminal digestion of protein (Min et al., 2003).

Plants such as *Acacia* species can be used to combat desertification, mitigating the effects of droughts, allowing soil fixation and enhancing the restoration of vegetation. In Burkina Faso, especially in sahelian area, *Acacia nilotica* and *Acacia raddiana* are used by most of livestock owners to feed small ruminant (Sawadogo, 2011) but their high tannin content may impair rumen fermentation and thus impair their nutritional value (Goel and Makkar, 2012). Thus, the objective of this experiment was to investigate the *in vitro* effect of both *Acacia* on ruminal fermentation, CH_4 production and rumen microbial community.

MATERIALS AND METHODS

The study was carried out at the Center for Nuclear Energy in Agriculture, University of São Paulo (CENA/USP), Piracicaba, Brazil. The animals were always treated in accordance with the guidelines of the Internal Commission for Environmental and Ethics in Experimentation with Animals of CENA/USP.

Plant material

Fresh leaves of *A. nilotica* var adansonii (Guill. et Perr.) O. Ktze and *A. raddiana* (Savi) were collected in December 2014 at Dori (14.04° North latitude and 0.03° West longitude) situated in North-East of Burkina Faso. The climate of the region, classified as sahelian, is marked by a long dry season from November to July and a short rainy season from August to October. The leaves were identified by reference to the herbarium of the "Centre National de la Recherche Scientifique et Technologique" in Ouagadougou, Burkina Faso. Then, harvested leaves were cleaned with water and dried at room temperature for a week according to the procedure of traditional healers in the country. They were also grind to powders before used for the experiment. Aroeira (*Schinus terebinthifolius* Raddi) was used as control for the quantification of tannins and phenols and Tifton-85 hay (*Cynodon spp*) was used as Control for rumen fermentation assay.

Animals

Three sheep breed Santa Inês (Ovis aries: 51.2 ± 7.9 kg), 2 to 3 years old, from the North of Brazil were used as inoculum donors. All animals were fitted with rumen cannula giving access to the rumen. Diets were formulated with forage (Tifton-85 hay) and concentrate containing 70% ground maize grain and 30% soybean meal, approximately 1.5% of individual animal's live weight. All animals had free access to water and mineral mixture.

Inoculum

Two type of ruminal content were collected before the morning feeding: rumen fluid and rumen solids. Fluids and solids have been placed separately: liquid phase was stored in pre-warmed thermal flasks and the solid phase in plastic bags in coolers with thermal packs heated to 39°C. After collection, the ruminal content was transported immediately to the laboratory. Once in the laboratory, 250 mL of liquid ruminal and 250 mL of solids were mixed in a blender for about 10 s, and then the mixture was squeezed through a cotton cloth as described by Bueno *et al.* (2005). The filtrate was put into flask in a water bath at 39°C under CO_2 saturation. A small quantity of the mixture (inoculum) was collected to measure pH to make the whole inoculum to add in with bottle.

***In vitro* rumen fermentation technique and sampling**

The in vitro gas production was measured using a semi-automatic system (Bueno et al., 2005). 0.5 g of each sample (*A. nilotica*, *A. raddiana* and Tifton-85 hay as internal standard was put in filter bags (Ankon® F57 filter bags). These bags were placed in 12 160 mL -glass bottles (3 plants substrates x 4 replicates of each sample of plant substrate were used. The fourth inoculum was prepared by mixing the blended (fluid and solid) ruminal contents of the three donors animals) and then, 50 mL of buffer solution, 25 mL of the inoculum solution previously prepared were added.

The bottles were then sealed and kept in an incubator at 39°C for the pressure measurements. The pressure in the bottles was measured by a pressure transducer and data logger (Press data 800, LANA/CENA-USP/Piracicaba, SP) at regular intervals of 2, 4, 8, 12 and 24h of incubation. In these intervals, 2 ml of gas were removed in each bottle using a 5 mL syringe and stored in 12 mL -vacuum tubes for determination of the concentration of CH_4 in the total gas produced during incubation.

After 24h of incubation, the fermentation was stopped by placing the bottles in cold water, the bottles were then opened and the bags were removed and treated with a neutral detergent solution for the determination of Neutral-detergent fibre (NDF) degradability (NDFD) and true organic matter degradability (TOMD) values (Soltan et al., 2012). The pH of the remaining contents of the bottle was measured and a small quantity was sampled to analyze SCFA (short chain fatty acids), ammonia nitrogen $N\text{-}NH_3$, microbial profiles and protozoa. For $N\text{-}NH_3$, three kinds of solutions were prepared: Sodium tetraborate solution (5%), acid sulfuric solution (0,01%) and acid boric solution (0,1). For protozoa count: 2 mL of ruminal solution were mixed in 2 mL of formaldehyde solution. For SCFA, 1.5 mL of ruminal fluid was centrifuged at 11000 rpm for 40 mn at 4°C. 800 µL of supernatant were transferred in a tube containing 100 µL of 2-ethyl-butyric acid (9.09 mmol/L) and 200 µL of formic acid (88%).

Analytical methods and calculations

Feeds were analyzed by determining dry matter (DM), mineral matter (MM) and CP according to AOAC (1995). NDF and acid-detergent fibre (ADF) were determined sequentially without addition of amylase according to Mertens (2002). The total phenol (TP) was determined by the Folin-Ciocalteau reagent method (Makkar, 2003) and total tannins (TT) were estimated as the difference in TP concentration before and after the treatment with insoluble polyvinylpolypirrolidone (Makkar et al., 1993), using tannic acid as standard. Condensed tannin (CT) concentrations were determined by the butanol-HCl method (Makkar, 2003) using leucocyanidin as standard.

Methane concentration was determined according to Longo *et al.* (2006) using a gas chromatograph (Shimadzu 2010, Tokyo, Japan) equipped with a Shincarbon ST 100/120 micro packed column using an external calibration analytical curve (0, 30, 60, 90 and 120; mL /l) prepared with pure CH_4 (99,9% of purity) as described by Salam *et al.* (2010). Net gas production (NetGP) and net methane output (NetCH4) values were calculated relative to DM, OM and NDF. Gas production was calculated according Soltan *et al.* (2012): V=7.365 x P; where 7.365 is the regression slope; V is volume (mL) and P is the pressure (psi) measured. The conversion efficiency of methane (CH4effic) value in relation to the NetGP volume, considering NetGP as 100%. CH_4 (mL)=[(total gas (mL) + 85 mL)*(%CH_4/100)] where 85 mL is volume of the headspace. The partitioning factor (PF) is calculated as the ratio of substrate (truly degraded dry matter) *in vitro* (mg) to the volume of gas (mL) produced by it.

The extraction of total DNA in the liquid phase of bottle´s content samples was performed using the PowerLyzer ™ PowerSoil (MoBIO) commercial kit. Quantitative Polymerase Chain Reaction (qPCR) analyses were performed using specifics primers as described by Denman and Mc Sweeney (2006), Denman *et al.* (2007). The relative abundance of microorganisms *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *rumen fungi* and *methanogenic archaea* was calculated using the ΔΔCt method (Livak and Schmittgen, 2001). The concentration of $N\text{-}NH_3$ was determined by micro-Kjeldahl method (AOAC, 1990) using sodium

tetraborate, boric acid and sulfuric acid solution. One mL of ruminal solution was put in tubes and disposed in the distiller (Kjeltec unit). During distillation and for each tube, 15 mL of sodium tetraborate solution were added in the distiller then, 10 mL from the distillation liquid were recovered in each small flask containing previously 10 mL of boric acid solution. The solutions contained in the flasks were then titrated with sulfuric acid. For protozoa, the stained sample was kept overnight and protozoa were counted microscopically following the procedure described by Kamra *et al.* (1991). SCFA analysis were carried out using gas chromatography with column Stabilwax according to Getachew *et al.* (2002). Likewise, external standard solution containing known concentrations of each SCFA (acetic, propionic, isobutyric, butyric, valeric and isovaleric acids) was prepared for calibration of the integrator. One µl of sample was injected into a Shimadzu 2010 gas chromatograph coupled with a flame ionization detector (FID), with a GP 10% SP-1200/1 H_3PO_4 80/100 Chromosorb WAW column.

Statistics

The data were subjected to analysis of variance using the general linear models procedure of SAS (2000).The means were compared using Tukey test and the differences between means with $P<0.05$ were considered statistically different, while differences between means with $0.05<P<0.10$ were accepted as tendencies to difference. Redundancy analysis (RDA) was used to determine the most influence variables among the comparison of two animal-diet and control based on similarity profiles. Environmental variables were normalized into a common range scale and combined with qPCR values. The comparison for microbial abundance profile and metadata were carried out using the R programming language and vegan package.

RESULTS

A. nilotica had higher concentrations in MM, CP, NDF, ADF and ADL than *A. raddiana* (Table 1). Regarding the phenolic profiles, *A. raddiana* had concentration in TP and TT much more higher than *A. nilotica* (39-40% and 5-7% respectively), but the concentration in CT was higher in *A. nilotica* than in *A. raddiana* (5% against 0.3%).

A difference was found between the two species of *Acacia* for the degradability of organic matter (*A.raddiana* > *A.nilotica*, $P<0.001$) (Table 2). In contrast, NDF degradability and total net GP production was similar for both plants ($P>0.05$). As a consequence the PF was higher for *A.raddiana* than for *A. nilotica* ($P<0.001$). The Net CH_4 production was much higher with *A. nilotica* than with *A. raddiana* (4.52 and 2.83mL /g DM, respectively, $P < 0.05$). However, when expressed relative to DOM or NDF they were similar.

Table 1. Chemical composition (g/kg DM) of the two species of *Acacia*

Chemical composition	*A. nilotica*	*A. raddiana*
DM	918.4	905.6
MM	119.8	47.2
OM	880.2	952.9
CP	158.8	120.4
NDF	519.7	272.1
ADF	338.3	118.4
ADL	250.4	70.9
CT	53.6	3.4
TT	54.2	391.6
TP	76.7	401.3

DM= Dry Matter, MM= Mineral Matter, OM= Organic Matter, CP= Crude Protein, NDF= Neutral Detergent Fiber, ADF= Acid Detergent Fiber, ADL= Acid Detergent Lignin, CT= Condensed Tannins (g of leucocyanidin /kg DM), TT= Total Tannin and TP= Total phenol (g of tannic acid /kg DM).

No difference was observed between the species ($P>0.05$) for N-NH_3 and SCFA (acetate, propionate and butyrate) (Table 3). The pH value was higher for *A. nilotica* than for *A. raddiana* ($P<0.001$).

Difference was observed between *A. raddiana* only and control ($P<0.05$) for rumen methanogens, *Ruminococcus flavefaciens, Fibrobacter succinogenes, protozoa* count and between both *Acacia* and control for rumen *fungi*. No significant difference was observed between both *Acacia* ($P>0.05$) for rumen *fungi* (Table 4).

Redundance analysis based on microbial abundance profile highlighted three clusters, one with *A. raddiana* treatment, one with *A. nilotica* treatment, and other with control treatment. *A. raddiana* treatment presented greater degradability of organic matter (OMD) and acetate to propionate ratio (A:P), that were associated with dissimilarity between the three clusters (Fig. 1). Where the OMD and A:P increased rumen *R. flavefaciens* and fungal communities abundance ($P<0.05$) and decreased methanogenic archaeal and *F. succinogenes (*$P<0.05$).

Table 2. Degradability, total gas and methane production, and partition factor during 24h-rumen fermentation of *A.raddiana*, *A.nilotica* and control

Parameters	Control	*Acacia nilotica*	*Acacia raddiana*
TOMD (g/kg DM)	323.26±15.24 [a]	603.71±10.78 [b]	808.90±10.78 [c]
NDFD (g/kg DM)	248.53±39.71	328.73±28.08	330.88±28.08
Net GP (mL /g DM)	103.22±5,6	96.79±4.0	97.34±4.0
Net CH_4 (mL /g DM)	4.09±0.52 [a]	4.52±0.36 [a]	2.83±0.36 [b]
CH_4 effi (%)	3.94±0.38 [a]	4.64±0.26 [a]	2.83±0.36 [b]
Net GP (mL /g DMO)	33.41±4.99 [a]	58.40±3.52 [b]	78.93±3.52 [c]
Net CH_4(mL /g DMO)	1.33±0.36 [a]	2.72±0.25 [b]	2.33±0.25 [bc]
Net GP (mL /g NDF)	25.69±5.33	31.77±3.77	32.89±3.77
Net CH_4 (mL /g NDF)	1.03±0.25	1.48±0.17	0.99±0.17
PF (mg of TDOM / mL of gas)	1.34±0.166 [a]	2.54±0.12 [b]	3.62±0.12 [c]

a,b means significantly different ($P < 0.05$). TOMD = True organic matter degradability, NDFD = Neutral detergent fiber degradability, CH_4 = Net Methane, Net GP = gas production, CH_4 effi = methane efficiency, PF = Partition Factor,

Table 3. Fermentation end-products and pH from the rumen fermentation of the two species of *Acacia*

Parameters	Control	*Acacia nilotica*	*Acacia raddiana*
N-NH_3 (mg/100 mL)	35.13±2.06	36.26±1.45	32.58±1.45
pH	6.68±0.02 [a]	6.72±0.01 [a]	6.61±0.01 [b]
Acetate (mmol/l)	35.58±1.09	34.71±0.77	34.40±0.77
Propionate (mmol/l)	8.69±0.34	8.7±0.24	8.16±0.24
Butyrate (mmol/l)	6.41±0.25 [a]	5.42±0.18 [b]	5.41±0.18 [bc]
Acetate/proporionate ratio	4.10 ±0.1	3.99 ±0.07	4.22 ±0.07

N-NH_3= Ammonia nitrogen

Table 4. Relative quantification ($100*(2\Delta Ct)-1$) of genes from rumen microbial community expressed as proportion of genes from total bacteria of different microbial population from substrates incubated *in vitro*

Parameters	Control	*Acacia nilotica*	*Acacia raddiana*
Protozoa ($x10^5$/ mL)	9.75±1.03 [a]	7.82±0.76 [ab]	5.75±0.76 [b]
Rumen fungi	1.00±0.07 [a]	0.72±0.07 [b]	2.42±0.07 [c]
Rumen methanogens	1.00±0.01 [a]	0.97±0.01[ab]	0.74±0.01 [b]
Ruminococcus flavefaciens	1.00±0.26 [a]	1.57±0.26 [ab]	2.09±0.26 [b]
Fibrobacter succinogenes	1.00±0.05 [a]	0.02±0.05 [b]	0.12±0.05 [b]

a,b,c means significantly different

DISCUSSION

In sahelian area of Burkina Faso, *Acacia* species are local resources important to feed animals. Yet, the concentrations and types of tannins largely present in *Acacia* could be anti-nutritional, but also positive to fight some diseases as parasitism or reduce excessive methane production. In particular CT have the potential to manipulate rumen fermentation by modulating protein degradation while inhibiting rumen CH_4 production. In many previous studies, *Acacia spp* have been reported to have antinutritional values and antimethanogenic activities. For instance, data have been recently produced for leaves, fruits or seed of *A. nilotica* (Boufennara et al., 2013; Tshabalala et al., 2013; Pal et al., 2015), *A. tortilis* (pal et al., 2015; Hassan et al., 2016), *A. senegal* (Pal et al., 2015), *A. concinna* (Patra et al., 2006); *A. albida* (Boufennara et al., 2013), *A. drepanolobium (Rubanza* et al., *2005), A. sieberiana* (Mlambo, 2009); *A. polyacantha* (Rubanza et al., 2005, 2007). Our study aimed to compare *A. nilotica* and *A. raddiana* commonly used for ruminant nutrition in sahelian area of Burkina Faso.

The results of the present study showed two very contrasted species in terms of chemical composition and phenol/tannin profiles. In terms of nutritional

value, *A. raddiana* showed lower CP content than *A. nilotica* (159 and 120 g/kg DM, respectively) but above the minimum for the nutritional requirements. According to Van Soest (1994), rumen microbiota requires a minimum of 70-80g crude protein /kg DM to optimize breakdown of cell wall components below which feed intake is reduced. Like all the legumes, the *Acacia* species contain high CP showing that the leaves of *A. nilotica* and *A. raddiana* would be a good source of protein to improve productivity of ruminant. Different values of CP were obtained in many previous studies with the same species. Boufennara *et al.* (2013) recorded higher CP content with *A. nilotica* (243g/kg DM) compare to our results. Pal *et al.* (2015) recorded for *A. nilotica* similar result with our result (159g/kg DM) but those recorded with *A. raddiana* were higher (153g/Kg DM). Despite having a lower CP content, *A. raddiana* showed more interesting fiber profile for ruminant nutrition when compared to *A. nilotica* which contain three times more ADL. *A. nilotica* contains more fiber than *A. raddiana.* This result was consistent with those recorded by Pal *et al.* (2015), but contrasted with the result of Rubanza *et al*. (2005) with the same species. MM values of the two *Acacia*, harvested in the same time were different with higher values for *A. raddiana* compared to *A. nilotica*. The differences of nutritional parameters could be the result of many factors as climate, type of soil, species, age of shrubs and stage of development. Tannins and phenol content of both species of *Acacia* were particularly interesting. Our result showed two contrasted plants in tannin content: *A. nilotica* was richer in CT than *A. raddiana*, while *A. raddiana* was richer in TT and TP than *A. nilotica*.

A. raddiana was more digestible than *A. nilotica* probably due to its lower fiber content, in particular in lignin and lower CT content. According to Patra and Saxena (2010), tannins have been implicated for their inhibitory effects on feed digestion, microbial population and enzyme activity in many experiments. In our study, the high level in TT contained in *A. raddiana* seems not to have impaired digestion. This could be due to the type of tannins (rather hydrolysable and not condensed). In the same time, the total GP production was similar among the two species. As a consequence, the PF which is OM truly degraded per ml of total gas produced was significantly higher for *A. raddiana* than for *A. raddiana*. This result suggests than degraded OM is less wasted as gas and more incorporated into microbial mass with *A. raddiana*. According to Blümmel *et al*. (1997), gas quantity produced in the rumen is inversely correlated to microbial yield, thus the PF value reflects variations in microbial biomass yield (Baba et al., 2002).

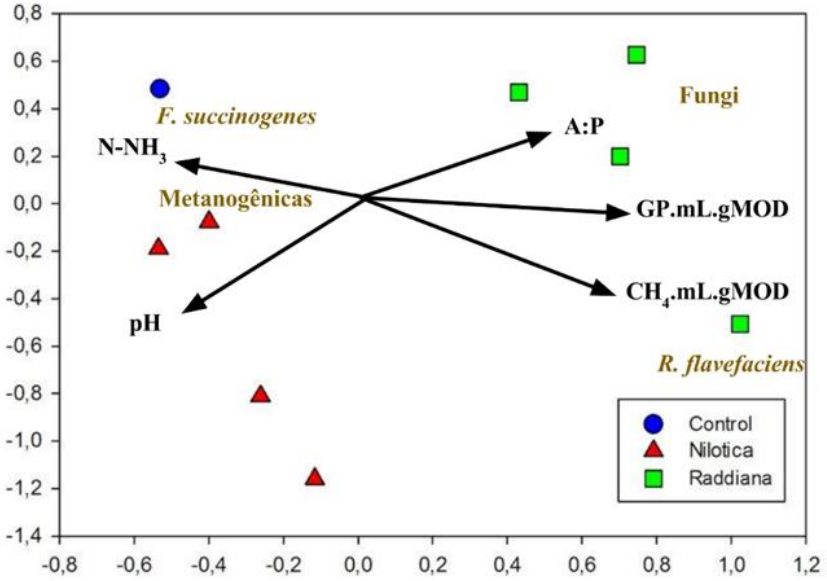

Fig 1. Distance-based redundancy analysis (RDA) of rumen microbial communities (*F. succinogenes, R flavefaciens*, archaea methanogens and fungi). Superimposed onto the ordination are correlations with two diferents diet (*A. raddiana* and *A. nilotica*) and control (Tifton-85), organic matter degradability (OMD) and acetate to propionate ratio (A:P) and methane production *in vitro*(CH4). Significant correlations are as $P<0.05$.

However the net CH_4 production (g/kg DM) was significantly lower with *A. raddiana* than with *A. nilotica*. This result contrasted with the result obtained by Pal *et al*. (2015) who observed for *A. nilotica* a greater inhibition of CH_4 production compared to *A. tortilis*. Tannins in general seem to act as methane mitigating, but effects between source (plant species) and experiments are still variable (Jayanegara et al., 2012). So, Patra *et al*. (2006), Abdalla *et al*. (2012), Bhatta *et al*. (2012), Anantasook *et al.* (2014) showed a decrease of CH_4 formation when tannin rich plant was added to a control diet. According to Makkar (2005), TP and TT were then good parameters to predict *in vitro* CH_4 formation. In our study, it seems that the TT content was more important to decrease CH_4 production than just CT content. Thus, tannins content in *A. raddiana* should be consisting mainly of hydrolysable tannins (HT) were also very active in this activity. This is consistent with recent work on the effect of ellagitannins on CH_4 production (Baert et al., 2016). HT had a greater effect in reducing methane emission with less adverse effect on digestibility than those of CT (Jayanegara et al., 2015). The action of tannins may be attributed to the direct inhibitory effects on methanogens and indirectly effects by decreasing fiber degradation (Patra and Saxena, 2010). These two types of tannins i) bind to fibers and decrease their degradability by ruminal microorganisms, diminishing the production of H_2, which is the limiting substrate of CH_4 by methanogens (Theodoridou et al., 2012, Jayanegara et al., 2015), ii) decrease H_2 in the rumen and affect protozoa-associated methanogens (Morgavi et al., 2010). It is interesting to note that the protozoa content in our study tended to be lower with *A. raddiana*, what could have played a role on CH_4 production. We observed that the CT-rich plant (*A. nilotica*), decreased dramatically the concentration of anaerobic fungi which primarily degrade the fiber components and the population of fibre-degrading bacteria (*F. succinogenes* and *R. flavefaciens*). On the other hand, HT-rich plant (*A. raddiana*) decreased the population of methanogens, the principal actors of methanogenesis. Ruminal protozoa as major H_2 producers host a certain proportion of the methanogens, and this association of protozoa and methanogens therefore contributes to CH_4 emissions (Beauchemin et al., 2008). In our study, lower concentrations protozoa and methanogens are consultant with the significant decrease of CH_4 production (*A. raddiana*). This observation is in compliance with those of Bhatta *et al*. (2012), as well as Goel and Makkar (2012) who reported the unidirectional relationship between protozoal numbers and methanogenesis.

Ammonia released during rumen fermentation is partly used for microbial protein synthesis. But, this production should not be in excess in the rumen as the surplus is excreted in the urine as urea. No significant difference was observed for NH_3 production but the values tended to be lower with *A. raddiana* than with *A. nilotica* consistent with lower CP content. According to Devant *et al.* (2000), ruminal ammonia N concentration depends on the amount of degradable protein. In this study, it seems that the effect of tannins to bind protein and reduce proteolysis in the rumen was not different between the two plants. The 5%-CT in *A. nilotica* seems to have a similar anti-proteolytic effect compared to the 39%-HT in *A. raddiana*. Similarly, difference on SCFA production was observed between the two species for butyrate production compared to control group. As the acetate/proprionate ratio (A:P) was similar for *A. raddiana* and *A. nilotica*, it is suggested that the HT effect was more important that the fermentation pathway to explain the differences observed in CH_4 production.

CONCLUSION

This study realized with *A. nilotica* and *A. raddiana* showed two contrasted plant in tannins and fiber contents which affect degradability of OM and CH_4 emission. *A. nilotica* had high lignin and CT contents that constituted an important constraint limiting the digestibility of plant material. The fermentation of *A. raddiana* led to lower protozoa and methanogens concentrations in the rumen than *A. nilotica* with a lower CH_4 production. The (A:P) ratio was not affected and the fact that, *A. raddiana* was more digestible than *A.nilotica* was rather connected to the fiber profile than tannin content. Our results indicate that TT had been the main driver to reduce CH_4 production. The difference of CP observed for both plants did not affect ammonia (NH_3-N) production. So, the inclusion of *A. raddiana* leaves as ingrediente of sheep diet should be an interesting tool to increase fiber digestion in the rumen, improving feed efficiency and reduce CH_4 production.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

Association of Official Analytical Chemists (AOAC), 1990. Association of Official Analytical Chemists, Official Methods of Analysis, vol. II, 15th ed. Arlington, VA, USA, 1141 p.

Association of Official Analytical Chemists (AOAC), 1995. Official Methods of Analysis, 16th ed, (Association of Official Analytical Chemists, Washington, DC, USA), 16 p.

Abdalla, A.L., Louvandini, H., Sallam, S.M.A.H., Bueno, I.C.S., Tsai, S.M., Figueira, A.V.O. 2012. In vitro evaluation, in vivo quantification, and microbial diversity studies of nutritional strategies for reducing enteric methane production. Tropical Animal Health and Production. 44: 953-964. DOI: 10.1007/s11250-011-9992-0

Anantasook, N., Wanapat, M., Cherdthong, A. 2014. Manipulation of ruminal fermentation and methane production by supplementation of rain tree pod meal containing tannins and saponins in growing dairy steers. Journal of Animal Physiology and Animal Nutrition. 98: 50-55. DOI: 10.1111/jpn.12029

Baba, A.S.H., Castro, F.B., Ørskov, E.R. 2002. Partitioning of energy and degradability of browse plants in vitro and the implications of blocking the effects of tannin by the addition of polyethylene glycol. Animal Feed Science and Technology. 95: 93-104.

Baert, N., Wilbert, F.P., Karonen, M and Salminen J.P. 2016. A study of structure-activity relationship of oligomeric ellagitannins on ruminal fermentation *in vitro*. Journal of Dairy Science. 99: 8041-8052. DOI: 10.3168/jds.2016-11069.

Beauchemin, K.A., Kreuzer, M., O'Mara, F., McAllister, T.A. 2008. Nutritional management for enteric methane abatement: a review. Australian Journal of Experimental Agriculture. 48: 21-27. DOI: 10.1071/EA07199.

Belem A.M.G, Nikiema Z.L., Sawadogo L., Dorchies Ph. 2000. Parasites gastro-intestinaux des moutons et risques d'infestation parasitaire des pâturages en saison pluvieuse dans la région centrale du Burkina Faso. Revue de Médecine Vétérinaire. 151: 437- 442.

Bhatta, R., Baruah, L., Saravanan, M., Suresh, K.P., Sampath, K.T. 2012. Effect of medicinal and aromatic plants on rumen fermentation, protozoa population and methanogenesis *in vitro*. Journal of Animal Physiology and Animal Nutrition. 97: 446-456. DOI: 10.1111/j.1439-0396.2012.01285.x.

Blümmel, M., Makkar, H.P.S., Becker, K. 1997. In vitro gas production: a technique revisited. Journal of Animal Physiology and Animal Nutrition. 77: 24-34.

Blümmel , M., Becker, K. 1997. The degradability characteristics of fifty-four roughages and roughage neutral-detergent fiber as described by in vitro gas production and their relationship to voluntary feed intake. British Journal of Nutrition. 77: 757-786. DOI: 10.1079/BJN19970073.

Boufennara, S., Bouazza, L., Bodas, R., Boussebou, H., López, S. 2013. Nutritive evaluation of foliage from some *Acacia* trees characteristic of Algerian arid and semi-arid areas. In: Ben Salem H, López-Fran cos A.(eds.). Feeding and management strategies to improve livestock productivity, CIHEAM/INRAT/OEP/IRESA/FAO. pp. 63-68.

Bueno, I.C.S., Cabral Filho, S.L.S., Gobbo, S.P., Louvandini, H., Vitti, D.M.S.S., Abdalla, A.L. 2005. Influence of inoculum source in a gas production method. Animal Feed Science and Technology. 123-124: 95-105. DOI: 10.1016/j.anifeedsci.2005.05.003.

Denman, S.E and Mc Sweeney, C.S. 2006. Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. FEMS Microbiology Ecology. 58: 572-582. DOI: 10.1111/j.1574-6941.2006.00190.x.

Denman, S.E., Tomkins, N.W., Mc Sweeney, C.S. 2007. Quantitation and diversity analysis of ruminal methanogenic populations in response to the anti-methanogenic compound bromochloromethane. FEMS Microbiology Ecology**.** 62: 313-322. DOI: 10.1111/j.1574-6941.2007.00394.x.

Devant, M., Ferret, A., Gasa, J., Calsamiglia, S., Casals, R. 2000. Effects of protein concentration and degradability on performance, ruminal fermentation, and nitrogen metabolism in rapidly growing heifers fed high-concentrate diets from 100 to 230 kg body weight. Journal of Animal Science. 78: 1667-1676. DOI: 10.2527/2000.7861667x.

Getachew, G., Makkar, H.P.S., Becker, K. 2002. Tropical browses: contents of phenolic compounds, in vitro gas production and stoichiometric relationship between short chain fatty acid and in vitro gas production. Journal of Agricultural Science. 139: 341-352. DOI: 10.1017 S0021859602002393

Goel, G., Makkar, H.P.S. 2012. Methane mitigation from ruminants using tannins and saponins, a status review. Tropical Animal Health and

Production. 44: 729-739. DOI: 10.1007/s11250-011-9966-2.

Hassan E., Ahmed M. R. 2016. Chemical composition and nutritional evaluation of the seeds of *Acacia tortilis* (Forssk.) Hayne ssp. *raddiana*. Food Chemistry**.** 200: 62-68. DOI: 10.1016/j.foodchem.2016.01.019

Jayanegara, A., Leiber, F., Kreuzer, M. 2012. Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from in vivo and in vitro experiments. Journal of Animal Physiology and Animal Nutrition. 96: 365-375. DOI: 10.1111/j.1439-0396.2011.01172.x.

Jayanegara, A., Goel, G., Makkar, H.P.S., Becker, K. 2015. Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population in vitro. Animal Feed Science and Technology. 209: 60-68. DOI: 10.1016/j.anifeedsci.2015.08.002.

Kamra, D.N., Sawal, R.K., Pathak, N.N.,Kewalramani, N., Agarwal, N. 1991. Diurnal variation in ciliate protozoa in the rumen of blackbuck (*Antilope cervicapra*). Letters in Applied Microbiology. 13: 165-167. DOI: 10.1111/j.1472-765X.1991.tb00598.x

Livak, K.J., Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2 (-ΔΔCt) method. Methods. 25: 402-408. DOI: 10.1006/meth.2001.1262.

Longo, C., Bueno, I.C.S., Nozella, E.F., Goddoy, P.B., Cabral Filho, S.L.S., Abdalla, A.L. 2006. The influence of head-space and inoculums dilition *in vitro* ruminal methane measurement. International Congress Serie. 1293: 62-65. DOI: 10.1016/j.ics.2006.03.017.

Makkar, H.P.S., Blümmel, M., Borowy, N.K., Becker, K. 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. The Journal of Food Science and Technology. 61: 161-165. DOI: 10.1002/jsfa.2740610205.

Makkar, H.P. 2003. Quantification of tannins in tree and shrub foliage: a laboratory manual. Animal Production and health Section. Joint FAO/IAEA Division of nuclear Technique in Food and Agriculture. International Atomic Energy Agency, Vienna, Austria.

Makkar, H.P.S. 2005. In vitro gas methods for evaluation of feeds containing phytochemicals. Animal Feed Science and Technology. 123-124: 291-302. DOI: 10.1016/j.anifeedsci.2005.06.003.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing beakers or crucibles: collaborative study. Journal of the Association of Official Analytical Chemists. 85: 1217-1240.

Min, B.R., Barry, T.N, Attwood, G.T, McNabb, W.C. 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Animal Feed Science and Technology. 106: 3-19. DOI:10.1016/S0377-8401(03)00041-5.

Mlambo, V., Mould, F. L, Smith, T., Owen, E., Sikosana, J. L. N., Mueller-Harvey, I. 2009. *In vitro* biological activity of tannins from *Acacia* and other tree fruits: Correlations with colorimetric and gravimetric phenolic assays. South African Journal of Animal Science. 39: 131-143.

Morgavi, D.P., Forano, E., Martin, C., Newbold, C.J. 2010. Microbial ecosystem and methanogenesis in ruminants. Animal. 4: 1024-1036. DOI: 10.1017/S1751731110000546.

Pal, K., Patra, A.K., Sahoo, A., Kumawat, P.K. 2015. Evaluation of several tropical tree leaves for methane production potential, degradability and rumen fermentation in vitro. Livestock Science. 180: 98-105. DOI: 10.1016/j.livsci.2015.07.011.

Patra, A.K., Kamra, D.N., Agarwal, N. 2006. Effect of plant extract on in vitro methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo, Animal Feed Science and Technology. 128: 276-291. DOI: 10.1016/j.anifeedsci.2005.11.001.

Patra, A.K., Saxena J. 2010. A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. Phytochemistry. 71: 1198-1222. DOI: 10.1016/j.phytochem.2010.05.010

Rubanza, C.D.K., Shem, M.N., Otsyina, R., Bakengesa, S.S., Ichinohe, T., Fujihara, T. 2005. Polyphenolics and tannins effect on in vitro digestibility of selected *Acacia* species leaves. Animal Feed Science and Technology. 119: 129-142. DOI: 10.1016/j.anifeedsci.2004.12.004

Rubanza, C.D.K., Shem, M.N., Otsyina, R., Bakengesa, S.S., Ichinohe, T., Fujihara, T. 2007. Effects of *Acacia nilotica*, *A. polyacantha* and *Leucaena leucocephala* leaf meal supplementation on performance of Small East African goats fed native pasture hay basal forages. Small Ruminant Research. 70: 165-173. DOI: 10.1016/j.smallrumres.2006.02.008.

Sallam, S., Bueno, I., Nasser, M.E., Abdalla, A.L. 2010. Effect of eucalyptus (*Eucalyptus citriodora*) fresh or residue leaves on methane emission in vitro. Italian Journal of Animal Science. 9: 299-303. DOI: 10.4081/ijas.2010.e58.

Sawadogo, B.C. 2011. Valorisation des connaissances locales en élevage au Burkina Faso : Pratiques et contraintes de l'élevage des petits ruminants par diagnostic participatif à *Borguiendé* dans la province du Soum. Rapport de fin de stage de 1ère année/Cycle des Conseillers d'Élevage. École Nationale de l'Élevage et de la Santé Animale (E.N.E.S.A), Ouagadougou, Burkina Faso. pp. 25.

Soltan, Y.A., Morsy, A.S., Sallam, S.M.A, Louvandini, H., Abdalla, A.L. 2012. Comparative in vitro evaluation of forage legumes (*Prosopis*, *Acacia*, *Atriplex*, and *Leucaena*) on ruminal fermentation and methanogenesis. Journal of Animal Feed Science. 21: 759-772. DOI: 10.22358/jafs/66148/2012.

Theodoridou, K., Aufrere, J., Niderkorn, V., Andueza, D., Le Morvan, A., Picard, F., Baumont, R. 2011. In vitro study of the effects of condensed tannins in sainfoin on the digestive process in the rumen at two vegetation cycles. Animal Feed Science and Technology. 170: 147-159. DOI: 10.1016/j.anifeedsci.2011.09.003.

Tshabalala, T., Sikosana, J.L.N., Chivandi, E. 2013. Nutrient intake, digestibility and nitrogen retention in indigenous goats fed on *Acacia nilotica* fruits treated for condensed tannins. South African Journal of Animal Science. 43: 457-463. DOI: 10.4314/sajas.v43i4.2.

Van Soest, P.J. 1994. Nutritional ecology of the ruminant. Cornell University Press Patra, A. K., Saxena, J., 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. Journal of the Science of Food and Agriculture. 91: 24–37.

Zabré Geneviève, Kaboré Adama, Bayala Balé, Tamboura H. Hamidou, Belem Adrien Marie Gaston, Niderkorn Vincent, Costa Junior Martin Livio, Louvandini Helder, Hoste Hervé. Botanical and Ethnoveterinary Surveys of Two *Acacias* (*Acacia raddiana* and *Acacia nilotica*) Exploited in Small Ruminant Rearing in Sahelian Area of Burkina Faso. Animal and Veterinary Sciences. 5: 63-68. DOI: 10.11648/j.avs.20170505.11.