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Short Communication

In vitro digestion characteristics of various combinations of elephant grass hay, gliricidia hay or silage, soybean meal and corn meal in rations for sheep

Características de la digestión in vitro de varias combinaciones de heno del pasto elefante, heno o ensilaje de gliricidia, harina de soya y harina de maíz en raciones para ovinos

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Abstract

This study examined fermentation rates and kinetics of sheep rations based on combinations of elephant grass hay, gliricidia (*Gliricidia sepium*) hay or silage, soybean meal and corn meal using in vitro techniques. Three rations were prepared, namely: Control (elephant grass hay + soybean meal + corn meal); gliricidia hay (elephant grass hay + soybean meal + corn meal + gliricidia hay); and gliricidia silage (elephant grass hay + soybean meal + corn meal + gliricidia silage). A fixed ratio of roughage:concentrate of 55:45 was maintained for all rations, which were isocaloric and designed to support sheep gains of 200 g/day. The gliricidia replaced 57.6% of the soybean meal in the rations containing gliricidia and 81.8% of the elephant grass hay. Fermentation rates and kinetics, in vitro dry matter digestibility (IVDMD) and degradability of the rations were evaluated. Rations containing gliricidia as both hay and silage had higher (P<0.05) IVDMD than the Control ration (67.8 and 66.2 vs. 59.8%). The degradability of the ration containing gliricidia hay was higher (P<0.05) than that of the gliricidia silage ration (57.8 vs. 50.5%), whereas the Control showed an intermediate value (54.4%). The ration containing gliricidia hay and the Control produced more gas in the first 24 h than the ration containing gliricidia silage, and the gliricidia hay ration showed the shortest colonization time. Peak gas production occurred for the ration with gliricidia silage later than for the other rations. The study showed that substituting soybean meal with preserved gliricidia can result in higher digestibility of sheep rations. Feeding studies with animals are now warranted to verify these laboratory findings.

Keywords: Degradability, digestibility, gas production, *Gliricidia sepium*, sheep farming, tropical legumes.

Resumen

En el estudio se determinaron las tasas y la cinética de fermentación ruminal de raciones para ovinos basadas en combinaciones de heno de pasto elefante, heno o ensilaje de gliricidia (*Gliricidia sepium*), harina de soya y harina de maíz, utilizando técnicas in vitro. Se prepararon tres raciones: Control (heno de pasto elefante + harina de soya + harina de maíz); heno de gliricidia (heno de pasto elefante + harina de soya + harina de maíz + heno de gliricidia); y ensilaje

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de gliricidia (heno de pasto elefante + harina de soya + harina de maíz + ensilaje de gliricidia). Se mantuvo una proporción fija de forraje:concentrado de 55:45 para todas las raciones las cuales fueron isocalóricas y diseñadas para producir ganancias diarias de 200 g peso vivo de ovinos. En ambos tratamientos con gliricidia el 57.6% de la harina de soya fue reemplazado y el 81.8% del heno de pasto elefante. Se evaluaron las tasas y cinética de la fermentación ruminal, la digestibilidad in vitro de la materia seca (IVDMD) y la degradabilidad de las dietas. Las raciones que contenían gliricidia como heno y ensilaje tuvieron una IVDMD más alta (P<0.05) que el Control (67.8 y 66.2 vs. 59.8%). La degradabilidad de la ración con heno de gliricidia fue mayor (P<0.05) que la de la ración con ensilaje de gliricidia (57.8 vs. 50.5%), mientras que el Control presentó un valor intermedio (54.4%). La ración con heno de gliricidia y el Control produjeron más gas en las primeras 24 horas que la ración con ensilaje de gliricidia, y la ración con heno de gliricidia mostró el menor tiempo de colonización. La producción máxima de gas ocurrió en la ración con ensilaje de gliricidia más tarde que en las otras raciones. El estudio mostró que sustituyendo la harina de soya por forraje preservado de gliricidia puede resultar en una digestibilidad más alta de raciones para ovinos. Para corroborar estos resultados obtenidos a nivel de laboratorio se requieren estudios de alimentación con animales.

Palabras clave: Degradabilidad, digestibilidad, ganadería ovina, *Gliricidia sepium,* producción de gas.

Introduction

Pasture-based production systems are limited primarily by variations in climatic conditions, which directly interfere with plant growth (Euclides et al. 2019), resulting in negative impacts on animal performance, especially during the dry season (Emerenciano Neto 2018; Braga et al. 2019). One possible solution is to conserve forage in periods of high availability to be used during times of scarcity (Bueno et al. 2018), along with feeding of protein supplements.

The use of high-protein feedstuffs in sheep rations is a common practice worldwide and soybean meal ranks highly as a protein source. Since soybeans are also used for human consumption, costs of this product for feeding livestock are high. Identifying a less expensive plant-derived protein would be of great benefit to ruminant production worldwide.

Shrub legumes are possible options as alternative sources of fodder which are high in protein, e.g. gliricidia (*Gliricidia sepium*), which grows well in tropical climates and is relatively drought-tolerant. Since its chemical and productive characteristics are similar to those of other leguminous species, it is a viable option for animal feeding, especially in regions where water deficit is a constant problem (Fernandes et al. 2017; Santana et al. 2019; Fernandes et al. 2020). Incorporating it in rations should allow a reduction in the level of soybean meal required to supply the protein needs of the sheep.

As a prelude to conducting feeding trials with animals, which also are expensive, in vitro studies in the laboratory can provide preliminary data on likely outcomes from feeding various rations. The in vitro gas production method described by Theodorou et al. (1994) consists of incubating samples of feedstuffs in bottles attached to a gas meter. According to Tagliapietra et al. (2010), the gas released from feedstuffs inoculated with rumen fluid reflects microbial activity, as gas is a product of fermentation.

We conducted this study to obtain preliminary data on the fermentation rates and kinetics, dry matter digestibility and degradability of 3 sheep rations made up of mixtures of hay of elephant grass (*Cenchrus purpureus* syn. *Pennisetum purpureum*), gliricidia as hay or silage, soybean meal and corn meal using in vitro techniques.

Materials and Methods

The experiment was conducted at the Laboratories of Animal Nutrition and Rumen Fermentation at the Department of Animal Science (DZO), Federal University of Sergipe (UFS), Aracaju, Sergipe. It was set up as a completely randomized design with 3 treatments, i.e. 3 rations formulated for sheep, namely: Control – basal ration of elephant grass hay-soybean meal-corn meal; gliricidia hay – elephant grass hay-gliricidia hay-soybean meal-corn meal with only 42.6% of the soybean meal supplement contained in Control; and gliricidia silage – elephant grass hay-gliricidia silage-soybean meal-corn meal with 42.6% of the soybean meal supplement contained in Control. These rations were formulated for sheep to achieve an estimated dry matter intake of 3.5% of body weight and 200 g/day liveweight gain (NRC 2007) and are described in Table 1. Since the roughage:concentrate ratio was fixed at 55:45, including gliricidia hay or silage reduced percentage of elephant grass hay and soybean meal in the ration, while percentage of corn meal was increased. The concentrations of crude protein in elephant grass hay, gliricidia hay and gliricidia silage, soybean meal and corn meal were, respectively: 12.9; 15.7; 17.5; 42.5 and 8.4% on a dry matter basis.

Table 1. Proportions of ingredients and nutrient concentrations in the experimental rations.

	Ration		
	Control	GH	GS
Ingredient (% DM basis)			
Elephant grass hay	55.0	10.0	10.0
Gliricidia hay	-	45.0	-
Gliricidia silage	-	-	45.0
Soybean meal	23.5	10.0	10.0
Corn meal	21.5	35.0	35.0
Nutrient concentration (% DM)			
Organic matter	93.9	94.7	93.9
Crude protein	21.7	18.3	18.3
Hemicellulose	28.2	30.3	27.0
Cellulose	28.6	22.0	21.2
Lignin	2.6	6.6	8.2

GH = Gliricida hay; GS = Gliricidia silage.

The gliricidia used to produce the hay and silage was obtained from trees at approximately 12 months after planting, by cutting and selecting tender branches (≤8 mm thick) with leaves, from the EVA (Interdisciplinary Space of Agroecological Experience) area adjacent to the Department of Animal Science (DZO) at UFS. The forage was then chopped to produce an average particle size of 2 cm, spread on plastic sheeting in the sun and turned every 30 min. After 2 days, the hay was bagged. The elephant grass was cut approximately 5 cm above ground level at 45 days growth and the hay-making process was the same as that for gliricidia.

Three experimental PVC silos 10 cm in diameter and 30 cm long with PVC caps at each end were used, and were sealed with metal clips. The fresh chopped gliricidia was compacted in these silos to a specific mass of 600 kg/m^3. To ensure anaerobic conditions in the silos, adhesive tape was used to promote better sealing than with the metal clips alone. The silos were weighed before and just after sealing. There was a layer of sand at the bottom of the silo to retain effluents and prevent contamination of the silage; a permeable mesh between the silage and the sand layer prevented contact between the sand and the silage.

In vitro gas production from the various rations was determined in accordance with the methodology described by Theodorou et al. (1994). To this end, after being dried in a forced-air oven, ration samples were ground through a Wiley mill with a 5-mm sieve.

Rumen fluid was collected from 3 sheep maintained on a diet of corn, soybean meal and fresh gliricidia and samples were mixed/bulked to form the rumen inoculum, which was filtered through gauze and stored in a thermos previously heated in water at 38 ºC. A constant flow of CO_2 was maintained during the preparation process.

For each ration 5 samples were incubated in penicillin-type glass bottles with a capacity of 100 mL. Each bottle contained 670 mg of sample in 67 mL of incubation solution. The incubation solution was prepared as described by Theodorou et al. (1994) using cysteine-HLC as a reducing agent (Mould et al. 2005).

Rumen fluid formed 20% of the incubation solution. A constant flow of CO_2 was maintained. Each bottle was inoculated manually by using a graduated syringe, and bottles were closed with rubber corks (14 mm), sealed with an aluminum seal and kept in a water bath at 39 ºC. In addition to the bottles with the samples, an additional 4 bottles containing incubation medium without samples (blanks) were evaluated.

Gas production was measured for a period of 48 h, and the pressure within the bottle was recorded with a digital manometer coupled to a 3-way valve. Immediately after the pressure readings, gas volume was measured using a graduated syringe attached to the valve. The syringe plunger was extracted until the transducer pressure returned to zero.

Gas volume and pressure data were tabulated to obtain the linear, quadratic and cubic statistical models and then determine the correlation between gas volume and pressure reading using Excel software. The model considered satisfactory was that which showed the highest R^2 value ($R^2 = 0.95$).

The following equation was obtained:

$$y = -0.382x^2 + 6.087x - 0.772,$$

where:

y is the final gas volume in mL; and

x is the gas pressure in kilopascal at the respective times.

Gas production on each occasion was corrected for the average gas production from bottles containing the incubation medium without diet samples. Mean gas production volumes for each treatment at the respective incubation times were adjusted to the 2-compartment logistic model proposed by Pell and Schofield (1993), as follows:

$$TV = Vf1/(1 + \exp(2 - 4*c1*(T - L))) + Vf2/(1 + \exp(2 - 4*c2*(T - L))),$$

where:

TV = total gas volume (mL/100 mg DM) accumulated by time T;

Vf1 = gas volume (mL) of the rapidly digested fraction;

c1 = degradation rate of the rapidly digested fraction (L/h);

L = lag time, or fiber colonization time (h);

Vf2 = gas volume (mL) of the slowly digested fraction; and

c2 = degradation rate of the slowly digested fraction (L/h).

After 48 hours of incubation, the contents of the bottles (which had been tared previously) were filtered through non-woven fabric ('TNT') of known dimensions. Dry matter degradability was determined based on the difference between the constant weight obtained by drying at 105 ºC and the weight of the incubated material. To determine the in vitro dry matter digestibility (IVDMD), the residue was washed with neutral detergent and weighed after drying at 105 ºC in a forced-air oven for 24 h and expressed as a percentage of the initial weight of the sample. The ash concentration in the residue was determined by method 942.05 of AOAC (1990).

The parameters estimated by the mathematical model were obtained using iterative non-linear methods. Results were adjusted by least-squares estimates, adopting the Marquardt method, by the PROC NLIN procedure of SAS statistical package (SAS Institute Inc., Cary, NC, USA). Data pertaining to degradability, digestibility and residual ash were subjected to analysis of variance and means were compared by Tukey's test at the 5% significance level.

Results

Rations containing gliricidia had higher IVDMD levels than the Control (P<0.001; Table 2). While degradability of the ration containing gliricidia hay was higher than that of the ration containing gliricidia silage (P = 0.015), degradability of the Control was intermediate.

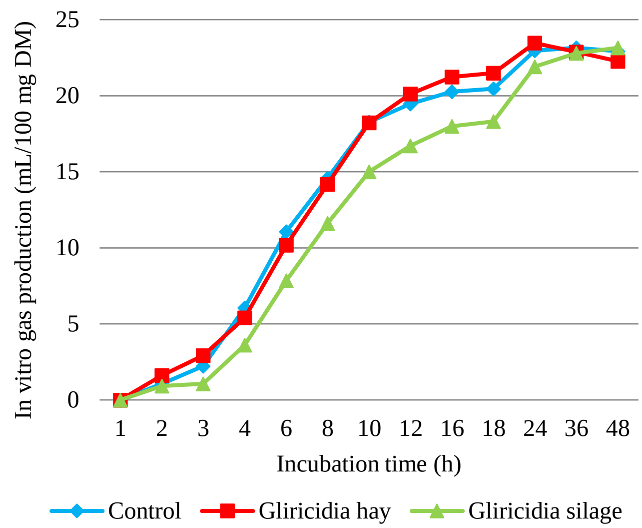
Table 2. In vitro dry matter digestibility (IVDMD) and degradability of rations comprised of varying combinations of elephant grass hay, gliricidia hay or silage, soybean meal and corn meal designed to produce 200 g/d gain in sheep.

	Ration			P value	s.e.m.
	Control	GH	GS		
IVDMD (%)	59.8b	67.8a	66.2a	<0.001	0.70
Degradability (%)	54.4ab	57.8a	50.5b	0.015	1.59

Means within rows followed by different letters differ (P<0.05) according to Tukey's test. Control (elephant grass hay + soybean meal + corn meal); GH: gliricidia hay treatment (elephant grass hay + soybean meal + corn meal + gliricidia hay); GS: gliricidia silage treatment (elephant grass hay + soybean meal + corn meal + gliricidia silage).

Significant (P<0.05) differences in accumulation of gas over time were observed for the different rations. The Control treatment and the ration containing gliricidia hay produced more gas in the first 24 h than the ration containing gliricidia silage, while the ration containing gliricidia hay showed the shortest colonization time. Peak gas production occurred later for the ration containing gliricidia silage (Figure 1).

The ration containing gliricidia silage produced the smallest volume of gas from the highly soluble fraction (12.4 mL/100 mg of incubated DM) and showed the longest fiber colonization (lag) time (3.8 h). This ration also produced the largest volume of gas from the lowly soluble fraction (10.7 mL/100 mg of incubated DM).



Control: $GP = 15.6185/\{1 + \exp^{[2+4*0.1925*(2.8873-Time)]}\} + 7.4599/\{1 + \exp^{[2+4*0.05*(2.8873-Time)]}\}$, $R^2 = 0.99$;
Hay: $GP = 15.5116/\{1 + \exp^{[2+4*0.1608*(2.7619-Time)]}\} + 7.2305/\{1 + \exp^{[2+4*0.0677*(2.7619-Time)]}\}$, $R^2 = 0.99$;
Silage: $GP = 12.4415/\{1 + \exp^{[2+4*0.2037*(3.8231-Time)]}\} + 10.6961/\{1 + \exp^{[2+4*0.0438*(3.8231-Time)]}\}$, $R^2 = 0.99$.

Figure 1. In vitro gas production for Control (elephant grass hay + soybean meal + corn meal), gliricidia hay treatment (elephant grass hay + soybean meal + corn meal + gliricidia hay) and gliricidia silage treatment (elephant grass hay + soybean meal + corn meal + gliricidia silage) following incubation in buffered rumen fluid.

Discussion

This study suggests that using gliricidia hay or silage to replace some of the soybean meal and elephant grass hay components of an elephant grass-soybean meal-corn meal ration (Control) for growing sheep could result in an increase in IVDMD of the ration, while degradability of the ration might be improved only in the case of gliricidia hay.

The lower IVDMD of the Control ration could be a function of a higher proportion of cellulose provided by the elephant grass hay in this ration (Table 1), since this component reduces both the level and rate of fiber degradation (Díaz et al. 2018). In general, tropical forages are poorly digestible (Gerdes et al. 2000; Oliveira et al. 2017), which is due mainly to the high concentration of cell wall components such as cellulose and low concentration of potentially digestible compounds such as non-fibrous carbohydrates, proteins, ether extract, vitamins and minerals (Oliveira et al. 2017). In the case of elephant grass hay the material used was whole plant material cut at 45 days of age, while the gliricidia was comprised of only fine stems and leaves. Inclusion of gliricidia in the ration allowed the proportion of soybean meal to be reduced, while the proportion of corn increased, increasing the supply of soluble carbohydrates to the population of microorganisms, which may have increased microbial activity.

The higher degradability of the ration including gliricidia hay could be a consequence of longer degradation time for the gliricidia hay, given the shorter colonization time on the particles by the incubated microorganisms. On the other hand, the lower degradability of the ration containing gliricidia silage may be a reflection of fermentation of carbohydrates during the ensiling process, since soluble carbohydrates serve as substrates for the growth of anaerobic bacteria. This, in turn, prompts a decline in the pH of the medium, resulting in the preservation of the material (Gomes et al. 2018; Santana et al. 2019). As a consequence, the levels of non-fibrous carbohydrates (NFC) (a dietary component with higher rumen degradation rates; Oliveira et al. 2017) decrease during the ensiling process (Ribeiro et al. 2014). Conversely, the higher gas production during the initial 24 h of fermentation from both the Control ration and that containing gliricidia hay was likely a consequence of their higher NFC concentration in comparison with the gliricidia silage diet, resulting in increased IVDMD. Oliveira et al. (2017) measured in vitro gas production from a range of forage plants and observed that higher fermentation rates within the first hours of incubation were detected in plants with higher soluble carbohydrate concentrations.

The different results for digestibility found in this study between the Control ration and rations containing gliricidia (hay or silage) demonstrate the beneficial effects of this plant, since incorporating gliricidia in the ration increased the amount of digested material without affecting gas production.

Since NFCs are considered the main substrate for lactic fermentation within the silo, their concentration decreases throughout the ensiling process (Zardin et al. 2017). The ration containing gliricidia silage showed longer fermentation times and higher gas production from the fibrous material, which is considered a slowly degraded component.

The time taken by dietary microorganisms to colonize the material influences gas production, since this parameter determines how long the rumen microorganisms will act on the substrate. Thus, peak production may be achieved sooner or later (Díaz et al. 2018). The shorter colonization time observed in the ration containing gliricidia hay allowed the microorganisms to act for a longer period, with peak production occurring sooner in this treatment.

The ration containing gliricidia silage showed the lowest values for fermentation parameters, which may be explained by its higher lignin and lower hemicellulose concentrations (Table 1). Lignin present in the cell wall complexes itself to carbohydrates (mainly hemicellulose) through covalent bonds, forming a mechanical barrier to rumen microorganisms, thus reducing the fermentation of carbohydrates (Oliveira et al. 2017; Díaz et al. 2018). This fact also explains why the Control and gliricidia-hay rations presented shorter lag phases, higher gas volumes and higher rates of gas production from rapidly digested fractions.

Conclusions

Incorporating gliricidia as hay or silage in traditional sheep rations based on elephant grass hay, soybean meal and corn meal should allow a reduction in amounts of elephant grass and more importantly soybean meal in the ration. These rations should also be more digestible than the traditional ones, which should result in better animal performance. Added to this, reducing the proportion of expensive soybean meal should lower the cost, while increasing the corn meal component would cancel out some cost advantages.

Further studies with sheep to determine feed intakes and animal performance on these or similar rations are needed to confirm if these laboratory findings can be reflected in improved production. Other shrub legumes might also be used depending on availability. If feeding studies are successful, the likely financial benefits to farmers would depend on the relative costs of the ration components, i.e. elephant grass hay, gliricidia hay or silage and soybean meal and corn meal.

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