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In vitro rumen fermentation and digestibility of silages of *Sorghum bicolor* and *Cajanus cajan*

Fermentación y digestibilidad ruminal *in vitro* de ensilados de *Sorghum bicolor* y *Cajanus cajan*

Esaú Pérez-Luna[1*ID], Samuel Albores-Moreno[2 ID], Alexis Ruíz-González[4 ID], Sergio Espinosa-Villafuerte[5 ID], José Alayón-Gamboa[3 ID], Benigno Ruíz-Sesma[6 ID]

[1]Universidad Autónoma de Chiapas, Facultad de Ciencias Agronómicas, Carretera Ocozocoautla-Villaflores km. 84.5. C.P. 30470. Chiapas, México. [2]El Colegio de la Frontera Sur, Departamento de Agricultura, Sociedad y ambiente, Ganadería Sustentable y Cambio Climático, Carretera Panamericana y Periférico Sur s/n. C.P. 29290. Barrio María Auxiliadora, San Cristóbal de Las Casas, Chiapas, México. [3]El Colegio de la Frontera Sur, Departamento de Conservación de la Biodiversidad, Avenida Rancho, Polígono 2-A, Ciudad Industrial. C.P. 24500. Lerma, Campeche, México. [4]Département des Sciences Animales, Université Laval, Québec, QC, Canada. [5]Consultor de Qualisilo S. A. de C.V., Calle Lyon número 160, Fraccionamiento La Salle segunda sección. C.P. 29070. Tuxtla Gutiérrez, Chiapas, México. [6]Universidad Veracruzana, Área Biológico Agropecuaria, Ingeniería en Sistemas de Producción Agropecuaria. *Author for correspondence: Esaú Pérez Luna. Universidad Autónoma de Chiapas (UNACH), Facultad de Ciencias Agronómicas, Carretera Ocozocoautla-Villaflores km. 84.5. C.P. 30470 Chiapas, México. Email: eperezl@unach.mx, samuel.albores@ecosur.mx, alexis.ruiz-gonzalez.1@ulaval.ca, sergio.zootecnista82@gmail.com, jalayon@ecosur.mx, ruizsb71@gmail.com

ABSTRACT

The dual-purpose cattle production system is the most predominant in the tropics, characterized by an extensive grazing system of native or introduced grasses with low digestibility and high concentrations of structural carbohydrates, causing with this a low productive efficiency of the systems, in this scenario, it is necessary to look for alternatives that improve productive efficiency. The objective was to evaluate the anaerobic fermentation and rumen digestibility *in vitro* of dry matter (DIVDM) of silages of *S. bicolor* and *C. cajan*. Four substrates (sorghum, sorghum + *C. cajan*, sorghum + *C. cajan* + inoculant) and four anaerobic fermentation times (0, 30, 60 and 90 days) were used in the treatments, in a completely randomized design with a 4x4 factorial arrangement, with three replications. The average for DM, OM, CP, NDF and ADF was 373.9±41.43; 960.58±7.59; 87.82±24.95; 588.73±51.17; 371.76±61.39 g kg^{-1} DM. For DIVDM it was 56.32±9.05 %. Significant differences ($P<0.05$) were found for fermentation parameters and DIVDM. The maximum volume of gas was presented in treatments containing only sorghum and sorghum + *C. cajan* + inoculant. The fractions of rapid fermentation were higher ($P<0.05$) in the treatments sorghum + *C. cajan* + inoculant. The fractions of medium and slow fermentation were higher in sorghum and sorghum + *C. cajan* + inoculant. It is concluded that the *C. cajan* plant alone or combined has a negative effect on DIVDM.

Keywords: sorghum, *Cajanus cajan*, inoculant, anaerobic fermentation, *in vitro* digestibility.

RESUMEN

El sistema de producción bovino de doble propósito es el más predominante en el trópico, caracterizado por un sistema de pastoreo extensivo de gramíneas nativas o introducidas con baja digestibilidad y altas concentraciones de carbohidratos estructurales, provocando con esto una baja eficiencia productiva de los sistemas, ante este escenario, es necesario buscar alternativas que mejoren la eficiencia productiva. El objetivo fue evaluar la fermentación anaeróbica y digestibilidad ruminal *in vitro* de la materia seca (DIVMS) de los ensilados de *S. bicolor* y *C. cajan*. En los tratamientos se utilizaron cuatro sustratos (sorgo, sorgo + *C. cajan*, *C. cajan*, sorgo + *C. cajan* + inoculante) y cuatro tiempos de fermentación anaeróbica (0, 30, 60 y 90 días), en un diseño completamente al azar con un arreglo factorial 4x4, con tres repeticiones. El promedio de MS, MO, PC, FDN y FDA fue 373.9±41.43; 960.58±7.59; 87.82±24.95; 588.73±51.17; 371.76±61.39 g kg^{-1} de MS. Para la DIVMS fue de 56.32±9.05%. Se encontró diferencias significativas ($P<0.05$) para los parámetros de fermentación y DIVMS. El volumen máximo de gas se presentó en los tratamientos que contenían únicamente sorgo y sorgo + *C. cajan* + inoculante. Las fracciones de rápida fermentación fueron mayores ($P<0.05$) en los tratamientos sorgo + *C. cajan* + inoculante. Las fracciones de fermentación media y lenta fueron mayores en sorgo y sorgo + *C. cajan* + inoculante. Se concluye que la planta de *C. cajan* sola o combinada presenta un efecto negativo en la DIVMS.

Palabras clave: sorgo, guandú, inoculante, fermentación anaeróbica, digestibilidad *in vitro.*

INTRODUCTION

Cattle raising is one of the main activities of the agricultural sector in Mexico, which is carried out under different conditions and production systems (García *et al.,* 2015). In this sense, one of the most common production systems, is dual-purpose cattle production under grazing of extensive grassland regions (Estrada *et al*., 2018) with native or introduced grasses (Kú *et al*., 2013). However, the nutritive quality of forages shows a low concentration of crude protein and high concentration of structural carbohydrates, which induces low digestibility (Piñeiro *et al*., 2017). In addition, due to climatic conditions, forage biomass produced is limited, particularly during the dry season (low rainfall and high temperatures), which has an impact on forage availability favoring seasonality in milk and meat production (García, 2018).

This has prompted the search for new forage materials or feeding strategies that meet the nutritional needs of animals and with this establish a uniform harvesting system ensuring a constant production level throughout the year. In addition to adverse climatic conditions, inadequate management of native and introduced pastures in tropical regions has been considered one of the main problems limiting the productivity of livestock enterprises (Kú *et al*., 2013). An animal load higher than the carrying capacity of the pasture and the inefficient management of the forage produced, cause the pasture overutilization and lead to overgrazing favoring the invasion of weeds or grasses of lower forage value, and severe nutritional deficiencies in the soil (Villanueva, 2015).

Recently, strategies have been proposed to counteract these effects (seasonality of forage production, low nutritional quality and effects on the soil); such as paddock segregation, conservation of forage surpluses during the rainy season, in addition, all

agroforestry, agropastoral, agrosilvopastoral modalities or through the intensive silvopastoral system (SSPi) (González *et al*., 2015).

In recent years, it has suggested the establishment of Integrated Agricultural Production Systems (SIPA, according its acronyms in Spanish) that are based on agricultural, livestock and forestry activities, carried out in the same area. They are associated crops, succession or rotation, which seek synergistic effects between the components of the agroecosystem, contemplating environmental suitability, producer valorization and economic viability (Balbino *et al*., 2011). Consequently, the soil-plant-climate-animal interrelationship is improved, which contributes to specifically determine the habitat of each of the organisms that make up the biological and energy flow systems, whether they belong to agricultural, livestock or forestry systems (Torres *et al*., 2007).

SIPA can integrate livestock-forestry activities (IGF) or silvopastoral: it integrates the livestock component (grazing and animal) and forestry, in consortium. This system is more focused on areas with crop planting restrictions, including only the forestry and livestock components in the same area. These can be used as a feeding strategy for grazing, cutting and hauling, and can be conserved (silage) for use during periods of low water (Mannetje, 1982).

Chiapas, there are soil and climatic characteristics for the establishment and use of this strategy, which will improve cattle production. However, little information has been generated on the associations of tropical forages that have been recently integrated into ruminant feeding systems.

Therefore, it is essential to investigate the crop-livestock or agro-pastoral association: a production system that integrates the agricultural and livestock components in rotation, consortium or succession in the same area and in the same agricultural year or for several years, in sequence or interspersed. Due to those important aspects of management, establishment, production and forms of conservation of new forages through silage are unknown (Balbino *et al*., 2011). Silage helps to maintain foliages with low DM and nutrient losses, maintaining good palatability by cattle (Gómez *et al.,* 2015). In this sense, the ensiling of associations between grasses and legumes has become important because, through this technique, the contribution of protein to the rumen system is increased, in addition to allowing the use and conservation of forage that could be used during the dry season (Contrera *et al*., 2008).

Studies indicate that the chemical composition of the silage of the association *S. bicolor* L. Moench Cv RB Cañero and *C. cajan* Cv Caqui, presents nutritional potential for feeding ruminants (Pérez-Luna *et al*., 2019). However, in the scientific literature, there is still no information concerning fermentation characteristics and nutritive value, in addition, the optimal utilization age of these substrates in ruminant feeding is unknown. Therefore, the objective of the present study was to evaluate the degradation and *in vitro* fermentation potential of *S. bicolor* L. Moench Cv RB Cañero and *C. cajan* cv Caqui silage in an integrated production system.

MATERIAL AND METHODS

Location of the study area

The experiment was conducted at Ranch "Loma Bonita", Villaflores municipality, Chiapas. Mexico, with predominantly warm climate intermediate subsumel Aw" 1 (w) (i') g with rainfall in summer, measured rainfall of 1200 mm, with an average annual temperature of 22 °C (INEGI, 2017).

Crop establishment and management

For the establishment of sorghum and pigeon pea crops, a soil layer of approximately 5 cm was mobilized for weed control. Sorghum was sown at 2.5 cm depth (two seeds per point) with a precision planter, at 50 cm distance between rows and 4 cm between plants, with a density of 250 000 plants per ha^{-1}; for pigeon pea, at 50 cm distance between rows and 14 cm between plants, with a density of 71 428 plants per ha^{-1}.

Crops were broadcast fertilized with the formula 300-150-50 for sorghum and 100-150-50 N-P-K for pigeon pea. N was distributed at 50% in two applications at 5 and 35 days after planting.

Weeds and chemical control of pests and diseases were controlled sporadically at concentrations recommended by the distributor. During the development of the crop, only *Melanaphis sacchari* was found in the sorghum; for this purpose, Cypermethrin was used at a rate of 1.5 L ha^{-1}, each time the presence of this pest was observed.

Production of micro silos

Crops were cut and chopped to a uniform particle size (2 to 3 cm in length) using a double-furrow silage machine. At the time of harvesting, samples were taken from each of the treatments and were deposited in plastic bottles (Pet), with 1L capacity, extracting from each of them the greatest amount of oxygen. It was to ensure good anaerobic fermentation of the forage.

The silage was made 85 days after sowing. After passing each substrate and its combinations in their different ensiling times according to the assigned treatment, the samples of each treatment were collected, were dried and were ground, and were sent to the Bromatology laboratory of the College of the Southern Frontier for dry matter (DM) analysis. Samples were placed in a forced air oven at a temperature of 60 °C for 48 h and Organic matter (OM) were placed by sample combustion in a muffle at 600 °C for 6 h. Crude protein (CP) was determined by the Kjeldahl method (CP, AOAC, 1996; ID 954.01) as well as neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest *et al*., 1991), (Table 1). In the Herbivore Ecology Laboratory of the same Center, fermentation tests were carried out with the *in vitro* gas production technique described by Menke & Steingass (1988) y Theodorou *et al*. (1994).

In vitro degradation and fermentation technique

The gas production technique proposed by Menke & Steingass (1988) and modified by Theodorou *et al*. (1994) was used to quantify the gas produced by *in vitro* fermentation during 72 h of incubation. The treatments were sorghum, sorghum+pigeon pea, pigeon pea and sorghum+pigeon pea+inoculant (Sill-All 4x4 Lallemand) samples, of 4 ensiling times (0, 30, 60 and 90 days), resulting in 16 possible combinations (Table 1), each combination was repeated 3 times. The inoculum consisted of a composite sample of rumen fluid (RF) obtained from three sheep (40 kg LW) by esophageal gavage and fed a forage-based diet. Prior to RF extraction, the animals were fasted for 14 hours. RF was filtered through eight layers of gauze and were mixed in a 1:9 ratio with a reduced mineral solution (Menke & Steingass, 1988; Krishnamoorthy *et al*., 2005).

For each combination, 500 mg of dry, ground sample was weighed and placed in 125 mL amber flasks, to which 90 mL of ruminal inoculum was added and was kept under constant CO_2 flow. Then they were hermetically sealed with rubber stoppers and aluminum ring (Theodorou *et al*., 1994). Flasks were incubated at 39 °C in a water bath. The gas pressure in each flask was measured with a manometer (Metron, Mode:63100, Mexico) at 0, 2, 4, 6, 6, 8, 8, 12, 16, 20, 24, 24, 30, 36, 42, 48, 60 and 72 h after incubation (Blümmel & Ledzien, 2001).
Gas pressure values were transformed to gas volume using a linear regression equation: $V= (P+0.0186) (0.0237)^{-1}$ (Elmasry *et al*., 2016).

Variables evaluated

In vitro gas production

The maximum volume (Vm, mL g^{-1}), fermentation rate (S, h^{-1}) and lag phase (L, h^{-1}) of gas production were estimated with the logistic model $Vo = Vm/(1+ e^{(2-4k(t-L))})$ (Schofield & Pell, 1995), using the NLIN program (SAS, 2006). Volumes were also obtained for time intervals of 0 to 8 (Vf_{0-8}), 8 to 24 (Vf_{8-24}) and 24 to 72 (Vf_{24-72}) h of incubation. These volumes were used to estimate the fractions (mg g^{-1} of forage) of fast (FF), medium (FM) and slow (FS) substrate fermentation, according to the linear regression equations proposed by Miranda *et al*. (2015): $FF=V_{f0-8}/0.4266$, $FM=V_{f8-24}/0.6152$, $FS=V_{f24-72}/0.3453$. The sum of the three fractions was considered as the total fermentable fraction (FT). In addition, the index of gas emission potential per gram of digestible organic matter (IPEGF; mL g^{-1} DOM) was estimated.

Table 1. Distribution of treatments

Substrates	Days of silage	Repetitions		
Sorghum	0	R1	R2	R3

	30	R1	R2	R3
	60	R1	R2	R3
	90	R1	R2	R3
Sorghum + Pigeon pea (S+PP)	0	R1	R2	R3
	30	R1	R2	R3
	60	R1	R2	R3
	90	R1	R2	R3
Pigeon pea (PP)	0	R1	R2	R3
	30	R1	R2	R3
	60	R1	R2	R3
	90	R1	R2	R3
Sorghum +Pigeon pea+ Inoculant (S+PPG+I)	0	R1	R2	R3
	30	R1	R2	R3
	60	R1	R2	R3
	90	R1	R2	R3

In vitro digestibility of dry matter (DIVDM)

At the end of the incubation period (72 h), the residues of each sample were obtained by filtering in a flask and a Buchner funnel with filter (F/rapid filter paper MOD.617 Code P.V.NO.1034), and the DIVDM was estimated, by drying at 60 °C for 48 h. With the initial and residual DM, the DIVDM (%) was calculated (Monforte *et al*., 2005).

Design and statistical analysis

Fermentation variables (Vm, S, L, FF, FM, FS, FT, IPEGF, DIVDM) were analyzed with the general linear model using the SAS statistical package SAS versión 9 (2006), under a completely randomized design with a 4x4 factorial arrangement, with repeated measures over time. Three replicates per treatment were used. Means of the 16 combinations were compared using Tukey's multiple comparisons and statistical tests were considered significant when $P<0.05$ (Montgomery, 2013). A Pearson correlation analysis was performed for chemical components (CP, NDF, and ADF) and fermentation variables; and a linear regression analysis for DIVDM and total fermentable fraction.

RESULTS

Chemical composition

The chemical characterization of the treatments is presented in Table 2. The average DM, OM, CP, NDF and ADF were 373.9±41.43; 960.58±7.59; 87.82±24.95; 588.73±51.17; 371.76±61.39 g kg^{-1} DM. For DIVDM it was 56.32±9.05 %.

Table 2. Proximal chemical composition of experimental treatments (g/kg)

TREATMENT	DM	OM	CP	NDF	ADF

S 0	439.0	953.2	62.1	605.5	339.6
S 30	399.0	966.2	80.5	538.8	286.0
S 60	379.1	964.4	73.4	541.1	294.5
S 90	355.3	960	69.8	586.9	369.6
S+PP 0	376.3	970.3	53.0	484.5	295.4
S+PP 30	350.7	966.3	78.8	626.1	354.0
S+PP 60	349.0	962.4	82.1	622	373.2
S+PP 90	306.2	964.2	80.8	610	382.4
PP 0	379.6	956.2	112.5	587.7	404.5
PP 30	372.2	952.6	135.8	657.8	489.7
PP 60	401.8	950	130.8	637.3	468.2
PP 90	444.5	944.1	122.9	624.5	450
S+PP+I 0	358.3	969.3	64.2	499.2	309.1
S+PP+I 30	407.4	966	81.7	581.9	374
S+PP+I 60	377.7	966.7	80.2	568.3	351.9
S+PP+I 90	286.8	957.3	96.5	648.1	406.1
Mean	373.93	960.58	87.82	588.73	371.76
SD	41.43	7.59	24.95	51.17	61.39

In vitro gas fermentation kinetics

For the variables Vm and S no interaction between factors was found, however, the variable L did show an interaction between factors (Table 3). Substrates with the highest Vm were sorghum with 317.37, 297.8, 294.57, 264.17 mL g^{-1}, respectively, at times of 0, 30, 60 and 90 days of anaerobic fermentation and the combination of sorghum+pigeon pea + inoculant with 289.47, 269.63 and 266.6 mL g^{-1}, at times of 0, 30 and 60 days of anaerobic fermentation, respectively. On the other hand, treatments that were characterized by an average gas production were sorghum+pigeon pea and pigeon pea with silage times of 0, 30, 60 and 90 days; Table 3).

Table 3. Parameters of gas production kinetics, fermentation fractions, *in vitro* dry matter digestibility and potential gas emission rate (IPEGF) of silage of *Sorghum bicolor* L. Moench Cv RB Cañero and *Cajanus cajan* Cv Caqui with different ensiling times

Treat	Parameters	Fermentation fractions	DIVDM	IPEGF

	Vm (mL g^{-1})	S (h^{-1})	L (h)	FF	FM	FS	FT	(%)	mL g^{-1} DMD
				---------- mg g^{-1} ----------					
S 0	317.4^{a}	0.036^{bcd}	4.6^{a}	152.1^{c}	243.4^{a}	388.9^{a}	784.4^{a}	67.2^{ab}	473.8^{ab}
S 30	294.6^{ab}	0.038^{abcd}	4.8^{a}	139.8^{cde}	233.3^{a}	349.6^{ab}	722.7^{ab}	72.1^{a}	408.3^{ab}
S 60	297.8^{ab}	0.039^{abcd}	4.2^{ab}	155.4^{c}	236.7^{a}	334.3^{abc}	726.5^{ab}	66.6^{ab}	447.2^{ab}
S 90	264.2^{abcde}	0.040^{abcd}	4.3^{ab}	137.4^{cde}	215.6^{ab}	287.6^{bcd}	640.5^{bcde}	60.4^{abc}	437.3^{ab}
S+PP 0	247.9^{bcdef}	0.043^{a}	3.0^{dc}	168.6^{bc}	197.3^{bc}	227.6^{cde}	593.6^{bcdef}	63.2^{abc}	393.4^{b}
S+PP 30	240.3^{cdef}	0.041^{abcd}	3.9^{abc}	138.2^{cde}	190.5^{bc}	256.1^{cde}	584.7^{cdefg}	60.7^{abc}	396.1^{b}
S+ PP 60	219.5^{defg}	0.041^{abcd}	3.2^{dc}	137.4^{cde}	173.4^{cd}	225.6^{def}	536.3^{defgh}	54.2^{cde}	407.2^{ab}
S+ PP 90	244.1^{bcdef}	0.039^{abcd}	3.5^{bcd}	143.9^{cde}	186.5^{bc}	266.2^{cde}	596.7^{bcdef}	54.3^{cde}	448.1^{ab}
PP 0	214.7^{efg}	0.042^{ab}	2.7^{d}	148.9^{cd}	168.8^{cd}	197.1^{ef}	514.8^{efgh}	55.7^{bcde}	385.9^{b}
PP 30	185.3^{g}	0.040^{abcd}	3.3^{dc}	111.9^{de}	144.3^{de}	197.1^{ef}	453.3^{gh}	44.3^{ef}	418.0^{ab}
PP 60	166.9^{g}	0.042^{abc}	3.2^{dc}	109.4^{e}	128.9^{e}	170.7^{f}	409.0^{h}	39.5^{f}	446.5^{ab}
PP 90	196.7^{fg}	0.041^{abcd}	2.8^{d}	137.4^{cde}	147.7^{de}	194.1^{ef}	479.2^{fgh}	46.5^{def}	423.8^{ab}
S+ PP +I 0	289.5^{abc}	0.037^{abcd}	0.0^{f}	262.4^{a}	185.9^{bc}	268.3^{cde}	716.6^{abc}	58.2^{bcd}	498.7^{ab}
S+ PP +I 30	269.6^{abcd}	0.036^{cd}	1.4^{e}	199.0^{b}	183.6^{bc}	284.5^{bcd}	667.2^{abcd}	59.3^{bc}	455.2^{ab}
S+ PP +I 60	266.6^{abcde}	0.035^{d}	0.9^{ef}	206.4^{b}	181.9^{c}	277.4^{bcd}	665.8^{abcd}	53.6^{cde}	499.0^{ab}
S+ PP +I 90	245.1^{bcdef}	0.036^{cd}	1.6^{e}	172.7^{bc}	171.7^{cd}	269.3^{cde}	613.7^{bcde}	45.4^{ef}	540.1^{a}
SE	10.33	0.0011	0.180	7.42	6.35	14.95	25.44	2.31	27.35
Substrate	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0003
Days silage	0.0007	0.4980	0.0001	0.0001	0.0005	0.1408	0.0014	0.0001	0.1695
Substrate *Days silage	0.0983	0.1357	0.0011	0.0001	0.0708	0.0122	0.0771	0.0089	0.5941

FACTOR A	Substrates				SE
	Sorghum	Sorghum + Pigeon pea	Pigeon pea	Sorghum + Pigeon pea + Inoculant	
Vm (mL g^{-1})	293.47^{a}	237.96^{c}	190.90^{d}	267.70^{b}	6.54
S (h^{-1})	0.0383^{b}	0.0410^{a}	0.0414^{a}	0.0360^{c}	0.0006
FM (mg g^{-1})	232.236^{a}	186.931^{b}	147.435^{c}	180.800^{b}	4.09
FT (mg g^{-1})	718.50^{a}	577.83^{c}	464.07^{d}	665.82^{b}	15.96
IPEGF (mL g^{-1} MSD)	441.67^{b}	411.20^{b}	418.56^{b}	498.28^{a}	13.80
FACTOR B	Times of silage (fermentation days)				SE
	0	30	60	90	
Vm (mL g^{-1})	267.358^{a}	247.450^{b}	237.708^{b}	237.533^{b}	12.71
S (h^{-1})	0.0399^{a}	0.0387^{a}	0.0392^{a}	0.0389^{a}	0.0008
FM (mg g^{-1})	198.87^{a}	187.92^{ab}	180.23^{b}	180.37^{b}	9.71
FT (mg g^{-1})	652.34^{a}	606.98^{ab}	584.40^{b}	582.51^{b}	32.05
IPEGF (mL g^{-1} DMD)	437.98^{a}	419.42^{a}	449.98^{a}	462.34^{a}	16.54

[a, b, c]= Means with different column letters are different (P<0.05); Vm= Maximum volume; S: fermentation rate; L: lag phase; FF= fast fermentation fraction; FM= medium fermentation fraction; FS= slow fermentation fraction; FT= total fermentable fraction; DIVDM= *in vitro* digestibility of dry matter; IPEGF: Potential gas emission rate per unit of digestible organic matter

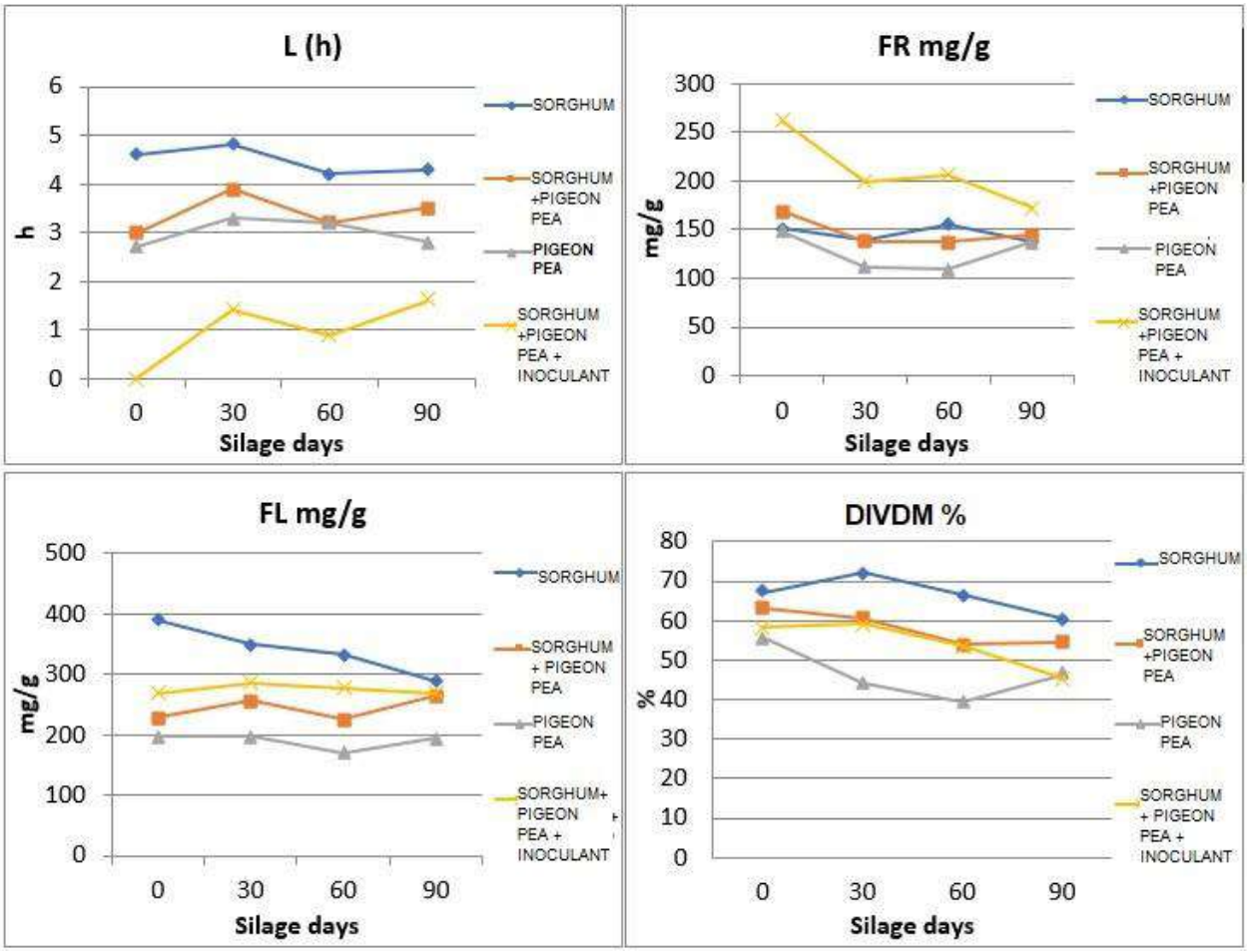

Figure 1. Interaction of substrate and silage days on L, FF, FS and DIVDM variables

On the other hand, the highest fermentation rate (S) was observed for S+PP 0, and the lowest (P=0.0001) was observed for S+PP+I 60, in the rest of the treatments no significant statistical difference was found.

The lowest lag phase (L) was observed in the S+PP+I mixture substrate at 0, 30, 60 and 90 days of ensiling, respectively. In addition, the intermediate L was found to be similar between PP 0, PP 90, S+PP 60, S+PP 0, PP 60 and PP 30, while the highest lag phases were observed in S 0 and S 30, and were similar between S 90, S 60, S+PP 30 and S+PP 90 (Table 3).

Anaerobic fermentation time (AF)

The maximum volume (Vm) of gas production and lag phase (L, h) (Table 3) was significantly (P<0.05) affected by AF times (Table 3). However, S did not show significant changes (P>0.05) between AF times. The fermentation time with the highest Vm was at 0 days AF (267 mL g^{-1}), which decreased with increasing AF days (Table 3).

The lowest lag phase (L) was observed at 0 days of fermentation (2.5962 h). Likewise, an intermediate L was found at 60 and 90 days AF (2.91 and 3.08 h) respectively) while a higher L was observed at 30 days AF (3.35 h).

Fermentable fractions, digestibility and gas emission potential index

The S+PP+I 0 substrate mixture presented the highest value of the fast fermentable fraction (0-8 h) (P<0.05), followed by S+PP+I at 30, 60 and 90 days of ensiling, respectively (Table 3). Likewise, it was found that S+PP+I 90 was statistically similar to all treatments containing sorghum substrate at different ensiling times, as well as PP 0 and PP 90 (Table 3). On the other hand, the mean fermentation fraction (8-24 h) was also found to differ (P<0.05) between treatments. The highest mean fermentation was found for S at 0, 30, 60 and 90 days of ensiling, respectively. Treatments PP 90, PP 30 and PP 60 showed lower mean DM fermentation.

For the slow fermentation fraction (24-72 h), differences (P<0.05) were also found between treatments. The highest fermentation value was observed in S 0, followed by S 30 and S 60. While the treatments containing sorghum as substrate alone or combined, presented similar slow fermentation fractions (Table 3). In addition, the pigeon pea treatments alone in their different ensiling days presented lower slow fermentation values.

The treatment that presented (P<0.05) the highest total fermentation fraction (FT) was S 0, followed by S 60, S 30, S+PP+I 0, S+PP+I 30, S+PP+I 60, which showed similar FT (Table 3). The rest of the treatments showed the lowest TF (Table 4).

The DIVDM and potential fermented gas emission rate (IPEGF) differed (P<0.05) among treatments (Table 3). An interaction between substrates and days of ensiling was found, the highest DIVDM was obtained in treatments S 30, S 0, S+PP 0, S+PP 30, S 90 and S+PP 60 (Table 3). The treatments with the lowest (P<0.05) IPEGF were S+PP 30, S+PP 0 and PP 0. While S+PP+I 90 produced the highest amount of gas per unit of fermented organic matter (Table 3).

The highest FM, FS, FF and DIVDM were obtained in sorghum, followed by sorghum + pigeon pea and sorghum + pigeon pea + inoculant (Table 3) and pigeon pea had lower FM, FS, FT and DIVDM (FM= 147.43 mg g^{-1}, FS=189.77 mg g^{-1}, FT=464.07 mg g^{-1} and DIVDM=46.48%).

Substrates showing the lowest IPEGF were sorghum, sorghum + pigeon pea and pigeon pea (441.67, 411.20 and 418.56 mL g^{-1} DOM). While the substrate that produced the

highest amount of gas per unit of fermented organic matter was sorghum + pigeon pea + inoculant (Table 3).

The highest FF, FM, FT and DIVDM occurred in the treatment without AF (0 days; FF= 182.98 mg g^{-1}, FM=198.87 mg g^{-1}, FT=652.34 mg g^{-1} and DIVDM=61.06%). While at 30, 60 and 90 days AF, they showed lower FF, FM, FT and DIVDM (Table 3). On the other hand, it was observed that the slow fermentation fraction (FS) and IPEGF did not show significant changes between AF times.

Relationship between chemical composition and *in vitro* gas production kinetics

The concentration of CP, NDF and ADF of different treatments was negatively correlated with maximum volume (Vm), fast and mid-fermentation fractions (FF, FM), total fermentation fractions (TF) and DIVDM (Table 4); while FT was negatively correlated with CP and ADF (Table 4).

However, when correlating CP and DIVDM of the pigeon pea composite treatments, it presented a negative correlation of -0.85 (P=0.0005).

DISCUSSION

Chemical composition

CP concentrations in *C. cajan* cv Caqui on the different AF days (Table 2) are above the threshold CP requirement (110-120 g kg^{-1} DM) for an average level of grazing animal production (NRC, 2016). Sorghum silage, sorghum + pigeon pea, sorghum + pigeon pea + inoculant (Sill-All 4x4 Lallemand) in their different days of anaerobic fermentation exceed the critical level (60-80 g kg^{-1} DM) that negatively influences voluntary intake (Orskov, 1992) and microbial activity in the rumen (Van Soest *et al*., 1991).

The NDF content of T2, T3, T5 and T13 was below the concentration of 550 g kg^{-1} DM which limits CV and nutrient digestibility in animals (Van Soest, 1965) and it is characterized as a high quality feed, having a concentration close to 450 g kg^{-1} DM NDF (Girma *et al.,* 2015). However, T1, T4, T6, T7, T8, T9, T10, T11, T12, T14, T15, T16 can be considered as medium quality substrates as they contain a concentration ranging between 450 and 650 g kg^{-1} DM NDF (Table 1). In addition, they have high concentrations of NDF (T1, T4, T6, T7, T8, T9, T10, T11, T12, T14, T15, and T16) and could be related to climate, temperature, radiation, precipitation and relative humidity (Méndez *et al*., 2020).

Table 4. Correlation matrix between chemical composition, gas production constants and *in vitro* digestibility of dry matter

	CP	NDF	ADF
V	-0.82924	-0.58985	-0.84829
	<.0001	0.0162	<.0001
S	0.27733	0.02217	0.234
	0.2984	0.935	0.3831
L	-0.03253	0.17503	-0.08863
	0.9048	0.5167	0.7441
FF	-0.52165	-0.60738	-0.49812
	0.0382	0.0126	0.0496
FM	-0.79137	-0.51604	-0.83235
	0.0003	0.0407	<.0001
FS	-0.68033	-0.33237	-0.69951
	0.0037	0.2085	0.0026
FT	-0.81092	-0.56184	-0.82585
	0.0001	0.0235	<.0001
DIVDM	-0.78719	-0.65437	-0.88745
	0.0003	0.006	<.0001
IPEGF	-0.1406	0.05275	-0.02326
	0.6035	0.8462	0.9318

[A] correlation coefficient; [B] Correlation significant at P<0.05. CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; DIVDM: *in vitro* digestibility of dry matter; S: fermentation rate; L: lag phase; Vm: maximum volume; FF: fast fermentation fraction; FM: mid fermentation fraction; FS: slow fermentation fraction; FT: total fermentable fraction; IPEGF: potential gas emission rate per unit of digestible organic matter

Kinetics of gas fermentation *in vitro*

The preservation of pastures using the AF technique is one of the feeding strategies that sustains production systems in the tropics during the dry season. This method of preservation allows maintaining the desirable nutritional characteristics of the fodder through anaerobic lactic acid fermentation (Contrera-Govea *et al*., 2008). The combination usefulness in silos of associations between grasses and legumes has gained importance because, through this technique, the contribution of protein to the rumen system is increased, making it possible to take advantage of, conserve and improve the fermentation and degradation conditions of substrates in the rumen (Gómez *et al.,* 2015). In this regard, Boddey *et al*. (2009) point out an increase in the sustainability of integrated systems that is achieved with the incorporation of legumes into the system. The use of shrub legumes such as pigeon pea, both for production in the consortium with annual crops such as maize or sorghum, as well as in association with grasses for direct grazing, are an option that aims to improve the protein content of the animals' diet.

In this sense, the higher Vm obtained in T1, T3, T2 and T4 are related to the higher digestion of structural carbohydrates present in the biomass (NDF), which cause a slow onset of fermentation (L), (Table 3), showing a delay in the adhesion of microorganisms

and increasing the fermentation rate (S), (Table 3) (Kibon & Orskov, 1993; Albores *et al*., 2018). Additionally, it has been reported that sorghum silage shows a potential to be a feeding strategy through AF and shows no differences (P>0.05) in AF times as shown in the present study (Rendón *et al*., 2014). On the other hand, the higher Vm found in T13, T14 and T15 could be related to the inoculum (Sil-all 4X4 Lallemand) that was added to the substrate and to the AF time that decreased the attachment time (L) of the microorganisms, improving the fermentation rate (S), (Table 3) (Rodríguez *et al*., 2013). On the other hand, *C. cajan* and its combinations with sorghum and additives have been studied mainly in the feeding of non-ruminants, so there is little information about its use in ruminants, and even less about its rumen degradation. In this context, the present study shows that the treatments with low total gas production were the substrates with sorghum + pigeon pea and pigeon pea (T5, T16, T8 and T6, T7, T9, T12, T10 and T11; Table 3). This low Vm is related to a high concentration of NDF and ADF (Table 2) and a rapid onset of fermentation, due to their CP contents, which upon degradation release ammonium carbonate and NH_3 that is used by the bacteria for microbial protein synthesis (Cajarville *et al*., 2012), decreasing the amount of gas produced (Table 3); (González *et al*., 1998).

Fermentable fractions, digestibility and rate of fermentable gas emission potential

The timing of energy availability and NH3 release in the rumen depends on the rate and potential availability of nutrients contained in the forages (FR, FM and FS; Table 3). In grasses, tree foliage and mixtures of these substrates for animal feeding, the rates of synchronization are chemically more complex and critical compared homogeneous feeds (Rosales & Gill, 1999); and it is common to observe an excess of nitrogen and poor to moderate synchronisation between nitrogen fermentation and DM fermentation. However, when these are combined in a silo, it is expected to improve the conditions of synchronization rates by increasing fermentable fractions and DM degradation (Nsahlai *et al*., 1995). In this regard, the findings on rapid to moderate onset of DM fermentation process with T13, T15, T14, T16, T5 (Table 3) could be due to increased fermentation of its soluble fraction containing sugars. In addition, fermentation of starch, cellulose and pectins (Miranda *et al*., 2015) that favored rapid microbial action and fermentation initiation (FR) as a consequence of the interaction in the concentration of CP, NDF, ADF and the Sil-all 4X4 Lallemand inoculum added to treatments (Table 2). It is probably conserved the amount of non-structural carbohydrates such as sugars and starch (Cajarville *et al*., 2012).

While T1, T3, T2, T4, T14, T15, T13 and T16, showed a higher starch component degradation and cellulose concentration (FM and FL; Tables 2 and 3), which could be influenced by the combination between sorghum and pigeon pea and the AF time (30 and 90 days), (Table 3). It directly influences the time in which microorganisms begin the degradation of substrates (Rodríguez *et al*., 2013).

The identification of treatments with slow fermentation, such as T6, T7, T8, T9, T10, T11 and T12, can be useful to incorporate them in balanced diets as a source of energy, and to synchronize them in mixtures with other substrates with fast fermentation to take advantage of the excess NH_3 they provide. The type of substrate and the time of FA (Table 3) (Cajarville *et al*., 2012) influenced them.

The higher DIVDM found in T2, T1, T3, T5, T6, T4 and T6 (Table 4) is related to the lower concentration of cellulose in the plant (Table 2 and 3), (Girma *et al*., 2015). Probably a higher energy concentration in anaerobic fermentation favored a rapid onset of fermentation sustaining high fermentation rates resulting in a higher total gas volume (Table 3). The lower DIVMS of T12, T16, T10 and T11 (Table 4) is associated with their higher proportion of NDF and ADF (Table 2) that limit fermentative activity (Table 3); because it chemically binds structural carbohydrates, limiting their digestion (Moore & Jung, 2001). Probably the AF time and the combination of substrates did not allow a level of synchronization to improve digestion (Table 3) (Rendón *et al*., 2014). In this regard, when observing the behavior of the data obtained for the variable CP and DIVMS, we proceeded to correlate the treatments containing pigeon pea, in which a correlation of -0.85 was found between CP and DIVMS, indicating that the CP content of this plant negatively affects the *in vitro* digestibility of dry matter. This behavior may be due to the presence of secondary metabolites present in this plant, which probably affected the microbial population or its fermentative activity.

The efficiency of DM degradability shows the potential for gas production (IPEGF) as an indication of the degree to which it occurs (Miranda *et al*., 2015; Bayssa *et al*., 2016). In this study T6, T5 and T9 have a high potential to be used as a mitigation strategy for gas production, as the least amount of gas was produced per unit of DM fermented (Vélez *et al*., 2015). This phenomenon can be explained by the high concentrations of cellular constituents (Table 2) that affect fermentation by possibly inhibiting microorganisms, without affecting the activity of enzymatic degradation on DM (Carballa *et al*., 2015).

Considering the above, it is concluded that sorghum plant silage has a potential in ruminant feeding, as it improves *in vitro* DM fermentation, especially the fast fermenting fractions (30 days).

Pigeon pea plant alone or in combination has a negative effect on *in vitro* dry matter digestibility.

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Errata Erratum

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