Chemical composition and *in situ* ruminal degradability of dry matter in mixed silages of *Tithonia diversifolia*: *Pennisetum purpureum* cv. Cuba CT-169, inoculated with VITAFERT

D. Gutiérrez [1], A.Morales[2], A. Elías[1], R. García López [1] and Lucía Sarduy[1]

[1] *Instituto de Ciencia Animal, Apartado Postal 24, San José de las Lajas, Mayabeque, Cuba*

[2] *Universidad de Holguín "Oscar Lucero Moya", Ave. 20 Aniversario s/n. Gaveta Postal 57, Holguín, Cuba*

Email: delfin@ica.co.cu

The chemical composition and *in situ* ruminal degradability of dry matter in mixed silages was analyzed, using different proportions of *Tithonia diversifolia* and *Pennisetum purpureum* cv. Cuba CT-169, inoculated with VITAFERT. A completely randomized design was used, with a 4 x 4 factorial arrangement. Treatments consisted on replacing four levels of *P. purpureum* with *T. diversifolia* (20:80, 40:60, 60:40 and 80:20 % weight HB), with the proportional inclusion of four levels of VITAFERT (0; 4.5; 6.0; 8.0 % weight HB) in the mixture. At the end of fermentation period (62 d), the chemical composition (% DM, % CP, % ash, % NDF) and *in situ* ruminal degradability of dry matter were determined. For that purpose, two bovine females (Holstein x Zebu) were used, with 475 kg of mean liveweight, with a fistula in the rumen and fed *ad libitum* with grass forage and a commercial concentrate at a rate of 6 g. kg LW^{-1}. The bags were incubated during a dynamics of fermentation (8, 16, 24, 48, 72 and 96 h). The results showed an increase in the content of CP and ash, and a reduction of NDF in all the mixtures inoculated with VITAFERT. However, the levels 4.5 and 6.0 % of VITAFERT reached the best response. The combination of 20:80 Tithonia:Pennisetum with 4.5 % of VITAFERT in the mixture had the highest degradability(48 %) in less time (30 h), degradation speed (c=13 % h^{-1}) and effective degradability (EDk0.02= 42.49 % , k0.04= 38.32 %).

Key words: *Tithonia, silages, effective degradability, proportions*

Due to its forage potential and its natural distribution, many shrub and tree species, under tropical conditions, are used for feeding ruminants (Toral 2005 and Escalante 2006). However, the seasonality of its production, as a consequence of the marked climatic variations, favors an important reduction of its availability and nutritional quality (Sánchez 2005).

There is an excess of forage biomass during the rainy season and, due to the need of using this material all the year, its efficient conservation is essential (Ojeda *et al.* 2006). According to Simón and Soca (1998), there are desiduous trees that lose their leaves due to flowering or the season. It is convenient, according to these authors, to use this produced biomass and avoid, through the cut, the expiring of these leaves. This action would increase the availabilities of regrowth during periods of scarcity.

For fighting this seasonal effect, it is necessary to use the production of green mass from the rainy period. In this sense, the use of silages is a traditional technique used for preserving the surplus of pastures and forages, balancing the nutrient content of diets and increasing cattle productivity (Bernal 2007). This practice is still valid for the current tropical conditions of Latin America and Cuba.

Producing mixed silages, based on tree plants, as a conservation technique that determines the quality of the silage (Ojeda *et al.* 2006), would allow to use simultaneously the yield and the fermentative potential of grasses. The reduction of water content of grass is also a practice that tends to increase the concentration of DM and nutrients of the silage (De Figueiredo and Marais 1994). The microbial inoculants stimulate the fermentation efficiency, assure the predominance of lactic bacteria and contribute to the material preservation (Bernal 2007).

Over the last years, there has been few studies developed in Latin America in which the silages of forage trees in mixtures with tropical grasses are evaluated, and their digestibility and chemical composition are analyzed. However, the dynamics and effect of these silages on ruminal fermentation has been studied (Gutiérrez *et al.* 2003 and Clavero and Razz 2008). Even though, studies on the most adequate levels of inclusion of trees on the mixture continue to be scarce. This type of contribution would allow to use mixed silages as an strategy for animal feeding during times of few availability of feed.

The objective of this study was to determine the effect of different proportions of *Tithonia diversifolia*, ensiled with *Pennisetum purpureum* cv. Cuba CT-169 and inoculated with VITAFERT, regarding the chemical composition and *in situ* ruminal degradability of DM.

Materials and Methods

The forage plants *Tithonia diversifolia* and *Pennisetum purpureum* cv. Cuba CT-169 were used in the study, with 40 and 80 d old, respectively, planted in forage areas from the Instituto de Ciencia Animal (ICA), settled on a typical red ferrallitic soil, with fast drying and constant profile (Hernández *et al.* 1999), without fertilization and no irrigation.

An experimental design, completely at random, with a 4x4 factorial arrangement, was used, with four proportions of *T. diversifolia* and *P. purpureum* (20:80, 40:60, 60:40 and 80:20% weight HB) and four increasing levels of VITAFERT(0; 4.5; 6.0; 8.0 %

weight HB). For each combination, five microsilos were prepared, for a total of 80 experimental units.

Procedure for producing silage. Both forages, harvested and fresh, were grounded separately in a forage mill, until they reached a particle size between 2 and 3 cm. Once grounded, they were dried under the sun for diminishing humidity up to around 30 % (27 %, 6 h), according to the methodology proposed by Reyes *et al.* (2008). The different combinations of forages and levels of VITAFERT were mixed and flattened by layers, using a tamping tool. Later, they were inserted in the microsilos, produced with tubes of PVC (24 cm x 10 cm), with a capacity for 450 g of fresh forage. Once finished the production process, each microsilo was covered and sealed hermetically. They were stored in a facility, out of the reach of rodents and other sources of danger. After the time settled for the fermentation (62 d after its production), the silos were opened, the final product was weighed and the contribution of DM was determined. In this moment, a random sample of around 180 g (± 67 % of finished product) was extracted from each microsilo for being analyzed in the laboratory.

Production of VITAFERT. This product was obtained from the fermentation of a mixture with final molasses of sugarcane, soy, maize, urea, ammonium sulfate and mineral formulas. Yogurt was used as microbial inoculum (Elías and Herrera 2008).

Chemical indicators. A proximal chemical analysis was performed to the samples of original material and silages. DM (%), ash (%) and CP (N x 6.25) were determined according to the methodology described by AOAC (1995). The NDF was stated according to Goering and van Soest (1970) in the laboratories of analytical services from ICA (LASAICA) (table 1).

In order to determine *in situ* ruminal degradability of DM, the nylon bag technique, described by Orskov and McDonald (1979), was used. The effective ruminal degradability was determined according to McDonald (1981) and the passage rate (k = 0.02, 0.04) from the rumen was stated according to NRC (1989). A total of 192 nylon bags (17.0 x 8.0 cm) were used, with a porosity of 50 µm of diameter. Each bag received 5 g of the ensiled material, grounded up to 2 mm. The bags were incubated in rumen per duplicate, in order inverse to incubation time (0, 8, 16, 24, 48, 72 and 96 h) for being removed all at the same time (Nocek and Russell 1988). Two bovine females (Holstein x Zebu) were used, with a permanent fistula in the rumen, with 475 kg of mean liveweight, and kept in individual pens. They were offered grass forage *ad libitum*, a commercial cattle concentrate (65 % wheat, 30 % soy, 2.4 % zeolite, 1 % common salt, 1 % mineral salts, 0.6 % phosphate) at a rate of 6 g. kg LW^{-1}, only in one time(8:30 h). Water was offered at will.

Table 1. Chemical composition of the material used in the different mixtures

Plant	DM (%)	CP (%)	TP (%)	Ash (%)
T. diversifolia	14.80	16.52	-	14.24
P. purpureum	19.20	5.72	-	5.00
VITAFERT[1]	9.70	4.80	2.88	5.19

[1]Chemical and microbiological composition: it is formed by concentrations of yeast and Lactobacillus ranging between 10^7-10^8 cfu and 10^9-10^{10} cfu, respectively. Concentrations of 450-600 mmol.L^{-1} of lactic acid and 225-230 mmol.L^{-1} of acetic acid.

Statistical analysis. An analysis of variance was performed to the values of *in situ* ruminal degradation of DM, according to a completely random design, with factorial arrangement, and the test of Duncan (1955) was applied. All data was processed using the statistical package INFOSTAT (Balzarini *et al.* 2001). The program NEW WAY version 5.0 WINDOWS® (Chen 1997) and linear regressions contained in the statistical program STATGRAPHICS plus v-5.1 were used to determine the fit of model parameters and statistical criteria.

Results and Discussion

Table 2 shows the chemical composition of the different forage mixtures and combination with VITAFERT. There was a quality improvement, regarding CP, ash concentration (%) and reduction of NDF (%) with the addition of VITAFERT to the forage mixtures, with respect to its no inclusion (0 %). These results confirm the information given by Cárdenas *et al.* (2003), who stated that the forage preservation is improved by the use of tree forage in the mixture with grasses inoculated with microorganisms, although in this study, the levels of inclusion 4.5 and 6.0 % of VITAFERT evidenced a superior response. It is also confirmed the reports of Nguyen *et al.* (2005), who refer that when using tree foliages in the preservation of tropical grasses, nutrient deficiencies are corrected, the content of protein and DM increases and the NDF decreases, as it happened in this study.

Protein values were in correspondence with the participation and inclusion level of trees in the mixture, which confirms the stated by Pinto *et al.* (2010). These authors refer that the inclusion of trees improves quality, increases the levels of protein and fermentable carbohydrates, as well as its contribution to silage preservation, considering that the used grass (*Pennisetum purpureum*) showed low protein levels (5.7 %). The values reached surpass (> 11 % CP) those informed by of Kato *et al.* (2006) in maize silages mixed with *Leucaena leucocephala*, *Acacia boliviana* and, in different proportions, with *Morus alba* (Boschini 2003), also in silages of *Pennisetum purpureum* cv. Taiwán mixed with *Guazuma ulmifolia*, *Lisyloma latisiliquum*, *Piscidia piscipula* and *Albizia lebbeck* (Cárdenas *et al.* 2003). These results are also superior to the informed by Alpízar *et al.* (2014) (11.51 % CP) in mixtures ensiled with 75 % of mulberry:sorgum.

The increase in the values of CP may be also connected

Table 2. Chemical composition of the different ensiled mixtures

Proportion	Niveles VITAFERT %	DM (%)	CP (%)	NDF (%)	Ash (%)
20 % *T. diversifolia* 80 % *P. purpureum*	0	37.50	7.79[a]	84.33[b]	9.81[a]
	4.5	37.50	18.17[c]	47.98[a]	27.41[c]
	6.0	38.39	19.66[c]	52.88[a]	28.10[c]
	8.0	39.37	12.92[b]	80.76[b]	12.11[b]
	SE±	0.58	0.67***	2.22***	0.57***
40 % *T. diversifolia* 60 % *P. purpureum*	0	31.70[a]	10.54[a]	80.41[c]	11.70[a]
	4.5	32.58[ab]	20.92[c]	50.55[a]	30.66[d]
	6.0	33.95[bc]	18.87[b]	47.54[a]	28.52[c]
	8.0	35.08[c]	18.88[b]	74.21[b]	16.21[b]
	SE±	0.52**	0.48***	2.00***	0.47***
60 % *T. diversifolia* 40 % *P. purpureum*	0	28.90[a]	13.92[a]	66.30[b]	19.19[a]
	4.5	29.83[a]	22.73[c]	44.66[a]	30.21[b]
	6.0	32.42[b]	21.92[c]	44.48[a]	28.80[b]
	8.0	32.83[b]	20.47[b]	63.80[b]	20.21[a]
	SE±	0.68**	0.48**	1.63***	0.09***
80 % *T. diversifolia* 20 % *P. purpureum*	0	33.45[a]	16.82[a]	69.83[b]	17.94[a]
	4.5	34.83[ab]	25.58[c]	45.80[a]	29.69[d]
	6.0	36.68[b]	22.79[b]	44.25[a]	27.54[c]
	8.0	37.00[b]	16.18[a]	64.31[b]	22.17[b]
	SE±	0.71**	0.51***	2.21***	0.59***

[abcd]Values with different letters differ at P<0.05 (Duncan 1955)
**P<0.01 ***P < 0.001

to the type of silage (predried), which contributes to the increase of DM, soluble carbohydrates and ammonium nitrogen, and reduces the deamination of amino acids, essential elements for succeeding in silages (Michelena *et al.* 2002 and Ruiz *et al.* 2009). The response of the bacterial inoculant (yeasts, lactobacilli), as a consistent improver of efficiency in fermentation and recovery of DM, confirms the information stated by Bolsen (1999) in studies developed with the use of biological additives on silages. This is added to the acceptability that could have had the grounding of Pennisetum, because of its thick stem, and the action of the microbial inoculum, elements that favor the conservation (Spitaleri *et al.* 1995).

Another element to be considered is the content of DM, which showed a square increase ($Y = 48.62-0.63X + 0.006 X^2$, $R^2 = 0.63$ SE± =2.04, $P < 0.0001$) with the participation of trees, being 55 % and 31.47 % the optimal values of inclusion in the mixture and contribution to DM, respectively. Mixture 20:80 showed an increase ($P < 0.0001$) in the concentration of DM, according to the results of the interaction between the factors proportion x levels (table 3). These results seem to indicate that a higher amount of substratum was proportionate in order to achieve increases of DM degradability. Similar performance was reported by Valentín *et al.* (2003) in mixed silages with Taiwán (*Pennisetum purpureum*) as basis, and the inclusion of equal parts of different tree species (*Guazuma ulmifolia*, *Lisyloma latisiliquum*, *Albizia lebbeck* and *Piscidia piscipula*).

Other authors (Tjandraatmadja *et al.* 1993) have reported the square response in grass silages with different inclusion levels of tropical trees. However, they also refer that the content of DM depends on the tree species involved in the mixture and its concentration.

In situ ruminal degradability of the DM tended to improve with the addition of the inoculum, according to the statistical results of the interaction between the factors proportion x levels, specifically when using 4.5 and 6.0 % of VITAFERT in the mixtures, with values between 37 and 45 % (table 4). These increases may have been associated to the increase of cell wall solubility, as a result from the increment of enzymatic activity of inoculated microorganisms within VITAFERT, and their functional effect on ensiled material, ash availability and nitrogen (Elías 1983). The concentration of short chain fatty acids within VITAFERT could also have influenced, as growth stimulators of microorganisms and their activity with a proper dose (Sosa 2010 and Gutiérrez 2012).

According to Chaucheyras Fonty (2001), the effect of cultures of microorganisms is very variable. The results found in this study, referred to the nitrogen content, informed as protein, follow a growing tendency with the inclusion of Tithonia in the mixture with Pennisetum, regarding the values of mixture. This is logical because of the high proportion of nitrogen and the fermentation at

Table 3. Mean values of DM (%)in the mixtures

Proportion Tithonia: Pennisetum	VITAFERT levels (%)				SE± Sign.
	0	4.5	6.0	8.0	
20:80	37.50^{fg}	37.50^{fg}	38.34^{fg}	39.37^{g}	0.64***
40:60	31.70^{b}	32.58^{bc}	33.95^{cd}	38.05^{de}	
60:40	28.94^{a}	29.83^{a}	32.42^{bc}	32.83^{bc}	
80:20	33.45^{bcd}	34.83^{de}	36.68^{ef}	37.00^{e}	

^{abcdefg}Values with different letters differ at P<0.05 (Duncan 1955)
***P < 0.001

Table 4. *In situ* ruminal degradation of DM

Proportion Tithonia: Pennisetum	VITAFERT levels (%)				SE± Sign.
	0	4.5	6.0	8.0	
20:80	14.77^{a}	45.08^{e}	40.47^{de}	9.22^{a}	0.66***
40:60	27.92^{bc}	37.41^{de}	40.32 ^{de}	10.18^{a}	
60:40	24.81^{b}	34.61^{cd}	37.15 ^{de}	26.52^{bc}	
80:20	32.72^{bcd}	31.90^{bcd}	39.41 ^{de}	37.03^{de}	

^{abcde}Values with different letters differ at P<0.05 (Duncan 1955)
***P < 0.001

ruminal level, expected due to the nature of Tithonia, according to Mahecha and Rosales (2005) and Pérez *et al.* (2009). The results from DM degradation are controversial and can be justified by losses in DM (2.6 percentage units), maybe soluble carbohydrates, and by the decrease in the fiber concentration, as well as by the increase in the concentration of N-NH_3, mainly during the first hours, which provokes variations during the rest of the ruminal fermentation kinetics. Likewise, the accumulation of ammonium in the rumen for limiting protein synthesis could have influenced (Bolíva and Ibrahim 2013) and, as result, the decrease of enzymatic activity of microorganisms (Monteils *et al.* 2012, Enríquez *et al.* 2013 and Romero *et al.* 2013).

The mixture 20:80 Tithonia: Pennisetum, with 4.5 % of VITAFERT, degraded 48 % in the lowest time (30 h). It was also observed that, during the extension of ruminal fermentation, differences among treatments were reduced (figure 1). This indicates that, in a short period of permanence of the silage in the rumen, it degraded a high percentage of material. This situation would favor animal intake and nutrition.

Another aspect that could be related to the kinetic performance of the curve is the stimulating activity of VITAFERT, associated to the existence of live cells, more active in the ruminal flow, at the beginning and during the extension of the degrading kinetics. Besides, there is a contribution of this biological product in

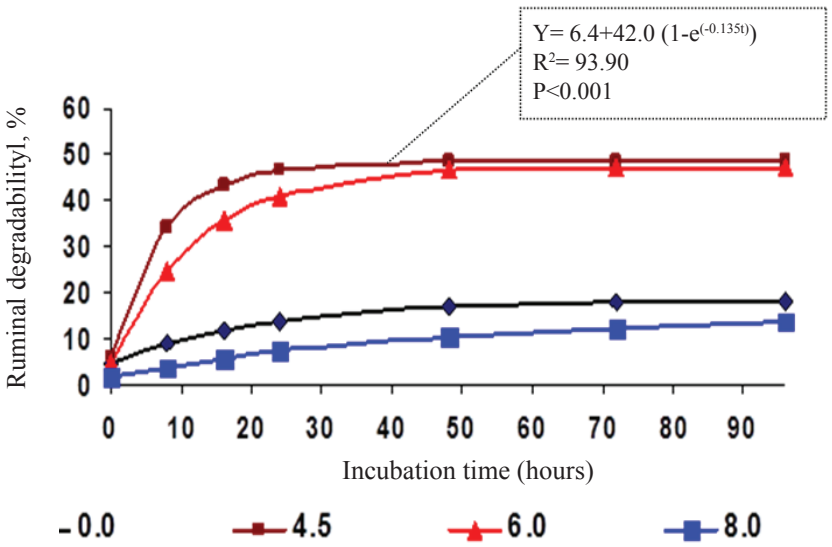

Figure 1.Curve of *in situ* ruminal degradation of DM, for the mixture of 20 % Tithonia: 80 % Pennisetum, according to the model proposed by Orskov and McDonald (1979).

Table 5. Values of effective degradability (ED) of DM

Parameters	VITAFERT levels	Proportion Tithonia:Pennisetum			
		20:80	40:60	60:40	80:20
ED, % (k= 2,0 % h)	0.0	13.83	28.55	24.23	31.06
	4.5	42.49	34.36	34.18	30.95
	6.0	38.89	38.72	35.09	37.84
	8.0	8.64	10.06	24.75	30.62
ED, % (k= 4,0 % h)	0.0	11.64	19.19	19.01	21.38
	4.5	38.32	29.02	26.40	25,.21
	6.0	33.23	33.21	30.11	32.50
	8.0	6.37	7.06	17.81	24.24
c (h^{-1})	0.0	0.046	0.017	0.039	0.013
	4.5	0.135	0.071	0.040	0.054
	6.0	0.080	0.083	0.078	0.081
	8.0	0.023	0.023	0.014	0.038
t ½ (h)		14.75	22.25	23.37	23.24
R^2		94.70	99.45	96.36	98.73
SE±		6.60	3.73	10.82	6.89
Sign.		***	***	***	***

*** P < 0.001

peptides and amino acids within the true protein (TP) and carbonated chains that could have been used as energy sources for initial microbial growth (Elías and Herrera 2008).

Values of effective degradability (42.49 and 38.32 %), as a variable indicating the real degradability of DM (Pinto *et al.* 2010), the high degradation rate (0.13 h) and the decrease of mean time of material permanence (t ½) (14.75h), demonstrate that the mixture 20:80 Tithonia: Pennisetum, with 4.5 % of VITAFERT achieved the best response of all (table 5).

In this mixture, the increase in the degradation constant (c) had a marked effect on the disappearance of ruminal DM, and it was lees affected with variations of the rates of passage, regarding the rest of the mixtures. This performance could be attributed to the lowest content of the soluble fraction, evidenced by the low time of retention in the rumen.

It seems that there were higher changes in the microbial activity of the rumen with this combination, effect that provoked an increase of the fermentative capacity of structural carbohydrates, when degrading complex carbonated chains and releasing simple chains, which are used by cellulolitic bacteria (Elías 1983 and Galina *et al.* 2008).

The results evidenced the possibility of applying the predrying technique with forages *Tithonia diversifolia*: *Pennisetum purpureum* cv. Cuba CT-169 to be ensiled. The inclusion of the levels 4.5 and 6.0 % of VITAFERT in the mixture increases the content of CP and ash concentration, and reduces the NDF. The combination 20 % Tithonia: 80 % Pennisetum, with 4.5 % of VITAFERT had the highest effective degradability and degradation speed.

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