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Ensilability and chemical composition of silages made with different mixtures of noni (*Morinda citrifolia* L.)

Ensilabilidad y composición química de ensilajes elaborados con diferentes mezclas de noni (*Morinda citrifolia* L.)

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Abstract

The ensilability and chemical composition of silages made with different proportions (100:0; 90:10; 80:20; 70:30; 60:40 and 50:50%) of foliage (fresh and pre-wilted) and noni fruit (fresh) was evaluated. Some fermentative patterns of the original forages and the chemical content of the silages were determined. Correlations between the parameters of quality, chemical composition and the fruit levels in the mixture were made and adjusted the same one to polynomial equations. A higher inclusion of fruits with both fresh and pre-wilted foliage of noni decreases the pH and $\text{NH}_3\text{-N Nt}^{-1}$ in the silage. In addition, the increase in the level of fruit increased the concentration of water soluble carbohydrates (WSC) and the ratio of WSC/PC. Moreover, the pre-wilted foliage increased the dry matter (DM) content and its combination with fruit levels between 30 to 40% decreased the fibrous compounds and crude protein (CP) concentration in the silage. According to a cubic model used, the pre-wilted foliage and fruit proportions showed a higher R^2 for DM, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent-lignin (ADL) and cellulose, while CP and hemicelluloses were higher in the fresh plant material. The results showed that the pre-withered process (foliage) and inclusion levels of 30 and 40% of fruits in the mixture improved the quality of the silage intended for animals.

Keywords: fermentation; foliage; food preservation; fruit; noni; wilted.

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Resumen

Se evaluó la ensilabilidad y la composición química de ensilajes elaborados con diferentes proporciones (100: 0; 90:10; 80:20; 70:30; 60:40 y 50: 50%) de follaje (fresco y presecado) y la fruta de noni (fresca). Se determinaron algunos patrones de fermentación de los forrajes originales y el contenido químico de los ensilajes. Se hicieron correlaciones entre los parámetros de calidad, la composición química y los niveles de fruta en la mezcla y se ajustaron a ecuaciones polinomiales. Una mayor inclusión de frutas con follaje fresco y pre-secado de noni, disminuyó el pH y el $\text{NH}_3\text{-N}$ en el ensilaje. Además, en incremento en el nivel de fruta aumentó la concentración de carbohidratos solubles en agua (CSA) y la relación de CSA/PB. También, el follaje pre-secado aumentó el contenido de materia seca (MS) y su combinación con niveles de fruta entre 30 a 40%, disminuyó los compuestos fibrosos y la concentración de proteína bruta (PB) en el ensilaje. Según un modelo cúbico utilizado, las proporciones de follaje y frutos previamente presecado, mostraron un R^2 mayor para MS, fibra detergente neutra (FDN), fibra detergente ácida (FDA), lignina ácido detergente (LAD) y celulosa, mientras que PB y hemicelulosa, fueron superiores en el material vegetal fresco. Los resultados mostraron que el proceso pre-secado (follaje) y los niveles de inclusión de 30 y 40% de las frutas en la mezcla, mejoraron la calidad del ensilaje destinado a los animales.

Palabras clave: conservación de alimento; fermentación; follaje; fruta; secado; noni.

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I. Introduction

The production of non-ruminant animals in the tropical area is limited by several factors; the most important of these factors is the obtaining of necessary nutrients to supply the nutritional requirements of these animals; in many cases conventional feeds are used, which contain large percentages of cereals and protein sources such as soybeans and fishmeal (Santana *et al.*, 2015). The high costs of some conventional raw ingredients by the boom in the production of agrofuel and other extrinsic and intrinsic factors of the market has a direct impact on animal production, mainly in this geographical area (Martínez *et al.*, 2015).

On the other hand, trees and bush have a great diversity and adaptation to various ecosystems, these feeds are considered to be technically, biologically and economically viable alternatives to animal feeding (Vandermeulen *et al.*, 2018). Noni (*Morinda citrifolia* L.) is a perennial plant, native to Southeast Asia, it grows in tropical and subtropical regions, it is identified by its large leaves, straight stem and yellow fruits (Sang *et al.*, 2001). This plant has been widely studied by their medicinal properties, is considered as one of the main sources of biologically active compounds (Arunachalam *et al.*, 2018). The fruit are rich sources of water-soluble polysaccharides such as fucose, xylose, mannose, galactose and fructose, minerals such as potassium, sulfur, calcium and phosphorus and amino acids such as aspartic acid, glutamic acid and isoleucine (Potterat *et al.*, 2007). However, as feedstuff, few researches have been done in animal production systems (Sakarkar & Deshmukh, 2011; Ali *et al.*, 2016).

Although this tree offers big kindness for sustainable animal production systems and can also be well adapted to different climatic conditions (Rivera *et al.*, 2012). There are still some limitations for use in animal production systems, such as incomplete characterization of their nutrient contents and lack of knowledge of the effect of noni on digestive and metabolic processes. Therefore, it is necessary to look for technological alternatives that could allow diminishing the anti-nutritional factors and achieving a better nutritive value.

The silage is one of the main technological processes for the preservation of animal feeds, through a fermentative method in the absence of air (Canibe &

Jensen, 2011; Ke *et al.*, 2015; Kung *et al.*, 2018), which propitiates positive nutritional changes and allows an easier and cheaper feeding (Amer *et al.*, 2012; Borreani *et al.*, 2018; Grant & Ferraretto, 2018). As a result, the objective of this work was to determine the ensilability and chemical composition of silages made with different proportions (100:0; 90:10; 80:20; 70:30; 60:40 and 50:50%) of foliage (fresh and pre-wilted) and noni fruit (fresh).

II. Materials and methods

The foliage and noni fruits were harvested in May/2016 the experimental areas at the Study Center of Animal Production at the University of Granma, Bayamo, Cuba, characterized by a flat topography and brown soil with carbonate (Hernández *et al.*, 1991). The annual rainfall is 1218 mm and the annual average temperature is 25.8 °C. During the growth of the plants, irrigation and fertilizer were not applied.

Foliage was collected by hand, formed by leaves and young stems of 120 days of regrowth, as well as, fruits individually from the trees were selected. For the harvest of fruit, their maturation state (yellow pale color and firmness from very hard to fairly hard) was considered (Rosalizan *et al.*, 2010).

Raw materials before ensiling were homogenized at 1mm in laboratory Cullison silos. The foliage for 3 hours with a density of 15 kg m⁻² was pre-wilted (Santana *et al.*, 2010). Raw materials before ensiling in the laboratory were homogenized to remove all the air and assure a correct compaction density. The silos in the absence of light at room temperature (28 ± 3 °C) and hermetically covered were placed for 21 days. The silages made with different proportions (100:0; 90:10; 80:20; 70:30; 60:40 and 50:50%) of foliage (fresh and pre-wilted) and noni fruit (fresh).

A. Chemical analysis

In order to determine ensilability, 50 g samples from each experimental variant was taken and 200 ml of distilled water was added. Then, samples into ambient temperature for 48 hours were incubated and pH quantifications at 0; 14; 18; 22; 26; 38 and 48 hours post incubation was done (Pieper *et al.*, 2017). Also, water soluble carbohydrates (WSC) contents was determined in fruit (fresh) and foliage (fresh and wilted) samples of according to Wiseman and Irvin (Wiseman & Irvin, 1957).

After 21 days of ensiling and immediately at the opening of the silos, samples for determination of pH, ammonia nitrogen/total nitrogen ratio ($\text{NH}_3\text{-N Nt}^{-1}$) and proximate chemical analysis were taken. Also, 30 g fresh silages in 270 ml of distilled water in a blender was stirred (AKA-DDR, T03259, Germany) for 2 min, filtrated through cheese cloth, acidified with 2% of orthophosphoric acid (v/v) and stored at -5 °C. In this silage extract, pH (PHSJ-4A, China) and $\text{NH}_3\text{-N Nt}^{-1}$ before acidification were measured, this last according to Conway (1947).

Samples for proximate analysis by quintupled were analyzed. Dry matter (DM) and crude protein (CP) were determined according to AOAC (2011). The neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent-lignin (ADL), cellulose, and hemicellulose were determined according to the method of Van Soest *et al.* (1991). The DM of the silages was carried out according to the method proposed by Weissbach & Berg (1977).

B. Statistical analysis

A general linear means (GLM) model was applied. An analysis of factorial ANOVA, correlations and regressions to investigate the effects of treatments on the fermentative process parameters and chemical composition was carried and

Duncan's test was applied to determine mean differences according to the statistical software SPSS version 22.0.

To establish the functional relationship between fruit proportions versus the fermentative process parameters and nutrition composition, the equations (linear, quadratic and cubic) were analyzed and the descending method was used. In order to select the best-fitted equation, it was considered the values of R^2 , significance level, standard error of estimate, the significant contribution of the terms of the equation and low indetermination coefficient ($1-R^2$).

III. Results

Figure 1 shows the pH during incubation of the mixtures made with fresh and foliage pre-wilted and different levels of fresh fruit. The higher inclusion of fruit provoked a decrease of the pH both in fresh and foliage pre-wilted, although being more evident in the first case. Apparently, stability is achieved after 22 hours of incubation (Figure 1). The lowest pH values were found at 48 hours between 3.9 and 4.1 for fresh materials and between 4.4 and 4.2 in the pre-wilted foliage at the same period (Figure 1).

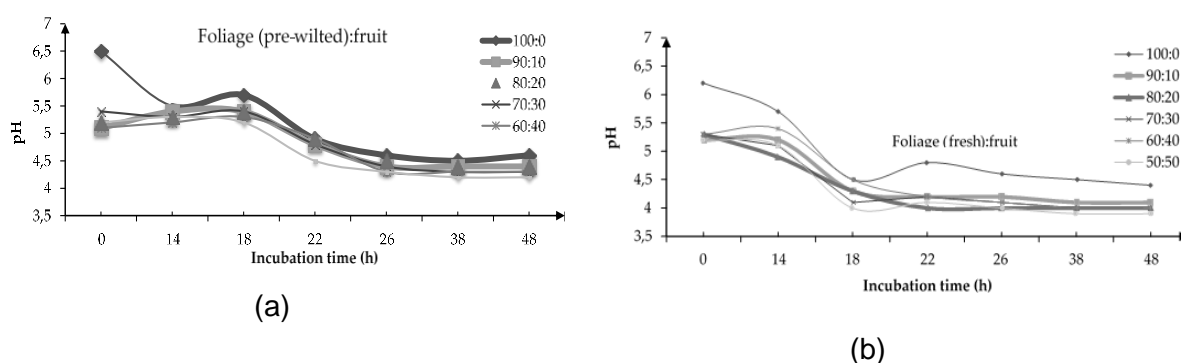


Fig. 1. Changes in pH during incubation of the mixture made with different mixtures of noni (*Morinda citrifolia* L.) pre-wilted (a) and fresh (b).

A comparison between fresh foliage and pre-wilted foliage with fruit levels for pH and NH₃-N results during incubation are shown in the Figure 2. Under the same fruit inclusion levels, the pre-wilted foliage increased the pH and decreased the NH₃-N concentration (Figure 2).

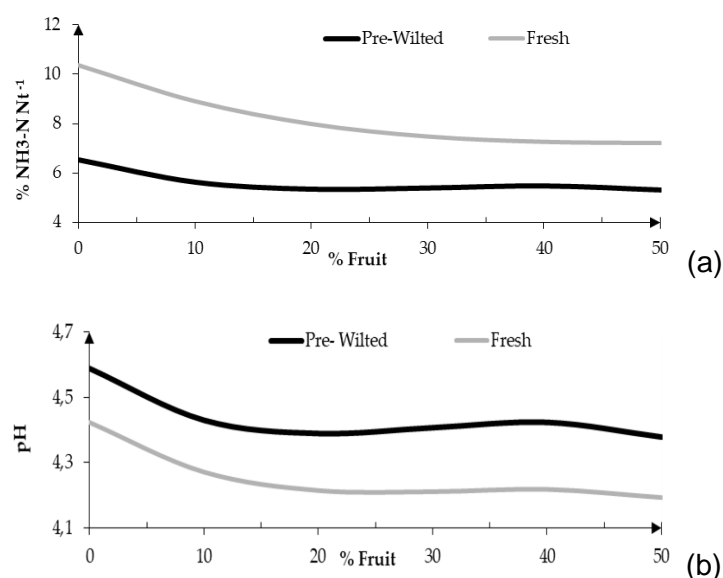


Fig. 2. pH (a) and NH₃-Nt⁻¹ (b) during incubation of the mixture made with different mixtures of noni (*Morinda citrifolia* L.).

The pre-wilted foliage showed higher content of DM ($p < 0.05$), however, drying in the foliage did not influence the protein content, which was lower in the fruit ($p < 0.05$). Also, the highest percentage of WSC was quantified in the fruit ($p < 0.05$), as well as a WAS/PC ratio more favorable (Table I).

Table I. DM, CP, WSC content and WSC/CP ratio of fresh and foliage pre-wilted and fruit of noni, as indicators of their fermentative potential.

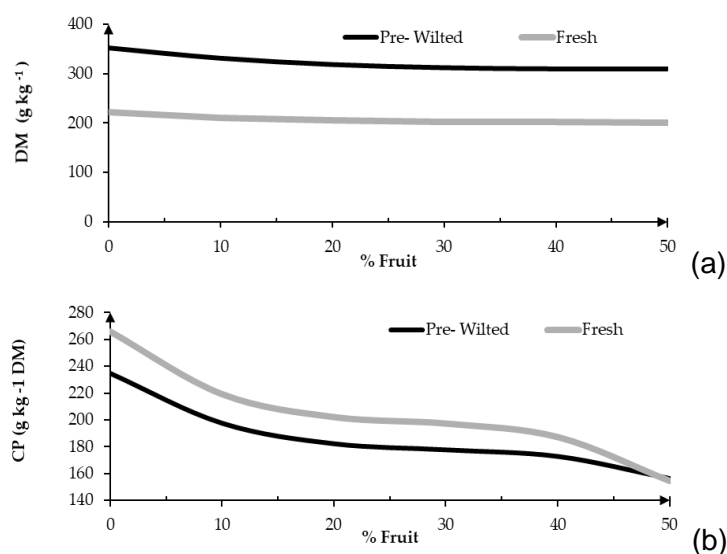
		DM	CP	WSC	WSC/CP
		g kg ⁻¹	g kg ⁻¹ DM		
Foliage	Fresh	246.7 ^b	185.5 ^a	34.3 ^c	0.18 ^c
	Pre-wilted	359.8 ^a	189.5 ^a	44.6 ^b	0.23 ^b
Fruit	Fresh	126.9 ^c	134.2 ^b	62.7 ^a	0.46 ^a
	SEM±	0.279	0.274	0.133	0.014

Vegetal material (1)	<0.001	0.030	<0.001	<0.001
Pre-wilted (2)	<0.001	<0.001	<0.001	<0.001
1x2	<0.001	0.229	<0.001	0.001

a,b,c Means with different superscripts within in the same column, differ significantly ($P<0.05$) according to Duncan's multiple range test.

In this sense, the pre-wilted foliage increased the DM content in all cases, although there was a tendency to decrease to a higher inclusion of fruit. However, fresh foliage in same inclusion of fruit has more CP concentration than pre-wilted foliage, although they were similar in the proportions of 50:50 foliage (Fresh and pre-wilted): fruit (Figure 3).

The use of pre-wilted foliage with inclusions of fruit had a higher NDF content when compared to fresh foliage, mainly with 10% fruit with 345 g kg⁻¹, although only up to 40% of fruit that decreased gradually. Likewise, a similar trend was observed for the contents of ADF and ADL in the mixtures, which had a higher content with 20 % (259.81 g kg⁻¹) and 10% (128.73 g kg⁻¹) of fruit, respectively and a decrease in its fibrous content from 30% fruit (Figure 3).



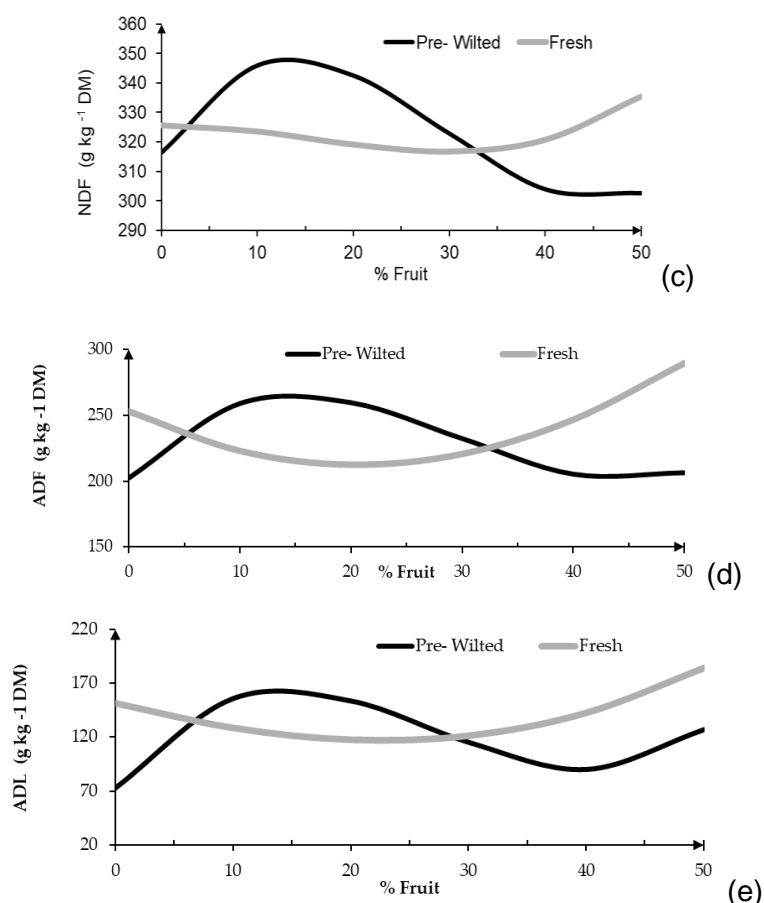


Figure 3. DM (a), CP (b), NDF (c), ADF (d) and ADL (e) contents of the silages made with different mixtures of noni (*Morinda citrifolia* L.).

Table II shows the functional relationship between fruit proportions versus the fermentative process parameters at 21 days. All the variables in the study were adjusted using a cubic model, mainly the $\text{NH}_3\text{-N Nt}^{-1}$ in the fresh foliage with a high R^2 .

Table II. Model $a+b*\text{Fruit}+c*\text{Fruit}^2+d*\text{Fruit}^3$ quantifying the response from some fermentative process parameters to the different fruit levels evaluated in the fresh and pre-wilted foliage.

Variables	Coefficients of cubic model								R ²	
	a		b		c		d			
	Estimate	SEM±	Estimate	SEM±	Estimate	SEM±	Estimate	SEM±		
pH	F	4.424	0.024	-0.021	0.005	0.0007	0.0002	0.000007	0.00001	0.76
	W	4.590	0.022	-0.022	0.004	0.0009	0.0002	-0.00001	0.00003	0.75

NH ₃ -N Nt ⁻¹	F	10.35	0.184	-0.177	0.036	0.0033	0.0018	-0.00002	0.00002	0.90
	W	6.548	0.131	-0.132	0.025	0.0046	0.0013	-0.00005	0.00002	0.75

F: Fresh foliage; W: Wilted foliage.

Moreover, the functional relationship between fruit proportions versus chemical composition at 21 days are presented in Table III. The pre-wilted foliage showed a higher R² for DM, NDF, ADF, ADL and cellulose, while CP and hemicelluloses were higher in the fresh plant material according the using a cubic model.

Table III. Model $a+b*\text{Fruit}+c*\text{Fruit}^2+d*\text{Fruit}^3$ quantifying the response from some nutrients to the different fruit levels evaluated in the fresh and pre-wilted silage.

Nutrients		Coefficients of cubic model								R ²
		a		b		c		d		
		Estimate	SEM±	Estimate	SEM±	Estimate	SEM±	Estimate	SEM±	
DM	F	222.1	0.259	-0.153	0.050	0.0041	0.002	-0.00004	0.00003	0.85
	W	352.2	0.394	-0.283	0.077	0.0060	0.004	-0.00006	0.00005	0.93
CP	F	266.1	0.908	-0.673	0.176	0.023	0.009	-0.00030	0.00011	0.91
	W	234.8	1.158	-0.517	0.225	0.016	0.011	-0.00020	0.00015	0.76
NDF	F	325.6	0.617	0.005	0.119	-0.003	0.006	0.00007	0.00008	0.41
	W	316.3	1.236	0.516	0.240	-0.025	0.012	0.00028	0.00015	0.60
ADF	F	253.1	0.963	-0.398	0.187	0.010	0.009	-0.00001	0.00001	0.84
	W	202.4	0.716	0.940	0.139	-0.042	0.006	0.00047	0.00009	0.89
ADL	F	151.4	1.191	-0.276	0.230	0.004	0.011	0.00005	0.00015	0.72
	W	72.65	0.562	1.417	0.109	-0.067	0.005	0.00080	0.00007	0.98
Cellulose	F	101.6	0.744	-0.122	0.145	0.056	0.007	-0.00060	0.00009	0.18
	W	129.7	0.561	-0.476	0.109	0.024	0.005	-0.00030	0.00007	0.84
Hem	F	72.53	0.726	0.403	0.141	-0.013	0.007	0.00008	0.00009	0.85
	W	113.9	1.197	-0.424	0.232	0.017	0.011	-0.00020	0.00015	0.34

F: Fresh foliage; W: Wilted foliage.

A functional relationship was found between fruit proportions versus the fermentative process parameters and nutritional composition (Tables II and III), these results allow to predict the fermentative potential and the chemical composition of the silages made with fresh and pre-wilted foliage and fruit levels up to 50% of the proportion; as previously reported the pre-wilted positively

influences the fermentation process, since the value of R^2 were mostly high, which ensured a good fit of the mathematical model, achieving good parameters of quality and the process fermentative, with a nutritional high value (Table III). Finally, our results suggest a direct relationship of noni fruit levels and pre-wilted on the quality fermentative and the chemical composition of the mixed silage of noni.

IV. Discussion

The use of noni fruit exerted a rapid decrease in the pH values, mainly when the fruit levels in the mixture are increased compared with the pure forages (Figure 1). The biological rapid fermentation indicated a high potential acidity of noni fruit, which is favorable for a natural conservation process. Probably due to the content of water soluble carbohydrates (Table I), citric acid and other organic acids in the fruit, which increase with the maturation state. Other authors have reported a similar effect on silages made with different mixtures of orange fruit wastes and *Leucaena* foliage (Revuelta *et al.*, 2008).

The variation of $\text{NH}_3\text{-N Nt}^{-1}$ is an indicator of proteolysis processes. It is clear the decrease of protein destruction when fruit levels were augmented in the mixture (Figure 2b). After that, a 20% decrease or stabilization the protein destruction was observed. The lowest values in NH_3 were obtained in wilted silages (from 6.54 to 5.55%), which can be considered excellent, because showed an ammonia-nitrogen concentration of up to 8% (Pineda *et al.*, 2016) (Figure 2).

The ammonia concentrations must not be higher than 110-120 g kg^{-1} of total nitrogen, in well-preserved silages (Boschini & Pineda, 2016). Pitt *et al.* (1985) showed mathematical models on the influence of the pH and nitrogen content on the proteolysis. This could have happened in this study, because when increased

the fruit levels in the mixture was observed a decrease of CP (Figure 3b). Although there was an increase of the pH levels, Rolz *et al.* (2010) reports a decrease of NH_3 , when higher level of coffee pulp was added to the mixture, besides the positive influence of the pre-wilted in the silage process.

On the other hand, Zambello *et al.* (2004) have reported a minimum DM concentration range from 250 to 270 g kg⁻¹ for plant materials to silage, which demonstrates that the DM concentration interfere directly in the fermentation process within the silo. In this sense, Boschini & Pineda (2016) have reported that the optimal values to obtain good silage are between 330 and 370 g kg⁻¹. In this sense, the DM ranges between 303.9 and 352.2 g kg⁻¹ in the silages where the material was pre-wilted, and between 200.3 and 222.1 g kg⁻¹ in the silages without previous drying (Figure 3a). All the treatments showed a tendency to decrease when the fruit levels were increased in the mixture (Figure 3), mainly by the humidity levels of the material. Nevertheless, all the values that were obtained in the silages with the material pre-wilted are considered of silage of good quality ($\geq 30\%$ of DM) (Ozkul *et al.*, 2011).

The decrease of the protein when the fruits were incorporated in the silages (Figure 3b), could be attributed to the degradation of this nutrient during silage, due to a high concentration of non-protein nitrogen (Salem *et al.*, 2013). As shown the WSC is majority in the fruit (Table I), this could provoke a higher buffering capacity in the foliage, which could be lowered when mixed with fruit (Wang *et al.*, 2015). It is observed that the pre-drying process increases the contents of all variables measured in the fruits and foliage (except the CP). It is necessary to highlight that the WSC/CP rate improves with the pre-drying. Apparently, the fermentation process and the chemical composition of the noni mixtures will depend on the plant material and the foliage drying process according to table I.

There is a highly significant relationship ($p < 0.05$) between these two factors for the chemical composition, with the exception of the protein content.

Moreover, the changes in CP of the fruit may be related with the fermentation processes that happen during the drying of rich materials in WSC (Potterat & Hamburger, 2007). There is a clear decrease of the pH in the mixtures (Figure 2a) compared to pure silages (0% fruit), meanwhile, all the pH values obtained with the fresh materials were significantly lower (from 4.18 to 4.25) than wilted silages (from 4.38 to 4.42). The pH is associated directly with the content of DM; a high DM provokes an appropriate fermentation by the levels of lactic acid. Likewise, the physiologic dryness limits the quantity of its synthesis and inhibits the development of the microorganisms, obtaining levels of pH above 4.2 (Wang *et al.*, 2015)

The fibrous components such as NDF, FAD and LAD (Figure 3) in the pre-wilted silage present an increase until a point (120 g kg^{-1}) starting from which begins a decrease of the same one. The opposite happens in the fresh silage, where it presents a decrease (200 g kg^{-1}) and stops, then increases the values of the fibrous fraction, possibly as effect of the acidic and enzymatic hydrolysis of the hemicellulose, because the enzymes of the vegetable cells and microorganisms present in the fermentation process can break the cellular structures, breaking the chemical links of structural carbohydrates, mainly the cellulose (Zhao *et al.*, 2016). This is very convenient for feeding non-ruminant animals such as pig and poultry, which do not assimilate ingredients with high levels of fiber (Jha & Berrocoso, 2016).

V. Conclusions

Results from the present study indicated that fruit levels in a proportion of 30% in the mixture and pre-wilted process of the foliage improved the fermentation process and chemical composition (Except to CP) of the silage noni foliage.

Thus, the increase in the level of fruit increased the concentration of WSC and the ratio of WSC/PC. Moreover, the pre-wilted foliage increased the concentration of nutrients and its combination with fruit levels decreased the fibrous compounds and CP concentration in the silage. Likewise, a high statistical significance was found between the interaction of the factors, the material of the plant and the pre-drying for DM and WSC, except for CP.

Also, all the variables in the study were adjusted using the cubic models, with high values the R^2 , as much for the fresh silages as for the pre-wilted. In this sense, the pre-wilted foliage and fruit proportions showed a high dependence between the variables, such as NDF, ADF, ADL and cellulose, while CP and hemicelluloses were higher in the fresh plant material. It is recommended in vivo studies of the best silages as animal feed, preferably in non-ruminant animals.

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