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Scientific Paper

Effect of the inclusion of *Musa* sp. on the conservation of *Morus alba* Linn.

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Abstract

Objective: To evaluate the effect of the inclusion of different levels of *Musa* sp. on the nutritional and fermentative quality of silages of *Morus alba* Linn.

Materials and Methods: A completely unrestricted design was used, with four treatments: T1-100 % *M. alba*; T2-85 % *M. alba*: 25 % *Musa* sp.; T3-70 % *M. alba*: 30 % *Musa* sp. and T4-55 % *M. alba*: 45 % *Musa* sp. The silages were made in 5-kg plastic bags during 40 days. At the moment of opening, the bromatological characteristics of the silages were measured, as well as the fermentation indicators. A variance analysis was used and correlations were done between all the bromatological variables and the fermentation indicators.

Results: The dry matter content decreased at a rate of 2,4 % per each increase of *Musa* sp. in the different treatments. The inclusion of 45 % of *Musa* sp. in the mixture generated a growth of 47,7 and 58,0 % in the content of non-fibrous carbohydrates and pectins, respectively, 27 % of starch and 1,1 megacalories more of net lactation energy per each kilogram of dry matter. Meanwhile the contents of neutral detergent fiber and digestibility of the neutral detergent fiber were reduced by 22 and 23 %, respectively, when including 45 % of *Musa* sp. in the diet. In addition, there was improvement in the fermentation indicators, because the lactic acid content increased and pH and ammoniacal nitrogen were reduced in the silages.

Conclusions: The inclusion of 0 and 15 % of *Musa* sp. in the *M. alba* silage showed the highest values of protein, ash and fiber; while in the treatments of 30 and 45 % improved the forage conservation process and favorable fermentation indicators were obtained, as well as reduction in the quantity of fiber and increase of non-fibrous carbohydrates.

Keywords: silage, animal nutrition, shrubs

Introduction

Ruminant feeding in the tropics is based, mainly, on the utilization of pastures and forages as the main source of nutrients (López-Herrera *et al*., 2019). Nevertheless, their nutritional composition is variable, and some factors influence it, such as cultivar, physiological age (Elizondo-Salazar, 2017) and seasonal conditions of rainfall (Ribera *et al*., 2017).

This situation forces to search for technological alternatives for ruminant feeding, which allow an adequate nutritional contribution, at low cost and, jointly, the increase in productive yields.

The use of trees and forage shrubs allows the adequate contribution of nutrients (Cardona-Iglesias *et al*., 2017), mainly of protein (Franzel *et al*., 2014). Nevertheless, if this type of resources is used, the diet should be complemented with energy sources that optimize the utilization of the forage nutrients (Jiménez-Ferrer *et al*., 2015).

Morus alba Linn. is an adequate forage resource for the supplementation of ruminants, because it produces higher quantity of digestible nutrients compared with other forages, it shows good quantity of biomass per year and appropriate adaptation to the environmental conditions (Torres-Navarrete *et al*., 2019).

According to Mohapatra *et al*. (2010), square banana (*Musa* sp.) belongs to the group ABB of the triploid hybrids *Musa acuminata* x *Musa balbisiana*. They have shown good capacity for their conservation through silage, especially when they are used in unripe state (Happi *et al*., 2008). In addition, they constitute a source rich in non-fibrous carbohydrates, with 81,5 % of DM (López-Herrera *et al*., 2017) and 80,1 % of starch (López-Herrera *et al*., 2019).

Silage is achieved through a fermentation process induced by bacteria that transform carbohydrates into organic acids, such as lactic acid,

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which decreases the pH of the medium (Yitbarek and Tamir, 2014). This process occurs under anaerobic conditions (Sánchez-Ledezma, 2018). This technique has allowed the conservation of diverse types of materials that can be later used in ruminant feeding (Arce *et al*., 2015 and López-Herrera *et al*., 2016).

The objective of this research was to evaluate the effect of the inclusion of different levels of *Musa* sp. on the nutritional and fermentation quality of silages of *M. alba* Linn.

Materials and Methods

Location. The study was conducted at the experimental farm Santa Lucía (FESL, for its initials in Spanish), property of the School of Agricultural sciences of the National University of Costa Rica. This facility is located between the coordinates 10° 1' 20'' North latitude and 84° 06' 45'' West longitude, at an altitude of 1 250 m.a.s.l., in Barva, Costa Rica.

Climate. The zone shows annual rainfall of 2 403 mm. Relative humidity is 80,5 % and mean annual temperature, 20,3 ºC, with minimum of 15,4 ºC and maximum temperatures of 25,2 ºC (IMN, 2020). The laboratory analyses were carried out at the Research Center on Animal Nutrition of the University of Costa Rica, located in Montes de Oca.

Forage materials. The *M. alba* forage was harvested in the plantation of the FESL, in plots designated for that purpose. It was established with asexual seed, with a density of 25 000 plants/ha. During the experimental period only weed control was carried out. The forage was harvested at 75 days of age and 40 cm of height. It was also subject to a dehydration process during 48 h in a greenhouse, in order to reduce the moisture content before elaborating the silages. The square banana fruits were obtained from the agroecological farm Vocaré, located in the Upala Canton, at 120-180 m.a.s.l., with average rainfall of 2 500 mm per year, average temperature of 26 ºC, relative humidity of 80-90 % and solar radiation of 3-5 h per day (Barrientos and Chaves, 2008). The nutritional composition of the materials used is shown in table 1.

Treatments and experimental design. A completely randomized unrestricted design was used, with four treatments: T1-100 % *M. alba*, T2-85 % *M. alba*: 25 % *Musa* sp. (SB) SB, T3-70 % *M. alba*: 30 % SB and T4-55 % *M. alba*: 45 % SB. All of them were subject to conservation through the microsilo ensiling technique. To each treatment 6 % weight/weight of sugarcane molasses, without being diluted, and artisanal bacterial inoculant, were added. The latter was elaborated by fermentation in the farm, from milk serum, milk and molasses + Lactobacillus 1,0 x 10^9, at dose of 1 L/t in fresh. Each ensiled treatment was repeated four times, for which the experiment had 16 experimental units in total.

Experimental procedure. The materials were separately chopped through an electrical engine chopper until obtaining an average particle size of 2,5 cm. They were manually mixed with the unripe square banana fruits. For vacuum packing the silage, polyethylene bags were used, with capacity for 5 kg and thickness of 0,063 mm. the material was manually compacted in the bags, extracting the air from them through a vacuum cleaner. Then, they were sealed with adhesive plastic tape and were put in a green bag for silage. A room was used as storehouse, where they were protected from bird attack and from routine works that could affect the ensiling process.

Table 1. Nutritional composition of the feedstuffs used for preparing the ensiled treatments (%).

Nutrient	*Musa* sp. (SB)	*M. alba*
CP	5,1	16,3
NDF	8,7	46,2
ADF	5,9	32,8
Lignin	5,1	7,28
NFC	86,3	24,9
Total starch	84,3	1,0
TND	89,2	60,3

CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, NFC: non-fibrous carbohydrates.

Sampling and experimental variables. After 40 days of fermentation, a sample of each experimental unit was sent for performing the analysis of nutritional quality. Dry matter, crude protein (CP = N x 6,25), ethereal extract and ash were determined by the methods indicated in AOAC (1998); while non-fibrous carbohydrates were estimated according to Detmann and Filho (2010). In addition, the starch content was determined by high production liquid chromatography (HPLC) in an Agilent 1260 Infinity chromatographer, with a column Agilent Hi-Plex H. (Ewen, 2011). The methodology proposed by Van Soest *et al*. (1994) was applied for the concentration of neutral detergent fiber, acid detergent fiber and lignin. The digestible neutral detergent fiber (dNDF) and total digestible nutrients were estimated according to the technique suggested by Detmann *et al*. (2008) and the energy fractions were calculated through the equations described in the charts of requirements for dairy cattle (NRC, 2001). From each microsilo a sample of 0,6 kg was taken to determine the silage quality through the fermentation indicators pH and lactic acid. For the pH a potentiometer with hydrogen electrode was used, according to the methodology proposed by WHO (2003). For the lactic acid, the method indicated by Ewen (2011), through HPLC, was followed.

Statistical analysis. The data were tabulated, and then processed through a variance analysis, after testing the normality and variance homogeneity assumptions by the statistical program INFOSTAT Professional (Di-Rienzo *et al*., 2019). Besides, a linear and quadratic regression analysis was carried out to determine the change rate in the nutritional variables, in case the effect of the treatment was significant. The correlation among all the bromatological variables and the fermentation indicators was also analyzed through Pearson's correlation coefficient. In all the information analysis significance was declared when p < 0,05. For the comparison between means Tukey's test was used with confidence level of 95 %.

Results and Discussion

Dry matter and fermentation indicators. The pH showed significant differences (p < 0,001), due to the substitution by SB fruit. The pH means in the silages showed linear decrease, as the quantity of SB increased. The treatment with the highest pH value was the one with *M. alba* without fruit; while those with the lowest pH were the ones of *M. alba* with 30 and 45 % of inclusion, without differences between them (table 2).

In the lactic acid, the inclusion of SB generated an inversely proportional effect, so that the treatment with the highest quantity of SB was the one that produced the lowest amount of lactic acid.

Although the SB decreased the pH values in the silages, the means of the different treatments showed adequate values for the silages of tropical plants and with high nitrogen content, such as legumes (Kung and Shaver, 2001).

The reduction in the final pH value, when increasing the quantity of SB, can be explained by the content of soluble carbohydrates present in the unripe fruit. According to López-Herrera (2017), it is 6,2 % DM, which is added to the one contributed by molasses and the forage to favor fermentation and pH decrease. In all the treatments the pH was similar to that referred by López-Herrera *et al*. (2017), and lower than the one reported by López-Herrera *et al*. (2019). In both studies, SB was used in the mixtures for silages. The above-referred differences are due to the quantity of carbohydrates present in the ensiled mixtures, and to the forage type used for their elaboration.

The moisture content was affected linearly, and it was directly proportional to the quantity of SB that was used. The treatment with the lowest dry matter (DM) content was 45 % of SB (table 2), because SB

Table 2. Dry matter content, pH and lactate.

Forage	SB	Dry matter, %	pH	Lactate, %
M. alba	0	43,5[c]	4,4[c]	6,8[b]
	15	38,9[b]	4,2[b]	6,9[b]
	30	34,6[a]	4,1[a]	6,2[ab]
	45	32,8[a]	4,0[a]	5,4[a]
SE ±		0,554	0,022	0,215
P - value		<0,001	<0,001	0,001

Different letters in the same column differ p < 0,05 has a lower DM content compared with mulberry, which was even dehydrated before ensiling. The DM reduction was 2,40 points for each 10 % increase in the quantity of SB present in the mixture.

It has been determined that the increases in moisture can influence negatively the final quality of the silage, because they promote secondary fermentation processes (chlostridial), formation of effluents and lactate reduction (Callejo-Ramos, 2018). This effect could be observed when recording the performance of the lactate levels (table 2). As the moisture content, the final concentration of lactate in the silage decreased. In addition, a correlation (ρ = 0,71; P = 0,002) was obtained between DM and concentration of lactic acid (ρ= 0,71; P= 0,002). There was also correlation between the concentration of lactic acid and pH (ρ= -0,64; P < 0,007).

This performance is in agreement with the results reported in other studies, in which it is recommended that the forages with high moisture content can be exposed to dehydration before the ensiling process (Han *et al*., 2006). Although there was decrease in the concentration of lactic acid, the values in all the treatments were within the acceptable ones (2-8 % DM) for the silages of legume species (Kung and Shaver, 2001).

Lactic acid was higher than the report by Kung *et al.* (2003), who found values of 3,61 and up to 4,45 % in alfalfa (*Medicago sativa* L.) silages. These differences could have been influenced by the DM content of mulberry forage and concentration of soluble sugars in the mixture, which can increase or decrease lactic acid production in the silo (López-Herrera and Briceño-Arguedas, 2017). It can be inferred that forage wilting is a strategy that allows the adequate conservation of forages, low pH values and ammoniacal nitrogen; besides the reduction of the populations of chlostridial bacteria, which coincides with reports by Nishino *et al*. (2012).

Intracellular components. The crude protein (CP) content of the silages showed significant differences, due to the inclusion of SB in the mixture, and this decrease occurred at a rate of 1,1 percentage points for every 10 % of SB inclusion. This was due to the lower content of this fraction in SB with regards to the forage protein (table 1), similar performance to the one described by López-Herrera *et al*. (2017). These authors found reductions of this fraction, when increasing the inclusion of unripe fruit of *Musa* sp.

None of the treatments showed CP concentrations lower than 7 %, which allows to consider that these mixtures are appropriate for the supplementation of ruminants, as complement of a balanced diet. It is estimated that the reduction of 7 % in the protein content could generate deficiencies in nitrogen metabolism in the rumen, because the value of this nutrient in the diet is reduced, which compromises the adequate functioning of the rumen (Calsamiglia *et al*., 2010).

In this research, the silages showed higher CP contents than the ones referred by Alpízar *et al.* (2014) in *Sorghum bicolor* (L.) Moench and *M. alba* silages. They were also higher than those reported by Lazo-Salas *et al.* (2018) in silages of pineapple (*Ananas Comosus* L.) crown and SB. Nevertheless, they were similar to the ones found by Montero-Durán (2016) in legume silages with SB, and to those obtained by López-Herrera *et al*. (2019) in silages of pasture, urea and SB. These differences in the protein content are due to the characteristics of each species.

The ethereal extract was not affected by the inclusion of SB in the mulberry silages (table 3). It is possible that the values of this fraction were similar in both species, so that the inclusion did not generate differences in the silages.

The inclusion level of SB showed significant differences in the content of non-fibrous carbohydrates

Table 3. Intracellular components of the *M. alba* silages with SB (% DM).

Forage	SB	Crude protein	Ethereal extract	Non-fibrous carbohydrates	Total starch	Ash
M. alba	0	18,0[c]	2,6	35,3[a]	0,4[a]	10,5[c]
	15	17,1[bc]	2,7	39,3[a]	8,2[b]	10,4[bc]
	30	15,6[ab]	2,9	44,3[b]	17,9[c]	9,9[b]
	45	13,6[a]	2,7	48,6[c]	27,4[d]	8,9[a]
SE ±		0,482	0,177	0,967	0,836	0,130
P - value		<0,001	0,872	<0,001	<0,001	<0,001

Different letters in the same column differ p < 0,05 among the treatments (table 3), so that at higher quantity of SB the concentration of non-fibrous carbohydrates of the ensiled mixture was higher. There was increase of this fraction, as the level of inclusion of SB increased. This performance is related to the quantity of such fraction found in the SB (table 1).

In the established treatments, the concentrations of this fraction were higher than the ones obtained by Cubero *et al*. (2010) with silages of *Zea mays* L. This suggests that the mixtures showed a favorable energy content for their utilization by ruminants, especially because this fraction is composed by starch, which can be transformed into propionate at rumen level, and increase the daily volume of milk (Owens and Basalan, 2016).

Starch is the main component of non-fibrous carbohydrates in the SB fruit, because it represents 97,7 % of this fraction (table 1). Its concentration in the ensiled treatments was affected by the inclusion of SB, and increased as the quantity of SB in the mixture increased (table 3). The increase in the starch concentration was of an average rate of 6 % for each increase of 10,0 % in the mixture. Thus, the treatment with 45 % of inclusion of SB obtained the highest concentration (27,4 % DM), similar to that reported by Der Bedrosian *et al*. (2012) in *Z. mays* silages (32,0 % DM).

Starch is the main compound of carbohydrates of the *Musa* sp. fruit in unripe state, which is in agreement with the results obtained by Barrera *et al*. (2010). Nevertheless, its content can vary among the *Musa* species and the geographical zones (Ravi and Mustaffa, 2013), for which bromatological analysis should be conducted before the utilization of these materials in the silages. In addition, its use should be balanced in the diet to prevent large quantities of starch in the rumen and its adverse effects, such as rumen acidosis (Humer *et al*., 2017).

The mineral content of the ensiled mixtures was affected by the SB inclusion level (table 3). When increasing the quantity of SB in the mixture, the concentration of the mineral fraction decreased.

According to Montero-Durán (2016), SB has low ash content (5 %), which contributed to reduce this variable in the silages. This performance coincides with the results obtained by López-Herrera *et al*. (2017) in silages of *Cenchrus purpureus* (Schumach.) Morrone and Pelipita banana (*Musa ABB*).

According to Hoffman (2005), the decrease in the mineral fraction is favorable, because ash lacks energy contribution. This increases the quantity of fermentable organic matter in the rumen, which can contribute to the energy feeding of the ruminants. In addition, there was a negative correlation between the ash content and digestible nutrients (ρ= -0,97; P < 0,001).

Components of the cell wall and energy. The content of neutral detergent fiber (NDF) of the silages was affected by the SB inclusion level. The NDF decreased as the quantity of SB in the ensiled mixtures increased (table 4). This performance can be due to the low quantity of fiber shown by SB compared with *M. alba* (table 1). The reduction occurred at a rate of 17 percentage points, as average, for each increase of 10 % in the mixture.

The decrease of fiber in the mixtures is favorable, because high contents in the forage can affect the voluntary intake and productivity of the animals (Combs, 2014), due to the physical filling caused by the distention of the rumen walls (Sousa, 2017).

Less fiber quantity in the rumen could affect the intake of physically active fiber, because the one in the forage is substituted by a less effective material for the stimulation of rumination and contains more starch. This situation could decrease the lactic solids, and cause risk of rumen acidosis (Izumi *et al*., 2018).

Table 4. Concentration of the cell wall components (g/100 g DM) and energy (Mcal NE_L/kg DM) of the ensiled mixtures.

Forage	SB	NDF	dNDF	Lignin	Pectin	TDN	NE_L
M. alba	0	33,5[c]	19,9[c]	4,6	5,4[a]	65,6[a]	1,5[a]
	15	30,6[b]	18,1[b]	4,4	6,1[ab]	66,9[a]	1,5[a]
	30	27,4[a]	16,2[a]	4,2	6,5[ab]	68,8[b]	1,6[b]
	45	26,1[a]	15,3[a]	4,3	8,5[b]	70,3[b]	1,6[b]
SE ±		0,620	0,365	0,175	0,699	0,408	0,010
P - value		<0,001	<0,001	0,292	<0,043	<0,001	<0,001

NDF: neutral detergent fiber, dNDF: digestibility of the neutral detergent fiber, TDN: total digestible nutrients, NE_L: net lactation energy

Different letters in the same column differ (p < 0,05)

According to the fiber contents found in this study, all the silages had good quality. However, none of the treatments with SB should be used as forage basis, but as complement in a balanced diet, with adequate contribution of effective fiber.

There was decrease in the lignin content, as the level of inclusion of SB in the mixtures increased (table 4). Nevertheless, that difference was not significant, although the lignin content of the SB fruit was different to that of *M. alba* (table 1).

According to Mohapatra *et al*. (2010), the *Musa* husks can contain 5-8 % lignin, when they are in unripe state, which allows to make this assumption in their performance.

The lignin values in this study were lower than the ones found by Montero-Durán (2016) in silages of *Cratylia argentea* Desv. and *Erythrina poeppigiana* Walp. with SB, although higher than those reported by Roa and Galeano (2015) in silages of *Trichanthera gigantea*, with 30 and 60 days of conservation (3,3 and 3,5 % DM, respectively). These differences in the lignin content can be due to the forage type, season and harvest age of the material (López-Herrera and Briceño-Arguedas, 2016).

Valente *et al.* (2016) stated that lignin is a compound of the structural tissues that make up fiber and has been related to the availability of the other components of the cell wall. Thus, besides the fiber concentration and effectiveness, the fiber quantity that is utilized as energy by the ruminant is important. Combs (2014) indicates that fiber digestibility can affect dry matter intake and animal productivity, because it has higher impact on production, if it is compared to the digestibility of any other nutrient.

The digestibility of the neutral detergent fiber (dNDF) of the silages was affected by the SB inclusion level (table 4). The dNDF was reduced, as the quantity of SB in the ensiled mixture increased. This decrease in the quantity of dNDF is due to the direct reduction of NDF by higher quantity of SB in the mixture; in addition to the fact that lignin remains constant. This increases its proportion in the fibrous fraction and thus, affects its digestibility. The reduction in the concentration of dNDF as percentage of DM occurs, as average, at a rate of 1,1 % for each increase of 10 % in the mixture. However, there were no differences among the treatments, when the dNDF was expressed as percentage of the NDF, because the equation of estimation of the dNDF considers the concentration of total fiber, lignin concentration and relation of both in the material (Sampaio *et al.,* 2012).

The pectin content was affected by the SB inclusion level (table 4). This fraction increased as the quantity of SB in the silages increased. These differences could be related to the concentration of pectins in the SB fruits compared with *M. alba*. According to Mohapatra *et al*. (2010), the peels of unripe SB can contain 10-21 % of pectins, which could explain their increase in the silages. This same effect was in the research conducted by Lazo-Salas *et al.* (2018), who worked with silages of pineapple crowns with SB. It was also recorded in the work by Álvarez-Brito (2017), who used silages of shrub legumes with SB. With regards to the latter, there is more similarity in the data.

The energy content of the silages increased as the quantity of SB in the mixture increased with significant differences (table 4). The treatments with 30 and 45 % were the ones with the highest energy content, without differences between them. These changes in the energy content are due to the energy content of SB (table 1).

In this study, the energy value for the treatment without SB was higher than the one reported by Boschini-Figueroa (2006), who indicated that at 70 days of regrowth *M. alba* shows 57,7 % of TDN and 1,22 Mcal/kg DM of net energy for milk production. This is due to the fact that in the ensiling process the fiber content is reduced, which increases the quantity of nutrients that are utilizable by the animal (López-Herrera *et al*., 2017). The incorporation of SB to the mixture increased significantly the final energy content of the silages.

When utilizing the energy requirements for the production of one kilogram of milk, 4 % of fat according to the requirement tables of NRC (2001) (117 g CP; 0,75 Mcal NE_L/kg), it was determined that each kilogram in fresh of the studied silages has the potential to produce 0,38 – 0,86 kg of milk, 4 % fat.

These differences are mainly due to the DM content in each forage, because the treatment without SB was the one that showed higher potential of silage production (0,67-0,86 kg milk/kg FM) compared with the one that included 45 %, which showed a potential for milk production of 0,38-0,70 kg milk/kg FM of silage. This means that although the SB increases the energy content in the silage, the reduction in the DM content causes the animal to consume more quantity of silage to reach the same potential of a drier material. This effect was obtained with dehydrated *M. alba*, for which the influence of the fresh forage on quality and the potential for milk production should be analyzed. The silages generated in this research are good quality materials for their utilization in the supplementation of dairy cattle.

Conclusions

The inclusion of 0 and 15 % of *Musa* sp. in the *M. alba* silage showed the highest values of protein, ash and fiber. Meanwhile, in the treatments of 30 and 45 %, the process of forage conservation was improved and favorable fermentation indicators were obtained, as well as reduction of the fiber quantity and increase of non-fibrous carbohydrates, which propitiated the increase of energy contribution of the silages.

As higher quantity of *Musa* sp. was used, there was reduction of the protein content, and DM contribution, which affected the productive potential of the ensiled mixtures. Thus, each silage should be balanced in a diet that allows to cover the deficiencies of each mixture.

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Authors' contribution

- Daniel Rojas-Cordero. Conducted the field experiments, their design and setting up. Carried out the data recording and processing, manuscript writing and revision.
- Andrés Alpízar-Naranjo. Conducted the field experiments, their design and setting up. Carried out the data recording and processing, manuscript writing and revision. Participated in the advisory of the research.
- Miguel Ángel Castillo-Umaña. Conducted the field experiments, their design and setting up. Carried out the data recording and processing, manuscript writing and revision. Participated in the advisory of the research.
- Michael López-Herrera. Conducted the field experiments, their design and setting up. Carried out the data recording and processing, manuscript writing and revision. Participated in the advisory of the research.

Conflicts of interests

The authors declare that there is no conflict of interests among them.

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