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Nutritive characterization of Musa *spp* and its effects on *in vitro* Rumen fermentation characteristics

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Abstract. This research aims to study the effect of nutritive value of *Musa spp* on animal feed. Residues of banana culture, leaves and stems, could be used as a fibre source for animal feeding, especially in Banana producing areas, such as Macaronesia Archipelagos, avoiding wastes and supplementing periods of scarcity of food.
Musa spp were collected and dried at 65 °C in an oven with controlled air circulation. The pseudostems were divided in three different portions and chemical composition, *in vitro* digestibility, and *in vitro* gas production were determined. Regarding dry matter results, they were low (16.54% in leaves and 6.54% DM% in pseudostem), crude protein raging 11.25 DM% in leaves and 7.25% in pseudostem. Concerning fiber values, NDF is higher in leaves (70.07 DM%) than in pseudostems (52.11 DM%) and ADL is higher in leaves (9.90 DM%) comparing with pseudostems (6.21 DM%). *In vitro* DM digestibility is low, (24.42% in leaves and 42.69% in pseudostem), corroborating the NDF values. Cumulative gas production was recorded at 4, 8, 12, 24, 48, 72, and 96 h of incubation. The results showed that the gas production in leaves was lower (11.36 mL 200 mg^{-1} DM) when compared to pseudostem (23.81 mL 200 mg^{-1} DM), being so in accordance with the digestibility results.
The current study suggested that this by-product can be used in animal feed, however, it will be necessary to carry out tests to improve its nutritional value, namely with NaOH and/or with Urea, being a promising strategy for improving ruminant feed efficiency.

Key words: animal science, *in vitro* digestibility, gas production, *Musa spp*, ruminants.

INTRODUCTION

The global increase in demand for food, especially protein, raises the need for more efficient and sustainable animal feeding strategies. The use of the most different food resources to meet nutritional requirements in ruminants is undergoing a wide discussion nowadays. The use of alternative feeds for ruminants is a strategy aimed to reduce feed costs and overcoming the pasturage scarcity problem occurring at critical times.

Livestock production in direct grazing regions often see themselves confronted with periods of pasture production shortage, motivated by normal production curves of pasture or adverse weather conditions strongly influencing grass production. With the current concepts of production, in developing countries, the uses of cereals in diets for animals are rare and expensive due to the direct competition as a source for human food. Thus, there is a need to maximize the use of available resources, which include consuming non-conventional resources (Ramírez-Restrepo & Barry, 2005).

There are several advantages of using non-conventional food in animal production. The main advantage of using alternative products is to reduce the cost of food by using locally available resources. The economic value of these products is often much smaller than conventional feed. Other advantages of using these resources are to reduce competition for food between humans and animals. The uses of food waste, such as fruit, cassava and sweet potatoes are excellent sources of fermentable carbohydrates, being an advantage for ruminants because they possess the ability to use inorganic nitrogen.

The ability of ruminants to efficiently use fibre makes it possible to explore agricultural by-products and wastes from tropical crops, like banana, which may also provide energy and protein.

Banana is one of the most famous and useful plants in the world. Practically all parts of the plant, can be used. Nowadays, people have devoted themselves to forest protection and found reasonable ways to use agricultural and forest residues. This is triggered by the rapid increase in consumption of wood fibre-based products, leading to illegal logging activities due to the reduction in permitted wood resources.

Banana tree is a natural resource, widely produced in tropical and subtropical countries all over the world (Li et al., 2010; Aziz et al., 2011; Sango et al., 2018), and for properly development, it requires constant heat, high humidity and proper water distribution.

These conditions are registered in the range between the 30° parallels of north and south latitude, in the regions where the temperatures are between the limits of 15 °C and 35 °C. However, there is the possibility of cultivation above 30° latitude north and south latitudes, since the temperature and the water system are suitable, with a typical banana plant tropical (Moreira, 1999). Banana is the most consumed fruit in the world, with a production of approximately 113 million tons in 2016. Canary Islands, Azores and Madeira, have climatic conditions to produce subtropical and some tropical crops, where there is work for years, adapting cultural techniques in order to optimize the different cultural systems, allowing to know deeply not only the culture, but also the differences to be taken into due account in the subtropical regions.

According to Batabunde (1992), all parts of banana tree can be all used, except the roots and baby trees. He also states that, around 30–40% of total banana production is available to animal feed as result of being rejected for exportation, suffer damages in the field or simply do not have the dimensions to be sold. FAO (2020) projections for world banana production, are that it should rise 1.5% a year, reaching 135 million of tons in 2028.

A major part of a banana tree is the fruit, so banana stem is rarely used. Generally, after collecting the fruits, the banana trees will be cut directly, since it can only have fruit once. After that, the trees are left there to rot. But it should, because the remaining parts could be used for ruminants feeding, since they contain all kinds of nutrients, and people just waste it.

In the leaves, the highest crude protein is found, followed by fruit peel and pseudostem. Both pseudostem and leaf contain moderate amounts of fibre but are higher than banana peel. The high contents of tannin in the leaf and fruit peel reduced their protein and dry matter digestibility and their value as ruminants. The low crude protein and high moisture contents of pseudostem reduced dry matter intake potential by ruminants.

Since leaves have low digestibility and stem has low dry matter and crude protein, to obtain high animal production from ruminants fed banana wastes, it should be supplemented with protein concentrate feeds.

One way to mitigate the factors caused by climate changes would be the use of alternative foods during scarcity periods. However, in order to optimize the use of these foods in animal feed, it is important to establish a balance between the nutrients in the diet to guarantee efficiency in ruminal fermentation processes and optimization of microbial growth, which will result in the maximization of fiber digestion and in the improvement of productive performance.

However, it is important to know the chemical composition and its nutritional value in the rational use of these foods, considering the level that can be included in the diet, with the objective of obtaining balanced diets that meet the nutritional requirements of the animal, maximizing its consumption (Clementino, 2008).

Therefore, the aim of this study was to study the effect of nutritive value of *Musa spp* on animal feed, to see if it could be an effective way to help in periods of scarcity of food.

MATERIALS AND METHODS

The present study was conducted in the Animal Nutrition Lab '*Prof. Doutor Gourlay Young do Amaral*', Department of Agricultural Sciences, University of the Azores, located in Angra do Heroísmo, Terceira, Azores, Portugal. The plant, manually harvested, in Terceira Island, was chopped and the pseudostem was divided in three portions and were dried in an air oven at 65 °C for 72 h.

Dried samples were then ground through a 1-mm screen using a Retsch mill (GmbH, 5657 HAAN, Germany). These grind samples were analysed for dry matter (DM, method 930.15), crude protein (CP, method 954.01) and total ash method (942.05), according to the standard methods of AOAC (1995). Briefly, the dry matter content o was determined by placing samples in an air oven at 105 °C for 24 h. The ashes were evaluated by igniting samples in a muffle furnace at 500 °C for 12 h. Crude protein was determined by standard micro-Kjeldahl method, using digestion equipment (Kjeldatherm System KT 40, Gerhart Laboratory Instruments, Bonn, Germany) and an automated Kjeltec 2300 Auto-analyser apparatus for distillation and titration (Foss Electric, Copenhagen, Denmark). The acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF), was be determined according to Goering & Van Soest (1970). *In vitro* digestibility was be determined using the Tilley & Terry (1963) method, modified by Alexander & McGowan (1966), and the liquid of the rumen was obtained from the local slaughterhouse (IAMA), as described by Borba et al., (2001).

Regarding *in vitro* gas production (GP) technique, which simulates the rumen fermentation process, and it is used to evaluate the potential of feeds to produce greenhouse gas, each assay was repeated three times (runs). Blanks were used for each inoculum to measure the fraction of total gas production due to substrate in inoculum and these values were subtracted from the total to obtain net GP. All treatments, for each assay, were incubated simultaneously in all runs (Menke et al., 1979).

The preparation of buffer solutions and rumen inoculum was as described by Menke & Steingass (1988).

The initial gas volume was recorded after 4, 8, 12, 24, 48, 72 and 96 hours of incubation.

This gas production represents the kinetic of the rumen apparent GP and is expressed by the McDonald (1981) equation. Gas production profiles were obtained after adjusting the data to the exponential equation of Ørskov & McDonald (1979):

$$p = a + b\ (1 - exp -^{c\ t})$$

where p is the gas production at time t; *the values of a and b represent constant values in the exponential equation*; $a+b$ the total potential gas production (mL g^{-1} DM), and c the rate constant.

The fermentation constants a, b and c were calculated by a suitable curve method using Neway Software Program (Rowett Research Institute, Aberdeen, UK) that was developed by Chen (1997).

For Statistical Analysis, ANOVA was performed, followed with posthoc least significant difference test by IBM SPSS version 24 Statistics Program software (SPSS Inc. Chicago, IL). For all analyses, a P value of < 0.05 was considered statistically different

RESULTS AND DISCUSSION

In the present study, the effect of nutritive value of *Musa spp* for animal feed was analysed and the results are presented at Table 1, as well as chemical composition values.

Table 1. Chemical composition and nutritive value of *Musa spp*

Treatment	DM (%)	100g DM						DMD (%)
		CP	NDF	ADF	ADL	EE	Ash	
Leaves	16.54[a] (± 0.58)	11.25[a] (± 1.18)	70.07[a] (± 2.28)	42.38[a] (± 1.63)	9.90[a] (± 0.63)	2.69[a] (± 0.42)	17.74[a] (± 0.94)	22.69[a] (± 1.27)
Pseudostem Total	6.54[b] (± 0.25)	7.25[b] (± 0.66)	52.10[b] (± 0.51)	30.62[b] (± 1.29)	6.57[a] (± 1.10)	1.36[b] (± 0.09)	24.07[b] (± 1.95)	40.39[b] (± 3.10)

DM – Dry Matter; CP – Crude Protein; NDF – Neutral Detergent Fibre; ADF – Acid Detergent Fiber; ADL – Acid Detergent Lignin; EE – Extract Ether; DMD – In vitro Dry Matter Digestibility. [a,b] different superscript within the same column indicates significant differences, $P \leq 0.05$.

Our results, regarding DM shown significant differences between leaves and pseudostems. For the CP, it shown significant differences between these two parameters ($P < 0.05$). Concerning NDF values, it shown significant differences between leaves and pseudostems, and the higher value was found in leaves (70.07%). These results and the DMD results are in accordance, showing that the pseudostem has a lower NDF value 52.10% vs the 70.07% in leaves and the DMD in leaves is 22.69% but in pseudostem is 40.39%. About ADF, it also shown significant differences between leaves and pseudostems. EE also shown significant differences between them, such in Ash and DMD. ADL shown no statistically significant differences among treatments ($P > 0.05$).

In most parameters, leaves presented the higher values, except for ashes and DMD. This is normal, since leaves has more NDF than pseudostems, so they have lower digestibility. On the other side, pseudostems have less protein, at the limit of ruminal activities and that is way their digestibility is not so high to.

40% of banana trees are considered as waste in the field, producing about 60–80 tons ha^{-1} year^{-1} of banana pseudo stems (Salehizadeh et al., 2017) which as low nutritive value and high-water content, around 93.4%, but based on DM, the nutrient value of CP is 6.5% and the nutrient value of lipid is 1.5% (Tuan, 2004). These values agree with the results of our study, in which the pseudostem values are like that and lower than the ones presented in leaves. According to Cordeiro et al., 2004, pseudostem has high percentage of ash content (14%), but low lignin content (12%), when compared with other plants. Furthermore, the lignocellulose is around 60% up to 85%, 50% of cellulose, over 17% lignin and 4% ash. Regarding leaves, the author states that they have around 26% cellulose, 17% hemicellulose, and 25% lignin (Jayaprabha, et al., 2011; Reddy & Yang, 2014). Another statement regarding the composition of pseudo stem, from Okelana, 2001, is that crude fibre of central portion of pseudo stem is about 20% and 40% in the external portion. Its ash content is from 14–30% and the fat ranges from 18–22%. Nevertheless, the quality of stem can be improved if a silage with other foliage or with yeast is done, as growth promoter, in order to improve protein content, feed intake and live weight gain as well (Ty et al., 2012; Tien et al., 2013; Manivanh & Preston 2015). Also, Chedly, & Lee, stated that, ensiling can also reduce some previously unpalatable products, into useful to livestock, by simply changing the chemical nature of the feed (Chedly & Lee, 1998).

Oliveira et al., (2014) studied the effect of the use of banana pseudostem hay in feeding small and large ruminants and concluded that due to the low protein content, its inclusion in the diet of these animals must be accompanied by supplementation of CP (crude protein).

Cumulative gas production as affected by substitution of ruminant diet with *Musa spp* is shown in Table 2 and Fig. 1.

Table 2. Effect of *Musa spp* on cumulative gas production (mL 200 mg^{-1} DM) and gas kinetics

Treatment	Incubation Time (hour)							Gas kinetic parameters			
	4	8	12	24	48	72	96	a	b	c	Lag Time (hr)
Leaves	1.49	2.19	2.86	4.67	7.59	9.75	11.36	-1.36	35.21	0.0188	2.1
Pseudostem	1.45	3.64	5.66	10.87	15.33	20.37	23.81	0.91	31.84	0.0192	1.5

a = gas production from the immediately soluble fraction (mL 200 mg^{-1} DM); b = gas production from the insoluble fraction (mL 200 mg^{-1} DM); c = gas production rate constant for the insoluble fraction (mL h^{-1}).

In vitro gas production technology simulates the rumen fermentation process and has been used to evaluate the potential of food gas production, and it was recorded at 4, 8, 12, 24, 48, 72 and 96 h of incubation. The results showed that compared with the pseudostem (23.81 mL 200 mg^{-1} DM), the gas production in the leaves (11.36 mL 200mg-1DM) was lower, so it was in line with the digestibility results.

When we observed the Lag time (hr), we can see that pseudostem, is the first to begin his fermentation (1.5 hr after placing the sample to incubate), when compared to the leaves.

The gas production constant rate (c) is also higher in pseudostems, which results in a greater gas production.

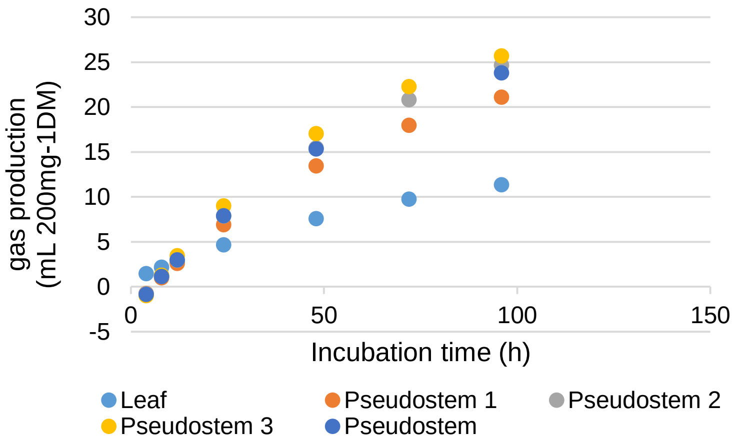

Figure 1. Pattern of in vitro gas production (fitted with exponential model) on incubation of *Musa spp* treated with NaOH, in buffered rumen fluid.

CONCLUSIONS

The study suggested that this by-product can be used in animal feed, however, further research is essential to describe more appropriate techniques to improve the quality of banana pseudo stem, such as to carry out tests to improve its nutritional value, namely with NaOH and/or with Urea.

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