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In vitro Digestibility of Whole Forage Re-Shoots from Two New Sugar Cane Cultivars (*Saccharum* spp. C97-366 and C99-374)

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ABSTRACT

In vitro digestibility of two new sugar cane cultivars for animal forage was determined. Three runs were made using gas from cattle feces as inoculum. The plant's integral fraction was studied at 6, 8, and 11 months of re-shoot. Forage cultivar My5514 was used as witness. The Ørskov and McDonald´s model (1979), transformed by Correa (2004), was used to determine the parameters. Simple variance analyses were carried out, and the differences between the means for $P < 0.05$, were determined by Tukey. The results showed that the two new cultivars produced similar *in vitro* gas volumes, and even higher (8 and 11 months of age) than the My5514 forage cultivar. At 8 months, the new cultivars had the best *in vitro* gas production values. The values achieved for the *in vitro* gas production, speed (0.011 h -1 -0.033h -1), and gas production potential (32.7 ml-52.1 ml), were high. Moreover, the lag had low values (2.10 h-4.57 h).

Key words: *sugar cane, cultivars,* in vitro *gas, re-shoot age, forage*

INTRODUCTION

The limitations of bovine production in the dry season, in the province of Camagüey and other similar areas in Cuba are associated with poor grass availability (quantity and quality). Accordingly, usually costly supplementation practices must be implemented in order to improve productivity of cattle systems (Fernández *et al*., 2013).

Therefore, the inclusion of sugar cane as a nutritional alternative for the dry season must be considered, due to its advantages over other species, and their friendly integration possibilities with other practices.

Researchers, specialists, and technicians at the Phyto Breeding Department of mid-eastern ETICA, in Camagüey, and the Center for Animal Production Development (CEDEPA), at the *Ignacio Agramonte Loynaz* University of Camagüey, began to make a previous selection (the sugar cane selection scheme stages in Cuba) of all the genotypes with phenotypic features for forage production. The results included 106 individuals that were evaluated for determination of forage qualities.

Based on the previous results, studies were continued with two new clones that were better than the rest, due to their forage features.

Considering the advances made in ruminant nutrition, awareness on the nutritional value of forages became critical. It is a very important component in bovine diets, and also an inexpensive, feasible and sustainable choice (León *et al*., 2012); it is a key element to consider at the time of choosing a forage type for production.

The purpose of this paper was to evaluate *in vitro* digestibility through the gas production technique at three different ages of whole forage re-shooting of new sugar cane cultivars (Saccharum spp. C97-366 and C99-374), in comparison with My5514 commercial variety.

MATERIALS AND METHODS

The experiment was made at the mid-eastern Camagüey Regional Sugar Cane Research Station (ETICA), in the municipality of Florida. The soils are brown with carbonates, according to Hernández *et al*. (1999), on the 21º 31´ north latitude, and 78º 04´ west longitude, 57.08 meters above sea level.

The average mean temperature is 26.7 ºC, with a maximum average of 31.4 ºC, and minimum average of 21.6 ºC . The annual rainfall average is 1 236.78 mm (80 % between May and October). The average relative humidity is 75.6 % (Florida´s agro-meteorological station, 2011).

The plantation was started in November 2009, using 252 three-bud chunks, distributed at 28 sticks per furrow. The plantation setting was 0.40 m x 1.60 m; Cuba Libre was the plantation method. A plantation density of 12 buds per linear meter was achieved. Crop handling were based on MINAZ-INICA (20007) regulations. The set up cut was made at 11 months, in October 2010.

The experiment was arranged by random blocks, with three treatments (cultivars C99.374; C97-366; and witness My5514, and three replicas, totalling nine experimental plots, in an area of 26.4 m^2 (3 furrows 5.5 m long each, with 1.60 m between them).

For forage sample collection, cuts were made at 6; 8; and 11 months, after the set up cuts. Cuts were made at ground level, with a machete.

Three representative samples of the integral fraction were used (stem + knot), of 1 ± 0.4 weight kg . The stem and knot per cent was considered, so the sample kept the same original proportion. All the samples achieved were placed in polyethylene bags for immediate transportation to the lab. Then, they were dried in a stove at 65 ºC, with forced air circulation for 48 h (AOAC, 1995), and hammer-crushed before going through 1 mm sift. The samples were properly preserved in glass jars with polished top, until analysis.

In vitro gas production was used to determine digestibility, according to Menke and Steingass (1988), in the Laboratory for Agro-environmental Control (LABCA), at the Center for Animal Production Studies (CEDEPA), *Ignacio Agramonte Loynaz* University of Camagüey, Cuba. Fresh bovine feces (less than 2h) were used as inoculum, dissolved in mineral buffer (mma), at a proportion of 1:3 (feces-mma). Mma was prepared as described by Martínez *et al*. (2004). In all the experiments, 300 mg of dry samples were placed in 100 ml syringes (FORTUNA®, Häberle Labortechnik. Germany), previously heated to 30 ºC, and Vaseline-lubricated plungers.

Each syringe was filled with 30 (± 1) ml of the inoculum-buffer mixture, and then were put in warm bath to 30 (± 0.5) ºC, vertically, and partially submerged in water. They were carefully shaken before submersion and at the time of reading (3; 6; 24; 48; 72; and 96 h of incubation). Three runs were made, corresponding to the whole sugar cane fraction, at ages 6; 8; and 11 months, respectively, for each cultivar. Three syringes containing the inoculum-buffer solution were used in every run as targets to correct gas production. Additionally, other three syringes containing 300 mg of dry and milled Guinea grass (Panicum maximum), were used as standards to correct differences between runs. The standard sample of Guinea grass was achieved according to Martínez *et al*. (2005).

The speed values for gas production (c), and the lag phase were determined with Excel, 2010, according to equation suggested by Correa (2004).

for $t \leq L$ $V = 0$

for $t > L$ $V = b^* (1 - \exp(-c^*t))$

Where:

V: volume gained in ml/300 mg of dry samples.

t: time in hours.

b: volume when $t \rightarrow \infty$.

c: specific growth speed of gas volume at the exponential phase.

Previously configured Microsoft Excel, 2010 was used to process the primary gas production data.

A database with the information gathered was made, and data normality was evaluated during statistical processing. Mean and standard error were determined for each case. Simple variance analysis were performed, and the mean differences were determined using the Tukey test for $P < 0.05$. SPSS 15.0 (2006) for Windows, was used for statistical analysis.

RESULTS AND DISCUSSION

Figure 1 shows cultivar influence on *in vitro* gas production, for 24; 48; 72; and 96 h of whole forage incubation, at 6; 8; and 11 months, respectively.

It shows that at 6 months, statistically significant differences were observed only between cultivars at 24 h of incubation; the C97-366 cultivar had the greatest *in vitro* gas production value. At 8 months of age, no statistically significant differences were observed between cultivars at 24 h of incubation. However, at 48; 72; and 96 h, the C99-374 cultivar had statistically significant differences in relation to the other cultivars studied.

Only the C97-366 cultivar was not statistically different at 96 h of incubation. At 11 months, statistically significant differences were observed at 24; 48; 72; and 96 h after starting runs. The new C99-374 and C97-366 cultivars reached the highest *in vitro* gas production during the study. Only the C97-366 cultivar showed no statistically significant differences, in comparison with My5514, at 24 h of run.

The results observed confirm that the two new cultivars have adequate *in vitro* gas volumes, similar or higher than My5514 during the three runs performed at 6; 8; and 11 months.

Figure 2 shows the behavior of *in vitro* gas production by the cultivars evaluated, at 6; 8; and 11 months.

At six months, the lag and *in vitro* gas production potential values ranged between 2.10 and 2.96 h, and 37.54 and 52.09 ml, respectively; whereas the speed values for gas production ranged between 0.011-0.024 h - l.

The values achieved in the lag phase were very similar between cultivars. The gas production potential for C99-374 showed the highest value, preceded by My 5512 and C97-366. The gas production speed for the C97-366 cultivar was the highest, preceded by My5514 and C99-374.

At 8 months, the lag phase and the *in vitro* gas production potential ranged between 4.26 and 4.57 h, and 39.72 and 45.51 ml, respectively; whereas the values for gas production speed ranged between 0.028-0.033 h - l.

The values achieved for the lag phase were very similar between cultivars, as well as for gas production potential and gas production speed, with a key role for C00-374 and My5514.

At 11 months, the lag phase and *in vitro* gas production potential values ranged between 2.48 and 3.27 h, and 32.73 and 36.64 ml, respectively; whereas the values for gas production speed ranged between 0.023 and 0.24 h - l-

The values observed for the lag phase, the gas production potential, and the gas production speed, were very similar between cultivars.

The results achieved in the lag phase for the study are lower than the reports made by Martínez *et al*. (2008) in a dynamic study of *in vitro* gas gained production, and parameters for better model adjustment (Correa, 2004), including 13 tropical forages. In turn, the gas production potential values are above the reported by the previous author. In terms of gas speed, the values observed are within the range published by the previous author.

González *et al*. (2012) published higher values for the lag phase regarding the parameters for *in vitro* gas production; and lower values for gas production potential in a study of sugar cane bagasse ammonified with urea, as an activator of ruminal fermentation.

Generally, the lag phase values observed in all the runs may be considered low, which shows the cultivars´ abilities to provide fermentable organic matter to the microorganisms in the rumen. These results owe to the fact that the sugar cane samples are composed of easily degradable, soluble carbohydrates. Moreover, the feces collected for the inoculum in the runs, came from bovines daily fed with milled sugar cane in their diet; hence, the microorganisms were adapted to it, and began degradation of samples readily (Tscherning *et al*., 2002; Posada and Noguera, 2005; Martínez *et al*, 2008; González *et al*., 2012; León *et al*., 2012).

The values for gas speed and gas production potential may be considered high, caused by the chemical composition of sugar cane, with high contents of easily degradable, soluble carbohydrates in the rumen. Furthermore, the samples studied belonged to the plant´s whole fraction, in which the stem accounts for 49 and 84 % of the total samples in the three ages evaluated. Considering that organ is in charge of storing sugars produced by the leaves through photosynthesis, they must provide enough fermentable organic matter for the rumen´s microorganisms.

Figure 3 shows the effect of re-shoot age on *in vitro* gas production, at 24; 48; 72; and 96 h of incubation of whole sugar cane, including the three cultivars in the study.

Besides, it shows that My5514 had the highest *in vitro* gas production at 8 months, thus statistically differing from 6 and 11 months, respectively, in the four times evaluated during the run. C97-366 and C99-374 had similar behaviors.

The results show that at 8 months the three cultivars reached the peaks for use as animal feeds, at a time when they can be best consumed by ruminants (Preston, 1998; Pate *et al*., 2002).

This behavior may be associated to a plant´s higher PB content and less fiber at 8 months, in comparison to the 11 months treatment. Moreover, in spite of higher fiber content than the 6

months, it also has higher soluble PB and carbohydrate contents that make feed degradation easier.

Although the results of the study, indicating that at 8 months the cultivars are better used by the animals, logic points using it at 11 months, when the plants have produced more green and fresh biomass (Stuart, 2002; Suárez *et al*., 2006; Ruíz *et al*., 2009); it means better nutrient yields (PB, P, K) per unit. As a result, a greater number of animals can be fed. Furthermore, the rootstock lasts longer, with fewer cuts for forage, which may take place in the next dry season. Hence, the plant has more time to produce better biomass. Accordingly, higher income is perceived by the agricultural systems, by saving land preparation and seed expenses.

CONCLUSIONS

The two new cultivars had similar, even higher, *in vitro* gas levels at 8 and 11 months of re-shoot, using My5514.

The cultivars showed the highest production values of *in vitro* gas, speed and potential at 8 months of re-shoot.

The values obtained for *in vitro* gas production, speed, and production potential were high, and the lag phase had low values.

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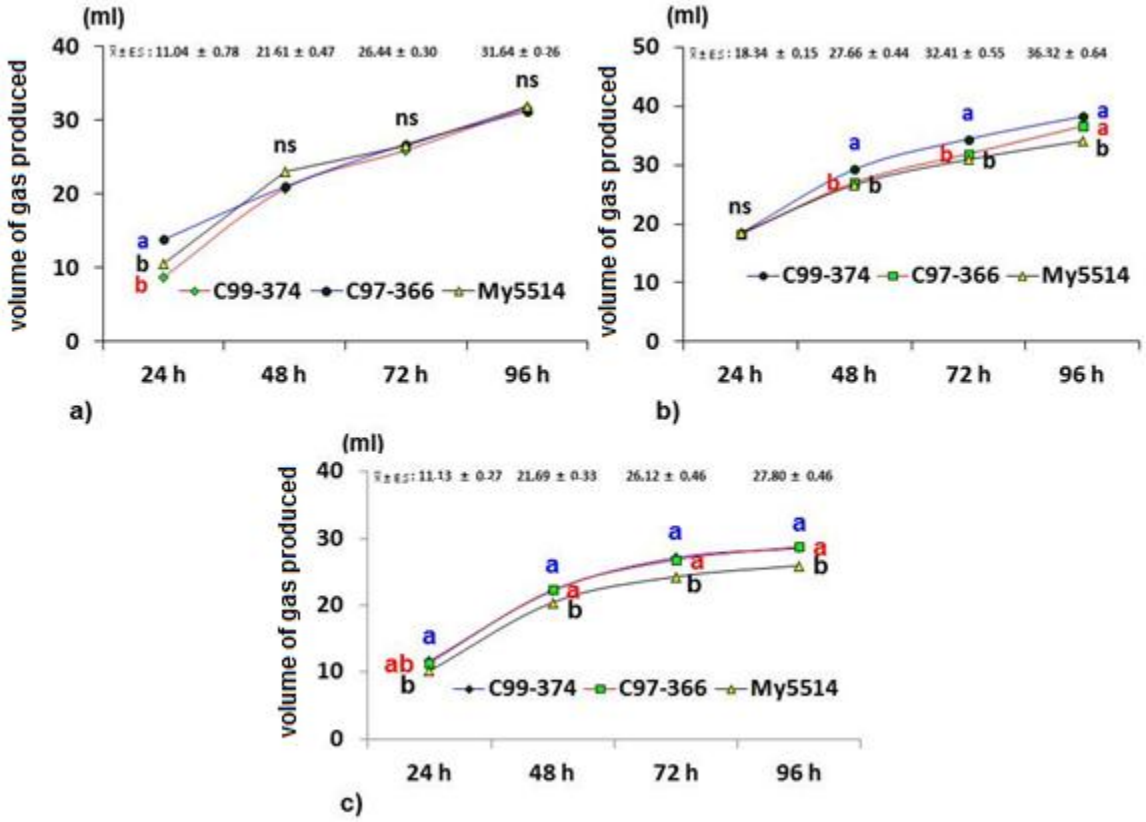

Fig. 1. Cultivar effect on *in vitro* gas production at 24 h, 48 h, 72 h and 96 h at ages: a) six months b) eight months c) eleven months

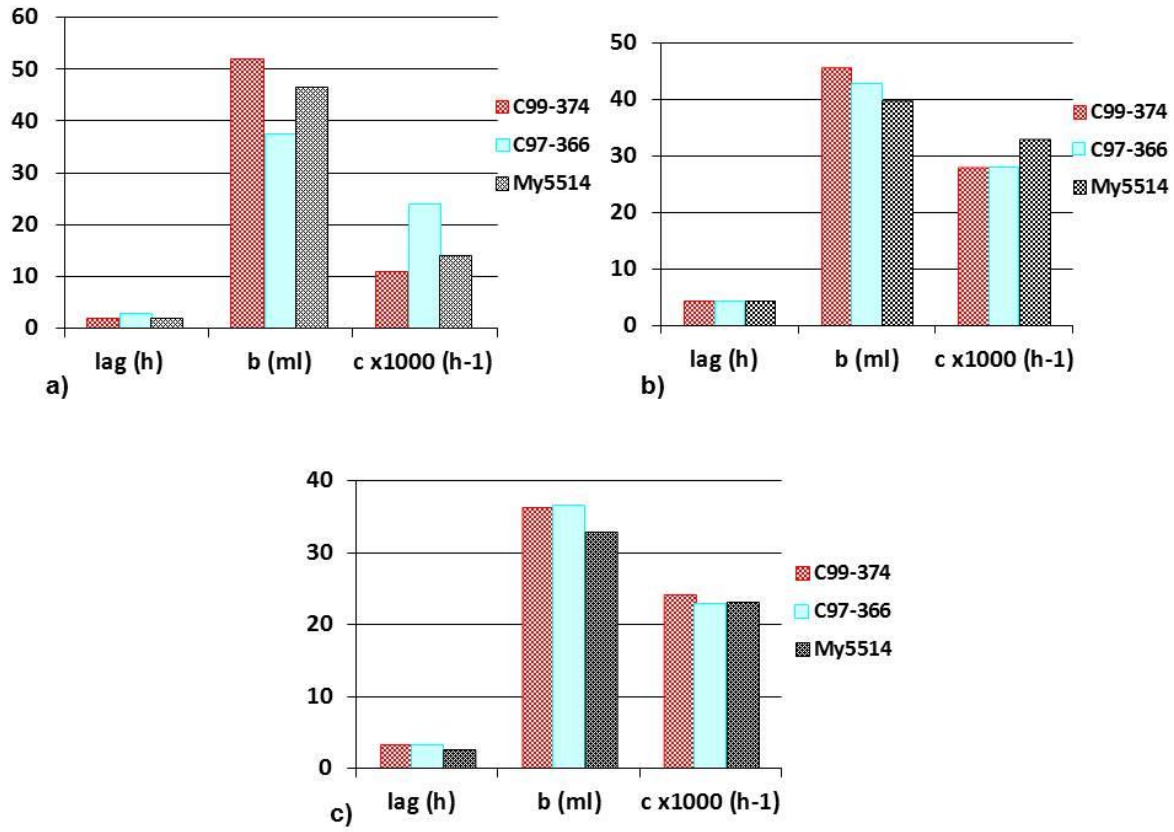

Fig. 2. Behavior of *in vitro* gas production parameters of the cultivars evaluated at different ages: a) six months, b) eight months c) eleven months

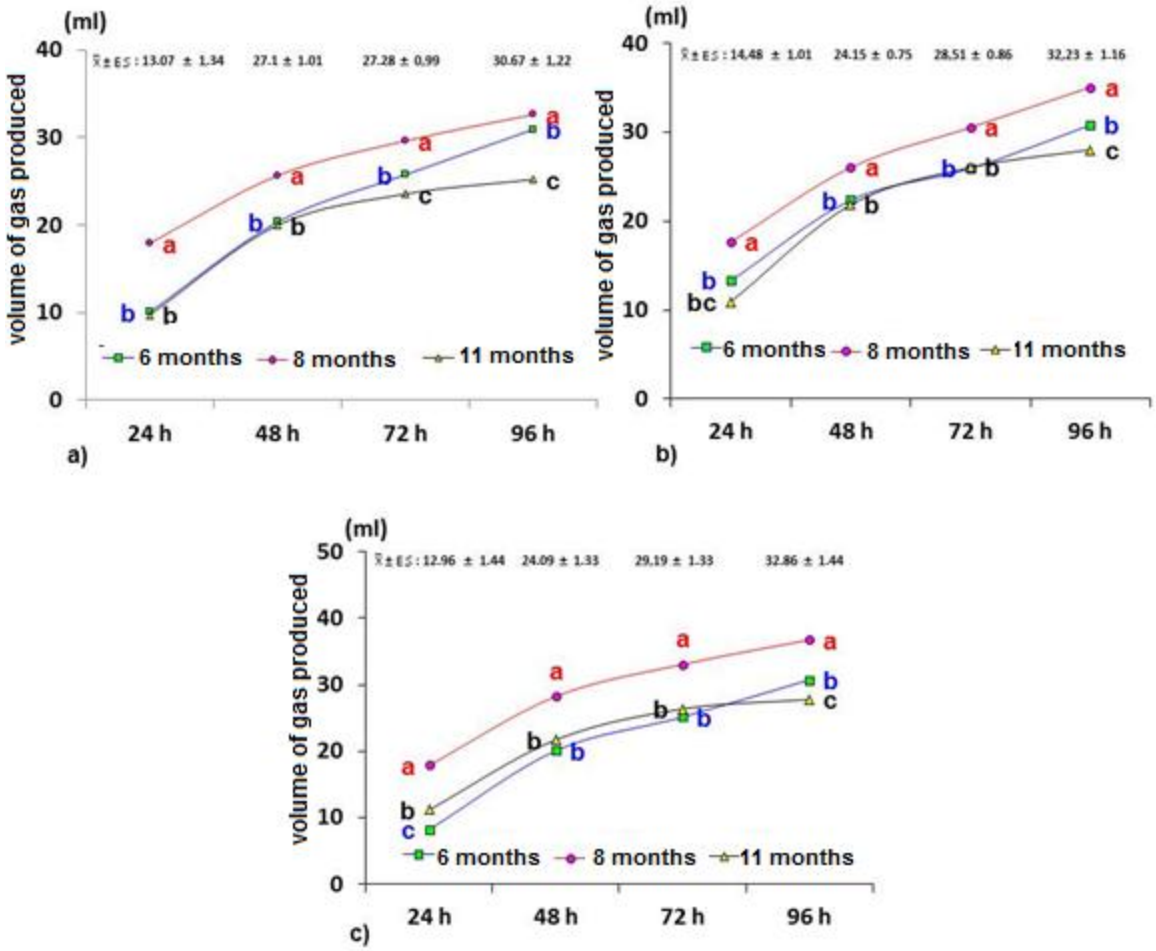

Fig. 3. Effects of re-shoot age on *in vitro* gas production at 24 h, 48 h, 72 h and 96 h for cultivars: a) My5514 b) C97-366 c) C99-374