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IN VITRO GAS PRODUCTION AND DIGESTIBILITY OF *MUCUNA* BEAN

C.A. Sandoval Castro[1]*, P. Herrera[2], C.M. Capetillo Leal[1] and A.J. Ayala Burgos[1]

[1] *Animal Nutrition Department, University of Yucatan, P.O. Box 4-116, Mérida, Yucatán 97100, México. E-mail: ccastro@tunku.uady.mx*

[2] *Estación Experimental "La Iguana". Venezuela*

**Corresponding author*

SUMMARY

In vitro gas production techniques simulate the rumen fermentation process and they have been used to evaluate the potential of feeds to supply nutrients to ruminants. Thus, beans and husks from *Mucuna* (*Mucuna* pruriens) were evaluated using an *in vitro* gas production technique. *In vitro* dry matter (IVDMD) and organic matter (IVOMD) digestibility of beans were high at 97.94 ± 0.35 and 96.02 ± 1.31%. The IVDMD and IVOMD of husks were lower, 78.96 ± 1.69 and 78.85 ± 1.75%, respectively. Energy value for ruminants was estimated from the digestible organic matter contained in the dry matter and was 13.90 and 11.14 (MJ kg DM^{-1}) for beans and husks, respecttively, showing the high potential of both *Mucuna* fractions. The gas production profiles were described with the equation: mL gas.g $DM^{-1} = a + b\ (1 - e^{-ct})$ (where: a = intercept, b = potential gas production and c = rate of gas production), yielding the following parameters for husks and beans, respectively; a = -32.5 ± 3.96 and -37.11 ± 4.084; b= 264.6 ± 4.88 and 249.6 ± 4.084 and c= ($\%.h^{-1}$) 3.053 ± 0.1700 and 4.208 ± 0.1909. These profiles are similar to those of a good starchy feed or a bean. The anti-nutritional factors did not show any detrimental effect on the *in vitro* fermentation. The results indicate that *Mucuna* has a potential to replace conventional energy sources (e.g., maize and sorghum) in ruminant diets. In addition to the beans, the husks can be incorporated into the diet without expecting major problems due to their high digestibility.

Key words: *Mucuna*, digestibility, *in vitro* gas production, chemical composition, ruminant.

INTRODUCTION

The velvet bean (*Mucuna pruriens*) is grown by an increasing number of smallholder tropical farmers. It is a prolific plant, producing high amounts of and husk and forage. In the early 20th century, the pods and forage were commonly used for feeding pigs and ruminants in the southern USA (Eilittä and Sollenberger, 2002) and both grain and foliage have been used as ruminant feed (Kay, 1978; Vivanco, 1998). *Mucuna*'s chemical composition compares well with commonly used cereals as sorghum (Sandoval *et al.*, 2000a) or protein sources (e.g. whole soybean) (Parr, 1988). Although anti-nutritional factors have been detected in *Mucuna*, there is not enough evidence on the potential detrimental effect of these compounds when *Mucuna* is fed to ruminants.

In vitro methods for evaluation of feeds are an important tool as they allow quick assessment of nutritional value and potential deleterious activity of any anti-nutritional compound present in the material. An *in vitro* gas production technique simulates the rumen fermentation. It is similar to the ruminal process as gas (CO_2 and CH_4) is produced from the carbohydrate fermentation. In addition, some CO_2 is released from the bicarbonate buffer used in the *in vitro* media (Blümmel and Makkar, 1997). In Germany, the Hohenheim *in vitro* gas test is routinely used for estimating *in vivo* digestibility and metabolizable energy for ruminants (Blümmel and Makkar, 1997).

Therefore, the objective of the present work was to assess potential detrimental effects of *Mucuna* beans and husks on rumen microbial population through the *in vitro* gas production technique.

MATERIALS AND METHODS

In vitro gas production

Rumen digesta samples were obtained from two steers and pooled together in order to achieve a homogenous inocula. Rumen digesta was collected at 08:00h (before the morning feeding), placed in a container that was sealed immediately, and transported to an adjoining laboratory. The preparation of buffer solutions and rumen inocula was as described by Menke and Steingass (1988).

The method of Theodorou *et al.* (1994) was used for the gas production procedure, except for the measurement of residues which is detailed below.

Measurements of pressure and gas production were done at 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 48, 60, 72 and 96 h. Each sample was replicated four times in 125 mL serum bottles with 1.0 g sample and 60 mL of medium.

To determine the residual substrate, at the end of the incubation period, the supernatants of each serum bottle were filtered using vacuum filtration into pre-weighed filter paper (Whatman No. 54). The particles were then washed with 150 mL of distilled water and dried over night at 60 °C. (Previous observations in our laboratory showed that the filter paper utilized had similar characteristics as a crucible porosity 1.)

Dry matter loss was calculated as the difference between the DM weight of the sample at the start of the incubation and the weight of the residue DM remaining at the end of the incubation, after correcting for the blank. Then, to determine ash content, the residues were kept at 550 ºC for 24h and OM fermentation calculated.

Analyses of data

Gas production profiles were obtained after fitting the data to the exponential equation of McDonald (1981):

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

and, the multiphasic model of Groot *et al.* (1996):

$$y = Vf_i * (1 + (b_i / x)^{c\,l})^{-1}$$

using the GraphPad Prism 2.0 package (GraphPad Prism, 1994-1995).

Energy value for ruminants (ME, MJ.kg DM^{-1}) was estimated from digestible organic matter in the dry matter (DOMD) as:

$$ME\ (MJ\ kg\ DM^{-1}) = 0.0157 * DOMD$$

The DOMD was calculated as AFRC (1993):

$$DOMD\ (g\ kg^{-1}) = DOM\ (1000 - ash) / 1000,$$

where:
DOM = digestible organic matter.

Chemical analyses

Ash, DM and crude protein (CP) contents of each feed were determined in duplicate using methods from the Association of Official Analytical Chemists (AOAC, 1980). Neutral and acid detergent fibre (NDF and ADF) were determined as described by Goering and Van Soest (1970).

RESULTS

The chemical composition of *Mucuna* beans and husks is presented in Table 1. As expected, the grain had higher CP content and lower fibre content than the husks. Total gas production and the kinetics of *in vitro* fermentation is presented in Table 2. The husk produced a higher amount of gas than the grain ($P<0.05$). Grain dry matter (IVDMD) and organic matter (IVOMD) digestibility of beans were 97.94 ± 0.35 and 96.02 ± 1.31%, respectively, and were higher ($P<0.05$) than the IVDMD and IVOMD of husks, which were 78.96 ± 1.69 and 78.85 ± 1.75%, respectively. Energy value for ruminants was estimated from the digestible organic matter contained in the dry matter and was 13.90 and 11.14 (MJ kg DM^{-1}) for beans and husks, respectively, showing the high potential of both *Mucuna* fractions.

Both exponential and monophasic models were able to describe the fermentation process (Table 2). However, the monophasic model gave a better fit. When the model was extended and a multiphase fermentation process was analyzed, no additional fermentation phases were detected.

DISCUSSION

Mucuna husk had a lower CP content than most tropical grasses in the region (6-12 %), but it had a higher *in vitro* digestibility (Sandoval *et al.*, 2000), probably due to a lower concentration of fibrous components. The grain had a protein content comparable to most leguminous forage trees of the Yucatán peninsula, such as *Guazuma ulmifolia, Leucaena leucocephala,* and *Brosimun alicastrum* (16-27%) (Lizárraga *et al.*, 2001a). The CP content was also higher than that of common cereal grain like sorghum (9.8%), but was lower than that of a common protein feedstuff, such as soybean meal (48%) (Sandoval *et al.*, 2000a).

In vitro digestibility of *Mucuna* bean was superior to most local forage tree species and it was comparable to those achieved by sorghum and commercial feed mixture (85-90%). The digestibility of the husk was higher than that of most tropical grasses (50-60%) (Sandoval and Mendoza, 2000). The relatively low content of fibre can facilitate the colonization of the feed by the rumen microbial population, which in turn might induce higher fermentation rates, therefore improving digestibility (Van Soest, 1994). As the fermentation process is partially regulated by the fibrous content of the feeds, the grain fermented faster than the husk (4.4 vs 3.5 % h^{-1}).

Total gas production was also higher than that of common tropical grasses (Sandoval and Mendoza, 2000) and forage trees (Lizárraga *et al.*, 2001b), but similar to concentrated feed mixtures and grasses (Sandoval *et al.*, 2000b), suggesting a potential fermentation efficiency similar to concentrates, and the potential of *Mucuna* to be incorporated in conventional feed mixtures. No apparent deleterious effect was observed on the microbial activity. The fermentation proceeded with a normal pattern and high gas production and *in vitro* digestibility was achieved. This result might indicate that the anti-nutritional factors contained in *Mucuna* were either not present in quantities high enough to alter the fermentation process or that rumen microbes may quickly metabolize the anti-nutritional factors to non-toxic compounds.

Table 1. Chemical composition, *in vitro* dry matter (IVDMD) and organic matter (IVOMD) digestibility (%) and metabolizable energy content of *Mucuna pruriens* beans and husks.

Component	Beans	Husks
Dry matter	98.29	98.85
Crude protein	27.34	4.84
Ash	3.44	5.78
Ether extract	2.41	16.82
Neutral detergent fibre	40.79	58.87
Acid detergent fibre	ND	37.49
Lignin	ND	11.22
IVDMD	97.94 ± 0.35	78.96 ± 1.69
IVOMD	96.02 ± 1.31	78.85 ± 1.75
ME (MJ.kg DM^{-1})	13.90	11.14

ND: No determined. ±E.E., ME content for ruminants estimated as 15.4 $MJ.kg^{-1}$ digestible organic matter in the dry matter.

Table 2. *Mucuna* beans and husks *in vitro* gas production coefficients for an exponential and a monophasic model.

Coefficient	Beans	Husks
Exponential model		
A	-37.1 ± 4.08	-32.5 ± 3.96
B	249.6 ± 4.08	264.6 ± 4.88
k ($\%.h^{-1}$)	4.21 ± 0.191	3.05 ± 0.170
R^2	0.990	0.985
Sx.y	7.045	8.794
Monophasic model		
Vf	211.3 ± 2.058	224.5 ± 3.093
B	20.05 ± 0.284	25.33 ± 0.489
C	2.10 ± 0.053	2.08 ± 0.060
k ($\%.h^{-1}$)	4.493	3.528
R^2	0.997	0.996
Sx.y	3.555	4.767

a, b, k: equation parameters from $y=a+b(1-e^{-kt})$, Vf, B, c: equation parameters from $y = Vf_i * (1+(b_i / x)^{c_i})^{-1}$ ±E.E. Sx.y= residual square deviation.

Although both the monophasic (Groot *et al.*, 1996) and exponential (Orskov and McDonald, 1979) models gave similar estimation of fermentation rates (k), the monophasic model was more accurate. The exponential model had the additional disadvantage of providing negative “a” values, which are difficult to interpret in biological terms. The apparent inadequacy of the model might arise from its origin as a tool for describing *in situ* rumen degradation (i.e., dacron bag technique). Thus, it is preferable to analyze *Mucuna in vitro* gas production data with an alternative model like that of Groot *et al.* (1996).

This study suggests that *Mucuna* beans and husks have a potential fermentation efficiency similar to that of conventional concentrates, and therefore *Mucuna* could be incorporated in feed mixtures to replace conventional energy sources (e.g., maize and sorghum) in ruminant diets. In addition, due to the high digestibility of the husks, they too can be incorporated into the diet without major problems.

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