IN VITRO GAS PRODUCTION OF TROPICAL PASTURE LEGUMES.

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ABSTRACT
The objective of this experiment was to use an *in vitro* gas production technique to study the fermentation dynamics of tropical legumes associated with *Brachiaria spp*. pastures. Three samples of the legumes Arachis (*Arachis pintoi*), Centro (*Centrosema pubescens*), Stylo *(Stylosanthes guianensis*) and Siratro (*Macroptilium atropurpureum*) with neutral detergent fibre (NDF) and crude protein (CP) concentrations of 452, 494, 528, 529 and 191, 160, 164 and 125 g/ kg DM, respectively, were incubated for *in vitro* gas production measurements. Solubles-corrected gas volumes were fitted to a first-order exponential model. There were significant differences ($p<0.01$) in all constituents and the parameters reflecting the fermentation dynamics of the legumes. Arachis had the highest nutritive quality as reflected by its higher CP, gas production and NDF digestibility, while Siratro had the lowest. Apart from Arachis, the tropical legumes produced less total gas, less gas from the NDF fraction, and had shorter lags and lower NDF digestibilities than the *Brachiaria spp*. pastures they were associated with. The rate of gas production of the legumes was higher than that observed for tropical grasses. The relationship between gas production and NDF digestibility was improved substantially by correcting the gas volumes for the fermentation of solubles ($r^2 = 0.91$ vs. 0.63).

KEYWORDS
in vitro gas production, *Arachis pintoi*, *Centrosema pubescens*, *Stylosanthes guianensis*, *Macroptilium atropurpureum*, tropical legumes, nutritive value, Costa Rica

INTRODUCTION
Introduction of tropical legumes into pasture swards is a widely used strategy to improve animal performance and the overall sustainability of tropical grazing systems. However, an adequate nutritional characterisation of tropical legume species is required to accurately predict diet selection, digestion and animal performance of ruminants grazing mixed pastures. Standard nutritional information, such as digestibility and crude protein, is available for tropical pasture legumes (Minson, 1988), but few estimates of their dynamics of fermentation are available. Due to their relatively low cost, reproducibility and the increasing number of dynamic systems to predict animal responses to nutrients, *in vitro* gas production techniques are now widely utilised to obtain kinetic descriptions of forage quality. The aims of the present experiment are 1) to use an *in vitro* gas production technique to study the fermentation dynamics of tropical legumes associated with *Brachiaria spp*. pastures and 2) to investigate the relationship between solubles-corrected gas production and NDF digestibility of tropical legumes.

METHODS
Three samples of the legumes Arachis (*Arachis pintoi*), Centro (*Centrosema pubescens*), Stylo *(Stylosanthes guianensis*) and Siratro (*Macroptilium atropurpureum*), associated with 21-day rotationally grazed *Brachiaria spp.* pastures, were collected from a humid lowland tropical region of Costa Rica during the wet season. Plant material was collected at ground level before grazing, samples were oven dried at 60°C for 48 h and ground through a 1 mm screen for analyses. All samples were analysed for NDF using a modified micro-technique (Pell and Schofield, 1993), and for CP using standard micro-Kjeldahl nitrogen determinations. *In vitro* gas production incubations were performed as described by Jessop and Herrero (1996). Measurements were taken at 1, 2, 3, 4, 6 and 8 h; thereafter every 4 h until 60 h, and then at 72, 78, 96, 102 and 168 h. Cumulative gas volumes were corrected for fermentation of soluble material by subtracting the gas produced up to 4 h (Herrero and Jessop, 1996) and fitted to the model $GAS = B(1-\exp^{-c(t-lag)})$, where B is the asymptote gas production from the fermentation of NDF (ml), c is the fractional rate of gas production (/h), and lag is the lag phase before fermentation of NDF begins (h)(Jessop and Herrero, 1996). NDF residues were collected at the end of the incubations for the estimation of NDF digestibility (D). Data were studied by regression and analysis of variance.

RESULTS AND DISCUSSION
The chemical composition and *in vitro* gas production dynamics of the four tropical legumes are presented in the Table. Even when managed under the same grazing regimes, there were significant differences ($p<0.01$) across all measured constituents and parameters. Arachis had the highest nutritive quality as reflected by its CP concentration, total gas production, B fraction and NDF digestibility, while Siratro had the lowest. Stylo and Arachis had significantly higher rates of gas production than Centro and Siratro ($p<0.01$), implying that their potentially digestible NDF fraction was of a very high quality. With the exception of Arachis, the tropical legumes produced less total gas, less gas from the NDF fraction, and had shorter lags and lower NDF digestibilities than the *Brachiaria spp*. pastures they were associated with (Jessop and Herrero, 1996). However, the legumes had a higher rate of gas production (c) than those observed for tropical grasses (Jessop and Herrero, 1996). The low digestibilities of Stylo and Siratro reported here, agree with the estimations of dry matter digestibility obtained by Minson (1988) for a range of tropical legumes. Lascano and Thomas (1988) report similar values for Arachis.

The ability to distinguish between the gas produced from the NDF or the soluble fraction is essential for appropriate characterisation of the fermentation dynamics of forages (Herrero and Jessop, 1996). The data from this experiment provides further evidence of the improved accuracy of the relationship between NDF digestibility and *in vitro* gas production, when using the gas produced up to 4 h as a correcting factor for the fermentation of soluble material in forages (Herrero and Jessop, 1996)(see Figure). The regression using the solubles corrected gas production data was $D = 0.0163(\pm 0.00024)$ B. It explained significantly more variance in NDF digestibility (91% vs. 63%, $p<0.0001$) than when using the total gas volumes as predictors, and had a non-significant intercept, suggesting that only NDF was being fermented. This suggests that the use of this correction method can provide accurate predictions of NDF digestibility from gas production measurements.

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Table 1
Chemical composition and *in vitro* gas production dynamics of tropical pasture legumes.

legume	NDF (g/kg DM)	CP (g/kg DM)	total gas (ml)	B (ml)	c (/h)	lag (h)	D
Arachis	452[a]	191[a]	48.0[a]	39.3[a]	0.0621[a]	4.4[a]	0.67[a]
Centro	494[b]	160[b]	42.5[b]	30.9[b]	0.0593[b]	3.2[b]	0.49[b]
Stylo	528[c]	164[b]	44.0[b]	32.7[c]	0.0656[a]	3.6[c]	0.52[c]
Siratro	592[d]	129[c]	33.8[c]	27.7[d]	0.0418[c]	3.8[d]	0.41[d]
SEM	14.9	7.2	1.51	1.41	0.00279	0.15	0.027

[a,b,c,d] Means with different superscripts indicate significant differences ($p<0.01$).

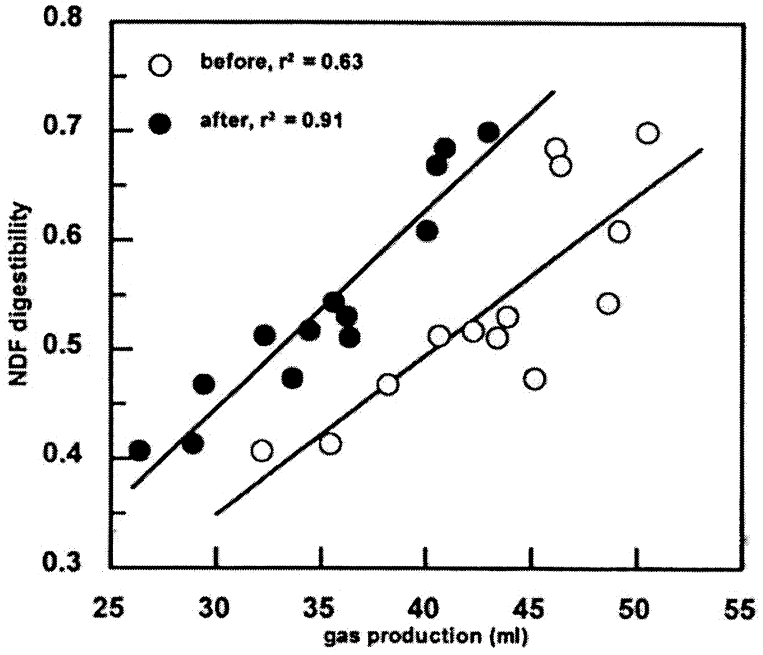

Figure 1
Comparison of the relationship between NDF digestibility and *in vitro* gas production of tropical legumes before and after correction for the fermentation of solubles.