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In vitro gas production and substrate digestion relationship for a buffer free of indirect gas production

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Introduction

In vitro gas production (GP) technique assumes that the produced gas during fermentation is proportional to dry matter disappearance (DMD). This has been demonstrated for the conventional system (Brooks and Theodorou, 1997), which use bicarbonate as buffer. However, the relationship between GP can be different according to the substrate (i.e. forages and concentrates). During the fermentation, different feed fractions are digested along the time and consequently the relationship between GP and DMD could change during the incubation for the same feed. The aim of this study was to verify if the relationship GP/DMD is constant along the time (incubation) for fresh alfalfa incubated at different pH and using a buffer system without indirect GP.

Material and methods

Fresh alfalfa samples were incubated at four pH (6.8, 6.3, 5.8 and 5.3). A pre-blooming alfalfa was sampled by hand-plucking, milled fresh with dry ice and conserved at -18 ºC until used for chemical and in vitro studies. Samples were analyzed by dry matter (DM). Incubation was carried out according to the technique of Brooks and Theodorou (1997) modified by Wawrzkiewicz and Danelón (2004) and replacing the buffer carbonate-bicarbonate by a phosphate-citrate buffer (Gomori, 1955). Duplicate samples of alfalfa were weighed (c.a. 3.5 g wet matter). Rumen fluid inoculum (50:50, solid : liquid fractions) was collected from two rumen cannulated cows grazing alfalfa pasture and supplemented with a concentrate (6.4 kg DM/day, 160 g/kg DM of CP, containing 300 mg of monensin per cow/day) and 15 kg of maize silage (as fed). Samples were incubated in duplicate in three independent periods and blanks were included (bottles with inoculum but without feed). The pressure was measured at 1, 2, 4, 6, 9, 12, 16, 20, 24, 30, 36 and 48 h and gas volume was extracted with a syringe (final pressure 0 psi). The relationship between pressure and volume was fitted to a linear model and GP was estimated from pressure data. Using the data from GP and DMD, the relationship between GP and DMD along the time was calculated. The results of GP and DMD were corrected by blanks (but not by quantity of incubated substrate) and were analyzed by regression analysis according to the following model:

$$GP\ (ml) = H + DMD\ (g) + Error$$

where H is the time. Additionally, the relationship GP/DMD was analyzed according to the model:

GP (ml)/DMD (g)= pH + H + Error

Data were analysed according to a complete block design (blocking by incubation) and orthogonal contrasts (linear and quadratic) were performed to examine the effects of pH. Statical significance was declared at $P \leq 0.05$.

Results and discussion

For GP (ml) model, the best fit was obtained classifying by H factor (Figure 1, Table 1) where H = 6 was smaller than 0 ($P < 0.01$) and H > 6 was not significant. The DMD (slope) was significant ($P < 0.01$) and different from 0.

The relationship GP/DMD was affected by acidity (0.15a, 0.13ab, 0.12b and 0.12b ml/g DM respectively for Tr 6.8, Tr 6.3, Tr 5.8 and Tr 5.3; different letters differ; $P < 0.05$) and by H (0.06b, 0.15a, 0.16a y 0.17a ml/g DM respectively for 6, 12, 24 y 48 h; $P < 0.01$).

Table 1. Gas production in function of digested substrate.

Parameter	Estimation	Standar Error	Significance
H = 6	-37.94	8.105	**
H > 6	-12.13	11.031	NS
DMD	0.18	0.023	**
Residual standar deviation	10.009		
R^2	0.87		

NS – Not significant, * $p < 0.05$, ** $p < 0.01$

The smaller GP/DMD registered at 6 h agrees with the negative intercept (Table 1). These parameters indicate that part of the material measured as digested at 6 h was really lost during the filtrate. Also, the smaller GP/DMD at pH 5.3 suggests that during the first hours the less pH generated a higher hydrolysis and losses by filtrate.

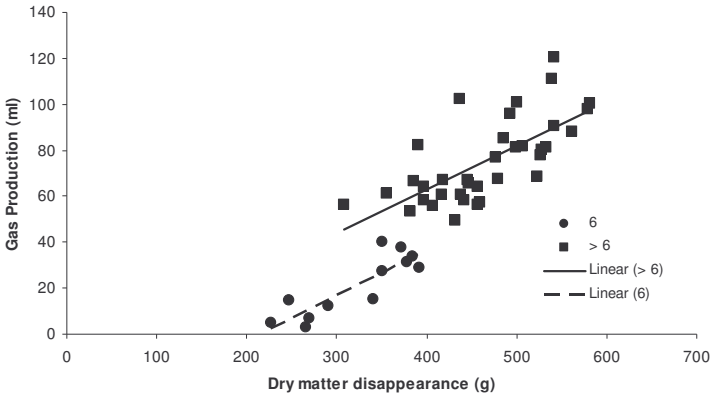

Figure 1: Gas production (ml) in function of Dry matter disappearance (g)

Conclusion

The phosphate-citrate buffer system allowed maintaining a constant proportion between GP and DMD along the incubation. Nevertheless, the measurement system of residual DM did not guarantee the total DM recovery at early times.

References

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