EFECT ON MICROBIAL GROWTH IN THE RUMEN AND FERMENTATION KINETICS OF FEEDSTUFFS UNDER DIFFERENT LEVELS OF NITROGEN

Sérgio Oliveira Dias Santo Freire *

INTRODUCTION

A new report from the Food and Agriculture Organization of the United Nations (Steinfield et al., 2006) considers livestock production as one of the major causes of the world's most pressing environmental problems. To optimize the rumen microbial system, feeds must be characterized according to their ingestive and degradation behaviour in the rumen (Tamminga, 1996). Improving the efficiency of feed nitrogen (N) utilization is the most effective means to reduce nutrient losses (Jonker et al., 2002). The challenge is to establish the minimal amount of protein required by dairy cows to achieve optimal, but not necessarily maximal, milk production.

This study was designed to investigate the minimal request of N for normal microbial growth using in vitro gas production technique, in different feedstuffs. As well as, the effects of different levels of N on the in vitro fermentation kinetics.

MATERIALS AND METHODS

FEED SAMPLES AND CHEMICAL ANALYSIS

Using the gas production technique, six feedstuffs (maize, maize gluten feed (MGF), soybean meal (SBM), citrus pulp, tapioca and wheat) were evaluated. About 500 mg of sample were weighed into 300 ml serum bottles and incubated with 60 ml of buffered rumen fluid (RF). Each sample was incubated in RF mixed with an anaerobic buffer/mineral solution in different dilutions, 1:2 and 1:9 (v/v), with or without N present in the buffer (NN or N-free incubations). The dilution of RF with buffer had the aim to reduce the content of N, and thus obtain different levels of it in the medium. With the purpose to maintain the buffer capacity in N-free incubations, ammonium bicarbonate in

Table 1 - Chemical composition (%) of substrates used for in vitro experiments.

Sample	Maize	MGF	SBM	Citrus	Tapioca	Wheat
DM *	875.7	860.7	909.4	840.6	871.3	870.0
Crude protein (%)	9.6	21.5	47.0	6.5	3.0	13.0
NDF (%)	14.5	42.5	16.1	37.0	15.4	12.0
ADF (%)	2.6	9.9	8.0	19.0	6.4	2.6
Starch+Sugar (%)	75.0	25.5	14.5	25.7	75.0	71.0

DM = dry matter; NDF = neutral detergent fibre; ADF = acid detergent fiber; *- g/kg;
MGF = maize gluten feed; SBM = soybean meal; citrus = citrus pulp.

the buffer was replaced with sodium bicarbonate on bicarbonate equivalents.

Pressure values were corrected for the quantity of substrate organic matter (OM) incubated and gas released from blanks (i.e., gas productions in buffered RF without sample).

Gas production of all samples were analysed in duplicate. Cumulative gas was expressed as millilitre of gas produced per mg of OM incubated (Figures 1a-4a). The rate of gas production (Figures 1b-4b) was calculated by first derivate from the cumulative gas production profiles (Cone et al., 1997). The numerous combinations between and within different samples, dilutions and N presence or absence, as well as its interactions, were investigated with multiple analysis (SPSS, 2007).

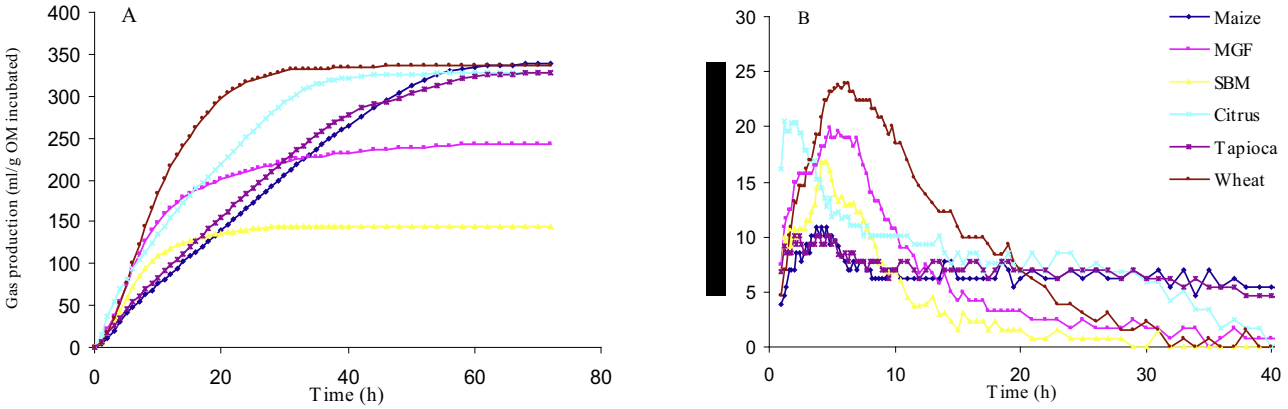

Figure 1 - Cumulative gas production profile (ml/g OM incubated) (A) and rate (ml/g OM/h) (B) in 1:9 dilutions without N in the medium.

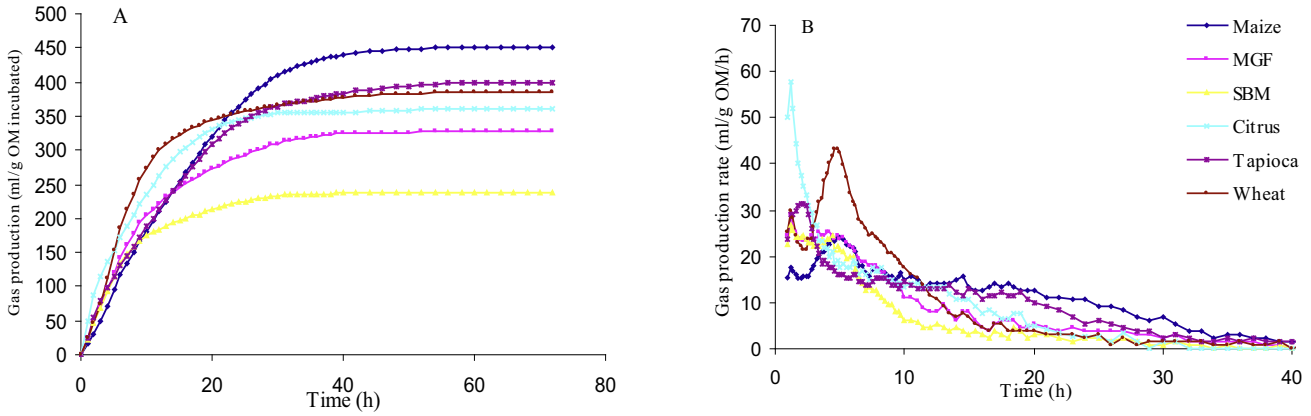

Figure 2 - Cumulative gas production profile in (ml/g OM incubated) (A) and rate (ml/g OM/h) (B) 1:2 dilutions without N in the medium.

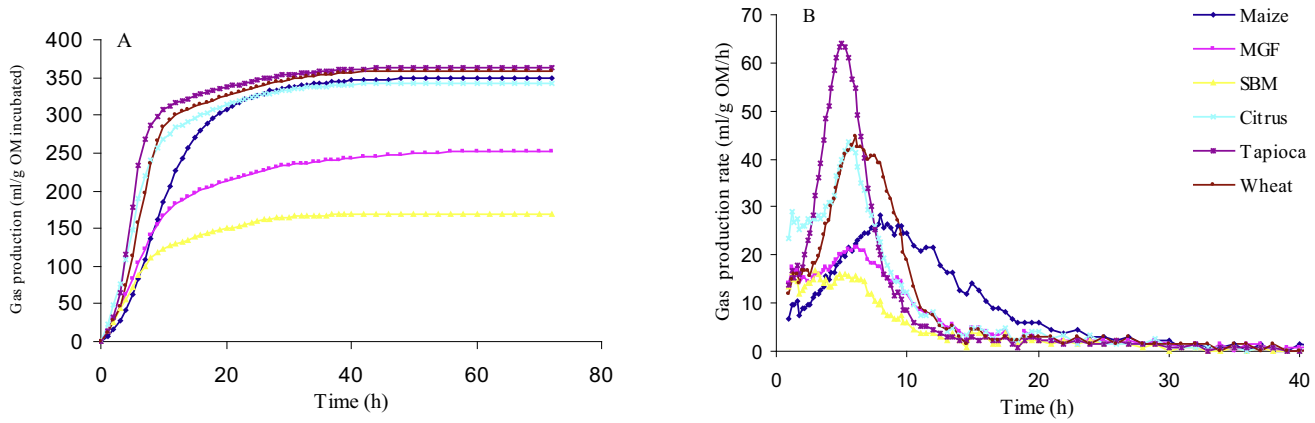

Figure 3 - Cumulative gas production profile (ml/g OM incubated) (A) and rate (ml/g OM/h) (B) in 1:9 dilutions with N in the medium.

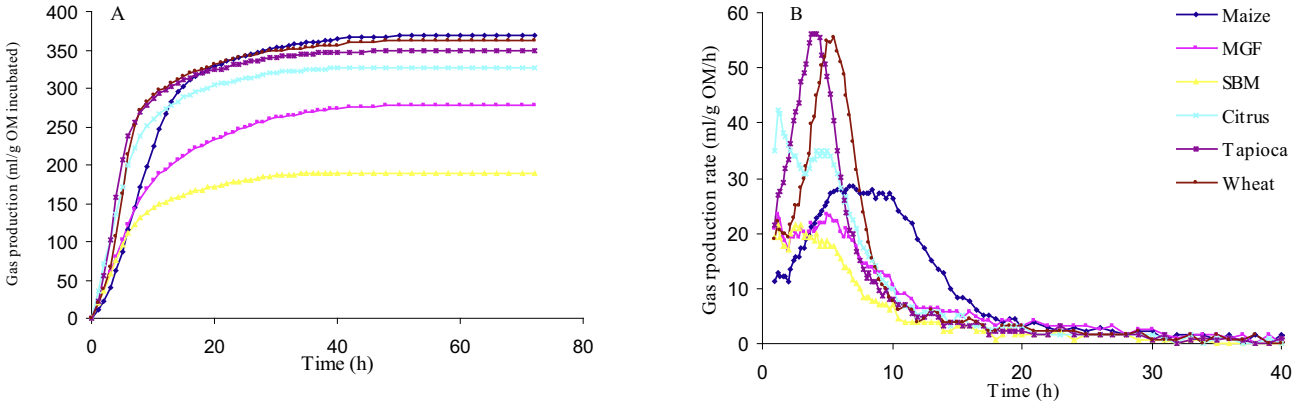

Figure 4 - Cumulative gas production profile (ml/g OM incubated) (A) and rate (ml/g OM/h) (B) in 1:2 dilutions with N in the medium.

RESULTS

Analysing gas production curve profiles it was possible to discern variations in fermentation kinetics and total gas produced (TGP) among various feeds (Figures 1–4). There were significant differences ($P<0.05$) in the cumulative gas production between samples, dilutions and NN or N-free. As expected, the gas production curves presented three phases. An initial phase of rapid gas production, followed by a phase with decreasing growth and finally by a phase in which the rate of gas production slows and reaches zero. Looking for the figures that represented the fermentation rates, in general the samples that showed higher peaks also showed faster decreases. The highest values were showed mainly in the NN incubations associated with the starchy feeds.

Within N-free medium it could be see that gas production in the 1:2 dilutions was always higher than the 1:9 dilutions, at 72 h of incubation. However within NN medium, gas production from citrus pulp and tapioca was higher in 1:9 dilution, contrarily to what was observed for maize, MGF, SBM and wheat (Table 2). Thus the results showed that with or without N that TGP increased with the decrease of the dilutions rate, except for citrus and tapioca in NN. Comparing within dilutions it is possible to discern that 1:9 NN always presented higher values of TGP than N-free. Already for 1:2, the NN produced always less gas at the end of the incubations. Verifying the Table 2 it is possible see that 1:2 incubations didn't need so much time to produce half of the total gas. The same was observed for the NN incubations relatively to N-free medium.

Interactions between sample x dilution and dilution x N were identified. In the interaction dilution x N is possible to see that on average 1:2 N-free produced more 47.5 ml/OM incubated than 1:2 NN ($P<0.05$). Already looking for 1:9 occur the opposite with NN present on average more 19.4 ml/OM incubated. The mean difference between dilutions is significant ($P<0.05$), with 1:2 dilution created on average more 40.3 ml/g OM incubated. Also the presence or absence of N in the medium led to significant differences ($P<0.05$). On average N-free produced more 14.1 ml/g OM incubated.

Table 2 -Gas volumes produced (ml/g OM incubated) in experiment 1 at 72 hours of incubation with different dilutions, incubated in N-free or NN and time needed to produced half of TGP (h).

	Time incubation 72 h dilution		**Time for produce 50% of total gas (h) dilution**	
Sample	1:9	1:2	1:9	1:2
Maize				
N-free	339,4	450,2	25	13
NN	349,7	369,4	9,5	8,5
MGF				
N-free	242,3	327,9	7,5	7
NN	252,7	278,3	7,25	6,75
SBM				
N-free	144,9	237,8	6	5,25
NN	169,3	189,5	5,75	5
Citrus Pulp				
N-free	327,5	361,7	13	6,5
NN	341,4	327,4	5,5	4,8
Tapioca				
N-free	327,3	399,0	21	11
NN	363,3	349,2	5	4,4
Wheat				
N-free	337,5	385,0	9,25	6,25
NN	359,2	362,7	6,5	5,25

DISCUSSION

In generally it is possible to see that the feedstuff used which are known to contain a high proportion of soluble carbohydrates or protein, were readily available and rapidly fermented. The inexistence of a lag time, even in 1:9 dilutions, can be explained by the fact that the number of microorganisms was not too low. At the same time it indicated that the feeds used provided easily fermentable com-

ponents. Groot et al. (1996) found a rapid rate of subtract digestion following incubation in the case of fermentation of soluble feedstuff components, which do not require colonization, under high microbial densities. Also the diet of the donor animals can influence the gas production profile (Cone et al., 1996).

The three phases showed by gas production curves are in agreement with Groot et al. (1996) who reported, that the first phase of the curve represents the gas production caused by fermentation of the soluble fraction, in the second place the gas production caused by fermentation of the non-soluble fraction and the last part like the gas production caused by microbial turnover.

Nitrogen associated with the inoculum may be insufficient to support degradation (Mould et al., 2005). Starchy feed ingredients showed a large response to N addition, while SBM or MGF, richer in crude protein demonstrated a response much more limited. Dryhurst and Wood (1998) reported that supplementation response to be dependent on both N content of the feed and its degradability. This is in accordance with the results, tapioca a highly degradable substrate with a low N content, showed the greatest response to supplementation. Already for maize the inherent degradability of this material, limited the response.

The constant level of fermentation rates showed in N-free incubations may represent the level of microbial activity that can be supported by N in the RF inoculum plus the sample. In contrast, N supplemented gas production rate profiles showed considerably highest peaks and then a quick decline. Groot et al. (1996) reported that a rapid decline of the rate can occur after depletion of the substrate component, which is most likely in the case where samples have a large amount of soluble components. For insoluble components, associated with the cell-wall fraction of substrates, the rate is more likely to decrease slowly when either chemical or structural barriers are encountered. Despite the N-free relatively to NN incubations presented rates much more limited, her constancy, sometimes, resulting at the end of the 72 hours of incubation on more gas produced. A fast or slow fermentation can be a tool for diet formulations. This higher fermentation can be regarded as more microbial activity and so more microbial biomass being formed.

The more gas achievable in 1:2 N-free relatively to 1:9 N-free can be explained by an increase on N in the medium, in addition to some increase in the OM. Greater number of microorganisms presence in 1:2 could also have influenced the results. This assumption was related by Jessop and Herrero (1998) cit. by Nagadi et al. (2000) who reported that if microbial activity is low, this would become a limiting factor and a significant proportion of degraded carbohydrate would be incorporated into new microbial matter rather than being fermented to products that gave rise to gas production.

Krishnamoorthy et al. (1991) report a direct relation between the volume of gas produced and the microbial biomass. At first sight was expected for a greater gas production in NN, though contrary to expectations this didn't occur. Melaku et al. (2003) suggested that this phenomenon could be due to the rapid rate of gas production leading to substrate exhaustion and limitation on the extent of gas production. Velocity of microbial growth affects the relationship between microbial growth and end products (Naga and Harmeyer, 1975).

It is well established that relationship between short chain fatty acids production and microbial biomass is not a constant (Leng, 1993), the explanation for which resides in the variation of biomass production per unit ATP generated (Blümmel et al., 1997a).

Other possibility to justify the results was found. Several authors have shown that less gas is produced from feeds high in propionate precursors relative to that in feeds high in acetate and butyrate precursors (Getachew et al., 1998; Williams, 2000). Substrates with proportionally higher gas volumes had comparatively low biomass yields (Blümmel et al., 1997a). Gas production may be used to predict in vitro microbial biomass yield if the amount of substrate truly degraded is known (Blümmel et al., 1997b). Despite no attempt was made to determine the composition of the gas produced, there are reports that rapidly fermentable carbohydrates yield relatively higher propionate as compared to acetate, and the reverse takes place when slowly fermentable carbohydrates are incubated (Getachew et al., 1998). Such changes in volatile fatty acids pattern could arise from either a shift of biochemical pathways within the microbes present or a shift in types of microbes present (Russell et al., 1979).

CONCLUSIONS

It was clearly demonstrated that the gas production technique is a useful tool for predicting the fermentative capacity of the samples used. Likewise, the apparatus utilized is sensibly to the different amounts of N used in the medium. This may allow it's utilization as an instrument to formulate rations according to the productive performance of the target animals.

The results also show that the N increase in the solutions with the aim to suppress the N deficits seems to origin different pathways in fermentation. To determine the minimal request of N more dilutions should be investigated. As

well as, the relation between volatile fatty acids produced and the substrate truly degraded.

REFERENCES

Blümmel, M; Makkar, H.; Becker, K. 1997a. In vitro gas production: a technique revised. J. Anim. Physiol. Anim. Nutr. 77: 24-34.

Blümmel, M.; Steingaß, H.; Bekker, K. 1997b. The relationship between in vitro gas production, in vitro microbial biomass yield and ^{15}N incorporation and its implications for the prediction of voluntary feed intake of roughages. Br. J. Nutr. 77: 911-921.

Cone, J.; van Gelder, A.; Driehuis, F. 1997. Description of gas production profiles with a three-phasic model. Anim. Feed Sci. Techn. 66: 31-45.

Cone, J.; van Gelder, A.; Visscher, G; Oudshoom, L. 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. Anim. Feed Sci. Techn. 61: 113-128.

Dryhurst, N.; Wood, C. The effect of nitrogen source and concentration on in vitro gas production using rumen micro-organisms. Anim. Feed Sci. Techn. 71: 131-143.

Getachew, G.; Blümmel, M.; Makkar, H.; Becker, K. 1998. In vitro gas measuring techniques for assessment of nutritional quality of feeds: a review. Anim. Feed Sci. Techn. 72: 261-281.

Groot, J.; Cone, J.; Williams, B.; Debersaques, F.; Lantinga, E. 1996. Multiphasic analysis of gas production kinetics for in vitro fermentation of ruminant feeds. Anim. Feed Sci. Techn. 64: 77-89.

Jonker, J.; Kohn, R.; High, J. 2002. Dairy herd management practices that impact nitrogen utilization efficiency. J. Dairy Sci. 85: 1218-1226.

Krishnamoorthy, U.; Steingass, H.; Menke, K. 1991. Prelimirary observation on the relationship between gas production and microbial protein synthesis in vitro. Arch. Anim. Nutri. 41:521-526.

Leng, R. 1993. Quantitative ruminant nutrition - a green science. Aust. J. Agric. Res. 44: 363-380.

Melaku, S.; Peters, K.; Tegegne, A. 2003. In vitro and in sacco evaluation of selected multipurpose trees, wheat bran and Lablabpurpureus as potential feed supplements to tef (Eragrostis tef) straw. Anim. Feed Sci. Techn. 108: 159-179.

Mould, F.; Morgan, R.; Kliem, K.; Krystallidou, E. 2005. A review and simplification of the in vitro incubation medium. Anim. Feed Sci. Techn. 123-124: 155-172.

Naga, M.; Harmeyer, J. 1975. Gas and volatile fatty acid production at different rates of rumen microbial protein synthesis in vitro. J. Anim. Sci. 40: 374-379.

Nagadi, S.; Herrero, M.; Jessop, N. 2000. The influence of diet of the donor animal on the initial bacterial concentration of ruminal fluid and in vitro gas production degradability parameters. Anim. Feed Sci. Techn. 87: 231-239

Russell, J.; Sharp W.; Baldwin, R. 1979. The effect of ph on maximum bacterial growth rate and its possible role as a determinant of bacterial competition in the rumen. J. Anim. Sci. 48: 251-255.

SPSS. 2007. Version 15. SPSS Inc. Chicago, Illinois, USA.

Steinfeld, H.; Gerber, P.; Wassenaar, T.; Castel, V.; Rosales, M.; Haan, C. 2006. Livestock's long shadow environmental issues and options. Food and agriculture organization of the united nations. Rome.

Tamminga, S. 1996. A review on environmental impacts of nutritional strategies in ruminants. J. Anim. Sci. 74: 3112-3124.

Williams, B. 2000. Cumulative gas-production techniques for forage evaluation. In D.I. Givens, E. Owen, R.F.E. Axford, H.M. Omed. (eds.). Forage evaluation in ruminant ruminant nutrition. CABI Publishing. Wallingford, UK. pp. 189-213.

* Mestre em Produção Anima