Session 20, abstract number 0534
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In vitro gas production of fresh alfalfa under different pH

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Key words: in vitro gas production, fresh alfalfa, ruminal environment.

Introduction

Ruminal acidity can affect digestive process (especially cell wall) through its effects on microorganisms. It is normally accepted that a pH value of 6.2 is the threshold below which organic matter (OM) and fibre digestion are depressed. Mould et al. (1984) concluded that the fiber digestion of several forages was affected under pH 6.0 – 6.1, but this threshold would depend on forage quality. However, de Veth and Kolver (2001) incubating fresh samples of ryegrass (Lolium perenne) found that digestion was affected under pH 5.8. Similar studies about the effect of ruminal acidity on fresh alfalfa digestion are unknown. In vitro gas production technique describes the digestion kinetic and can be used to predict the nutritive value of feeds. The aim of this work was to assess the fresh alfalfa digestion under different conditions of ruminal pH using the in vitro gas production technique.

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), ashes, crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre ash free (ADF). The OM was calculated by difference between DM and ash. 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) and conserved at -18 ºC up to 2 hours before incubation. Rumen fluid inoculum (50:50, solid : liquid fractions) was collected from two rumen cannulated Holstein dairy cows grazing a 100% alfalfa pasture. Each incubation bottle was filled with 99 ml of inoculum (rumen fluid to incubation medium ratio was 1:10). 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 gas production was estimated from pressure data. From the raw data set peak rate (ml/g OM h), time to peak rate (h) and lag time (h) were estimated. 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

The chemical composition of alfalfa (pre-blooming) was: DM 190 g/kg of wet matter, 296 g/kg DM of CP, 84 g/kg DM of ashes, 269 g/kg DM of NDF and 149 g/kg DM of ADF. The initial pH values of the incubation medium fluctuated between 6.2 – 6.3, (Treatment [Tr] 6.8); 6.0 – 6.1, (Tr 6.3); 5.7 – 5.8, (Tr 5.8) and 5.4 – 5.7, (Tr 5.3) and differences between treatments were significant ($P < 0.05$) at 6, 12, 24 and 48 h.

Table 1: In vitro gas production characteristics of fresh alfalfa

Variables	Treatments[2]				SEM[1]	Significance[3]		
	6.8	6.3	5.8	5.3		Treat	Lineal	Cuad.
Gas production (ml/g Organic matter)								
6 h	46 a	39 ab	23 ab	5 b	4.7	**	**	NS
12 h	104	100	106	91	14.6	NS	NS	NS
24 h	148	110	124	113	10.3	NS	NS	NS
48 h	190 a	148 ab	145 ab	118 b	12.2	*	**	NS
Lag time (h)	3.3 b	4.0 b	4.0 b	7.0 a	0.55	*	**	NS
Rate of gas production								
Peak rate (ml/g OM-h)	18.1	20.2	21.8	15.4	2.97	NS	NS	NS
Time to peak rate (h)	6.0 c	7.0 bc	8.5 ab	10.7 a	0.41	**	**	NS

[1] Standar error of the mean.
[2] Different letters in the same row differ ($p<0,05$);
[3] Treat, treatments; linear and quadratic contrasts; NS = $p>0,05$; *, $p<0,05$; **, $p<0,01$.

Cumulative in vitro gas production increased linearly ($P < 0.05$) with pH at 6 and 48 h ($P < 0.05$; Table 1) and gas production at 48 h decreased under pH 5.7 – 5.8 (Tr 5.8). The lag time increased linearly with the reduction of pH ($P < 0.05$) showing a negative effect of acidity on ruminal microbes activity. Time to peak rate also increased when the pH decreased ($P < 0.01$; Table 1), as a probable consequence of higher lag time. However, no differences in peak rates were observed among treatments ($P > 0.05$).

Conclusions

The digestion of fresh alfalfa was affected below pH 5.7 – 5.8 (Tr 5.8) and the highest digestion was observed at pH 6.2 – 6.3 (T 6.8). The lowest gas production was registered in Tr 5.3 and it was associated to higher lag times and lower digestion rates. This suggests that digestion of fresh alfalfa would be limited by a ruminal pH lower than that commonly reported in the literature (pH 6.2).

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