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# Effects of water extracts from chicory and BHT on the *in vitro* rumen degradation of feeds

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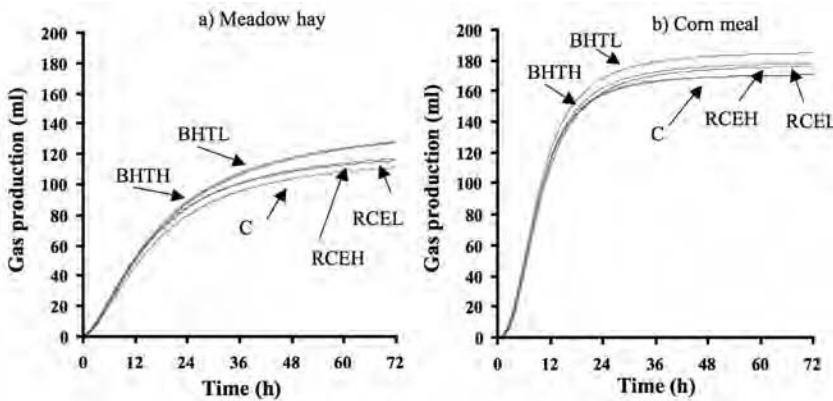
**ABSTRACT** - Effects of Butyl-Hydroxyl-Toluene (BHT) and of Red Chicory Extract (RCE) on kinetics of gas production (GP) and rumen degradability values (OMd, NDFd and *in vitro* true OM degradability - IV-TOMD) of two feeds (meadow hay and corn meal) were evaluated using an *in vitro* automatic batch system. For each feed 2 increasing dosages (0.15 and 1.5 mg/g of feed) of BHT and RCE and a Control (C) were tested in 4 replications and 2 incubations. First incubation lasted 72h, the 2<sup>nd</sup> one was stopped at the times on which half of GP was produced ( $t_{1/2}$ ), which were 9 and 16 h for corn and hay, respectively. From the supernatants of the 2<sup>nd</sup> incubation, VFA, NH<sub>3</sub>, N content of the residual NDF were analysed and the microbial N balance was computed. The 2 feeds significantly affected rumen fermentation parameters; BHT significantly increased asymptotic GP,  $t_{1/2}$  and IVTOMD ( $P < 0.01$ ), decreased the proportion of butyrate ( $P < 0.01$ ) but did not affect microbial N balance; RCE did not influence any of the parameters measured with respect to C, except for a significant increase of the estimated N available for microbes at the higher dosage.

*Key words:* Gas production, *In vitro* rumen degradability, Natural extracts, Antioxidants.

**Introduction** – In the North East area of Po valley red chicory is enjoying a great success and in the year 2005 the local market demand reached about 250,000 ton/year. Increasing amounts of by-products are made available. Red chicory has been shown to contain considerable amounts of phenolic compounds with antioxidant properties (Rossetto *et al.*, 2005). Extraction of bioactive substances from by-products is receiving growing interest for human and animal nutrition, also for the opportunity to replace synthetic compounds. Some studies suggested that natural extracts from vegetables can be used to manipulate the rumen fermentations, selecting or promoting the growth and the activity of microbes, changing the amount and the ratio of the end products of fermentation (Naziroğlu *et al.*, 2002; Busquet *et al.*, 2006; Alexander *et al.*, 2008). However, limited data are available about the effect of antioxidants and natural extracts on rumen fermentations. This study was aimed to screening the effect of Red Chicory Extracts (RCE) and Butyl-Hydroxyl-Toluene (BHT) on some parameters of rumen fermentations when incubated *in vitro* with different feeds.

**Material and methods** – *In vitro* rumen fermentations were conducted using an automatic batch gas production (GP) system (RF, Ankom Technology®) for 72 h at 39°C. In each jar, 25 ml of rumen fluid, collected from 3 dry cows, 50 ml of buffer (Menke *et al.*, 1979) and 0.55 g/batch of meadow hay or corn meal, milled at 1 mm, were used. For each feed 5 treatment groups were tested in 4 replications: 2 increasing dosages (0.15 and 1.5 mg/g of feed) of BHT (BHTL; BHTH, respectively) and RCE (RCEL; RCEH, respectively) and a Control (C) group. Four blanks without feeds were also included. Note that the maximum dosage of BHT permitted by law in compounds feeds is 0.15 mg/g. Chicory extracts were achieved as described by Rossetto *et al.* (2005). GP at various times ( $t$ ) was measured by mean of a pressure detector every minute. GP kinetics were fitted with the model:  $GP = A/[1 + (t_{1/2}/t)^2]$ , where A is the asymptotic GP,  $t_{1/2}$  is the time at which half of the asymptotic GP is

Figure 1. Kinetics of gas production (72h) of meadow hay (a) and corn meal (b) samples incubated with 0 (C), 0.15 (L) and 1.5 (H) mg/g feed of BHT or Red Chicory Extract (RCE).



tion for the 2 feeds (9 and 16 h for corn and hay, respectively) were used to establish the times for stopping a 2<sup>nd</sup> incubation performed with the same criteria described above. The supernatant fractions, obtained from the 2<sup>nd</sup> incubation, were analysed for volatile fatty acids (VFA), NH<sub>3</sub> and N content of the undegraded NDF and the microbial N balance was computed (Grings *et al.*, 2005). Data were analyzed for the effects of feeds, additives at different dosages and their interactions by ANOVA.

**Results and conclusions** - The GP kinetics are graphically described in Figure 1. The kinetic parameters and the degradability values are given in table 1. In general, the residual variability within treatment was low (coefficients of variation always lower than 7%, except for c). Hay and corn significantly differed ( $P<0.01$ ) for almost all the various kinetic and degradability parameters. With respect to Control, BHT significantly increased asymptotic GP,  $t_{1/2}$  and IVTOMD, without any difference between the two dosages. No significant influence of RCE was observed on the various parameters of GP and degradability parameters with respect to Control. In agreement with litera-

Table 1. Kinetics of gas production (GP), OM, NDF and in vitro true OM degradability of feeds incubated with 0 (C), 0.15 (L) and 1.5 (H) mg/g feed of BHT or Red Chicory Extract (RCE).

	Feed			Additive				Root MSE	
	Hay	Corn	Control C	BHT		RCE			
				L	H	L	H		
Kinetic of gas production:									
c		1.59 <sup>B</sup>	2.43 <sup>A</sup>	2.04	1.91	1.82	2.12	2.16	0.27
A	ml	132 <sup>B</sup>	176 <sup>A</sup>	147 <sup>B</sup>	161 <sup>A</sup>	167 <sup>A</sup>	148 <sup>B</sup>	148 <sup>B</sup>	11
$t_{1/2}$	h	16.0 <sup>A</sup>	8.7 <sup>B</sup>	12.0 <sup>B</sup>	13.0 <sup>A</sup>	13.4 <sup>A</sup>	11.8 <sup>B</sup>	11.3 <sup>B</sup>	0.8
Degradability (72 h):									
OMd	%	66.3 <sup>B</sup>	95.8 <sup>A</sup>	81.4	80.6	80.1	82.1	80.8	1.3
NDFd	"	59.6 <sup>B</sup>	83.8 <sup>A</sup>	71.2	73.4	72.7	70.6	70.6	1.9
IVTOMD	"	70.0 <sup>B</sup>	98.0 <sup>A</sup>	83.5 <sup>B</sup>	84.4 <sup>A</sup>	84.7 <sup>A</sup>	83.8 <sup>ab</sup>	83.6 <sup>b</sup>	0.4
TOMD/GP	mg/ml	2.51	2.70	2.70	2.47	2.52	2.68	2.68	0.20

Numbers on the same row for feeds or additives with different letters significantly differed: A,B  $P<0.01$ ; a,b  $P<0.05$ . c=sharpness of the curve profile; A= asymptotic GP;  $t_{1/2}$ =ime at which half of the asymptotic GP has been formed; TOMD/GP= truly degraded OM/GP at 72h.

produced, c is a constant representing the sharpness of the switching characteristics of the curve profile. At the end of incubation OM, NDF and the *in vitro* true OM degradability (IVTOMD) were computed from chemical analysis of feeds and residues as proposed by Grings *et al.* (2005). The  $t_{1/2}$  values resulting from the first incubation

**Table 2.** VFA profile and microbial N balance of hay and corn incubated for 9 and 16 h ( $t_{1/2}$ ), respectively, with 0 (C), 0.15 (L) and 1.5 (H) mg/g feed of BHT or Red Chicory Extract (RCE).

		Feed		Control C	Additive				Root MSE
		Hay	Corn		BHT		RCE		
					L	H	L	H	
Acetate (Ac)	%	73.5 <sup>A</sup>	66.6 <sup>B</sup>	66.7 <sup>B</sup>	74.5 <sup>A</sup>	74.6 <sup>A</sup>	68.2 <sup>B</sup>	66.4 <sup>B</sup>	0.6
Propionate (Pr)	"	15.9 <sup>A</sup>	20.8 <sup>B</sup>	17.6 <sup>b</sup>	19.0 <sup>a</sup>	19.5 <sup>a</sup>	17.9 <sup>b</sup>	17.6 <sup>b</sup>	0.8
<i>n</i> -Butyrate (Bu)	"	7.2 <sup>A</sup>	9.6 <sup>B</sup>	13.1 <sup>A</sup>	2.9 <sup>B</sup>	2.4 <sup>B</sup>	10.6 <sup>A</sup>	13.0 <sup>A</sup>	1.1
Others VFA	"	3.4 <sup>A</sup>	3.0 <sup>B</sup>	2.8 <sup>B</sup>	3.6 <sup>A</sup>	3.5 <sup>A</sup>	3.3 <sup>AB</sup>	3.0 <sup>AB</sup>	0.3
(Ac+Bu)/Pr ratio		6.4 <sup>A</sup>	4.8 <sup>B</sup>	5.7 <sup>a</sup>	5.4 <sup>b</sup>	5.3 <sup>b</sup>	5.7 <sup>a</sup>	5.7 <sup>a</sup>	0.2
Microbial N balance:	mg/jar								
N from feed (F)		4.3	6.8	5.6	5.6	5.6	5.6	5.6	-
N from NH <sub>3</sub> at t=0 (NO)		10.4	10.4	10.4	10.4	10.4	10.4	10.4	-
N from NH <sub>3</sub> at $t_{1/2}$ (Nt)		10.4 <sup>A</sup>	5.4 <sup>B</sup>	9.3 <sup>a</sup>	8.9 <sup>a</sup>	7.4 <sup>ab</sup>	8.1 <sup>ab</sup>	5.8 <sup>b</sup>	1.6
N content of NDF at $t_{1/2}$ (N_NDF)		1.2 <sup>B</sup>	1.9 <sup>A</sup>	1.5	1.3	1.7	2.0	1.3	0.5
Microbial N at $t_{1/2}$		3.1 <sup>B</sup>	9.9 <sup>A</sup>	5.2 <sup>b</sup>	5.8 <sup>b</sup>	6.9 <sup>ab</sup>	5.9 <sup>b</sup>	8.6 <sup>a</sup>	1.9

<sup>A,B</sup>  $P < 0.01$ ; <sup>a,b</sup>  $P < 0.05$ . Microbial N at  $t_{1/2}$  was computed as:  $(F + NO) - (Nt + N\_NDF)$ .

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16 h ( $t_{1/2}$  for hay) of incubation was similar to the value measured at the beginning of incubation, while for corn after 9 h of incubation the amount of N from ammonia was reduced by half, with respect to the initial value. The estimated amount of N available for microbial growth for hay was about 3 times lower than that observed for corn at  $t_{1/2}$ . BHT significantly decreased the proportion of butyrate and significantly increased the remaining VFA ( $P < 0.01$ ), but no significant effects were observed for the microbial N balance, with respect to C. RCE did not influence any of the parameters measured with respect to C, except for a significant increase ( $P < 0.05$ ) of the estimated N available for microbes at the higher dosage. In conclusion, the results of this work did not evidence a significant effect of RCE on rumen fermentation when incubated with different feeds at different dosages, whereas BHT significantly influenced GP kinetics, degradability parameters and VFA profile.

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ture, the ratio between the truly degraded OM and GP at 72h (TOMD/GP) ranged from 2.47 to 2.70 mg/ml. The former parameter was not significantly influenced both by feeds and additives.

As expected, results of the 2<sup>nd</sup> incubation showed significant differences of VFA profile between hay and corn ( $P < 0.01$ ) (Table 2). Hay produced higher proportions of acetate and lower proportions of propionate and *n*-butyrate with respect to corn. At  $t_{1/2}$  hay and corn significantly differed also for the microbial N balance. With hay, amount of N in form of ammonia found after