Reprod. Nutr. Dev. 40 (2000) 431–440

Original article

Effects of *Lavandula officinalis* and *Equisetum arvense* dry extracts and isoquercitrin on the fermentation of diets varying in forage contents by rumen microorganisms in batch culture

Laurent-Philippe BROUDISCOU*, Bernadette LASSALAS**

Département de l'Élevage et Nutrition des Animaux,
Institut National de la Recherche Agronomique, Paris, France

(Received 27 September 1999; accepted 10 August 2000)

Abstract — The short-term actions of *Lavandula officinalis* and *Equisetum arvense* dry extracts, and of isoquercitrin, flavonoid present in *Equisetum arvense*, on in vitro fermentation by rumen microbes were studied in batch culture. The orchard grass hay : barley ratios in the three experimental diets were 100:0, 75:25, 50:50 on a DM basis. The production rates of all volatile fatty acids except isobutyrate were strongly influenced by the composition of the diet and to a lesser extent, by plant extracts, with significant interactions between both factors. When hay was the only substrate, the addition of *L. officinalis* and *E. arvense* enhanced the fermentation rate by 50%, through an increased release of acetate and propionate. On the contrary, with the two other diets, the fermentation rate was strongly lowered by isoquercitrin. Gas outputs were not significantly influenced by plant extracts.

isoquercitrin / plant-extract / rumen / microorganism / in vitro

Résumé — Effet des extraits secs de prêle et de lavande et de l'isoquercitrine sur la fermentation de rations de teneurs en fourrage variables par les micro-organismes du rumen en mini-fermenteur. L'influence à court terme d'extraits de *Lavandula officinalis* et d'*Equisetum arvense*, et celle de l'isoquercitrine sur les fermentations par les microbes du rumen ont été étudiées en mini-fermenteurs. Les proportions, en MS, de foin de dactyle et d'orge dans les trois rations expérimentales étaient 100:0, 75:25, 50:50. Les productions de tous les acides gras volatils sauf l'isobutyrate ont été fortement influencées par la ration, puis par l'extrait végétal, avec des interactions significatives entre facteurs. Avec 100 % de foin, les extraits de *L. officinalis* et *E. arvense* ont augmenté les fermentations de 50 %, par une production accrue d'acétate et de propionate. Au contraire, avec les deux autres rations, les fermentations ont été diminuées par l'isoquercitrine. Les productions de gaz n'ont pas été significativement modifiées par l'apport d'extrait végétal.

isoquercitrine / extrait végétal / rumen / micro-organisme / in vitro

* Correspondence and reprints: INRA-INAPG, UMR Physiologie de la Nutrition et Alimentation, 16 rue Claude Bernard, 75231 Paris Cedex 05, France.
E-mail: Laurent.Broudiscou@inapg.inra.fr
** Present address: URH, INRA Clermont-Theix, 63122 St-Genès-Champanelle, France.

1. INTRODUCTION

In a social context increasingly opposed to the use of additives such as ionophores in ruminant nutrition, new commercial products of plant origin have been proposed to farmers for reasons of greater social acceptance in European countries. However, the actual potential of manipulating rumen function with plant extracts containing high levels of secondary metabolites has to be assessed. The recent screening test of 13 plant extracts in dual-outflow fermenters led to the selection of two extracts, for further evaluation. *Lavandula officinalis* tended to increase the extent of fermentation, and *Equisetum arvense* tended to inhibit methane production by rumen microorganisms [2].

The objectives of this study were (1) to test the hypothesis of a short-term action of *L. officinalis* and *E. arvense* extracts on carbohydrate fermentation by rumen microbial populations unaccustomed to these additives; a major *E. arvense* flavonoid, isoquercitrin, was also tested; (2) to detect a possible interaction with the composition of the diet, with the proportion of hay in our experimental diets varying from 100 to 50% on a dry matter basis; (3) to assess the ability of a batch in vitro technique to screen plant extracts.

Table I. Composition of plant extracts.

Plant	*L. officinalis*	*E. arvense*
Part	flowers	aerials
Solvent	ethanol 30%	water
DM (%)	97.7	95.0
OM (%DM)	91.6	84.1
N (%OM)	0.82	1.05
Phenolic compounds[a]		
Type	Luteolin-7-glucoside	Isoquercitrin
% DM	9.8	> 0.3

[a] Data provided by the manufacturer.

Table II. Composition of feeds.

	Hay	Barley
Dry matter (DM g·kg^{-1})	921	911
Composition of DM (g·kg^{-1} DM)		
Organic matter	913	924
Crude protein (N × 6.25)	148	129
Starch	traces	627
Neutral detergent fiber	634	157
Acid detergent fiber	342	60

2. MATERIALS AND METHODS

2.1. Experimental strategy

Two factors were combined within a full factorial design which was replicated three times: (1) the additive, with four levels (no additive, *L. officinalis* extract, *E. arvense* extract, isoquercitrin); (2) the proportion of hay in the experimental diet, with three levels (100, 75, 50% DM). All three additives were tested at similar input rates as in [2], that is 20 mg extract and 200 µg isoquercitrin per 24 h incubation. *L. officinalis* and *E. arvense* dry extracts were purchased from AMI SA (Paris, France), isoquercitrin from ExtraSynthèse SA (Genay, France). The characteristics of both products are detailed in Table I. The diets were composed of ground orchard-grass hay and barley, with 200 mg per incubation. The composition of feeds is given in Table II. Three rumen-cannulated sheep were used as donors, each being adapted to one of the three experimental diets. In each run, 3 additional incubations, one per inoculum type, contained no feed. They were used as blanks in the calculation of the 12 fermentation balances issued from the combination of experimental factors. Our experimental design required a total of 45 incubations in 3 runs and thus generated 36 observations.

2.2. Incubation procedure

Incubations were conducted in 120 mL serum bottles sealed by gas-tight rubber stoppers (Sodipro, Echirolles, France) and kept in a water bath at 39 °C (±0.5). Fermentation gases were allowed to expand in 100 mL glass syringes on the top of the bottles. This small working-volume, short-term, batch incubation device appears similar to a number of others which have been proposed over the past few years, as initiated by Menke et al [14]. However, the main purpose of these systems was the estimation of ruminant feed degradability through in vitro gas production kinetics. Our batch system is more concerned with the quantitative study of overall production by rumen microbes of all gaseous and liquid metabolites from specified substrates, with no rise of gas pressure.

Before each incubation run, a fresh Simplex-type buffer was prepared by mixing 0.4 L of a Coleman buffer solution, 0.6 L of deionised water and 1 mL of 1 $g \cdot L^{-1}$ resazurin solution [28]. The Coleman buffer solution was made of K_2HPO_4 12.7 $g \cdot L^{-1}$, KH_2PO_4 10.0 $g \cdot L^{-1}$, NaCl 1.3 $g \cdot L^{-1}$, $MgSO_4$ (7 H_2O) 0.18 $g \cdot L^{-1}$ and $CaCl_2$ (2 H_2O) 0.12 $g \cdot L^{-1}$. The Simplex buffer was degassed by boiling for 15 to 20 min on a hot plate stirrer. After cooling under a CO_2 atmosphere, the solution was supplemented with $NaHCO_3$ (7.5 $g \cdot L^{-1}$) and with a 20 $g \cdot L^{-1}$ cysteine-HCl solution (11 $mL \cdot L^{-1}$). The buffer was kept under CO_2 until colourless (pH of 6.7) prior to storage in an air-tight polyethylene terephtalate glycol (PETG) bottle.

Each bottle was inoculated under a CO_2 atmosphere with 40 mL of a mixture of 3 volumes of filtered rumen fluid and 5 volumes of Simplex buffer. Fermentation broths were alternately subjected to gentle stirring for 30 s, using a magnetic bar at 300 rpm, followed by a resting period of 4 min. At the end of the incubation period, the composition of fermentation gases was determined and the fermentation broths were sampled for soluble end-product analysis.

2.3. Analytical methods

Volatile fatty acids (VFA) were determined by capillary GC. A WCOT fused silica capillary column (25 m × 0.25 mm I.D, film thickness 0.20 μm, Chrompack), with CP-Wax 58 as the stationary phase, was used. The operating conditions were as follows: N_2 as the carrier and make-up gas, initial oven temperature 130 °C, slope of 15 $°C \cdot min^{-1}$ up to 220 °C, injector temperature 220 °C, detector temperature 250 °C. A volume of 0.1 mL of isocaproate 10 $g \cdot L^{-1}$ was added as an internal standard to 1 mL of sample. The individual concentrations were quantified from the corresponding peak surfaces corrected for the internal standard. Fermentation gases were analysed by gas chromatography as described in [3].

2.4. Calculations and statistical analyses

The production of VFA has been expressed per 200 mg of experimental substrates. The amount of hexoses theoretically fermented in 24 h incubation (HF) was estimated from net productions of individual VFA, as described in [5]:

$$HF\ (\mu mol) = (C2 + C3)/2 + C4 + C5 + C6.$$

The results were analysed with a mixed-model using a GLM procedure [15]. The terms in the model were diet, additive, run and diet × additive. Diet sums of squares were partitioned into linear and quadratic effects. One factor, run, was random; the additive being fixed. When the level of significance of the interaction term was equal to or higher than 0.05, multiple comparisons of means for the additive factor were performed according to the Tukey method [15].

3. RESULTS

One observation corresponding to the combination "isoquercitrin + hay 75%" was withdrawn from the data set because of technical failure. As a consequence, the GLM procedure was conducted on 35 observations.

VFA productions are presented in Table III and Figure 1. Once corrected for endogenous substrates, the levels of production of all VFA except isobutyrate were strongly influenced by the composition of the diet and, to a lesser extent, by the addition of plant extracts. C2 and C3 outputs were similarly altered by the treatments, through a complex pattern involving the interaction of the diet with the additive (Fig. 1). The amounts of C2 and C3 produced from the 100% hay diet were increased by the plant extracts: strongly by *L. officinalis* (60% and 37% respectively) and *E. arvense* (59% and 40% respectively), to a lesser degree by isoquercitrin (29% and 15% respectively). On the contrary, with medium and high grain diets, incubations with isoquercitrin led to smaller productions of both VFA, compared to the three other treatments which did not differ. As a consequence, the production of C2 and C3 varied as a curvilinear function of hay proportion in controls while it decreased nearly linearly with increasing hay proportions in all other incubations. The hierarchy among the three additives remained unchanged whatever the diet. The production of C4 did not show any significant interaction between both factors. It increased with the dietary proportion of non-structural carbohydrates in a curvilinear way, as shown in Figure 1. This increase was slightly inferior with isoquercitrin. The amount of HF followed the same trend as

Table III. Effects of additives (A) and grain:forage ratio (D) on acetate (C2), propionate (C3), isobutyrate (IC4), butyrate (C4), valerate (C5) and caproate (C6) productions and on the amount of hexoses theoretically fermented (HF) in 24 h incubations (μmol).

Response		C2	C3	IC4	C4	C5	C6	HF
Source	DF	P < F	P < F	P < F	P < F	P < F	P < F	P < F
Run	2	0.92	0.001	0.18	0.36	0.015	0.059	0.70
D	1	0.000	0.000	0.21	0.000	0.000	0.000	0.000
D^2	1	0.41	0.72	0.43	0.050	0.61	0.86	0.33
A	3	0.008	0.000	0.40	0.030	0.008	0.27	0.002
D × A	3	0.10	0.041	0.61	0.25	0.083	0.89	0.052
RSD		89.4	17.9	1.69	12.8	1.31	0.718	59.8
Diet		Means						
50% hay		658.2	182.4	0.390	140.1	11.45	4.03	573.5
75% hay		572.5	167.2	1.416	107.2	8.63	3.06	488.3
100% hay		414.1	150.9	1.278	52.9	5.88	1.99	344.3
Additive								
Control		532.0^{ab}	162.0	1.476	99.4	8.48^{ab}	3.22	459.3^{ab}
Isoquercitrin		452.9^{a}	141.9	0.096	87.8	7.36^{a}	2.79	392.7^{b}
E. arvense		607.6^{b}	184.1	1.144	108.2	9.56^{b}	3.32	516.4^{a}
L. officinalis		587.4^{ab}	176.5	1.251	102.7	9.08^{ab}	2.74	495.8^{a}

Values with different letters are significantly different ($P < 0.01$).

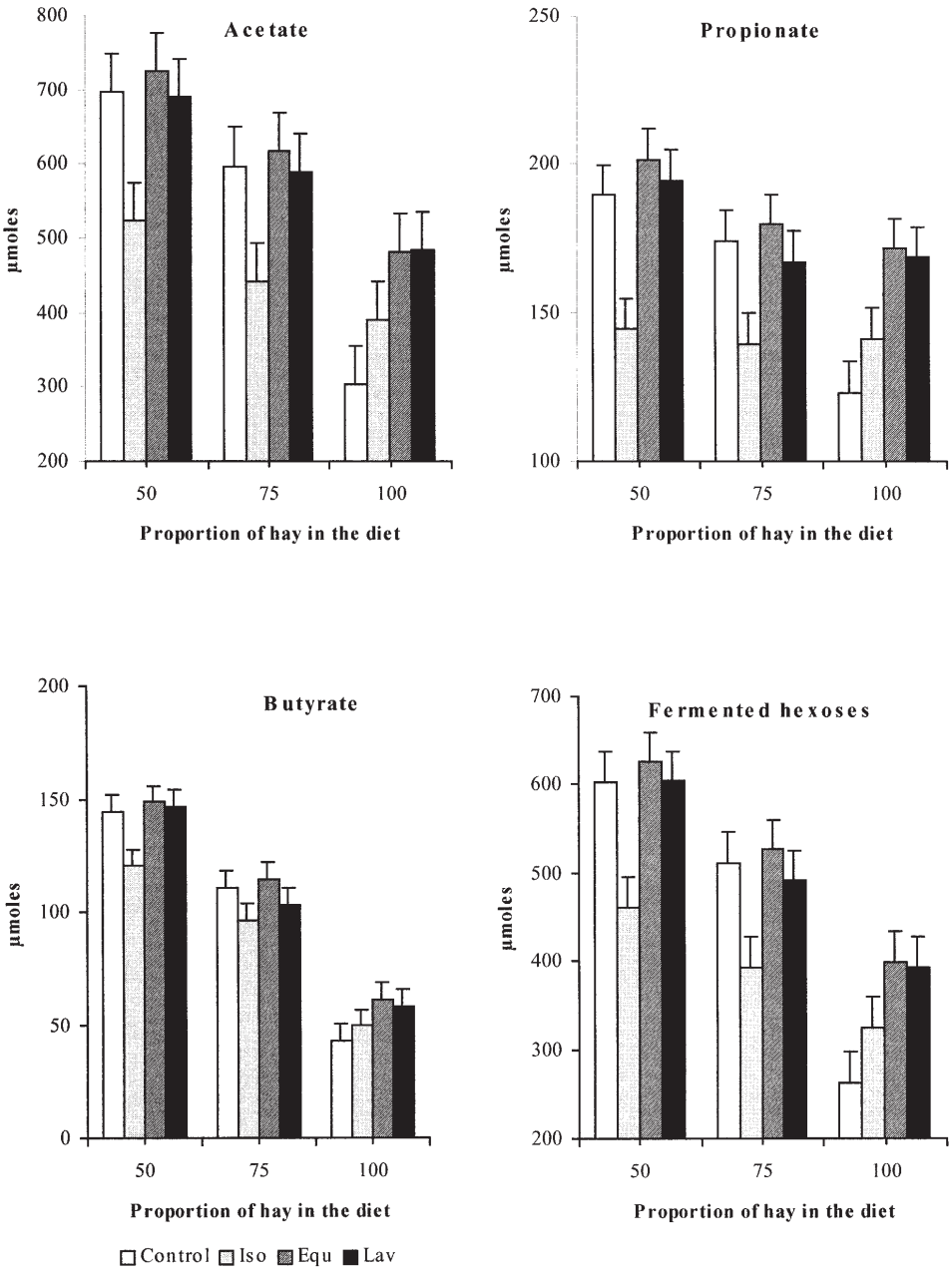

Figure 1. Effects of additive – *L. officinalis* (Lav), *E. arvense* (Equ) plant extracts, isoquercitrin (Iso) – and hay dietary proportion on volatile fatty acid productions and fermentation of hexoses after 24 h incubation. Error bars: SEM.

C2 and C3 productions, owing to their relative importance in the pool of VFA (Fig. 1). It decreased with the proportion of grain in the experimental diet, in a linear way when a plant extract was present and in a curvilinear one otherwise, as shown by the interaction term D × A close to significance. When hay was the only substrate, the addition of *L. officinalis* and *E. arvense* increased the fermentation yield by 50%. On the contrary, with the two other diets, the fermentation yield was strongly lowered by isoquercitrin. Data on the fermentation pattern are given in Table IV. The sole active factor was the composition of the diet, which modified the relative productions of C3 and C4 in a curvilinear way, mostly between 75% and 100% of hay. Individual gas productions are presented in Table V. The average fraction of gas output rates accounted for by endogenous substrate degradation equalled 45% (SE of 7.4%). The outputs of CO_2 and CH_4 were mainly influenced by the diet, and increased linearly with the amount of dietary non-structural carbohydrates. Their relative productions were not clearly modified by any treatment. The relative production of H_2 was significantly influenced by the diet, in a curvilinear way, and reached a maximum with a proportion of hay comprised between 75% and 100%.

4. DISCUSSION

The differences among diets in the composition of VFA were consistent with the observations of Beuvink and Spoelstra [1] who reported comparable differences in the relative amounts of C2, C3 and C4 produced from 24 h fermentation of rice starch and cellulose in a batch in vitro system. In the control flasks, the fermentation pattern with

Table IV. Effects of additives (A) and grain: forage ratio (D) on acetate (C2), propionate (C3), isobutyrate (IC4), butyrate (C4), valerate (C5) and caproate (C6) relative productions in 24 h incubations (mol per 100 mol fermented hexoses).

		C2	C3	IC4	C4	C5	C6
Source	DF	P < F	P < F	P < F	P < F	P < F	P < F
Run	2	0.62	0.19	0.13	0.19	0.41	0.021
D	1	0.34	0.000	0.069	0.000	0.10	0.22
D^2	1	0.83	0.054	0.72	0.012	0.30	0.66
A	3	0.53	0.78	0.49	0.25	0.77	0.035
D × A	3	0.39	0.54	0.88	0.29	0.13	0.051
RSD		9.11	5.90	0.403	2.09	0.325	0.159
Diet		Means					
50% hay		114.3	32.1	0.05	24.5	2.00	0.71
75% hay		117.1	34.3	0.27	22.1	1.77	0.63
100% hay		118.0	45.2	0.36	15.8	1.77	0.63
Additive							
Control		113.0	39.0	0.34	21.1	1.94	0.76
Isoquercitrin		115.7	37.0	0.03	21.7	1.85	0.70
E. arvense		118.1	36.5	0.24	20.3	1.83	0.63
L. officinalis		118.9	36.6	0.27	20.0	1.79	0.53

Table V. Effects of additives (A) and grain: forage ratio (D) on methane (CH_4), carbon dioxide (CO_2) and hydrogen (H_2) net and relative (mol per 100 mol fermented hexoses) productions in 24 h incubations.

Response		Net production (μmol·d^{-1})			Relative production (mol·100 mol^{-1} HF)		
		CH_4	CO_2	H_2	CH_4	CO_2	H_2
Source	DF	P < F	P < F	P < F	P < F	P < F	P < F
Run	2	0.79	0.013	0.023	0.39	0.073	0.14
D	1	0.000	0.001	0.084	0.36	0.11	0.045
D^2	1	0.36	0.57	0.000	0.23	0.59	0.035
A	3	0.002	0.92	0.99	0.51	0.12	0.21
D × A	3	0.11	0.30	0.039	0.026	0.15	0.45
RSD		16.15	328.2	0.326	11.39	120.5	0.103
Diet							
50% hay		254.8	1706	1.21	45.4	303.1	0.215
75% hay		202.3	1494	1.61	41.8	309.0	0.334
100% hay		158.3	1183	0.97	49.7	384.6	0.303
Additive							
Control		193.1[a]	1513	1.27	48.6	386.5	0.304
Isoquercitrin		192.9[a]	1442	1.23	48.9	377.6	0.330
E. arvense		214.7[b]	1405	1.25	42.1	267.2	0.241
L. officinalis		218.7[b]	1479	1.25	43.8	305.2	0.261

Values with different letters are significantly different ($P < 0.01$).

the intermediate diet was close to the one observed in our batch system validation trial using a 70% meadow hay and 30% pelleted barley diet, which led to the production of 557 μmol of C2, 171 μmol of C3 and 100 μmol of C4 per 200 mg of substrate DM (Broudiscou and Lassalas, unpublished data). The change in the fermentation pattern clearly occurred, in a curvilinear way, for a proportion of hay higher than 75%. The low input levels of plant extracts in the present work were identical to those applied in a previous screening experiment in continuous culture [2], with a view to easing data comparison. Isoquercitrin was thus introduced at a concentration of 11 μM, while phenolics were frequently tested in batch systems at much higher concentrations, for example 10 mM [17]. As a consequence, the corresponding input of organic matter was also kept at a minimum.

The interactions observed between the diet and additive factors have always discriminated the control from the treatments with the plant extracts. All three additives appeared to be beneficial to the microbial community adapted to forage. They enhanced the extent of fermentation of the 100% hay diet above the value expected from the mere degradation of the additional organic matter brought into by *L. officinalis* and *E. arvense* extracts. Isoquercitrin and luteolin-7-glucoside were probably submitted to bacterial hydrolysis and their sugar moiety fermented into VFA during the 24 h incubation. Isoquercitrin has been shown to be a source of carbon and energy for *Enterococcus casseliflavus* and *Eubacterium ramulus*, both isolated from

human faeces [22]. In the same way, a major fibrolytic bacteria in the rumen, *Butyrivibrio fibrisolvens*, was isolated in a medium containing rutin as the sole energy-yielding substrate [11]. However, they can only account for a negligible part (less than 4%) of the increase in fermented hexoses observed when the additives were present in the 100% hay diet. On the contrary, the data on both other diets suggest that this stimulation of microbial fermentation linked to structural polysaccharide degradation was counterbalanced by a detrimental action on the microbial species relying on amylolysis.

Yet the modes of action of isoquercitrin and *L. officinalis* and *E. arvense* extracts on the rumen microbial population are still unclear and experimental data are scarce. In a recent paper, Scehovic [21] extracted the buffer-soluble contents of about a hundred plant species harvested in natural grasslands (mono- and dicotyledons). He quantified their individual effect on rumen bacteria during 4-h batch incubations. this study revealed strong interspecific differences and stressed how difficult it was to relate the stimulating or inhibiting actions of plants to the presence of particular phytochemicals. One may at least advance three hypotheses to explain the effects of our additives: (1) inhibitory or stimulatory action of flavonoids on some rumen microorganisms; (2) effect of the degradation products of flavonoids; (3) in the case of *L. officinalis* and *E. arvense* extracts, direct action of other secondary metabolites. Flavonoids are known to interact directly with microorganisms, in a positive as well as in a negative way. Legume plants flavonoids are engaged in the transcription of nodulation genes in symbiotic bacteria, in a highly specific interaction [6]. On the opposite, an antibacterial effect of flavonoids has been evidenced in pure cultures [18, 26, 27]. Flavonoids can alter the bioenergenetic status of the bacterial membrane: uncoupling of the energy transducing cytoplasmic membrane by flavonoids present in propolis has been reported [16]. The degradation products of flavonoids are also good candidates for modifying the microbial metabolism in the rumen. The bacterial ring fission of aglycone flavonoids leads to the production of phenolic acids, for instance 3,4-dihydroxyphenylacetic acid from isoquercitrin [22] and quercetin [29], and phenylacetic acid from naringenin [29]. Some of these simple phenolic compounds may interact with the biosynthesis of aromatic amino acids: both biosynthesis pathways are linked through cinnamic acid. Analogous molecules, phenylpropanoic acid and phenylacetic acid, have been reported to enhance cellulose degradation by, and growth of several strains of *Ruminococcus albus* [23, 24]. Finally, the action of secondary compounds other than flavonoids must be considered for the two dry plant extracts, even if the biochemical compositions of these products were too complex to be characterised and if the main goal in the present work was to assess the nutritional interest of plant extracts of known botanical origin. From phytochemical databases, one can note that 40 organic substances other than sugars, fatty acids or amino acids have been identified in *E. arvense* [6]. Amongst them, several phenolic acids, caffeic acid, gallic acid, vanillic acid and their related hydrolysable tannins such as tannic acid, and one sterol, β-sitosterol, are at least partly soluble in water and express an antibacterial activity [7, 19, 20]. However, the data on 100% hay diet suggest that, if present, tannins were not in sufficient amounts to reduce cell-wall degradability or notably lower the availability of ammonia to cellulolytic bacteria.

The changes observed in fermentations yield when the plant extracts and isoquercitrin were added to incubations reflected the differential influences of these additives on rumen bacteria and probably the combined effects of different secondary metabolites. It is well known that the antibacterial activity of flavonoid-rich products depends on the bacterial species and was found to be more effective against

gram-positive strains than on gram-negative ones [9, 16]. One must note that the short-term exposure in our trial excluded any significant change in the specific composition of the microbial population. The results suggest that *E. arvense* extract supplied the bacteria linked to cell-wall degradation with stimulatory compounds other than isoquercitrin, unless isoquercitrin action was matrix-dependent. Simultaneously, the inhibition of a number of bacterial species linked to amylolysis could lead to the changes in VFA production pattern. Supporting this hypothesis is the fact that important amylolytic bacteria in the rumen, *Selenomonas ruminantium* and *Prevotella* sp., are also known as major propionate producers [8, 25]. The release of propionate has been most strongly affected by the interaction in our essay. At the same time, butyrate production was not altered, in agreement with the fact that one of the cellulolytic bacterial species in the rumen, *Butyrivibrio fibrisolvens*, is considered as a major butyrate producer.

In dual outflow fermenters receiving a 50:50 hay-barley diet for seven days, the addition of *L. officinalis* and *E. arvense* dry extracts altered microbial fermentation in two different ways. *L. officinalis* appeared to increase the amount of fermented OM, while *E. arvense* tended to inhibit methanogenesis. Even if this former essay was based on an experimental design allowing some confounding between main effects and interactions, the discrepancy between our long-term and short-term data raises questions about the significance of batch incubation results in plant extract studies. Short term in vitro techniques have already been used to assess the influence of secondary metabolites on rumen fermentation [10, 13]. A gas-test procedure based on the use of PEG 6000 has even been proposed to evaluate the biological activity of tannins [12]. However, our discrepant observations stress a possible adaptive response of the rumen microbial community to our plant extracts. Such specific microbial adaptations to tannins, either tolerance or detoxification capability, have been observed in the rumen ecosystem [4]. On this assumption, the use of continuous cultures from the early screening step on is preferable to batch short-term cultures in studies on the nutritional interest of plant compounds.

ACKNOWLEDGEMENTS

The authors would like to thank L. L'Hotelier for taking care of the experimental animals.

REFERENCES

[1] Beuvink J.M.W., Spoelstra S.F., Interactions between substrate, fermentation end-products, buffering systems and gas production upon fermentation of different carbohydrates by mixed rumen microorganisms in vitro, Appl. Microbiol. Biotechnol. 37 (1992) 505–509.

[2] Broudiscou L.-P., Papon Y., Broudiscou A.F., Effets de 13 extraits végétaux riches en flavonoïdes sur les fermentations et la méthanogénèse dans des cultures continues de microorganismes du rumen, in: 19th International Conference on Polyphenols, Lille, 1–4 Septembre 1998, Groupe Polyphenols, Bordeaux, 1998, pp. 589–590.

[3] Broudiscou L.P., Papon Y., Broudiscou A., Effects of inorganic nitrogen and amino acids on the degradation of ammonia-treated barley straw and proteosynthesis in a continuous culture of rumen microbes, Anim. Feed Sci. Technol. 77 (1999) 149–162.

[4] Dawson K.A., Rasmussen M.A., Allison M.J., Digestive disorders and nutritional toxicity, in: Hobson P.N., Stewart C.S. (Eds.), The Rumen Microbial Ecosystem, 2nd ed., Chapman & Hall, London, 1997, pp. 631–660.

[5] Demeyer D.I., van Nevel C.J., Methanogenesis, an integrated part of carbohydrate fermentation and its control, in: McDonald I.W., Warner A.C.I. (Eds.), Digestion and Metabolism in the Ruminant, University of New England Publishing Unit, Armidale, 1975, pp. 366–382.

[6] Gough C., Galera C., Vasse J., Webster G., Cocking E.C., Denarie J., Specific flavonoids promote intercellular root colonization of *Arabidopsis thaliana* by *Azorhizobium caulinodans* ORS571, Mol. Plant Microbe Interact. 10 (1997) 560–570.

[7] Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants, CRC Press, Boca Raton, 1992.

[8] Hespell R.B., Biotechnology and modifications of the rumen microbial ecosystem, Proc. Nutr. Soc. 46 (1987) 407–413.

[9] Ivancheva S., Manolova N., Serkedjieva J., Dimov V., Ivanovska N., Polyphenols from Bulgarian medicinal plants with anti-infectious activity, Basic Life Sci. 59 (1992) 717–728.

[10] Khazaal K., Ørskov E.R., The in vitro gas production technique: an investigation on its potential use with insoluble polyvinylpolypyrrolidone for the assessment of phenolics-related antinutritive factors in browse species, Anim. Feed Sci. Technol. 47 (1994) 305–320.

[11] Leedle J.A.Z., Hespell R.B., Differential carbohydrate media and anaerobic replica plating technique in delineating carbohydrate utilising subgroups in rumen bacterial populations, Appl. Environ. Microbiol. 39 (1980) 709–719.

[12] Makkar H.P.S., Becker K., A bioassay for polyphenols (tannins), in: Vercauteren J., Cheze C., Dumon M.C., Weber J.F. (Eds.), Proceedings of the 18th International Conference on Polyphenols, Vol. 1, Groupe Polyphenols, Bordeaux, 1996, pp. 197–198.

[13] Makkar H.P.S., Blummel M., Becker K., In vitro effects of and interactions between tannins and saponins and fate of tannins in the rumen, J. Sci. Food Agric. 69 (1995) 481–493.

[14] Menke K.H., Raab L., Salewski A., Steinglass H., Fritz D., Schneider W., The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro, J. Agric. Sci. Camb. 93 (1979) 217–222.

[15] Minitab, User's Guide 2: Data Analysis and Quality Tools, Minitab Inc., State College, 1998.

[16] Mirzoeva O.K., Grishanin R.N., Calder P.C., Antimicrobial action of propolis and some of its components: the effects on growth, membrane potential and motility of bacteria, Microbiol. Res. 152 (1997) 239–246.

[17] Murray A.H., Iason G.R., Stewart C., Effect of simple phenolic compounds of heather (*Calluna vulgaris*) on rumen microbial activity in vitro, J. Chem. Ecol. 22 (1996) 1493–1504.

[18] Padmavati M., Sakthivel N., Thara K.V., Reddy A.R., Differential sensitivity of rice pathogens to growth inhibition by flavonoids, Phytochemistry 46 (1997) 499–502.

[19] Pizzorno J.E., Murray M.T., A Textbook of Natural Medicine, John Bastyr College Publications, Seattle, Washington, 1985.

[20] Recio M.C., Rios J.L., Villar A., A review of some antimicrobial compounds isolated from medicinal plants reported in the literature 1978–1988, Phytother. Res. 3 (1989) 117–125.

[21] Scehovic J., Evaluation in vitro de l'activité de la population microbienne du rumen en présence d'extraits végétaux, Rev. Suisse Agric. 31 (1999) 89–93.

[22] Schneider H., Schwiertz A., Collins M.D., Blaut M., Anaerobic transformation of quercetin-3-glucoside by bacteria from the human intestinal tract., Arch. Microbiol. 171 (1999) 81–91.

[23] Stack R.J., Hungate R.E., Opsahl W.P., Phenylacetic acid stimulation of cellulose digestion by *Ruminococcus albus* 8, Appl. Environ. Microbiol. 46 (1983) 539–544.

[24] Stack R.J., Cotta M.A., Effect of 3-phenylpropanoic acid on growth of, and cellulose utilization by, cellulolytic ruminal bacteria, Appl. Environ. Microbiol. 52 (1986) 209–210.

[25] Stewart C.S., Flint H.J., Bryant M.P., The rumen bacteria, in: Hobson P.N., Stewart C.S. (Eds.), The Rumen Microbial Ecosystem, 2nd ed., Chapman & Hall, London, 1997, pp. 10–72.

[26] Villanueva V.R., Bogdanovsky D., Barbier M., Gonnet M., Lavie P., Sur l'identification de la 3,5,7-trihydroxy flavon (galangine) à partir de la propolis, Ann. Inst. Pasteur 106 (1964) 292–302.

[27] Villanueva V.R., Barbier M., Gonnet M., Lavie P., Les flavonoides de la propolis. isolement d'une nouvelle substance bacteriostatique: la pinocembrine, Ann. Inst. Pasteur 118 (1970) 84–87.

[28] Williams A.G., Coleman G.S., The Rumen Protozoa, Springer-Verlag, New York, 1992.

[29] Winter J., Moore L.H., Dowell V.R. Jr, Bokkenheuser V.D., C-ring cleavage of flavonoids by human intestinal bacteria., Appl. Environ. Microbiol. 55 (1989) 1203–1208.