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Effect of Yeast Culture and Phenolic Acids on the Physiology of Rumen Fermentation Determined in vitro

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Summary

The effect of p-hydroxybenzoic acid (HBA), syringic acid (SYA) and yeast culture (YS) on rumen fermentation *in vitro* has been investigated. Meadow hay was used as a substrate and rumen fluid as an inocula. The yeast culture Levucel contained $5x10^8$ yeast cells *Saccharomyces cerevisiae* per 1 g of dry matter and was used in the amount of 0.5 g/l of the medium. The following combinations of additives were used: hay without additive, hay + YS, hay with 1, 5 or 10 mmol HBA or SYA, and hay + YS with 1, 5 or 10 mmol HBA or SYA. The test tubes were incubated for 96 hours at 39 °C. The results showed that 1 mmol HBA had a significant effect on yeast efficacy. This was manifested in the increased degradability of hay dry matter ($P<0.05$) and enhanced total gas production ($P<0.05$). SYA in the same amount combined with yeast had a similar effect on gas production ($P<0.05$), but hay dry matter degradability was not affected. The results showed a slight effect of phenolic acids and yeast culture on hay rumen fermentation *in vitro*.

Key words
In vitro – Fermentation – Yeast culture – Phenolic acids – Rumen

Introduction

Yeast supplementation of fodder or fermentation media can affect the course of rumen fermentation (Wallace and Newbold 1993). It can promote digestibility (Erasmus *et al.* 1992, Mpofu and Ndlovu 1994), change both the production and ratio of volatile fatty acids (Wiedmeyer *et al.* 1987, Martin *et al.* 1989) and gases (Martin and Nisbet 1992, Carro *et al.* 1992), as well as other parameters of rumen fermentation. During forage plant cell development, phenolic acids and lignin are deposited in the maturing cell wall in specific structural conformations. Lignin is the key element that limits cell-wall digestibility (Jung and Allen 1995). Degradation of plant materials is accompanied by a rise of phenolic compounds, mostly as degradation products of lignin. Some of them are noted for their antimicrobial actions (Jurd *et al.* 1971, Davidson and Branen 1981), ability to inhibit cellulose degradation (Akin *et al.* 1988, Fukushima *et al.* 1991), as well as methane production (Martin 1988). However, there are still others, e.g. p-hydroxybenzoic (HBA) and syringic acid (SYA), which stimulate rumen microorganisms (Borneman *et al.* 1986). The objective of the present study was to determine if these acids have any effect on the activity of *Saccharomyces cerevisiae* yeast and to determine their influence on rumen fermentation.

Material and Methods

The experiment was conducted *in vitro* and the degradability of hay dry matter, as well as the production of gases and volatile fatty acids (VFA) were determined. Meadow hay with a particle size of 0.15–0.4 mm and sulphate cellulose were used as substrate. Hay and cellulose degradability was estimated by the method of Mellenberger *et al.* (1970). For fermentation, 60 ml glass test tubes with rubber stoppers were used. The substrate was weighed into the test tubes in amounts of 0.25 g for 17.5 ml of strained rumen fluid taken from rumen fistulated sheep fed meadow hay *ad libitum* and ground barley in a ratio of 80:20. McDougal's buffer (1948), pH 6.8, was then added into the test tubes. A commercial yeast culture (YS) Levucel (Lallemand, Canada) was used

containing $5x10^8$ of *Saccharomyces cerevisiae* yeast cells per 1 g. Yeast activity was determined by cultivation and corresponded with the data of the supplier. P-hydroxybenzoic and syringic acids were obtained from commercial sources (Sigma). In each incubator, control test tubes contained rumen fluid and buffer with no substrate. Additional tubes containing rumen fluid, buffer and cellulose were used as the control of rumen fluid cellulolytic activity. In other tubes, meadow hay with the following combinations of additives was used: hay without additive, hay + YS, hay with 1, 5 or 10 mmol HBA or SYA and hay + YS with 1, 5 or 10 mmol HBA or SYA. Yeasts were used in the amount of 0.5 g/l (i.e. 17.5 mg per tube). Three replicates were used for each combination, including the controls, and all six incubations were completed. The tubes were stored in an incubator at 39 °C for 96 h. During this time, the rubber stopper of each tube was stabbed by an injection needle and the amount of total gases produced was measured by means of rubber tubing connected to a gas flowmeter. The degradability was determined from the difference of substrate weight before and after incubation. The VFAs were determined by gas chromatography (Cottyn and Boucque, 1968) using crotonic acid as internal standard on Perkin-Elmer equipment. The differences among the mean values of the parameters observed were statistically analyzed by Student's t-test.

Table 1
Influence of yeast culture and p-hydroxybenzoic acid on in vitro rumen fermentation.

	Cellulose	Hay	Hay+YS	Hay + 1HBA	Hay + 5HBA	Hay + 10HBA	Hay+YS + 1HBA	Hay+YS + 5HBA	Hay+YS +10HBA*	SE
Degradability (%)	93.3^c	64.0	66.5	64.6	64.3	66.2	67.4^a	66.0	65.5	0.9
Total gas (ml)	60.6^a	52.6	55.4	54.1	52.4	51.3	58.1^a	55.3	50.6	2.2
Total VFA (mmol l^{-1})	97.8^b	78.3	83.1	77.2	79.4	74.7	78.2	80.7	78.8	2.9
Acetate (mol%)	59.7^c	69.2	68.6	69.2	69.0	69.1	68.9	68.8	68.7	0.3
Propionate (mol%)	31.0^c	18.5	18.5	18.5	18.6	18.5	18.4	18.3	18.3	0.4
Iso-butyrate (mol%)	0.85	0.97	1.02	0.92	0.94	0.95	0.97	1.03	0.99	0.06
Butyrate (mol%)	6.7^c	8.9	9.2	9.0	9.0	9.0	9.2	9.3	9.7	0.2
Iso-valerate (mol%)	0.99^b	1.37	1.49	1.34	1.33	1.39	1.44	1.51	1.50	0.08
Valerate (mol%)	0.73^a	1.05	1.17	1.05	1.07	1.07	1.16	1.13	1.14	0.06
A/P ratio	1.93^c	3.75	3.72	3.74	3.73	3.73	3.76	3.77	3.76	0.09

SE, standard error
a, b, c, significantly different from hay in the same row ($P<0.05$, $P<0.01$, $P<0.001$)
YS, yeast culture
HBA, p-hydroxybenzoic acid
* numbers 1, 5 and 10 mean milimols

Results

The parameters measured during cellulose fermentation naturally differed from those obtained during hay fermentation (Table 1). The degradability, total gas and total VFA production as well as the molar % of propionate were higher and the molar % of acetate, butyrate, iso-valerate, valerate and the A/P ratio were lower for cellulose. The hay degradability and gas production after yeast supplementation showed a tendency to higher values, but the differences were not significant. HBA did not affect the observed parameters at any concentration. The yeast additive in combination with 1 mmol HBA significantly increased hay dry matter degradability ($P<0.05$) and gas production ($P<0.05$). Addition of 5 and 10 mmol HBA + YS had no significant effect on either parameter. No significant changes in total VFA production or in the molar % of the individual acids were found after the application of either additives (Table 1).

Syringic acid (1 mmol + YS) significantly increased gas production ($P<0.05$) (Table 2). Hay dry matter degradability was not significantly affected and was similar to that observed after 5 or 10 mmol SYA supplement + YS. The addition of SYA without YS had no effect. Yeast culture without SYA significantly increased gas production ($P<0.01$). Total VFA production and the molar % of individual acids were also not significantly affected by any of the additives used. However, a certain tendency was noted to higher values, mainly of total VFAs, which positively correlated with hay degradability and gas production (Table 2).

Table 2
Influence of yeast culture and syringic acid on in vitro rumen fermentation.

	Cellulose	Hay	Hay+YS	Hay + 1SYA	Hay + 5SYA	Hay + 10SYA	Hay+YS + 1SYA	Hay+YS + 5SYA	Hay+YS +10SYA*	SE
Degradability (%)	93.4[c]	64.5	66.0	64.4	63.8	64.0	66.5	66.4	66.7	1.0
Total gas (ml)	61.4[b]	48.1	55.9[b]	49.7	48.9	47.6	53.7[a]	51.9	53.6	1.8
Total VFA (mmol l^{-1})	95.8[b]	77.9	79.8	77.3	76.7	75.2	78.0	76.9	77.0	4.3
Acetate (mol%)	59.7[c]	69.2	68.3	69.4	69.4	69.8	69.1	68.7	69.1	0.5
Propionate (mol%)	31.1[c]	18.6	18.7	18.7	18.3	18.2	18.2	18.2	18.2	0.4
Iso-butyrate (mol%)	0.87	0.97	1.00	0.92	0.99	1.01	0.97	1.06	0.97	0.09
Butyrate (mol%)	6.6[c]	8.9	9.4	8.9	8.9	8.7	9.1	9.3	9.1	0.3
Iso-valerate (mol%)	1.02[a]	1.32	1.51	1.35	1.33	1.30	1.48	1.53	1.46	0.11
Valerate (mol%)	0.71[b]	1.07	1.17	1.05	1.06	1.03	1.15	1.17	1.13	0.06
A/P ratio	1.93[c]	3.72	3.67	3.78	3.81	3.86	3.82	3.78	3.82	0.09

SE, standard error
a, b, c, significantly different from hay in the same row ($P<0.05$, $P<0.01$, $P<0.001$)
YS, yeast culture
SYA, syringic acid
* numbers 1, 5 and 10 mean milimols

Discussion

A combined effect of yeasts and phenolic acids on hay fermentation *in vitro* was not generally observed in our experiments. The yeast additive did not significantly affect the degradability of dry matter even though a tendency to higher values was observed. Similar results were obtained after the addition of yeasts to a diet consisting of 80 % hay and 20 % barley (Zeleňák *et al.* 1994) as well as in the experiments of Martin *et al.* (1989) and Carro *et al.* (1992) with low-concentrate diets. This is in agreement with the findings of Williams (1989) who claimed that yeasts have no effect on the roughage degradation in diets whose composition is optimal for cellulolysis and contains not easily fermentable saccharides.

HBA and SYA addition in amounts of 1, 5 or 10 mmol into the fermentation media had no effect on dry matter degradability. Both acids are formed in association with acid complexes isolated from the cellular walls of roughages (Jung and Fahey 1983) and are cross-bound to some polymers such as lignin-hemicelluloses, glycan-suberin and hemicellulotic chains (Whitmore 1982). They are released from the cellular walls by C1 cellulases (Hartley and Jones 1976) and alkali treatment of straw (Salomonsson *et al.* 1978). Even though it is generally known that phenolic compounds have antimicrobial properties (Davidson and Branen 1981) and inhibit cellulose degradation by ruminal microorganisms (Jung and Fahey 1983, Burrit *et al.* 1984), HBA and SYA belong to those phenolic substances that have no pronounced antimicrobial effect. They are rather known by their positive effect on ruminal microorganisms (Borneman *et al.* 1986).

A combination of yeast and HBA at a concentration of 1 mmol significantly increased dry matter degradability ($P<0.05$), which could be ascribed to the cumulative action of both supplements used. Hay degradability after application of 5 or 10 mmol HBA with YS was insignificantly elevated only to the level achieved with YS alone.

The yeast supplement and 1 mmol HBA or SYA resulted in higher total gas production. However, in the case of syringic acid this increase was lower than that after yeast alone which indicates that this effect can rather be attributed to yeast then to a combination of YS + SA. The combination of YS with 1 mmol of HBA, however, can be considered to be effective. Generally, gas production exhibited higher variability over an insignificant range than did degradability. This can be explained by the fact that gas production is a sensitive parameter of fermentation and is better related to phenolic assays than dry matter degradation (Khazaal *et al.* 1993a, 1993b).

Neither total VFAs nor the molar % of the individual acids were affected by the additives used. A numerical response of VFAs to yeast during *in vitro* fermentation has also been recorded by other authors (Dawson *et al.* 1990, Hession *et al.* 1992). In our previous experiments there were no changes in the concentration of total VFAs using a low-concentrate diet. However, significant changes occurred in the

molar % of individual VFAs, although both the experimental conditions and diet compositions were different. The results showed a slight effect of phenolic acids and yeast culture on hay *in vitro* fermentation.

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