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The effect of pectinolytic yeasts on rumen microflora

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ABSTRACT

The effect of pectinolytic yeast culture Kluyveromyces marxianus CCY 51–1–1 on growth (total anaerobes, cellulolytic, amylolytic and pectinolytic bacteria) and enzyme activities (cellulase, amylase, pectinase and urease) of the rumen microflora were investigated. Significant increase of cellulase and α – amylase activities were found in experiment with 24 hours incubation *in vitro* in rumen liquid from calves or rams. Enhanced cellulase, α – amylase, pectinase activities and a decrease in urease activity were observed after three weeks of addition of yeast cultures to the diet of sheep. No significant effect on the rumen bacterial counts was observed in either type of the experiments. The concentration of yeast supplement was stable for eight hours when cultivated *in vitro* in rumen liquor.

KEY WORDS: pectinolytic yeast, rumen microflora, enzyme activity

INTRODUCTION

Previous studies have suggested that yeast culture supplements can have a significant influence on the performance of ruminants. Benefitial effects of these supplements have been associated with their abilities to alter rumen function (Dawson et al., 1990). Many biotechnological companies (Alltech from USA – Yea Sacc; United Molasses from England – Diamond V; Lallemand from Canada – Biosaf) use preparations of yeast culture Saccharomyces cerevisiae for these purposes. The aim of our experiments was to investigate the effect of another yeast species, the pectinolytic yeast Kluyveromyces marxianus, on the rumen microflora activity.

MATERIAL AND METHODS

Yeast supplement: In both types of experiments was used strain Kluyveromyces marxianus CCY 51–1–1, obtained from Czechoslovak collection of yeasts in

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Bratislava. Yeasts were cultivated in malt extract medium and used in liquid form.

Rumen fermentation cultures: Rumen liquor from three milk fed calves (fed by 10 l milk replacer, 300 g concentrate mixture and hay to appetite) and two rams (fed by the same high fiber diet as sheep in *in vivo* trial) respectively, were withdrawn by stomach tube and mixed 1:2 (one part of rumen liquor and two parts of medium) with medium containing: $NaHCO_3$ 0.98%, $Na_2HPO_4 \cdot 12H_2O$ 0.7%, KCl 0.058%, $CaCl_2$ 0.004%, $MgSO_4 \cdot 7H_20$ 0.012%, yeast extract 0.05%, vitamine solution (B – complex Spofa and Kanavit Biotika, CSFR) 0.1%, mineral solution (Caldwell, Bryant 1966) 0.1%, urea 0.05%, glucose, fructose and sucrose 0.5%, pH 6.5 and replaced into 100 ml flasks with rubber stoppers. All manipulations with rumen contents were made under carbon dioxide. Into six flasks 1 ml of yeast culture (10^5 colony forming units – c.f.u.) were added. All flasks (control group of six flasks was without yeasts) were cultivated 24 hours at 38°C.

In vivo feeding trial: Eight sheep (35–40 kg) were fed (2.5 kg) twice a day by high fiber diet containing hay (85%), ground barley (15%) and mineral supplement. Four of them received yeast culture supplement (10^9 c.f.u. – total dose in 5 ml) twice a day (experimental group) and four sheep were the control group (without yeasts or medium). The samples were collected two times during the third week of experiment, 3 h after feeding.

Microbiological techniques: Total anaerobes were determined on the RGCA medium (Bryant, Burkey 1953); cellulolytic, amylolytic, pectinolytic and xylanolytic bacteria were counted in the RGCA medium modified to contain 0.4% P – cellulose, 1% maize starch, 1% pectin and 0.2% Remazol brilliant blue – xylan (Farkas et al., 1985) respectively, as a sole source of carbon. All media were incubated in 38°C. Live yeasts were enumerated on malt extract agar supplemented with chloramphenicol (100 $\mu g \cdot ml^{-1}$) and penicilin (400 $IU \cdot ml^{-1}$).

Enzyme activities: Endoglucanase activity was measured by method of releasing reducing sugars (Somogyi, 1952) from P – cellulose. Results are expressed in μg glucose$\cdot ml^{-1} \cdot h^{-1}$. Activity of pectinolytic enzymes were determined by viscosimetric measurement (Wojciechowicz, 1971). Results are expressed as per cent decrease of the relative viscosity of the 0.5% pectin solution in samples of rumen fluid before incubation and after 48 h incubation at 38°C. α – amylase activity was estimated by blue starch colour Spofa test. Results are expressed in $nkat \cdot ml^{-1}$. The urease activity was measured with Cook (1976) method.

RESULTS

The survival of live yeast supplement (10^5 yeasts) *in vitro* in rumen calves or rams fermentation cultures was investigated in first series of experiments (Table 1). The concentration of yeasts was stable for up to 6 or 8 hours of cultivation in calf or ram rumen fluid, respectively, while after 24 h cultivation the yeast counts decreased in comparison with that determined immediately after inoculation. The concentrations of live yeast in samples of rumen fluid before inoculation were lower than 1.00 c.f.u. per ml.

TABLE 1

Concentration of live yeast Kluyveromyces marxianus during 24 h cultivation ***in vitro*** in the rumen fluid of calves and rams (means ± SEM of 6 observations).

Time (hour)	Concentration of live yeast in 1 ml (log 10 ± SEM)	
	Calves	Rams
before inoculation	<1.0	<1.0
0	5.34 ± 0.04	5.45 ± 0.02
2	4.43 ± 0.10	5.05 ± 0.02
4	5.18 ± 0.06	5.57 ± 0.04
6	5.26 ± 0.02	5.56 ± 0.03
8	ND	5.42 ± 0.12
24	2.62 ± 0.22^a	4.87 ± 0.02^b

ND – not determined, ^{a,b} – $P < 0.01$

The effect of the live yeast culture supplement on bacterial counts and enzymes activities at 24 h incubation *in vitro* in rumen liquor from rams are shown in Table 2. No significant effect of yeast supplementation on bacterial counts of total anaerobes, that is, pectinolytic or cellulolytic bacteria was observed. However, there was a significant increase of the cellulase ($P < 0.01$) and α – amylase ($P < 0.001$) activity in experimental group.

Similar results were obtained from an analogous experiment with 24 h incubation *in vitro* rumen liquor from milk fed calves (Table 3).

On the basis of those preliminary results the next series of *in vivo* feeding trials were carried out. Total anaerobes, pectinolytic, cellulolytic, amylolytic and xylanolytic bacteria concentrations and cellulase, α – amylase, pectinase and urease activities are shown in the Table 4. Addition of yeast culture to the diet resulted in significant increase of cellulase, amylase and pectinase activities ($P < 0.05$) and decrease of urease activity ($P < 0.01$).

TABLE 2

The effect of pectinolytic yeast Kluyveromyces marxianus on the 24 h fermentation ***in vitro*** in rumen liquid from rams fed a hay–concentrate diet

	Group	
Indices	experimental	control
Bacteria species	Bacterial count (log 10 ± SEM)	
Pectinolytic bacteria	8.12 ± 0.12	8.23 ± 0.23
Cellulolytic bacteria	6.89 ± 0.05	6.88 ± 0.06
Total anaerobes	8.79 ± 0.10	8.73 ± 0.14
Enzymes	Enzyme activity	
Cellulase ($\mu g \cdot ml^{-1} \cdot h^{-1}$)	5.93 ± 0.01^{a}	3.48 ± 0.01^{b}
Amylase ($nkat \cdot ml^{-1} \cdot h^{-1}$)	4.98 ± 0.08^{A}	3.75 ± 0.08^{B}
Urease ($\mu mol \cdot ml^{-1}$)	13.73 ± 1.62	15.41 ± 0.56
Pectinase (%)	58 ± 4	65 ± 4

Means ± SEM of 6 observations, ^{a,b} – $P < 0.01$, ^{A,B} – $P < 0.001$

TABLE 3

The effect of pectinolytic yeast Kluyveromyces marxianus on the 24 h fermentation ***in vitro*** in rumen liquid from calves fed on a hay–concentrate and milk replace diet.

	Group	
Indices	experimental	control
Bacteria species	Bacterial count (log 10 ± SEM)	
Pectinolytic bacteria	8.13 ± 0.09	8.02 ± 0.02
Cellulolytic bacteria	7.20 ± 0.32	7.12 ± 0.35
Total anaerobes	8.51 ± 0.25	8.31 ± 0.31
Enzymes	Enzyme activity	
Cellulase ($\mu g \cdot ml^{-1} \cdot h^{-1}$)	5.52 ± 0.95^{a}	2.16 ± 0.52^{b}
Amylase ($nkat \cdot ml^{-1} \cdot h^{-1}$)	10.08 ± 0.30^{A}	6.76 ± 0.22^{B}
Urease ($\mu mol \cdot ml^{-1}$)	8.06 ± 0.98	10.16 ± 0.44

Means ± SEM of 6 observations, ^{a,b} – $P < 0.05$, ^{A,B} – $P < 0.01$

DISCUSSION

The ability of yeast culture supplements based on Saccharomyces cerevisiae to alter ruminal microbial population has been reported recently. Addition of yeast to diets of non lactating cows tended to increase the number of total anaerobic bacteria and increased number of cellulolytic bacteria (Wiedmeier et

TABLE 4

The effect of pectinolytic yeast Kluyveromyces marxianus after 21 days on the rumen fermentation of sheep given a hay–concentrate diet.

Indices	Group	
	experimental	control
Bacteria species	Bacterial count (log 10 ± SEM)	
Pectinolytic bacteria	7.74 ± 0.19	7.69 ± 0.14
Cellulolytic bacteria	6.62 ± 0.16	6.87 ± 0.17
Amylolytic bacteria	7.40 ± 0.18	7.29 ± 0.18
Xylanolytic bacteria	6.72 ± 0.12	6.50 ± 0.09
Total anaerobes	8.25 ± 0.13	7.85 ± 0.19
Enzymes	Enzyme activity	
Cellulase ($\mu g \cdot ml^{-1} \cdot h^{-1}$)	8.64 ± 0.22^a	7.61 ± 0.24^b
Amylase ($nkat \cdot ml^{-1} \cdot h^{-1}$)	4.95 ± 0.15^a	4.39 ± 0.17^b
Urease ($\mu mol \cdot ml^{-1}$)	49.41 ± 3.6^A	72.2 ± 6.4^B
Pectinase (%)	65 ± 6	49 ± 3

Means ± SEM of 6 observations, ^{a,b} – $P < 0.05$, ^{A,B} – $P < 0.01$

al., 1987). The similar results have been described by Harrison et al. (1988) with cows which were fed a diet of 40% corn silage and 60% concentrate. The concentration of anaerobic bacteria tended to be higher while cellulolytic bacteria concentration was significantly greater in cows fed S. cerevisiae than in cows receiving control diet. However, the increase in the number of cellulose degraders in the rumen did not effect the level of fibre digestion.

The results reported in this paper did not confirm with the mode of action of S. cerevisiae (Wallace, 1992), for there were no increases in the counts of viable rumen bacteria in the rumen liquor of any experimental groups *(in vitro* or *in vivo)*. There were however significantly higher enzyme activities (with the exception of urease which was reduced), in all the experimental groups in both types of our experiments *(in vitro* and *in vivo)*.

The concentrations of live yeast cells in the rumen have been reported to be low, but they were dependent on the type of diet consumed by the animal (Lund, 1974). Similary, the strain of S. cerevisiae used has also been shown to be important (Newbold and Wallace, 1992). The pectinolytic yeast K. marxianus used by ourselves did not growth extensively in the rumen, but after eight hours cultivation in the rumen fluid its concentration was maintained at approximately the same level as immediately after supplementation. Dawson et al. (1990) also have reported similar behaviour of S. cerevisiae yeast in the rumen of steers and in continuous cultures. Live yeast cultures present in the rumen may influence fermentation processes their own metabolic activities.

CONCLUSIONS

Supplementation of a pectinolytic yeast culture to diets of calves and sheep changes the enzyme activity (cellulase and α – amylase or urease and pectinase) of rumen microflora. No effect was found on rumen bacterial counts. These results suggest that live yeast culture supplement stimulate rumen microbial activities.

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STRESZCZENIE

Wpływ drożdży pektynolitycznych na mikroflorę żwacza

W doświadczeniu *in vitro* i *in vivo* badano wpływ kultury drożdży Kluyveromyces marxianus CCY 51-1-1 na wzrost beztlenowców bakterii celulolitycznych, amylolitycznych i pektynolitycznych oraz na aktywność enzymatyczną (celulazy, amylazy, pektynazy i ureazy) mikroflory żwacza. Stwierdzono istotne zwiększenie aktywności celulazy i α-amylazy w doświadczeniu *in vitro*, w którym przez 24 godz. płynną treść żwacza cieląt i owiec inkubowano z dodatkiem drożdży. Zwiększenie aktywności celulazy, α-amylazy oraz pektynazy, a obniżenie aktywności ureazy wykazano w 3 tygodnie po dodaniu kultur drożdży do dawki dla owiec. Nie stwierdzono wpływu dodatku drożdży na liczebność bakterii żwacza, a zawartość drożdży w ciągu 8 godz. podczas inkubacji płynnej treści żwacza *in vitro* była stała.