IN VITRO EFFECTS OF BACITRACIN AND MONENSIN ON OVINE RUMEN FERMENTATION*

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The effect of bacitracin on production of volatile fatty acids (VFA) and gases was investigated in *in vitro* incubations of the rumen fluid of a sheep and compared with that of monensin. Starch and xylan were used as substrates. In cultures with starch both antibiotics at 8 mg/l significantly increased production and molar proportion of propionate and decreased molar proportion and production of butyrate and methane. High amount of hydrogen gas accumulated in bacitracin-treated cultures. In cultures with xylan, both antibiotics significantly decreased production of methane. Again, bacitracin increased production of hydrogen. Both antibiotics increased non-significantly proportion of propionate at the expense of acetate and butyrate. In general, effects of both additives were more apparent on starch than on xylan. Fermentation shifts in cultures with bacitracin were less pronounced than those in cultures with monensin. It can be concluded that bacitracin is less efficient rumen modifier than monensin.

rumen fermentation; bacitracin; monensin

INTRODUCTION

Bacitracin is a polypeptide antibiotic produced by *Bacillus licheniformis* and *Bacillus subtilis*. It consists of several compounds active against cell wall biosynthesis in many grampositive bacteria (Glasby, 1979). In the USA, bacitracin is one of five antibiotics approved for the prevention of liver abscesses in feedlot cattle (Nagaraja, Chengappa, 1998). Ruminal effects of bacitracin were investigated by several authors. Russel and Strobel (1988) and Van Nevel and Demeyer (1992) showed in *in vitro* experiments that bacitracin increased production of propionate and decreased that of methane. Acetate and butyrate production was less influenced. Also Spires and Froetschel (1992) concluded that bacitracin was a propionate enhancer. Moreover, authors showed that bacitracin completely inhibited the lactate production. Rumen bacteria particularly sensitive to bacitracin are *Butyrivibrio fibrisolvens, Eubacterium limosum* and *Ruminococcus flavefaciens* (Wang et al., 1969). In a feeding trial, bacitracin increased weight gains of feedlot steers by 4.1% and decreased feed/gain ratio by 4.7% (Spires, Olmsted, 1992). According to Spires and Olmsted (1992), bacitracin was less efficient performance enhancer than laidlomycin, a semisynthetic ionophore compound. It can be assumed that bacitracin, thank to its high molecular mass (1488), is not absorbed from the digestive tract. Thus, bacitracin cannot influence the intermediary metabolism of animals and does not leave detectable residues in tissuess (Nagaraja, 1995).

Monensin is an ionophore antibiotic produced by *Streptomyces cinnamonensis*. It inhibits growth of rumen grampositive bacteria and "gramnegative" bacteria with a grampositive structure of the cell wall (e.g. *Butyrivibrio fibrisolvens*). Since the mid-seventieth, monensin has been successfully used as a performance enhancer in cattle. In the EU, monensin is an approved growth promotant for young cattle up to the age of six months. Its ruminal effects include the increase of propionate production with a corresponding decrease in acetate, butyrate and methane production (reviewed by Schelling, 1984). Contrary to bacitracin, monensin encounters little difficulty in crossing ruminant gut (molecular mass of 671). Consequently, mixing errors and misuse situations in the field may cause intoxication of cattle by monensin (Potter et al., 1984).

Bacitracin and monensin differ in the mode of action. Bacitracin inhibits the synthesis of peptidoglycan in grampositive bacteria whereas monensin interferes with the ions flux through the cell membrane (Henning, 1982). The purpose of our study was to compare effects of bacitracin and monensin on production of fermentation end-products in *in vitro* incubations of the rumen fluid of a sheep.Two different substrates were used: starch and xylan.

MATERIALS AND METHODS

An adult rumen-canulated sheep (*ca* 80 kg body weight) was fed hay (300 g) and commercial concentrate (300 g) at 9.00 h and 16.00 h. The composition of feeds was reported earlier (Mbanzamihigo et al., 1996). Rumen contents were sampled 2 h after the morning feeding and filtered through a metal sieve (pore size

* The authors are grateful to the European Training Foundation for an individual mobility grant to Dr. Milan Marounek.

I. Effects of bacitracin and monensin on production of volatile fatty acids (VFA) and gases from starch and xylan in *in vitro* incubations of the rumen fluid of a sheep

Metabolite		Treatment		
		control	bacitracin	monensin
a) Starch-supplied cultures				
Total VFA	(mmol.l^{-1})	41.2 ± 3.2	33.3 ± 4.7	43.7 ± 6.2
Acetate	(mol.%)	54.7 ± 7.2	57.7 ± 3.3	45.3 ± 3.3
Propionate	(mol.%)	15.4 ± 2.0^{a}	38.0 ± 3.2^{b}	49.9 ± 3.7^{b}
Butyrate	(mol.%)	26.8 ± 8.4^{a}	4.3 ± 0.6^{b}	3.9 ± 1.0^{b}
Valerate	(mol.%)	0.8 ± 0.5	0	0.9 ± 0.3
Isoacids	(mol.%)	2.3 ± 0.9^{a}	0^{b}	0^{b}
Hydrogen	(mmol.l^{-1})	0.025 ± 0.011^{a}	3.32 ± 1.28^{b}	0.026 ± 0.006^{a}
Methane	(mmol.l^{-1})	17.8 ± 1.3^{a}	6.0 ± 0.7^{b}	4.5 ± 0.3^{b}
b) Xylan-supplied cultures				
Total VFA	(mmol.l^{-1})	58.5 ± 1.4	51.3 ± 3.2	55.1 ± 4.1
Acetate	(mol.%)	65.2 ± 2.9	61.8 ± 2.4	55.4 ± 2.8
Propionate	(mol.%)	27.2 ± 2.0	30.8 ± 2.8	40.1 ± 4.2
Butyrate	(mol.%)	6.4 ± 1.9	5.7 ± 2.1	3.2 ± 1.4
Valerate	(mol.%)	0.8 ± 0.7	0.8 ± 0.7	1.0 ± 0.7
Isoacids	(mol.%)	0.4 ± 0.3	0.9 ± 0.7	0.3 ± 0.2
Hydrogen	(mmol.l^{-1})	0.004 ± 0.002^{a}	0.084 ± 0.022^{b}	0.004 ± 0.002^{a}
Methane	(mmol.l^{-1})	10.8 ± 0.2^{a}	7.4 ± 0.7^{b}	1.8 ± 0.7^{c}

Average values of five experiments ± SEM
^{abc} Values in the same row with different superscripts differ significantly ($P < 0.05$)

1–1.5 mm) under CO_2 atmosphere. Rumen fluid was diluted five-fold with a buffer solution (Burroughs et al., 1950) at pH 7.2. The buffer contained NH_4HCO_3 (1.41 g/l) as N source. Monensin (Elanco Animal Health, Brussels) or bacitracin (US Biochemical Corp., Cleveland) were dissolved in ethanol or 50% ethanol acidified with 6M HCl (10 µl/ml), respectively. The additives (0.4 mg) were placed in the empty gas-tight incubation flasks. The solvent (0.2 ml) was evaporated under stream of CO_2, then diluted rumen fluid (50 ml) was added. The final concentration of the additives was 0 or 8 ppm. Flasks were filled with CO_2 and incubated in a shaking waterbath at 39 °C for 22 h. The incubations were done in duplicate. Substrate was 0.5 g of wheat starch (Sigma) or 0.5 g of oat-spelt xylan (Fluka). Blank incubations were carried out by incubating rumen fluid without substrate and with or without antibiotics. At the end of the incubation, 2 ml of the fermentation gas were sampled with a gas-tight syringe and analysed for methane and hydrogen by adsorption gas chromatography (Marty, Demeyer, 1973). Incubation was then stopped by adding 1 ml of 5 M H_2SO_4, incubation fluid centrifuged and filtered. Total VFA in filtrate were estimated by titration after steam distillation (Friedemann, Brook, 1938). Their molar composition was determined by gas liquid chromatography (Ottenstein, Bartley, 1971). In total, five identical incubations were carried out.

One-way analyses of variance and Bonferroni test, where appropriate, were performed to evaluate effects of antibiotics on *in vitro* rumen fermentation. To compare starch and xylan fermentation in control cultures, Students *t*-test was used. The statistical significance was declared at $P < 0.05$.

RESULTS

Table I presents data on the effects of antibiotics on production of VFA and gases from starch and xylan in *in vitro* rumen incubations. The values were corrected for metabolites production in corresponding incubated blanks. In starch-supplied cultures of the rumen fluid monensin non-significantly decreased total VFA production. Bacitracin and monensin significantly increased production and molar proportion of propionate and decreased molar proportion and production of butyrate and methane. High amount of molecular hydrogen accumulated in cultures with bacitracin. Both additives inhibited production of isoacids. Molar proportion of acetate was not significantly affected.

In cultures with xylan, bacitracin significantly decreased production of methane and increased production of hydrogen. Other fermentation shifts were not significant. Monensin significantly decreased methane production and non-significantly increased production and mo-

lar proportion of propionate at the expense of acetate and butyrate.

DISCUSSION

Although literature on effects of antimicrobials in the rumen is extensive, comparative studies are less frequent. Previous works compared bacitracin and monensin using corn meal or timothy hay (Russell, Strobel, 1988), and detergent-washed hay or soluble starch (Van Nevel, Demeyer, 1992) as substrates. Neither hemicelluloses nor other plant cell wall components were tested in this respect. Russell and Strobel (1988) concluded that bacitracin and monensin produced effects on fermentation that were strikingly similar and suggested that any grampositive antibiotic may produce fermentation effects similar to those of monensin. Both Russell and Strobel (1988) and Van Nevel and Demeyer (1992) found that bacitracin was less inhibitory to fibre digestion than monensin. In this study, effects of bacitracin on propionate and methane production were less pronounced than those of monensin. Effects of both additives were more apparent on starch than on xylan. Bacitracin strongly increased production of molecular hydrogen in cultures with starch (133 times more than in controls), and, to a lesser extent, also in cultures with xylan. On the contrary, the hydrogen accumulation in control and monensin-treated cultures was negligible. The accumulation of hydrogen gas in cultures with bacitracin indicates insufficient hydrogen uptake in alternative hydrogen-utilizing reactions. Fermentation of xylan in control cultures produced significantly more acetate and propionate and less butyrate and methane than fermentation of starch. Monensin had no apparent effect on total VFA production in xylan-supplied cultures, in spite of the fact that xylan is an important component of fibre. This is in agreement with a previous finding that monensin at 10 mg/l significantly decreased total VFA production from cellulose by 42–46%, but had no influence on VFA production from wheat hemicellulose (Šimůnek et al., 1989).

It can be concluded that fermentation shifts in cultures supplied with bacitracin were less pronounced than those in cultures with monensin. Contrary to monensin, in bacitracin-treated cultures with starch a significant part of metabolic hydrogen was lost as hydrogen gas, instead of used in propionate production. Future experiment should find out if the hydrogen accumulation in treated cultures occur also with other non-ionophore antibiotics.

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Received for publication on April 26, 2001
Accepted for publication on November 5, 2001

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Účinky bacitracinu a monensinu na bachorovou fermentaci ovce *in vitro*.

Scientia Agric. Bohem., *33*, 2002: 59–62.

Bacitracin a monensin jsou antimikrobiální látky s různým mechanismem účinku, které mění stechiometrii bachorové fermentace. Předností bacitracinu je to, že z trávicího traktu není vstřebáván. Nezanechává tudíž rezidua ve tkáních a ani nehrozí otrava při předávkování. Srovnávali jsme účinky bacitracinu a monensinu při inkubacích bachorové tekutiny ovce se škrobem a xylanem. V kulturách se škrobem obě antibiotika významně zvýšila produkci i molární zastoupení propionátu za současného snížení produkce butyrátu a metanu. Bacitracin mnohonásobně zvýšil produkci plynného vodíku, která byla v ostatních kulturách velmi malá. V kulturách s xylanem obě antibiotika významně snížila produkci metanu. I zde bacitracin zvýšil produkci plynného vodíku. V těchto kulturách obě aditiva nevýznamně zvýšila molární podíl propionátu na úkor acetátu a butyrátu. Účinek monensinu i bacitracinu byl výraznější při růstu mikroorganismů na škrobu než na xylanu. Vliv aditiv na celkovou produkci těkavých mastných kyselin byl malý (statisticky nevýznamný), nezávisle na substrátu. Změny fermentační stechiometrie v kulturách s monensinem byly větší než v kulturách s bacitracinem. Bacitracin se tudíž monensinu v řadě ohledů podobá, jeho účinek je však slabší.

bachorová fermentace; bacitracin; monensin

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