Adaptation effects of ionophores on rumen fermentation

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The effect of monensin on the *in vitro* degradation of cellulose was investigated using ruminal fluid obtained from sheep adapted and unadapted to the antibiotic. *In vivo* ruminal adaptation to monensin was also examined over a six-week treatment period. Results showed that monensin severely inhibited total VFA production when cellulose was offered as substrate to unadapted ruminal organisms. There was only a small decrease when the rumen inoculum was obtained from sheep fed monensin for 32 days. Addition of monensin to the diet resulted in higher propionate and lower butyrate concentrations when compared with values obtained from sheep fed diets without monensin. The addition of monensin decreased dilution rate and increased rumen volume.

Die uitwerking van monensin op die *in vitro* afbraak van sellulose is ondersoek deur gebruik te maak van rumenvloeistof wat verkry is van skape wat aangepas en nie aangepas is nie. *In vivo* grootpensaanpassing by monensin is ook ondersoek tydens 'n ses-weke behandelingstydperk. Resultate het getoon dat monensin die totale produksie van vlugtige vetsure ernstig onderdruk het wanneer sellulose as substraat by onaangepaste grootpensorganismes gebruik is. Daar was slegs 'n klein verlaging wanneer entmateriaal van die grootpens van skape verkry is wat 32 dae monensin gevoer is. Byvoeging van monensin by die rantsoen het hoër propionaat en laer butiraatkonsentrasies tot gevolg, in vergelyking met waardes verkry van skape wat nie monensin gevoer is nie. Byvoeging van monensin verlaag verdunningstempo en verhoog grootpens volume.

Keywords: Monensin, rumen fermentation, volatile fatty acids, cellulose degradation, adaptation

Introduction

Ionophore antibiotics such as monensin are widely used as feed additives for ruminants and increase feed efficiency by influencing ruminal fermentation. However, knowledge of their mode of action still has many gaps. One question that has not been resolved is the adaptation of rumen microbes to the antibiotics. Monensin has been shown to inhibit *in vitro* cellulose digestion when the inoculum was obtained from animals unadapted to the antibiotic (Simpson, 1978; Henderson, Stewart & Nekrep, 1981). Furthermore, results obtained with unadapted organisms may not reflect *in vivo* effects. The aim of the present study was to investigate the effect of monensin on the *in vitro* degradation of cellulose using ruminal fluid obtained from sheep adapted and unadapted to the antibiotic. Since most *in vivo* rumen fermentation studies are of short duration, only 14 – 21 days (Dinius, Simpson & Marsh, 1976; Lemenager, Owens, Shockey, Lusby & Totusek, 1978), adaptation was investigated over a six-week treatment period.

Methods

Four rumen-fistulated sheep, which had never been previously exposed to dietary antibiotics, were fed 600 g hay and 400 g coarsely ground concentrate per day. Hay was given at 08h30 and 16h15, and the concentrate was delivered by an automatic feeder every 2 hours. Prior to the experiment this ration was fed for four weeks. The experimental period of 11 consecutive weeks consisted of four periods: Period I (three weeks), no addition of monensin; period II (two weeks), addition of monensin at a low level (20 mg/day); period III (four weeks), addition of monensin at a high level (40 mg/day) and period IV (two weeks), no addition of monensin. Monensin sodium (Rumensin^R) was mixed to concentrates which would achieve low and high levels of intake. Each experimental week consisted of five consecutive days. On days two and five, rumen fluid samples were collected through the fistula at 08h00, 10h00 and 12h00 and analysed for pH, buffer capacity, volatile fatty acids (VFA), L-lactic acid, D-lactic acid (08h00 samples only) and ammonia. The determination of VFA was performed by gas chromatography using Chromosorb 101 as column packing whereas D- and L-lactic acid and ammonia were determined enzymatically. Unfortunately, samples from one day of period I were lost. On day three of week one, three, six and seven, the rumen volume and dilution rate of the liquid phase were determined by the injection of 150 ml Cr-EDTA solution containing 2,80 g Cr/l.

Two trials were conducted to assess the influence of monensin on cellulose degradation by determining the VFA and NH_3 concentrations in batch culture fermentation flasks. Each flask contained 15 ml of rumen inoculum, 40 ml of a phosphate-bicarbonate buffer and 350 g of a cellulose supplement with 15 mg of urea. Rumen inoculum was taken from each sheep at 08h00 in weeks three (period I) and eight (period III). In each trial four fermentations were performed per animal; two control flasks without additional monensin and two flasks each with 100 μg of monensin added. After 24 h of fermentation, microbial activity was stopped by adding 0,15 ml H_3PO_4 (20% w/v solution) to a 1,0 ml sample of the incubation medium. The samples were centrifuged at 11 000 *g* for 15 min and the supernatants were analysed for VFA and NH_3. The production of metabolites was calculated as the difference between quantities present before and after fermentation.

The significance of differences was determined by Student's paired *t* test (one-sided) unless otherwise stated.

Results and Discussion

Effect of monensin on VFA, lactate and NH_3 concentration *in vivo*

The data of the three samplings on each sampling day were averaged and this pooled value was used to calculate a mean value per period and per animal. Addition of monensin to the diet resulted in higher ($P < 0,05$) propionate concentra-

Table 1 Effect of monensin on ruminal fermentation. Data from the 08h00, 10h00 and 12h00 samplings are pooled. Mean values ± SEM (n = 4) are presented.

	Period			
Concentration[a]	I control	II 20 ppm monensin	III 40 ppm monensin	IV control
Total VFA	67,9 ± 4,1	71,1 ± 3,2 [c]	70,9 ± 2,7 [c]	78,0 ± 5,0
Acetate	40,1 ± 2,3	42,6 ± 1,7 [c]	42,7 ± 1,6 [c]	51,3 ± 3,4
Propionate	15,4 ± 1,4	18,4 ± 1,1 [bc]	18,7 ± 0,8 [bc]	14,3 ± 0,7
n-Butyrate	9,3 ± 0,4	6,8 ± 0,5 [bc]	6,6 ± 0,2 [bc]	9,3 ± 0,9
Acetate/ propionate	2,70 ± 0,08	2,34 ± 0,09[bc]	2,31 ± 0,04[bc]	3,62 ± 0,27
L-lactate	0,09 ± 0,01	0,06 ± 0,01[b]	0,13 ± 0,02[bc]	0,06 ± 0,01
Ammonia	10,67 ± 0,09	10,18 ± 0,30[c]	13,02 ± 0,24[b]	13,65 ± 0,35
pH	6,51 ± 0,02	6,50 ± 0,02	6,53 ± 0,02	6,45 ± 0,02
Buffer capacity	10,00 ± 0,28	9,70 ± 0,24	9,83 ± 0,23	9,58 ± 0,27

[a] Concentrations are expressed as mmol per litre; buffer capacity is expressed as the number of mmol H^+ required to cause a given drop in pH of 100 ml rumen fluid.

[b]Significantly different from control period I

[c]Significantly different from control period IV

tions and lower ($P<0,01$) n-butyrate concentrations (Table 1). Both acetate and total VFA levels of periods II and III were unaffected when compared with period I but, in contrast, were depressed $P<0,01$ and ($P<0,025$, respectively) when compared with period IV. Monensin did not affect n-valerate and branched-chain VFA levels (data not shown). The monensin-induced depression of the n-butyrate levels noted in our experiment agrees with others although most *in vivo* experiments have shown marked depressions in acetate levels along with increases in propionate owing to monensin (Dinius *et al.*, 1976; Poos, Hanson & Klopfenstein, 1979). The lack of a control group of animals in the present study could possibly account for this. Monensin decreased the dilution rate from 7,9 to 6,2 %/h ($P<0,025$) whereas the rumen volume was increased from 6,3 to 7,5 l ($P<0,05$) (Table 1). Similar results were obtained by Lemenager *et al.*, (1978). Values for L-lactate and ammonia did not change consistently and no significant changes were observed for the pH and buffer capacity (Table 1).

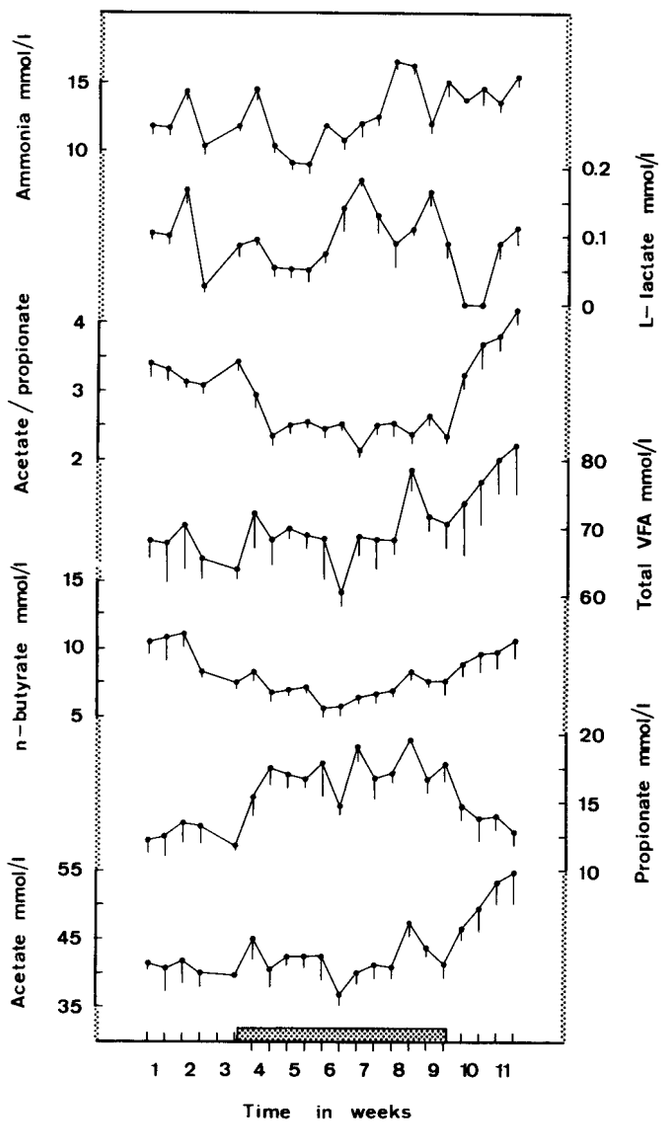

Figure 1 Effect of monensin on ruminal fermentation at the 08h00 sampling. The stippled block on the time axis indicates the period when monensin was mixed with the feed. Vertical bars represent SEM (n = 4).

The influence of monensin was most pronounced at 08h00 before the hay was fed. At this time the rumen fluid was characterized by a high pH and a high proportion of acetate when compared with values 2 and 4 hours later. Values for the 08h00 samples are shown in Figure 1. No clear indications of adaptation to the antibiotic were noted and no differences in efficacy between the doses of monensin were observed. However, addition to and removal of monensin from the feed elicited sudden changes, as seen from the acetate to propionate ration. The levels of L-lactate were extremely low after the removal of monensin from the feed, both at 08h00 (Figure 1) and at 10h00 and 12h00 (data not shown). However, as the L-lactate levels fluctuated greatly during the experimental period, more work should be done to confirm the decrease in L-lactate after the removal of monensin from the feed. Surprisingly D-lactate levels were not affected.

Effect of monensin on *in vitro* cellulose degradation

Monensin severely inhibited the production of total VFA ($P<0,001$), and especially that of propionate ($P<0,001$) when cellulose was offered as substrate to non-adapted ruminal organisms (Table 2). However, fermentations using rumen inoculum from sheep fed monensin for 32 days elicited a small, albeit significant ($P<0,025$), decrease in the total VFA production. It should be noted that in the absence of added monensin VFA production increased (total VFA: $P<0,05$ and propionate: $P<0,025$; Student's t test; two-sided) in period III (7,4 mmol) in comparison with period I (6,3 mmol). In the trial conducted with unadapted bacteria (period I) the NH_3-level increased slightly ($P<0,05$) owing to the presence of monensin which may suggest an

Table 2 Effect of monensin on breakdown of cellulose *in vitro*. Mean values of the production of VFA and ammonia ± SEM (n = 4) are presented.

Source and treatment of ruminal fluid[a]		Production (mmol)				
		Acetate	Propionate	n-butyrate	Total VFA	Ammonia
Unadapted	+	3,3 ± 0,2	2,0 ± 0,1	0,6 ± 0,1	6,3 ± 0,5	1,4 ± 0,1
(Period I)	−	1,7 ± 0,1[b]	0,6 ± 0,1[b]	0,3 ± 0,1[b]	2,7 ± 0,3[b]	1,6 ± 0,1[b]
Adapted	+	4,1 ± 0,4	2,8 ± 0,2	0,4 ± 0,1	7,4 ± 0,7	0,9 ± 0,1
(Period III)	−	3,2 ± 0,4[b]	2,7 ± 0,5	0,3 ± 0,1[b]	6,3 ± 1,0[b]	0,8 ± 0,1

[a] − , no addition of monensin to the fermentations; + , addition of 100 μg monensin per fermentation flask
[b] Significantly different from monensin-treated fermentation of the same trial

inhibition of the microbial protein synthesis. In contrast, in fermentations carried out with adapted bacteria, NH_3-levels were much lower ($P < 0,025$; Student's t test; two-sided), irrespective of the absence (0,9 mmol) or presence (0,8 mmol) of additional monensin. This agrees with findings of other workers that monensin does not inhibit protein synthesis of adapted bacteria. (Herod, Bartley, Davidovich, Bechtle, Sapienza & Brent, 1979).

Since ionophores have been shown to inhibit the growth of important cellulolytic bacteria (Henderson *et al.*, 1981), a reduction of fibre digestibility would be expected. *In vitro* work with non-adapted bacteria showed monensin to be indeed, a potent inhibitor of cellulolytic activity (Simpson, 1978). However, our *in vitro* data using monensin-adapted inocula demonstrated that cellulose degradation is barely affected by monensin, whereas using unadapted bacteria, cellulose degradation was severely inhibited. These findings are consistent with the results of Poos *et al.*, (1979) who observed a decreased *in vivo* fibre digestibility after feeding monensin for two weeks but not after about seven weeks, in spite of enhanced propionate production throughout. Similarly, on a forage diet, monensin did not affect fibre digestibility in animals that received monensin for 21 days (Dinius *et al.*, (1976). The *in vivo* cellulose disappearance in monensin-adapted steers determined after nine and 13 days did not alter (Lemenager *et al.*, 1978).

In conclusion, the degradation of cellulose *in vitro* by bacteria unadapted to monensin, does not reflect the long-term effects of monensin *in vivo*. Thus, to conduct studies *in vitro* on the influence of ionophores on cellulose degradation and on other microbial processes (Herod *et al.*, 1979), micro-organisms should be obtained from ruminants that are adapted to the feed additives.

References

DINIUS, D.A., SIMPSON, M.E. & MARSH, P.B., 1976. Effect of monensin fed with forage on digestion and the ruminal ecosystem of steers. *J. Anim. Sci.* 42, 229.

HENDERSON, C., STEWART, C.S. & NEKREP, F.V., 1981. The effect of monensin on pure and mixed cultures of rumen bacteria. *J. Appl. Bact.* 51, 159.

HEROD, E.L., BARTLEY, E.E., DAVIDOVICH, A., BECHTLE, R.M., SAPIENZA, D.A. & BRENT, B.E., 1979. Effect of adaptation to monensin and lasalocid on rumen fermentation *in vitro* and the effect of these drugs on heifer growth and feed efficiency. *J. Anim. Sci.*, 49 (Suppl. 1), 374.

LEMENAGER, R.P., OWENS, F.N., SHOCKEY, B.J., LUSBY, K.S. & TOTUSEK, R., 1978. Monensin effects on rumen turnover rate, twenty-four hour VFA pattern, nitrogen components and cellulose disappearance. *J. Anim. Sci.* 47, 255.

POOS, M.I., HANSON, T.L. & KLOPFENSTEIN, T.J., 1979. Monensin effects on diet digestibility, ruminal protein, bypass and microbial protein synthesis. *J. Anim. Sci.* 48, 1516.

SIMPSON, M.E., 1978. Effects of certain antibiotics on *in vitro* cellulose digestibility and volatile fatty acid production by ruminal micro-organisms. *J. Anim. Sci* 47 (Suppl. 1), 429.