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EFFECT OF TYLOSIN ON RUMEN FERMENTATION IN VITRO

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Abstract

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The effects of tylosin on production of end-products of carbohydrate metabolism, utilization of lactic acid and degradation of protein were investigated in *in vitro* experiments with rumen inocula. Tylosin non-specifically inhibited the rumen fermentation with inocula from non-adapted cows, but most of its effects disappeared when inocula wera taken from adapted wethers. Production of lactic acid was severely depressed by tylosin, no matter whether inocula originated from non-adapted or adapted animals. Some other tylosin-induced metabolic effects differed from those of ionophore compounds, in spite of the fact, that both tylosin and ionophores are potent gram-positive antibiotics.

Cattle, wethers, rumen fluid, incubation

Tylosin is a macrolide antibiotic produced by *Streptomyces fradiae* that is a potent inhibitor of the proteosynthesis in many gram-positive bacteria. Several researchers have demonstrated that continuous low-level feeding of tylosin reduced the incidence and severity of liver abscesses, increased average daily gains and improved feed conversion of feedlot cattle (Brown et al. 1973; Heinemann et al. 1978; Potter et al. 1985). The liver-protecting effect tylosin is based on its inhibitory influence on *Fusobacterium necrophorus* (previously recognized as *Sphaerophorus necrophorus*), a bacterium that was isolated from 95% of the abscess specimens in experiments of Brown et al. (1973). The combination of monensin and tylosin has been cleared for commercial use as the feed additive in the cattle industry in the USA nowadays and similar ionophore /tylosin combinations are being tested (Gill and Owens 1984; Strasia and Jordan 1985).

Although the addition of tylosin to ruminant diets seems to be promising, little information is currently available on its effect on the rumen fermentation. Purser et al. (1965) found that volatile fatty acid (VFA) distribution was modified towards more butyrate and less propionate in tylosin-fed wethers. Rumen protozoal numbers were increased following antibiotic supplementation while no differences were observed in the total viable bacteria counts. Similar effects of tylosin on the molar composition of VFA were found by O' Connor et al. (1970) *in vitro*. Nagaraja and Taylor (1987) mentioned briefly that tylosin increased acetate and decreased propionate in their batch culture fermentations. The susceptibility and resistance of pure cultures of rumen bacteria to tylosin have been reported in several studies (El Akkad and Hobson 1966; Wang et al. 1969; Nagaraja and Taylor 1987; Marounek et al. 1988). Tylosin inhibited the growth of rumen gram-positive bacteria and those bacteria which often stain gram-negatively, but possess cell walls with gram-positive ultrastructure, such as *Butyrivibrio fibrisolvens* and the ruminococci.

The purpose of this work was to define better effects of tylosin on rumen fermentation *in vitro*. We aslo watend to test the hypothesis of Rusell and Strobel (1988) who suggested that any gram-positive antibiotic can produce fermentation shifts, similar to those of monensin. Their experiments showed that bacitracin, a polypeptide gram-positive antibiotic, produced fermenta-

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tion effects that resembled very much alternations in rumen stoichiometry observed with ionophores. Our in vitro experiments were performed using rumen fluid from both non-adapted and adapted animals.

Material and Methods

a) General remarks

Samples of rumen contents were taken from rumen fistulated animals (two cows or two wethers), two hours after the morning feed. The animals were fed twice a day. The cows weighing about 550 kg were fed with 2 kg of concentrate, 6 kg of meadow hay and 10 kg of maize silage per day. The wethers, weighing 50 kg, were fed with 0.8 kg of meadow hay and 0.4 kg of a commercial concentrate mixture, supplemented with 200 mg of tylosin (Elanco) per day for two months. Rumen fluid was prepared from whole rumen contents by straining through two layers of chese cloth. Its average pH was 6.70 and 6.67 for cows and wethers, resp. The *in vitro* incubations were carried out as deseribed previously (M a r o u n e k et al. 1983). Tylosin was added at 0-2-5-10 and 20 mg/l, final concentration. Each experimental or control arrangement was incubated in four replicates.

Six experiments were performed with the rumen fluid from non-adapted cows (No. 1–6) and eight experiments with the rumen fluid adapted wethers (No. I–VIII). Two experiments (No. 5 and No. V) were performed at low pH values, all others in the physiological pH interval (pH 6–7).

b) Incubations

E x p e r i m e n t 1. Glucose (1.5 g) was dissolved in 100 ml of Mc Dougall buffer containing sources of nitrogen, vitamins and microelements, then 50 ml of rumen fluid were added. The cultures were incubated anaerobically at 39 °C for 6 hours.

E x p e r i m e n t 2. Maize starch (3 g) was suspended in 100 ml of buffer and the 50 ml of rumen fluid were added. The cultures were incubated 8 hours.

E x p e r i m e n t 3. Whatman filter paper No. 1 was ground and added (3 g) to 100 ml of buffer and 50 ml of rumen fluid. The incubation time was 20 hours.

E x p e r i m e n t 4. Citrus pectin (Genu Pektin from the Pectin Factory Ltd., Copenhagen, commercial quality) was dissolved in buffer (2 g/100 ml) and 50 ml of rumen fluid were added. The cultures were incubated 7 hours.

E x p e r i m e n t 5. In this experiment 1.5 g of sucrose and 3 g of soluble starch were added to 100 ml of buffer and 50 ml of rumen fluid. The pH was kept between 5 and 6, to achieve a high production of lactic acid. The incubation time was 7 hours.

E x p e r i m e n t 6. Sodium lactate was dissolved in buffer (50 mmol/l), then rumen fluid was added (100 ml + 50 ml). The incubation time was 7 hours.

E x p e r i m e n t s 1–6 were perfomed using the same substrate and incubation conditions, except that adapted wethers instead of non-adapted cows served as donors of inocula and the lowest concentration of tylosin (2 mg/l) was omitted.

E x p e r i m e n t VII. In this experiment we estimated the effect of tylosin on production of VFA and methane from the mixture of sodium carboxymethylcellulose (Koch Light Laboratories, Ltd, UK), maize starch and glucose in ratio 9:9:2. The mixed substrate (27 g) was added to 600 ml of buffer and 300 ml of rumen fluid. Tylosin was added at 10 mg/l, final concentration into one incubation vessel, whereas control received no tylosin. Fermentation gas was collected in calibrated glass cylinders, equipped with three-ways valves. The incubation was completed after 11 hours.

E x p e r i m e n t VIII. Casein from Fluka (1.5 g) and glucose (1.5 g) were added to 100 ml of buffer and 50 ml of rumen fluid. The cultures were incubated 6 hours.

c) Analyses

Total VFA were estimated by titration after steam distillation. Their molar composition was determined by gas liquid chromatography using a column of the Inerton AW (0.25–0.32 mm) with 10 % Reoplex 100 (Lachema, Brno, Czechoslovakia); lengt 1.8 m. A Chrom 4 gas chromatograph (Laboratory Instruments, Prague) equipped with a FI detector was used. The separation was carried out at 125 °C and an inlet pressure of 150 kPa. The same column and de-

tector were used for the determination methane at room temperature and at an inlet pressure of 40 kPa. Lactate was oxidized to acetaldehyde and measured in microdiffusion chambers (Conway 1957). Protein was measured according to Lowry (1951).

Results

Effects of tylosin on production of VFA and lactate from carbohydrates are shown in Figs. 1–4. Tylosin had little effect on production of butyrate in experiments with inocula taken from non-adapted cows (Fig. 1). The total VFA, acetate and propionate were decreased in tylosin-treated cultures. The molar ratio acetate to propionate was lowered by tylosin in cultures with starch and pectin, but increased in cultures with glucose and cellulose. Contrary to this, the total VFA and their molar composition were unchanged in experiments with inocula from adapted wethers. The addition of tylosin resulted in considerably lower production of lactic acid, both with non-adapted and adapted cultures (Figs. 1 and 3). The utilization of lactic acid was lowered in tylosin-treated cultures with inocula from cows and to a smaller extent also in cultures with inocula from adapted wethers (Figs. 1 and 3). Tylosin also diminished production of methane and degradation of protein (Figs. 4 and 5). These experiments were performed using adapted inocula only.

Discussion

In our experiments tylosin inhibited rumen fermentation *in vitro* with inocula taken from non-adapted animals, but most of its effects disappeared when inocula originated from adapted animals. This adaptation effect was probably caused by selection of bacterial strains resistant to tylosin, although other mechanism can not be excluded, e. g. the inactivation of the antibiotic, the alteration of its target site or the block in transport. The most pronounced effect of tylosin was its inhibitory influence on the production of rumen lactate, that persisted in animals adapted to high doses of the antibiotic. Tylosin was much more effective in this respect than monensin in our previous experiments (Marounek et al. 1988). This lactate-depressing property can be related to the inhibitory effect of tylosin on gram-positive bacteria, that are principal lactate producers in the rumen. Surprisingly, the microscopic appearance of the rumen fluid was not much changed after adaption and gram-positive bacteria were still present. The decrease in methane production was proportional to the decrease in VFA (Fig. 4); this finding supports the assumption that tylosin has little direct effect on methanogens.

Russel and Strobel (1988) stated that any gram-positive antibiotic may produce effects similar to those of monensin. In our experiments tylosin, a potent gram-positive antibiotic, exerted only partially effects on the molar composition of VFA, that are typical for monensin and other ionophores. Neither penicillin, another gram-positive antibiotic, resembled monensin in experiments of O'Connor et al. (1970) and Beede and Farlin (1977).

In conclusion, the addition of tylosin to monensin-supplemented or monensin--free diets may be benefitial for ruminants, thanks to its liver-protecting, lactate-depressing and protein-sparing effects.

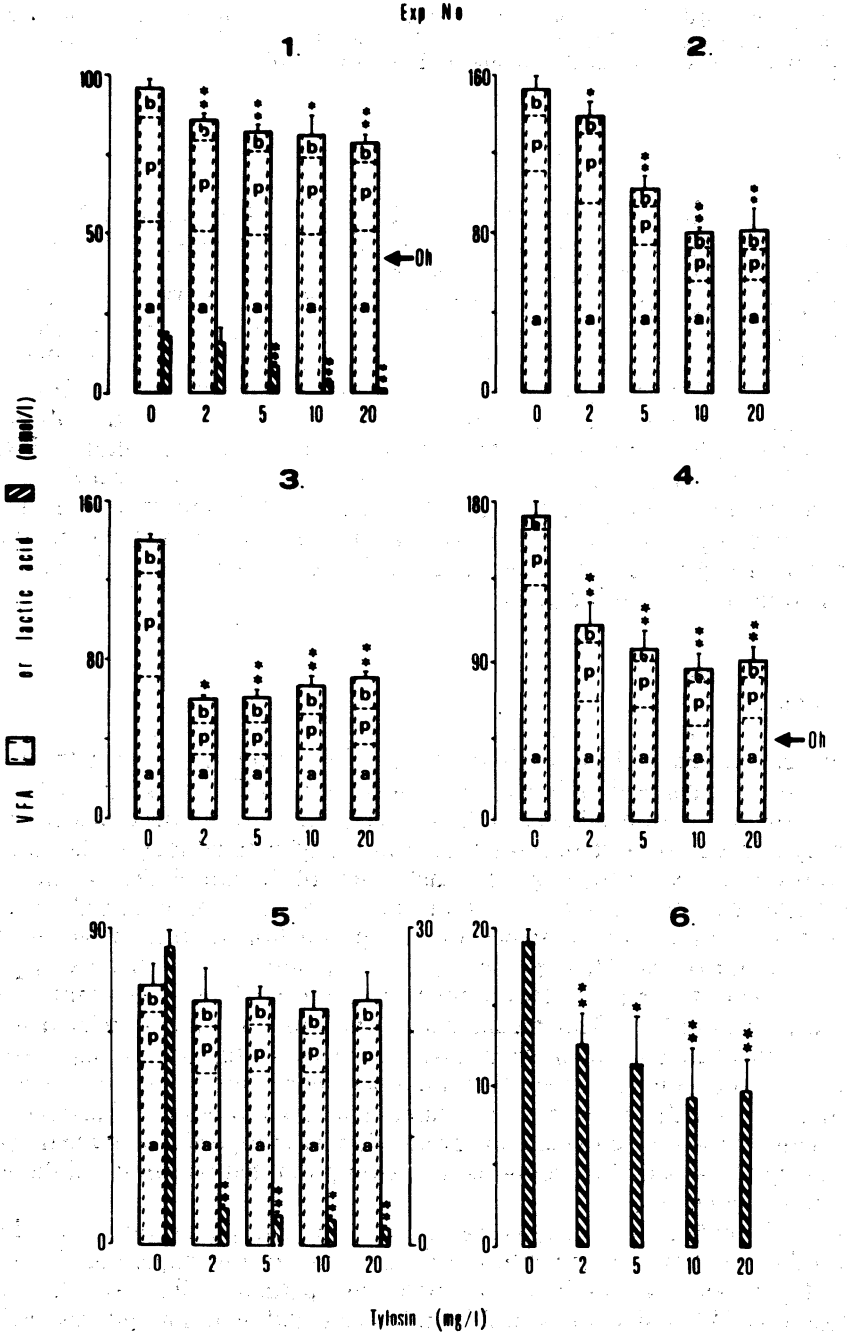

Fig. 1 Effect of tylosin on *in vitro* rumen fermentation with inocula from non-adapted cows. Substrates were glucose (1), starch (2), cellulose (3), pectin (4), sucrose and starch at low pH (5) and lactate (6).
a – acetate, p – propionate, b – butyrate
* – $P < 0.05$, ** – $P < 0.005$

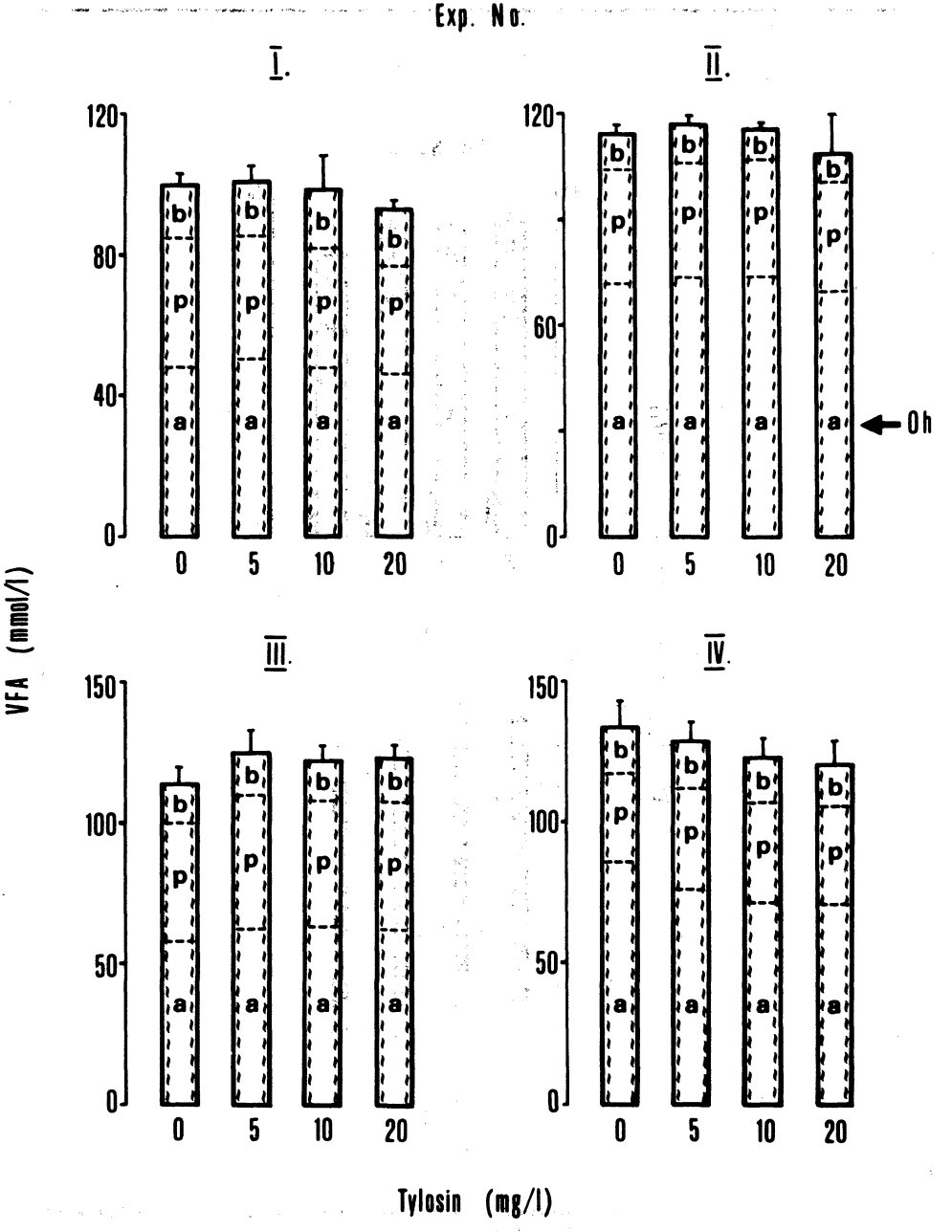

Fig. 2. Effect of tylosin on *in vitro* rumen fermentation with inocula from adapted wethers. Substrates were glucose (I), starch (II), cellulose (III) and pectin (IV).
a — acetate, p — propionate, b — butyrate

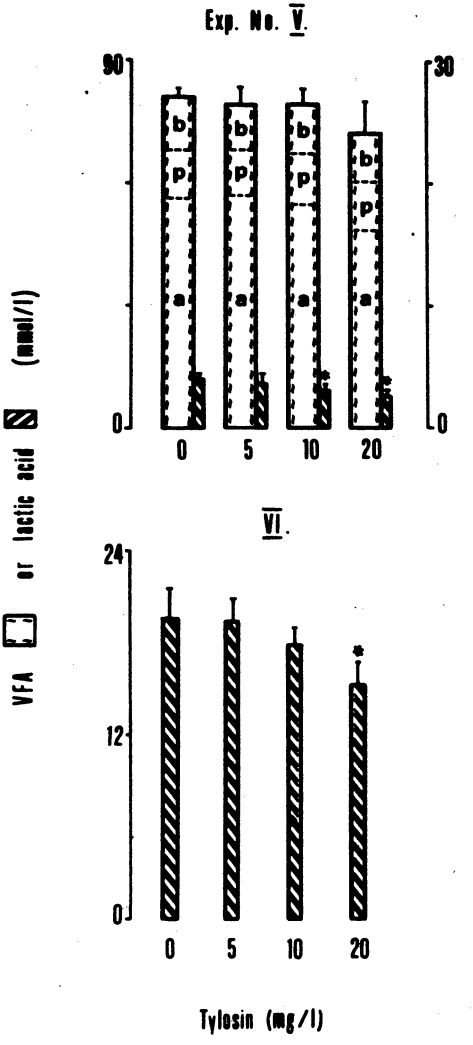

Fig. 3. Effect of tylosin on *in vitro* rumen fermentation with inocula from adapted wethers. Substrates were glucose and starch at low pH (V) and lactate (VI).
a — acetate, p — propionate, b — buryrate * — $P < 0.025$

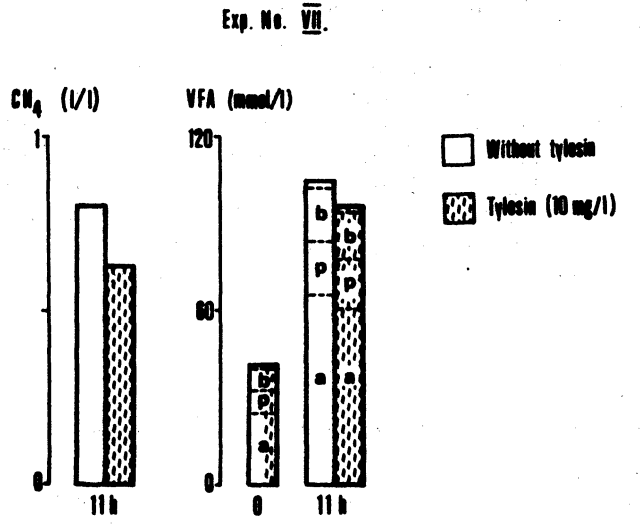

Fig. 4. Effect of tylosin on *in vitro* production of methane and VFA from glucose with inocula from adapted wethers.
a — acetate, p — propionate, b — butyrate

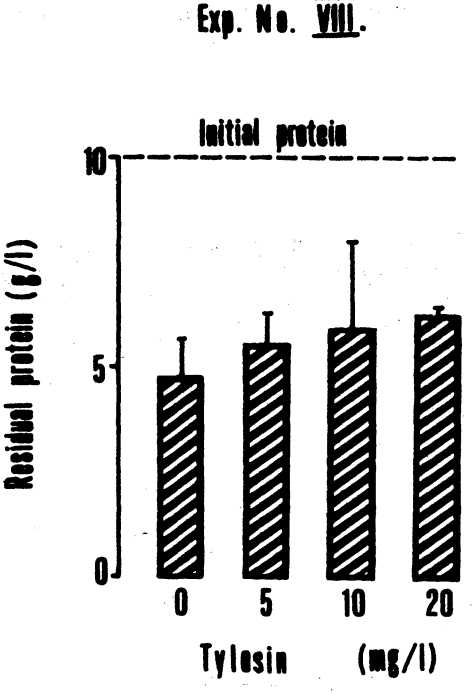

Fig. 5. Effect of tylosin on *in vitro* fermentation of casein with inocula from adapted wethers.

Účinek tylosinu na bachorovou fermentaci *in vitro*

Zjišťovali jsme účinek tylosinu na tvorbu konečných produktů metabolismu sacharidů, utilizaci kyseliny mléčné a odbourání proteinu v pokusech in vitro s bachorovou tekutinou. Tylosin nespecificky inhiboval bachorovou fermentaci bylo-li inokulum vzato z neadaptovaných krav. Jeho vliv na fermentaci byl malý, bylo-li inokueum vzato ze skopců adaptovaných na příjem tylosinu. Produkce kyseliny mléčné byla tylosinem za všech okolností výrazně potlačena. Přestože tylosin, podobně jako ionofory, inhibuje růst grampositivních bakterií, nejsou jím způsobené posuny ve složení metabolitů zcela stejné.

Воздействие тилозина на ферментацию рубца ин витро

Устанавливали воздействие тилозина на образование конечных продуктов метаболизма сахаридов, утилизацию молочной кислоты и расщепление протеина в ходе экспериментов в пробирках с жидкостью рубца. Тирозин неспецифически ингибировал ферментацию рубца при получении инокуляционного вещества у неадаптированных коров. Его влияние на ферментацию было небольшим при получении данного вещества у холощеных баранов, приспособленных к приему тилозина. Продукция молочной кислоты при всех обстоятельствах была тилозином существенно приторможена. Несмотря на то, что тилозин подобно ионофорам ингибирует рост граммположительных бактерий, вызванные им сдвиги в составе метаболитов не являются совершенно одинаковыми.

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