

CONTROLLING METHANE PRODUCTION WITH VIRGINIAMYCIN

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SUMMARY

An experiment was conducted to determine the effect of virginiamycin (VM) in sheep fed hay with or without a grain supplement. Measurements were made of rumen fluid volatile fatty acid concentrations, protozoal numbers and methane production at 3 hours after feeding. Animals fed barley grain had higher concentrations of propionic acid relative to acetic and butyric. There was a further increase in the ratio of propionate to acetate + 2 butyrate from 0.25 to 0.55 with the inclusion of VM. The production of methane and numbers of protozoa in the rumen increased 2 to 3 fold in animals fed grain without VM. VM maintained protozoal numbers and methane production relatively constant despite the change in diet. It is suggested that the primary action of VM was on the pattern of volatile fatty acid production, and that this has secondary effects on the protozoal population and methane production.

Keywords: methane, virginiamycin, protozoa

INTRODUCTION

Up to 10% of the diet energy is lost from the body as methane in ruminants (Czerkawski 1986). This gas is thought to be a contributing factor to the greenhouse effect. There has been extensive research on the subject of methane inhibition in ruminants (Czerkawski 1986), as methane production represents a loss of energy which could be converted to useful end products such as propionate.

It has been suggested that the feed additive virginiamycin (VM) may act as an inhibitor of methane production (Van Nevel *et al.* 1984; Godfrey *et al.* 1995; Nagaraja *et al.* 1995a, 1995b), since propionate levels have been found to increase when VM is added to the diet. Again, it has been shown that protozoal numbers are lower with the addition of VM (Murray *et al.* 1992). This is to be expected since the link between protozoa and methane production is well established (Van Nevel *et al.* 1992). Lower numbers of *Entodinium* were found in sheep fed grain with VM (Nagaraja *et al.* 1995a), but these authors were not able to identify whether the inhibition of methane was due to inhibition of protozoa, a direct toxic effect on methane producing bacteria, or through altering the pattern of rumen fermentation towards propionate and thus directing metabolic hydrogen away from methane production.

Many inhibitors of methane have been shown to be effective for periods of around 3 weeks (Czerkawski 1986), when fermentation patterns return to normal. This suggests that the methane producing bacteria can adapt to the inhibitors (Czerkawski 1986). When VM is included in the diet for extended periods protozoa numbers and volatile fatty acid (VFA) production have been found to return to pre-inclusion levels (Nagaraja *et al.* 1995a) in the same way as reported for other more specific methane inhibitors (Czerkawski 1986).

The aim of this study was to determine the effect VM has on the pattern of rumen fermentation, with respect to methane production and protozoal numbers, in sheep fed diets based on cereal chaff and grain.

MATERIALS AND METHODS

Animals and housing

Twenty-two crossbred lambs weighing approximately 35 kg were treated against parasites (Ivomectin, Merck Sharp and Dohme, Australia) before being housed in individual pens with grating floors. All animals were fed a basal ration consisting of 800 g of oats chaff plus 1% urea for 5 days before being weighed and randomly allocated to treatment groups.

Dietary treatments

There were 4 experimental groups in a 2 x 2 factorial design consisting of 2 levels of grain (0 and 400 g/d) and two levels of VM (0 or 20 mg/kg). There were 5 sheep per group. Sheep given no grain were fed at the rate of 800 g chaff /day throughout the experiment. The sheep which received grain were fed the same ration of chaff for the first 13 days before changing the diet to 400 g chaff and 400 g barley grain per day. A solution of urea (30% w/v) was applied to both chaff and grain to supply 10 g urea/kg. Diets

containing VM were prepared by adding the compound in the form of a wettable powder formulation (Pfizer Animal Health, Australia). These diets were fed from day 14 to day 35. Each morning the required amount of VM wettable powder was mixed with water and sprayed on to the feed while turning in a mixer.

Rumen sampling

A total of 10 samples of rumen fluid was taken from each sheep: sample 1 was taken on day 1 (before any dietary treatments started); and samples 2 to 10 were taken on days 13, 14, 15, 16, 20, 23, 27, 30 and 34 respectively. Samples of rumen fluid were taken via stomach tube 3 hours after feeding. Each sample of approximately 60 mL was divided into sub-samples as follows: 13 mL taken into a 20 mL syringe for measurement of methane production; 4 mL mixed with 16 mL of 10% formol saline for protozoal counting; and 13 mL into bottles containing 2 drops of concentrated H_2SO_4 and then stored at -20°C for measurement of VFA.

Measurements

Methane Thirteen mL of rumen fluid were incubated at 39°C for 20 hours in a 20 mL syringe that was shaken periodically. The gas produced over the 20 hour time period was then transferred to a 10 mL syringe and 1 mL of 1 M NaOH was added to absorb the CO_2 from the gas sample. The gas remaining, mostly methane and nitrogen, was then passed through an oxidising chamber where the methane was oxidised to CO_2 and collected in 5 mL of a solution containing 5% NH_4Cl + 2 mL 20% $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$. The barium carbonate precipitate was then dried and weighed to determine the quantity of methane carbon produced per mL of rumen fluid.

Volatile fatty acids These were measured by gas-liquid chromatography with isocaproic acid as an internal standard (Erwin *et al.* 1961).

Statistical analysis Analysis of variance with repeated measures using Statview 4.5 for the Macintosh was used to test for treatment and time effects.

RESULTS

There was no apparent effect of any treatment on feed intake, animal health or welfare associated with the sudden introduction of grain.

In the animals fed chaff without addition of VM there were no significant changes in protozoa numbers or in the pattern of fermentation during the experiment. The addition of VM to the chaff on day 14 increased ($P < 0.05$) the ratio of propionate to acetate plus 2 butyrate from 0.28 to 0.36 in 24 hours but did not change protozoa numbers or methane production.

In sheep fed grain without VM from day 14 of the trial, methane production more than doubled (Figure 1a). Methane production was also significantly higher in the rumen fluid of animals fed grain on its own than when it was fed with VM ($P < 0.01$). The inclusion of VM resulted in reduced methane production for 3 consecutive days after grain introduction ($P < 0.05$).

Rumen protozoa numbers were significantly lower in rumen fluid from sheep fed grain with VM compared to those fed grain on its own ($P < 0.01$) (Figure 1b). Protozoal numbers in sheep fed grain with VM remained at a relatively constant level following the introduction of grain. In the sheep fed grain on its own however, protozoa numbers almost doubled during the first 4 days following the introduction of grain. Differences between protozoal numbers in grain fed sheep with or without VM were statistically ($P < 0.05$) significant on days 14 and 15 and the difference decreased over the rest of the experimental period to be similar by day 34 (Figure 1b). Methane production was significantly correlated to protozoal numbers over the 35 day experimental period ($R^2 = 0.65$).

There were significant changes in the ratio of propionate to acetate + 2 butyrate as a result of grain in the diet (from 0.25 to 0.30) and a much greater increase ($P < 0.01$), to 0.55, due to the inclusion of VM with the grain (Figure 1c).

DISCUSSION

There was a large increase in methane production and protozoa numbers with grain feeding in the absence of VM. This increase in methane production was probably due to the increase in the amount of fermentable carbohydrate available for fermentation in the rumen fluid. There was an increase in protozoa numbers in the rumen and, due to the close association of methane producing bacteria and protozoa (Vogels *et al.* 1980), increased methane production would be expected.

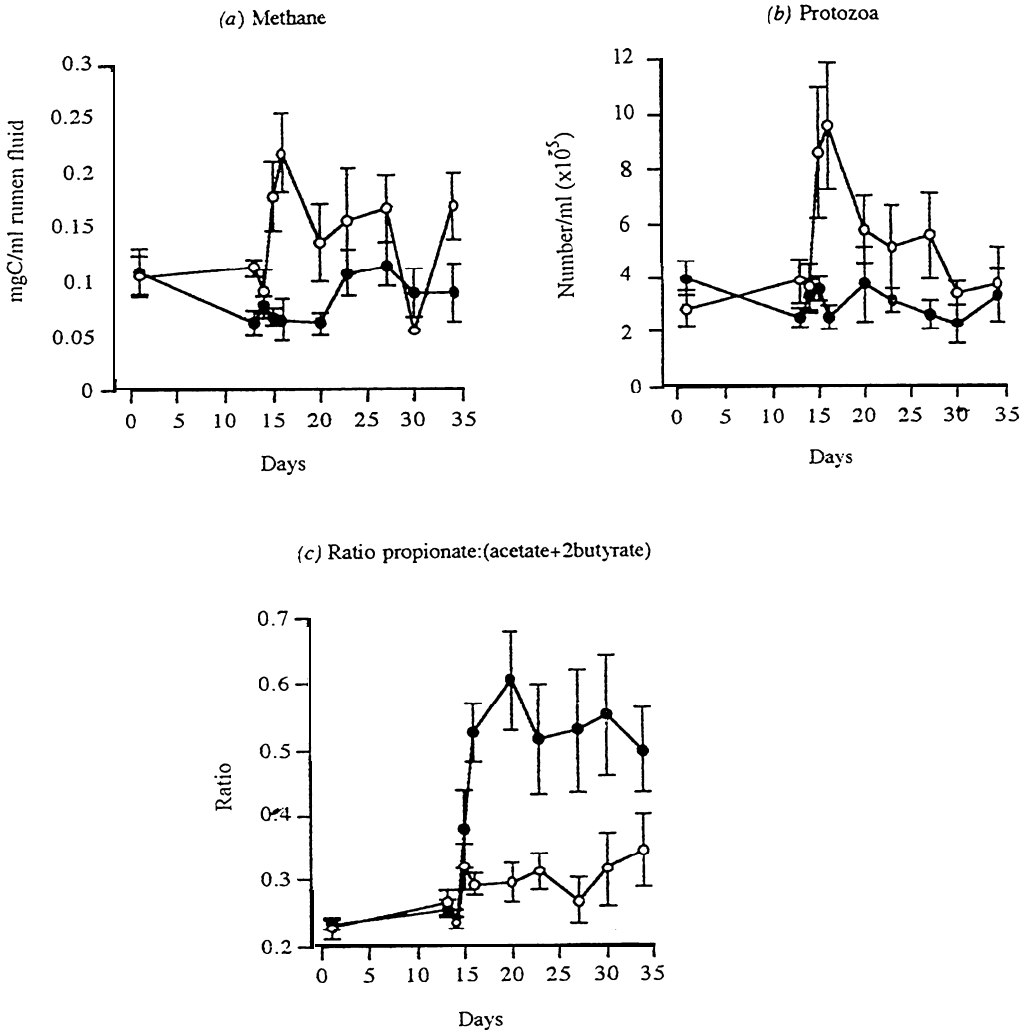


Figure 1. The effect of suddenly introducing grain to the diet of sheep previously fed oat chaff on: (a) methane production, (b) protozoal numbers and (c) ratio of propionate to (acetate + 2 butyrate) in rumen fluid. The grain was fed without treatment (○) or with virginiamycin (●)

Virginiamycin maintained methane production and protozoal numbers relatively constant with the introduction of grain feeding. This observation is consistent with findings of Nagaraja *et al.* (1995a, 1995b). The inhibition of protozoa could be due to a number of reasons, but is not well understood. It could be caused by VM inhibiting bacteria used to supply protozoal energy, or it could be due to a

decreased rate of fibre digestion (Van Nevel 1992), thus depleting energy sources protozoa would need to increase rapidly.

The method of inhibition of methane production remains unclear. It was found that VM increased propionate concentrations when added to chaff alone but did not change methane levels. When added to the grain diet however, significantly higher increases in propionate concentrations were observed as seen in Figure 1c. This suggests that the primary action of VM may be to increase propionate levels and thus reduce the amount of hydrogen available for methane production. The significant correlation found between protozoa numbers and methane suggests a link, but does not identify the primary action of VM.

Over the 34 days of the experiment, methane production tended to rise in the VM treatment group, whilst the ratio of propionate to acetate + 2 butyrate decreased. This would suggest some adaptation of the rumen microbial ecosystem to the presence of VM, as the fermentation patterns observed were similar to the non VM treatment group. The proportion of propionate was however still significantly higher in the VM treatment group than the non VM treatment group at the end of the experiment. This pattern of fermentation indicates that the sheep in the VM treatment group would derive more metabolisable energy from the fermentation of the grain than those sheep not receiving VM.

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