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Effect of Dietary Monensin or Chlortetracycline on Methane Production from Cattle Waste

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Wastes from feedlot cattle fed finishing diets containing either monensin, chlortetracycline, or no antibiotic were investigated as substrates for methane production. We used continuously mixed anaerobic fermentors with 3-liter working volumes at 35 and 55°C; these fermentors were fed once per day. Within a few days after waste from animals fed monensin was added, the volume of methane produced began to decrease in the 55°C fermentors. After 9 days of daily feeding, methane production was severely inhibited, the pH dropped from 7.6 to 5.9, and the concentration of volatile acids increased from 543 to 6,300 mg/liter (as acetate). Although additions of waste from cattle fed monensin were discontinued after 9 days, the fermentors did not resume gas production within 8 weeks. The addition of waste from cattle which had been fed chlortetracycline reduced the methane production rate approximately 20%; however, pH and volatile acid values were comparable to control fermentor values after 40 days. Similar effects were observed with the 35°C fermentors. In a batch fermentation experiment in which 50-g portions of volatile solids from waste of animals fed monensin, chlortetracycline, or no antibiotics were added to fermentors, monensin delayed the onset of methane production for about 40 days, but then these fermentors began to produce methane at a rate comparable to the control rate. The ultimate methane yields from the three types of waste after 180 days were not significantly different. These studies indicate that monensin has a detrimental effect on the conversion of feedlot wastes to methane, unless microorganisms can be adapted to the levels that are present in these wastes.

Monensin in ruminant diets increases feed efficiency (14, 15). One mechanism by which monensin is postulated to alter rumen fermentation for this increased efficiency is by selecting for a monensin-resistant microbial population that produces more propionate, less acetate and butyrate, and less methane than the microbial population in untreated animals (4, 19). Feeding monensin has been shown to reduce feed intake initially by as much as 15 to 30%. Consumption then gradually returns to approximately 90% of control values after 30 days of feeding (12). This characteristic presumably is due to the changing microflora in the rumen.

Herberg et al. (8) have shown that [^{14}C]monensin administered orally to steers is excreted rapidly and quantitatively in the feces. Donoho et al. (5) found that monensin is metabolized to many different compounds in steers and rats. Their results suggest that this antibiotic is not degraded extensively in the alimentary canals of ruminants by their microbial populations, but is degraded as a result of absorption and metabolism at the tissue level by steers and rats. To our knowledge, no published information is available concerning the effect that monensin and its metabolites, which are found in feedlot waste, have on the anaerobic fermentation of this type of waste to methane.

Chlortetracycline (aureomycin) is a broad-spectrum antibiotic that is used routinely for prevention of liver abscesses in feedlot animals. It was of general interest to determine the influences of this antibiotic on methane production from the waste of beef cattle fed this antibiotic. Hungate et al. (10) observed a small reduction in the amount of methane produced when rumen contents were incubated in the presence of chlortetracycline.

The objective of this study was to determine the effect that wastes from feedlot cattle fed either monensin or chlortetracycline had on methane production at 35°C (mesophilic) and 55°C (thermophilic). Our results indicated that the waste from cattle fed monensin completely inhibited methane production, whereas the waste from cattle fed chlortetracycline had only a minimal effect.

MATERIALS AND METHODS

The fermentation vessels used in this study were 4-liter Pyrex aspirator bottles with 3-liter working vol-

umes, as previously described (21, 22).

Waste was collected from steers that were fed three different rations. The control ration (without antibiotics) contained (on a dry matter basis) 87.9% corn, 7.0% corn silage, 3.25% soybean meal, and a vitamin-mineral supplement. The chlortetracycline or monensin rations were the same as the control ration, except that 10.8 g of chlortetracycline per kg or 22 g of monensin per kg of dry ration was included. These concentrations are within the ranges normally prescribed for beef cattle. Three steers were assigned to each ration. The animals were confined to indoor metabolism stalls on concrete floors. Waste (feces and urine) was collected daily and stored at 4°C until about 200 kg was accumulated. The waste was dispensed into screw-capped plastic bottles and stored at −20°C until 1 day before use, as described previously (22).

Methane production in fermentors fed once per day was initiated by adding 3.0% control waste plus a 20% inoculum from a methane-producing fermentor. To obtain fermentor steady-state conditions, six fermentors at 35°C and six at 55°C were fed waste without antibiotics at 6% volatile solids (VS) (organic matter) on a 9-day retention time (RT) for three volume turnovers, or 27 days. Each fermentor was then sampled on 3 successive days. After sampling, the contents of all fermentors at the same temperature were intermixed and two fermentors per temperature received waste from animals fed monensin, two per temperature received waste from animals fed chlortetracycline, and two per temperature continued to receive the control waste. Samples were taken daily after additions of waste from cattle fed monensin or chlortetracycline were initiated. The data reported represent averages of two fermentors.

Duplicate batch fermentors at 55°C were started by adding 2 liters of mineral solution (22) to each fermentor, along with 50 ml of inoculum (1.5 g of VS) from another fermentor at the same temperature and 50 g of VS from either control waste, waste from cattle fed monensin, or waste from cattle fed chlortetracycline. The 50 g of VS was added randomly over a 20-day period. The amount added each day depended on the stability of the fermentor, which was based on pH. The pH of each fermentor was measured periodically, and NaOH was added to maintain the pH between 6.5 and 7.0. These batch fermentors were operated for 180 days, and the methane yields were measured periodically.

Gas volume was determined by syphon displacement of a 20% NaCl–0.5% citric acid solution, as previously described (21). Total solids, VS, and total volatile acids (salicic acid method) were measured by previously published methods (1). Individual volatile fatty acids (VFAs) were measured by methods described previously (7, 17, 21). The influent waste was adjusted to 6% VS before VFA analyses began. The VFAs in the fermentors were determined from samples of the effluent.

The most probable number (MPN) of methanogenic bacteria and the total number of viable bacteria per milliliter of fermentor fluid from the 55°C fermentors were determined before adding waste from cattle fed monensin, chlortetracycline, or no antibiotic and after 1 RT (9 days). Anaerobic techniques for the preparation and use of media were essentially those of Hungate (9), as modified by Bryant (2). The methanogenic MPN medium contained (in grams per 100 ml): clarified fermentor effluent, 30.0; sodium formate and sodium acetate, 0.2 each; ammonium chloride, 0.027; mineral solution 3, 5.0 (20); Pfennig metals solution, 1.0 (11); resazurin, 0.0001; $NaHCO_3$, 0.75; and cysteine hydrochloride–$Na_2S \cdot 9H_2O$ solution, 2.0 (11). Fermentor effluent was collected as needed from the control fermentor 6 h after waste was added. It was clarified by autoclaving at 121°C for 5 min, strained through eight layers of cheesecloth, and centrifuged at 45,000 × *g* for 30 min. If not used immediately, this preparation was autoclaved at 121°C for 15 min and stored at 4°C. Just before use in media, it was centrifuged at 12,500 × *g* for 5 min. Media were prepared under a 50% H_2–50% CO_2 gas phase and dispensed in 5-ml amounts into tubes (18 by 150 mm). The agar roll tube medium used to determine bacterial counts contained the following constituents (in grams per 100 ml [final concentration]: clarified fermentor effluent, 40.0; glucose, cellobiose, soluble starch, maltose, and glycerol, 0.02 each; Trypticase, 0.2; yeast extract, 0.05; Pfennig metals solution 1.0 (16); hemin and reazurin, 0.0001 each; mineral solution S2, 5.0 (16); cysteine-hydrochloride solution, 2.0 (20); $NaCHO_3$, 0.75; agar, 3.0; and VFA solution, 0.3 (20). This medium was prepared under an 80% N_2–20% CO_2 gas phase and dispensed in 5-ml amounts into tubes (18 by 150 mm). Anaerobic dilution solution was prepared as previously described (3).

Fermentor fluid samples for bacterial counts were obtained 6 h after waste was added. The 200-ml samples were blended in a Waring blender for 1 min and diluted in a 10-fold dilution series through 10^{-10}. The 10^{-3} to 10^{-10} dilutions were used to inoculate triplicate tubes of the methanogenic MPN medium. The 10^{-7} to 10^{-9} dilutions were used to inoculate four replicate agar roll tubes to determine the viable counts. Both culture media were incubated for 2 weeks at 55°C with tubes in an upright stationary position. The gas phase in each MPN medium tube was analyzed, and if methane was detected, the tube was recorded as positive. The three-tube MPN procedure (1) was used to determine the MPN of methanogenic bacterial cells per milliliter of fermentor fluid.

RESULTS

Figure 1 shows methane production in 55°C fermentors which initially were stabilized with waste containing no antibiotic and then received waste from cattle fed monensin or chlortetracycline. The volume of methane began to decrease within a few days after the waste from cattle fed monensin was added, and after 12 days (day 44) essentially no methane was produced. Daily waste additions to the monensin-containing fermentors were stopped after 1 RT (day 41). These fermentors did not resume methane production within 8 weeks. The fermentors receiving the waste from cattle fed chlortetracycline showed a decrease of approximately 20% in the rate of

methane production compared with the controls.

From the data in Fig. 1 one might assume that methane production was decreasing before monensin was added. This is somewhat misleading because unfortunately we did not determine the rate of methane production on days 31 and 32. However, Fig. 2 shows that all fermentors had similar concentrations of acid on day 33; this indicates the stability of the fermentors up to that time.

The percentages of methane and carbon dioxide produced in the 55°C monensin and chlortetracycline fermentors are shown in Fig. 3. The percentage of methane in the fermentors receiving the waste from cattle fed monensin began to decrease within 2 days and decreased to approximately 35% of the total gas content after 1 RT. The carbon dioxide percentage increased to approximately 70% during this same period. No hydrogen was detected in the monensin fermentors at any time. The ratio of methane to carbon dioxide in the chlortetracycline and control fermentors remained relatively constant.

Figure 2 shows the effect of adding waste from cattle fed monensin or chlortetracycline on the

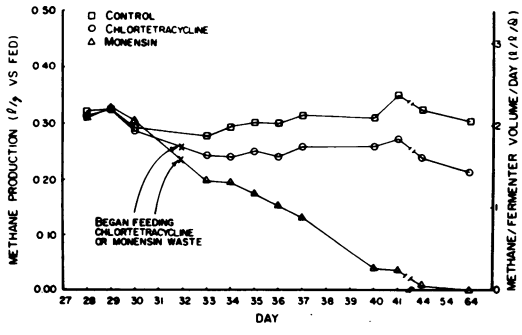

FIG. 1. *Methane production in 55°C fermentors which received waste from cattle fed monensin or chlortetracycline.*

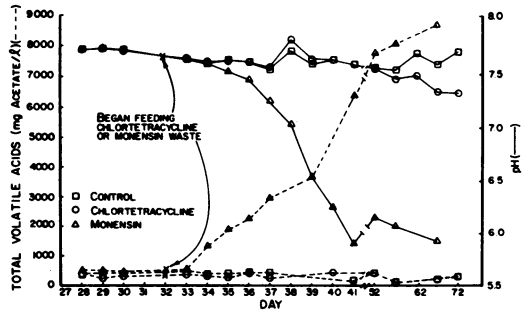

FIG. 2. *Concentrations of VFAs and pH's in 55°C fermentors which received waste from cattle fed monensin or chlortetracycline.*

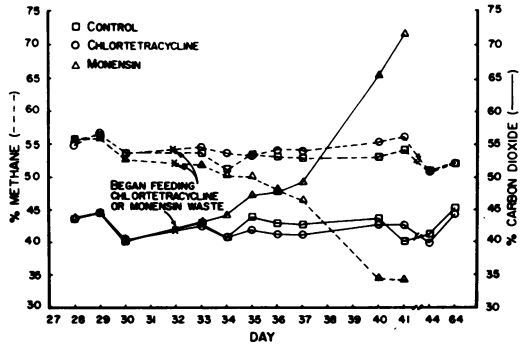

FIG. 3. *Percentages of methane and carbon dioxide in 55°C fermentors which received waste from cattle fed monensin or chlortetracycline.*

concentrations of VFAs and on the pH in 55°C fermentors. Within 2 days after waste from cattle fed monensin was added, the concentrations of volatile acids began to increase, and within 3 days the pH began to decrease. This trend continued until daily additions were stopped. By this time the concentration of the volatile acids had increased to 6,300 mg/liter (as acetate), and the pH had decreased to 5.9. The fermentors receiving the waste from cattle fed chlortetracycline showed no adverse effects on volatile acids and pH compared with controls.

Figure 4 shows the concentrations of the individual VFAs that accumulated in the 55°C fermentors which received waste from cattle fed monensin. The concentrations of acetate and propionate increased immediately. After 7 days of daily additions, butyrate began to accumulate significantly. The individual acids found in the chlortetracycline fermentors did not vary from the acids found in the controls (data not shown). The only acids detected in these fermentors were acetate (<5 mM) and valerate (<2 mM). The concentrations of the significant individual acids (>5 mM) detected in the influent wastes from cattle fed no antibiotic, cattle fed monensin, and cattle fed chlortetracycline were as follows: acetate, 41.3, 32.7, and 31.2 mM, respectively; propionate, 11.1, 9.3, and 8.9 mM, respectively; butyrate, 10.8, 5.5, and 8.7 mM, respectively; and lactate, 9.4, 10.4, and 13.5 mM, respectively.

The trends observed in the 35°C fermentors were similar to those observed in the 55°C fermentors. The waste from cattle fed monensin totally inhibited methane production within 12 days, and the waste from cattle fed chlortetracycline reduced the rate of methane production approximately 20%. Of interest in the 35°C fermentors was the fact that no adverse effects were observed for 4 or 5 days after additions of waste from cattle fed monensin began, compared with the 2-day period in 55°C fermentors.

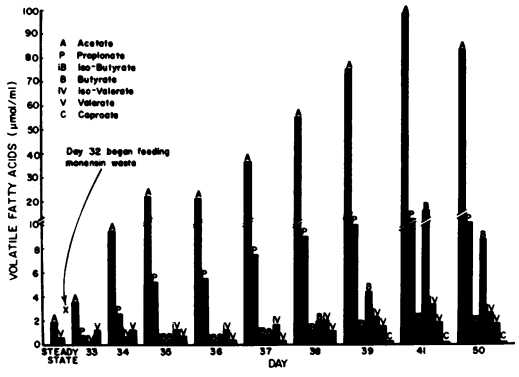

FIG. 4. *Concentrations of individual VFAs that accumulated in 55°C fermentors which received waste from cattle fed monensin.*

The methane yields from the 55°C batch fermentors are shown in Fig. 5. These data show that monensin significantly delayed the initiation of methane production, but eventually methane was produced at a rate comparable to the control rates. Nearly one-third of the methane was produced in the control and chlortetrayline fermentors after 25 days of incubation, but only minimal amounts were produced in the monensin fermentors after 40 days of incubation. However, the methane yields in the monensin fermentors after 180 days were only slightly less than the yields in the controls.

The average MPN of methanogenic bacteria per milliliter of fermentor fluid (based on four determinations from four different 55°C fermentors) before additions of waste from cattle fed monensin or chlortetracylcine was $2.5 \times 10^8 \pm 0.4 \times 10^8$ cells per ml, and the total viable count was $3.7 \times 10^9 \pm 0.5 \times 10^9$ cells per ml. After waste from cattle fed monensin and waste from cattle fed chlortetracycline were added for 9 days (1 RT) the methanogenic MPN ranges were 1.4×10^8 to 1.9×10^8 and 1.9×10^8 to 2.4×10^8 cells per ml, respectively, whereas the total viable count was 2.6×10^9 to 3.3×10^9 and 2.0×10^9 to 2.4×10^9 cells per ml, respectively (based on two determinations). There was no significant reduction in the methanogenic or viable count due to monensin or chlortetracycline.

DISCUSSION

These studies indicate that methane production is totally inhibited in 35 and 55°C conventional fermentors when these fermentors are fed at moderately high solids loading rates (6% VS) and short RTs (9 days) with beef feedlot waste containing monensin. Methane production from waste containing chlortetracycline is reduced 20%, although this amount would probably be less in a practical situation because chlortetracycline is not fed to feedlot cattle on a daily basis, but is normally fed intermittently for the prevention of liver abscesses. Monensin is incorporated into rations and is fed on a daily basis.

In 1978 more than 80% of the cattle in feedlots were fed monensin (13). This indicates the potential negative impact of this antibiotic on the feasibility of converting feedlot waste to methane. However, based on the data from the batch fermentors (Fig. 5), there is some positive potential for fermentation of this waste to methane. From the data in this experiment we are not sure whether bacterial populations develop that are resistant to monensin or whether monensin is chemically inactivated after 40 to 50 days in a 55°C fermentor. Data from Eli Lilly & Co., manufacturers of monensin, indicate that a concentration of 0.1 to 0.2 μg of monensin per g in soil samples is deactivated in 14 days when incubated with animal feces and in 25 days when incubated without feces (6). However, this concentration may be small compared with the concentration present in waste from animals fed monensin. A longer time period, such as 40 to 50 days (as we observed in the batch fermentors), may be required to degrade this higher concentration.

The methanogenic bacteria may not be the primary target of action of monensin. Chen and Wolin (4) and Van Nevel and Demeyer (19) concluded that the effects of monensin on methanogenesis may be indirect; i.e., the primary inhibition may be an inhibition of the production of methane precursors, such as hydrogen and formate. Slyter (18) found that less hydrogen and formate accumulated in continuous cultures of rumen bacteria when monensin was added to

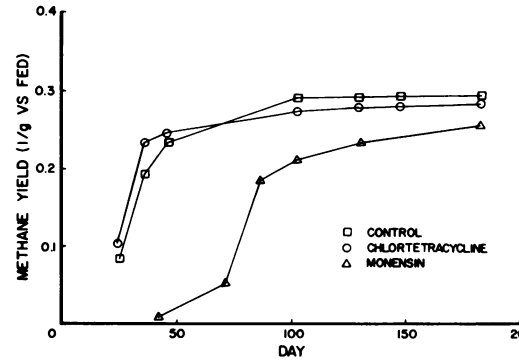

FIG. 5. *Methane yields in 55°C batch fermentors which received waste from cattle fed monensin or chlortetracycline.*

the cultures, compared with cultures to which no monensin was added. Hungate et al. (10) also concluded that this was the reason for diminished methane production when chlortetracycline was added to rumen contents. Our data on the number of methanogens present in the fermentors showed that the methanogenic population was not significantly decreased when waste from cattle fed monensin was added to the fermentors for 9 days, although methane production was minimal at this time. This lends some support to the conclusion described above.

Preliminary results indicate that after a gradual adaptation over a 3-month period at 55°C, rates of methane production from waste of cattle fed monensin (6% VS, 25-day RT) are comparable to control waste rates. Whether this RT can be shortened with longer adaptation periods is uncertain. When the 25-day RT fermentors were switched to an 18-day RT, the fermentors failed. We also took a 33% inoculum from 180-day batch fermentors and added it to a fermentor fed monensin-containing waste, but it also failed between the same RTs.

Further studies will be necessary to determine what bacterial populations are affected by monensin in feedlot waste fermentors. In general, very little is known about the populations in thermophilic fermentors. Unpublished results of Leedle and Bryant (R. Leedle, M. S. thesis, University of Illinois, Urbana, 1977) and our own work (V. H. Varel, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, I31, p. 89) indicate that the predominant isolates are gram-negative rods. Gram-positive bacteria are reportedly more sensitive to monensin than gram-negative bacteria (4). Whether microbial population shifts occur in methane-producing fermentors, as is postulated to occur in the rumen ecosystem, is unknown. Chen and Wolin (4) postulated that monensin acts in ruminants by selecting for a microbial community that produces proportionally more propionic acid than the community of microbes in untreated ruminants. The concentrations of acids which we observed in the fermentors receiving waste from cattle fed monensin cannot be compared directly with the rumen VFA pattern because we were not able to reach a steady-state equilibrium in the fermentors. Also, in rumens the VFAs are absorbed through the rumen wall, whereas in the fermentors the acids must be metabolized to methane and carbon dixoide. If the microorganisms in a methane-producing fermentor operating at a short RT (<10 days) can be adapted to monensin, it will be of interest to compare the predominant species of bacteria in fermentors fed waste containing monensin with the species in fermentors fed monensin-free waste.

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