# Effects of the antibiotic monensin and an inhibitor of methanogenesis on in vitro continuous rumen fermentations

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1. The effects of a methane inhibitor, ICI 111075, and a propionate enhancer, monensin, were studied using in vitro continuous fermenters.

2. Both compounds increased the yield of substrate energy, carbon and hydrogen in volatile fatty acids (VFA). This was mainly due to an increase in the molar proportion of propionic acid.

3. Improved yields of VFA were accompanied by reductions in methane production and microbial yield.

4. Since published information showed that monensin reduced rumen dilution rate in vivo an analogous in vitro system was proposed in which a high dilution rate control fermenter was compared with a monensin treated fermenter set to run at a low dilution rate.

5. Results showed that the general intrinsic microbial activity of the chemical manipulators was not affected by changes in dilution rate. Changing dilution rate in addition to chemical treatment however resulted in substantial modifications in the net effect on the fermentation.

6. The practical implications of reducing rumen dilution rate as a side effect of chemically manipulating the rumen fermentation could involve changes in food intake, increased importance of secondary fermentations and a reduced effect of nutrients not degraded in the rumen.

The use of chemicals to manipulate selectively the rumen fermentation, such that animal growth performance is improved, has been reviewed recently (Chalupa, 1977; 1979). Work to manipulate favourably energy transactions within the rumen has involved mainly two distinct classes of compound which either primarily inhibit methane production or enhance propionate production. Methane inhibitors generally also enhance propionate production since both processes are inversely related.

Numerous production trials have been done to demonstrate the growth-promoting efficacy of these chemicals. Whilst some trials have shown beneficial effects with methane inhibitors (Trei, Parish & Scott, 1971; Trei & Scott, 1971 *a, b*; Trei *et al.* 1972; Leibholz, 1975), others have shown little or no response (Sawyer *et al.* 1974; Cole & McCroskey, 1975). One of the major problems with this type of compound, which are mainly halogenated methane analogues, has been the adaptation of the rumen micro-organisms after prolonged administration (Johnson, 1974; Clapperton, 1977). It has also been concluded that level of feeding, level of concentrate and feeding frequency could affect the efficacy of methane inhibitors (Cole & McCroskey, 1975). The beneficial effects of the propionate enhancer, monensin, have been demonstrated extensively. Performance has been improved in cattle (Oliver, 1975; Davis & Erhart, 1976; Potter *et al.* 1976; Raun *et al.* 1976; Turner *et al.* 1977) and lambs (Nockels *et al.* 1978; Joyner *et al.* 1979). Avoparcin, another propionate enhancer, improves both food conversion and daily gain in cattle (Johnson *et al.* 1979). Interestingly, monensin had no beneficial effect in this trial.

Studies to explain the effects of chemical manipulators on the rumen fermentation in terms of fermentation balances have been limited. In vitro studies have shown the effects of chloral hydrate (Van Nevel *et al.* 1969), sodium sulphite, chloral hydrate and hemiacetal of chloral and starch (Marty & Demeyer, 1973) and monensin (Van Nevel & Demeyer, 1977; Short, 1978) on the fermentation of carbohydrate to volatile fatty acids (VFA), carbon dioxide, hydrogen and methane. The use of batch culture (Van Nevel *et al.* 1969; Marty

& Demeyer, 1973; Van Nevel & Demeyer, 1977) or semi-continuous batch culture (Short, 1978) may not be relevant to effects in the animal.

The development of a number of in vitro continuous fermentation systems has provided a method with which to study the rumen fermentation in detail under strictly-controlled conditions. One such system is Rusitec (Czerkawski & Breckenridge, 1977) which is both simple and reliable and at present provides a good in vitro simulation of in vivo conditions. In addition to being able to quantify the effects of manipulators by means of fermentation balances the ability to control dilution rate precisely enables the effects of the latter to be determined.

The importance of dilution rate in determining the pattern and efficiency of rumen fermentation has been shown by infusing artificial saliva into the rumen of sheep (Harrison et al. 1974; Harrison et al. 1975) and by the inclusion of mineral salts in the diet (Thomson et al. 1975; Thomson et al. 1978). Whilst dietary administration of monensin has produced substantial reductions in rumen dilution rate in cattle (Lemenager et al. 1978) and sheep (Allen & Harrison, 1979) other studies have shown no effect (Van Nevel & Demeyer, 1979). Using the facilities provided by multiple continuous fermenters the effects of rumen manipulators and dilution rate can be studied separately or in combination. In the instance of monensin the latter situation may theoretically simulate the in vivo conditions best.

The aim of this study was to use a continuous fermentation system to compare and quantify the effects of a methane inhibitor, ICI 111075 and a propionate enhancer, monensin. The role of dilution rate in modifying the efficacy of these rumen manipulators was also investigated.

## EXPERIMENTAL

### Fermentation system

The continuous fermenter used was based on the design described by Czerkawski & Breckenridge (1977). Modifications included having six fermenters in a single unit with food containers attached via metal rods to a single beam which oscillated 50 mm vertically at 8 cycles/min to agitate the fermentation (Fig. 1). The overflow pipes were adjusted to give a liquid volume of 1 l and a gas space of 150 ml. Buffer, pH 7 (Bales et al. 1976; but omitting the acetic acid) was pumped continuously into each fermenter by a six-channel peristaltic pump (Proportioning pump; Technicon Instruments Ltd, London). Effluent liquid and gases were collected as described by Czerkawski & Breckenridge (1977).

### General procedure

Rumen fluid was obtained from two fistulated heifers given a hay-concentrate diet, pooled and filtered through four layers of muslin cloth. Strained fluid (500 ml) was put into each fermentation vessel along with a nylon bag containing 50 g rumen solids and another bag containing the experimental diet of 10 g long hay and 2 g cattle concentrate. The gas phase was flushed with carbon dioxide (Expt 1) or high purity nitrogen (White Spot $N_2$, British Oxygen Co. Ltd, Worsley, Manchester) in Expts 2 and 3. No gross differences in fermentation pattern were apparent with the two gases. Each fermenter was filled to 1 l liquid volume by pumping buffer at the chosen experimental rate (cf. Czerkawski & Breckenridge, 1977). Subsequently the basic sampling and feeding procedures described by Czerkawski & Breckenridge (1977) were adopted. Gas samples (2 ml) were withdrawn from the fermenter headspace using a syringe and a hypodermic needle inserted through a rubber suba-seal located in the top of the fermentation vessel. These samples were then analysed for percentage composition. Experiments consisted of a 7 d acclimatization period followed by daily administration of chemical manipulators for 25 d.
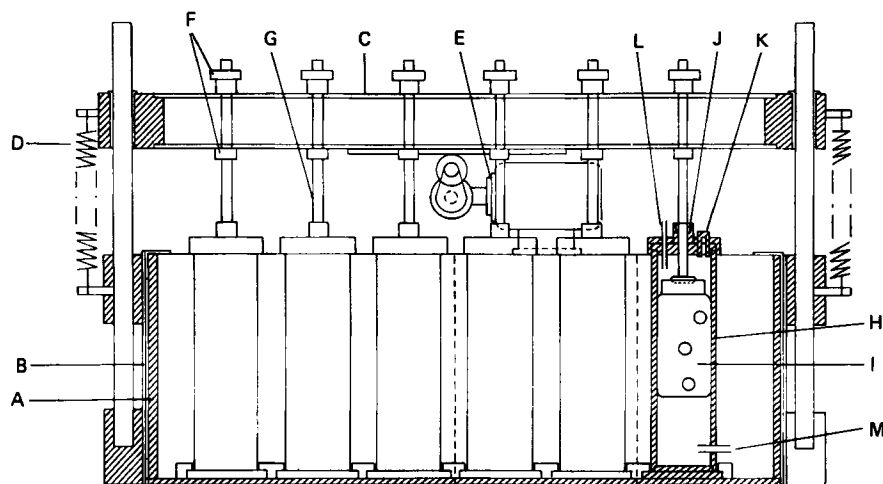
Fig. 1. Schematic diagram of a six vessel continuous fermentation system. A, Perspex tank; B, steel frame to support tank; C, oscillating beam; D, spring to aid downward movement; E, Parvalux motor/gear box; F, locking nuts; G, steel shaft; H, Perspex fermentation vessel; I, polyethylene bottle to contain nylon feed bags; J, gas-tight gland; K, suba-seal gas sampling gland; L, outlet pipe for liquid effluent and gases; M, inlet pipe for buffer.
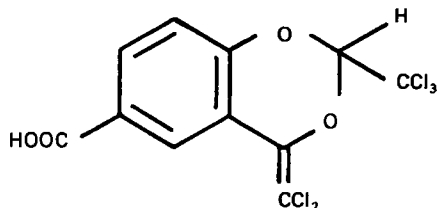


Fig. 2. ICI 111075, 2-trichloromethyl-4-dichloromethylene benzo[1,3] dioxin-6-carboxylic acid.

## Experimental procedures

*Expt 1.* Single fermenters were dosed daily with ICI 111075 (Fig. 2) at 2·0, 1·0 and 0·5 $\mu$g/ml or monensin at 0·5 $\mu$g/ml. Compounds were dissolved in ethanol and an equal volume of solvent was added to both control fermenters. All six fermenters were run at a dilution rate of 1·0/d. Total gas volume was not determined in this experiment and results were expressed as percentage composition.

*Expt 2.* Duplicate fermenters were dosed daily with ICI 111075 (2·0 $\mu$g/ml), monensin (0·5 $\mu$g/ml) or ethanol (controls). Dilution rate was 1·0/d.

*Expt 3.* Three fermenters were run at a dilution rate of 0·90/d and three others at 0·45/d. In each group single fermenters were dosed daily with ICI 111075 (2·0 $\mu$g/ml), monensin (0·5 $\mu$g/ml) or ethanol (control).

## Analytical methods

Gases were measured daily by a modification of the technique of Czerkawski & Clapperton (1968*a*). The basic apparatus was a Pye 104 Katharometer Power supply and Katharometer detector (Pye Instruments Ltd, Cambridge). Column 1 was 0·46 m × 6·4 mm glass packed with Porapak Q 80–100 mesh (Waters Associates Inc., Maple St, Milford, Mass. 01757, USA). The delay column was 7·62 m × 3·2 mm in copper tubing and the final column was

1·52 m × 6·4 mm in glass packed with Molecular Sieve 5A 80–100 mesh (Phase Separations Ltd, Deeside Industrial Estate, Queensferry, Clwyd CH5 2LR). The two separate columns and detector were housed in an insulated box at room temperature. The carrier gas was argon (50 ml/min). Samples were injected through a sampling valve with a loop of 1 ml volume. The detector signal was processed by a Spectra Physics SP4000 Chromatography Data System (Spectra Physics Ltd, St Albans, Herts AL1 5UF) which integrates negative (composite gas, $CO_2$, from column 1) as well as positive ($H_2$, oxygen, $N_2$, $CH_4$ from final column) peaks of virtually unlimited size. This eliminates the need to change polarity and attenuation. The equipment was standardized using a mixture of $CO_2–H_2–N_2–CH_4$ (25:25:25:25, by vol.).

VFA were measured daily by a modification of the technique of Cottyn & Boucque (1968). The apparatus was a Pye 104 gas–liquid chromatograph equipped with a flame-ionization detector, an S4 Autojector and a Spectra Physics SP4000 Chromatography Data System.

A glass column (1·52 m × 6·4 mm) packed with 100 g Carbowax 20M TPA/kg Chromsorb WHP 80–100 mesh (Field Instruments Co. Ltd, Twickenham, Middlesex TW1 4EG) was used. The column was operated at 150° with argon (50 ml/min) as carrier gas. Samples (0·6 ml) of fermenter liquid were pipetted into centrifuge-tubes (Eppendorf), mixed with 0·3 ml diluent (100 mM-crotonic acid in 50 ml orthophosphoric acid/l) and centrifuged (2 min, 14000 rev/min). The clear supernatant fraction was transferred to vials for direct injection of a standard 2 μl sample onto the column. The crotonic acid which has a retention time greater than valeric acid but less than caproic acid was used as internal standard. The equipment was standardized using a mixture of acetic (60 mM), propionic (20 mM), butyric (20 mM), isovaleric (10 mM) and valeric acid (10 mM) prepared in the same manner.

Microbial DNA was assayed daily by the diphenylamine reaction of Burton (1956). Duplicate 1 ml samples of effluent liquid were taken daily and centrifuged (14000 rev/min, 5 min). The supernatant fraction was discarded, 0·5 ml 0·25 M-perchloric acid added, mixed and centrifuged (14000 rev/min, 2 min). The supernatant fraction was discarded and 1 ml 0·25 M-perchloric acid mixed with the residue. The mixture was heated (70°, 30 min), centrifuged (14000 rev/min, 2 min) and the whole supernatant fraction taken for the diphenylamine reaction. Calf thymus DNA (0–200 μg/ml) was used as standard.

*Calculations*

The net gas volume produced was calculated by subtracting the effluent liquid volume (l) from the total gas volume (l) collected in the butyl rubber bag. Gas volumes were measured at atmospheric pressure but were not corrected to STP. Using the percentage composition of the gas phase in the fermenter headspace, the volume of $CO_2$, $H_2$ and $CH_4$ was calculated and converted to mmol/d. Results are means of single daily determinations.

Net VFA production (mmol/d) was calculated by multiplying the concentration of the individual VFA (mmol/l) in the effluent liquid by the volume of effluent collected per day. Results are means of single daily determinations over the 25 d treatment period.

Microbial matter produced (mmol/d) was calculated using DNA measurements and the following assumptions: (a) DNA contains 140 mg N/g (Ling & Buttery, 1978); (b) there are 32 mg DNA-N/g microbial-N (Allison, 1970); (c) there are 105 mg microbial-N/g total microbial dry weight (Allison, 1970); (d) the molecular formula of microbes is $C_6H_{9·85}O_{2·99}N_{1·20}$ (Demeyer & Van Nevel, 1975). Microbial yield was expressed in terms of g microbial-N/kg organic matter fermented ($OM_f$).

The organic matter fermented was taken as the dry weight of substrate lost from the nylon bags each day. As most of the organic matter consists of anhydro-glucose units results are calculated on the basis that all loss of substrate was due to the fermentation of hexose. Hexose was taken as having a molecular weight of 162 g and division of the organic matter fermented each day (g/d) by 162 gave the amount of hexose fermented (mmol/d).

Table 1. *Expt 1. Effect of ICI 111075 and monensin on the production of hydrogen, methane and volatile fatty acids (VFA) and the substrate fermented in a continuous in vitro fermentation system*

| | | ICI 111075 ($\mu$g/ml) | | | Monensin | |
|---|---|---|---|---|---|---|
| | Control | 0·5 | 1·0 | 2·0 | 0·5 $\mu$g/ml | LSD† |
| Gases (% composition) | | | | | | |
| H$_2$ | 0·14 | 0·14 | 0·33 | 0·51** | 0·03 | 0·22 |
| CH$_4$ | 8·04 | 4·79*** | 2·60*** | 0·70*** | 1·47*** | 1·58 |
| VFA (mmol/d) | | | | | | |
| Acetic | 30·93 | 31·33 | 33·27 | 31·18 | 27·26 | 3·75 |
| Propionic | 7·80 | 10·21* | 12·16*** | 13·54*** | 9·19* | 1·19 |
| Butyric | 3·12 | 3·04 | 3·31 | 3·31 | 1·82*** | 0·41 |
| Hexose fermented (mmol/d) | 30·77 | 30·52 | 29·82 | 29·08 | 25·00*** | 2·48 |

\* $P < 0.05$, \*\* $P < 0.01$, \*\*\* $P < 0.001$.
† Least significant difference.

The recovery of hexose energy in fermentation end-products was calculated using the heats of combustion of the respective products. The heat of combustion of hexose was taken as 2832 J/mmol. The energy content of the microbial matter was calculated as 3262 J/mmol assuming the average microbial composition was (1 kg): 400 protein, 100 lipid, 100 carbohydrate, 200 nucleic acids, 200 cell wall.

The recovery of metabolic hydrogen (2H) was calculated using the method described by Marty & Demeyer (1973). Lactate was not determined in samples of fermenter effluent liquid and was therefore not included in the equation to calculate 2H recovered in microbial cells.

Statistical analysis of the results was by an analysis of variance.

## RESULTS

*Expt 1. Dose response of methane inhibitor ICI 111075.* The production of gases, VFA and the amount of hexose fermented are summarized in Table 1 as means for the 25 d treatment period. The production of CH$_4$ was inhibited by 40, 68 and 91% at doses of 0·5, 1·0 and 2·0 $\mu$g ICI 111075/ml respectively and was accompanied by a progressive increase in H$_2$ production. Monensin treatment decreased both CH$_4$ and H$_2$ production.

ICI 111075 had no significant effect on acetate and butyrate production but increased propionate production above control values by 31, 55 and 73% at doses of 0·5, 1·0 and 2·0 $\mu$g/ml respectively. Total VFA production was increased slightly. Monensin reduced acetate and butyrate production by 12 and 41% respectively while propionate production was increased by 18%. Total VFA production, relative to the control, was reduced by 11%.

ICI 111075 had no significant effect on the amount of hexose fermented although there was a slight downward trend at the higher doses. Monensin, however, had an immediate and maintained depressant effect on hexose digestion, the over-all reduction being 19%.

*Expt 2. Comparative effects of ICI 111075 and monensin.* The net production of gases, VFA, microbial yield and the amount of hexose fermented are summarized in Table 2. ICI 111075 at 2·0 $\mu$g/ml reduced CH$_4$ by 82%, increased H$_2$ 5-fold but did not affect CO$_2$ production. Monensin at 0·5 $\mu$g/ml reduced CH$_4$ by 57%, H$_2$ by 15% and CO$_2$ production by 33%.

ICI 111075 increased the net production of acetate, propionate and butyrate by 15, 119

Table 2. *Expt 2. Effect of ICI* 111075 *and monensin on the net production of gases, volatile fatty acids (VFA) and microbial matter and the substrate fermented in a continuous in vitro fermentation system*

|  | Control | ICI 111075 | Monensin | LSD† |
|---|---|---|---|---|
| Gases (mmol/d) | | | | |
| $CO_2$ | 27·35 | 27·51 | 18·39*** | 1·77 |
| H | 0·13 | 0·65*** | 0·11 | 0·06 |
| $CH_4$ | 6·41 | 1·22*** | 2·75*** | 0·52 |
| VFA (mmol/d) | | | | |
| Acetic | 21·04 | 24·23*** | 19·47** | 1·11 |
| Propionic | 6·51 | 14·28*** | 8·09*** | 0·80 |
| Butyric | 3·92 | 4·16** | 2·54*** | 0·18 |
| Isovaleric | 0·69 | 0·58*** | 0·62*** | 0·04 |
| Valeric | 1·63 | 1·67 | 1·46*** | 0·07 |
| Total | 33·75 | 44·90*** | 32·18 | 1·92 |
| Hexose fermented (mmol/d) | 31·17 | 31·80 | 25·95*** | 0·71 |
| VFA/hexose fermented | 1·08 | 1·41*** | 1·24*** | 0·06 |
| Microbial yield (gN/kg $OM_f$‡) | 14·21 | 12·81 | 13·00 | — |

\* $P < 0.05$, \*\* $P < 0.01$, \*\*\* $P < 0.001$.
† Least significant difference.
‡ Organic matter fermented.

and 6% respectively. Since the total VFA production was increased by 33% the molar proportion of acetate fell from 0·62 to 0·54 whilst that of propionate rose from 0·19 to 0·32. In comparison monensin reduced the net production of acetate and butyrate by 8 and 35% respectively while propionate production increased by 24%. Total VFA production, however, was slightly depressed.

The amount of hexose fermented was increased slightly by ICI 111075 but was depressed by 17% with monensin. Both ICI 111075 and monensin improved the yield of VFA produced per unit hexose fermented by 31 and 15% respectively. (Table 2). The yield of microbial matter in terms of g microbial N per kg organic matter fermented was depressed by both treatments.

The recovery of hexose energy, C and $H_2$ in fermentation products is summarized in Table 3. ICI 111075 and monensin increased the yield of hexose energy in VFA by 32 and 13% above control values respectively. This was accompanied by reductions in energy lost as $CH_4$ of 82 and 49% and energy found in microbes by 10 and 8% for ICI 111075 and monensin respectively. The increased $H_2$ production associated with ICI 111075 treatment only constituted a very small loss of substrate energy. Similar values were obtained for the recovery of hexose C. The total recovery of metabolic H was only 69 and 73% with ICI 111075 and monensin respectively compared with a control recovery of 87%.

*Expt 3. Effect of dilution rate on the action of ICI 111075 and monensin.* The production of gases, VFA, microbial yield and the amount of hexose fermented are summarized in Table 4 (high dilution rate) and Table 5 (low dilution rate) as means for the 25 d treatment period. In control fermentations reducing the dilution rate from 0·90 to 0·45/d increased $CO_2$ and $CH_4$ production by 64 and 40% respectively. The total net production of VFA was reduced by 15% despite a 9% increase in the hexose fermented. Consequently the yield of VFA per unit hexose fermented decreased from 1·33 to 1·06. The molar proportion of acetate rose from 0·60 to 0·67 while the proportion of propionate fell from 0·21 to 0·18. The yield of microbial matter was reduced by 43% from 14·16 to 8·07 g microbial N per kg organic matter fermented.

Table 3. *Expt 2. Effect of ICI 111075 and monensin on the recovery of hexose energy, carbon and metabolic hydrogen in fermentation end-products*

| | Control | ICI 111075 | Monensin |
|---|---|---|---|
| Energy (% hexose energy) | | | |
| H | 0·01 | 0·05 | 0·01 |
| $CH_4$ | 6·40 | 1·21 | 3·30 |
| VFA | 49·40 | 65·12 | 55·72 |
| Microbial matter | 17·20 | 15·56 | 15·77 |
| Total | 72·99 | 81·94 | 74·80 |
| C (% hexose C) | | | |
| $CO_2$ | 14·62 | 14·41 | 11·83 |
| $CH_4$ | 3·43 | 0·65 | 1·77 |
| VFA | 47·50 | 62·49 | 53·80 |
| Microbial matter | 14·97 | 13·49 | 13·70 |
| Total | 80·52 | 91·04 | 81·10 |
| Metabolic H (% recovery)* | | | |
| H | 0·17 | 0·74 | 0·17 |
| $CH_4$ | 35·97 | 5·72 | 17·41 |
| VFA | 42·32 | 53·28 | 46·60 |
| Microbial matter | 8·68 | 9·54 | 9·29 |
| Total | 87·14 | 69·28 | 73·47 |

* Metabolic H calculated using the equations described by Marty & Demeyer (1973).

Table 4. *Expt 3. Effect of ICI 111075 and monensin on the net production of gases, volatile fatty acids (VFA) and microbial matter and the substrate fermented in a continuous in vitro fermentation system running at a high dilution rate (0·9/d)*

| | Control | ICI 111075 | Monensin | LSD† |
|---|---|---|---|---|
| Gases (mmol/d) | | | | |
| $CO_2$ | 25·83 | 23·72 | 17·71*** | 2·94 |
| $H_2$ | 0·02 | 0·23*** | 0·03 | 0·06 |
| $CH_4$ | 8·71 | 1·12*** | 4·87*** | 1·12 |
| VFA (mmol/d) | | | | |
| Acetic | 27·58 | 25·46 | 23·16*** | 2·36 |
| Propionic | 9·68 | 12·90*** | 10·83 | 1·22 |
| Butyric | 4·91 | 4·78 | 3·49*** | 0·38 |
| Isovaleric | 0·89 | 1·10* | 1·18** | 0·21 |
| Valeric | 1·55 | 1·53 | 1·27*** | 0·13 |
| Total | 45·77 | 46·26 | 40·67** | 3·72 |
| Hexose fermented (mmol/d) | 34·15 | 34·20 | 28·76*** | 1·16 |
| VFA/hexose fermented | 1·33 | 1·36 | 1·42 | 0·13 |
| Microbial yield (gN/kg $OM_f$‡) | 14·16 | 12·64 | 12·77 | — |

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Least significant difference.
‡ Organic matter fermented.

At the high dilution rate ICI 111075 reduced $CH_4$ by 87% and $CO_2$ by 8% but increased $H_2$ 12-fold. At the low dilution rate a similar pattern of effects was observed but with a much greater reduction in $CO_2$ and greater increase in $H_2$ which accompanied the inhibition of $CH_4$. At both dilution rates acetate was reduced by 7% while propionate was increased by 33 and 60% at high and low dilution rates respectively. Total VFA production was increased slightly and the hexose fermented was reduced slightly at both dilution rates but neither

Table 5. *Expt 3. Effect of ICI 111075 and monensin on the net production of gases, volatile fatty acids (VFA) and microbial matter and the substrate fermented in a continuous in vitro fermenter running at a low dilution rate (0·45/d)*

| | Control | ICI 111075 | Monensin | LSD† |
|---|---|---|---|---|
| Gases (mmol/d) | | | | |
| $CO_2$ | 42·33 | 33·81*** | 26·53*** | 2·73 |
| $H_2$ | 0·01 | 1·21*** | 0·02 | 0·20 |
| $CH_4$ | 12·19 | 2·01*** | 6·55*** | 0·98 |
| VFA (mmol/d) | | | | |
| Acetic | 25·06 | 23·56 | 19·98*** | 1·79 |
| Propionic | 7·18 | 11·49*** | 10·85*** | 0·95 |
| Butyric | 4·56 | 4·33 | 3·32*** | 3·46 |
| Isovaleric | 0·49 | 0·49 | 0·50 | 0·06 |
| Valeric | 1·20 | 1·63*** | 0·85*** | 0·14 |
| Total | 39·04 | 41·80* | 35·59* | 2·71 |
| Hexose fermented (mmol/d) | 37·18 | 36·14 | 30·30*** | 1·52 |
| VFA/hexose fermented | 1·06 | 1·16* | 1·20** | 0·09 |
| Microbial yield (gN/kg OMf‡) | 8·07 | 9·20 | 9·19 | — |

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Least significant difference.
‡ Organic matter fermented.

was statistically significant. The yield of VFA per unit hexose fermented (Tables 4 and 5) was not affected at the high dilution rate but showed a 9% improvement at the low dilution rate. The yield of microbial matter was reduced by 11% and increased by 14% at high and low dilution rates respectively.

Monensin reduced $CH_4$ production by 44 and 46% and $CO_2$ by 31 and 37% at high and low dilutions respectively. $H_2$ production was not increased. Acetate production was reduced by 16 and 20%, butyrate production reduced by 29 and 27% and propionate production increased by 12 and 51% at high and low dilution rates respectively. Total VFA production was reduced by 11 and 9% and the hexose fermented reduced by 16 and 19% at high and low rates respectively. Consequently the yield of VFA per unit hexose fermented (Tables 4 and 5) was improved by 7% at high dilution and by 13% at low dilution. The yield of microbial matter was reduced by 10% and increased by 14% at high and low dilutions respectively.

The recovery of hexose energy, C and $H_2$ in fermentation products is summarized in Table 6. In the low dilution rate control fermentation the recovery of hexose energy was 23% lower in VFA and 43% lower in microbial matter in comparison with a high dilution rate control. Losses of hexose energy as $CH_4$ increased by 28% and losses of hexose C as $CO_2$ were increased by 51%. The total recoveries of hexose energy were 84 and 65% in the high and low dilution rate controls respectively.

ICI 111075 increased the recovery of hexose energy in VFA by 6 and 16% at high and low dilution rates. Recoveries of energy in microbial matter were reduced by 10% at high dilution rate but increased by 15% at low dilution rate. Losses of hexose energy as $CH_4$ were reduced by 87 and 83% respectively. Similar patterns were recorded for C recoveries. Treatment with ICI 111075 reduced the total recovery of metabolic H by 22 and 35% relative to controls at high and low dilution rates respectively.

Monensin increased the yield of hexose energy in VFA by 8% at high dilution and by 16% at low dilution. Energy in microbial matter was reduced by 10% at high dilution but

Table 6. *Expt 3. Effect of ICI 111075, monensin and dilution rate on the recovery of hexose energy, carbon and metabolic hydrogen in fermentation end-products*

| | High dilution | | | Low dilution | | |
|---|---|---|---|---|---|---|
| | Control | ICI 111075 | Monensin | Control | ICI 111075 | Monensin |
| Energy (% hexose energy) | | | | | | |
| $H_2$ | * — | 0·01 | * — | * — | 0·08 | * — |
| $CH_4$ | 7·96 | 1·04 | 5·29 | 10·21 | 1·72 | 6·73 |
| VFA | 58·61 | 61·94 | 63·23 | 45·37 | 52·55 | 52·75 |
| Microbial matter | 17·17 | 15·38 | 15·46 | 9·77 | 11·20 | 11·12 |
| Total | 83·74 | 78·37 | 83·29 | 65·35 | 65·55 | 70·60 |
| C (% hexose C) | | | | | | |
| $CO_2$ | 12·61 | 11·58 | 10·28 | 18·98 | 15·60 | 14·57 |
| $CH_4$ | 4·27 | 0·53 | 2·80 | 5·45 | 0·92 | 3·62 |
| VFA | 56·61 | 59·46 | 60·79 | 44·04 | 50·54 | 50·92 |
| Microbial matter | 14·88 | 13·32 | 13·42 | 8·52 | 9·72 | 9·62 |
| Total | 88·37 | 84·89 | 87·29 | 76·99 | 76·78 | 78·73 |
| Metabolic H (% recovery)† | | | | | | |
| $H_2$ | 0·02 | 0·25 | 0·04 | 0·01 | 1·47 | 0·03 |
| $CH_4$ | 37·95 | 4·76 | 24·82 | 60·50 | 9·77 | 38·45 |
| VFA | 42·45 | 50·56 | 49·00 | 37·50 | 48·79 | 49·51 |
| Microbial matter | 8·89 | 9·22 | 9·31 | 8·74 | 9·23 | 9·53 |
| Total | 89·31 | 64·79 | 83·17 | 106·75 | 69·26 | 97·52 |

* $P < 0.005$.
† Metabolic H calculated using the equations described by Marty & Demeyer (1973).

increased by 14% at low dilution. Losses of energy as $CH_4$ were higher than those with ICI 111075 being only 34% lower than control at both dilution rates. Losses of hexose C as $CO_2$ were reduced by 18 and 23% at high and low dilution rates respectively. Again similar effects were seen with C recoveries. The total recoveries of hexose energy and C were not affected greatly by monensin treatment. Total recoveries of metabolic H were only reduced by 7 and 9% relative to controls at high and low dilution rates respectively.

## DISCUSSION

The ability to control strictly fermentation conditions is an important advantage when using a simple continuous fermenter to study the effects of rumen manipulators in vitro. A stable fermentation can normally be achieved in 5–7 d and the agreement between different fermenters is demonstrated by the following means ($\pm$ SE; mmol/d) calculated from single daily determinations for six fermenters over 5 d (Expt 2): total VFA 39·77$\pm$0·02, $CH_4$ 10·67$\pm$0·04 and hexose fermented 33·55$\pm$0·04.

Similar fermenters have been shown to behave in a manner comparable to the in vivo situation (Czerkawski & Breckenridge, 1977) and should provide a useful tool with which to study the quantitative effects of chemical manipulators.

Expt 1 showed that the effects of a $CH_4$ inhibitor, ICI 111075 could be maintained over a 25 d period in a dose-related manner. Inhibition of $CH_4$ was accompanied by increased levels of H and propionic acid and is, therefore, similar to the effects of other $CH_4$ inhibitors (Van Nevel et al. 1969; Trei, Parish, Singh et al. 1971; Marty & Demeyer, 1973). Monensin reduced substrate digestion and production of $CH_4$, acetate, butyrate and total VFA but increased propionate production. The profile of activity was comparable with other in vitro experiments (Richardson et al. 1976; Van Nevel & Demeyer, 1977; Slyter, 1979).

Expt 2 demonstrated that both manipulators increased the yield of hexose energy, C and

2H in VFA. ICI 111075 increased total VFA production, mainly as a result of increased propionate. A small increase in hexose fermented was also observed. Monensin, however, reduced total VFA production although the molar proportion of propionate increased. Since the amount of hexose fermented was substantially reduced the yield of VFA per unit hexose fermented was increased. These improvements were balanced by reductions in $CH_4$ production of 81 and 57% and reductions in microbial yield of 10 and 9% for ICI 111075 and monensin respectively.

Monensin has reduced microbial cell yields in batch culture (Van Nevel & Demeyer, 1977) and in semi-continuous batch culture (Short, 1978) using micro-organisms unadapted to the compound. Short (1978) using rumen fluid from sheep previously receiving monensin found that cell yield was not reduced by further addition of monensin. No indications of adaptation have been observed in the continuous fermenters over a 25 d period using the factors of microbial yield or substrate digestion.

ICI 111075 produced little effect on digestibility while monensin had a consistent inhibitory effect. Short (1978) found that monensin had no effect on cellulose digestion in rumen fluid from animals 'adapted' to monensin. Lemenager et al. (1978) found that monensin depressed digestibility in fluid from both control and monensin-treated steers but the effect was less in the latter instance where it was noted that the basal level of digestion was already lower than in the control group. This is in agreement with results (Davies & Stanier, unpublished results) obtained using fluid previously treated with monensin for a 25 d period in a continuous fermenter.

Other differences between ICI 111075 and monensin were: (a) the consistent reduction of $CO_2$ production with monensin which was not entirely due to the depression of hexose fermentation while the $CH_4$ inhibitor had little effect; (b) the large increase in $H_2$ production accompanying the depression of $CH_4$ production with ICI 111075 while monensin tended to reduce $H_2$ below control values, the latter being in agreement with Slyter (1979).

The results highlight the different effects these manipulators have on the rumen fermentation and show that both increase the yield of hexose energy in VFA which are likely to benefit animal performance.

Monensin treatment reduces rumen dilution rate by 31% in steers (Lemenager et al. 1978) and by 42% in sheep (Allen & Harrison, 1979). The effects of changing dilution rate have been demonstrated in vivo (Harrison et al. 1974; Harrison et al. 1975; Thomson et al. 1975; Thomson et al. 1978). Expt 3 was designed to show the effects of dilution rate on the action of ICI 111075 and monensin. In control fermenters reducing the dilution rate from 0·90/d to 0·45/d increased the hexose fermented but reduced the net production of VFA. Consequently the yield of VFA per unit hexose fermented was reduced by 20%. Losses of hexose as $CO_2$ and $CH_4$ were increased greatly at the low dilution rate. The conversion of VFA to $CO_2$ and $CH_4$ has been reported by Isaacson et al. (1975); Rowe et al. (1979) and A. Davies, M. L. Loughnan, J. V. Nolan and R. A. Leng, unpublished results. Rowe et al. (1979) isolated considerable numbers of Methanosarcina from sheep fed molasses-based diets. They concluded that their presence indicated that secondary fermentation was occurring in the rumen and that this could explain the conversion of acetate-C to $CO_2$. It was also noted that rumen dilution rates are very low on the diets used. These results are, therefore, comparable with those obtained in the fermenters and the secondary fermentation of VFA to $CO_2$ and $CH_4$ may well be the reason for the observed low yield of VFA which accompanied a large increase in $CO_2$ and $CH_4$ production at the low dilution rate. The molar proportions of VFA were not affected significantly by the dilution rates used but the yield of microbial matter was reduced by 43% at the low dilution rate. The latter was in agreement with Owens & Isaacson (1977).

The addition of ICI 111075 and monensin produced the expected effects on the

fermentation. The responses were similar at both dilution rates apart from a much greater increase in propionate production at the low dilution rate. Consequently the recovery of hexose energy and C in VFA showed a much greater improvement under these conditions. Both compounds increased the yield of microbial matter by 14% at the low dilution rate but reduced it by 10% at the high dilution rate.

Calculation of metabolic H recoveries using the established stoichiometry described by Marty & Demeyer (1973) and Demeyer & Van Nevel (1975) resulted in control recoveries of 89 and 106% at high and low dilution rates respectively. Monensin gave recoveries of 83 and 98% while ICI 111075 gave recoveries of only 65 and 69% at high and low dilution rates respectively. Clearly in the case of the $CH_4$ inhibitor the stoichiometric equations do not account for a large proportion of the metabolic H produced. The latter is in agreement with Marty & Demeyer (1973) who found that recoveries of metabolic H were low with a variety of $CH_4$ inhibitors. The recovery of metabolic H in VFA was increased by 19 and 15% relative to control with ICI 111075 and monensin respectively. At the low dilution rate this effect was greater, recoveries in VFA being increased by 30 and 32% with ICI 111075 and monensin respectively.

The theoretical hexose fermented calculated from the equations described by Marty & Demeyer (1973) gave values 24, 22 and 25% less than the observed values for control, ICI 111075 and monensin fermentations respectively, at a high dilution rate.

At the low dilution rate the theoretical hexose fermented was 40, 34 and 34% less than the observed values for control, ICI 111075 and monensin fermentations respectively. The theoretical calculations predicted a reduced fermentation of hexose at the low dilution rate relative to the high dilution rate when in fact a slight increase was observed. These results may indicate an overestimation of the observed hexose fermented or the formation of end-products not included in the equation to calculate the hexose fermented. Use of the calculated hexose fermented increases substantially the values for microbial yield giving a range of 13–22 g N/kg $OM_f$ over all treatments in Expts 2 and 3. This compares with a range of 8–14 g N/kg $OM_f$ using the observed values.

The experiment of Allen & Harrison (1979) showed that treating sheep with monensin increased total VFA production and organic matter digestion, but resulted in no change in VFA yield. The efficiency of microbial growth was markedly reduced. These results were compatible with the observed reduction in rumen dilution rate which accompanied treatment with monensin. In the present study an analogous system was proposed which could possibly predict the in vivo effects of monensin by comparing results of a high-dilution rate control fermenter with that of a low-dilution-rate-monensin-treated fermenter. The results show that quantitatively a combination of monensin treatment and a reduction in dilution rate cancel out the improved VFA yield observed with monensin treatment alone. The reduction in fermented hexose due to monensin was less in the combined treatment as a result of the increase in hexose fermentation at the lower dilution rate. Relative to a high-dilution-rate control microbial yield was depressed when monensin treatment was accompanied by a low dilution rate. The in vitro results are therefore comparable with the results of Allen & Harrison (1979) and suggest that where chemical manipulators of rumen fermentation also reduce dilution rate the net effect on the system may depend on the balance between often opposing shifts in the fermentation.

In summary the results show that the effects of two different types of rumen manipulator can be maintained, quantified and compared using a continuous fermentation system. The responses obtained were similar to those found in vivo. Using in vivo results the effect of dilution rate was demonstrated and it was shown that the net effect of chemical manipulators on the rumen fermentation can be modified substantially by additional effects such as a reduction in dilution rate. The practical implications of the results are likely to involve

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changes in food intake, increased importance of secondary fermentations and reduced effects of nutrients not degraded in the rumen.

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