APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1977, p. 251-257
Vol. 34, No. 3

Printed in U.S.A.

Effect of Monensin on Rumen Metabolism In Vitro

C. J. VAN NEVEL* AND D. I. DEMEYER
Laboratorium voor Voeding en Hygiëne, Rijksuniversiteit Gent, B-9230 Melle, Belgium

Received for publication 9 March 1977

The effect of Monensin (Rumensin, Eli Lilly & Co.) in incubations with mixed rumen microorganisms metabolizing carbohydrate or protein substrates was investigated. Monensin partly inhibited methanogenesis and increased propionate production, although the effect was not always statistically significant. Incubations with substrates specific for methane bacteria suggest that inhibition of methanogenesis by Monensin was not due to a specific toxic action on the methanogenic flora, but rather to an inhibition of hydrogen production from formate. Total and net microbial growth were considerably decreased by addition of Monensin, although the amount of substrate fermented was not altered, resulting in lowered values of microbial growth efficiency. In incubations with casein, Monensin lowered protein degradation in line with a lowered ammonia production, whereas a slight accumulation of α-amino nitrogen was observed. The results suggest that besides an influence of Monensin on the rumen carbohydrate fermentation pattern, another reason for the beneficial effects observed in vivo might be decreased food protein degradation in the rumen, altering the final site of protein digestion in the animal. Also, the possibility of a decrease in rumen microbial growth efficiency has to be considered when using Monensin as a food additive.

During the last two years, numerous papers in the literature have dealt with the effect of Monensin (Rumensin, Eli Lilly & Co.) on the performance of beef cattle fed different rations (e.g., 4, 17-19, 21). Monensin-treated animals show an improved feed efficiency, whereas in the rumen a shift in the molar proportions of volatile fatty acids towards propionic acid is observed (7, 17, 22). Methane production is depressed and rumen ammonia levels tend to be lower, indicating a decreased proteolysis and altered site of protein digestion (7; J. Thornton et al., J. Anim. Sci. 43:336, 1976). All these results suggest that the favorable effect of feeding Monensin may be related, at least partly, to its action on rumen fermentation. Other possible reasons, due to interactions with animal metabolism other than rumen metabolism, were reviewed earlier (21, 22). The in vitro experiments described here were conducted to study in more detail and in a quantitative manner the effects of Monensin on the metabolism of carbohydrate and protein substrates by mixed rumen microorganisms, including the aspect of microbial growth yield (protein synthesis). Since nothing is known about the possible mechanisms by which Monensin acts on rumen metabolism in general, and on methane production in particular, incubations with substrates specific for methane bacteria were done. Metabolic hydrogen recovery was calculated from fermentation balances, based on stoichiometric relationships between end products formed. Such balances not only provide a control on the accuracy of the experimental data, but often provide evidence for explaining the mechanism of action of additives influencing the fermentation pattern in the rumen (6, 14, 26–28).

MATERIALS AND METHODS

Animals. Rumen contents were obtained from a wether provided with a rumen fistula and kept indoors in a cage. The animal received 400 g of hay and 200 g of commercial concentrates (crude protein, 14%) twice daily. Samples were withdrawn from the rumen with the apparatus described by Hungate (9) after a fasting period of at least 24 h, so that effects of endogenous substrates would be minimal.

Incubation procedure. Rumen contents were filtered through four layers of surgical gauze, and 40-ml samples were transferred anaerobically to incubation flasks, provided with a rubber septum to permit gas sampling. Carbohydrate substrate (250 μmol each of cellobiose and maltose, reagent grade; Merck, Darmstadt) and 5 mg of nitrogen as NH_4HCO_3 (Merck, Darmstadt) were added, dissolved in 10 ml of Burroughs artificial saliva (3). Besides the experiments with pure carbohydrates as energy substrate, a series of incubations using ground concentrates as substrates (1.5 g/flask) was carried out. Samples (40 ml) of washed cell suspen-

sions of mixed rumen bacteria, prepared as described elsewhere (5), were incubated in a similar manner, but Hungate's buffer (10) was used instead of Burroughs saliva.

Incubations with substrates specific for methane bacteria were done with 2.5 mmol of sodium formate (Merck, Darmstadt) or a CO_2-H_2 (50%-50%) gas mixture (L'Air Liquide, Ghent). The gas volume of an incubation flask is about 110 ml. Unless stated otherwise, the incubation time was 2 h, and CO_2 (99.995% pure, L'Air Liquide) was used as incubation gas. Fermentation was stopped by injecting 1 ml of H_2SO_4 (10 N) at the end of incubation or before incubation (blank values). For incubations with protein, 250 mg of isoelectric casein (Difco, Detroit, Mich.), dissolved in 10 ml of Burroughs saliva, was used as the sole substrate, and incubation was for 3 h under CO_2. Here blank values were obtained by incubation of rumen fluid without any substrate. Crystalline Monensin (potency, 877 mg/g) was a gift from Eli Lilly Benelux, Brussels, and was added in ether solution in concentrations ranging from 0.5 to 25 μg/ml, comparable to that done in other recent work (22). Ether was removed from the incubation flask by evaporation before adding rumen fluid.

Analysis. Fermentation end products, methane, hydrogen, volatile fatty acids, lactic acid, and ammonia-nitrogen, were determined as described earlier (5, 25, 28). "Total" and "net" microbial growth was determined by measuring the incorporation of ^{32}P-labeled phosphate in microbial material and the decrease of ammonia-nitrogen in the ammonia pool, respectively (25).

Results are expressed in terms of nitrogen incorporated (N_{inc}) in microbial material per 100 μmol of hexose fermented (growth yield). From phosphorus incorporation (P_{inc}), N_{inc} was calculated as $P_{inc} \times 8.37 = N_{inc}$, based on the average N/P ratio in rumen microbial dry matter (25). In incubations with casein, protein degradation was determined by the biuret method (8) on the supernatant after centrifugation (20,000 $\times$ g, 15 min, 0°C) of a sample of incubation fluid. Therefore, in these incubations, fermentation was not stopped by injecting H_2SO_4, but during preparatory procedures incubation flasks were kept in an ice bath. α-Amino nitrogen was determined after deproteinization of 1 ml of incubation fluid with $HClO_4$ (0.6 N) on the supernatant, using the ninhydrin reagent described by Oddy (15) after elimination of NH_3 by evaporation to dryness in strong alkaline conditions.

Calculation of metabolic hydrogen recovery and fermentation balances. Hydrogen recoveries were calculated as described by Demeyer and Van Nevel (6). Distribution of metabolic hydrogen (2H) over reduced fermentation end products was calculated as follows: (i) for total hydrogen used in end products, $2\ H_u = 2P + 2B + 4M + H_2$; (ii) for percentage of 2H used in P = $(2P/2H_u \times 100$, in B = $(2B/2H_u) \times 100$, in M = $(4M/2H_u) \times 100$, and in H_2 = $(H_2/H_u) \times 100$, where P, B, M, and H_2 are net production (micromoles) of propionate, butyrate, methane, and hydrogen gas, respectively. The amount of hexose fermented during incubation was calculated as $C_6 = (A/2) + (P/2) + B$.

For casein incubations it was assumed that amino acids are degraded by deamination, followed by decarboxylation of the keto acids formed (R. A. Prins, *in* R. T. J. Clarke and T. Bauchop [ed.], *The Normal Flora of the Gut*, in press). A stoichiometric relationship between volatile fatty acids and ammonia production can be derived: fermentation of 1 mol of amino acids (AA) yields 1 mol of ammonia (NH_3) and 1 mol of acetic (A), propionic (P), isovaleric (IV), and valeric (V) acids and 0.5 mol of butyric acid (B). Per mole of valeric acid formed, a second mole of ammonia is formed by deamination of δ-aminovaleric acid. The relation can be written as: $NH_3 = AA = A + P + IV + 2V + (B/2)$ (D. I. Demeyer, Aggregaatsthesis, State University of Ghent, Ghent, Belgium, 1976).

Statistical analysis. Results were statistically analyzed by a t test on paired observations (24).

RESULTS

Effect of monensin on production of methane and volatile fatty acids. In Tables 1 and 2, the effect of Monensin on the fermentation pattern in incubations with rumen fluid, using concentrates or a mixture of cellobiose-maltose and ammonium bicarbonate as substrate, is illustrated. The overall effect is comparable to in vitro and in vivo results described earlier in the literature, although the increase in propionate formation was not always statistically significant (4, 7, 17, 21, 22). Although calculation of mean values indicates a lowered methane production after Monensin treatment (in 17 out of a total of 25 individual incubations), this effect was not statistically significant in most cases. Table 3 shows the distribution of metabolic hydrogen over the reduced fermentation end products and a shift to propionic acid was noted, but this was only significant at the lower Monensin concentrations. In the same table we present the hydrogen recoveries, calculated as described earlier (6). Hydrogen recovery tended to be lower in Monensin-treated incubations, although not statistically significant. Production of negligible amounts of lactic acid (<4 μmol/flask) or hydrogen gas (<2 μmol/flask) was observed in this series of incubations. This indicated that a normal type of rumen fermentation occurred.

Microbial growth yield. In the incubations with sugars (Table 2), total growth and net growth were determined, and values were expressed as growth efficiencies (milligrams of nitrogen incorporated per 100 μmol of hexose fermented). In the absence of Monensin, the range of values obtained was equivalent to 51.1 to 80.6 and 13.0 to 17.2 g of N_{inc} per kg of organic matter fermented for total and net growth, respectively, in reasonable agreement with other data obtained in vitro as well as in vivo (25).

TABLE 1. *Effect of Monensin on the fermentation pattern in incubations in vitro of rumen fluid with concentrates as substrate*[a]

Monensin (μg/ml)	Fermentation pattern											
	Acetic acid		Propionic acid		Butyric acid		Methane		C_6 fermented[b]		NH_3 production	
	Mean	t calculated	Mean	t calculated	Mean	t calculated	Mean	t calculated	Mean	t calculated	Mean	t calculated
0 (4)[c]	105[d]		56[d]		20[d]		43[d]		1,210		4.29[e]	
		2.80 (NS)[f]		6.18[g]		2.12 (NS)		1.45 (NS)		0.82 (NS)		4.45[g]
5 (4)	97		67		18		37		1,114		2.51	
0 (4)	105		56		20		43		1,210		4.29	
		5.00[h]		3.82[h]		2.19 (NS)		1.38 (NS)		2.58 (NS)		5.79[g]
25 (4)	89		71		18		39		1,017		2.38	

[a] A 40-ml amount of rumen fluid was incubated with 1.5 g of ground concentrates, as described in the text.
[b] Micromoles of hexose fermented, calculated from the production of acetic (A), propionic (P), and butyric (B) acid: C_6 fermented = (A/2) + (P/2) + B.
[c] Number in parentheses is number of incubations.
[d] Expressed as micromoles per 100 μmol of C_6 fermented.
[e] Milligrams of NH_3-N per incubation flask.
[f] NS, Not significant.
[g] Significant at $P < 0.01$.
[h] Significant at $P < 0.05$.

Efficiency for both total and net growth was significantly depressed by Monensin, and it seems that net growth was somewhat more inhibited than total growth (Table 4).

Mechanism of methane inhibition. To find out if a direct toxic action of the antibiotic on methane bacteria was responsible for the frequently observed inhibition of methane production, we carried out incubations with formate or a gas mixture of CO_2 and H_2, both substrates being specific for methane bacteria (16, 23) (Table 5). This assumes that methane production is a representative parameter for the indication of a toxic effect of a compound on methanogens. Methane production from CO_2-H_2 was not affected by Monensin, but with formate as substrate considerable inhibition was noted. Hydrogen gas accumulated but in amounts considerably below those expected from inhibited methane production (70 versus 1,600 μmol). This enables us to conclude that the methane-depressing property of Monensin is not due to a direct toxic effect on the methanogenic flora in the rumen, but rather to an inhibition of organisms decomposing formate to CO_2 and H_2, these gases being, quantitatively, by far the most important substrates for methane bacteria (12). Additional support for this theory is found in the fact that in incubations where methane production was depressed, we always found a lower hydrogen recovery compared with the blank (Table 6), indicating accumulation of a reduced end product other than methane, gaseous hydrogen, propionate, and butyrate, since these are involved in the calculation of a hydrogen balance. This unknown accumulated product may be formic acid.

Effect of Monensin on protein incubations. In these incubations, casein was added as the sole substrate to enable calculation of fermentation balances based on the stoichiometry of protein fermentation (see Materials and Methods). The results are summarized in Table 7. Monensin lowered protein disappearance and resulted in a slightly higher accumulation of α-amino nitrogen and a considerable depression in ammonia production. Calculated nitrogen recoveries were somewhat low, especially in the blank incubations, perhaps due to accumulation of peptide nitrogen (13) not recovered by the α-amino nitrogen determination. Addition of carbohydrates (cellobiose and maltose) as the energy source in these incubations did not influence the effect of Monensin on protein breakdown. In Table 8, the effect of Monensin on the fermentation pattern of casein is illustrated. A strong inhibition of methane and volatile fatty acid production is apparent. Actual ammonia

TABLE 2. *Effect of Monensin on methane and volatile fatty acid production in incubations of rumen fluid with cellobiose-maltose-NH_4HCO_3 as substrate*[a]

Monensin (μg/ml)	Fermentation end products									
	Acetic acid		Propionic acid		Butyric acid		Methane		C_6 fermented[b]	
	Mean	t calculated	Mean	t calculated	Mean	t calculated	Mean	t calculated	Mean	t calculated
0 (2)[c]	90[d]		33[d]		39[d]		64[d]		561	
		4.5 (NS)[e]		4.0 (NS)		5.0 (NS)		8.0[f]		6.1 (NS)
0.5	81		37		41		48		767	
0 (8)	104		55		22		48		535	
		0.3 (NS)		2.6[f]		0.2 (NS)		2.4[f]		0.9 (NS)
1.0	103		60		22		41		561	
0 (10)	107		60		17		40		526	
		1.0 (NS)		2.1[f]		0.9 (NS)		1.2 (NS)		0.3 (NS)
5.0	104		64		16		36		511	
0 (6)	104		63		17		39		526	
		0.2 (NS)		1.6 (NS)		0.7 (NS)		0.8 (NS)		0.7 (NS)
25.0	103		67		15		34		486	

[a] Incubation circumstances as described in the text.
[b] Micromoles of hexose fermented: (A/2) + (P/2) + B.
[c] Number in parentheses is number of incubations.
[d] Micromoles per 100 μmol of C_6 fermented.
[e] NS, Not significant.
[f] Significant at $P < 0.05$.

TABLE 3. *Effect of Monensin on distribution of metabolic hydrogen (2H) over reduced fermentation end products and hydrogen recovery in in vitro incubations*[a]

Monensin (μg/ml)	2H in reduced end products (%)							
	Methane		Propionic acid		Butyric acid		2H recovery (%)	
	Mean	t calculated	Mean	t calculated	Mean	t calculated	Mean	t calculated
0	64		15		20		108.5	
		181[b]		31[c]		44[c]		4.33 (NS)[d]
0.5	55		21		24		95.5	
0	54		33		13		100.5	
		2.8[c]		2.5[c]		1.43 (NS)		1.36 (NS)
1.0	48		38		14		96.4	
0	50		39		11		97.8	
		2.1 (NS)		1.8 (NS)		2.10 (NS)		0.95 (NS)
5.0	44		45		11		90.8	
0	49		41		10		93.0	
		1.6 (NS)		1.4 (NS)		3.08[c]		0.24 (NS)
25.0	40		48		12		91.0	

[a] Calculated as described in the text and by Demeyer and Van Nevel (6) from results in Table 2.
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] NS, Not significant.

production (522 μmol) was in reasonable agreement with the production calculated from volatile fatty acid production (483 μmol) (see Materials and Methods) for blank incubations, but considerable discrepancies were noted in the presence of Monensin. In the latter case, however, values for volatile fatty acid production showed abnormally high variation (Table 8). As with carbohydrate as substrate, significant amounts of lactate and hydrogen gas were never detected.

DISCUSSION

Although it might be expected that the beneficial effect of Monensin on beef cattle performance is at least partly due to its action on rumen metabolism, only a few papers in the literature have dealt with this subject thus far (7,

TABLE 4. *Influence of Monensin on rumen microbial growth yields in vitro*[a]

Monensin (μg/ml)	Total growth[b]		Net growth[c]	
	Mean value	t calculated	Mean value	t calculated
0	1.306		0.211	
		10.32[d]		8.25[d]
0.5	0.620		0.046	
0	0.947		0.262	
		3.54[e]		1.55 (NS)[f]
1.0	0.654		0.185	
0	0.828		0.279	
		2.45[d]		5.18[e]
5.0	0.583		0.134	
0	0.828		0.279	
		3.91[e]		8.39[e]
25.0	0.583		0.052	

[a] Determined in the incubations as presented in Table 2. Results are given in milligrams of nitrogen incorporated in microbial material per 100 μmol of hexose fermented [(A/2) + (P/2) + B].

[b] Nitrogen incorporation calculated from $^{32}PO_4^{3-}$ incorporation.

[c] Nitrogen incorporation calculated from NH_3 incorporation.

[d] $P < 0.05$.

[e] $P < 0.01$.

[f] NS, Not significant.

22). In the work described here, we tried to quantitate the effect of the antibiotic on rumen metabolism, using incubations in vitro to enable an exact determination of end products formed, as well as of microbial growth yields. The overall effect of the drug on the rumen fermentation pattern in vitro is comparable to results reported earlier and obtained in vivo (7, 17, 21). In our experiments, however, the inhibitory effect on methane production and the stimulation of propionic acid production were not always statistically significant.

Our results indicate that Monensin has no direct toxic effect on the methanogenic flora, since no effect on methane formation was obtained with gaseous carbon dioxide and hydrogen as the substrate. With formate as the substrate, inhibition of methanogenesis was obtained, whereas gaseous hydrogen was only found in amounts far below the amounts calculated from inhibited methane production: e.g., 70 versus 1,600 μmol at the 100-μg/ml level in the washed cell suspension experiment. This indicates that Monensin specifically inhibits formate decomposition, whether added as substrate or formed in the pyruvate lyase reactions during carbohydrate fermentation. A similar

TABLE 5. *Effect of Monensin on methane production from CO_2-H_2 or formate*[a]

Substrate	No. of incubations	Incubated medium[a]	Monensin added (μg/ml)				
			0	1	5	25	100
CO_2-H_2 (50%-50%)	4	SRF	100[b] (609)[c]	103 ± 8[b]	96 ± 5	108 ± 12	101 ± 8
	2	WCS	100 (311)	98 ± 4	98 ± 2	96 ± 2	91 ± 5
Formate	2	SRF	100 (719)	98 ± 2	82 ± 11	85[d]	74 ± 12
	2	WCS	100 (648)	97 ± 4	93 ± 0.2	77 ± 5	38 ± 3

[a] A 40-ml amount of strained rumen fluid (SRF) or washed cell suspension (WCS) was incubated for 2 h with 10 ml of Burroughs solution (SRF) of phosphate buffer (WCS) and Monensin under CO_2-H_2 (50%-50%). For incubations with formate, 2.5 mmol of sodium formate was incubated under CO_2 (100%).

[b] Relative amount of methane produced: mean value ± standard error (value without Monensin = 100).

[c] Number in parentheses is absolute amount of methane formed in micromoles per flask.

[d] One incubation only.

TABLE 6. *Effect of Monensin on hydrogen recovery (percentage) in incubations in which methanogenesis was inhibited*[a]

Monensin (μg/ml)				
0	0.5	1.0	5.0	25.0
112 (100)[b]	96 (73)	101 (75)		
105 (100)	95 (77)	104 (85)		
115 (100)		109 (91)	94 (72)	100 (81)
122 (100)		105 (78)	98 (73)	—[c]
90 (100)		81 (59)	76 (52)	73 (34)
88 (100)		85 (75)	74 (50)	73 (39)

[a] Incubations with cellobiose-maltose and NH_4HCO_3 as substrate (see Table 2), but here only incubations in which methane was inhibited are shown. Hydrogen recovery was calculated as described in the text.

[b] Number in parentheses gives methane production as a percentage of the blank value (without Monensin).

[c] Sample lost.

TABLE 7. *Effect of Monensin on casein fermentation in vitro*[a]

Monensin added (μg/ml)	N fractions						N recovery (%)
	NH_3-N formed (10)[b]		α-NH_2-N formed (6)[b]		Protein N degraded (4)[b]		
	Mean value	t calculated	Mean value	t calculated	Mean value	t calculated	
0	8.868[c]		3.643		15.975		78.3[d]
		9.88[e]		4.43[e]		4.73[f]	
5	3.834		5.948		10.825		90.4
0	8.868		3.643		15.975		78.3
		12.43[e]		4.19[e]		3.63[f]	
25	3.335		5.898		9.575		96.4

[a] A 40-ml amount of rumen fluid was incubated with 250 mg of casein as described in detail in the text.
[b] Number in parentheses is number of incubations.
[c] Milligrams of nitrogen per incubation flask (net values).
[d] Calculated as (NH_3-N + α-NH_2-N)100/protein nitrogen degraded.
[e] $P < 0.01$.
[f] $P < 0.05$.

TABLE 8. *Effect of Monensin on methane and volatile fatty acid production in incubations with casein*[a]

End product formed	μmol/incubation flask with Monensin added (μg/ml) at:		
	0	5	25
Methane	80 ± 8[b]	9 ± 3	6 ± 4
Acetic acid	181 ± 29	45 ± 49	−71 ± 51
Propionic acid	178 ± 14	41 ± 24	−12 ± 14
Butyric acid	66 ± 9	31 ± 11	15 ± 12
Isovaleric acid[c]	63 ± 7	12 ± 5	6 ± 5
Valeric acid	14 ± 6	1 ± 0.6	0

[a] Same incubations as presented in Table 7.
[b] Mean value ± standard error (net values).
[c] Includes both isovaleric and 2-methylbutyric acids.

mechanism for the effect on methane production of chlortetracycline was suggested earlier (11). Hungate et al. (12) estimated that about 18% of rumen methane is derived from formate. This rather small contribution of formate to the hydrogen formed as precursor for methanogenesis may explain the less pronounced inhibition of methane production found with carbohydrates as the substrate when compared with formate as the substrate. An increase in propionate production accompanying inhibition of methanogenesis reflects a shift from the flow of electrons from formate and methane to succinate or propionate as alternate electron sink products in the mixed microbial population (6, 29).

A thus far unknown but important property of Monensin is the inhibitory effect on microbial growth yield observed in our incubations, using two different methods measuring total and net microbial growth, respectively (25). As growth inhibition was not accompanied by a decrease in fermentation activity in terms of fermentation products formed, part of the energy (adenosine 5'-triphosphate) generated during fermentation and normally used for maintenance and growth of the microbes must be used for other purposes when Monensin is added. It is tempting to suggest that Monensin uncouples growth from fermentation. The mechanism of this action could be related to an altered cell membrane permeability, since Monensin is known to be an ionophore (20). Another very interesting effect of Monensin is the considerable decrease in protein degradation in incubations with casein. This effect is in line with the lowered rumen ammonia level found in vivo (7) and in our in vitro incubations with concentrates (Table 1). Accumulation of α-amino nitrogen as an intermediate indicates a faster rate of proteolysis than the rate of amino acid metabolism, a well-known phenomenon (1). The overall effect on casein fermentation suggests that deaminase activity is more inhibited than proteolysis, although it is possible that Monensin has an inhibitory effect on peptide transport into microbial cells, as it is known that some bacteria can only use NH_3 or peptides as nitrogen source (2). This is important in view of very recent work indicating that feeding a specific deaminase inhibitor 4,4'-dimethyldiphenyl iodonium chloride had a favorable effect on growth rate and feed efficiency of beef cattle fed a roughage diet with 11% crude protein (W. Chalupa et al., J. Anim. Sci. 43:316, 1976). All available information strongly suggests that besides the influence of Monensin on the fermentation pattern in the rumen, another reason for the beneficial effect of the drug may be inhibition of feed protein degradation in the rumen, thus altering the final site of protein digestion in the animal. This is, however, coupled to a decreased efficiency of rumen micro-

bial protein synthesis, which could be a disadvantage depending on the nature of the diet and the amount of protein escaping rumen degradation. For application of Monensin in ruminant feeding, the relative importance of both effects, i.e., the protein-sparing effect through lowered protein degradation in the rumen versus inhibition of microbial protein synthesis, should be investigated under in vivo conditions. It should be mentioned here that Dinius et al. (7) could not find any effect of Monensin on the number of rumen protozoa and total and cellulolytic bacteria. Finally, the possible depression of microbial growth efficiency becomes very important when feeding diets where non-protein nitrogen partly replaces protein, but Davis and Erhart (4) showed that Monensin still has a beneficial effect when feeding a finishing steer ration (11.9% crude protein) where 2% of the crude protein was replaced by urea.

ACKNOWLEDGMENTS

This investigation was supported by the I.W.O.N.L., Brussels.

The technical help of C. Vermander, G. De Decker, N. Faquaet, and G. Van de Velde is gratefully acknowledged. Thanks are due to R. Moermans from C.L.O. Ghent for statistical analysis of the results. Monensin was kindly supplied by Eli Lilly Benelux, Brussels.

LITERATURE CITED

1. **Blackburn, T. H.** 1965. Nitrogen metabolism in the rumen, p. 322-334. *In* R. W. Dougherty (ed.), Physiology of digestion in the ruminant. Butterworths, Washington, D.C.
2. **Bryant, M. P.** 1970. Normal flora—rumen bacteria. Am. J. Clin. Nutr. 23:1440-1450.
3. **Burroughs, W. N., A. Frank, P. Gerlaugh, and R. M. Bethke.** 1950. Preliminary observations upon factors influencing cellulose digestion by rumen micro-organisms. J. Nutr. 40:9-24.
4. **Davis, G. V., and A. B. Erhart.** 1976. Effect of Monensin and urea in finishing steer rations. J. Anim. Sci. 43:1-8.
5. **Demeyer, D. I., and H. K. Henderickx.** 1967. The effect of C 18 unsaturated fatty acids on methane production in vitro by mixed rumen bacteria. Biochim. Biophys. Acta 137:484-497.
6. **Demeyer, D. I., and C. J. Van Nevel.** 1975. Methanogenesis, an integrated part of carbohydrate fermentation and its control, p. 366-382. *In* I. W. McDonald and A. C. I. Warner (ed.), Digestion and metabolism in the ruminant. The University of New England Publishing Unit, Armidale, Australia.
7. **Dinius, D. A., M. E. Simpson, and P. B. Marsh.** 1976. Effect of Monensin fed with forage on digestion and the ruminal ecosystem of steers. J. Anim. Sci. 42:229-234.
8. **Herbert, D., P. J. Phipps, and R. E. Strange.** 1971. Chemical analysis of microbial cells, p. 209-344. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 5B. Academic Press Inc., London.
9. **Hungate, R. E.** 1950. The anaerobic mesophilic cellulolytic bacteria. Bacteriol. Rev. 14:1-49.
10. **Hungate, R. E.** 1969. A roll tube method for cultivation of strict anaerobes, p. 117-132. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 3B. Academic Press Inc., London.
11. **Hungate, R. E., D. W. Fletcher, and I. A. Dyer.** 1955. Effects of chlortetracycline feeding on bovine rumen microorganisms. J. Anim. Sci. 14:997-1002.
12. **Hungate, R. E., W. Smith, T. Bauchop, I. Yu, and J. C. Rabinowitz.** 1970. Formate as an intermediate in the bovine rumen fermentation. J. Bacteriol. 102:389-397.
13. **Mangan, J. L.** 1972. Quantitative studies on nitrogen metabolism in the bovine rumen. The rate of proteolysis of casein and ovalbumin and the release and metabolism of free amino acids. Br. J. Nutr. 27:261-283.
14. **Marty, R. J., and D. I. Demeyer.** 1973. The effect of inhibitors of methane production on fermentation pattern and stoichiometry in vitro using rumen contents from sheep given molasses. Br. J. Nutr. 30:369-376.
15. **Oddy, V. H.** 1974. A semiautomated method for the determination of plasma alpha amino nitrogen. Clin. Chim. Acta 51:151-156.
16. **Paynter, M. J. B., and R. E. Hungate.** 1968. Characterization of *Methanobacterium mobilis* sp.n., isolated from the bovine rumen. J. Bacteriol. 95:1943-1951.
17. **Perry, R. W., W. M. Beeson, and M. T. Mohler.** 1976. Effect of Monensin on beef cattle performance. J. Anim. Sci. 42:761-765.
18. **Potter, E. L., C. O. Cooley, L. F. Richardson, A. P. Raun, and R. P. Rathmacher.** 1976. Effect of Monensin on performance of cattle fed forage. J. Anim. Sci. 43:665-669.
19. **Potter, E. L., A. P. Raun, C. O. Cooley, R. P. Rathmacher, and L. F. Richardson.** 1976. Effect of Monensin on carcass characteristics, carcass composition and efficiency of converting feed to carcass. J. Anim. Sci. 43:678-683.
20. **Pressman, B. C., and N. T. deGuzman.** 1975. Biological applications of ionophores: theory and practice. Ann. N.Y. Acad. Sci. 264:373-386.
21. **Raun, A. P., C. O. Cooley, E. L. Potter, R. P. Rathmacher, and L. F. Richardson.** 1976. Effect of Monensin on feed efficiency of feedlot cattle. J. Anim. Sci. 43:670-677.
22. **Richardson, L. F., A. P. Raun, E. L. Potter, C. O. Cooley, and R. P. Rathmacher.** 1976. Effect of Monensin on rumen fermentation in vitro and in vivo. J. Anim. Sci. 43:657-664.
23. **Smith, P. H., and R. E. Hungate.** 1958. Isolation and characterization of Methanobacterium ruminantium. J. Bacteriol 75:713-718.
24. **Snedecor, G. W.** 1955. Statistical methods. The Iowa State College Press, Ames.
25. **Van Nevel, C. J., and D. I. Demeyer.** 1977. Determination of rumen microbial growth in vitro from ^{32}P labelled phosphate incorporation. Br. J. Nutr. 38:101-114.
26. **Van Nevel, C. J., D. I. Demeyer, B. G. Cottyn, and C. V. Boucqué.** 1973. Incorporation of linseed oil hydrolysate and sodium sulfite in rations for beef production. 2. Effect on rumen fermentation in vivo. Z. Tierphysiol. Tierernaehr. Futtermittelkd. 31:66-71.
27. **Van Nevel, C. J., D. I. Demeyer, B. G. Cottyn, and H. K. Henderickx.** 1970. Effect of sodium sulfite on methane and propionate in the rumen. Z. Tierphysiol. Tierernaehr. Futtermittelkd. 25:91-100.
28. **Van Nevel, C. J., H. K. Henderickx, D. I. Demeyer, and J. Martin.** 1969. Effect of chloral hydrate on methane and propionic acid in the rumen. Appl. Microbiol. 17:695-700.
29. **Wolin, M. J.** 1974. Metabolic interactions among intestinal microorganisms. Am. J. Clin. Nutr. 27:1320-1328.