ELIOPHYLIN AS A RUMEN FERMENTATION EFFICIENCY ENHANCER

CHAO-MIN LIU, L. JENSEN, J. W. WESTLEY, and D. SIEGEL

Roche Research Center, Hoffmann-La Roche Inc., Nutley, NJ. 07110 U.S.A.

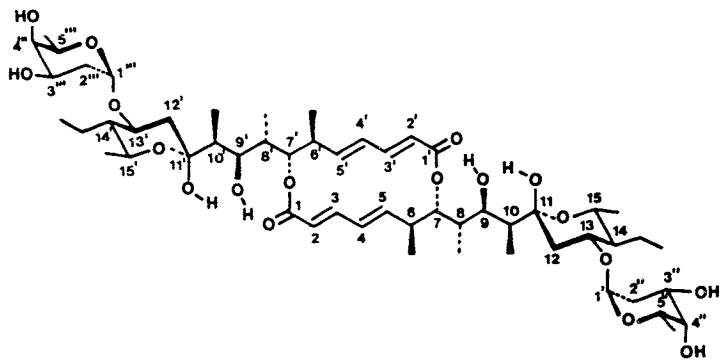
(Received for publication October 5, 1992)

In cattle, the positive correlation between enhanced propionate production in the rumen and an increase in feed conversion after the treatment of low concentration of lasalocid or monensin has been well documented[1~4]. RICHARDSON *et al*[5] showed that the increase in feed efficiency by monensin was a result of increased synthesis of propionic acid and decreased synthesis of acetic acid and *n*-butyric acid by microflora in the rumen. Becuase monensin increases feed efficiency and reduces feed intake, its inclusion in animal feed results in a significant savings in feed costs to the cattle industry. Both monensin and lasalocid are being marketed as cattle growth performance enhancer under the trade name Rumensin and Bovatec, respectively.

During our search for novel cattle growth performance enhancers from microbial sources using an *in vitro* rumen fermentation system[2], a Streptomyces culture was found to produce a substance which elicited unusually high level of propionate production in rumen fluid. Production of the active substance was carried out in a medium containing (in g/liter): tomato paste (Cantalina) 5.0, distiller's soluble 5.0, meat peptone (type-SB, Marco Development Corp., Hackensack, NJ) 5.0, debittered dried 1.0, and K_2HPO_4 1.0. The medium pH was adjusted to 7.0 before sterilization. The fermentation, carried out at 28°C in an aerated stirred fermenter, was harvested at 120 hours. The active compound was extracted from whole broth with an equal volume of ethyl acetate. After concentration, the solids which separated on standing, were collected and decolorized with charcoal and recrystallized from chloride-methanol-acetone. *Anal* calcd for $C_{54}H_{88}O_{18}$: C 63.26, H 8.65, O 28.09. Found: C 63.17, H 8.58, O 28.26. MF: $C_{54}H_{88}O_{18}$ MW: 1,025.28. The infrared, ultraviolet and NMR spectra of the substance were identical to those of elaiophylin[6].

Unlike polyether antibiotics monensin or lasalocid, elaiophylin is a macrolide antibiotic. Its activity as a rumen fermentation efficiency enhancer was evaluated *in vitro* by measuring the change in production of volatile fatty acids (VFA) (acetate (C_2), propionate (C_3) and *n*-butyrate (nC_4)) by rumen fluid samples from a fistulated bovine. The activity of the tested compounds is expressed by the molar ratio of propionate and acetate plus *n*-butyrate [C_3/C_2+nC_4] (VFA ratio) in comparison with that of the untreated control. To evaluate the efficacy of elaiophylin activity *in vivo*, the ratio of VFA produced in the rumen of fistulated animals was analyzed (three times a week) before and after oral medication (as a top dressing at 1 mg/kg daily). Lasalocid, an established VFA regulator with field proven effectiveness as a performance enhancing agent in cattle, was used as a positive control and Ro 2-7187, a glycopeptide identical to antibiotic A5415[7], served as a negative control.

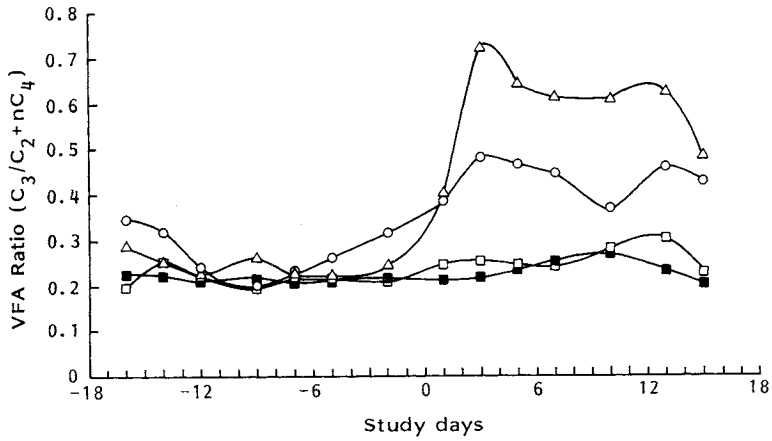
The *in vitro* effect of elaiophylin on VFA ratio and total VFA production in rumen fluid is shown in Table 1. Elaiophylin at cocentrations higher than 10 μg/ml stimulates propionate production at the expense of acetate and *n*-butyrate with activity comparable to that of lasalocid or antibiotic



Elaiophylin

Fig. 1. Change in VFA ratio in medicated animals over 30 day period.

■ Non-medicated control, △ lasalocid (positive control), ○ elaiophylin, □ Ro 2-7187 a lipopeitide (negative control).



Sixteen fistulated bovines were used with 4 animals in each test group. Each animal was medicated with a top dressing twice daily with 1/2 of the daily dosage of 1 mg/kg in the morning and 1/2 in the evening. Rumen fluid was collected three times a week and the VFA ratio in each samples were analyzed as described in Table 1.

Table 1. The effects of elaiophylin, lasalocid and antibiotic X-14873A on *in vitro* rumen fermentation.

Compound concentration (μg/ml) in rumen fluid	VFA molar ratios[b] $[C_3/(C_2+nC_4)]$			Total VFA production (μmoles/ml)		
	A[a]	B[a]	C[a]	A[a]	B[a]	C[a]
0.0 (control)		0.170			89.4	
0.010	0.161	0.170	0.159	107.2	100.0	80.2
0.032	0.164	0.171	0.167	97.9	100.3	93.3
0.10	0.149	0.176	0.162	86.4	100.9	97.2
0.32	0.149	0.176	0.162	79.9	101.4	94.3
1.0	0.166	0.192	0.170	98.8	100.7	7.3
3.2	0.192	0.250	0.179	79.3	104.0	98.9
10.0	0.255	0.296	0.268	103.1	97.8	91.9
32.0	0.286	0.351	0.457	81.2	92.0	84.6
100.0	0.326	0.407	0.679	89.0	80.2	70.3
320.0	0.443	0.547	0.689	72.9	66.2	75.2
1,000.0	0.474	0.526	—	64.2	67.0	—

The rumen fluid was obtained from a fistulated animal prior to the morning feeding. The animal was fed twice daily with 80% concentrate*: 20% rough (hay) ration (80/20 ration). The buffered rumen fluid was prepared as described[9]. Individual fermentations were carried out in a 250-ml Erlenmeyer flask. First, 0.75 g of 80/20 ration was mixed with 1 ml of compound dissolved in methanol and allowed to stand overnight. 60 ml of buffered rumen fluid were then added. The mixture in flasks was incubated at 39°C and shaken (120 oscillations/minute) for 4 hours. After incubation, the fermentation was stopped by mixing 2 ml of 25% (v/v) metaphosphoric acid with 6 ml of the fermentation fluid which was free of particles. The phosphoric acid treated mixture was left at room temperature for 30 minutes. The solution was clarified by centrifugation at 16,000 rpm for 10 minutes before analysing for volatile fatty acid contents by gas-liquid chromatography (Hewlett-Packard 5840A: Column, 1.8 m × 4 mm id glass packed with 10% SP1200/1% H_3PO_4 on 80/100 Chromosorb WAW; column temperature 140~145°C; N_2 carrier gas, 50 ml/minute) using α-methyl valeric acid as internal standard.

[a] A; Lasalocid, B; X-14873A, C; elaiophylin.

[b] Results expressed as μmol propionate/[μmol acetate + μmol *n*-butyrate].

* The concentrate in ration is composed of (in %): corn meal 15, cracked corn 70.475, soybean oil meal 7.5, alfalfa meal 5, limestone 0.8, Dical (Stauffer) 0.5, salt 0.6, Roche Vitamin PMX 0.1, trace minerals 0.05.

X-14873A, both are polyether antibiotics reported to exhibit cattle growth performance enhancing activity[4,8]. Elaiophylin also inhibits lactic acid production in the rumen fluid with $IC_{50}=2.2\,\mu g/ml$ (vs. $1.28\,\mu g/ml$ for lasalocid and $3.13\,\mu g/ml$ for monensin). The results of *in vivo* study on elaiophylin in comparison with lasalocid are presented in Fig. 1. Lasalocid produced an average increase of 250% in the ratio of propionate production in 4 animals, while elaiophylin caused an average increase of about 186%. These results are consistent with the observation of an *in vitro* study in which elaiophylin at concentration of 3 ppm of higher elevates the propionate production in rumen fermentation carried out in shake flasks. Although the optimum daily dose of elaiophylin in animal feed remains to be determined, the results presented demonstrate that elaiophylin is an effective rumen propionic acid production enhancer and suggest that this compound is a potential candidate as a cattle growth performance enhancer.

References

1) RUFF, M. D.: Veterinary applications. *In* Polyether Antibiotics. Naturally Occcurring Acid Ionophores. Vol. 1. Biology. *Ed.*, J. W. WESTLEY, pp. 303~332, 1982
2) PERRY, T. W.; W. M. BEESON & M. T. MOHLER: Effect of monensin on beef cattle performance. J. Anim. Sci. 42: 761~765, 1976
3) BRETHOUR, J. R.: Lasalocid for finishing steers. Abst. of 71st Ann. Meeting Am. Soc. Anim. Sci. pp 357~358, Tucson, July 29~Aug. 1, 1979
4) BROWN, R. E. & A. DAVIDOVICH: The performance response of finishing cattle fed graded levels of lasalocid. Abst. of 71st Ann. Meeting Am. Soc. Anim. Sci., p. 358, Tucson, July 29~Aug. 1, 1979
5) RICHARDSON, L. E.; A. P. RAUN, E. L. POTTER, C. O. COOLEY & T. P. RATHMACHER: Effect of monensin on rumen fermentation *in vitro* and *in vivo*. J. Anim. Sci. 43: 657~664, 1976
6) LEY, S. V.; D. NEUHAUS, & D. J. WILLIAMS: A conformational study of elaiophylin by X-ray crystallography and difference ^1H NMR methods; observation of a selective sign reversal of the nuclear Overhauser effect. Tetrahedron Lett. 23: 1207~1210, 1982
7) FUKUDA, D. S.; R. H. DU BUS, P. J. BAKER, D. M. BERRY & J. S. MYNDERSE: A54145, a new lipopeptide antibiotic complex: Isolation and characterization. J. Antibiotics 43: 594~600, 1990
8) LIU, C.-M.; J. W. WESTLEY, T. E. HERMANN, B. LA T. PROSSER, N. PALLERONI, R. H. EVANS & P. A. MILLER: Novel polyether antibiotics X-14873A, G and H produced by a *Streptomyces*: Taxonomy of the producing culture, fermentation, biological and ionophorous properties of the antibiotics. J. Antibiotics 39: 1712~1718, 1986
9) CHENG, E. W.; G. HALL & W. BRUUOUGHS: A method for the study of cellulose digestion by washed suspensions of rumen microorganisms. J. Dairy Sci. 38: 1225, 1955