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The effect of isoacids on ruminal fermentation : *in vitro* trials

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Summary. Isoacids (isobutyric, isovaleric and valeric acids) showed a stimulating effect on ruminal microflora fermentations, particularly on acetic, propionic and butyric acid production and on microbial protein synthesis. No effects were observed on production of isoacids but the acetic/propionic ratio was decreased by treatments.

Isoacids such as isobutyric, isovaleric and 2-methylbutyric acids, which derive from the catabolism of certain amino acids in the rumen, stimulate the cellulolytic activity of rumen bacteria and protein synthesis (Bentley *et al.*, 1955), either singly or in association (Dehority *et al.*, 1967). In this research we checked the effect of isobutyric, isovaleric and valeric acids and of two mixtures of them at two concentration levels (table 1) on the *in vitro* fermentative activity of rumen microorganisms.

TABL. 1. — *Effect of the lower level of isoacids* (24 replicates).

		Treatments mM/100 ml				
		0.74	0.64	0.64	0.68	0.68
		Iso Butyric	Iso Valeric	Valeric	Mix (1)	Mix (1)
Gas production ml	24.0 c	30.4 b	29.3 bc	28.7 bc	27.3 c	28.0 bc
VFA production mg/100 ml	102.3 a	148.1 bc	112.7 bc	144.0 bc	122.9 abc	119.5 abc
Acetic ac. mg/100 ml	66.1 abc	67.2 ab	46.9 c	65.9 abc	54.5 abc	66.1 abc
Propionic ac. mg/100 ml	33.2 abc	34.3 abc	26.5 c	35.6 abc	29.2 bc	27.7 abc
Butyric ac. mg/100 ml	11.4 abc	13.3 ab	9.1 c	13.2 ab	10.0 bc	11.4 abc
$C_2 + C_4/C_3$ Molar % ratio	3.58a	3.54ab	3.50ab	3.44b	3.59ab	3.54ab
Isobutyric ac. mg/100 ml	1.0 a	–	0.05a	1.25a	3.6 a	1.8 a
Isobutyric ac. mg/100 ml	5.9 a	4.8 a	–	6.7 ab	9.5 ab	6.1 ab
Valeric ac. mg/100 ml	1.8 a	7.0 ab	1.7 a	–	6.0 ab	6.8 ab
TCA proteins mg/100 ml	16.3 a	16.7 a	17.3 a	16.7 a	17.4 a	16.9 a

Means in the same row with different letters are statistically different for $P < 0.01$.

Material and methods. Six fermentations were carried out with four replicates for each treatment and for the untreated control. Thirty ml of inoculum (33 % rumen fluid and 66 % buffer) (Menke, 1979), were used for each fermentation syringe. The ruminal inoculum was taken before the first feeding of the morning (8 am), from three heifers fitted with a ruminal fistula and fed a ration of hay, corn silage and concentrate (40 : 40 : 20 and 13 % crude protein on dry matter basis). The substrate used (666 mg/100 ml of inoculum) had the following composition : cellulose 20.0 %, starch 67 %, glucose 8.0 %, urea 4.52 %, sodium sulphate 0.49 %. Three ml of fermented liquid were taken from each fermenter for the determination of the volatile fatty acid composition according to

the method of Jouany (1982). Gas production was measured directly from the syringes in comparison with the gas production of the untreated control. TCA precipitable proteins were determined on the total content of two syringes adding 5 ml of a 50 : 50 (w : v) solution of TCA. The sample was centrifuged for 30 min at 15,000 xg and the precipitate washed three times before nitrogen determination using the Kjeldhal method. Factorial analysis of variance and the studentized range test were applied to the data.

TABLE. 2. — *Effect of the higher level of isoacids* (24 *replicates*).

		Treatments mM/100 ml				
		1.48	1.28	1.28	1.36	1.36
	Control	Iso butyric	Iso valeric	Valeric	Mix (1)	Mix (1)
Gas production ml	14.7 a	26.4 b	26.2 bc	25.7 bc	25.8 bc	23.2 c
VFA production mg/100 ml	79.6 a	142.5 bcd	150.6 bc	144.5 bcd	175.8 b	141.5 bcd
Acetic ac. mg/100 ML	22.7 a	37.8 c	45.3 bc	43.7 bc	57.0 c	40.2 c
Propionic ac. mg/100 ml	13.1 a	20.7 c	24.6 bc	24.1 bc	28.0 b	21.2 c
Butyric ac. mg/100 ml	4.7 a	9.0 bc	9.3 bc	9.62 bc	11.4 b	8.4 b
$C_2 + C_4/C_3$ Molar % ratio	3.80 a	3.66 ab	3.56 b	3.55 b	3.63 ab	3.65 ab
Isobutyric ac. mg/100 ml	0.8 a	—	0.8 a	0.25 ab	2.8 ab	1.2 ab
Isovaleric ac. mg/100 ml	5.9 a	0.4 ab	—	14.4 ab	2.2 ab	1.1 a
Valeric ac. mg/100 ml	2.1 a	9.6 ab	2.0 a	—	2.1 ab	10 a
TCA proteins mg/100 ml	20.9 a	23.4 bc	22.9 bc	23.1 bc	23.6 c	23.5 bc

Means on the same row with different letters are statistically different for $P < 0.01$.

Results and discussion. Gas production after 6 h of fermentation was stimulated ($P < 0.01$) by all the treatments at both levels of isoacids and at the higher level gas production was on average greater. At the lower level (table 1) only isobutyric and valeric acid had a positive effect ($P < 0.01$), while at the higher level (table 2) all the treatments stimulated the volatile fatty acid production ($P < 0.01$). The greatest effects on production of propionic acid were given by isovaleric and valeric acid and mix 1, while butyric acid production was stimulated by all the treatments ($P < 0.01$) but most markedly by mix 1. An interesting fact to note is that only valeric and isovaleric acids were capable of modifying ($P < 0.01$) the $C_2 + C_4/C_3$ ratio, and, in particular, that valeric acid had the same effect even at the lower level of treatment. No effect on the isoacids produced could be seen. All the treatments, at the higher level, led to an increase ($P < 0.01$) of TCA precipitable proteins. The improvement in fermentative efficiency and in protein synthesis observed in different experiments would seem to be sufficient to explain the positive effect on milk production (Felix, Cook and Huber, 1980).

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