Original Research Paper

Effects of Heavy Metals and Arsenate on the Ovine Rumen Fermentation *in vitro*

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Abstract

The inhibitory effect of four trace elements on production of volatile fatty acids (VFA) and lactate was evaluated in cultures of rumen contents containing lead, mercury, cadmium and arsenic at 50, 100 and 250 µg/ml. The production of VFA in cultures containing Pb^{2+} , Hg^{2+} , Cd^{2+} and As^{5+} at 50 µg/ml was decreased by 15.9, 40.0, 29.1 and 35.6%, respectively. The effect of trace elements on molar profile of VFA was variable, which suggests a different toxic effect of elements tested on different groups of rumen microorganisms. Increasing concentrations of Pb^{2+} in rumen cultures decreased molar proportion of acetate and increased that of butyrate, whereas Hg^{2+} and Cd^{2+} increased molar percentages of acetate and decreased butyrate. Lactate concentration in cultures containing Hg^{2+} increased in a dose-dependent manner from 3.10 to 9.20 µmol/ml. Effects of other trace elements on lactate production were not consistent.

Keywords: Trace elements; rumen metabolites; inhibition; lead; mercury; cadmium; arsenic.

INTRODUCTION

Ruminant diets can contain high concentrations of trace elements by the consumption of contaminated feed. Sources of these trace elements include burning of fossil fuels, atmospheric pollution from the non-ferrous industry, contaminated feed phosphates and spreading of sewage sludge on pasture and agriculture land. Sheep on a poor pasture consume plant material with soil which can be contaminated as well (Healy and Ludwig, 1965). Toxic trace elements hinder rumen fermentation, thereby decreasing the productivity of ruminants. Forsberg (1977b) showed that the elements (and the concentrations causing 50% inhibition of *in vitro* rumen fermentation) in decreasing order of toxicity were Hg^{2+} (20 µg/ml), Cu^{2+} (21 µg/ml), Cr^{3+} (70 µg/ml), Ni^{2+} (160 µg/ml), Cd^{2+} (175 µg/ml), As^{3+} (304 µg/ml), and As^{5+} (1.61 mg/ml). Rumen microorganisms also modify the toxicity of trace elements either by decreasing the toxicity via sulphide production resulting in precipitation of heavy metals, or potentiating the toxicity by transformation of the element to a more toxic form, e.g. methylation of mercury (reviewed by Forsberg, 1977b). Ions of heavy metals may also inhibit activity of some ruminal enzymes (Faixova and Faix, 2002), furthermore the susceptibility of rumen bacteria to heavy metals may differ (Lenártová et al., 1998). Heavy metals are removed from the body via urine and faeces, or accumulate in bones and tissues (Phillips and Mohamed, 2011). Contamination of animal products by heavy metals represents a serious problem, therefore contents of

heavy metals in animal products have been monitored by veterinary authorities. The aim of our experiment was to compare the effect of lead, mercury, cadmium and arsenate on ovine rumen fermentation *in vitro*. Contrary to the study of Forsberg (1977b), the stress was laid on effects of these trace elements on production of ruminal metabolites, i.e. volatile fatty acids (VFA) and lactate.

MATERIALS AND METHODS

Two rumen-cannulated wethers were fed meadow hay *ad libitum* and ground barley (300 g) twice a day. Rumen contents were sampled 1.5 h after the morning feeding and mixed. Rumen contents (50 ml) were incubated in 300 ml serum bottles, diluted with 100 ml of the McDougall buffer (McDougall, 1949), containing urea at 0.6 g/l. Substrates were wheat cellulose (1 g) and hemicelluloses (0.5 g), prepared as described by Marounek et al. (1988), and citrus pectin (0.5 g). To provide for anaerobiosis, amorphous FeS was added at 80 mg per flask. Chlorides of Pb, Hg, Cd and sodium arsenate ($Na_2HAsO_4 \cdot 7 H_2O$) were dissolved in warm distilled water and added to incubation flasks to obtain concentrations of Pb, Hg, Cd and As of 50, 100 and 250 µg/ml. Cultures treated with arsenate were inoculated using rumen contents from other donors. Flasks were filled with CO_2 and incubated in duplicate in a shaking water bath at 39 °C for 16 h. At the end of the incubation, the pH was measured, incubation was stopped by adding 1 ml of 5 M H_2SO_4 , incubation fluid centrifuged and

Figure 1. Effects of trace elements on production of volatile fatty acids in cultures of ovine rumen contents

filtered. Total VFA in filtrate were estimated by titration after steam distillation (Friedemann and Brook, 1938). Their molar composition was determined by gas liquid chromatography (Ottenstein and Bartley, 1971). Lactic acid was oxidized to acetaldehyde and measured using microdiffusion chambers (Conway, 1957).

RESULTS AND DISCUSSION

Production of VFA was decreased in all treated cultures (Fig. 1). Effect of mercury was more pronounced than that of other trace elements. Inhibitory effect of lead was weak. Inhibitory effect of arsenate did not change when As concentration in cultures increased from 50 to 250 µg/ml. Effects of lead, mercury, cadmium and arsenate on molar composition of VFA were variable. Increasing concentrations of lead in rumen cultures decreased molar proportion of acetate and increased proportion of butyrate (Table 1). Mercury and cadmium increased molar percentages of acetate and decreased propionate. Effects of arsenate were similar, but less pronounced. Concentration of lactate in cultures treated with mercury increased in a dose dependent manner from 3.10 to 9.20

µmol/ml. Lactate concentrations in other cultures varied from 2.43 to 7.10 µmol/ml and effects of additives were not consistent.

Table 1. Effects of trace elements on the molar percentages of VFA produced in cultures of rumen contents

Element	Concentration (µg/ml)	Molar %			
		Acetate	Propionate	Butyrate	Valerate
Pb	0	47.5	41.3	8.8	2.4
	50	47.1	41.0	9.8	2.1
	100	43.9	43.3	10.6	2.2
	250	41.6	41.6	13.4	3.4
Hg	0	44.2	45.3	8.6	1.9
	50	56.6	25.7	13.7	4.0
	100	62.2	27.8	8.8	1.2
	250	63.2	28.4	6.5	1.9
Cd	0	46.1	41.2	10.3	2.4
	50	50.7	31.7	13.8	3.8
	100	53.9	27.6	14.5	4.0
	250	53.3	29.4	11.9	5.4
As	0	54.9	18.9	19.5	6.7
	50	63.1	13.5	16.4	7.0
	100	58.6	15.3	19.1	7.0
	250	58.9	15.9	18.4	6.8

Czech hygienic limits of the Pb, Hg, Cd and As content in compound bovine feedingstuffs are 5, 0.1, 1 and 2 mg/kg, respectively. Average concentrations of these elements reported by Czech veterinary authorities were lower: 154, 1.48 and 79 µg/kg, respectively (Anonymous, 2014). Lower, rather than higher trace element concentrations in the incubation fluid are thus relevant. The production of VFA in cultures containing Pb, Hg, Cd and As at 50 µg/ml was decreased by 15.9, 40.0, 29.1 and 35.6%, respectively. The elements in increasing order of toxicity based on the inhibition of VFA production at 50 µg/ml were Pb, Cd, As and Hg. The corresponding ranking of trace elements toxicity, based on the inhibition of gas evolution reported by Forsberg (1977b) was Pb, As, Cd and Hg. It should be mentioned that inorganic arsenic exists as arsenite (As^{3+}) and arsenate (As^{5+}), the former being more toxic to rumen microflora (Forsberg, 1977a).

The effect of trace elements on molar profile of VFA was very variable. This suggests a different toxic effect of trace elements on different groups of rumen bacteria. Forsberg (1977a) observed a greater resistance of *Megasphaera elsdenii* to arsenate as compared to that of *Bacteroides succinogenes*. The bacteria which most frequently exhibited the greatest sensitivity to effects of heavy metals were *Bacteroides succinogenes*, *Ruminococcus albus*, *Bacteroides amylophilus*, and *Eubacterium ruminantium* (Forsberg, 1977b). Lenártová et al. (1998) reported that *Selenomonas ruminantium* was tolerant to ten-times higher concentrations of Hg^{2+} than *Streptococcus bovis*. The authors assume that the production of sulphides prevented the toxic effects of Hg^{2+} to *Selenomonas ruminantium*. Bacteria dispose with metal-resistance mechanisms, frequently based on efflux of toxic ions that entered the cell, or are able to convert toxic ions to a less toxic and less available species (Silver, 1998). To elucidate the role of dietary factors in the defence of rumen microorganisms against environmental pollutants requires a further study.

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