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RUMEN LACTATE CONCENTRATION AS INFLUENCED BY ADDED POTASSIUM *IN VITRO*

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The influence of potassium (200, 500, and 1000 mM/liter) on rumen lactate concentration was determined by measuring lactic acid concentration, *in vitro*. Rumen fluid samples were obtained from a rumen-fistulated steer fed mainly concentrates plus low quality alfalfa hay. Three hundred or 1500 mg of substrate (a mixture of equal parts of glucose, alfalfa meal, and starch) were added to samples which were analysed for determining lactate concentrations. Incubation of samples was carried out for 2 or 4 hours at 39 C. Colorimetric method was used for lactate determinations. The response of lactate content to high levels of added potassium in rumen fluid samples was variable. A comparison with control samples indicated that samples with 500 and 1000 mM/liter of added potassium exhibited notable decline in lactate concentration, while the addition of 200 mM/liter potassium appeared to cause a progressive accumulation of lactic acid, *in vitro*. Incubation periods, potassium and substrate levels, all significantly ($P<0.01$) affected the lactate concentration in rumen fluid.

INTRODUCTION

The concentration of cations and anions in the animal body below certain levels results in deficiency effects whereas their concentration above certain levels may prove harmful to the organism. Whanger and Matrone (1966) suggested that deficiency of sulphur in the diet of sheep was responsible for a possible reduction in number of lactic acid fermenting microbes in the rumen, leading to lactate accumulation. Bunn and Matrone (1968) reported that incorporating alfalfa (5 per cent) or sodium bicarbonate (6 per cent) and potassium bicarbonate (4 per cent) or both in the basal urea diet of sheep, the metabolism of lactate was enhanced. Both the cations and alfalfa perhaps furnished nutrients or cofactors that stimulated the proliferation of microbes involved in acrylate pathway

Potassium (K) constitutes an important cation in ruminant rations. Its consumption especially by dairy animals on lush pastures may go as high

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as 500 g or more daily. Since high lactic acid concentration may lead to atypical fermentations resulting in lactic acidemia, the present work was initiated to find out if excessive content of K in rumen fluid affects the concentration and *in vitro* fermentation of lactate.

MATERIALS AND METHODS

A rumen-fistulated steer was used as rumen fluid donor. The animal was fed twice daily a ration consisting of rolled corn, liquid protein concentrate and low quality alfalfa hay. Free access to water was allowed at all times.

Rumen fluid samples were collected 2 to 3 hours post feedings, approximately 150 ml of fluid were squeezed out of well mixed rumen contents obtained by direct insertion of hand in the rumen. After straining through four layers of cheese cloth, an aliquot sample from every trial, designated as zero time was analysed for lactic acid and original K content.

Ninety-six milliliters of the strained rumen fluid were mixed with 64 ml of phosphate buffer solution adjusted to a final pH of 6.6 and prewarmed to a temperature of 39 C. Ten milliliters of this mixture were poured into each of the 16 glass incubation tubes (30 ml capacity). A substrate, containing equal parts of glucose, alfalfa meal and starch, was added to rumen fluid samples at two levels. Three hundred milligrammes of this substrate mixture were added to each of the tubes numbered one to eight, whereas, 1500 mg of the same substrate were added to each of the tubes with numbers 9 to 16.

Three levels of K viz., 200, 500, and 1000 mM/liter were used for adding potassium as chloride salt to rumen fluid samples. Thus tubes with numbers 3,4,11 and 12 contained 200 (K-200), tubes 5, 6, 13 and 14 contained 500 (K-500), and tubes 7,8,15 and 16 had 1000 (K–1000)mM/liter of added K. Incubation tubes with numbers 1,2,9 and 10 served as control (NK) samples. The experimental as well as control samples were run in duplicate.

Each sample was well mixed, then gassed with carbon dioxide for ten seconds, loosely stoppered and was placed in the incubator maintained at a temperature of 39 $\pm$ 1 C. The samples were shaken every thirty minutes. At the end of two hours incubation, eight of the 16 samples representing one from each duplicate set, were picked up at random. The rest of the eight samples were incubated for another two hours, thus, half the number of

samples was incubated for two and the other half for four hours. At the completion of the respective incubation periods 5 ml of 25 per cent metaphosphoric acid were added to each tube to stop further fermentative activity. Lactate concentration in these samples was determined according to the method of Barker and Summerson (1941). Potassium content of the original rumen fluid samples was detemined by using atomic absorption (Perkin-Elmer 303) spectrophotometer (Wilson, 1967).

RESULTS AND DISCUSSION

Table 1 shows the mean concentrations of lactate influenced by various levels of K (None, NK; 200, K-200; 500, K-500; and 1000 mM/liter, K-1000) added to rumen fluid samples. The results have been presented in a way that the effect of incubation periods and levels of substrate used in these experiments can also be observed. The range of average lactate concentrations obseved was 138 to 567 in K-200; 66 to 110 in K-500; and 38 to 48/mg per cent in K-1000 samples as compared to 85 to 291 mg per cent in control samples. All the samples containing 1500 mg substrate when incubated for 4 hours showed higher lactic acid concentration. Analysis of variance indicated that K levels, substrate levels, and incubation periods had a significant ($P<0.1$) effect on lactate levels.

TABLE 1. *Mean rumen lactate concentration* in vitro

Amount of potassium (K) added (mM/liter)	Incubation periods			
	2 hours		4 hours	
	Levels of substrate added (mg)			
	300	1500	300	1500
	Mean ± S.E.	Mean ± S.E	Mean ± S.E	Mean ± S.E
	.....mg lactate / 100 ml rumen fluid.....			
None (NK)	85 ± 11	179 ± 28	39 ± 6	291 ± 44
200 (K-200)	138 ± 8	265 ± 24	211 ± 25	567 ± 53
500 (K-500)	66 ± 5	78 ± 2	243 ± 34	110 ± 8
1000 (K-1000)	38 ± 4	40 ± 2	42 ± 3	48 ± 2

Mean lactate concentration and original K content in zero time rumen fluid samples were 1.04 mg/100 ml and 24 mM/liter, respectively.

With the possible exception of K-200 samples, increase in K added to rumen fluid samples, appeared to depress the activity of lactate producing microorganisms both in K-500 and K-1000. In the latter, due to higher K concentration, the inhibition seemed to be more drastic, presumably by involving additional microbial species responsible for lactate production. MacLeod and Onofrey (1957) reported that 800 mM/liter sodium or potassium when added to culture media, inhibited the growth of three marine organisms under study. It was observed by Bardos and Gordon (1953) that addition of the chloride salts of potassium, magnesium or calicum to a basal medium in slightly hypertonic concentration, inhibited the growth of *Lactobacillus leichmannii*. This inhibition was reversed by the addition of sufficient amount of B_{12}. With an increase in incubation time and higher levels of substrate, a variable rise in lactate content was noticeable in K-500 and K-1000 samples. This rise was more apparent in K-500 (due to low K concentration) than in K-1000 samles.

Samples (K-500) containing 300 mg substrate incubated for 4 hours exhibited a relatively higher rise (243 mg per cent) in lactic acid levels than in those samples (110 mg per cent) containing 1500 mg substrate and incubated for a similar period. This fall in lactate content seemed on the one hand to be the result of a depression in the activity of lactate producers and on the other hand to be due to the consequent suppression of lactate utilizing microogranisms.

A comparison of lactate concentration (table 1) in NK, K-200, K-500, and K-1000 samples containing 300 mg substrate when incubated for 2 or 4 hours, respectively, showed that lactic acid values fell from 85 to 39 mg per cent is control samples due to lactate fermentation by lactate utilizers. Contrary to this, a varying but increasing amount of lactic acid accumulation was evident in experimental samples containing various levels of K. This consequently appeared to indicate that lactate fermenting microor ganisms were also influenced by the inhibitory role of K. Normally, any lactate accumlation in the rumen is expected to decline to the original low levels after 1 to 3 hours (Waldo and Schultz, 1956). For the rumen fluid samples that contained 1500 mg. substrate, it may be stated that despite the inhibition arising out of higher levels of K, a rise in lactate values, though variable in magnitude, may be expected with an increase in concentration of substrate and incubation time.

No explanation seemed readily available for the behaviour shown by K-200 samples concerning high lactates levels. Roberts *et al.* (1949) in their studies of K metabolism in *Escherichia coli*, however, suggested that more glucose was utilized in the presence of potassium at low pH than at high pH. This might partly explain the situation obtained in K-200 samples, since in the latter, pH would be low as compared to samples containing 500 or 1000 mM K/liter.

Presentation of a complete picture of changes associated either with rumen microbial population or in its metabolic pattern in response to various levels of K added to rumen fluid samples seemed difficult. Further exploration of the problem, therefore, is need to provide conclusive evidence.

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