EFFECT OF POTASSIUM ON THE RUMEN MICROORGANISMS OF ANIMALS FED ON DIETS CONTAINING UREA[1]

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Four diets with varying levels of urea (0 to 2.2%) and potassium (0.83 to 1.20%) were fed in succession to a group of eight animals. Microbial concentration and rate of growth were depressed by the decrease of potassium intake in urea containing diets. The influence of low potassium intake was more obvious on the bacterial population. Two adult Rahmany sheep were fed on a semi-purified diet containing urea as the only source of nitrogen. The animals remained normal until the 8 week after which they showed symptoms of vitamin 8 deficiency. Potassium balance which was slightly positive (0.2 g/d) at the normal state was negative (-3.6 g/d) at the stage of the disorder.

Key words: Sheep, urea, vitamin B complex, potassium deficiency, purified diets

Sheep fed on a purified diet containing urea as the only source of nitrogen died after seven months with cerebrocortical necrosis as the main post-mortem finding (Naga et al 1975). When sheep on this diet were treated with vitamin B complex at the appearance of teeth gnashing, partial feed refusal or diurisis, they returned to normal.

Bacteria have a relatively high requirement for potassium for normal functioning and growth (Meyer et al 1968). There is also evidence that diets containing high levels of urea cause high urinary losses of potassium (Juhasz et al 1975). It is therefore possible that the development of vitamin B deficiency symptoms in sheep consuming a semi-purified diet containing urea as the major source of nitrogen, could be ascribed to a shortage of potassium which affected the ability of bacteria to grow and synthesize the B group vitamins.

Therefore, the following experiments were conducted to test this hypothesis.

Materials and Methods

Experimental Procedure

Experiment 1: Digestibility and nitrogen, potassium and sodium balance trials were conducted on eight ruminant animals (2 buffalo, 2 cattle, 2 sheep, 2 goats) fitted with rumen fistulas and fed on increasing levels of urea in diets I to IV as shown in table 1. Concentrations and growth rate of ruminal bacteria and protozoa in these animals on each diet were also estimated.

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Table 1:
Composition of the experimental diets

	Diet				
Ingredient (%)	I	II	III	IV	V
Wheat straw	64.4	62.8	61.5	62.3	47.2
Cottonseed cake	34.0	23.4	12.7	-	-
Rice starch	-	11.2	22.9	28.1	41.0
Urea	-	1.0	1.3	2.2	1.7
Cottonseed oil	-	-	-	.6	1.7
Molasses	-	-	-	1.8	-
Sucrose	-	-	-	-	2.2
Na Cl	.5	.5	.5	.7	.5
Ca CO_3	1.0	1.0	1.0	1.4	1.0
Mineral mixture[a]	-	-	-	2.8	4.5
Vitamins A & D	.1	.1	.1	.1	.2
Potassium	1.2	1.1	.9	.8	.8

[a] Contained (%); Calcium phosphate, 46.29; potassium carbonate, 29.83; magnesium sulphate, 15.28; sodium chloride, 7.03; ferrous sulphate, .85; zinc sulphate, .286; manganese sulphate, .19; cupper sulphate, .026; potassium iodide, .026; molybdenum oxide, .007; sodium borate, .17; cobalt chloride, .005; sodium florid. .009 and sodium selenate, .055.
[b] One g of this mixture contains 5000 and 500 I.U. of vitamins A and D_3 respectively.

Experiment 2: Two adult Rahmany rams fitted with permanent rumen fistulas were fed on diet V (table 1) so as to cover their maintenance requirements (NRC 1968). The ration was offered as two equal portions at 12 hr intervals. The animals had free access to water. The change to the experimental diet was made over a period of two weeks. After 8 weeks on this diet symptoms of vitamin B deficiency appeared. Rumen contents withdrawn at this stage were incubated in vitro with different levels of supplemental potassium (KCl) and the growth rates of bacteria and protozoa measured. Microbial growth was also measured at different intervals after feeding with and without added potassium. The animals were then given a vitamin B complex dose (table 2) which relieved the symptoms of the disorder. Five and six weeks later, signs of vitamin B deficiency reoccurred in both animals and they were treated by supplementing the ration with 2.5 g per head of K_2SO_4 given in two equal portions at 12 hr intervals with their food. The amount of potassium was calculated from the optimum level of potassium found from the in vitro trials. At different stages, concentrations and growth rates of rumen microorganisms, feed intake, dry matter digestibility and nitrogen, sodium and potassium balances were estimated.

Methods: Collections of faeces and urine in the digestibility and balance trials were made using bags and rubber funnels attached with harnesses as described by Naga et al (1975). Dry matter and nitrogen contents of the feed, faeces and urine were estimated according to the methods of the AOAC (1970). Bacterial and protozoal nitrogen in rumen contents were determined using diaminopimelic acid (DAPA) and aminoethane phosphoric acid (AEP) as markers. Estimations of DAPA and AEP were made according to the methods of Mason (1969) and el-Shazly et al (1975) respectively. Potassium and sodium were estimated in the HCl extracts of the ash of feed and excrete using a Pye Unicam atomic absorption spectrophotometer.

Table 2:
Composition of the vitamin B complex (mg dosed)

Vitamin	Amount (mg/ml)
B_1	8.0
B_2	1.6
B_6	0.4
Nicotinamide	16.0
Ca-pantothenate	4.0

The sheep were dosed with 5 ml of the solution

The measurements of the concentrations and rates of growth of rumen bacteria and protozoa were made as follows. About 250 g of rumen digesta containing about two thirds of fibrous material and one third liquor (Manal Zaki El-Din and el-Shazly 1969) were incubated in vitro for 1 hr under an atmosphere of CO_2 at 40° in a water bath. About five minutes elapsed between the sample withdrawal and incubation. Seven samples were taken over the 12 hr feeding period for incubation. At the start of the incubation of each sample, a subsample of about 50 g was taken and microbial activity stopped with 5 ml of 5N H_2SO_4. These subsamples were pooled within animals and analysed for bacterial and protozoal N. After the 1 hr incubation period another subsample was taken, killed, pooled and analysed as above. Growth rate was defined as the change in concentration of microbial nitrogen during incubation, and expressed as change per hr.

Results and Discussion

Table 3 shows that there was a tendency for the concentration and growth rate of microbial nitrogen in the rumen to be depressed by the decrease of potassium intake. This effect was more pronounced in bacteria than in protozoa. The exception was bacterial growth rate on diet IV which was higher than on all other diets, even although this diet furnished the lowest level of potassium. It is possible that the adequacy of sodium intake on diet IV compensated for the shortage of potassium.

The level of potassium in the diet which was required to sustain positive balance was higher in the present study (1.16%, table 3) than the values (.32, .34, .55 and .75%) recommended by other authors (Du Toit et al 1934;Telle et al 1964; Roberts and St Omer 1965; Hafez and Dyer 1969) on conventional diets. Juhasz et al (1976) reported that diets which increase the level of blood ammonia result in increasing potassium losses in the urine. Both urea and cotton seed cake have been found in this laboratory (unpublished results) to increase blood ammonia above the 600 mg/100 ml mentioned by Juhasz et al (1976). It seems that losses of sodium may also increase. In the present study up to 0.53% dietary sodium did not achieve a positive sodium balance (table 3), whereas 0.1 to 0.2% of sodium in conventional diets was adequate (Hafez and Dyer 1969; NRC 1968). The estimation of potassium and sodium requirements with urea feeding needs further examination.

Table 3:
Expt 1: Effect of the level of daily potassium intake on the bacterial and protozoal populations in the rumen (mean of 8 animals ±SE)

	Potassium			Sodium			Bacterial N		Protozoal N	
Diet No.	Intake (mg/ $W^{.75}$)	Balance (mg/ $W^{.75}$)	% in diet	Intake (mg/ $W^{.75}$)	Balance (mg/ $W^{.75}$)	% in diet	Concentration (mg/100g)	Growth (%/h)	Concentration (mg/100g)	Growth (%/h)
I	852	136	1.17	310	-276	.43	64.0	6.9	92.1	18.2
	(104)	(90)		(50)	(189)		(4)	(1.8)	(6.4)	(.7)
II	795	76	1.16	288	-383	.41	63.9	2.8	78.6	14.2
	(44)	(44)		(12)	(166)		(5.5)	(.7)	(9.9)	(.5)
III	696	-56	.92	416	-169	.53	39.5	1.3	73.2	10.0
	(77)	(56)		(31)	(85)		(2.9)	(.4)	(12.7)	(3.6)
IV	472	-152	.83	540	+ 67	.86	23.7	13.1	60.6	-1.1
	(75)	(185)		(25)	(107)		(1.1)	(3.1)	(1.7)	(9.4)

Table 4 shows the effect of feeding a semi-purified diet containing urea as the only source of nitrogen. The development of the vitamin B deficiency was accompanied by a severe depression in the growth rate of bacteria while that of protozoa was much less affected.

Table 5 shows that vitamin B had no direct effect on bacteria when it was added in vitro. Potassium administration to the animals suffering from vitamin B deficiency improved both the health of the animals and the growth rate of the ruminal microorganisms especially that of bacteria.

The effect of different levels of potassium is shown by table 6 which suggests that potassium was a limiting factor, although the means do not differ significantly. Optimal potassium supplement in the present study for bacterial growth was 20 mg/g rumen contents (table 7), although again the means do not differ significantly.

Table 4:
Expt 2: Concentration and rate of increase of bacterial and protozoal nitrogen in digesta from sheep depleted of potassium (mean of 2 sheep)

Nutritional state of the animals	Bacterial		Protozoal N	
	Concentration[1] (mg/100 g r.c.)	Growth rate [2] (%/h)	Concentration[1] (mg/100 g r.c.)	Growth rate [2] (%/h)
Normal	23.5	26.6	60.5	13.1
Deficient	20.6	- 22.1	19.3	6.4
After vit B treatment	26.6	- 11.1	14.8	15.5
Supplementation with potassium	20.2	25.1	14.8	52.1

[1]Digesta as taken from rumen
[2] Growth in vitro

Table 5:
The effect of vitamin B complex or potassium on in vitro rate of growth (± SE) bacteria obtained from the rumen of deficient sheep[1]

Bacterial growth	Treatment		
	Control[2]	Vitamin B_3	Potassium[α]
% per h	-6.1 (3.9)	-7.8 (1.8)	+5.8 (+4.6)

[1] Results are averages of two replicates on each of the two animals
[2] Without any additions
[3] The vitamin B solution indicated in table 2 was added in vitro at the rate of 0.1 ml/100 ml medium
[4] Potassium was added in vitro at the rate of 30 mg/100 ml medium in the form of potassium chloride

Table 6:
Effect of potassium added in vitro on the growth rate of bacteria obtained from sheep suffering from vitamin B deficiency[1]

Time after feeding(h)	Growth rate of bacteria (%/h)	
	Without added potassium	With added potassium[2]
0	- 11.2	1.8
1	37.5	3.1
2	+ 34.6	13.0
4	- 22.2	17.8
6	- 6.4	- 4.0
9	- 11.2	0.6
Mean (± SE)	- 8.9 ± 9.8	5.5 ± 3.3

[1] Results are averages of two animals
[2] Potassium was added to the in vitro medium at the rate of 30 mg/100 ml

Table 7:
Effect of different levels of potassium on the in vitro growth rates (± SE) of bacteria and protozoa obtained from the rumen of a vitamin B deficient sheep[1]

Level of potassium supplementation (mg/100 g R.C.)	Growth-rate (%/h) of:	
	Bacteria	Protozoa
0	12.4 (6.1)	40.0 (30.7)
10	27.0 (5.4)	16.2 (26.7)
20	58.2 (19.9)	35.0 (20.0)
30	51.6 (14.5)	19.6 (22.5)
40	34.6 (18.9)	17.6 (18.1)
60	26.3 (22.6)	45.8 (24.0)

[1] Results are averages of four replicates, two on each animal

Table 8:
Dry matter digestibility (DMD), nitrogen, sodium and potassium balances at various nutritional states of sheep given a semi-purified diet

Intake				Faecal(g/d)			Urinary (g/d)				Balance (g/d)[4]			
Feed	N	Na	K	N	Na	K	N	Na	K	Volume(litres)	N	Na	K	DMD %
										Normal (at the 4th week)				
851	8.3	8.3	5.2	3.2	2.2	1.1	4.7	10.2	3.9	1.2	.4	-4.1	.2	65.4
							Vitamin B complex deficient (at the 8th week)							
611	8.0	5.9	4.2	4.1	1.7	1.0	7.4	5.7	6.8	1.6	-3.5	-l.5	-3.6	59.1
							Normal after vitamin B treatment (at the 10th - week)[2]							
850	9.0	8.1	5.2	3.2	2.9			8.0	4.0	1.2	1.0	-2.8	0.2	69.4
							Normal after potassium treatment (at 15th week)[3]							
821	8.6	8.0	6.8	2.8	0.8	0.5	3.3	7.7	3.1	1.2	2.4	-0.5	2.0	69.8

[1] Results are averages of 7 consecutive days on each of the two animals
[2] A 5 ml dose of the vitamin B solution (table 2) given through the fistula
[3] After 5 weeks from the last dose of vitamin B the deficiency state reoccurred. Potassium supplement (2.5g K_2SO_4/head)was given at the third day of disorder appearance
[4] Difference includes losses in suint

It is interesting to relate the data in table 4 to those of table 8. Potassium balance at the normal state was 0.2 g/d (table 8). At the stage of deficiency where rumen bacteria were degenerating in vitro (table 4), potassium and nitrogen losses were greater. Roberts and Driedger (1966) reported that one of the main consequences of potassium deficiency is the marked decrease of feed intake and body weight losses.

In the present study, dry matter digestibility was also reduced. Campbell and Roberts (1965) found that potassium deficiency reduced nitrogen balance but had no effect on DM digestibility. While St Omer and Roberts (1967) found that potassium deficiency had no effect on nitrogen balance. These different effects may perhaps be explained by the degree of potassium depletion.

The negative balance of sodium which has been frequently observed in this study suggests that the addition of 1% NaCl to ruminant diets may not be adequate with diets containing urea.

The improvement of feed intake, DM digestibility and N and potassium balance in response to vitamin B treatment indicated that the disorders from which the animals suffered were probably due to vitamin B deficiency. This developed when the activities of the bacterial population were depressed, apparently as a result of potassium shortage. Potassium balance which was negative at the deficiency stage returned positive after the vitamin treatment (table 8) which indicates the adequacy of potassium intake to the host animal. However, the bacterial growth rate was not restored (in vitro) unless potassium intake was increased by about 30%. This requirement of the bacteria was not observed until the fourth week on the experimental diet (diet V). this occurred due to the exhaustion of the body other element (s), or that the prolonged feeding on the experimental diet had induced changes in the physiology or metabolism of the host animals.

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