CXLVIII. THE EFFECT OF A PHOSPHORUS DEFICIENCY ON THE PROTEIN AND MINERAL METABOLISM OF SHEEP

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MUCH work has been done on various aspects of the effects of aphosphorosis on both ruminants and other types of mammals, but no complete study of the effect on both protein and mineral metabolism has yet been recorded. The effect of a P-deficient diet on the general health, blood chemistry, appetite and mineral metabolism has been admirably reviewed by Theiler & Green [1931] and Schmidt & Greenberg [1935] and needs no further discussion. On the other hand, little attention has been paid to the protein metabolism. In a study on sheep Woodman & Evans [1930] found that the digestibility of the ration was unchanged by either Ca or P deficiency, similar results being recorded by Forbes [1937] on rats and Kleiber *et al.* [1936] on steers. A diminished utilization of food energy has, however, been recorded [Eckles *et al.* 1926; 1932; Theiler *et al.* 1924; Theiler, 1933, 1, 2; Kleiber *et al.* 1936], cows on P-deficient rations requiring 20% more digestible nutrients than are allowed in the feeding standards to maintain a constant live weight [Eckles & Gullickson, 1927].

The present study was undertaken to find if any disturbance of the protein metabolism followed the ingestion of a P-deficient diet.

Experimental

Four sheep were used, the animals being about 14 months old. The diets fed are shown in Table I. During periods 1 and 4 the rations fed were low in P, with a Ca : P (CaO : P₂O₅) ratio of about 5 : 1. In period 2 the ration was supplemented with Na₂HPO₄, giving a Ca : P ratio of approximately 1 : 1. In period 3 both P and Ca additions were made, the ratio again being about 5 : 1, although both the Ca and P intakes were far in excess of the actual requirements. Periods 1, 2 and 4 were of 6 weeks each, i.e. 2 weeks on transition from the previous diet and 4 weeks on experiment. Period 3 lasted only 2 weeks, analyses being carried out for the whole period.

Table I. *Quantities of food consumed per day, g.*

Food	Periods			
	1*	2	3	4
Beet pulp	400	500	500	350
Starch	200	250	250	250
Blood meal	50	50	50	50
Straw	150	200	200	100
Na ₂ HPO ₄	—	10	10	—
CaCO ₃	—	—	45	—
N intake	13.28	15.05	15.05	12.34
CaO intake	5.39	6.90	32.01	4.51
P ₂ O ₅ intake	1.11	6.43	6.43	0.79

* Sheep no. 2 received more food in periods 1 and 4.

Analyses of urine and faeces were carried out every 2 days, the methods being those used in previous experiments [Morris & Ray, 1939].

Results

Food intake. The quantity of food supplied per day was based on preliminary periods in which an excess was offered and accurate records kept of the actual intake. It will be seen from Table I that the food intake varied with the P content of the diet, the low P diets giving a lower food intake. This fact has been recorded by other investigators [Du Toit *et al.* 1931; Huffman *et al.* 1933; Martin & Peirce, 1934] and may possibly be the result of a diminished secretion of digestive juices with a decreased P intake. There was also a lowered state of general health of the sheep during the P-deficient periods, the sheep being ill-at-ease for the first 3 weeks and growing more lethargic till the end of the 6 weeks period. In period 3, that of the high Ca-high P diet, the animals were definitely "off colour" but the period was too short (2 weeks) for any change in the food intake to be recorded. Similar results with a high Ca diet have been recorded by Bethke [1932], Bethke *et al.* [1933] and Cox & Imboden [1936].

Weight changes. The effect of the diets on the gain in live weight is shown in Table II. In period 2, with the P supplement, the gains are slightly greater

Table II. *Live weight changes, kg.*

Sheep	Periods			
	1	2	3	4
1	+2.25	+2.00	+1.25	-2.50
2	+4.50	+1.35	+2.10	-4.20
3	+1.95	+4.05	+1.55	-2.85
4	+0.50	+3.00	+1.90	-4.85
Mean	+2.30	+2.60	+1.70	-3.60

than in period 1, though the differences are not statistically significant, while in the final low P period a marked loss in weight is found. In period 3 the gains are distinctly lower than in period 2. The present results confirm those of other investigators [Du Toit *et al.* 1931; Godden & Ray, 1937].

Protein digestibility. Table III shows the apparent and true digestibilities of the ingested protein. Only in period 4 is there any alteration in the apparent

Table III. *Digestibility of food nitrogen*

Period	Sheep	Faecal N g.	Intake of N g.	% apparent digestibility	Metabolic faecal N, g.	% true digestibility
1 (low P)	1	6.48	26.56	75.6	6.48	100
	2	7.91	28.16	71.8	7.30	97.8
	3	6.31	23.80	73.4	5.70	97.4
	4	6.63	26.56	75.0	6.08	97.9
2 (P supplement)	1	8.47	30.10	71.8	9.10	>100
	2	8.19	30.10	72.8	9.10	>100
	3	10.85	30.10	63.9	9.10	90.1
	4	8.18	30.10	72.8	9.10	>100
3 (P + Ca supplement)	1	8.90	30.10	70.4	9.10	>100
	2	7.05	30.10	76.6	9.10	>100
	3	8.05	30.10	73.2	9.10	>100
	4	7.51	30.10	75.0	9.10	>100
4 (low P)	1	7.39	24.68	70.0	6.08	94.7
	2	7.86	26.24	70.0	6.48	94.7
	3	7.71	24.68	68.8	6.08	93.4
	4	8.74	24.68	64.6	6.08	89.2

digestibility of the protein, a slight lowering being recorded. When the metabolic faecal N is taken into account, period 4 shows a definite lowering of the true digestibility while period 1 also shows a trend in this direction. It may be noted that in periods 2 and 3, when adequate amounts of P and Ca were fed, the metabolic faecal N calculated from the dry matter intake, using the results of Hutchinson & Morris [1936, 1, 2], is greater than the actual amount of N excreted. Mitchell [1934] claims that in such cases the metabolic faecal N figure must be taken and that the error lies in the analytical figure obtained for the actual N excreted. In the present experiment the actual faecal N excreted is lower than the calculated metabolic N so consistently during periods 2 and 3 that some other explanation must be sought. It is possible that, with a completely adequate diet, the apparent biological value of the protein fed may alter the metabolic faecal N, the higher the biological value the lower being the N excretion. In the present instance blood meal was being fed as the chief source of protein and this has been shown to have a high biological value for ruminants [Morris & Wright, 1933, 1, 2; Morris *et al.* 1936].

From the results it is doubtful if there is any significant difference in the apparent digestibility of the protein ingested. There is, however, no doubt that a deficiency of P in the diet causes a significant lowering of the true digestibility of the protein.

Urinary partition of nitrogen

(i) *Total N.* A P-deficient diet has a marked effect on the total urinary N output (Table IV). In periods 1 and 4, although the N intake is lower than in periods 2 and 3, the urinary output is, on the whole, higher, showing a decreased retention of absorbed N (see also Table VII). In period 3 the N excretion is significantly higher than in period 2, although the N intake is the same. This indicates that the Ca : P ratio, together with the total P intake, has a definite influence on the protein metabolism.

Table IV. *Partition of urinary N*

Period	Sheep	Total N g.	Urea N g.	NH ₃ N g.	Amino- acid N g.	Hip- puric acid N g.	Pre- formed crea- tinine g.	Crea- tine g.	Uric acid g.	Purine base g.	Allan- toin g.
1 (low P)	1	12.77	8.09	1.77	0.21	0.33	1.44	0.64	0.32	0.39	6.45
	2	10.67	5.16	1.64	0.25	0.25	1.51	0.87	0.37	0.35	5.85
	3	12.99	8.04	1.12	0.51	0.36	1.26	0.79	0.36	0.40	6.15
	4	9.49	4.49	0.46	0.33	0.13	1.11	0.69	0.35	0.41	4.72
2 (P supplement)	1	9.06	4.15	1.73	0.61	0.23	1.72	0.53	0.38	0.43	2.72
	2	10.44	5.47	1.37	0.32	0.29	1.69	0.81	0.39	0.48	3.07
	3	8.47	4.63	0.90	0.19	0.40	1.53	0.89	0.41	0.36	2.55
	4	9.86	4.94	0.81	0.32	0.40	1.53	0.91	0.42	0.48	2.89
3 (Ca + P supplement)	1	10.50	3.54	3.44	0.87	0.30	1.16	0.34	0.26	0.72	4.42
	2	12.21	6.57	2.17	0.51	0.21	1.12	0.55	0.29	0.59	5.02
	3	11.02	6.24	1.09	0.18	0.46	1.37	0.73	0.34	0.64	4.70
	4	11.04	6.21	1.04	0.34	0.40	1.29	0.57	0.32	0.62	4.82
4 (low P)	1	13.10	7.62	0.27	0.49	0.50	1.66	0.83	0.40	0.29	5.84
	2	14.87	9.52	0.53	0.21	0.66	1.72	0.79	0.39	0.20	6.24
	3	11.58	5.80	0.41	0.33	0.54	1.38	0.59	0.39	0.52	5.25
	4	11.12	6.46	0.43	0.38	0.52	1.38	0.68	0.38	0.39	4.94

The present results confirm those found by Godden & Ray [1937]. The possibility that the excess urinary N is due to a diminished utilization of food energy must, of course, be borne in mind.

(ii) *Exogenous metabolism.* The total exogenous output is unaffected by any alteration in the diet (Table IV). In all periods this amounts to slightly more than 70% of the total N. There are, however, considerable variations within each group. The highest value, i.e. in sheep 1, period 3, was due to faecal contamination of the urine. The observed variations in the amino-acid N output are normal for ruminants [Morris & Wright, 1933, 1, 2; Hutchinson & Morris, 1936, 1, 2].

The extremely low ammonia-N output in period 4 for all animals cannot at present be explained. This decrease is outside the normal range of variations and may possibly be the cumulative effect of periods 3 and 4, during both of which the Ca : P ratio was abnormal.

(iii) *Creatinine-creatinine metabolism.* The creatinine output, particularly when expressed as % of total N, is markedly higher in period 2 than in the remaining three periods. The lowest values occur in period 3.

The creatine output remains unchanged in total amount but the percentage is lower with the low P diets. In period 3 there is both a total and percentage decrease. This appears readily explicable, since P is intimately connected with the creatine metabolism, both varying in the same direction.

(iv) *Purin metabolism.* In the P-deficient periods both uric acid and purine base excretions, when expressed as % of total N, are lower than in the normal period. In period 3 the uric acid decreases but the purine base increases markedly.

The P deficiency causes a marked increase in the urinary output of allantoin, the diet with the abnormal Ca : P ratio having a less striking effect in the same direction. It is interesting to note that Terroine [1936] obtained similar results and claimed that this increase is due to synthesis of allantoin from ingested protein and is not due to an increased catabolism of body tissue.

(v) *Sulphur metabolism.* Details of the S metabolism are shown in Table V. In the P-deficient periods the urinary S output is greatly diminished with a correspondingly large increase in the N : S ratio. In period 3, although the S output remains constant (as compared with period 2) the N has increased, with a corresponding slight increase in the N : S ratio.

Table V. *S balance*

Period	Sheep	Urine N g.	Urine S g.	N : S ratio
1 (low P)	1	12.77	1.45	8.8
	2	10.67	1.47	7.3
	3	12.99	1.80	7.2
	4	9.49	0.99	9.6
2 (P supplement)	1	9.06	2.64	3.4
	2	10.44	2.73	3.8
	3	8.47	2.80	3.0
	4	9.86	2.69	3.7
3 (Ca + P supplement)	1	10.50	2.72	3.9
	2	12.21	2.71	4.5
	3	11.02	2.84	3.9
	4	11.04	2.52	4.4
4 (low P)	1	13.10	1.09	12.0
	2	14.87	1.28	11.6
	3	11.58	0.94	12.3
	4	11.12	1.10	10.1

To account for the diminished excretion of urinary S the possibility must be considered that, in a diet deficient in P, S may be able to replace P to some extent in the metabolic reactions in the body. No evidence on this point is, however, available.

Table VI. *S output in faeces*

Period	Sheep	Wt. dry faeces g.	N in faeces g.	S in faeces g.	S g. per kg. dry faeces	N : S of faeces
2	1	251.70	8.47	1.55	6.16	5.4
	2	293.00	8.19	1.54	5.25	5.3
	3	314.70	10.85	1.55	4.92	7.0
	4	281.50	8.18	1.66	5.89	4.9
	1	349.18	8.90	1.94	5.56	4.6
	2	315.85	7.05	1.89	5.98	3.8
	3	337.23	8.05	1.95	5.78	4.1
	4	330.00	7.51	1.88	5.69	4.0
4	1	156.67	7.39	0.93	5.93	7.9
	2	210.04	7.86	1.13	5.37	7.0
	3	197.30	7.71	1.03	5.22	7.5
	4	353.32	8.74	1.80	5.09	4.9
		Period		2	3	4
Mean S g. per kg. dry faeces of all 4 animals				5.56	5.75	5.40
Mean N g. per kg. dry faeces of all 4 animals				30.21	23.64	32.22

The faecal S excretion and the N:S ratio in the faeces are shown in Table VI. It will be seen that the S output varies directly with the dry weight of the faeces, and not, as in the case of the N output, with the dry matter of the ingested food. Further work on this subject has been carried out which shows that the result is definitely not due to any inherent error in the analytical technique used.

Nitrogen balance. The N balance (Table VII), although positive in each period, is markedly lower in periods 1 and 4 with a P-deficient diet. This agrees with the weight gains previously noted.

Table VII. *N balance and biological value*

Period	Sheep	N intake g.	N output g.	Daily balance g.	Biological value
1 (low P)	1	26.56	19.25	3.66	60
	2	28.16	18.58	4.79	72
	3	23.80	19.30	2.25	53
	4	26.56	16.12	5.22	72
2 (P supplement)	1	30.10	17.53	6.29	78
	2	30.10	18.63	5.74	76
	3	30.10	19.32	5.39	78
	4	30.10	18.04	6.03	75
3 (Ca + P supplement)	1	30.10	19.40	5.35	73
	2	30.10	19.26	5.42	70
	3	30.10	19.07	5.52	72
	4	30.10	18.55	5.78	72
4 (low P)	1	24.68	20.49	2.10	54
	2	26.24	22.73	1.76	53
	3	24.68	19.29	2.70	60
	4	24.68	19.86	2.41	60

The apparent biological value of the protein has been calculated. The formula used is the customary one

$$1 - \frac{U - E}{I - (F - M)},$$

where U = urinary N, E = endogenous urinary N, I = intake, F = total faecal N and M = metabolic faecal N. The figures for M and E were taken from the results of Hutchinson & Morris [1936, 2]. It is clear that an appreciably lower figure is

recorded with the P-deficient diets. The apparent biological value for period 3 is lower than that for period 2 and appears to substantiate the claim that in this period the results are transitional between those for the normal and the P-deficient periods.

The results of these determinations of the biological value are important in that they show the impossibility of calculating biological values from the results of experiments in which all food factors except the protein are not present in sufficient amount.

Nitrogen metabolism. A résumé of the results in the present experiment shows, therefore, that the exogenous metabolism remains unaltered during the feeding of a P-deficient diet. Since the endogenous metabolism is represented by the creatinine, creatine, purine and S metabolism, it would appear that, with a P-deficient ration, or with a ration containing an abnormally large Ca : P ratio, the endogenous metabolism is diminished. That this will eventually lead to active tissue catabolism appears probable from the work of Theiler, Du Toit and others. In the present instance, however, the excess urinary N found on feeding diets deficient in P is due to the ingested protein and is exogenous in origin. The excess allantoin must, therefore, also arise from the ingested protein, as Terroine has claimed.

The primary effect of a P-deficient diet is, therefore, a slowing-down of tissue formation with a consequent deamination and excretion of the excess of amino-acids. The decreased protein digestibility is probably the result of a diminished secretion of digestive juices and enzymes, which appear to use P in their metabolic reactions.

Mineral metabolism. The Ca and P balances are shown in Tables VIII and IX. With a P-deficient diet there was a loss of both Ca and P. When the supplement of P was added (period 2) a positive balance was recorded.

Whereas the urinary Ca increased in periods 1 and 4, the urinary P almost disappeared owing to the very low intake. In any period the amount of P excreted in the urine is only about 1% of the total P excreted. The Ca excretion varies markedly. In the normal period 2 the Ca excretion in the urine is only about 0.5% of the total output, whereas in the P-deficient periods it is about 5%. In period 3, with the abnormal Ca : P ratio, the urinary Ca is about 1.2%

Table VIII. *CaO balance*

Period	Sheep	CaO intake in 2 days g.	CaO output in 2 days			Daily balance g.
			Faeces g.	Urine g.	Total g.	
1 (low P)	1	10.78	10.88	0.46	11.34	-0.28
	2	12.02	13.42	0.46	13.88	-0.93
	3	9.50	11.14	0.60	11.74	-1.12
	4	10.50	11.26	0.66	11.92	-0.71
2 (P supplement)	1	13.80	12.04	0.08	12.12	+0.84
	2	13.80	12.60	0.06	12.66	+0.57
	3	13.80	12.70	0.04	12.74	+0.53
	4	13.80	11.92	0.06	11.98	+0.91
3 (P + Ca supplement)	1	128.04	118.92	2.56	121.48	+3.28
	2	128.04	119.08	1.76	120.84	+3.60
	3	128.04	122.76	1.50	124.26	+1.89
	4	128.04	124.52	1.16	125.68	+1.18
4 (low P)	1	9.02	11.50	0.56	12.06	-1.52
	2	10.22	12.42	0.44	12.86	-1.32
	3	9.02	11.40	0.68	12.08	-1.53
	4	9.02	11.40	0.46	11.86	-1.42

Table IX. P_2O_5 balance

Period	Sheep	P_2O_5 intake in 2 days g.	P_2O_5 output in 2 days			Daily balance g.
			Faeces g.	Urine g.	Total g.	
1 (low P)	1	2.22	2.54	0.02	2.56	-0.17
	2	2.24	3.52	0.02	3.54	-0.65
	3	1.90	3.70	0.02	3.72	-0.91
	4	2.08	3.26	0.02	3.28	-1.10
2 (P supplement)	1	12.86	12.42	0.10	12.52	+0.17
	2	12.86	11.30	0.16	11.46	+0.70
	3	12.86	11.68	0.60	11.74	+0.56
	4	12.86	11.04	0.20	11.24	+0.81
3 (P + Ca supplement)	1	12.86	10.96	0.11	11.07	+1.79
	2	12.86	12.86	0.11	12.97	-0.11
	3	12.86	13.45	0.05	13.50	-0.64
	4	12.86	13.19	0.04	13.23	-0.37
4 (low P)	1	1.58	3.14	0.02	3.16	-0.79
	2	1.84	3.78	0.02	3.80	-0.98
	3	1.58	3.58	0.00	3.58	-1.00
	4	1.58	3.30	0.02	3.32	-0.87

of the total. This agrees with the theory of Cayla [1935] that an excess of Ca over P in the diet may lead, whether the P intake is deficient or not, to a deficiency of both Ca and P. This may possibly be due to a precipitation of insoluble Ca salts in the alimentary tract, whereby they are not absorbed and utilized.

SUMMARY

1. A complete study has been made of the effect of a P-deficient diet on the protein and mineral metabolism of sheep.

2. An increased output of urinary N was noted in the periods of P deficiency and when a diet with an abnormally high Ca : P ratio was fed, although the food intake was lower.

3. The exogenous N metabolism was unaffected by a P-deficient diet, but the endogenous metabolism was diminished.

4. In the P-deficient periods there were losses of both Ca and P, which were remedied by the addition of a supplement of P.

5. The proportion of the total Ca excreted found in the urine rose, in the P-deficient periods, from 0.5 to 5%.

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