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THE EFFECT OF RUMINAL AND DUODENAL APPLICATION OF DIFFERENT LEVELS OF CALCIUM AND PHOSPHORUS TO SHEEP ON SEMI-PURIFIED DIETS*

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OPSOMMING: DIE EFFEK VAN RUMINALE EN DUODENALE AANWENDING VAN VERSKILLENDE PEILE VAN KALSIUM EN FOSFOR BY SKAPE

Nege behandelings van Ca_oP_o, Ca_r, Ca_d, P_d, $(CAP)_r$, Ca_dP_r, $(CaP)_d$ en Ca_rP_d (o = geen supplementasie; r = aan rumen en d = aan duodenum) is ondersoek. P-supplementasie onderdruk Ca-absorpsie en -retensie en omgekeerd. P-absorpsie in die duodenum is konsentrasie-afhanklik en Ca- en anorganiese P-konsentrasie in die rumen is afhanklik van die inname: Ca meer so as P. Serum-Ca was betreklik konstant terwyl serum anorganiese P (P_i) beduidend deur die behandelings beïnvloed is. Ca- en P-onderhoudsbehoeftes kan as 3,0 en 1,8 g/dag en die totale endogene verlies 0,5 en ongeveer 0,34 g/dag onderskeidelik beskou word. Die Ca/P verhouding vir die rumen-mikro-organismes is nie krities nie maar die Ca-peil mag gewoonweg meer krities as die P-peil in die rumen wees. 'n Wye Ca/P verhouding vir herkouers is aan te beveel. Konsentrasies van P_i en vetsure in rumenvloeistof is betekenisvol gekorreleerd sowel as rumen-Ca met vetsure en TCA-N.

SUMMARY

The effect of different levels and combinations of Ca and P consisting of nine treatments as Ca_oP_o, Ca_r, P_r, Ca_d, P_d, $(CaP)_r$, Ca_dP_r, $(CaP)_d$ and Ca_rP_d were investigated with an unrestricted semi-purified diet (r = to the rumen; d = to the duodenum, and o = no supplementation). P supplementation, irrespective of site, depressed Ca absorption and retention and *vice versa*. P absorption is favoured by high concentrations in the duodenum, absorption thus being concentration dependent. Ca and inorganic P (P_i) concentration in rumen liquor is intake dependent; the former more so than the latter. The serum Ca was relatively stable despite treatment differences, whereas serum P_i concentration was significantly affected by treatments. Serum Ca and P_i are negatively correlated ($r = -0{,}5307$). Ca_r resulted in the highest concentration of serum Ca but depressed serum P_i more effectively than e.g. Ca_oP_o and Ca_d. The Ca and P maintenance requirements were found to be 3,0 and 1,8 g/day and the endogenous loss 0,5 and approximately 0,34 g/day respectively. The Ca/P ratio for the rumen micro-organisms is not critical but the rumen Ca level may usually be of a more critical nature in contrast to rumen P_i levels, and thus, favouring a wider ratio than is usually recommended for non-ruminants. Concentrations of rumen liquor P_i and VFA were found to be significantly associated as well as rumen Ca with VFA and TCA-N, respectively.

Although intensive research has been carried out on the role of Ca and P in general on a wide variety of animals, conflicting and varying results for the requirements, ratios and metabolism of these minerals in ruminant nutrition indicate the need for more controlled and precise work on these elements with ruminants. As a result of the complex interrelationships between Ca and P the earlier research was generally aimed at the integrated role of these two minerals and Mg for the sheep and its ruminal micro-organisms as a single function. During the past decade, however, more and more attention has been given to the mineral requirements of the micro-organisms of the rumen, but the animal, the mammalian component of the system, as a separate function is still being neglected. The lack of knowledge of the specific role of Ca and P in ruminant nutrition is probably due to the modifying influence of the rumen.

The object of this investigation was as a first step, to differentiate between the host animal and the rumen environment as regards the Ca and P requirements, ratio and balances. To achieve this, different levels and combinations of Ca and P were supplied to the whole system (rumen and sheep) and to the sheep only, by by-passing the rumen by infusing the minerals *per duodenum*. Mg was also measured as an additional mineral parameter but is not reported on in this paper. The effect of the experimental elements on (a) the activity of the rumen micro-organisms, and (b) on the sheep were therefore investigated independently and compared with the replete total system. The complex conjugation of the two systems was, however, simultaneously demonstrated.

The field covered by the investigation is rather wide, but it facilitates a general survey of the problems of Ca and P nutrition of the total and individual components of the ruminant system, and serves to indicate the direction for further more detailed and more specific research.

Procedure

(a) MATERIAL

(i) Animals: Five four-tooth (full-grown) Merino wethers were fitted with permanent ruminal and duodenal cannulae (Jarrett, 1948; Phillipson, 1952). The duodenal cannula was inserted immediately caudal to the common opening of the bile and pancreatic ducts. Metabolism crates fitted with polyethylene urine collection trays and galvanised iron feed bins were used.

(ii) During the preliminary trial periods metropolitan scheme water was available *ad lib.* During collection periods deionised water was offered.

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(iii) Rations: The composition of the basal ration (DM basis) is given in Table 1. One kg of feed was offered daily at 09h00, but, when necessary, this quantity was increased so as to ensure *ad lib.* feed intake. The basal mineral mixture is presented in Table 2. The major components were B.P. grade. All trace minerals were adequately supplied and the composition of the trace mineral mixture approximates that as described by Moir & Harris (1962).

Table 1

The percentage composition of the basal ration

Component	%
Oat hulls	80,23
HCl precipitated casein	5,00
Corn starch	5,00
Cane sugar	5,00
Urea	1,77
Mineral mix + Vitamins	3,00
TOTAL	100,00

Table 2

Percentage composition of the basal mineral mixture

Component	%
KCl	14,71
K_2SO_4	12,90
$KHCO_3$	21,41
$NaHCO_3$	5,42
NaCl	13,67
$MgSO_4.7H_2O$	25,03
Trace mineral + vit.	6,86
TOTAL	100,00

(b) METHODS

Supplementary Ca was provided in the form of $CaCO_3$ and supplementary P as a 1:1 mixture of K_2HPO_4 and KH_2PO_4. Supplements of Ca and/or P were given either to the rumen in the ration and/or directly *per duodenum* thus bypassing the rumen. The various treatment combinations of supplementary Ca and P administration are presented in Table 3.

Table 3

Experimental treatments

Treatment	Site of supplementation		Abbreviated definition
	Rumen	Duodenum	
A Basal (control)	–	–	Ca_oP_o
B Basal plus	Ca	–	Ca_r
C Basal plus	P	–	P_r
D Basal plus	–	Ca	Ca_d
E Basal plus	–	P	P_d
F Basal plus	Ca+P	–	$(CaP)_r$
G Basal plus	P	Ca	Ca_dP_r
H Basal plus	–	Ca+P	$(CaP)_d$
I Basal plus	Ca	P	Ca_rP_d

An incomplete Latin square design consisting of nine treatments, nine periods and five sheep was used. The experiment was commenced during March, 1964. At the end of Period 5 one sheep (No. 2) died and was replaced. After the termination of the experiment, No. 2 continued to receive the treatments for incompleted periods (1 to 5) so that eventually it received the full complement of treatments. From Period 6 the intake of No. 5 deteriorated gradually, and the last four periods (6 to 9) were most unsatisfactory. Consequently, Sheep 5 was rested, allowed to recuperate and thence repeated the treatments concerned. These subsequent periods for the two sheep were numbered 10 to 14. Consequently the experiment was completed in the pattern as is shown in Table 4.

Each period was of 28 days' duration consisting of seven days repletion, 14 days preliminary feeding and seven days collection.

The oat hulls were purchased from commercial firms and were generally of very low mineral content (Ca 0,054%; P 0,025%; Mg 0,056%; N 0,28% and crude fibre 28,0%). The chemical composition of the experimental diets found by analysis is given in Table 5. The basal +Ca+P ration, (i.e. the same as $(CaP)_r$) also served as the repletion diet. The sheep were also fed on this ration for six weeks before the commencement of the experiment.

Duodenal infusions

The quantities infused were based upon intake data from preliminary trials of about six weeks' duration in which DM intakes of the sheep were from 1 000 to 1 300 g. These initial quantities for the duodenal infusions, once decided upon, were subsequently kept constant but for decreasing them considerably when inappetence occurred. Thus duodenal supplementary Ca was normally provided daily as 22,5 g $CaCO_3$, (9 g Ca) suspended in 1,5 *l* deionised water and the daily P was supplied as a mixture of 9,9 g

Table 4

The plan of the experiment as completed

Periods	Experimental months	Treatments				
		Sheep				
		1	2	3	4	5
1	March	E	–	I	B	G
2	April/May	H	–	D	I	E
3	June	I	–	C	F	A
4	July	B	–	E	G	F
5	July/August	G	–	B	H	D
6	August	D	I	F	C	–
7	September	F	G	H	A	–
8	October	A	C	G	D	–
9	November	C	F	A	E	–
10	December		A			–
11	January		E			I
12	January/ February		D			H
13	February		B			C
14	March		H			B

Table 5

The percentage chemical composition of the diets on DM basis

Component	Basal	Ca, P and K in supplemented diets
Ca	0,066	0,730
P	0,068	0,335
Mg	0,130	
K	1,14	1,69
Na	0,29	
Fe	0,039	
Cu	0,0018	
Mn	0,0047	
Co	0,0008	
Mo	0,00008	
Zn	0,0029	
S	0,24	
I, Se, B	traces	
N	1,79	
Crude Fibre	25,0	
Ash	7,2	7,5

K_2HPO_4 plus 9,9 g KH_2PO_4 (4 g P) dissolved in 1,5 *l* deionised water (pH = 6,7). The rate of infusion into the duodenum was approximately 1,5 *l* per 22 hours.

The pumping unit

In order to facilitate the application of the duodenal treatments, a locally constructed unit was geared to rotate a cam and a roller pump. The unit pumped the $CaCO_3$ suspension and the fluids concerned from polyethylene bottles (serving as reservoirs set up above the unit) through polyethylene tubes (3 mm internal diameter), interrupted with a glass drip tube, to the sheep. The bottles containing the $CaCO_3$ suspended in 1 500 ml H_2O and the bottles containing $CaCO_3$ + K_2HPO_4 + KH_2PO_4 mixture were fitted with stirrers to ensure a homogenous flow of the suspension to the sheep. An equal quantity of deionised water was pumped into all sheep not receiving duodenal experimental treatments in order to maintain a similar fluid status.

Parameters

(1) DM and water intake, DM digestibility and balance of N, Ca, P and Mg.

(2) Rumen function as reflected by:

(a) pH of rumen digesta measured immediately after sampling.
(b) Total volatile fatty acid (VFA) concentration in rumen digesta.
(c) The concentration of trichloroacetic acid precipitated nitrogen (TCA-N) in rumen digesta.
(d) Cellulose digestion as determined by the cotton thread technique described by Moir & Harris (1962) and Nel (1968).

(3) The mean retention time (MRT) of the ingesta as estimated by the stained particle technique described by Balch (1950) and Castle (1956) and was calculated as proposed by Graham & Williams (1962).

(4) Blood serum Ca, P and Mg levels.

(5) Concentration of Ca, P and Mg in rumen fluid and urine.

Bodyweights of the experimental animals were recorded at the beginning and end of each period.

Sampling

The collected urine was acidified daily with concentrated HCl to pH 3–4. A 10% aliquot was taken and bulked prior to analysis. Faeces were collected each morning at 09h00, 1/10 aliquots taken, dried to constant mass in a forced draught oven, DM determined and then bulked. One tenth of the orts and samples of the rations mixed were also taken, bulked, sub-sampled, ground and stored.

Rumen liquor

Samples of rumen contents were taken not less than two hours after removal of orts on the morning following the completion of the collection period. Samples (about 120 ml) were withdrawn from the ventral region of the rumen. After the measurement of the pH of the samples they were strained through a single layer of Bolting silk (Henry Simon's Bolting silk, XX). From each strained rumen liquor (SRL) sample, approximately 25 ml was sub-sampled and centrifuged (CRL) at high speed (10 000 *g* for 20 minutes). A relatively clear supernatant fluid, which was free from micro-organisms, was carefully decanted into 30 ml McCartney bottles for subsequent mineral analysis. The remaining portion of the SRL was stored in a deep freeze for total VFA and TCA-N analyses, Inorganic P analysis of the CRL was performed on the same day as sampling. The remaining CRL was also stored in the deep freeze for subsequent Ca and Mg analyses.

Blood

Samples were taken immediately prior to rumen sampling. Approximately 25–30 ml of blood was obtained by jugular puncture and allowed to coagulate. The clear serum was decanted and inorganic P analysis performed immediately. The remaining serum was deep frozen until Ca and Mg analyses could be carried out.

Analytical

All analyses were carried out in duplicate except in the case of TCA-N for which there was insufficient sample.

CALCIUM AND MAGNESIUM: Analyses and preparation of samples for Ca and Mg in rumen liquor, blood serum, urine, feeds and faeces were performed as described by Nel (1967a), Nel & Moir (1967) and Nel & Moir (1968a).

PHOSPHORUS: The inorganic P in blood serum was performed using the "heteropoly blue" method as described by Boltz & Lueck (1958). However, the molybdovanadophosphoric acid method (Boltz & Lueck, 1958) was used for all other P analyses. Solids were ashed and subsequently dissolved as described by Nel (1967a).

TOTAL VFA, TCA-N AND NITROGEN. The analysis for total VFA content of strained rumen fluid was conducted by the steam distillation (McAnally, 1944) as modified by Barcroft, McAnally & Phillipson (1944). TCA-N was determined as described by Cline, Hershberger & Bentley (1958). Analyses of total nitrogen in faeces, feeds and urine were performed by the method outlined by the AOAC (1960).

Statistical Analysis

The change in the experimental plan (Table 4) as a result of the misfortune with Sheep 2 and 5, complicated

the statistical analysis considerably. The statistical solution of the results was obtained by the method of least squares as described by Harvey (1960). Analyses of variance were carried out and for significant effects Duncan's Multiple Range test (Kramer, 1957) was applied. Data expressed in percentage within the range 0–100 were transformed using the Angular Transformation of Stevens (1953). The analysis by the method of least squares permitted testing for the effects of sheep, treatments and periods but the data of Periods 10 to 14 had to be sacrificed. Alternative analyses of variance were, however, also carried out where the observations of Periods 10–14 (Table 4) were included, assuming no period effect. These analyses of variance were used as check on the interpretation of the former statistical analyses.

Results

The means of the body mass on the final day, the mean daily water and dry matter intakes during collection periods, the mean percentage of dry matter digestibilities and the mean apparent $^1/2$-Life ($^1/2$-L) of the cotton threads suspended in the rumen are presented against the different treatments in Table 6. The body mass changes were not considered to be of any particular significance but the animals were weighed regularly as a check on their general welfare. The water intakes in Table 6 are the voluntary intakes. The sheep received in addition 1,5 *l* of water with the infusions. The drymatter (DM) consumption during collection periods differed significantly ($P < 0,05$) between sheep only. The DM masses of the duodenal supplements were included in the DM intake for calculation of the digestion coefficients as given in Table 6. The transformed DM digestion coefficients revealed no significant difference between treatments and periods. If all the treatments are considered in the "alternative statistical analysis" then Treatments Ca_OP_O and P_r > Ca_r, Ca_rP_d and P_d ($P < 0,05$). This difference in significance is possibly due to differences in degrees of freedom in favour of the alternative analysis.

The mean mass losses of cotton thread bundles between the different intervals in the rumen were not significant. The means of only the apparent $^1/2$-Life are, however, given in Table 6 as an indication of the cellulolytic activity in the rumen. The grand mean $^1/2$–L of the bundles, was 36,8 hours. There were significant differences between animals for the intervals (h) 24–36 ($P < 0,01$); 0–36 ($P < 0,05$) and 0–48 hours ($P < 0,05$) as well as for the apparent $^1/2$–L ($P < 0,05$). There also was a significant period effect on the cotton thread digestion for the interval 24–36 h in as much that the digestion during Periods 3 and 4 (June and July) was better than for Periods 2, 9 and 1 (April, November and March).

The Effects of Calcium

(1) Ca balances

The Ca balance data are presented in Table 7. The percentages of Ca absorbed and retained are the weighted means. Each figure represents the mean of five sheep. From the data it can be seen that negative balances were obtained for Treatments Ca_OP_O, P_r and P_d. Positive balances were obtained for all sheep in the remainder of the treatments except for Sheep 3 which gave a negative value of Treatment Ca_d.

The Ca retention obtained with Treatments Ca_r, $(CaP)_d$, Ca_dP_r, Ca_rP_d, $(CaP)_r$ and Ca_d was significantly better than with Treatments P_r and P_d ($P < 0,05$). A notable feature from the percentages of absorption for the different treatments in Table 7 is the larger negative values of Treatments P_r and P_d than the other negative values. The high P levels in Treatments P_r and P_d apparently depressed the absorption of Ca below that of Treatment Ca_OP_O. Statistically these treatments do not differ significantly. Treatments Ca_r, $(CaP)_d$, Ca_dP_r, $(CaP)_r$ and Ca_rP_d, however, differ significantly from both Treatments P_r and P_d ($P < 0,05$) and Treatments Ca_d from P_d ($P < 0,05$).

(2) The correlations and regression of Ca output and faecal Ca on Ca intake

To be able to make some deductions and estimates from the sets of data of Ca intake, Ca output and faecal Ca excretion, the product-moment correlation coefficient (r) between these variables was generally calculated. The regressions by the method of least squares and the regression equations were also determined where appropriate.

(a) Ca output cf. Ca intake

In Fig. 1 the results of the Ca output (y) as the dependent variable were plotted against the Ca intake. The correlation coefficient $r = 0,9799$ is significant ($P < 0,001$). The regression equation for the line of best fit for the plotted data is

$$y = 0,8242x + 0,4999 \ (SE = \pm 0,6626).$$

In Fig. 1 the line of best fit crosses the line of equilibrium (broken line) at a point which indicates that a Ca intake of 3 g/day is required for Ca equilibrium. The daily total endogenous loss of Ca obtained from the above regression is 0,5 g and the retention is 18%.

(b) Faecal Ca cf. Ca intake

In similar fashion the following correlations and regression equations of the different dependent variables (y) in g/day were calculated on the Ca intake (x) in g/day.

(i) Faecal Ca excretion (y) in g/day for all treatments $r = 0,9811$ ($P < 0,001$)

$$y = 0,8236x + 0,4495 \ (SE = \pm 0,6462)$$

The endogenous loss of Ca is 0,45 g/day. Subtracting this figure from the former suggests that the endogenous loss of Ca by way of the kidneys is a negligible 0,05 g/day. From the point of equilibrium it is estimated that 2,2 g Ca

Table 6

The means of the live mass, the daily H_2O and DM intake, the DM digestibility and the apparent $^1/_2$-L of cotton threads in the rumen for the different treatments

Treatments	Body mass	H_2O intake	DM intake	DM digest.	Apparent $^1/_2$–L
	kg	ml	g	%	h
Ca_oP_o	49,50	771	881	63,7	41,9
Ca_r	44,90	510	891	57,2	36,0
P_r	45,00	743	737	62,8	40,1
Ca_d	47,20	502	661	60,2	38,6
P_d	44,80	1301	979	56,7	38,0
$(CaP)_r$	49,80	705	932	58,9	35,6
Ca_dP_r	48,20	940	945	59,6	37,7
$(CaP)_d$	44,20	866	801	59,2	29,4
Ca_rP_d	46,80	1218	1151	56,9	33,7
$\bar{X}$	46,71	840	886	59,5	36,8
SD	–	± 476	± 289	± 4,2	± 11,9
CV%	–	56,7	32,6	7,05	32,3
Significance					
Sheep	–	**	*	*	*
Periods	–	**	NS	NS	NS
Treats	–	NS	NS	NS?	NS

Table 7

The balance of Ca for the different treatments

Treatments	Intake	Faecal excr.	Apparent Absorp.	Apparent absorp.	Urinary excr.	Retained	Retained
	g/day	g/day	g/day	%	g/day	g/day	%
Ca_oP_o	0,582	0,786	–0,204	–35,05	0,073	–0,277	–47,59
Ca_r	6,506	5,980	0,526	8,08	0,219	0,307	4,72
P_r	0,486	0,946	–0,460	–94,65	0,021	–0,481	–98,97
Ca_d	9,436	8,308	1,128	11,95	0,074	1,054	11,17
P_d	0,646	1,000	–0,354	–54,80	0,045	–0,399	–61,76
$(CaP)_r$	6,805	6,202	0,603	8,86	0,019	0,584	8,58
Ca_dP_r	9,624	8,618	1,006	10,45	0,019	0,987	10,26
$(CaP)_d$	8,928	6,976	1,952	21,86	0,018	1,934	21,66
Ca_rP_d	8,402	7,578	0,824	9,81	0,028	0,796	9,47
$\bar{X}$	5,713	5,155	0,558	9,76	0,057	0,501	8,76

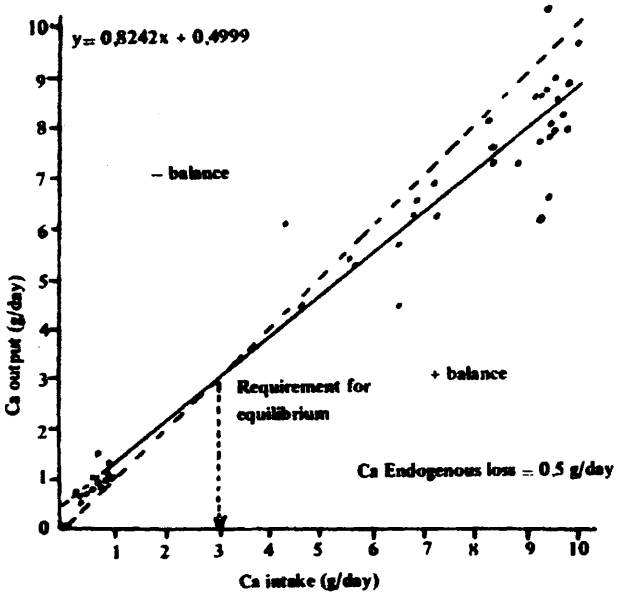


Fig. 1. *The correlation between and regression of Ca output as g/day (y) and g Ca intake per day (x) for all treatments*

is daily required to balance the faecal loss only. The "true" absorption is 18%.

(ii) A separate regression equation was calculated for treatments Ca_r, Ca_d, $(CaP)_r$, Ca_dP_r, $(CaP)_d$ and Ca_rP_d constituting the high level Ca treatments.

$r = 0{,}8620$ ($P<0{,}01$)
$y = 8099x + 0{,}5683$ (SE = $\pm 0{,}7885$)

The endogenous faecal loss is 0,57 g/day, the required daily intake to balance this loss is 2,8 g Ca and the absorption is 19%.

(iii) The regression for treatments Ca_oP_o, P_r and P_d constituting the low level treatments is

$r = 0{,}6384$ ($P<0{,}05$)
$y = 0{,}7480x + 0{,}4832$ (SE = $\pm 0{,}2189$)

The Ca faecal loss is 0,48 g per day, the daily requirement to balance the faecal loss is 1,88 g and the true absorption is 25%.

(3) Concentrations of Ca

The concentrations of Ca in urine, rumen liquor and blood serum are presented in Table 8. The urinary concentration of Ca as a result of Treatment Ca_r is higher than all other treatments ($P<0{,}01$) and Treatment Ca_d is higher than P_r and Ca_dP_r ($P<0{,}05$).

The statistical analysis of the transformed data of the rumen fluid reveal that the treatments involving ruminal supplementation of Ca give a concentration of Ca in centrifuged rumen fluid higher than any other treatments ($P<0{,}01$). The order of Ca concentration in the rumen fluid for the different treatments was Ca_rP_d, $(CaP)_r$, Ca_r, Ca_oP_o, Ca_dP_r, P_r, P_d, Ca_d and $(CaP)_d$. The Ca concentrations in rumen fluid as a result of Treatments Ca_rP_d, $(CaP)_r$ and Ca_r were significantly higher than those of Treatments Ca_oP_o, Ca_d, Ca_dP_r, P_r, $(CaP)_d$ and P_d, the other treatments not being significantly different. The mean Ca contents of blood serum for the different treatments are ranked in the following order: Ca_r, Ca_oP_o, Ca_d, Ca_rP_d, $(CaP)_r$, Ca_dP_r, P_d, P_r and $(CaP)_d$.

The statistical analysis of the data (angularly transformed) showed significant treatment differences which are as follows:

(i) $Ca_r > Ca_d$, Ca_rP_d, $(CaP)_r$, $(CaP)_d$, Ca_dP_r, P_r and P_d ($P<0{,}01$);
(ii) $Ca_oP_o > (CaP)_r$ ($P<0{,}05$), $(CaP)_d$, Ca_dP_r, P_r and P_d ($P<0{,}01$);
(iii) $Ca_d > (CaP)_d$ ($P<0{,}05$), Ca_dP_r, P_r and P_d ($P<0{,}01$);
(iv) Ca_rP_d and $(CaP)_r > Ca_dP_r$, P_r ($P<0{,}05$), and P_d ($P<0{,}01$); and
(v) $Ca_dP_r > P_d$ ($P<0{,}01$).

(4) Correlation between serum Ca and rumen fluid Ca

The correlation coefficient was calculated between the concentration of Ca in serum (mg%) and the concentration of Ca in rumen fluid (mg%) and a significant $r = 0{,}4538$ ($P<0{,}01$) was found. The regression of serum Ca concentration (y) in mg% on the rumen liquor Ca concentration (x) in mg% is $y = 0{,}1048x + 7{,}9659$ (SE = $\pm 0{,}7358$). Several logarithmic functions were also tested as fits to these data but the linear regression was a better fit than the curvilinear functions tested.

The effects of Phosphorus

(1) P balances

The weighted means of the percentages of P absorbed and retained are presented in Table 9. Negative balances were obtained for Treatments Ca_oP_o, Ca_r and Ca_d which were treatments of low P intake. The retention obtained with Treatments Ca_dP_r and P_r is, however, also negative, a surprising result in the light of a very high intake of P. The statistical analysis of the retention data reveals that the percentage retention of P in Treatment $(CaP)_d$ is significantly higher than that in Treatments Ca_oP_o, Ca_r ($P<0{,}05$) and in treatment Ca_d ($P<0{,}01$). With Treatments P_d, Ca_rP_d, $(CaP)_r$, Ca_dP_r, P_r, Ca_oP_o and Ca_r significantly more P was retained than with Treatment Ca_d.

The percentage absorption of P also showed significant treatment differences as follows:

(i) $P_d > (CaP)_r$, Ca_oP_o, Ca_r and Ca_d;
(ii) $(CaP)_d$, P_r, Ca_dP_r and $Ca_rP_d > Ca_oP_o$, Ca_r and Ca_d;
(iii) $(CaP)_r > Ca_oP_o$ and Ca_d; and
(iv) Ca_oP_o and $Ca_r > Ca_d$.

Table 8

The concentrations of Ca in urine, centrifuged rumen liquor and blood serum

Treatments	Urine	Rumen liquor	Serum
	mg%	mg%	mg%
Ca_oP_o	8,79	2,71	9,12
Ca_r	20,86	6,47	9,46
P_r	1,58	2,46	7,66
Ca_d	6,57	2,28	8,71
P_d	3,14	2,45	7,98
$(CaP)_r$	1,78	8,17	8,52
Ca_dP_r	1,35	2,60	8,04
$(CaP)_d$	1,58	1,84	7,52
Ca_rP_d	2,07	8,57	8,61
$\bar{X}$	5,30	4,17	8,40
SD	7,56	3,53	0,82
CV%	142,6	84,7	9,72
Significance			
Sheep	**	NS	*
Periods	NS	NS	**
Treatments	**	**	**

Table 9

The balance of the total P for the different treatments

Treatments	Intake	Faecal excr.	Apparent absorp.	Apparent absorp.	Urinary excr.	Retained	Retained
	g/day	g/day	g/day	%	g/day	g/day	%
Ca_oP_o	0,598	0,674	–0,076	–12,7	0,016	–0,092	–15,4
Ca_r	0,606	0,739	–0,133	–21,9	0,013	–0,146	–24,1
P_r	2,468	1,565	0,903	36,6	0,931	–0,028	– 1,1
Ca_d	0,448	0,623	–0,175	–39,1	0,043	–0,218	–48,7
P_d	4,400	3,042	1,358	30,9	0,913	0,445	10,1
$(CaP)_r$	3,122	2,748	0,374	12,0	0,177	0,197	6,3
Ca_dP_r	3,166	2,809	0,357	11,3	0,552	–0,195	6,2
$(CaP)_d$	4,278	2,753	1,525	35,6	0,687	0,838	19,6
Ca_rP_d	4,782	3,858	0,924	19,3	0,181	0,743	15,6
$\bar{X}$	2,652	2,090	0,562	21,19	0,390	0,172	6,47

(2) **Correlation and regression of P output and P intake**

(a) **P total output on P intake**

As for Ca, the correlation coefficients and the regressions were calculated for P output cf. intake. The correlation between P output and P intake is r = 0,9755 (P< 0,001) and the regression of P output (y) in g/day against P intake (x) in g/day is y = 0,8049x + 0,3432 (SE = ± 0,327). A curvilinear function was also fitted to these data and the equation, $y = 0{,}1598 + 1{,}054x - 0{,}0476x^2$ is approaching significance ($F_{0,05} = 4{,}07$ and calculated F = 4,01) and is a better fit to the data than the linear regression. The scattergram and the lines of best fit are shown in Fig 2. From Fig. 2 the daily requirements for equilibrium are 1,8 and 2,3 g P and the estimated total endogenous losses of P 0,34 and 0,16 g/day as obtained by the linear and curvilinear functions, respectively.

The correlations and regressions for the two populations of the P observations (high and low levels) were also calculated. The regression for Treatments P_r, P_d, $(CaP)_r$, Ca_dP_r, $(CaP)_d$ and Ca_rP_d of P output (y) in g/day on P intake (x) in g/day is y = 0,7020x + 0,7671 (SE = ± 0,3723). The curvilinear function, $y = 0{,}0209x^2 + 0{,}5577x + 0{,}9872$ was also tried as a fit to these data but was found to be insignificant. The correlation coefficient is 0,9124. The estimated P intake required for balance in this case is 2,6 g P/day and the endogenous loss 0,77 g P/day. These two figures are higher than those previously found when the P total output was plotted against P intake for all treatments together.

The regression, P output (y) and P intake (x), for Treatments Ca_oP_o, Ca_r and Ca_d (low levels of P) is y = 0,9135x + 0,1992 (SE = ±0,1015) The estimated daily endogenous loss in this case is only 0,20 g P and the estimated P intake for equilibrium is 2,3 g.

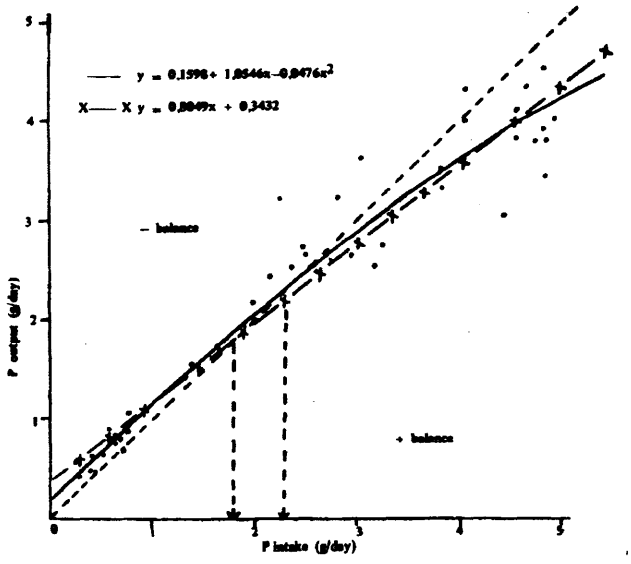


Fig. 2. *The regression of P output (y) on the P intake (x) for all treatments*

(3) **Concentrations of P**

The concentrations of the total P in urine, inorganic P in centrifuged rumen liquor and in blood serum are given in Table 10.

(a) **Urinary concentration of total P**

Considerable differences in the urinary P concentration occurred between sheep (Table 10). The data also reveal significant treatment effects; Treatment P does not differ from P_r but does from Treatments $(CaP)_d$ (P< 0,05), Ca_dP_r, Ca_rP_d, $(CaP)_r$, Ca_d, Ca_r and Ca_oP_o (P< 0,01); Treatment P_r is higher than Treatments $(CaP)_r$, Ca_d, Ca_r (P< 0,05) and Ca_oP_o (P< 0,01), and, Treatment $(CaP)_d$ > Ca_oP_o (P< 0,05).

(b) **Inorganic P in centrifuged rumen liquor**

Significant differences were found between sheep and between treatments. The treatment effects upon values of P_i in rumen contents are as follows:

Treatment $P_r > Ca_d$ (P< 0,05), Ca_oP_o and Ca_r (P< 0,01); in Treatments Ca_dP_r and $(CaP)_r > Ca_oP_o$ and Ca_r and Treatments $(CaP)_d$, P_d and $Ca_d > Ca_r$. The implication of these significant differences is that site of application of P, singly and in combination with Ca, had no influence on the concentration of P_i in the rumen suggesting efficient recycling of P_i to the rumen.

(c) **Inorganic P in blood serum**

The effects of treatments differ significantly(P< 0,01); Treatments P_r and $(CaP)_r > Ca_oP_o$, Ca_d and Ca_r; Treatments Ca_dP_r and $P_d > Ca_d$ and Ca_r and Treatments Ca_rP_d and $(CaP)_d > Ca_r$. The concentration of P_i in blood serum as a result of Treatment Ca_r was significantly less than the others in every instance (P < 0,01); in all other cases the differences were significant at the 5% level.

(4) **Correlation between serum P_i and rumen liquor P_i**

The correlation coefficient between the concentration of serum P_i and the concentration of rumen liquor P_i is r = 0,5421 (P< 0,01).

Correlations between Ca, P and Mg

The following correlation coefficients were calculated between concentrations (mg%) of Ca, P and Mg alternately combined as bivariates.

(1) **Blood serum**

(i) Ca and P_i, r = –0,5307 (P< 0,01)

(ii) Ca and Mg, r = 0,2859 (NS)

(iii) P_i and Mg, r = 0,0778 (NS)

Table 10

The concentration of total P in urine, P_i in rumen liquor and blood serum

Treatments	Urine	Rumen liquor	Serum
	mg %	mg%	mg %
Ca_oP_o	1,40	45,96	5,75
Ca_r	1,40	27,60	4,18
P_r	86,38	93,36	8,16
Ca_d	4,63	63,24	5,52
P_d	100,34	72,28	7,45
$(CaP)_r$	17,55	78,06	7,51
Ca_dP_r	40,70	84,46	8,04
$(CaP)_d$	81,10	72,34	7,95
Ca_rP_d	12,98	62,90	7,30
$\bar{X}$	38,51	66,7	6,87
SD ±	52,21	25,59	1,69
CV%	135,58	38,37	24,49
Significance			
Sheep	**	*	NS
Periods	NS	NS	NS
Treatments	**	**	**

Table 11

The means of the ratios of Ca/P, Ca/Mg and P/Mg in centrifuged rumen liquor and in blood serum

Treatments	Rumen fluid			Blood serum		
	Ca/P	Ca/Mg	P/Mg	Ca/P	Ca/Mg	P/Mg
Ca_oP_o	0,057	0,74	12,98	1,65	3,65	2,32
Ca_r	0,146	1,82	9,22	2,35	3,92	1,71
P_r	0,026	0,73	27,65	0,96	3,36	3,59
Ca_d	0,044	0,83	26,15	1,61	3,52	2,23
P_d	0,027	0,70	23,02	1,09	2,92	2,71
$(CaP)_r$	0,085	2,06	21,51	1,15	3,53	3,13
Ca_dP_r	0,028	0,71	26,37	1,05	3,13	3,16
$(CaP)_d$	0,025	0,58	22,55	0,95	3,15	3,32
Ca_rP_d	0,099	2,93	26,70	1,18	3,27	2,78
$\bar{X}$	0,041	1,23	21,90	1,33	3,38	2,78
CV%	70,3	24,5	53,6	38,17	12,86	29,14
Significance						
Sheep	NS	NS	NS	NS	**	*
Periods	NS	NS	NS	NS	**	NS
Treatments	**	**	NS	**	**	*

(2) **Rumen liquor**

(i) Ca and P_i, r = –0,1033 (NS)

(ii) Ca and Mg, r = 0,4338 (P<0,01)

(iii) P_i and Mg, r = –0,0943 (NS)

(3) **Serum and rumen liquor**

(i) Rumen Ca and serum P_i, r = –0,0182 (NS)

(ii) Rumen Ca and serum Mg, r = 0,1899 (NS)

(iii) Rumen Mg and serum Ca, r = 0,2961 (P<0,05)

(iv) Rumen P_i and serum Mg, r = –0,0761 (NS)

(v) Rumen P_i and serum Ca, r = –0,5412 (P<0,01)

(vi) Rumen Mg and serum P_i, r = – 0,0185 (NS)

The ratios of Ca, P and Mg

The means of the ratios of Ca/P, Ca/Mg and P/Mg in centrifuged rumen liquor and in blood serum are presented in Table 11.

The ratios in rumen liquor

Treatment effects in Table 11 are highly significant. Treatment P_d has a lower ratio of Ca/P than Ca_d, Ca_oP_o, $(CaP)_r$ and Ca_rP_d while Treatments Ca_dP_r, $(CaP)_d$ and P_r< Ca_d, Ca_oP_o, $(CaP)_r$, Ca_rP_d and Ca_r indicating the mobility and recycling of P.

The Ca/Mg ratio of the three Treatments Ca_rP_d, Ca_r and $(CaP)_r$ are considerably higher than all others (P< 0,01). Treatment Ca_rP_d is significantly higher than Treatment $(CaP)_r$.

The ratios in blood serum

The data concerning the Ca/P ratio in serum showed significant treatment differences (P< 0,01). The Ca/P ratio in Treatment Ca_r is higher than all other treatments and Treatments Ca_d and Ca_oP_o are higher than Treatments Ca_dP_r, $(CaP)_r$, P_r and P_d. The Ca/Mg ratio data (Table 11) differ significantly between sheep, between treatments and between periods (P <0,01). Treatment Ca_r resulted in Ca/Mg ratios in blood serum that are significantly higher than those of all other treatments. Likewise, those of Treatments Ca_d and Ca_oP_o are greater than those of all other treatments. Likewise, those of Treatments Ca_d and Ca_oP_o are greater than those of Ca_dP_r and P_d and Treatments Ca_rP_d, P_r, $(CaP)_r$, $(CaP)_d$ and Ca_dP_r are significantly higher than Treatment P_d. It is noteworthy that the coefficient of variation of the Ca/Mg ratio in both the rumen liquor and the serum was the lowest in each case (24,5% and 12,86% respectively).

The values of P/Mg ratio for Treatment P_r are significantly higher than those of Treatments Ca_d (P< 0,05), Ca_oP_o and Ca_r (P<0,01); furthermore, the ratios in the following groups of treatments differed significantly: (1) Treatments Ca_rP_d and $(CaP)_d$ > Treatment Ca_r (P< 0,05) and, (2) Treatments $(CaP)_r$ and Ca_dP_r > Ca_oP_o and Ca_r (P < 0,05).

Experimental effects on nitrogen

(1) **Nitrogen balances**

To test the effects of the treatments on nitrogen balances the relevant data are tabulated in Table 12. The percentage apparent N digestibility differs significantly between sheep, treatments and periods. Treatment P_d was significantly lower than all other treatments. The percentage N retention, however, did not differ significantly.

The correlation coefficient between N output (y) and N intake (x) is r = 0,9336 and the regression equation is y = 0,7579x + 1,2793 (SE = ± 1,521). The total daily endogenous N excretion is estimated as 1,3 g/day and the daily N requirement for equilibrium is 5,3 g/day.

Treatment effects on other Parameters

The treatment means for the pH of the rumen contents, VFA and TCA-N concentration in strained rumen liquor and the rate of passage given as the mean retention time (MRT) of stained particles are compiled in Table 13.

The parameters as presented in Table 13 failed to show any significant effect. The possible association between these parameters and between other variables were determined by means of correlation coefficients. Significant correlations only are reported here. Logarithmic and quadratic transformations were also carried out for all these correlations but only those transformed values that improve the correlation coefficient are reported.

The VFA concentration (mmole%) in rumen liquor is significantly correlated with

(i) DM intake (g/day), r = 0,536 (P<0,01)

(ii) Rumen fluid P_i concentration, r = –0,490 (P<0,01)

(iii) Rumen fluid Ca concentration $r_{quad.}$ = 0,442 (P<0,01).

The following significant correlation coefficients between TCA-N concentration (mg%) and other variables were calculated:

(i) (a) Rumen liquor Ca concentration, r=0,453 (P< 0,01)

(b) Rumen liquor Ca concentration (quadratic transformation), $r_{quad.}$ = 0,615 (P< 0,001)

(ii) (a) VFA concentration, r = 0,655 (P< 0,001)

(b) VFA concentration (quadratic transformation) $r_{quad.}$ = 0,728 (P< 0,001).

Discussion

Digestion of DM and cellulose

The finding in the alternative statistical analysis that the DM digestibility in treatments Ca_oP_o and P_r was sig-

Table 12

The mean nitrogen balance data

Treatments	Intake	Faecal excr.	Apparent absorp.	Apparent absorp.	Urinary excr.	Retained	Retained
	g/day	g/day	g/day	%	g/day	g/day	%
Ca_oP_o	15,76	3,12	12,64	80,20	9,70	2,94	18,65
Ca_r	15,96	3,52	12,44	77,94	9,85	2,59	16,23
P_r	13,18	3,06	10,12	76,78	9,12	1,00	7,59
Ca_d	11,84	2,75	9,09	76,77	7,99	1,10	9,29
P_d	17,54	4,62	12,92	73,66	9,82	3,10	17,67
$(CaP)_r$	16,68	3,64	13,04	78,18	9,06	3,98	23,86
Ca_dP_r	16,90	4,37	12,53	74,14	11,36	1,17	6,92
$(CaP)_d$	14,30	3,48	10,82	75,66	7,59	3,23	22,59
Ca_rP_d	20,60	5,20	15,40	74,76	11,46	3,94	19,13
$\bar{X}$	15,86	3,75	12,11	76,36	9,55	2,56	16,14

Table 13

The means of rumen pH, VFA concentration (mmole%), TCA-N concentration (mg%) and MRT (h) of stained particles

Treats	pH	VFA	TCA-N	MRT
		mmole%	mg%	h
Ca_oP_o	6,29	10,65	71,85	78,7
Ca_r	6,33	11,21	95,45	67,8
P_r	6,41	8,73	73,77	80,9
Ca_d	6,49	8,07	68,77	80,4
P_d	6,44	8,91	62,03	72,0
$(CaP)_r$	6,67	7,98	64,82	78,3
Ca_dP_r	6,31	9,80	77,53	79,4
$(CaP)_d$	6,46	9,09	71,46	75,2
Ca_rP_d	6,42	10,61	70,02	65,5
$\bar{X}$	6,42	9,40	72,85	75,4
SD ±	0,29	2,36	26,30	13,45
%CV	4,47	25,1	36,1	17,84

nificantly higher than that in the Ca_r, Ca_rP_d and P_d treatments is at variance with that of Davison & Woods (1963). They claimed that maximal digestibility of energy was obtained at 0,6% Ca of the DM intake. The depressing effect upon DM loss by the supplementation of Ca to the rumen (Ca_r and Ca_rP_d) and P to the duodenum (P_d and Ca_rP_d) may, however, be ascribed to other components in the DM consumed than energy. This is also borne out by the fact that the control group Ca_oP_o showed the poorest cotton thread digestion but the highest DM digestion.

The DM digestibility may also be influenced by the rate of passage of the food through the alimentary tract (Pearce & Moir, 1964). No significant differences were found, however, in the mean retention time (MRT), as a measure of the rate of passage, between the different treatments (Table 13). On closer inspection, if the means are ranked, it will be noticed that in the two sets of data for the MRT and for the DM digestibility, Treatments Ca_r, P_d and Ca_rP_d occupy the lowest ranks (7, 8 and 9). A possible association between these two variables (DM digestibility and the MRT), was confirmed by a highly significant correlation ($r = 0,532$, $P < 0,01$). It appears therefore, that the addition of Ca to the rumen (Ca_r) and P_i to the duodenum (P_d) may accelerate the passage of food through the alimentary tract thus reducing dry matter digestibility. This possible effect is nullified where Ca and P are combined at the one site. In view of the fact that P_i is recycled to the rumen in substantial quantities (McDougall, 1948; Clark, 1953; Tomas, Moir & Somers, 1967) any adverse effect of the Ca in Ca_rP_d, could possibly have been reduced by the P available from the duodenum. As this was not the case, the elevated P concentration in the duodenum must act directly through the animal. The manner in which any Ca effect could operate is not clear.

Although the evidence for these effects is not conclusive, further work is probably justified in view of widespread recommendations for the use of $CaCO_3$ in ruminant rations. If, for example, elevated rumen Ca increased the rate of passage of concentrate or grain rations, not only could more protein and hexose escape fermentation, thus enhancing food efficiency, but acidosis may be reduced, thus, possibly maintaining intake. The fact that possible treatment differences in the MRT were not found is perhaps due to the large variability, due to sampling error in particular, with the consequent lack in sensitivity in the statistical tests. The positive relationship between these bivariates clearly demonstrates the importance of measuring the rate of passage of food in investigations of this nature.

The Effects of Calcium

Absorption of and requirement for Ca

The method of estimating the endogenous loss by means of a regression equation (extrapolation) was applied by Tyler & Willcox (1942) on laying and non-laying hens. Mitchell (1962) used the same method for estimating endogenous Ca and Ca requirements in human beings. A condition for valid extrapolation is linearity in particular beyond the range of the data. Therefore logarithmic and quadratic transformations were performed as a check on linearity.

The endogenous Ca loss did not differ to any considerable extent between high and low level Ca intakes. The endogenous loss of Ca is thus relatively independent of Ca intake. This is in agreement with the findings of Visek, Monroe Swanson & Comar (1953) who showed that even large increases in the dietary Ca did not affect the endogenous Ca materially. The levels of endogenous loss of Ca as reported by the A.R.C. (1965) are a few times higher than those of the present investigation. They reported values of 20–60 mg Ca/kg body mass (0,94 – 2,82 g Ca/day/sheep) as determined by isotope methods as against these values of from 9,6 to 12,1 mg Ca/kg body mass (0,45–0,57 g Ca/day/sheep).

The maintenance requirement of 3 g Ca/day (with a mean DM intake of 886 g/day) for Merino sheep of approximately 47 kg live mass (Fig. 1) agrees well with the value recommended by the N.R.C. (1957) of 3,2 g Ca per day. It must, however, be realised that the value as obtained in this trial was obtained at the point of equilibrium where, broadly speaking 50% of the observations will be negative and the balance positive. The figure of 3 g Ca/day therefore does not involve any safety margin. This value thus can be arbitrarily increased by say, 0,5 to 1 g Ca/day *viz.* an intake of 3,5 to 4 g Ca/day to ensure fairly general positive balances. This latter value agrees with the optimal Ca level of intake of 4 g/day for growing lambs suggested by Davison & Woods (1963). The figure of 3 g Ca for equilibrium obtained in this investigation represents roughly 0,3% of the DM intake which approximates the 0,27% of the N.R.C. on the AIR DRY basis. The present values do not imply that it is the ideal concentration for, say, good growth response. In fact, Wise, Ordoveza & Barrick (1963), working with Hereford calves, suggested a Ca concentration in the diet of 0,81% as giving the best growth response. This concentration is at least twice as high as that obtained here for maintenance of full-grown sheep. If the faecal endogenous loss of Ca is assumed to be 0,5 g/day the mean true absorption of Ca calculated from the grand mean of Table 8 will be 18,5% which is in close agreement with the 18% as derived from the regression of faecal Ca excretion (y) on the Ca intake (x). This figure, however, also includes the negative values. If the negative values are ignored the true absorption of Ca approaches 27%. Seeing that the Ca intake levels in the current research represent levels far in excess of optimal levels, the excess being excreted by way of the faeces, the figure of 27% absorption is an underestimate of the true absorption.

Treatment Ca_oP_o represents a condition where the animals were subjected to both Ca and P stress. The true absorption in this case will be:

$$100\ (0,582 - 0,786 + 0,5) \div 0,582 = 50,9\%$$

This result is in agreement with some reported values (Luick, Boda & Kleiber, 1957; Tillman & Brethour, 1958a, c). Kleiber & Luick (1956) reported true digesti-

bilities of 56% in one cow and 17% in another. The value of 50,9% attained in this study for Treatment Ca_OP_O implies an adaptation mechanism by means of more efficient absorption under conditions of Ca stress in the absence of high P. The true absorption for treatment P_r is only 8,2%. This apparent depression of Ca absorption by P is perhaps due to chemical association between these two elements resulting in insoluble complexes. In similar fashion Myburgh & Du Toit (1970) claimed that excessive intakes of $MgSO_4$ may result in losses of Ca from the body.

Concentrations of Ca (Table 8)

(a) **Urinary Ca concentration**

Significant differences are:

Ca_r > all other treatments (P<0,01)
Ca_d > P_r and Ca_dP_r (P <0,05)

High Ca concentrations administered to the rumen in the absence of added P, resulted in a very high concentration of Ca in urine. This finding is supported by Nicolaysen, Eeg-Larsen & Malm (1953) who found with human beings that ingestion of P depressed Ca urinary excretion.

Although the variation between animals and even between days was large the level of the renal Ca excretion in sheep may be considered as an individual constant of genetic origin, conditioned by endogenous factors. This was also found by Nicolaysen *et al.* (1953) with rats and human beings. The site of this control may be at the different absorption sites or may be in the kidneys.

The observation in this investigation, however, lends support to the findings of Otto (1932), Hansard, Comar & Plumlee (1952) and Kleiber & Luick (1956) who consider the Ca faecal excretion as the principal pathway of Ca loss The grand mean for the urinary Ca concentration with a daily intake of 5,71 g and 5,12 g faecal Ca is only 5,3 mg% (0,057 g/day). It can therefore be concluded that in general the Ca renal excretion is negligible. This view is also held by Lofgreen (1960). The total Ca urinary excretion varies from 0,006 to as high as 0,390 g/day. It will thus be unwise to ignore Ca urinary excretion in balance studies as was proposed by Hansard *et al.* (1952) and Kleiber *et al.* (1956).

(b) **Ca concentrations in rumen fluid**

All the treatments where Ca was supplied to the rumen *viz.* Ca_rP_d, $(CaP)_r$ and Ca_r showed a significantly higher Ca concentration than all other treatments. This implies *inter alia*, (1) that ruminal Ca concentration is Ca intake dependent and (2) that the Ca administered to the duodenum was poorly recycled to the rumen. The recycling of Ca to the rumen by way of the saliva as found by McDougall (1948) and Tomas (1965) is in fact negligible and is thus support for the present evidence. From Tomas' work it was calculated that only 0,25 g of Ca/day will enter the rumen as against 5 g of P. The latter is also confirmed by Hill (1962).

The mean rumen Ca for the lower range of treatments (Ca_OP_O, P_r, Ca_d, P_d, Ca_dP_r and $(CaP)_d$) is 2,39 mg%; that for the higher range (Ca_r, $(CaP)_r$ and Ca_rP_d) 7,74 mg% and that for all observations 4,17 ± 3,53 mg% of Ca (P< 0,05). Although these values represents a relatively large difference in concentration it apparently had no influence on the microbial activity as no significant treatment difference could be found in dry matter digestibility nor in the TCA-N and VFA concentration in rumen liquor (Table 13). These findings, therefore, lend support to those of Hubbert, Cheng & Burroughs (1958) who showed rumen microbes to be tolerant to large variations in Ca concentration in the rumen. They claimed the approximate minimum and maximum Ca requirements for rumen micro-organisms to be 50 to 450 ppm (5,0 and 45,0 mg%) as the lower and upper limit, respectively. The range in the present investigation approximates that of Hubbert *et al. (loc. cit.).*

The Ca concentration in rumen fluid is subject to many factors. In the first instance it is clearly dependent on Ca intake (Annison & Lewis, 1962), as was contended earlier but will also be dependent on those factors causing changes in the rumen volume and pH. Thus, for instance Nel & Moir (1968b) showed that a high starch diet has an influence on both the pH and the Ca concentration in the rumen.

(c) **Ca concentration in blood serum**

Treatments Ca_OP_O and Ca_r, respectively, resulted in the highest concentrations of Ca in serum (Table 8), but these two treatments did not differ significantly. Ca_r gave values of serum Ca concentration that were significantly higher than all the remaining treatments, indicating that Ca_r in the absence of supplemental P created conditions favourable for high serum Ca. The control treatment (Ca_OP_O), on the other hand, also gave a high serum Ca concentration, which is significantly higher than $(CaP)_r$, $(CaP)_d$, Ca_dP_r, P_r and P_d. This effect is evidence of considerable bone mobilisation triggered by either the deficiency of Ca or P.

The differences between the other treatments prove that, in the first instance, Ca gave rise to serum Ca levels higher than those where Ca and P were infused jointly into the duodenum and also when Ca_dP_r was applied. The latter infers that P even in this case, exhibits an effect on blood Ca despite the difference in site of application. It is also clear that the application of P only, irrespective of site, had a depressing effect on serum Ca in all cases. Ca supplied to the rumen (with or without P) is more effective in raising the blood Ca levels than when Ca (with or without P) was infused into the duodenum.

The lowest value for serum Ca concentrations (Table 8) was for Treatment $(CaP)_d$ with 7,52 mg% and the highest value the 9,46 mg% of Ca_r. In general it can be concluded that the serum Ca concentration is relatively stable The Ca concentrations in serum were, however, generally lower than those reported by Dukes (1955),

Rose, Irving & Hetherington (1963), Payne & Leech (1964) and Long, Ullrey, Vincent & Zutaut (1965). The serum P_i concentrations, on the other hand, were considerably higher than reported values (Malan, 1930; Watson, 1933; Long *et al.*, 1965) and, therefore, the relatively low serum Ca levels may perhaps be partly explained by the negative association between the concentrations of serum Ca and serum P_i.

The effects of phosphorus

P balances

The retention of P, when Ca was infused duodenally (Ca_d), was significantly lower than all other treatments indicating that high concentrations of Ca in the duodenum, with low P intakes, are detrimental to absorption and retention of P. This inference is substantiated by the fact that $(CaP)_d$ gave the best retention of P of all treatments but this was only significantly better than that for Ca_oP_o, Ca_r and Ca_d (Table 9). The mean true absorption of P for all treatments is approximately 29% if the endogenous loss is assumed to be 0,20 g/day. The true absorption is 45% for the high level treatments at an endogenous loss of 0,77 g/day. Published values are very variable but published values by Tillman & Brethour (1958a, b, c) are of the same order as found in this investigation.

With the P and Ca concentrations fed in these experiments it is evident that Ca supplementation to the rumen $(CaP)_r$, and to the duodenum (Ca_dP_r) depressed the absorption of dietary P as compared with P_r. P infused into the duodenum was significantly better absorbed than $(CaP)_r$ but not better than Ca_dP_r. In addition to these differences the alternative analysis showed the P in $(CaP)_d$ to be significantly better absorbed than in $(CaP)_r$. The essential consequence is that P given at the duodenal level was better absorbed on a percentage basis than ruminally administered P under these conditions. The difference in absorption between these two environments appears to be due to P concentration as a result of the larger dilution factor in the rumen as against that of the duodenum supporting the contention that P absorption is concentration dependent as reported by Wright (1955) and McHardy & Parsons (1956).

The differences in the endogenous loss of P (faecal and urinary) are very variable and the indications are that the level of endogenous P excretion is to a large extent dependent on the level of P intake. This finding supports the claim of Preston & Pfander (1964) that 'metabolic faecal P increases progressively with increasing P intake'. The urinary excretion with most of the high level P treatments was excessive which is contrary to the normal situation in sheep. The urinary excretion is usually negligible (Watson, 1933). The levels of the total endogenous excretion of P in the present investigation (0,34; 0,77; 0,20; 0,16 g P/day) are lower than most reported values (Tillman & Brethour, 1958a, c; Schroder & Hansard, 1958; Lueker & Lofgreen, 1961; A.R.C., 1965) but agree with the values (0,267; 0,347 and 1,356) obtained by Preston & Pfander (1964). A.R.C. (1965) reported values of 24 to 77 mg P/kg live mass.

The requirements of P for equilibrium in this experiment were found to be 1,8; 2,6 and 2,3 g P per day/sheep. It is clear, however, that these requirements were complicated by differences in the efficiency of P utilisation between high and low level intakes of P. The highest value *viz.* 2,6 g/day/sheep is identical with the recommendation of the N.R.C. (1957). The present figure, however, represents the requirement for equilibrium on the average which implies that only 50% of the individuals will be in positive balance. Gallup & Briggs (1950) recommended P intakes of 2,4 – 2,9 g/day/100 lb sheep to secure general positive balances. Their lower value approximates the 2,6 g/day/sheep of this experiment and is almost identical with the recommendation of Beeson, Johnson, Bolin & Hickman (1944). If the 2,6 g P/day is expressed in terms of the DM intake (886 g/day) a concentration of 0,29% P of the consumed DM is obtained. If the absolute P intake is arbitrarily increased to 3 g/P/day to secure general positive balances the resultant P concentration in the DM is 0,34%. Both these concentrations are higher in general than reported concentrations (N.R.C., 1957 e.g. 0,23%) but 0,34% dietary P is identical with the recommendation of Wise *et al* (1963). A P intake of 3 g/day is, however, still lower than the 3,8 g P/day for maintenance as was suggested by Gueguen (1962). The question arises whether it is advisable to express P requirement as a concentration of the DM intake without defining the required minimum DM intake to facilitate P equilibrium. According to the findings of Barrow & Lambourne (1962) the faecal excretion of P is independent of the level of feed intake. It is possible that the absolute P requirements are reasonably constant within certain limits of the variation in feed intake. As it is convenient for general application, expressing the P requirement as a concentration of the feed will remain popular; the absolute P requirement must, however, also be taken into account, particularly where a limited feeding regime is practised.

Concentrations of P

(a) **Urinary concentration:**

The mean concentration of P in the urine of 52,2 mg% is considerably higher than the 3 mg% reported by Robbins, Kunkel & Crookshank (1965). The Ca supplementation in the different combinations of Ca and P *viz.* $(CaP)_d$, Ca_dP_r, Ca_rP_d and $(CaP)_r$, results in a depression of the P concentration in urine, irrespective of the site of application of Ca and P.

The effect of infusing P into the rumen had a less pronounced effect on the urinary P concentration than duodenal infusings of P. The P excretion as a result of P_d and P_r, in particular the former, was excessive and kidney damage was evident in some cases. A better balance of Ca thus also prevents blood P_i rising above the renal threshold level for P; this mechanism is most probably exhibited at the sites of absorption or in promoting storage. It is clear, however, that urinary P excretion cannot be ignored as negligible as was suggested amongst others, by Barrow & Lambourne (1962) and Gueguen (1962).

It was earlier shown that P supplementation affects the urinary Ca excretion in the same fashion as urinary P excretion is affected by Ca supplementation. Their influence on each other is thus reciprocal.

(b) **Inorganic P in centrifuged rumen liquor**

From the significant treatment differences it is observed firstly, that all treatments where P was administered to the rumen gave significantly higher rumen fluid P_i than those treatments where no Ca and no P, or where only Ca, was supplied to the rumen. Essentially this result implies that ruminal P_i concentration is dependent on P intake, a finding which is diametrically opposed to the conclusion of Clark (1953) who concluded that ruminal P is independent of dietary P in both sheep and cattle. The results of Tomas (1965), however, support the present finding. In fact, from the abovementioned differences it is also evident that even duodenally infused P (P_d and $(CaP)_d$) resulted in P_i concentrations in the rumen which are higher than the P_i concentration as a result of Ca_r. It did not, however, differ significantly from, for instance, Ca_oP_o in this statistical analysis but it did in the alternative analysis. It, therefore, can also be postulated that high Ca application to the rumen depresses P_i levels in the rumen. The lowest mean P_i concentration (27,60 mg% P_i) was obtained as a result of Ca_r. On the other hand, the highest mean concentration of P_i resulted from P_r *viz.* 93,4 mg % (Table 10).

The grand mean was 66,7 ± 25,59 mg% of P_i concentration and the range 125,2 – 20,0 mg%. These values agree with values forwarded by Tomas (1965) and Garton (1951) who found ranges of 78,9 – 28,2 mg% P_i and 164 – 35 mg%, respectively. They are, however, in general higher than those for total P reported by Clark (1953).

The large differences in the ruminal P_i concentrations failed, however, to result in any significant difference in DM intake, DM digestion and cotton thread digestion. The correlation between VFA concentration and P_i concentration in rumen liquor is $r = 0{,}490$ ($P<0{,}01$).

It is also evident from the results that P_i is fairly well recycled to the rumen, a mechanism which guarantees a relatively high concentration of P_i in rumen contents (Clark, 1953; Hill, 1962; Tomas, 1965) and that the microbial population in the rumen is always well supplied with P. From this reasoning it is also clear that micro-organisms are to a large extent independent of dietary P for their P requirements. It is clear that the animal system would be affected first under a marginal P deficiency. During this experiment it was frequently observed that the rumen P_i levels rise during inappetence as a result of the relatively constant flow of salivary P_i and the concurrent decrease in rumen volume. McDougall (1948), for instance, reported the P concentration in parotid saliva to be of the order of 81 mg% and Tomas (1965) calculated the daily total P flowing into the rumen to be 5 g. Thus it can be suggested that the inappetence and depraved appetite associated with conventional P deficiency is caused not only by a ruminal but by an animal effect. This finding supports the ultimate conclusion of Clark (1953). The results reported here indicate that hardly any P deficiency can possibly be so severe as to cause a P deficiency in the rumen as far as the requirements of the rumen microbes are concerned. This view is opposed to that held by Preston & Pfander (1964) who concluded that the P requirement of the rumen micro-organisms is greater than that of the host animal. This, in fact, is borne out by the published required P concentrations for the rumen micro-organisms *in vitro viz.* 40–80 ppm (4,0 – 8,0 mg% P) as was suggested by Anderson, Cheng & Burroughs (1956) in their *in vitro* studies with cellulose digestion. These values are considerably lower than the lowest mean values obtained in the present investigation.

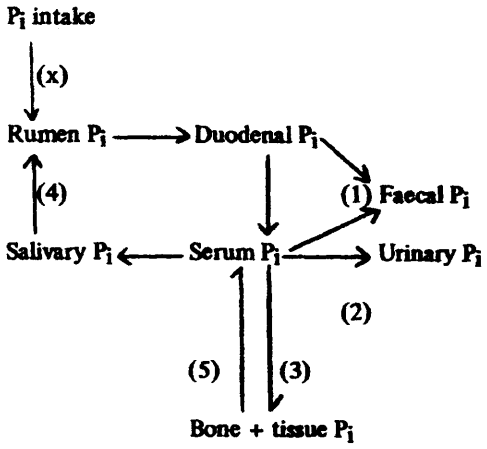
(c) **P_i concentration in serum**

The maximum value of P obtained in serum was 10,0 mg% and the lowest 3,10 mg%. Treatment P_r resulted in the highest mean serum P_i level (8,16 mg%) and Treatment Ca_r in the lowest (4,18 mg%). Considering the low P intake on the P deficient treatments (see Table 9), the P_i level in the serum was in general, surprisingly high compared with those quoted in the literature for comparable diets.

Clark (1953) for instance, reported a mean P_i value of 2,6 mg% in 'blood' (presumably plasma). The 'normal' sheep contained a mean of only 3,7 mg%. These values are much lower than the present values and are, in fact, lower than most published values (Malan, 1930; Watson, 1933; Dukes, 1955, Rose *et al.*, 1963; Payne & Leech, 1964; Long *et al.*, 1965; Myburgh & Du Toit, 1970). The general trend of the serum P_i concentrations in the present investigation tend to be higher than have been reported. The mean nevertheless, falls within the range as reported by Dukes (1955; 3–8 mg%). The lowest concentration of serum P_i found in the literature, was the 1,4 mg% of Du Toit, Malan & Groenewald (1931). No explanation can be offered for the rather high values in particular for the low level P treatments. A further feature of the observations of Table 10 is the relatively high value of 5,75 mg% obtained for Ca_oP_o which is an indication of bone mobilisation.

The consequence of the significant differences is that all P treatments (with or without Ca) resulted in higher P_i concentrations than the other treatments. There is further a reasonable agreement between the order of P_i in serum and that in rumen liquor indicating an association between these bivariates. Furthermore, the addition of P to the rumen is as, or even more efficient than addition to the duodenum in raising the serum P_i concentration. No direct support for this view was found in the literature.

The significant ($P< 0{,}001$) correlation coefficient between serum P_i and rumen fluid P_i is at variance with the view of Clark (1953) who claimed that it is 'obvious that there is no correlation coefficient between the blood phosphorus figures and the phosphorus content of the rumen.' If the situation is examined in the light of the following diagram, however, the various possibilities suggest themselves



As P_i intake increases to maximum metabolic capacity outputs (1), (2) and (3) increase so that intake P_i is the dominant feature and rumen P_i will appear to be a major factor controlling serum P_i notwithstanding the recycling of salivary P_i (4), controlled by serum P_i level, to the rumen. As intake P_i diminishes to a deficiency state, output (1) will diminish towards endogenous output levels, output (2) will tend towards zero, but (3) is reversed, (5) to become an input into the system, and the relative values of (x) and (5) will determined the nature of any apparent relationship.

Ratios of Ca, P and Mg in rumen liquor and blood serum

There is a large variation in the Ca/P ratios in centrifuged rumen liquor (Table 11). The range is 0,552 – 0,018 and the grand average 0,041 ± 0,058. This mean ratio is substantially narrower than usually found in conventional diets for sheep. From this fact it is clear that the conventional recommended ratios of Ca and P in the diets of ruminants are not important at all, in particular with respect to the welfare of the rumen micro-organisms. Although treatment differences in Ca/P ratios in the rumen were found in that Treatments Ca_r, Ca_oP_o, $(CaP)_r$ and Ca_rP_d gave ratios of 0,04 to 0,15 which are much wider than most of the other treatments, these differences failed to show any significant bearing on the percentage of DM digestibility, cotton thread weight loss, and the concentration of TCA-N or VFA in the rumen environment. Consequently, it is clear that the rumen microbes are adaptable to a wide range of ratios favouring perhaps a narrow Ca/P ratio as is normally the case in the rumen. Barth & Hansard (1962) with *in vitro* techniques reported that a Ca/P ratio of 4 will depress availability of P in the rumen whereas a ratio of 6 inhibited cellulose digestion. Although diets with these ratios (4 and 6) were reported to be about ideal for ruminants (Wise *et al*, 1963), they will certainly rarely if ever occur in the rumen under natural conditions. This is a further demonstration that the rumen is a completely different system from the animal, each with its own separate set of requirements.

The mean Ca/P ratio in the serum *viz.* the animal aspect as distinct from the rumen, is 1,33 ± 0,51 and a range of 3,35 – 0,74. These ratios vary considerably from those in rumen liquor so that it can be concluded that the Ca/P ratios in serum will be relatively independent of Ca/P ratios in the rumen contents but will presumably be primarily dependent on the relative amounts of Ca and P absorbed. Ross (1962), Chandler & Cragle (1962) and Bronner (1964) are of the opinion that the main sites of absorption of Ca and P are in the abomasum and the small intestine.

These findings lend support to the findings of Wise *et al.* (1963) who suggested that Ca/P ratios for ruminants are not critical as distinct from non-ruminants. From the results of this experiment there is no ground to oppose the findings of Wise *et al.* (1963) who suggested wider Ca/P ratios for ruminants than are conventional. They favoured, by implication, a ratio of 4 to 6. A wider ratio is advocated than that recommended by usual standards (N.R.C., 1957, 1964).

Lewis, Burkitt & Wilson (1951) claimed reduced weight gain when excess Ca was fed to steers, whereas Haag, Jones & Brandt (1932) concluded that a ratio of 10,5:1 was no more detrimental to ruminants than one of 7,6. The reasons why the current wide ratios had no detrimental effect are clear from the results concerning the relative concentrations of Ca and P_i in rumen liquor. In the rumen the Ca requirement is directly intake dependent and the requirement for Ca is more critical relative to high concentrations of P_i. It is, therefore, not surprising that for the sake of the rumen environment relatively more Ca is required and that the Ca/P ratio is less critical than can be the case for non-ruminants.

The significant treatment differences in the Ca/Mg ratios in rumen fluid (Table 11) are caused by variation in the numerator and is thus of no particular consequence. The mean Ca/Mg ratio in serum is wider than that in centrifuged rumen liquor.

The P/Mg ratios in rumen liquor are many times higher than the ratios in serum. Treatment Ca_r resulted in the lowest mean P/Mg ratio in both rumen liquor and serum. This action of Ca is largely due to its depressing effect on the P concentration in serum as well as by an elevating effect, but to a lesser degree, on the Mg concentration in rumen liquor.

It must be realized, however, that a change in the quantity of one mineral does not only involve a change in its ratio with one other mineral but also its ratio with all other minerals.

Experimental effects on nitrogen and on the concentrations of VFA and TCA-N in rumen fluid

Nitrogen: The N daily requirement of 5,3 g for equilibrium and 1,3 g endogenous N as estimated from the regression of N output on N intake are in agreement with the figures of Smuts & Marais (1938). The metabolic N as obtained here agrees well with the 34 mg/kg body weight forwarded by Harris & Mitchell (1941).

VFA: The mean concentration (9,40 mmole% – Table 13) approximates that of Hemsley & Moir (1963) sampled at

T_O (6,8 mmole %). The association between the VFA and Ca concentrations in rumen liquor ($r_{quad.}$ = 0,442; P < 0,01) lends support to the previous suggestion that the concentration of soluble Ca in the rumen might be more critical than the other variables investigated. Emery, Brown & Thomas (1964) also found that $CaCO_3$ increased the fatty acid and lactic acid content of the 'rumen ingesta'.

TCA-N: The mean ruminal concentration of TCA-N (Table 13) is 73 mg% which agrees closely with the 75 mg% reported by Hemsley & Moir (1963). No reference in the literature concerning the significant relationship between the concentrations of TCA-N and Ca in rumen liquor could be found

This association is in accordance with the above-mentioned view as far as Ca and VFA is concerned. These associations would point to a relatively high requirement for Ca in the rumen environment. This single aspect, however, must be considered in relation to the complexity of the rumen system – a series of interlocking systems (Moir, 1965).

General conclusions

It was found by Rotensten (1938) that the Ca retention in rats is dependent on their previous Ca level of intake. The consequence of this finding with relation to the present work is that the Ca balance studies may have been affected by residual effects from period to period, the animals having been conditioned by their previous dietary treatment.

The duration of collection and especially preliminary periods in mineral research are extremely important. For the type of experimental design as was used in the current research the duration of repletion periods to condition the sheep to a state of equilibrium for the next experimental phase, was most important to counteract residual effects. It is also questionable whether the Latin square type design can be recommended for research of this nature particularly with regard to residual effects. A factorial design for this type of experiment would be commendable providing that the number of animals and infusions involved could be handled. This design necessitates teamwork.

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