636.2:591.132/.133

MEDEDELINGEN LANDBOUWHOGESCHOOL
WAGENINGEN • NEDERLAND • 69-11 (1969)

OBSERVATIONS ON THE DIGESTION AND ABSORPTION OF FOOD ALONG THE GASTRO-INTESTINAL TRACT OF FISTULATED COWS

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(Received 31-III-1969)

H. VEENMAN & ZONEN N.V. – WAGENINGEN – 1969

1. THE RATE OF FLOW OF DIGESTA AND THE NET ABSORPTION OF DRY MATTER, ORGANIC MATTER, ASH, NITROGEN AND WATER

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INTRODUCTION

Knowledge of the composition and flow rate of digesta at suitable points along the gastro-intestinal tract is indispensable in the quantitative study of segmental net absorption of protein, fat, minerals, vitamins, water etc. Much of the information on the composition of ruminant digesta has been obtained from slaughter experiments. However, tremendous post-mortem differential shedding of alimentary epithelium, especially in the first part of the small intestine, has been reported by BOYNE et al. (1956) and BADAWY et al. (1957) in sheep and VAN 'T KLOOSTER (1967) in cows. The desquamated cells greatly increased the dry matter and nitrogen content of the digesta and may also influence the concentration of inorganic elements. In slaughtered animals the pH of the first part of the small intestine has almost invariably been found too high.

Once successful techniques for the insertion and maintenance of cannulae into the alimentary tract had been developed, several workers began to study the composition and flow rate of digesta through the duodenum of live sheep (PHILLIPSON AND ASH, 1965; BRUCE et al., 1966), calves (SMITH, 1962) and goats (RIDGES AND SINGLETON, 1962). Attention was paid less frequently to digesta in other sections of the alimentary tract of calves and sheep (HOGAN and PHILLIPSON, 1960; BRUCE et al., 1966; TOPPS et al., 1968; GOODALL and KAY, 1965). Little or no information is available on the composition and flow rate of digesta in the various segments of the alimentary tract of cows except that of Russian workers as compiled by SINESHCHEKOV (1965) and of VAN 'T KLOOSTER (1967).

By using inert reference substances we have measured the flow of fluid out of the reticulo-rumen and the flow of digesta in the proximal duodenum, distal duodenum and terminal ileum of two fistulated dairy cows, fed on each of 5 different rations. The composition of representative samples of feed, digesta and faeces was determined. This paper describes the flow rate of digesta and the fate of dry matter (DM), organic matter (OM), ash, nitrogen (N) and water in the alimentary tract. It also contains information on the eletrical potential difference (PD) across the gut wall and on the pH of digesta. Following papers describe the digestion of the cell-wall constituents of roughages (GAILLARD and VAN 'T KLOOSTER, 1969) and the fate of Na, K, Ca, Mg, and P in the digesta of the same experiments (ROGERS and VAN 'T KLOOSTER, 1969). A report on the fate of the different N fractions is in preparation (VAN 'T KLOOSTER and BOEKHOLT, 1970).

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EXPERIMENTAL

Animals: Two Friesian cows, Zwartschoft (Z) and Witschoft (W), were fitted with T-piece cannulae in the proximal duodenum, distal duodenum and terminal ileum. The surgical technique was similar to that of MARKOWITZ et al. (1964). Six weeks later each cow was fitted with a rumen cannula as described by VAN 'T KLOOSTER and ROGERS (1970). All cannulae were of hard plastic.

At the start of the trial Z was 4 years old, weighed 462 kg and was yielding about 12 kg milk per day. W was 3 years old, weighed 420 kg and was yielding about 13 kg milk per day. Both animals remained in good health during the trials. At the end of the 4-month trial period the milk yields of Z and W had declined by 3 kg to 9 and 10 kg per day.

Diets: Various rations were composed, mainly with a view to bring about large differences in flow rate of fluid and digesta through the reticulo-rumen and duodenum respectively. Table 1 shows the composition of the 5 rations. In experiments 1 and 2 the same rations were offered, but in experiment 2 an additional 110 g of potassium (K) were dosed into the rumen twice daily at the end of feeding. The K solution was a 5:1 mixture of KCl and K_2CO_3. After 3 days on high K, W refused her hay and had to be taken off the experiment. Two weeks later the experiment was repeated with this cow. This time only 82.5 g K were dosed instead of the original 110 g. Although she took a long time to eat her ration, she refused only small amounts of hay the second time.

TABLE 1. The composition of the rations expressed as kg offered per day.

	Period 1		Period 2		Period 3		Period 4		Period 5	
	Z	W	Z	W	Z	W	Z	W	Z	W
Hay	8,00	7,00	8,00	7,00	3,00	3,00	3,00	3,00		
Cellulose					4,00	3,00	4,00	3,00		
Grass									73,25	64,69
Maize meal	3,43	3,43	3,43	3,43			0.30	0,30		
Extr. soya meal	0,86	0,86	0,86	0,86						
Extr. linseed meal	0,86	0,86	0,86	0,86						
Extr. coco-nut meal	0,86	0,86	0,86	0,86	1,00	1,00	0,25			
Extr. groundnut meal					0,50	0,50	0,25			
Maize gluten					1,50	1,50	1,00	1,00		
Maize gluten meal						0,50		0,50		
Maize starch							1,30	1,30		
Mineral mixture					0,20	0,20	0,20	0,20		

In experiments 3 and 4, only 3 kg of hay were fed per day; 4 and 3 kg of cellulose (paper pulp) were mixed with the concentrate mixture and fed as a wet mash twice daily to Z and W respectively. The protein intake in experiment 3 was about 15% above the standard given by the Central Animal Feeding Office in the Netherlands. In experiment 4 the protein intake was about 15% below this standard. The ration in experiment 4 had a very low lysine content.

In experiment 5 an all-grass ration was fed. The young spring grass was mown once every 4 days, mixed, sampled and stored in plastic bags (7.5 kg per bag), first at –15 °C and 4-5 hours later, when the outside layers had frozen, at –5 °C. The stored grass was fed, one bag at a time throughout the day from about 7 a.m. to 9 p.m. All refusals were weighed and sampled separately.

Indicators: In each experiment three indicators were used viz. PEG, Cr_2O_3 and Cr-EDTA. One hundred g PEG in 1 l water were mixed thoroughly throughout the rumen contents twice daily after feeding. At the same time 17g Cr_2O_3-paper were put into the rumen. The daily doses of PEG and Cr (as Cr_2O_3) were 200 and 9.738 g respectively. Dosing was started 5 or more days before each experimental period. Once daily[1], at approximately 2 p.m., about 8 l of fluid were withdrawn from the rumen of each cow. One litre of Cr-EDTA solution (BINNERTS et al., 1968), containing about 2.500 g Cr, was mixed with the 8 l of rumen fluid. The 9 l were returned to the rumen within 5 minutes. Great care was taken in the attempt to spread the indicator dose throughout the rumen contents.

The mean daily rate of flow (F) of fluid out of the reticulo-rumen was calculated from the rate of dilution of Cr-EDTA (VAN 'T KLOOSTER and ROGERS, 1970), by plotting log Cr concentration against time. The mean rumen fluid volume (V) at the time of dosing was calculated by dividing the daily dose of indicator (D) by the concentration of Cr immediately after dosing (C_0) and corrected for Cr remaining in the rumen at the end of each day. C_0 was estimated by mathematical extrapolation to time zero. The daily flow rate was calculated (HYDÉN, 1961) with the formula: $F = 24 \times 2{,}30 \times bV$, where b = regression coefficient. In this formula V can be replaced by $D/10^a$ where $a = \log C_0$. The standard deviation for F is then given by:

$$s_F = 24.\ 2{,}30.\ D.\ \sqrt{\text{var } b/10^a}.$$

PEG and total Cr were used to estimate the rate of flow of digesta past each of the intestinal cannulae by dividing the mean daily dose of indicator by the mean concentration in representative bulk samples of digesta. Appropriate corrections were made for sample losses. The total daily passage of a nutrient past any of the intestinal cannulae was calculated by multiplying the concentration of nutrient in the bulk sample by the flow rate. In these calculations the flow rate used was the mean of two values, one based on total Cr (mainly Cr_2O_3) and the other on PEG.

Experiments: In experiments 1, 3 and 4 the preliminary feeding period lasted for 10 days and in experiment 5 for 5 days. In experiment 2 the same ration as in experiment 1 was fed. Extra K was dosed. Collection and sampling of digesta started 3 days later. After a further 3 days, faeces collections were started. The experimental period normally lasted 7 days, except in experiment 1 (5 days). Rumen fluid, contents from the proximal duodenum, distal duodenum and

[1]) Dosing with Cr-EDTA was twice daily in experiment 4.

ileum were sampled each day for 5 days (120 h) of the experimental period. In experiment 4 the distal duodenum was not sampled and in experiment 2 (W) no samples of intestinal digesta were taken.

Sampling techniques: Balance harnesses were used to obtain separate collections of faeces and urine, which were weighed, thoroughly mixed and sampled each day of the experimental period. Phenol was added as a preservative. Great care was taken in sampling food, digesta, faeces etc. and every effort was made to obtain representative bulk samples.

Rumen fluid was sampled at 3 hour intervals, beginning 1.5 hours after the administration of Cr-EDTA. A hollow plastic rod (length about 68 cm, diameter 2.5 cm), perforated at the tip, was inserted into the rumen. The fluid collecting at the tip was siphoned into a 1 l plastic bottle. Fluid was collected from several areas of the rumen until the 1 l bottle was almost full. A sample of 100 ml was taken. The remaining 800-900 ml was returned to the rumen. Eight samples of 100 ml were removed in this way from the rumen each day for 5 days. Twenty ml aliquots were removed from each sample, bulked and acidified for mineral analysis. The remaining 80 ml was retained for Cr-EDTA analysis.

Duodenal contents. The contents of the proximal duodenum were sampled every 2 hours for 5 days (60 samples from each cow). The contents of the distal duodenum were sampled every 4 hours (30 samples from each cow). At the appropriate time, about 750 ml of digesta were collected in a 1 l plastic bottle, mixed and sampled (50 ml). The samples from each cannula were bulked separately in a plastic bucket and stored at 4 °C. Phenol (1 g per l) was added.

Ileal contents. 100-150 ml digesta were removed every 2 hours for 5 days. A sample (50 ml) was taken, transferred to its appropriate bucket and stored at 4 °C with phenol. There was often a time lag of 5–20 minutes and sometimes up to 30 minutes before a flow of ileal digesta commenced. At the end of each experimental period a 3 l bulk sample of ileal digesta (60 samples) was available from each cow.

Analytical Methods

Indicators. When the fluid part of the digesta was used for PEG analysis a correction for DM was taken into account. PEG and total Cr were analysed as described previously (VAN 'T KLOOSTER et al., 1969). Soluble Cr (Cr-EDTA) was estimated as outlined by BINNERTS et al., (1968) after proper dilution of the samples. Subsequent work showed the necessity of a correction factor to be applied in the case of soluble Cr.

N, DM and ash. Total N was estimated by the Kjeldahl method using a Se mixture and $CuSO_4$ as catalysts. DM and ash were determined in the usual manner.

pH and PD. The pH of digesta was measured with a Beckman pH-meter (G.S.) immediately after withdrawing a sample from the intestine. The same pH-meter was used as a millivoltmeter to measure the PD between blood (+) and gut lumen (–). The PD was measured as described by CARE and VAN 'T KLOOSTER (1965), using PVC tubing in the jugular vene and saturated KCl-agar

bridges in the intestinal lumen. The pH and PD were measured on the day immediately after the experimental period had ended.

RESULTS

The faecal recovery of the indicators is shown in table 2. The recovery of PEG and total Cr varied from 95–104% and 94–108% respectively. The overall mean recovery was 99%.

TABLE 2. The recovery of the indicators from faeces. Values are expressed as % of dose. Appropriate corrections for losses have been made.

	Experiment					
	1	2	3	4	5	Mean
Zwartschoft PEG	97	95	96	100	102	98
Total Cr	102	101	100	97	98	100
Witschoft PEG	99	–	95	97	104	99
Total Cr	108	–	99	94	100	100

Tables 3 and 4 show the mean flow rate of fluid out of the reticulo-rumen and the flow of digesta through the proximal and distal duodenum, terminal ileum and faeces. Considerable variations in the mean flow rates were seen between experiments. The flow rates of fluid to the omasum varied from 190–272 l and 162–218 l per day for Z and W respectively. The flow rates of digesta through the duodenum were of the same order, viz. 154–265 and 165–267 kg per day respectively. The amounts of digesta reaching the caecum in the different experiments were only 1/3 (experiment 1) to 1/5 (experiment 5) of the amounts passing the duodenum.

TABLE 3. The mean flow rates of rumen fluid and proximal duodenal digesta.

	Period 1		Period 2		Period 3		Period 4		Period 5	
	Z	W	Z	W	Z	W	Z	W	Z	W
Rumen fluid (l per day)	228,6	186,4	263,8	164,8	190,0	161,8	233,3	181,0	271,6	218,5
± S.D.	5,6	3,0	6,4	3,5	5,4	4,8	–	–	8,3	8,2
Rumenfluid (l/kg d.m.)	18,9	16,5	20,9	14,8	20,3	18,3	24,8	21,5	24,5	22,3
Proximal duodenum (kg per day)	203	175	235	–	154	202	198	165	265	267

The highest flow rates through the rumen and duodenum were seen on the grass ration (experiment 5). On the hay and concentrate rations the flow rates were intermediate and on the semi-synthetic rations of experiment 3 and 4 both high and low flow rates were seen.

TABLE 4. The quantities (kg) of digesta, dry matter (DM), ash and nitrogen (N) passed per day through

	Period 1				Period 2			
	total	DM	ash	N	total	DM	ash	N
Zwartschoft								
intake[1]		12,11	0,84	0,302		12,63[2])	1,23[2])	
intake water	52				70			
duodenum proximal	203	7,94	1,60	0,330	235	8,99	1,95	
duodenum distal	217	8,84	1,86	0,361	237	9,27	1,94	
ileum terminal	72	5,08	0,89	0,125	73	5,20	0,89	
faeces	27,55	3,83	0,43	0,102	30,57	3,90	0,45	
Witschoft								
intake[1])		11,27	0,76	0,283		11,12[2])	1,01[2])	
intake water	49				56			
duodenum proximal	175	6,94	1,35	0,287				
duodenum distal	181	7,67	1,48	0,307				
ileum terminal	56	4,19	0,69	0,109				
faeces	26,39	3,43	0,37	0,092	26,53	3,10	0,38	

[1] total intake plus indicators and minus refusal
[2] including the extra potassium salt

TABLE 6. The amounts of DM. OM, ash and N absorbed from or secreted into (–) the stomachs, duodenum, The latter value is equivalent to the apparent digestibility. All values are expressed as a percentage

		DM					OM				
		stomachs	duod.	s/i	l/i	total	stomachs	duod.	s/i	l/i	total
Ration 1	Z	34	–7	31	10	68	44	–6	25	7	70
	W	38	–6	31	6	69	47	–6	26	4	71
Ration 2	Z	29	–2	32	10	69	38	–2	26	8	70
Ration 3	Z	48	–7	31	6	78	56	–4	24	4	80
	W	38	–9	41	5	75	51	–9	31	5	78
Ration 4	Z	37	–6[1])	27	11	69	49	–4[1])	20	6	71
	W	41	–9[1])	33	6	71	53	–8[1])	25	3	73
Ration 5	Z	42	–8	35	6	75	56	–8	27	7	82
		39	–14	35	6	76	56	–13	33	6	82
Mean of	Z	38	–6	31	9	72	49	–5	24	5	75
all rations	W	39	–9	37	6	73	52	–9	28	4	75

[1] Estimates
[2] Possible artefact due to en-masse sand excretion

the duodenum and ileum with the mean quantities ingested and excreted in the faeces.

Period 3				Period 4				Period 5			
total	DM	ash	N	total	DM	ash	N	total	DM	ash	N
	9,35	0,57	0,236		9,42	0,49	0,162		11,10	1,06	0,332
44				46				89			
154	4,90	1,16	0,263	198	5,91	1,40	0,216	265	6,38	1,97	0,335
165	5,52	1,32	0,276					269	7,28	2,09	0,356
35	2,59	0,50	0,075	77	3,93	0,82	0,084	52	3,42	0,89	0,102
11,15	2,02	0,29	0,063	21,85	2,84	0,28	0,074	18,15	2,70	0,87	0,074
	8,82	0,59	0,256		8,42	0,48	0,148		9,80	0,87	0,295
37				40				64			
202	5,45	1,42	0,292	165	4,94	1,23	0,208	267	5,98	2,01	0,270
206	6,29	1,55	0,296					282	7,39	2,25	0,313
31	2,66	0,47	0,085	45	2,96	0,55	0,076	49	2,94	0,81	0,098
12,83	2,15	0,30	0,072	17,90	2,39	0,26	0,066	17,94	2,31	0,70	0,067

small intestines (s/i) and large intestines (l/i). The total net absorption from the entire tract is also given. of the intake.

ASH					N				
stomachs	duod.	s/i	l/i	total	stomachs	duod.	s/i	l/i	total
–91	–31	116	55	49	–9	–11	79	7	66
–79	–16	104	42	51	–1	–7	69	6	67
–59	1	85	36	63					
–102	–28	143	36	49	–11	–6	85	5	73
–140	–21	182	29	50	–14	–2	82	5	71
–187	–32[1])	151	110	42	–33	–7[1])	88	6	54
–158	–27[1])	169	62	46	–40	–9[1])	98	7	55
–86	–12	114	2	18[2])	–1	–6	76	9	78
–130	–28	165	12	19[2])	8	–14	73	10	77
–105	–20	121	48	44	–14	–8	83	7	68
–117	–18	142	39	46	–12	–8	81	7	68

TABLE 5. The daily flow of DM, OM, ash and N in the digesta of the proximal duodenum (D_1), distal duodenum (D_2), ileum and faeces. All values are expressed as a percentage of the intake.

		DM				OM				ASH				N			
		D_1	D_2	Ileum	Faeces	D_1	D_2	Ileum	Faeces	D_1	D_2	Ileum	Faeces	D_1	D_2	Ileum	Faeces
Ration 1	Z	66	73	42	32	56	62	37	30	191	222	106	51	109	102	41	34
	W	62	68	37	31	53	59	33	29	179	195	91	49	101	108	39	33
Ration 2	Z	71	73	41	31	62	64	38	30	159	158	73	37				
	W								27				37				
Ration 3	Z	52	59	28	22	43	48	24	20	202	230	87	51	111	117	32	27
	W	62	71	30	25	49	58	27	22	240	261	79	50	114	116	33	29
Ration 4	Z	63	69[1])	42	31	51	55[1])	35	29	287	319[1])	168	58	133	140[1])	52	46
	W	59	68[1])	35	29	47	55[1])	30	27	258	285[1])	116	54	141	150[1])	51	45
Ration 5	Z	58	66	31	25	44	52	25	18	186	198	84	82[2])	101	107	31	22
	W	61	75	30	24	44	57	24	18	230	258	93	81[2])	92	106	33	23
Mean of all rations	Z	62	68	37	28	51	56	32	25	205	225	104	56	114	121	39	32
	W	61	70	33	27	48	57	29	25	217	235	93	54	112	120	39	32

[1]) Estimates.
[2]) Possible artefact due to en-masse sand excretion

Net absorption of water. Tables 3 and 4 indicate that slight net absorption of water occurred from the stomachs in most cases. There was a net secretion of about 8 l water into the duodenum. Net absorption of water from the small intestine was very high, viz. about 155 l or 76% of the water that passed the distal duodenum. Absorption continued from the large intestine from which about 33 l (64% of the water which passed the terminal ileum) was absorbed.

DM of intestinal digesta. Although considerable diurnal variation was observed in the consistency of duodenal digesta, variation in the DM content of the bulk-samples was small between cows. The consistency of ileal digesta was thicker and less variable than that of duodenal digesta. The mean DM content of the digesta in proximal duodenum, distal duodenum and ileum were 3.13% (2.24–3.96), 3.42% (2.62–4.24) and 6.89% (5.13–8.66) respectively.

Table 4 shows the daily intake of DM and the amounts which passed the 3 intestinal sampling points and appeared in the faeces. Table 5 shows the DM flow at each point expressed as a percentage of the DM intake. Table 6 shows the segmental absorption of DM expressed as a percentage of the DM ingested. The average net absorption (as percentage of the intake) was: stomachs, 38.5%; duodenum, –7.5%; small intestine, excluding duodenum, 34.0%; large intestine, 7.5%; total net absorption 72.5%. The faecal values have not been corrected for the small losses at sampling times. Table 7 shows the segmental absorption as a percentage of the digestible DM.

The fate of OM in the intestines is summarized in tables 5, 6 and 7. The sto-

TABLE 7. Net absorption of digested DM and OM in the stomachs, the small intestine (s/i), including the duodenum, and the large intestine (l/i) in percentages of the apparent digested DM and OM.

		DM			OM		
		stomachs	s/i	l/i	stomachs	s/i	l/i
Ration 1	Z	50	35	15	62	28	10
	W	55	35	10	66	28	6
Ration 2	Z	42	43	15	55	34	11
Ration 3	Z	61	32	8	72	23	5
	W	51	42	8	66	29	5
Ration 4	Z	53	30	17	69	22	9
	W	58	33	10	73	22	5
Ration 5	Z	56	35	9	69	23	8
	W	51	41	8	68	25	7
Mean		53	36	11	67	26	7

TABLE 8. The pH of duodenal[1]) and ileal digesta and the potential difference (PD) between the blood and the lumen of the alimentary tract.

	Period 1				Period 2				Period 3				Period 5			
	Rumen	D_1	D_2	Ileum	Rumen	D_1	D_2	Ileum	Rumen	D_1	D_2	Ileum	Rumen	D_1	D_2	Ileum
Zwartschoft																
pH		2.45	3.21	7.62		2.50	3.35	7.90		2.78	3.41	7.38		2.31	3.08	7.46
Mean PD (mV)	20.8	5.6	8.6	(11.7)	29.9	18.9	17.7	(21.7)	17.2	10.4	10.6		31.4	18.1	18.0	
Range PD (mV)	19–22				28–32	16–21	15–22		16–18	8–14	9–12		29–32	15–22		
Na meq/l																
or meq/kg	111	57	73		102	56	56		127	64	78		95	50	63	
K meq/l																
or meq/kg	23	29	27		45	41	41		17	24	22		48	39	36	
Witschoft																
pH		2.47	3.41	7.45		2.62	3.28	7.45		2.88	3.47	7.65		2.87	3.88	7.57
Mean PD (mV)	23.6	4.4	4.8	(12.1)	32.8	10.9	11.6	(25.8)	20.9	10.2	10.9		31.2	14.6	16.5	
Range PD (mV)	22–25	–	–		31–35	8–13	10–15		20–22	8–12	8–14		29–33	10–17	15–19	
Na meq/l																
or meq/kg	113	60	73		93	62[2])			118	63	73		98	57	70	
K meq/l																
or meq/kg	25	29	27		51	40[2])			20	22	20		49	36	33	

[1]) D_1 = proximal duodenum
D_2 = distal duodenum.
[2]) Values based on samples taken over 24 hours only.

machs absorbed more than twice as much OM as the entire small intestine.

Ash of intestinal digesta and faeces. At any one sampling point, between cow differences in ash content of wet digesta were small. Differences between diets were also small except in the case of ileal digesta which tended to have a higher ash content in experiments 3 and 5 than in 1, 2 and 4. The mean ash content and ranges of proximal duodenal, distal duodenal and ileal digesta were 0.76% (0.70–0.83), 0.81% (0.75–0.89) and 1.35% (1.06–1.52) respectively.

The ash content of faeces varied widely between diets. A very high ash content was seen on diet 5. For faeces the variation between cows was greater than for intestinal digesta. The means and ranges for Z and W were 2.34% (1.30–4.77) and 2.10% (1.42–3.93) respectively.

Table 4 shows the daily intake of ash, the amount which passed the 3 intestinal sampling points as well as the amounts that appeared in the faeces. Table 5 shows the ash flow at each point expressed as a percentage of the ash intake. Table 6 shows the segmental absorption of ash expressed as a percentage of the ash ingested. It shows that large amounts of ash were added to the stomachs by saliva and gastric juice and that the additions greatly exceeded absorption. On average, net absorption (as a percentage of intake) was: stomachs, –111%; duodenum, –19%; small intestine, excluding duodenum, 131%; large intestine, 44%; total net absorption, 45%.

In experiment 5 little difference was seen between the amounts of ash in the ileum and faeces (table 6). This is thought to have been an artefact.

The fate of N in the intestines. The N content of digesta, collected at any one point, varied widely. The ranges observed in the proximal duodenal, distal duodenal and ileal digesta were 0.101–0.171%, 0.111–0.170% and 0.109–0.277% respectively. Table 4 shows daily intake of N and the amounts which passed the 3 sampling points and appeared in the faeces. Table 5 shows the N flow at each point expressed as a percentage of the intake. Table 6 shows the segmental absorption of N expressed as a percentage of N ingested. The amount of N recovered from the proximal duodenum was the same as or more than the amount fed except in experiment 5 (Table 5). In experiment 4 the increase was substantial. This diet was low in lysine and the total N intake was 15% below the standard. The average net absorption (as a percentage of the intake) was: stomachs –13.5%, duodenum, –7.5%; small intestine, excluding duodenum 82%; large intestine, 7%; total net absorption 68%.

pH values of intestinal digesta are shown in table 5. The pH at any one site did not change significantly between experiments. Z tended to have lower values than W but the differences were not significant. The increase in pH from proximal to distal duodenum to ileum were highly significant. Mean values ±SD were 2.42±0.24, 3.26±0.32 and 7.57±0.21 respectively in Z and 2.75±0.33, 3.47±0.38 and 7.55±0.11, respectively, in W.

The PD's between blood (+) *and digesta* (–) are shown in table 8. Values for the PD between blood and ileal digesta were unreliable in experiments 1 and 2 and were omitted in the other experiments. The mean PD between blood and rumen fluid ranged from 17.2 to 32.8 mV. The K concentration in rumen fluid ranged

from 17 to 51 meq per l. Variation in Na concentration was small i.e. 127 to 93 meq per l. In the 8 experiments in which the PD was measured, a roughly linear relationship existed between PD across the rumen wall and log K concentration in the rumen fluid, as expressed by the equation PD = −19.2 + 30.1 log K concentration. PD's across the duodenal wall tended to be higher in Z than in W, except in experiment 3 when they were the same in both cows (table 5). No consistent difference was seen between the PD across the proximal and distal parts of the duodenum. The ranges of the means in each of 8 experiments were 4.4–18.9 mV across the wall of the proximal duodenum and 4.8–18.0 mV across the wall of the distal duodenum.

Corresponding ranges for K concentration were 22–41 and 20–41 meq per kg respectively and for Na concentration 64–50 and 78–56 meq per kg. A plot of log K concentration against PD gave the same general trend as was found for the rumen, but there was much greater scatter of points.

Discussion

The faecal recovery of Cr and PEG from faeces in the different experiments was almost 100% after correction for losses (table 2). Therefore the total dose, after correction for losses, must have passed all sections of the intestine. Results of experiments with sheep had shown that the recovery of indicators from gut contents may vary considerably from day to day; (Van 't Klooster et al., 1969; MacRae and Armstrong, 1969). It was therefore considered advisable to base the calculations on two independent indicators in an attempt to reduce the error which might otherwise occur in spite of very frequent sampling. The total flow of digesta, based on PEG exceeded the flows based on the mean of both indicators by only 1.5, 1.8 and 3.5% for the proximal duodenum, distal duodenum and ileum respectively.

The rate of flow of fluid out of the reticulo-rumen was estimated by determination of the rate of dilution of the water soluble indicator Cr-EDTA (Binnerts et al., 1968). The technique was essentially similar to that based on the rate of dilution of single doses of PEG (Hydén, 1961; Poutiainen, 1968; Warner ans Stacy, 1968) or ^{51}Cr-EDTA (Downes and MacDonald, 1964; Hogan, 1965; Stacy and Warner, 1968).

Saliva is the main source of rumen water. Factors which influence saliva secretion may influence, therefore, the flow of fluid out of the reticulo-rumen. Kay (1960) found that salivation was related to bodyweight. Two dietary factors known to influence salivation are the DM intake and the physical character of the ration (Bailey and Balch, 1961; Kaufmann and Orth, 1966; Putnam et al., 1966; Poutiainen, 1968). The DM intake and physical character of the diets were similar in experiments 3 and 4, but much higher flow rates were found on ration 4 in which some of the concentrate had been replaced by maize meal in order to lower the protein intake. The cows ate this ration greedily. It is possible that the higher flow rate reflected an increased salivation due to increased palatability following the addition of maize meal.

BAILEY and BALCH (1961) reported that in cows eating a variety of diets the highest flow rates of saliva were observed on grass diets. The highest flows of fluid through the reticulo-rumen of our cows were also associated with a grass diet. If the flow of fluid through the reticulo-rumen is expressed as l per kg DM intake (table 3), values of 15–25 l per kg are found for our various diets as compared with values of 18–40 l per kg in the experiments of BAILEY (1961). The intake of DM in Bailey's experiments was much lower than in ours. This suggests that the amount of saliva added per kg DM intake varies widely and decreases strongly with increasing feed intake (PUTNAM et al., 1966).

A water soluble indicator can be used to measure the rate of flow of only the fluid phase of digesta leaving the reticulo-rumen. It does not measure the rate of flow of total digesta. As the mean DM content of digesta from the proximal duodenum was only 3.1 %, a comparison between the rate of flow of fluid out of the reticulo-rumen and the rate of flow of total digesta in the proximal duodenum would be almost the same as comparing the flow of fluid in both areas.

In 7 out of 9 experiments the flow of digesta through the proximal duodenum was equal to or slightly less than the flow of fluid out of the reticulo-rumen. Apparently the volume of gastric juice secreted in the abomasum in these experiments was sufficient to replace most if not all of the water absorbed in the omasum. Similar results have been reported by SPERBER et al. (1956) for cows and by OYAERT and BOUCKAERT (1961) and HOGAN (1964) for sheep. In 2 experiments with W an appreciably increased flow through the duodenum was found (table 3). In these cases the secretion into the abomasum appeared to have exceeded the absorption from the omasum.

There is a lack of quantitative data on the secretion of bile, pancreatic juice and juice of Brunner's glands in cows. In sheep HARRISON and HILL (1962) estimated the total secretion into the duodenum at about 1.1 ml per kg per hour. If this value is applicable to cows, one would expect about 12 litres of fluid to be secreted into the duodenum of our cows. Net additions into the duodenum of about 20 g N and 780 g DM were found in our cows. If the mean values for N and DM contents of bile (HARRISON, 1962) pancreatic juice (DUKES, 1960; TAYLOR, 1962) and juice of Brunner's glands (DUKES, 1960) are used, one would expect a secretion of fluid of 16 l or more to account for the increases in N and DM observed. The observed addition of fluid (about 8 kg) was only about half the expected increase. This can be explained partly by backflow between the 2 duodenal cannulae and partly by water absorption from the duodenum. The detection of bile acids in digesta from the proximal cannula confirmed that backflow occurred. Water absorption from the duodenum has not been definitely proved because of the uncertainty about the composition of the secretion products.

The mean net water absorption (about 188 l) from the intestines in the present experiments agrees well with the value of 220 l given by SINESHCHEKOV (1965) when 250 l chyme entered the duodenum of cows. In our data the water absorbed from the intestines was equivalent to about 90 % of the weight of digesta entering the duodenum. A similar value (88 %) was reported by SINESHCHEKOV.

The overall digestibility of DM was high (72.5%) in our experiments. When the DM excretion was corrected for sampling losses, the mean digestibility was calculated at 71%. The stomachs absorbed amounts equivalent to 38% of the intake, the intestines 34%. On diets of much lower digestibility (55–60%) SINESHCHEKOV (1965) reported that the stomachs absorbed 40% and the intestines 15–20%, but that the percentage absorption from the intestines increased by a factor of 1.5–2 on diets of high digestibility. Our results are in good agreement with this. The relative importance of the stomachs, small and large intestines in the absorption and digestion of food is shown in tables 6 and 7. On average, these areas absorbed 53, 36 and 11% of the digestible DM respectively. In sheep, BADAWY and MACKIE (1964) and TOPPS et al., (1968) found values which were higher in the stomachs and lower in the intestines, but such differences can be attributed to differences in the composition of diets. In goats on diets of about 60% DM digestibility, RIDGES and SINGLETON (1962) found that 58% of the digestable DM was absorbed from the stomachs and 42% from the intestines. DM absorption from the intestines is influenced not only by total DM digestibility (SINESHCHEKOV, 1965) but also by the dietary N intake. This can be deduced from experiments with sheep by CLOETE (1964) and BRUCE et al., (1966). In their experiments, the addition of protein to rations composed of low quality hay or hay plus maize increased the relative absorption of DM and OM from the small intestine. This is quite understandable as the absorption of N is almost fully restricted to the small intestine.

The stomachs were major importance in the assimilation of OM. The relative absorption of digestible OM from the stomachs, the small and large intestine was 67% (55–73), 26% (22–29) and 7% (5–11) respectively. In our experiments the absorption of crude protein (Nx6.25) could account for 56% of the OM absorbed from the small intestine.

Absorption of minerals distal to the ileal fistula (ROGERS and VAN 'T KLOOSTER, 1969) was not appreciably different in experiment 5 compared with the other experiments, yet table 6 shows that the absorption of ash was very low from this segment in experiment 5. It is felt that the value for ash absorption from the large intestine in that experiment was an artefact caused by massive and sudden excretion of foreign-body sand. The data confirm the major importance of the small intestine in the absorption of inorganic substances and that absorption from the large intestines was appreciable in spite of the low values caused by the artefact in experiment 5.

In general, the amount of N flowing through the proximal duodenum was greater than the amount ingested (tables 5 and 6). An exception was formed by the grass ration where the same amount or less N in the duodenum was found. In these experiments the estimated mean flow rate of saliva was about 159 l per day. At total N levels of 8–18 mg per 100 ml saliva (BAILEY and BALCH, 1961; EMERY et al., 1960) the total flow of salivary N to the rumen would have been 13–29 g per day. KAY (1963) suggested that the addition of N with saliva amounted to about 10% of the N intake, but that diffusion of urea from blood to the rumen would be even greater. In the present experiments the intake was

252 g. If saliva N amounted to about 25 g per day and transrumenal inflow was about 30 g per day, the total inflow of N (feed plus endogenous) to the stomachs would indicate that N absorption occured in most experiments, especially on the grass diets. High concentrations of NH_3 have been found in the rumen of sheep eating grass (HOGAN, 1964). It is known that the absorption of NH_3 from the rumen is largely governed by its concentration. Our data confirm, in general, that absorption of N (as NH_3) from the stomachs is appreciable on grass diets but is small on stall diets.

The major importance of the entire small intestine for N absorption is shown by the very high absorption (75 %) of dietary N which occured from this segment (table 5). Further absorption (equivalent to 7% of the N intake) apparently occurred from the large intestines. However, N estimation on faeces was done on material dried at 60 °C whereas ileal contents were freeze dried. It has been shown that losses of 0–8 % of faeces N may occur on drying at 60 °C. Unless similar losses of N occur in the freeze-drying process, the apparent absorption from the large intestine may have been overestimated. Small absorption of N from the large intestines of sheep has been reported by GOODALL and KAY (1965) and CLARKE et al. (1966). Details of the fate of the different N fractions of digesta will be given in a separate paper (VAN 'T KLOOSTER and BOEKHOLT, 1970).

The pH values of duodenal digesta were much lower than those obtained from slaughtered animals, (DUKES, 1960; KOLB, 1965). Our values agree with those obtained from in-vivo studies in cows (SINESHCHEKOV, 1965; VAN 'T KLOOSTER, 1967), calves (TOPPS et al., 1968) and sheep (HARRIS and PHILLIPSON, 1952; HARRISON and HILL, 1962; TOPPS et al., 1968). The pH in the distal duodenum was consistently somewhat higher than in the proximal part, but indicated that the secretion products (bile, pancreatic juice and Brunner's juice) entering the duodenum had relatively poor neutralizing capacity on the acid abomasal digesta (HARRISON and HILL, 1962).

A positive relationship between PD across the rumen wall and K concentration in rumen fluid has been reported by DOBSON (1956), DOBSON and PHILLIPSON (1958), HARRISON et al., (1964) and FERREIRA et al., (1966). Although a positive relationship is evident in our data (PD = −19 + 30 log K conc.), the slope (b) of the regression deviates markedly from the theoretical value of 61 mV at 39 °C for a 10-fold increase in the concentration of a freely diffusing ion. This indicates that factors other than K concentration may play an important role in determining the PD across the rumen wall.

SCOTT (1965) confirmed the conclusion of BARRY et al. (1964) that metabolizable energy was necessary to maintain the PD across the intestine of sheep. In experiments with rat intestine, KOHN et al. (1968) found that l-amino acids (except lysine and arginine) and a few d-amino acids increase the PD when present in mucosal fluid. In the present experiments the regression of PD across the duodenum on log K concentration in duodenal digesta showed a

positive relationship but the scatter of points was much wider than was the case for the rumen. This indicates that other unidentified substances may be of great importance in the regulation of PD across the duodenal wall.

Summary

Five different diets were fed to each of two fistulated lactating cows in metabolism stalls. Cr-EDTA was used to measure the rate of flow of fluid out of the reticulo-rumen. Cr_2O_3 and PEG were used to measure the rate of flow of digesta in the proximal and distal duodenum and terminal ileum. Representative samples of feed, digesta and faeces were analysed for their DM, ash and N content.

The rate of flow of fluid out of the reticulo-rumen (162–272 l per day) was of the same order as the digesta flow through the duodenum (154–267 kg per day). The rate of flow of digesta through the distal duodenum and ileum averaged 213 (165–282) and 55 (31–77) kg per day respectively.

The stomachs, small and large intestines absorbed 53, 36 and 11% of the digestible DM respectively. The relative absorption of digestible OM from the stomachs, entire small intestine and large intestine was on average 67, 26 and 7% respectively. Only small amounts of N were absorbed from the stomachs. The absorption of crude protein could account for more than half of the OM absorbed from the small intestine.

The addition of DM, OM, N, ash and H_2O to the duodenal digesta was attributed to bile, pancreatic juice and the juice of Brunner's glands.

The large intestines absorbed appreciable net amounts of water and ash, but relatively little OM, DM and N. The pH of digesta and the PD across the wall of the tract were measured. The results are given and discussed briefly.

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2. THE DIGESTION OF THE CELL-WALL CONSTITUENTS OF ROUGHAGES

BLANCHE D. E. GAILLARD AND A. TH. VAN 'T KLOOSTER

INTRODUCTION

With the aid of fistulated animals it is possible to study in vivo the composition of digesta from different parts of the alimentary tract of ruminants. The digestion of various nutrients has been investigated with these methods. As regards the cell-wall constituents research was mainly concerned with the digestion of cellulose usually in connection with the digestion of other nutrients, (BRUCE et al., 1966; CLOETE, 1966; TOPPS et al., 1968). Many of these experiments are carried out with a single feed or by comparing the composition of digesta reaching the duodenum in one group of sheep with the composition at the end of the small intestine in another group of animals. BAILEY (1967) measured the disappearance of hemicellulose as well as of cellulose from the rumen of cows at different times after feeding. PORTER and SINGLETON (1966) investigated the digestion of pentosan from hay using two sheep with duodenal fistulae.

Our aim was to follow the digestion of the cell-wall polysaccharides cellulose and hemicellulose at different parts of the digestive tract of the same animal when given mixed rations and also roughage only. This paper describes the results obtained with two fistulated cows given two different normal rations and two semi-synthetic rations rich in cellulose. One of the synthetic rations was low in protein and extremely low in lysine.

Lignin has often been discussed as a reference substance in digestion trials. The main disadvantage in this respect is the sensitivity of the lignin determination to the pretreatment of the samples as drying at elevated temperatures tends to give higher lignin values. It is also uncertain whether the lignin determined in the digesta and in the faeces still has the same composition as the lignin determined in the food. As in our experiments other markers were used for the calculation of the passage rate of the digesta at different points along the alimentary tract, it seemed a good opportunity to check the 'apparent' lignin digestibilities at the same time.

EXPERIMENTAL

Animals: Two Friesian cows (Z and W), three and four years old and weighing 462 and 420 kg respectively were used. Both cows had fistulae in the rumen, proximal duodenum, distal duodenum and terminal ileum (VAN 'T KLOOSTER and ROGERS 1969).

Feeding and sampling: The composition of the rations is given in table 1 in part I. Feeding times were 7.15 a.m. and 5.00 p.m. The experimental periods

lasted for 7 days, the preliminary periods for 10 days, except in experiment 5 where a 5 day preliminary period was taken. Contents from the proximal duodenaland ileal fistulae were sampled every second hour during 5 days of the experimental period (VAN 'T KLOOSTER and ROGERS, 1969). No samples were taken from gut contents of cow W in experiment 2. Bulk samples of each animal were freeze dried before analysis. Faeces were collected totally and sampled daily.

Indicators: In each experiment polyethyleneglycol (PEG) and chromium-sesquioxide (Cr_2O_3) were used as indicators. At feeding times 100 g PEG and 17 g Cr_2O_3-paper, containing 4.864 g of Cr_2O_3 were brought directly into the rumen. Both indicators were used for the calculation of the total amounts of cellulose and hemicelluloses that daily passed the different fistulae.

Chemical analyses: Hemicelluloses, cellulose and lignin were determined as described by GAILLARD (1966): Total cell-wall substances were first prepared from the samples by boiling with a neutral detergent solution. The hemicelluloses content was estimated by hydrolysis with N sulphuric acid followed by a sugar determination in the filtrate. To the percentage of sugars thus calculated was added the amount of anhydro-uronic acid found by titration. Cellulose was determined by hydrolysing the residue with 72% sulphuric acid and subsequent estimation of the sugars in the filtrate. The amount of lignin was found by subtracting ash from the final hydrolysis residue.

Protein was determined by the macro Kjeldahl method.

PEG was estimated according to HYDÉN (1961) and Cr_2O_3 as described earlier (VAN 'T KLOOSTER et al., 1969).

RESULTS AND DISCUSSION

The amounts of the food consumed in the five rations, of the digesta passing by the duodenum and terminal ileum and of the faeces are given in table 1. In table 2 the corresponding amounts of hemicelluloses, cellulose and lignin are given with the different percentages of each of these components digested at the different stages. The contribution of microbial cell-wall polysaccharides in this respect is small (PORTER and SINGLETON, 1965; BAILEY, 1967) and may therefore be neglected.

In all 5 experiments cellulose and hemicelluloses showed similar digestibilities, the values for cellulose always being somewhat higher than the corresponding ones for hemicelluloses. The digestibilities of hemicelluloses did not increase any more after passing the duodenal fistula. For cellulose a slight increase was found in most of the experiments. This shows that the breakdown of the cell-wall polysaccharides as such is almost fully restricted to the reticulorumen. This is in agreement with the findings of PORTER and SINGLETON (1966), BRUCE et al. (1966) and TOPPS et al. (1968). It does not necessarely mean that the polysaccharides degraded in the rumen are completely broken down into monosaccharides and transferred into volatile fatty acids. It is possible that some of these polysaccharides pass on to the intestine in a partly degraded state.

TABLE 1. Amounts of food given daily and of corresponding digesta and faeces in kg air dry matter

	ration		duodenum	ileum	faeces
Experiment I					
cow Z	hay	7.656	8.894	5.592	4.220
	concentr.	6.000			
cow W	hay	6.692	7.636	4.606	3.790
	concentr.	6.000			
Experiment II					
cow Z	hay	7.656			
	concentr.	6.000	9.520	5.561	4.221
	K	0.220			
cow W	hay	6.214			
	concentr.	6.000	—	—	3.383
	K	0.165			
Experiment III					
cow Z	hay	2.820			
	concentr.	3.000	5.296	2.803	2.120
	cellulose	4.000			
cow W	hay	2.820			
	concentr.	3.500	5.884	2.845	2.280
	cellulose	3.000			
Experiment IV					
cow Z	hay	2.780			
	concentr.	3.100	6.586	4.407	3.015
	cellulose	4.000			
cow W	hay	2.780			
	concentr.	3.100	5.430	3.281	2.596
	cellulose	3.000			
Experiment V					
cow Z	grass	12.450	6.909	3.690	2.870
cow W	grass	11.000	6.574	3.151	2.458

Due to their lower degree of polymerization they may have become soluble in the neutral detergent solution and escape determination as cell-wall polysaccharides. The values mentioned in the tables have therefore to be interpreted as digestibilities of the intact cell-wall polysaccharides. Whether partly degraded polysaccharides pass into the intestine and to what extent they may be further digested during passage of the intestinal tract is at the moment being investigated.

Production (BRÜGGEMANN and GIESECKE, 1963) and absorption (MYERS, et al., 1967) of volatile fatty acids in the caecum and large intestine suggest that carbohydrates are fermented in the hind gut. However, quantitative information on the production of volatile fatty acids in the caecum and large intestine is lacking. It was found that only 4–5% of the organic matter in the ration disappeared in this section (VAN 'T KLOOSTER and ROGERS, 1969). That in the present experiments hardly any cellulose or hemicellulose is degraded after passing the abomasum shows that fermentation in the gut of intact cell-wall substances into volatile fatty acids is indeed of little importance. The possibili-

TABLE 2. Amounts of hemicelluloses, cellulose and lignin in food and corresponding digesta and faeces in grams and the percentages digested.

		hemicelluloses		cellulose		lignin	
cow		Z	W	Z	W	Z	W
Experiment I							
hay		1045.0	913.5	1698.1	1484.4	279.4	244.3
concentrate		544.8	544.8	277.8	277.8	78.6	78.6
total food		1589.8	1458.3	1975.9	1762.2	358.0	322.9
duodenum	g	603.0	500.9	654.6	543.7	305.1	255.8
% **digested**		**62.1**	**65.7**	**66.8**	**69.2**	**14.6**	**20.8**
ileum	g	602.3	538.3	619.6	504.8	279.0	243.2
% **digested**		**62.1**	**63.1**	**68.7**	**71.4**	**21.8**	**24.7**
faeces	g	628.8	533.6	579.8	542.0	321.1	294.1
% **digested**		**60.4**	**63.4**	**70.6**	**69.2**	**10.1**	**8.9**
Experiment II							
hay		1045.0	848.2	1698.1	1378.3	279.4	244.3
concentrate		544.8	544.8	277.8	277.8	78.6	78.6
total food		1589.8	1393.0	1975.9	1656.1	358.0	322.9
duodenum	g	502.7		725.4		300.8	
% **digested**		**68.4**		**63.3**		**16.0**	
ileum	g	586.7		672.9		298.6	
% **digested**		**63.1**		**65.9**		**16.6**	
faeces	g	515.8	473.2	596.0	473.3	333.0	286.5
% **digested**		**67.5**	**66.0**	**69.8**	**71.5**	**7.0**	**11.2**
Experiment III							
hay		449.5	449.5	603.5	603.5	113.4	113.4
concentrate		324.0	372.0	156.3	178.5	52.2	54.9
cellulose		232.0	174.0	2537.6	1993.2	36.2	27.2
total food		1005.5	995.5	3297.4	2685.2	201.8	195.5
duodenum	g	94.3	97.7	293.9	263.0	166.8	141.8
% **digested**		**90.6**	**90.2**	**91.1**	**90.2**	**17.3**	**27.5**
ileum	g	119.1	107.0	259.3	219.4	146.6	142.0
% **digested**		**88.2**	**89.4**	**92.1**	**91.8**	**27.3**	**27.5**
faeces	g	144.4	181.9	196.3	219.8	161.3	135.9
% **digested**		**85.6**	**81.7**	**94.1**	**91.8**	**20.0**	**30.5**
Experiment IV							
hay		409.5	409.5	570.5	570.5	152.1	152.1
concentrate		121.8	114.4	52.7	38.7	18.6	6.8
cellulose		232.0	174.0	2537.6	1903.3	37.6	28.2
total food		763.3	697.9	3160.8	2512.5	208.3	187.1
duodenum	g	308.7	221.0	692.6	456.7	175.1	117.8
% **digested**		**59.6**	**68.1**	**78.1**	**81.8**	**15.3**	**36.7**
ileum	g	359.2	259.2	850.6	384.9	150.3	98.8
% **digested**		**53.0**	**62.7**	**73.1**	**84.7**	**27.3**	**46.9**
faeces	g	306.0	266.6	624.1	415.9	110,6	148.8
% **digested**		**59.8**	**61.6**	**80.3**	**83.4**	**46.5**	**20.1**
Experiment V							
grass		1368.3	1208.9	1842.6	1628.0	193.0	170.5
duodenum	g	156.1	148.6	178.2	136.7	107.8	107.8
% **digested**		**88.6**	**87.7**	**90.3**	**91.6**	**44.1**	**36.8**
ileum	g	204.8	158.5	208.1	133.6	130.6	112.8
% **digested**		**85.0**	**86.9**	**88.7**	**91.8**	**32.3**	**33.8**
faeces	g	167.9	141.1	118.8	92.9	144.9	132.7
% **digested**		**87.7**	**88.3**	**93.5**	**94.3**	**24.9**	**22.1**

ty remains that a small amount of degraded cell -wall polysaccharides is converted here into volatile fatty acids.

The 5 experiments show some interesting differences in the apparent digestibilities of cellulose and hemicelluloses between the different rations. In experiments I, II and V the cellulose and hemicelluloses are mainly given as roughage. Due to the usual higher content of lignin in the hay (exp. I and II) the cell-wall polysaccharides are less accessable to the attack by microorganisms and are therefore less well digested than the cell-wall polysaccharides of the grass (exp. V).

In experiment III a small amount of hay is fed and a considerable part of the cellulose and hemicelluloses is given in an easily accessable form (paper pulp and concentrate). The digestibilities of the cellulose and hemicellulose polysaccharides therefore equal those of experiment V (grass ration).

Hardly any difference in digestibility of cell-wall polysaccharides was observed between experiments I and II where rations were given differing only in potassium contents. In experiments III and IV also about the same rations were fed. However, as far as crude protein is concerned, the animals were fed above standard in experiment III and below standard in experiment IV. Moreover the lysine content of the ration in experiment IV was very low by feeding maize gluten protein. Apparently the available protein and its quality were insufficient to promote a good activity of the microorganisms and hence depressed the polysaccharide digestion. This was more so with cow Z than with cow W.

The data for lignin digestion clearly show that lignin is far from reliable as a reference substance in digestion trials. This is probably due to differences in pretreatment of the samples and to the inaccuracy of the determination at the low lignin levels encountered in the different samples.

Summary

The digestion of the cell-wall polysaccharides at different parts of the alimentary tract was studied with two fistulated cows on various rations. It was found that digestion of hemicelluloses occurred in the rumen only. Cellulose digestion mainly occurred in the rumen but there was an indication that small amounts may be digested afterwards in the intestines. The influence on the digestibility of the level of protein and of lignification is discussed. Lignin was found unsuitable as reference substance in digestion trials.

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3. THE FATE OF Na, K, Ca, Mg, AND P IN THE DIGESTA

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INTRODUCTION

Secretion and absorption of nutrients is a continuous process which probably occurs all along the alimentary tract. In certain areas the outflow of nutrients from gut lumen to blood may exceed the inflow so that the net result is absorption. The reverse may exist in other areas. For a deeper understanding of the processes involved in absorption and secretion, it is desirable to identify the segments in which net absorption or net secretion occurs.

The proper use of inert reference substances in healthy fistulated animals offers a suitable method of estimating the flow of digesta past any point in the intestine. If representative samples are analysed for mineral content, the total amount of any mineral passing by a sampling point can be calculated. The net absorption or net secretion of the mineral between any two points is calculated by the difference between the total amounts of the mineral passing daily at each of these points. In studies on the sites of net mineral absorption, this technique has many important advantages over others (such as in-vitro work, slaughter techniques with or without the use of isotopes, arterio-venous techniques, etc.). Although some workers have studied the flow rates and composition of digesta in fistulated sheep and goats there is little information in the literature on these topics in fistulated cattle. SMITH (1962) studied absorption in fistulated milk-fed calves. The only studies reported for cows are those of SINESHCHEKOV and his team, (1962 and 1965) and VAN 'T KLOOSTER (1967).

By using the indicator technique we have studied the rate of flow of digesta and the fate of various nutrients in the gastro-intestinal tract of fistulated cows on varying intakes of dry matter, minerals and nitrogen. Data on the rate of flow of digesta and the fate of dry matter, ash, nitrogen and water are given in paper 1 (see p. 3 – VAN 'T KLOOSTER and ROGERS, 1969). This paper describes the fate of Na, K, Ca, Mg and P in the same experiments.

EXPERIMENTAL

Two fistulated lactating Friesian cows, Zwartschoft (Z) and Witschoft (W), were used in these experiments. Each cow had been fitted with cannulae in the rumen, proximal duodenum (D_1), distal duodenum (D_2) and terminal ileum (I_t).

Comprehensive details concerning the animals, experimental procedures, diets and sampling are given in the preceding paper 1.

Representative samples of saliva, rumen fluid, digesta from D_1, D_2, and I_t,

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TABLE 1. The intakes of Na, K, Ca, Mg and P expressed as g per 100 g DM ingested.

Intake g per 100 g DM	Zwartschoft					Witschoft				
	Experiment					Experiment				
	1	2	3	4	5	1	2	3	4	5
Na	0.22	0.21	0.30	0.32	0.27	0.20	0.20	0.32	0.33	0.26
K	1.61	3.29	1.04	0.80	3.75	1.54	3.01	1.17	0.87	3.74
Ca	0.61	0.58	0.68	0.66	0.64	0.60	0.63	0.73	0.74	0.64
Mg	0.20	0.20	0.17	0.15	0.12	0.20	0.20	0.21	0.16	0.12
P	0.40	0.39	0.40	0.30	0.49	0.40	0.41	0.46	0.36	0.48

faeces, urine, milk, drinking water, feed and feed refusals were analysed for Na, K, Ca, Mg and P, as described by VAN 'T KLOOSTER et al. (1969). Acidified saliva, clear rumen fluid, urine and drinking water were merely diluted before analysis. Feeds, feed refusals, intestinal digesta, ileal washings, faeces and milk were dried, ashed and extracted with HCl before dilution.

Samples of saliva, mainly of the parotid gland, were collected from sponges placed between the molars and the cheek. Samples were collected frequently in experiments 1, 3 and 5, but less frequently in experiment 2. No saliva was collected in experiment 4. Samples were bulked and acidified for each experiment.

The volume of saliva secreted daily was estimated by subtracting the daily intake of water in food and drink from the daily volume of fluid leaving the reticulo-rumen (POUTIAINEN, 1968). The differences in the mineral composition of saliva were appreciable between cows but were small between experiments. Therefore, the values were averaged seperately for each cow. The amount of minerals added by saliva to the rumen was calculated by multiplying the estimated volume of saliva secreted per day by the average mineral composition.

The mean daily amounts of digesta passing by each of the 4 cannulae were estimated using Cr-EDTA (rumen fluid) or Cr_2O_3 and PEG (intestinal digesta) as described in the preceding paper. The mean daily amounts of minerals passing by each of the 4 sampling points were calculated by multiplying the mean amount of digesta passing daily at each point by the mineral composition of the bulk sample collected at that point. The mean daily excretion of minerals in the faeces (F) was calculated in a similar manner.

If x grams of a mineral pass daily by one sampling point and y grams pass daily by a distal sampling point, (x−y) grams represents the net daily absorption of the mineral which has occurred between the two points (negative values represent net secretion). Net absorption from the stomachs (cardia to pylorus inclusive) is given by $(I + S - D_1)$, from the small intestine, excluding the duodenum, by $(D_2 - I_t)$ and from the caecum and large intestine by $(I_t - F)$, where I, S, D_1, D_2, I_t and F represent the daily amounts of a mineral present in the intake, saliva, proximal duodenum, distal duodenum, terminal ileum and faeces respectively (table 2). The location of the duodenal cannulae was such that the difference $(D_1 - D_2)$ would represent most, but not all, of the net flux in the

duodenum. Earlier work showed that some antiperistalsis occurred between the two cannulae, therefore the estimates of changes occurring within the duodenum are not as reliable as estimates for other areas. SCOTT (1967) and VAN 'T KLOOSTER and ROGERS (1970) found that only small amounts of Na and K are bound in rumen contents. It was, therefore, possible to make an approximation of the daily amounts of these minerals flowing out of the reticulo-rumen (R) by multiplying the flow of fluid by the concentration of Na and K in the fluid. Net absorption of Na and K from the reticulo-rumen was estimated as $(I + S - R)$ and from the omaso-abomasum as $(R - D_1)$. Because large amounts of Ca, Mg and P are bound in rumen contents (GARTON, 1951), similar estimates were not possible for these minerals. Study of their absorption from the anterior part of the tract was confined, therefore, to the area between the cardia and the pylorus.

Digesta were lost by sampling and by leakage. Amounts removed by sampling were 800, 900 and 600 ml per day of rumen fluid, duodenal and ileal digesta respectively. The daily amounts of minerals removed by sampling could be measured accurately. Loss by leakage from the intestinal cannulae was a problem only at sampling times. The ileal leakage, including the washings from the collection bottle, was stored seperately for analysis. It was necessary, however, to make an arbitrary estimate of the leakage from the rumen and duodenum, i.e. 700 and 200 ml per day respectively.

The faecal excretion of minerals was corrected for losses by sampling and leakage from the ileum. It was considered unnecessary to correct faeces values for losses from the rumen and duodenum.

As a check on the experiments, the apparent availabilities and balances were determined in the usual manner.

Blood samples were collected into heparinized bottles on a few occasions in periods 1, 2, 3 and 5. Plasma was diluted and the Ca and Mg levels were determined as other samples by atomic absorption flame spectrophotometry.

RESULTS

The mineral contents of the diets, expressed as a percentage of DM ingested, are shown in table 1. The total daily intakes of minerals are shown in tables 2 and 3. All intakes have been corrected for refusals. There were wide variations in the intakes of K, Mg and P due to different DM intakes and dietary composition of the rations. Na and Ca intakes were more constant.

The mineral balances are shown in table 3. Consistent relationships were not observed between mineral balances and dietary composition or intake. K balances, however, tended to be highest on the high K diets i.e. periods 2 and 5. The mean balances (g per day) for Z and W respectively were Na, –0.2 and –1.5; K, +4.4 and +4.2; Ca, –0.5 and +2.0; Mg, +0.2 and +0.7; P, +2.0 and +2.7.

The apparent availabilities are shown in tables 2 and 3. The values for intake and faecal excretion, given in table 2, are the means for 5 periods. The mean apparent availability (table 2) for each cow is based on these means. Table 3

TABLE 2. Daily intake of minerals, estimated addition of minerals by saliva, amounts which passed daily in rumen fluid, duodenum, terminal ileum and faeces and apparent availabilities (means for the five periods).

	Zwartschoft					Witschoft				
	Na	K	Ca	Mg	P	Na	K	Ca	Mg	P
Intake (g)	28.1	238.8	68.6	18.6	43.2	25.2	210.5	65.5	18.0	41.7
Saliva minerals (g)	611.1	57.3	3.8	1.0	59.1	496.5	44.5	2.1	0.6	56.7
Intake + saliva (g)	639.2	296.1	72.4	19.6	102.3	521.7	255.0	67.6	18.6	98.4
Rumen fluid (g)	598.0	267.3	–	–	–	450.9	229.8	–	–	–
Duodenum I (g)*	284.9	262.4	70.6	15.7	92.0	271.5	244.5	66.2	13.8	76.7
Duodenum 2 (g)*	350.3	254.7	70.1	15.7	90.4	347.0	238.3	66.0	13.9	72.5
Ileum (g)*	127.8	103.2	57.9	16.4	29.4	85.5	81.1	50.2	14.5	27.1
Faeces (g)	3.8	25.8	56.2	15.7	28.5	4.1	25.0	48.9	14.1	25.7
Apparent availability %	87	89	18	16	34	84	88	25	22	38

* Values for Witschoft contain an estimate for period 2.

shows the availabilities for each period. If the availabilities in table 3 are averaged a slight discrepancy arises between this average and the value given in table 2. This is to be expected because of the variation in intake of K, Mg and P, and the different ways in which the values would have been computed.

The mean mineral composition of saliva is given in table 4. Little variation in the composition was found between experiments. The saliva Na:K ratio ranged from 17:1 to 28:1, indicating normal Na status (DENTON, 1956; KEMP and GEURINK, 1966; JONES et al., 1967). The estimated daily saliva secretions and their relationship to the DM intake are shown in table 5. The mean estimated addition of minerals by saliva to the rumen is shown in table 2.

Table 2 also summarizes the overall mean daily intake of each of the 5 mineral substances by each cow. It shows the mean amounts estimated to pass with the fluid out of the reticulo-rumen, the amounts passing through the proximal and distal duodenum and terminal ileum and the corrected values for faeces. The data were treated as a whole because there appeared to be a consistent trend in the sites of mineral absorption. Although between-experiment variation occurred in the amounts of minerals absorbed in different areas, the data were not extensive enough to allow firm conclusions to be drawn about the effect of diet on segmental absorption.

Table 6 shows the mean total net absorption of Na, K, Ca, Mg and P from the digestive tract (I – F) and the mean net absorption or secretion between different points along the tract.

The total amount of a mineral entering the reticulo-rumen was taken as the amount ingested plus the estimated amount secreted with saliva (I + S). The amounts of Ca and Mg added by saliva were small (approximately 3.0 and 0.8 g per day respectively). Extremely large amounts of Na (overall mean of about 550 g) and large amounts of P (overall mean of about 58 g) were added by saliva. The overall mean dietary intakes of these elements were about 27 and 42 g respectively (table 2).

Net absorption of Na and K commenced from the reticulo-rumen (table 6).

TABLE 3. Intake of minerals, excretion in the faeces, total output, apparent availabilities and balances f

	Na					K				
	1	2	3	4	5	1	2	3	4	5
Zwartschoft:										
Intake g/day	26.1	26.7	28.6	29.7	29.4	194.7	414.2	97.6	75.3	412.:
In faeces g/day	6.6	4.2	2.4	3.6	1.9	26.7	38.4	12.3	17.8	33.:
Total output g/day	29.5	22.6	33.5	29.5	26.2	195.2	410.7	93.8	81.0	392.4
Apparent availability: %	75	84	92	88	93	86	91	87	76	92
Balance g/day	–3.4	+4.1	–4.9	+0.2	+3.2	–0.5	+3.5	+3.8	–5.7	+19.5
Witschoft										
Intake g/day	22.7	21.9	27.8	27.7	25.9	174.3	334.2	103.7	73.4	366.(
In faeces g/day	5.8	6.1	2.4	4.2	2.2	31.3	32.7	14.0	15.0	32.:
Total output g/day	23.4	23.6	28.9	33.0	25.4	174.8	326.4	96.0	78.3	355.7
Apparent availability: %	75	72	91	85	92	82	90	87	80	91
Balance g/day	–0.7	–1.7	–1.1	–5.3	+0.5	–0.5	+7.8	+7.7	–4.9	+10.

The overall mean amounts of Na absorbed were 41 and 71 g in Z and W respectively. These amounts correspond to 6.4 and 13.5% of the mean amount entering the reticulo-rumen. The net amounts of K absorbed were about 29 g and 25 g per day in Z and W respectively, i.e. about 10% of the amounts of K entering the reticulo-rumen. The amounts of K absorbed in the reticulo-rumen were highest on the high K intakes of experiment 2. The omaso-abomasum absorbed very large amounts of Na, i.e. 313 and 179 g per day in Z and W respectively. Results for K were inconclusive from this segment, net secretion occurring in W. Net K absorption from the stomachs (cardia to pylorus) was of minor importance.

Between the cardia and the pylorus the net absorption ($I + S - D_1$) of Ca was slight, 1 to 2 g per day, but that of Mg was high. Table 6 shows that the net absorption of Mg from the stomachs was greater than the total net absorption from the entire tract. This result occurred in each of the 9 experiments in which it could be measured. Net absorption of P was appreciable from this area.

The duodenum ($D_1 - D_2$) was a site of net Na secretion – 65 and 75 g per day in Z and W respectively. Slight net absorption of K occurred but the amounts were less than 3% of those entering the rumen. Ca absorption was negligible and Mg absorption did not occur at all (table 6). Small amounts of P_i were absorbed i.e. 2–4 g in Z and W respectively, but these amounts were almost negligible compared with the inflow to the rumen (about 100 g).

The small intestine was a major site of net Na absorption and the major site of net K, Ca and P absorption (table 6). While negligible net amounts of Mg were absorbed from the small intestine in some of the experiments, the overall picture showed a slight net secretion.

The large intestines were important in net Na and K absorption ($I_t - F$) but

: five experimental periods.

Ca					Mg					P				
1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
'3.3	73.4	63.7	62.0	70.5	24.7	24.7	16.3	14.1	13.5	47.9	48.6	37.8	27.9	53.5
0.4	57.3	52.2	55.1	55.9	20.5	21.7	12.3	12.4	11.4	31.9	29.8	25.5	20.7	34.5
'3.9	71.2	65.4	64.8	69.9	24.1	23.4	17.0	14.1	12.6	45.8	46.6	36.4	29.3	47.4
.8	22	18	11	21	17	12	25	12	16	33	39	33	26	36
0.6	+2.2	−1.7	−2.8	+0.6	+0.6	+1.3	−0.7	0	+0.9	+2.1	+2.0	+1.4	−1.4	+6.1
8.2	70.2	64.3	62.5	62.5	23.0	22.4	18.9	13.8	12.0	44.7	45.4	40.9	30.0	47.5
8.0	53.3	47.7	49.2	46.5	15.9	18.3	15.8	10.3	10.2	27.3	26.9	26.1	17.3	30.8
4.0	66.8	62.5	62.0	62.3	22.0	20.8	18.6	13.6	11.5	43.1	41.7	39.7	27.0	43.7
0	24	26	21	26	31	19	17	26	15	39	41	36	42	35
4.2	+3.4	+1.8	+0.5	+0.2	+1.0	+1.6	+0.3	+0.2	+0.5	+1.6	+3.7	+1.2	+3.0	+3.8

the amounts absorbed were only $\frac{1}{2}$ to $\frac{1}{3}$ of those absorbed in the small intestine. Table 6 shows that slight net absorption of Ca, Mg and P occurred from the large intestines, but if the flow of digesta through the terminal ileum is based on Cr_2O_3 alone (as distinct from the mean flow based on both PEG and Cr_2O_3) the estimates of net absorption of these elements from this segment become zero.

Table 4 shows the mean concentration and range of mineral substances in the saliva, rumen fluid, duodenal and ileal digesta, faeces, urine and milk.

The mineral concentrations in milk were fairly constant as demonstrated by the ranges in table 4, but consistent between-cow differences were seen in the concentrations of Na, Ca, Mg and P. K concentrations were similar in both cows. The variation in mineral output in milk was mainly due to individual differences in milk yield.

Plasma Ca levels were within the normal range in all samples examined. Plasma Mg was above 2.0 mg per 100 ml in all samples except in Z, period 5. In this case hypomagnesaemia due to the grass diet, was noted (table 7). Plasma levels decreased to 0.9 mg per 100 ml. W showed no such response. Neither Z nor W developed hypomagnesaemia on the high K diet (period 2) although Mg availability dropped in response to K supplementation (compare experiment 1 and 2 in table 3).

Table 7 shows the Mg intake, apparent availability, and excretion in the urine and the range of the plasma Mg level. No blood was examined during period 4. The lowest urine outputs of Mg corresponded with the lowest Mg availabilities, especially when the intake was low. However, daily urinary Mg outputs of less than 1 g were associated with hypomagnesaemia in only 1 out of 4 experiments.

TABLE 4. Mean concentrations and ranges of minerals in bovine saliva, digesta, faeces, milk and urine, over the 5 periods.

		Parotid saliva per l		Rumen fluid per l		Duodenum 1 per kg		Duodenum 2 per kg		Ileum per kg		Faeces per kg		Milk per kg		Urine per l	
	Mean	148.1	156.1	110.8	107.8	59.7	63.0	67.5	72.0	88.9	87.8	7.4	8.5	22.8	14.5	38.6	49.6
Na meq	Min.	140	151	95	93	50	57	56	70	59	68	5	5	21	14	12	20
	Max.	160	163	127	118	71	72	78	73	109	102	10	10	25	15	88	99
	Mean	8.2	6.3	26.5	31.7	30.7	27.0	31.3	27.1	43.5	40.7	30.2	31.0	40.5	41.9	238.7	252.5
K meq	Min.	8	6	10	13	20	21	22	20	28	28	21	21	40	41	77	137
	Max.	9	7	48	51	39	36	41	33	69	59	47	45	42	43	424	401
	Mean	1.0	.8	8.8	9.8	17.2	16.6	16.4	15.4	50.3	56.9	141.0	126.6	54.7	59.6	2.3	4.4
Ca meq	Min.	.8	.6	7	7	15	13	12	12	35	45	93	89	53	58	.1	.4
	Max.	1.1	.9	13	15	21	19	19	18	75	81	228	181	59	63	5.1	9.7
	Mean	.4	.3	5.4	6.5	6.3	5.4	6.3	5.4	22.3	26.1	60.6	59.3	7.6	9.6	8.7	16.0
Mg meq	Min.	.36	.25	4	4	3	3	3	3	13	17	46	46	7	8	5	5
	Max.	.50	.33	6	9	9	8	8	7	27	43	88	98	8	9	18	40
	Mean	10.5	13.3	12.3	12.8	14.5	12.8	13.5	11.6	16.6	19.9	46.0	42.9	31.1	35.7	2.8	1.3
P. mmol	Min.	9	12	8	10	11	10	10	9	9	14	30	31	29	31	1	0.4
	Max.	12	14	16	15	19	14	14	14	23	29	72	64	34	37	6	2.6

* Mean of 4 experiments.
** Mean of 3 experiments.

TABLE 5. Estimated flow of saliva, intake of DM and the relationship between flow of saliva and DM intake.

	Cow	Experiment 1	2	3	4	5	Mean
Saliva	Z	181	202	146	187	180	179
l per day	W	145	114	130	149	153	138
DM intake	Z	12.1	12.6	9.4	9.4	11.0	10.9
kg per day	W	11.3	11.1	8.8	8.4	9.8	9.9
Saliva flow	Z	15.0	16.0	15.5	19.9	16.3	16.5
l per kg DM intake	W	12.8	10.3	14.7	17.7	15.6	14.1

TABLE 6. Mean daily net absorption or net secretion (–) of mineral substances from different areas of the alimentary canal. The values (g/day) represent the mean of five experiments.

Mean daily net absorption (g)	Zwartschoft Na	K	Ca	Mg	P	Witschoft Na	K	Ca	Mg	P
Total net absorption from gastro intestinal tract	*24.3*	*213.0*	*12.4*	*2.9*	*14.7*	*21.1*	*185.0*	*16.6*	*3.9*	*16.0*
Reticulo-rumen	41.2	28.8	–	–	–	70.8	25.2	–	–	–
Omaso-abomasum	313.1	4.9	–	–	–	179.4	–14.7	–	–	–
Stomachs	354.3	33.7	1.8	3.9	10.3	250.2	10.5	1.4	4.8	21.7
Duodenum*	–65.4	7.7	0.5	0	2.0	–75.5	6.2	0.2	–0.1	4.2
Small intestine*[1]	222.5	151.5	12.2	–0.7	61.0	261.5	157.2	15.8	–0.6	45.4
Large intestine*	124.0	77.4	1.7	0.7	0.9	81.1	56.1	1.3	0.4	1.4

* Values for Witschoft contain an estimate for diet 2.
[1] Excluding duodenum.

TABLE 7. Mg intake, availability, excretion in the urine and plasma levels.

	Zwartschoft Experiment 1	2	3	4	5	Witschoft Experiment 1	2	3	4	5
Intake (g per day)	24.7	24.7	16.3	14.1	13.5	23.0	22.4	18.9	13.8	12.0
Availability %	17	12	25	12	16	31	19	17	26	15
In urine (g per day)	2.3	0.4	3.6	0.8	0.3	4.5	1.2	1.5	2.2	0.2
Range in plasma (mg per 100 ml)	2.4–3.2*	2.2	2.6–2.7	–	0.9–1.4	2.2–2.5	2.2–2.7	2.5–2.7	–	2.0–2.2

* One very high level.

Discussion

The daily saliva secretion was estimated by subtracting the intake of water in food and drink from the daily volume of fluid calculated to leave the reticulo-rumen. The latter was calculated by the rate of dilution of Cr-EDTA in rumen fluid. If net absorption of water from the rumen is negligible (Von Engelhardt, 1963), the estimates of saliva flow are correct. If net water absorption occurs (Warner and Stacy, 1968; Tsuda, 1964), the estimates of saliva flow are conservative. The saliva samples were mainly of parotid origin and probably contained Na and K concentrations which were similar to those of mixed saliva (Dobson et al., 1960). Therefore, the estimated additions of salivary Na and K to the reticulo-rumen and their estimated net absorptions from it are probably correct, but possibly conservative. The general results concerning the net absorption of Ca and Mg from the stomachs would not be influenced appreciably by differences in their concentration in parotid and mixed saliva and/or conservative estimates of saliva flow, because their concentrations in saliva are low.

The data confirm that Na is absorbed along the entire alimentary tract against both a concentration and electrical gradient. On average, between 6–14% of the Na entering the cardia was absorbed from the reticulo-rumen. Very large amounts of Na were absorbed from the omaso-abomasum. The average amounts, 313 and 179 g corresponded with 52 and 27% of the total Na entering the omasum. The abomasum is known to secrete large amounts of fluid containing Na, thus the absorption from the omasum was even greater than our figures indicate. This confirms the great importance of the omasum in the absorption of Na as reported in calves (Yang and Thomas, 1965; Perry et al., 1967) and sheep (Oyaert and Bouckaert, 1961).

The addition of Na to the duodenum was attributed to Na in bile, pancreatic juice and succus entericus. Strong net absorption followed from the small intestine where 223–262 g Na were absorbed i.e. 64–76% of the amount passing the distal duodenum. The importance of the small intestine as a major site of Na absorption has been reported previously for cows (Van Weerden, 1961; Van 't Klooster, 1967) and calves (Smith, 1962 and 1966; Yang and Thomas, 1965; Perry et al., 1967). Na absorption continued in the large intestines, where 124–81 g per day were absorbed. Although the large intestines were not quantitatively as important as the omasum and small intestines in Na absorption, the efficiency of absorption from the large intestines was highest when compared with other areas – only about 5% of the Na which passed the terminal ileum was eventually excreted in the faeces. Na absorption from the large intestine has been reported by Van Weerden (1961) and Van 't Klooster (1967) in cows, Smith (1962) and Perry et al. (1967) in calves, and Goodall and Kay (1965) and Bruce et al., (1966) in sheep.

The data support the finding that K absorption increases with increasing K intakes (Suttle and Field, 1967) and increasing K concentration in digesta (Scott, 1967) and that absorption was down its concentration gradient (Hydén, 1961). Our values indicated that the stomachs were of minor importance in net absorption except on high K intakes (Scott, 1967; Perry et al., 1967; Pou-

TIAINEN, 1968; OYAERT and BOUCKAERT, 1961). The jejunum and ileum absorbed massive amounts of K especially on the high K diets. The overall mean amount absorbed here was about 155 g per day, or about 63% of the total K which passed the distal duodenal cannula. Absorption continued strongly from the caecum and large intestine, as reported previously by VAN WEERDEN (1961) and VAN 'T KLOOSTER (1967) in cows, and PERRY et al. (1967) in calves.

Of the 70 g Ca that entered the cardia daily, only 3 g came from saliva. Even smaller net amounts – about 2 g – were absorbed between the cardia and the distal duodenum. KIMBERG et al. (1961) reported that Ca was actively absorbed from the duodenum of rats. PHILLIPSON and STORRY (1965), however, could detect net absorption neither from the duodenum of sheep nor from the rumen, even when Ca levels in the rumen fluid were increased to six times normal. Some net absorption of Ca from the stomachs and the duodenum was previously reported by one of us (VAN 'T KLOOSTER, 1967) in slaughter experiments with cows.

The jejunum and ileum were the major sites of net Ca absorption in both cows. This confirms the findings of VAN 'T KLOOSTER (1967) in cows, SMITH (1962) CRAGLE et al. (1964), YANG and THOMAS (1965) and PERRY et al. (1967) in calves and PHILLIPSON and STORRY (1965), SCOTT (1965) and VAN 'T KLOOSTER and CARE (1966) in sheep.

Saliva levels of P were given as 0.3–0.4 g per l (EMERY et al., 1960; BAILEY and BALCH, 1961). Our values were similar. The mean daily inflow of saliva P to the rumen was estimated at about 58 g. The results were based on the P levels of mainly parotid saliva which may overestimate the levels in mixed saliva (DOBSON et al., 1960) but unless the overestimate exceeded 25% some absorption of P occurred from the stomachs. CRAGLE et al. (1964) using the slaughter technique in cattle of various ages, reported P absorption from the abomasum. YANG and THOMAS (1965) using slaughtered calves, reported that absorption also occurred from the rumen and omasum. HYDÉN (1961) stated that P absorption from the reticulo-rumen of sheep was negligible. He quoted SCARISBRICK and EWER (1951), SPERBER et al. (1952) and WRIGHT (1955) that ^{32}P exchange between rumen fluid and blood occurred but that net flux was negligible.

Secretion of P into the first part of the small intestine with absorption in the remainder was reported by CRAGLE et al., (1964) and YANG and THOMAS (1965) in calves. The jejunum and ileum were found to be the main sites of P absorption in the present experiments, confirming the findings of VAN 'T KLOOSTER (1967) in cows and BRUCE et al. (1966) in sheep. A small net absorption also occurred from the duodenum.

Most authors agree that absorption of Mg in adult animals occurs mainly from the small intestine, with little or no net absorption from the stomachs or large intestines. Our results show that Mg absorption occurred from the stomachs, with little or no net absorption behind the proximal duodenum. This result was found in each of the 9 experiments in which it could be measured. The possibility of a consistent error in the Mg estimation of the digesta from the proximal and distal duodenum was not overlooked. The analytical technique

for feed, digesta and faeces was standard. Samples were repeatedly analysed after the results of the first experiment had indicated little or no difference between the amounts of Mg passing the proximal duodenum, distal duodenum, ileum and anus. Acid extracts of wet duodenal digesta were also analysed and results agreed well with the values obtained after ashing dried material.

Variations in the concentrations of mineral and indicator in digesta are appreciable and largely independent of each other (VAN 'T KLOOSTER et al., 1969). It is, therefore, very difficult to obtain representative samples in slaughter experiments. The difficulties are increased by very rapid post-mortem changes such as cell shedding, antiperistalsis and dilution of the indicator. Cell shedding could increase the amount of Mg in the beginning of the small intestine. One or a combination of these factors could cause a large over-estimation of the amount of Mg passing through this area. This could partly explain why no net Mg absorption was found from the stomachs in slaughter experiments in calves (PERRY et al., 1967) or cows (VAN 'T KLOOSTER, 1967). The use of ^{28}Mg in slaughter experiments had additional difficulties associated with the isotope i.e. recycling, poor mixing with digesta Mg, differences in physical form of the digesta Mg and the label, etc. Basing his results on this technique, FIELD (1961) reported that Mg absorption occurred from the middle third of the small intestine of sheep.

It is also difficult to explain the discrepancy between our findings and those which were based on perfusion of intestinal loops in-vivo (CARE and VAN 'T KLOOSTER, 1965; PHILLIPSON and STORRY, 1965; SCOTT, 1965; VAN 'T KLOOSTER and CARE, 1966; CARE et al., 1967). These workers concluded that the small intestine was the main site of net Mg absorption but SCOTT (1965) stated that 'carefully defined criteria of normality must be established... before any conclusions (from perfusion of gut loops) may be referred to the whole animal'. While net Mg absorption may occur from some loops of small intestine, it is possible that the net absorption from the whole of the small intestine may be small, due to net secretion or no change occurring in other areas. Net secretion or zero net flux into different sections of the small intestine has been reported frequently (FIELD, 1961; CARE and VAN 'T KLOOSTER, 1965; PHILLIPSON and STORRY, 1965; SCOTT, 1965; VAN 'T KLOOSTER and CARE 1966).

Studies on segmental net absorption of Mg in the fistulated bovine are very rare (SMITH, 1962; VAN 'T KLOOSTER, 1967). SMITH used very young milk-fed calves and found that the large intestine and small intestine, in that order, were the organs of greatest importance for net Mg absorption. In view of the absence of physiological development of the forestomachs in the calves, it is not surprising that their sites of net absorption of Mg should be similar to those in other monogastic animals, such as rats (CHUTKOW, 1963).

In a series of 4 experiments in a fistulated cow, VAN 'T KLOOSTER used Cr_2O_3 and PEG to measure the flow of digesta through the distal duodenum and terminal ileum. His results, based on Cr_2O_3, indicated that of the total net Mg absorption, 1/3 occurred form the stomachs and 2/3 from the small intestine. However, the results based on PEG indicated the reverse i.e. 2/3 from the sto-

machs and 1/3 from the small intestines. The latter results would support our present conclusion that the stomachs of the cow may be of major importance for net Mg absorption.

Net Mg absorption from the rumen appears to be negligible except at very high Mg concentrations in rumen fluid (STEWART and MOODIE, 1956; CARE and VAN 'T KLOOSTER, 1965; PHILLIPSON and STORRY, 1965). Ionization of Mg is maximal in the abomasum (STORRY, 1961b; VAN 'T KLOOSTER, 1967) and would appear to favour absorption by simple diffusion of ions against low potential differences (PD). However, CARE and VAN 'T KLOOSTER (1965) found no net absorption from the abomasum or duodenum of sheep. It is felt that little net absorption of Mg would occur from an organ so well designed for secretion. Of the remaining 2 compartments of the stomachs the omasum is the more likely site of absorption. Its ability to absorb large amounts of Na and H_2O (OYAERT and BOUCKAERT, 1961; YANG and THOMAS, 1965; PERRY et al., 1967), VFA (YANG and THOMAS, 1965; BOYNE et al., 1966), CO_2 (OYAERT and BOUCKAERT, 1961) and NH_3 (BOYNE et al., 1956; OYAERT and BOUCKAERT, 1961) is already demonstrated. It is now suggested that the omasum may be an organ of great importance in Mg absorption.

In these experiments the total Mg level in the fluid leaving the reticulo-rumen was 5–7 meq per l. Let us assume that the normal concentration of Mg ions in plasma is 1.2 meq per l and that the PD's across the omasal wall are similar to those across the rumen wall, viz. 17–32 mV (VAN 'T KLOOSTER and ROGERS page 3). For absorption to occur by passive diffusion of Mg ions through the omasal wall under these circumstances the concentration of Mg ions in omasal fluid would have to be 4.3–13.2 meq per l. Part of the Mg in rumen fluid is non-ionic. It would appear, therefore, that absorption of Mg from the omasum would involve a process not relying on simple diffusion of free ions (STORRY, 1961b) unless water absorption from the omasum was so strong that the concentration of Mg ions in the fluid leaving the reticulo-rumen increased two times or more.

SUMMARY

The sites of net mineral absorption from the digestive tract were studied in 2 fistulated lactating cows by using the inert reference substances Cr_2O_3, PEG and Cr-EDTA.

The reticulo-rumen absorbed appreciable net amounts of Na and K. The omaso-abomasum was very important in net Na absorption but not for K absorption. As a unit the stomachs were of minor importance in net K and Ca absorption. They absorbed at least some P and were the site of major importance for Mg absorption.

Negligible net absorption of K, Ca and P occurred from the duodenum. The net secretion of Na into this segment was ascribed to bile, pancreatic juice and juice of the Brunner's glands. The amount of Mg in the gut lumen was unchanged after passage through the duodenum.

The small intestine was the major site of K, Ca and P absorption and a

major site of Na absorption. Net Mg absorption did not occur from this segment.

The large intestine absorbed appreciable amounts of Na and K. Little or no net absorption of Ca, Mg or P occurred from this segment.

Net Mg absorption occurred mainly from the stomachs. Little or no net absorption occurred between the pylorus and the anus. The discrepancy between these findings and those of other workers is discussed. The ability of the omasum to absorb large amounts of other nutrients suggests that it may be of great importance for net Mg absorption also.

Acknowledgements

We gratefully acknowledge the encouragement and surgical assistance of Prof. A. M. Frens. We thank Mr. ir. H. A. Boekholt for co-operation in the digesta sampling and the pH and PD measurements, Messrs. G. Bangma, R. Terluin and J. Vogt for technical assistance and Mr. J. A. van Dijk and Miss H. G. Stienstra for analytical assistance.

One of the authors (P. R.) wishes to thank the Agricultural Institute of Ireland for permission and financial assistance to work abroad and the Netherlands Government and the International Agricultural Centre, Wageningen, for a grant during the final ten weeks.

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