

# Hypomagnesaemia in milking cows: intake and utilization of magnesium from herbage by lactating cows

A. KEMP, W. B. DEIJS, O. J. HEMKES

Institute for Biological and Chemical Research on Field Crops and Herbage, Wageningen, Netherlands

A. J. H. VAN ES

Laboratory of Animal Physiology, Agricultural University, Wageningen

---

## Summary

Magnesium metabolism trials have been carried out with milking cows on winter rations and on rations consisting of freshly cut herbage from pastures with different fertilizer treatments. Data of 39 cows are available in total (*table 1 and 2*).

The mean daily magnesium intake of the cows on herbage was 16,62 g/day. Differences in magnesium intake were mainly caused by variations in magnesium content of the herbage while differences in palatability also played a part. The intake of magnesium in the winter rations was considerably higher than in rations consisting of herbage.

On an average 83 % of the magnesium ingested was excreted in the faeces within a range of 67 % to 93 %. Consequently, the mean "availability" of herbage magnesium in these trials was 17 % within a range of 7 % to 33 %. The excretion of magnesium in milk was 0,12 g Mg per kg of milk and the urinary excretion of magnesium fluctuated from 0,40 g Mg to 4,24 g Mg/day with a mean value of 1,77 g Mg/day.

A close correlation existed between the serum magnesium levels and the excretion of magnesium in urine. Hypomagnesaemia occurred only when the daily excretion of magnesium in urine was lower than 1 g Mg (*fig. 1*). Since daily excretion of creatinine in urine is relatively constant, daily urinary excretion of magnesium may be approximately computed by means of determining the concentration of creatinine and magnesium in urine samples. This technique offers possibilities in grazing experiments.

The excretion of magnesium in urine was closely correlated with the amount of "available" magnesium above the magnesium secreted in milk. Hypomagnesaemia occurred only when the amount of "available" magnesium above the amount secreted in milk was lower than 1 g Mg/day. The level of the daily excretion of magnesium in urine appeared to be an even better measure for the magnesium status of the animal than the magnesium concentrations in the blood-serum since a decrease of magnesium excretion in the urine preceded a fall in the serum magnesium levels (*fig. 2*).

The magnesium retentions were limited and any excess of the amount necessary for maintenance, secretion of milk and retention was mainly excreted in the urine. The retention was zero when the amount of "available" magnesium above the amount secreted in milk was about 2,5 g Mg/day (*fig. 2*).

Consequently the daily requirement of "available" magnesium of a milking cow appeared to be about 2,5 g plus 0,12 g Mg for each kg of milk.

The "availability" of herbage magnesium increased as the herbage matured. In three experiments carried out with herbage from the same pasture, but cut in different stages of growth with crude protein contents of 26 %, 18 % and 14 %, the figures for "availability" of magnesium were 10 %<sub>0</sub>, 16 %<sub>0</sub> and 20 %<sub>0</sub> respectively (*table 3*). Moreover, the data suggest that heavy applications of nitrogen and potassium on pasture have an unfavourable influence on the "availability" of herbage magnesium.

A higher intake of potassium in the form of KCl was associated with a lower excretion of magnesium in urine and with a fall in the serum magnesium levels.

The rate of mobilisation of stored bone magnesium to prevent hypomagnesaemia seems to be insufficient in mature cows. The experiments reported here suggest that hypomagnesaemia in cows arises as a result of a shortage of magnesium due to the reduction in the dietary supply of "available" magnesium.

---

## 1. Introduction

As long as 30 years ago SJOLLEMA (1931) found that a fall in the magnesium concentrations in blood-serum was always attendant on the incidence of hypomagnesaemic tetany in milking cows. According to these results clinical symptoms of hypomagnesaemic tetany occurred only when the serum magnesium levels fell below 1,0 mg/100 ml.

By approaching the problem from this point of view hypomagnesaemic tetany does not occur when the serum magnesium levels remain within the normal range. Therefore, generally the magnesium concentration of the blood-serum has been chosen as the starting point of experiments. This is still more evident now, since subsequent experiments have shown that if, for instance, two cows of one herd are suffering from hypomagnesaemic tetany the serum magnesium concentrations of nearly all the other cows of the herd are low or very low (KEMP, 1958). In the Netherlands it is estimated that about 1 to 2 % of the milking cows is suffering from hypomagnesaemic tetany every year and we assume that in spring and in autumn when the frequency of tetany is highest about 30 to 50 % of the cows show low serum magnesium values. What harmful effect this might have is unknown but according to DE GROOT (1960) the possibility exists that it is important for the longevity of dairy cattle, because prolonged hypomagnesaemia should have a harmful influence on the function of the heart.

In literature no common opinion can be found as to the influence of the magnesium intake by the cows on the incidence of hypomagnesaemia. It is commonly held that hypomagnesaemia occurs on pastures with high magnesium contents of the herbage as well as on pastures low in magnesium, although there have been only few systematic investigations in this field. Recently, results of grazing experiments with lactating cows in different countries have shown that fertilizer treatment of pasture may have a striking effect on the serum magnesium levels (BARTLETT *et al*, 1954; KEMP, 1958; SMYTH *et al*, 1958 and HVIDSTEN *et al*, 1959). In all these experiments hypomagnesaemia and hypomagnesaemic tetany could be induced. Moreover, several experiments have shown that an oral administration of magnesium considerably reduces the incidence of hypomagnesaemic tetany in cattle and it has also been possible to maintain the serum magnesium levels within the normal range by high applications of magnesium on pasture (ALLCROFT, 1953; BARTLETT *et al*, 1954; PARR and ALLCROFT, 1957; SMYTH *et al*, 1958; SEEKLES and BOOGAERDT, 1956).

In a previous publication (KEMP, 1960) information has been given about the chemical composition of the herbage and the incidence of hypomagnesaemia. A significant positive correlation has been found between the magnesium contents of the herbage in the week preceding blood sampling and the serum magnesium levels. In 822 cows, 23 of which were suffering from hypomagnesaemic tetany with serum magnesium levels lower than 1,0 mg Mg/100 ml, hypomagnesaemia did not occur when

the magnesium contents of the herbage were higher than 0,20 % of Mg in the dry matter. In the light of these observations, the present paper describes results of metabolism experiments with milking cows which have been carried out in 1958, 1959 and 1960. A part of this work has already been reported (KEMP *et al*, 1961).

## 2. Experiments

In the years mentioned above 11 balance trials have been carried out with lactating cows which were fed indoors on a winter ration in one experiment and on freshly cut herbage in the other 10 experiments. In most of the trials four Friesian cows have been used which had been selected from the Friesian cattle in the Institute herd. The EXPERIMENTS 9, 10 and 11 have been carried out with 3, 2 and 2 cows respectively, consequently data of 39 cows are available in total. The major details of the experimental plan are given in TABLE 1. In the present experiments not only the magnesium balances were established but also data on other constituents were collected. However, this paper is mainly dealing with magnesium.

### *Experimental routine and sampling*

The herbage was mown in the morning, transferred to the digestion stall and mixed. As soon as its approximate dry-matter content was known, ordinarily within a few hours, the rations for one day (on Saturday for two days) were weighed out in such a way that the animals were given a constant amount of dry matter daily. These rations were computed according to feeding standards used in the Netherlands (C.V.B. 1956). When the bags were filled with the rations many small quantities of herbage were taken very carefully to obtain two representative herbage samples. The herbage of each of these samples was cut into pieces of 2—3 cm's length. From this material subsamples were taken after mixing in an amount equal to 0,5 % of the weight of the day ration and put into one of two sampling bottles. The subsamples of succeeding days were put into the same bottles. In this manner two composite samples were obtained from herbage fed during the experimental period. The samples were stored at about  $-20^{\circ}$  C.

The cows used in the experiments were in good condition and had not suffered from metabolic disorders in the preceding months. Usually two groups of two animals each with normal serum magnesium levels were composed in such a way that in both groups milk production, body weight, age and month of lactation were almost the same.

The animals were brought into the digestion stall some days prior to the preliminary period and in a few days they were accustomed to the experimental ration. One week before the beginning of the experimental period the animals were equipped with a device for the separate collection of faeces and urine, (VAN ES and VOGT, 1959), including urinal and harness. If no urinal was used, a separate collection was obtained by catheterizing the urine by means of a balloon catheter similar to the one used by CUNNINGHAM, *c.s.* (1955). This catheter was applied a few days prior to the experimental period.

In general the preliminary period lasted about 7 days, the experimental period 6 to 10 days. We prefer experimental periods of 8 to 10 days but not enough herbage was available on the experimental pastures during the EXPERIMENTS 4, 5 and 6. As it is preferred to have herbage at nearly the same stage of growth throughout the experiment, longer periods were not possible.

TABLE 1. Details of the experiments

Experiment	Cow Nr. and name	Duration of the periods		Ration	Intake of dry matter kg/day	Mean daily milk yield kg
		Prel. period	Exp. period			
1 (1958)	1 Klaske 12	14 days	7 days	<i>Winter ration</i> hay silage fodderbeets concentrates	14,16	18,44
	2 Rika 25	20. IV to 3. V	3. V to 10. V		14,91	24,71
	3 Janke 113				15,79	17,85
	4 Joh. Wipkje				15,38	18,48
2 (1958)	1	—	10 10. V to 20. V	<i>Herbage</i> crude protein <sup>4</sup> K <sup>1</sup> 22 3,7 k 22 2,2 k 22 2,2 K 22 3,7	10,49	14,97
	2 } the same				12,30	20,68
	3 } as in Exp. 1				12,54	16,97
	4 }				12,08	16,93
3 (1958) Same pasture as in Exp. 2	1	—	8 20. V to 28. V	k 20 2,0 K 20 3,7 K 20 3,7 k 20 2,0	10,85	14,83
	2 } the same				10,71	17,86
	3 } as in Exp. 1				12,19	16,45
	4 }				11,86	16,39
4 (1959)	1 IJbeltje 14	2	6	K 17 2,6 k 17 1,8 k 17 1,8 K 17 2,6	12,13	17,55
	2 Dientje J. M.	22. IV to 24. IV	24. IV to 30. IV		10,90	14,44
	3 Uret Joh. 19				11,90	17,66
	4 Johanna 4				9,30	12,81
5 (1959)	1	2	6	17 3,3 17 3,3 17 3,3 17 3,3	13,27	17,85
	2 } the same	30. IV to 2. V	2. V to 8. V		11,73	14,55
	3 } as in Exp. 4				12,77	18,79
	4 }				11,37	14,39
6 (1959) Same pasture as in Exp. 4	1	2	6	k 22 2,0 K 22 3,2 K 22 3,2 k 22 2,0	12,81	15,81
	2 } the same	8. V to 10. V	10. V to 16. V		11,22	14,32
	3 } as in Exp. 4				12,24	17,72
	4 }				10,23	15,15
7 (1959)	1 Klaske 12	7	10	N <sup>2</sup> 18 1,6 n 12 1,7 n 12 1,7 N 18 1,6	9,65	9,48
	2 Uret Joh. 19	14. IX to 21. IX	21. IX to 1. X		10,10	11,03
	3 Janna 3				8,00	6,20
	4 Uret Joh. 17				9,08	6,98
8 (1959)	1 Benedictus	7	10	KC1 <sup>3</sup> 13 1,6 KC1 13 1,6 13 1,6 13 1,6	10,46	9,76
	2 Dientje J. M.	5. X to 12. X	12. X to 22. X		9,38	5,07
	3 Janke 113				9,31	6,83
	4 Ymkje 10				11,52	8,99
9 (1960)	1 Dientje J. M.	10	10	26 4,1 26 3,0 26 3,0	12,58	18,60
	3 Sjoerdje 29 de L.	4. IV to 12. IV	12. IV to 22. IV		9,25	13,78
	4 Kl. Anke A.E.V.				11,69	20,65
10 (1960)	1 Sjoerdje 23 de L.	3	8	18 3,4 18 3,4	10,71	13,99
	2 Kl. Anke A.E.V.	23. IV to 26. IV	26. IV to 3. V		13,08	21,60
11 (1960)	1 } the same	—	9 3. V to 12. V	14 3,1 14 3,1	10,88	14,27
	2 } as in Exp. 10				13,33	20,36

1 K heavily dressed with potassium; k slightly dressed with potassium.

2 N heavily dressed with nitrogen; n slightly dressed with nitrogen.

3 Cow 1 and 2: KC1, 400 g/day in the form of pellets.

4 % in dry matter.

The starch equivalent of the dry matter of herbage was computed according to the principles of BROUWER and DIJKSTRA (see DIJKSTRA, 1954). We determined the crude fibre and ash content of the dry matter of herbage in the beginning of the preliminary period and computed the starch equivalent of the herbage. To account for the decrease in feeding value in the course of the experiment, as soon as the starch equivalent was known, a value slightly lower than this, but always the same was used to compute the rations.

During the experimental period, the milk, urine and faeces of every 24 hours were collected, weighed, mixed and sampled.

The day-samples were put into composite sampling bottles in amounts equal to a constant percentage of the total quantities of milk, urine and faeces. In most experiments the faeces were sampled in duplicate. The composite sampling bottles of milk and of faeces contained some formalin as a preservative, those of the urine some HCl. The composite samples of the faeces were stored at about  $-20^{\circ}$  C, those of milk and urine at about  $+3^{\circ}$  C. The first half of the day-ration of the first herbage was given the first day of the experimental period at 16,00 h, the second half the next day at 6,00 h. The collection of milk, urine and faeces started the second day at 6,00; 8,00 and 8,00 h respectively.

Residues of the feed, if considerable, were collected at the end of each 24 hours, if small at greater intervals. These residues were weighed, chopped and mixed and from the mixed material a constant percentage was put into another composite sampling bottle. A daily record was also kept of the water consumption.

Prior to the chemical analysis the samples of feed, feed residues and faeces were dried at  $75^{\circ}$  C. The contents of dry matter and crude protein of the faeces, however, were determined in the original sample.

Blood samples were taken twice a week from the jugular vein throughout the preliminary and experimental period.

### *Methods of analysis*

#### a. The determination of magnesium in herbage and faeces<sup>1</sup>

##### Reagents

Standard magnesium solution.

Dissolve 8,559 g of  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$  (= 1,4 g of MgO) in 1 l of distilled water. Diluting 50 ml of this solution to 1 l gives a standard solution containing 0,07 mg of MgO/ml.

##### Solution A

1 g of polyviol VO3/20 (BRUGGER, 1958), 1 g of carbocel Z.L.C.F. and 600 ml of water are mixed. The mixture is put aside until the next day and then heated up to  $70^{\circ}$  C until the solution is clear.

272 mg of  $\text{CaCO}_3$  is dissolved in an amount of hydrochloric acid just sufficient in a one litre volumetric flask. Add the above mentioned solution of polyviol-carbocel and make up to the mark with water.

##### Solution B

200 mg of Titan yellow is dissolved in nearly 1 l of water. 2 ml of the standard magnesium solution is added (1,4 mg of MgO/ml) and then it is diluted to 1 l.

<sup>1</sup> This method has been developed in collaboration with *W. R. Domingo* (Laboratory for Soil and Crop Testing, "Mariëndaal", Oosterbeek).

### Solution C

50 g of NaOH is dissolved in about 900 ml of water. 5 ml of a solution containing 861 mg of  $\text{Fe}_2(\text{SO}_4)_3 \cdot (\text{NH}_4)_2 \text{SO}_4 \cdot 24 \text{H}_2\text{O}$  in 100 ml of water is added. During this addition the solution has to be turned round continuously to prevent precipitation. After cooling dilute to 1 l and mix intensively.

Remark: The solutions A and B should be prepared one day before use, the solution C immediately before use.

### Procedure

A quantity of dried and ground herbage or faeces containing 1,0—5,0 mg of MgO (in most cases 1,5 g of dried herbage or 0,5 g of dried faeces) is ashed at 500° C. The ash is mixed with 5 drops of hydrochloric acid (25 %) and evaporated on a waterbath until dryness. After the addition of one drop of hydrochloric acid (25 %) the ash is dissolved in 100 ml of water in a volumetric flask. The solution is filtered through Mg-free filter-paper.

5 ml of the filtrate is pipetted into a 100 ml Erlenmeyer-flask, provided with a small iron rod in a plastic wrap. Subsequently 25 ml of solution A and 10 ml of solution B are added. By means of an automatic pipet 10 ml of solution C is added in about 17 seconds. At the same time the mixture is stirred magnetically.

The optical density is determined in a Beckman DU spectrofotometer at 538 m light-path 1 cm.

In the same way a calibration curve is determined starting with several quantities of the diluted standard solution (0,5—5,0 ml), made up to 5 ml with water.

Normal quantities of iron and aluminium do not disturb the reaction. In order to avoid the unfavourable effect of high contents of iron and/or aluminium, for instance caused by soil-contaminations; these elements are precipitated from the ash solution with ammonium hydroxide after the addition of ammonium chloride.

#### b. The determination of magnesium in urine

After adding 1 drop of hydrochloric acid (25 %) 20 ml of urine is evaporated in a porcelain crucible on a water-bath, dried at 100° C and heated over a flame until the beginning of destruction. The residu is ashed at 500° C. The determination of magnesium in the ash is carried out in the same way as under a.

#### c. The determination of magnesium in milk

After adding 1 ml of acetic acid (50 %) 15 ml of milk is treated in the same way as under b.

#### d. The determination of magnesium in blood-serum

The determinations are carried out according to the method of ORANGE and RHEIN (1951), starting with 2 ml of blood-serum.

As to the accuracy of the determination of magnesium from the magnesium contents in 2 separately treated samples of herbage and faeces, the coefficients of variation of the figures of the magnesium intake and the magnesium in faeces have been estimated at about 2 percent. Those of the figures of magnesium in milk or in urine were much lower. Therefore we assumed that the standard deviations of the figures for "availability" and for retention were about 3 units and 1 gram Mg respectively.

*Details of the rations fed*

Some details of chemical composition and previous fertilizer treatment of the swards used to provide herbage, and of the daily dry matter intake of the individual cows are given in TABLE 1.

In EXP. 1 all cows were fed on the same rations consisting of 5 kg of hay, 15 kg of silage, 20 kg of fodderbeets and 6 kg of concentrates. After finishing this experiment the experimental period of EXP. 2 commenced immediately without a preliminary period. Cow 1 and 4 were fed on freshly mown herbage heavily dressed with potassium and the rations of cow 2 and 3 consisted of herbage considerably lower in potassium. EXP. 3 started also immediately after the end of EXP. 2. In this experiment cow 2 and 3 consumed herbage high in potassium and cow 1 and 4 were fed on herbage low in potassium. In EXP. 1, 2 and 3 the same cows were used and at the end of the experimental period of EXP. 3, cow 2 showed slight symptoms of hypomagnesaemic tetany. The mean serum magnesium concentration of this cow in this period was 0,60 mg Mg/100 ml. In EXP. 2 cow 1 showed low serum magnesium levels which values recovered slightly during EXP. 3.

In each of the EXP. 4, 5 and 6 the same animals were used. In EXP. 4 the cows 1 and 4 were fed on herbage which was higher in potassium than the herbage which was given to the cows 2 and 3. After this experiment EXP. 5 commenced in which all cows received the same herbage from another pasture. EXP. 6 started immediately after EXP. 5 and cow 1 and 4 were fed on herbage with a lower potassium content than the herbage offered to cow 2 and 3. The short duration of the preliminary and the experimental periods of these trials was due to a shortage of herbage available on the experimental pasture. Cow 1 showed low serum magnesium levels in EXP. 6.

During EXP. 7 cow 1 and 4 were fed on herbage with a higher crude protein content than cow 2 and 3 (see TABLE 1). The herbage fed in this experiment was mown from the same pasture divided into two plots with low and high applications of nitro-chalk.

In EXP. 8 all the cows were fed on the same herbage. However, in the beginning of the preliminary period there was started in dosing cow 1 and 2 with 200 gram of KCl per head per day. This KCl was given in the form of pellets which were administered by means of a dosing gun. After three days the amount of KCl was increased to 400 g/day which dosage lasted until the end of the experimental period. KCl was given four times a day, i.e. at 6,45 h, 10,30 h, 14,30 h and 18,00 h.

In the EXP. 9, 10 and 11 herbage was given to the cows from the same pasture but in different stages of growth. The crude protein contents of the herbage fed in EXP. 9, 10 and 11 were 26 %, 18 % and 14 % respectively (see TABLE 1). The cows 1 and 2 in EXP. 10 and 11 were the same ones as cow 3 and 4 respectively in EXP. 9. Consequently it was possible in these trials to study the utilization of herbage magnesium in the same cows fed on rations consisting of herbage in different stages of growth.

### 3. Results

The results of the metabolism experiments carried out with 39 milking cows are given in TABLE 2. The values reported are daily means of the experimental periods.

With the 35 cows receiving only cut herbage, the intake of magnesium ranged from 10,3 g/day to 22,4 g/day, with the 4 cows receiving winter rations these values were 31,8 g/day to 34,4 g/day. The mean values were 16,62 g/day and 33,08 g/day

TABLE 2. The intake, excretion in faeces and urine, secretion in milk and retention of magnesium in lactating cows fed indoors on winter rations or freshly cut herbage

Experiment Nr.	Animal	Magnesium intake g/day <sup>1</sup> A	Magnesium in dry matter % A	Magnesium in faeces g/day B	Magnesium in urine g/day D	Magnesium in milk g/day D	Total excretion g/day	Retention g/day	"Availability" of Mg (% of inges- ted Mg not ex- creted in faeces) %	"Available" Mg above the quantity secreted in milk g/day A-B-D	Mean serum magnesium values mg/100 ml
1 Winter ration	1	13.81	0.212	25.83	3.14	1.88	30.85	0.96	19	4.10	2.15
	2	32.78		26.77	2.22	2.59	31.58	1.20	18	3.42	2.45
	3	34.13		27.86	5.39	1.82	35.07	-0.94	18	4.45	2.20
	4	33.59		25.88	4.24	1.99	32.11	1.48	23	5.72	2.35
2 Herbage	1 K	14.30	0.135	12.68	0.85	1.80	15.33	-1.03	11	-0.18	1.05
	2 k	20.52	0.162	16.62	1.34	2.58	20.54	-0.02	19	1.32	2.20
	3 k	20.58	0.162	16.57	2.00	2.05	20.62	-0.04	19	1.96	2.60
	4 K	16.66	0.135	14.12	1.42	2.13	17.67	-1.01	15	0.41	2.20
3	1 k	17.70	0.164	15.49	0.45	1.83	17.77	-0.07	12	0.38	1.35
	2 K	15.03	0.139	12.90	1.19	2.18	16.27	-1.24	14	-0.05	0.602
	3 K	16.90	0.139	13.80	1.57	2.14	17.51	-0.61	18	0.56	2.20
	4 k	19.57	0.164	15.15	2.10	2.27	19.52	0.05	23	2.15	2.10
4	1 K	13.33	0.108	11.55	1.01	2.13	14.69	-1.36	13	-0.35	2.35
	2 k	13.81	0.123	11.96	0.54	2.06	14.56	-0.75	13	-0.21	2.25
	3 k	14.99	0.123	11.86	2.00	2.21	16.07	-1.08	21	0.92	2.40
	4 K	10.33	0.108	8.79	0.64	2.04	11.47	-1.14	15	-0.50	2.00
5	1	18.06	0.136	16.63	0.52	2.12	19.27	-1.21	8	-0.69	1.80
	2	15.94	0.136	13.19	1.03	2.11	16.33	-0.39	17	0.64	2.75
	3	17.37	0.136	14.31	2.01	2.39	18.71	-1.34	18	0.67	2.65
	4	15.44	0.136	13.78	0.53	2.17	16.48	-1.04	11	-0.51	2.30
6	1 k	17.68	0.135	16.46	0.81	1.75	19.02	-1.34	7	-0.53	1.25
	2 K	13.83	0.122	11.15	1.73	2.04	14.92	-1.09	19	0.64	2.65
	3 K	15.10	0.122	10.05	3.34	2.21	15.60	-0.50	33	2.84	2.45
	4 k	14.13	0.135	11.35	1.59	2.20	15.14	-1.01	20	0.58	2.15
7	20.48	0.210	15.90	3.17	0.93	20.00	0.48	22	3.65	2.50	
	2 n	19.21	0.188	14.31	3.59	1.09	18.99	0.22	26	3.81	2.50
	3 n	15.26	0.188	12.41	2.20	0.78	15.39	-0.13	19	2.07	2.40
	4 N	19.25	0.210	15.86	1.65	0.77	18.28	0.97	18	2.62	2.20
8	1 KCI	20.39	0.192	16.64	3.15	0.95	20.74	-0.35	18	2.80	2.10
	2 KCI	18.34	0.192	15.80	2.26	0.62	18.68	-0.34	14	1.92	2.60
	3	18.12	0.192	14.35	3.12	0.87	18.34	-0.22	21	2.90	2.40
	4	22.43	0.191	18.73	3.04	0.96	22.73	-0.39	16	2.74	2.10
9	1 K	18.57	0.147	16.45	0.52	2.43	19.40	-0.83	11	-0.31	1.20
	3 k	14.04	0.152	12.71	0.48	1.91	15.10	-1.06	9	-0.53	2.20
	4 k	17.70	0.152	15.84	0.65	2.38	18.87	-1.17	11	-0.52	2.00
	1	13.29	0.124	11.53	0.40	1.86	13.79	-0.50	13	-0.10	1.90
10	2	16.31	0.124	13.45	0.98	2.13	16.56	-0.25	18	0.73	2.10
	1	12.9	0.112	10.00	0.69	1.71	12.40	-0.11	19	0.58	2.10
	2	15.16	0.112	11.97	1.26	2.05	15.28	-0.12	21	1.14	2.20

1 magnesium in drinking water included.

2 with slight clinical symptoms of hypomagnesaemic tetany.

respectively. Data of BROUWER and BRANDSMA (1953) showed a higher intake of magnesium in winter rations compared with rations only consisting of herbage which is in agreement with the data reported here. This higher intake of magnesium in winter rations has also been reported by ROOK (1958). As to the magnesium contents of the herbage offered to the animals, these values ranged from 0,108 per cent to 0,210 per cent in dry matter which concentrations are quite normal for conditions in our country. The herbage fed in EXP. 2, 3, 4, 6 and 9 shows the reducing influence of heavy potassium applications on pasture on the magnesium contents of the herbage. On an average the magnesium concentration of the herbage from the high potassium plots is about 14 per cent lower than the contents of the herbage low in potassium. These data emphasize the unfavourable influence of heavy potash applications on pasture on the intake of herbage magnesium by cows.

The greater part of the ingested magnesium is excreted in the faeces, namely 83 per cent as a mean of all values within a range of 67 per cent to 93 per cent. ROOK and BALCH (1958) found in metabolism experiments with herbage a range of these values of 73 per cent to 95 per cent and mean values of 82 per cent, 83 per cent and 82 per cent in three experiments respectively. There is a striking conformity between these results. According to the results of ROOK and BALCH (1958) as well as according to the data presented in this paper, there is a considerable variation in the proportions of the feed magnesium excreted in the faeces and therefore also in the figures for "availability", which is the percentage of ingested magnesium not excreted in the faeces. In the one experiment with a winter ration this "availability" was not found to be significantly higher than in rations consisting of freshly cut herbage. The mean "availability" of magnesium in all the individual cows was 17 per cent within a range of 7 per cent to 26 per cent with one exception of 33 per cent. The proportion of the feed magnesium not excreted in the faeces is not related with the amount of magnesium ingested.

Compared to the excretion of magnesium in faeces, the excretion of magnesium in urine is much lower. This urinary magnesium excretion is lower according to the magnesium concentration in the blood-serum being lower. SJOLLEMA (1932) already reported very low levels of urinary magnesium in cattle at pasture which were suffering from hypomagnesaemic tetany. In the data presented here the mean excretion of magnesium in urine was 1,77 g/day within a range of 0,40 g/day to 4,24 g/day. There are two exceptions, namely the excretions of magnesium of the cows 3 and 2 in the EXP. 1 and 3 respectively. It is possible that these high values, viz. 5,39 g/day and 1,19 g/day are due to the contamination of urine with faeces or an incorrect determination of magnesium. The fluctuations in the excretion of magnesium in urine were mainly caused by variations in the concentration of magnesium in the urine. However, the urine production may also play a part. For instance, the daily urine production may become half as much again or more if the amount of feed potassium is increased considerably.

The amount of magnesium secreted in the milk, on the contrary, is mainly dependent on the daily quantity of milk produced because the concentrations of magnesium in the milk of individual cows were within a relatively small range. The daily secretion of magnesium for all 39 cows was 1,77 g Mg per cow per day and the mean concentration was 0,12 g Mg per kg of milk. The lowest secretions of magnesium have been found in EXP. 7 and EXP. 8. The cows used in these experiments were at the latter half of their lactation and the daily milk yield was lower than that of the cows in the other experiments (TABLE 1).

The body retentions of magnesium in the individual cows fluctuated from  $-1,4$  g/day to  $+1,5$  g/day with a mean value of  $-0,5$  g/day. The lowest retentions were associated with the lowest magnesium intake and the lowest "availability" of the magnesium ingested. In the 5 cases in which cows were clearly suffering from hypomagnesaemia the magnesium balances were negative, although all negative magnesium balances reported here were not associated with hypomagnesaemia.

The serum magnesium levels of the experimental cows were mainly within the normal range, however there were some exceptions.

Hypomagnesaemia was found in cow 1 in EXP. 2 and 3 (herbage) while the serum magnesium concentrations of this cow remained within the normal range in EXP. 1 (winter ration). Cow 2 showed low serum magnesium levels and slight symptoms of hypomagnesaemic tetany in EXP. 3 (herbage high in potassium) while serum magnesium remained normal in EXP. 1 and 2 on winter ration and herbage low in potassium respectively. Hypomagnesaemia occurred also in cow 1 in EXP. 6 and in cow 1 in EXP. 9.

According to the afore mentioned there was a wide variation in urinary excretion of magnesium and the lowest amounts of magnesium excreted in the urine were associated with the lowest serum magnesium levels.

FIG. 1. Correlation between the daily amount of magnesium excreted in urine and the magnesium concentration in blood-serum

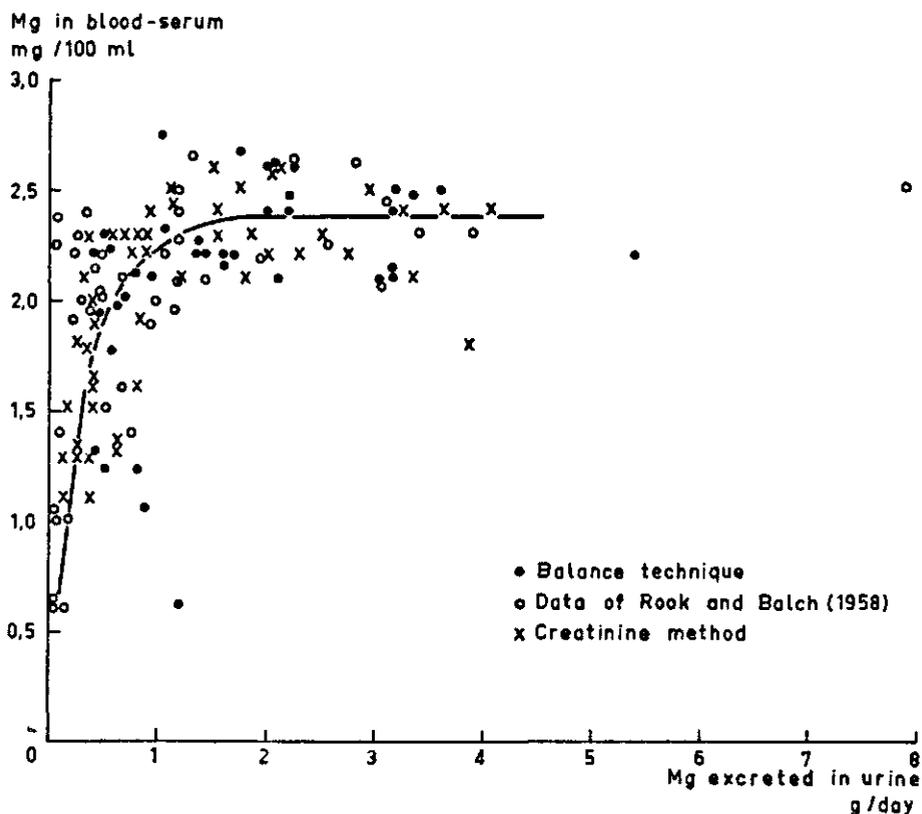


Fig. 1 shows this relationship very clearly. This figure has been composed with data of three sources. Firstly, data have been used on the metabolism experiments reported in table 2 and, secondly, the data of ROOK and BALCH (1958). Also data have been obtained from an experiment in which samples of blood and urine have been collected from a number of lactating cows of the "Droevendaal" experimental farm at Wageningen. One week before the cows were put to a pasture heavily dressed with liquid manure, a sample of blood and urine was taken from each cow; samples were also collected from the same cows a week after this data. These samples were analysed for magnesium. Also, in those of the urine the concentration of creatinine was determined. Since the daily amount of creatinine excreted in urine of dairy cows is relatively constant (DE GROOT and AAFJES, 1960) the daily excretion of magnesium could be computed approximately by means of the concentration of magnesium in the urine. Firstly, it is notable that the data of the different experiments were about localised within the same range; accordingly, there were no significant differences between these 3 groups of data. Hypomagnesaemia occurred only when the excretion of magnesium in urine was below 1 g Mg per day. In this respect it should be considered that a slight contamination of the urine with faeces containing per kg about 10 times as much magnesium as urine may play a part in some of the values of magnesium in urine. For instance, the lowest serum magnesium level in this figure viz. 0,6 mg/100 ml, was associated with an unreliable high magnesium excretion in the urine in consequence of contamination with faeces. Some of the other values may also be slightly too high, therefore the amount of 1 g Mg per day mentioned above may be lower. With excretions of magnesium in the urine higher than 1 g/day all serum magnesium levels were within the normal range.

#### 4. Discussion

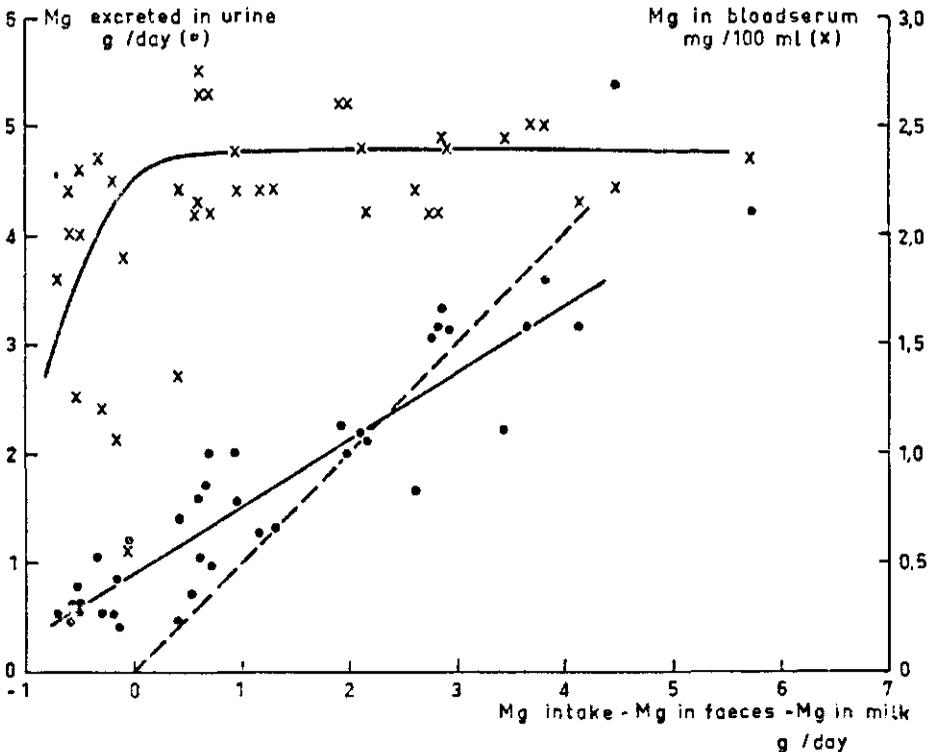
That part of the magnesium ingested which enters the blood may be used for maintenance, for production of milk and for retention. Any excess above the amount required for these purposes may be removed by means of the kidney. A considerable part of the magnesium secreted from the blood into the digestive tract (with saliva, gastric juice, bile and other fluids) is reabsorbed. According to FIELD (1960) the reabsorption amounts to about 75 %. For a mature cow the total volume of these secretions is about 200 l a day. Their magnesium contents average 1,0 to 0,5 mg/100 ml, therefore the amount of endogenous magnesium in the faeces may be estimated at  $200 \times 1,0$  to  $0,5 \text{ mg/100 ml} \times 25 \text{ per cent} = 0,5$  to  $0,25 \text{ g Mg per day}$ . The bile contributes only a small part of this amount, as the daily amount of bile produced is small (25 l/day according to MAYNARD, 1947) compared to the amounts of the other secretions and because the magnesium content of bile is not very high according to FIELD (1960). Even if the magnesium of bile should be less readily reabsorbed which might be possible since a part of the magnesium in bile may be considered to be excreted rather than secreted, the quantity not reabsorbed in all probability will be small, less than  $25 \text{ l} \times 3,0 \text{ mg Mg/100 ml} = 750 \text{ mg Mg/day}$ . The retention of magnesium appears to be limited, high retentions did not occur, but high excretions did. Obviously any excess of the amount necessary for maintenance, secretion of milk and retention was mainly excreted in the urine approximately giving a linear relation between "available" magnesium above the quantity secreted in milk and the urinary excretion of magnesium (FIG. 2). Also in the case

of negative retentions, the relation between "available" magnesium above the quantity secreted in milk and the amount of magnesium excreted in urine seems to be linear. The retention was zero when the former amount of magnesium was about 2,5 g/day. Obviously this amount is equal to the amount of magnesium required for maintenance and milk diminished by the amount of magnesium secreted in milk. The accuracy of this figure, of course, is low in view of its standard deviation of 1 g mentioned above and of possible unknown systematic errors.

In FIG. 2 the magnesium ingested minus magnesium in faeces minus magnesium in milk in g/day is plotted against the excretion of magnesium in urine in g/day. In case the former amount equals the latter, the retention is zero. At this point the regression line (solid line) intersects the dashed line for magnesium in urine equal to 2,5 g Mg/day. The perpendicular distance between the two lines obviously gives the values for negative or positive retention of magnesium. Consequently for a given figure on the abscis the differences between the corresponding ordinates of the dashed line and the solid line gives the positive or negative retention.

A regression analysis of magnesium excreted in urine on the amount of "available" magnesium above the quantity secreted in milk for the experiments reported here gave the following relationship:  $Mg \text{ in urine (g)} = 0,62 \times (Mg \text{ in food} - Mg \text{ in faeces} - Mg \text{ in milk}) + 0,90$ . The error attached to the regression coefficient 0,62 was  $\pm 0,04$ .

FIG. 2. Correlation between the amount of "available" magnesium above the quantity secreted in milk (g/day) and the amount of magnesium excreted in urine (g/day) and the serum magnesium concentrations (mg/100 ml)



If it is assumed that the amount of magnesium required for maintenance and milk, diminished by the amount of magnesium secreted in milk, is mainly excreted by the kidney and only 0.5 g Mg/day as endogenous faecal magnesium, then the amounts of magnesium excreted in urine lower than about 2.0 g Mg/day might be explained by an increased reabsorption of magnesium in the kidney from the urine or by a more efficient utilization of magnesium for maintenance. This would be in agreement with the fact that the serum magnesium levels begin to fall when the quantity of magnesium in urine is lower than 1 g/day and the retention of magnesium is lower than about — 1 g/day which also may be deduced from the data of FIG. 2. Therefore, the level of the daily excretion of magnesium in urine appears to be an even better measure for the magnesium status of the animal than the magnesium concentrations in blood-serum. However, in this respect it must be considered that according to FIG. 2, the amount of "available" magnesium above requirement for milk production may be lower than 1 g/day, while at the same time hypomagnesaemia as well as normal serum magnesium levels may occur. This suggests individual differences concerning the influence of stored magnesium in the body on the magnesium concentrations of blood-serum although the present data suggest also that the reserve of magnesium in the body of mature cows does not play an important part in maintaining the serum magnesium levels.

According to the afore mentioned our experiments about the origin of hypomagnesaemia suggest that hypomagnesaemia in cows arises as a result of a shortage of magnesium due to the reduction in the dietary supply of "available" magnesium. This emphasizes first of all the importance of a sufficient supply of "available" feed magnesium. This result is entirely in agreement with the findings of ROOK and BALCH (1958).

The present data give no conclusive evidence about the influence of fertilizer treatment of pasture and about the chemical composition of herbage on the "availability" of the magnesium ingested. However, the present data suggest that heavy dressings of pasture with potassium and/or nitrogen have an unfavourable influence in this respect. The mean figure for "availability" of magnesium was 16 per cent in 11 animals fed on rations high in potassium and/or nitrogen and the same was 20 per cent in 10 cows on rations low in potassium and/or nitrogen. This difference might be seen as an indication that high contents of potassium and/or nitrogen of the herbage are associated with a lower percentage of the magnesium ingested which is not excreted in the faeces. The exceptions mentioned before, i.e. cow 1 in EXP. 3 and cow 1 and 3 in EXP. 6 have not been included in these mean values. As to the influence of the chemical composition of herbage on the "availability" of magnesium, the data of EXP. 9, 10 and 11 show very clearly the effect of herbage cut in different stages of growth on the magnesium digestion. According to TABLE 1, the cows 3 and 4 in EXP. 9 were the same cows as cow 1 and 2 in the EXP. 10 and 11. During the EXP. 9, 10 and 11 these cows consumed herbage from the same pasture but in different stages of growth. TABLE 3 shows some data about the composition of the herbage ingested in percentages of dry matter and about the magnesium digestion. The figures are mean values of the two cows and detailed observations of each cow have been given in the TABLES 1 and 2.

The crude protein contents of the herbage fed in the three experiments decreased very clearly and also the magnesium contents were lower as the herbage matured. The percentage potassium remained the same. Normally potassium goes down as the herbage matures, but this decreasing trend was prevented by means of higher potas-

TABLE 3. Influence of freshly cut herbage in different stages of growth on the magnesium digestion in milking cows

Composition of the herbage consumed				
	Exp. 9	Exp. 10	Exp. 11	
Crude protein .....	25,9	17,8	14,0	
Magnesium .....	0,152	0,124	0,112	
Potassium .....	3,01	3,35	3,08	
Intake, excretion, secretion, retention and "availability" of herbage magnesium				
Magnesium intake .....	g/day	15,87	14,80	13,73
Excreted in faeces .....		14,28	12,49	10,98
Secreted in milk .....		2,15	2,00	1,88
Excreted in urine .....		0,57	0,69	0,98
Mg retention .....		-1,1	-0,4	-0,1
"Availability" of magnesium (%) .....		10	16	20
"Available" magnesium above the quantity secreted in milk, g/day .....		-0,6	+0,3	+0,9

sium applications on some strips of the experimental pasture. Consequently, from EXP. 9 to EXP. 11 increasing amounts of herbage were mown from these strips. The daily magnesium intake in the three experiments was low compared to the other experiments reported in this publication and the intake of magnesium in EXP. 11 was lower than in EXP. 9. This difference was due to a lower magnesium content of the herbage consumed in EXP. 11, although it was remarkable that the daily dry matter intake in EXP. 9 was lower than in EXP. 10 and 11 (TABLE 1) which was caused by higher feed residues in EXP. 9. The palatability of the herbage fed in this experiment seemed to be less. The amounts of magnesium excreted in faeces decreased very clearly and the differences in "availability" of the feed magnesium changing from 10 per cent in EXP. 9 to 16 per cent and 20 per cent in the EXP. 10 and 11 respectively, were very striking. These increasing values for "availability" were related with increasing urinary excretion of magnesium and with higher amounts of "available" magnesium above the quantity secreted in milk. The secretion of magnesium in the milk remained almost the same. The slight decrease of magnesium secretion in milk was caused by decreased magnesium contents of the milk. The magnesium retention changed from -1 to about zero in EXP. 9 and 11 respectively. The data of these experiments suggest that the utilization of herbage magnesium becomes better as the herbage matures which would lead to a better magnesium supply to the animal. Moreover these data emphasize the importance of a high "availability" of feed magnesium.

In another experiment carried out with two dry cows fed on a winter ration the influence of dosing the cows with potassium chloride on the magnesium excretion in urine has been studied. During the preliminary periods of 13, 5 and 6 days respectively and the three successive experimental periods A, B and C lasting 8 days each the two cows received the same ration consisting of hay and concentrates. During the preliminary and experimental period B both cows were dosed with 400 gram of KCl per head per day in the form of pellets which were given by means of a dosing gun. The excretion of magnesium in urine in the experimental periods A, B and C were for cow 1: 0,24 g; 0,13 g and 0,19 g Mg/day and for cow 2: 0,66 g; 0,44 g and 0,69 g Mg/day respectively. Consequently in this experiment the higher intake of KCl led to a lower urinary excretion of magnesium. This may be an indication that an increased potassium intake is related with a lower "availability" of

feed magnesium. Both cows showed subnormal serum magnesium levels which is in agreement with the low values for magnesium excretion in urine. The lowest serum magnesium concentrations have been found during the B periods in which potassium chloride was given.

Our experiments suggest also the existence of individual differences in utilization of feed magnesium. It is remarkable that all cows with serum magnesium levels ranging from 0,50 to 1,80 mg/100 ml showed very low percentages of magnesium not excreted in the faeces (7—14 per cent), while the other cows of the experiments receiving the same herbage showed normal serum magnesium concentrations ranging from 2,10 to 2,75 mg/100 ml and higher percentages of magnesium not excreted in the faeces (11—23 per cent). This is in agreement with the fact that it is generally assumed in practice that some cows are more susceptible to hypomagnesaemia than other cows, although, differences in magnesium intake and in mobilisation of bone magnesium may play a part here. In this respect it should be taken into consideration that a high yielding cow is not necessarily more susceptible to hypomagnesaemia than a cow with a low milk yield because a higher production in general will lead to a considerably higher intake of herbage.

The daily intake of herbage magnesium as well as the low "availability" and the considerable differences in the "availability" of herbage magnesium play an important part in the origine of hypomagnesaemia in mature cows. A shortage of "available" magnesium above the quantity required for milk production leads to a fall in urinary excretion of magnesium and after that hypomagnesaemia may occur. Especially for mature cows a sufficient daily supply of available magnesium seems to be necessary because the mature cow in contrast to the young calf (BLAXTER and MCGILL, 1956) is apparently unable to mobilize the stored body magnesium in the bones at least in sufficient quantities over the period during which hypomagnesaemia develops. As to the magnesium intake there is already a lot of information about factors influencing the magnesium content of herbage, although the dry matter intake may play also an important part here. However, the differences in magnesium intake and the differences in "availability" of the magnesium ingested are equally responsible for the dietary shortage of magnesium. Apart from between animal differences there are only some indications concerning the factors in the herbage influencing the "availability" of herbage magnesium. Although many factors may be of influence here, our experiments suggest that a high potassium and/or nitrogen concentration of the herbage is associated with a low "availability" of magnesium. Moreover the experiments presented in this paper about the "availability" of magnesium in herbage in different stages of growth show that the "availability" increases very clearly as the herbage matured. Since in these experiments the nitrogen content of the herbage as well as the phosphorus content of the herbage decreased considerably a possible explanation of this phenomenon may be for instance the formation of the insoluble magnesium ammonium phosphate in the gut and therefore a higher excretion of magnesium in the faeces associated with a lower utilization of the magnesium ingested.

#### ACKNOWLEDGEMENTS

We are indebted to ir. J. WIND for stimulating this investigation and for his contributions and his advice. Moreover we like to thank Mr. G. A. J. BUITING for technical assistance and Miss A. H. VAN ROSSEM for reviewing the English text.

The investigation was partly sponsored by the National Council for Agricultural Research of the Organization for Applied Scientific Research (T.N.O.).

## LITERATURE

- ALLCROFT, R. 1953 *Proc. 15th Vet. Congr., Stockholm 1*, 573.
- BARTLETT, S., B. B. BROWN, A. S. FOOT, S. J. ROWLAND, RUTH ALLCROFT and W. H. PARR 1954 *Brit. Vet. J.* 110, 3—19.
- BLAXTER, K. L., and R. F. MCGILL 1956 *Vet. Rev. and Ann.* 2, 1 : 35—55.
- BROUWER, E., en S. BRANDSMA 1953 *Meded. Landbouwh.school, Wageningen.* 53, 31—73.
- BRÜGGER, G. 1958 *Landw. Forsch.* 11, 202.
- CUNNINGHAM, H. M., G. L. FREDERICK and S. J. BRISSON 1955 *J. Dairy Sci.* 38, 997.
- DIJKSTRA, N. D. 1954 *Neth. J. agr. Sci.* 2, 273.
- ES, A. J. H. VAN, and J. E. VOGT 1959 *J. An. Sci.* 18, 1220.
- FIELD, A. C. 1960 *Conference Brit. Vet. Ass., London.*
- GROOT, TH. DE 1960 *Brit. Vet. J.* 116, 225.
- , and J. H. AAFJES 1960 *Brit. Vet. J.* 116, 1.
- HVIDSTEN, H., M. ÖDELIEN, R. BAERUG and S. TOLLERSRUD 1959 *Act. Agric. Scand.* 261.
- KEMP, A. 1958 *Neth. J. agric. Sci.* 6, 281—297.
- 1960 *Neth. J. agric. Sci.* 8, 281—304.
- 1960 *Conference Brit. Vet. Ass., London.*
- MAYNARD, L. A. 1947 *Animal Nutrition.* 2nd Edition.
- PARR, W. H., and R. ALLCROFT 1957 *Vet. Rec.* 69, 1041.
- ROOK, J. A. F., C. C. BALCH and C. LINE 1958 *J. agric. Sci.* 51, 189.
- ROOK, J. A. F., and C. C. BALCH 1958 *J. agric. Sci.* 51, 199.
- SEEKLES, L., en J. BOOGAERDT 1955 *Tijdschr. Diergeneesk.* 80, 331.
- 1956 *Tijdschr. Diergeneesk.* 81, 281.
- SIJLLEMA, B. 1931 *Landbouwk. Tijdschr.* 43, 67—77, 139—147, 593—610, 793—815.
- SMYTH, P. J., A. CONWAY and M. J. WALSH 1958 *Vet. Rec.* 70, 846.