STUDIES IN THE METABOLISM OF COBALT

Submitted as a Thesis for the Degree of Ph.D. of Edinburgh University,

by

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Contents

Introduction

The successful use of Cobalt, as a therapeutic measure in curing Bush Sickness and Morton Mains Disease in New Zealand, Enzootic Marasmus in Australia, Pining in Great Britain, Nakuruitis in Kenya and Salt Sickness of Florida, has shown that Cobalt is an essential component of the diet of ruminants for normal growth.

Filmer and Underwood (1935) discovered that Cobalt was the actual curative agent of Enzootic Marasmus which had been thought to be due to iron deficiency. This discovery was soon found to apply to Bush Sickness in New Zealand (Askew and Dixon 1936, Askew 1939). In Australia and New Zealand various workers subsequently demonstrated that top-dressing with Cobalt rich fertilisers increased significantly the cobalt content of the herbage (Underwood and Harvey 1938, Rigg 1940, Askew and Dixon 1937). Andrews and Prichard (1947) showed that cobalt sulphate can be distributed successfully and economically from the air by aeroplane in the top-dressing of hill pastures.

In Great Britain a very widespread wasting condition known as Pining has been proved due to cobalt deficiency. The symptoms of Pining are similar to those of general malnutrition. A pining lamb shows progressive debility, the growth is retarded and it soon presents a stunted and unthrifty appearance. The fleece becomes dry, lustreless and broken. The physical condition quickly deteriorates and a serous/

serous discharge from the eyes is also noticed. Finally the gait becomes inco-ordinated and as a result of extreme weakness, the animal is unable to rise.

Stewart, Mitchell and Stewart (1941) showed that this pining condition in sheep and cattle is due to cobalt deficiency of the herbage and soil. They found that a dressing of 2lbs. of cobalt chloride per acre produced a very marked increase in the cobalt content of the herbage and that pining in lambs could be cured and prevented by top-dressing the herbage with cobalt rich fertiliser. Stewart, Mitchell and Stewart (1942) confirmed their previous work noting, that, although cobalt chloride, as a fertiliser, is rapid in its action, it has a considerable residual effect. Stewart (1946) observed that if the first year grass was top-dressed with 2lbs. of cobalt sulphate per acre, the third year grass would still be adequate in its cobalt content. He presented experimental data to show that both dosing the lambs with cobalt and manuring the land with cobalt rich fertiliser produce the same result - healthy lambs of good average size but suggested that where soil type is satisfactory, cobalt manuring is the best method of overcoming cobalt deficiency on arable grass-land whereas feeding a mineral mixture enriched with cobalt, is the common method in use to overcome cobalt-deficiency on hill farms where it is impossible to top-dress the pasture. In many farms of Great Britain/

Britain, such methods of preventing pining are now in general use.

Although it has been conclusively proved that cobalt deficiency diseases in sheep and cattle can be overcome by feeding cobalt, little is known about the specific physiological rôle of cobalt in the animal body. Stewart and Holman (1944) substantiated the findings of Robscheit-Robbins and Whipple (1942) who found that cobalt did not affect haemoglobin formation; Stewart and Holman (1944) also presented data to prove that cobalt deficiency in itself does not cause anaemia.

In recent years, radioactive cobalt has been used in an attempt to unravel several aspects of cobalt metabolism in the animal body. Comar, Davis and Taylor (1946) reported that if cobalt was administered orally to a steer, very little was retained in the tissues. They also showed that significant amounts of injected cobalt appeared in the abomasum contents while none was found in the rumen. Comar, Taylor, Huffman and Ely (1946) found that cobalt supplements should be supplied every week if it was desired to maintain the cobalt level in the rumen contents of animals on feed deficient in cobalt. Comar and Davis (1947) reporting on tissue distribution of labelled cobalt administered to young calves, supported the view that the major function of cobalt in the ruminant is a localised action in the rumen.

From/

From our available knowledge we presume that only ruminants seem to suffer from cobalt deficiency. It is known that injection of cobalt is not as effective as oral administration in alleviating deficiency symptoms which might indicate that the metabolism of cobalt is associated with the complex process of the alimentary tract. Mackintosh (1945) observed that cobalt might be essential for the maintenance of the normal bacterial flora in the rumen and pointed out that cattle or sheep would seldom show cobalt deficiency symptoms until after they commence to ruminate. Stewart (1946) showed that lambs grazing cobalt dressed pasture, and therefore obtaining a dose of cobalt everyday, made greater live-weight gains and were in better health than were those which were being dosed once a week. Thomson and Gordon (1947) and Ray, Weir, Pope and Phillips (1947) expressed the opinion that cobalt might be concerned primarily with the biological processes in the rumen and suggested that cobalt might act upon some of the micro-organisms of the rumen concerned with the synthesis of B Vitamins.

From the above brief review of available literature, we can summarise as follows that non-ruminants apparently do not suffer from cobalt deficiency, that lambs and calves do not exhibit symptoms of cobalt deficiency until after the rumen has started to function and that injected cobalt, which does/

does not reach the rumen, is relatively inefficient in curing cobalt deficiency. This evidence, although indirect, would suggest that the rumen might be the principal seat of action of cobalt.

Many shepherds and farmers reported that lambs seem to eat down the cobalt dressed pasture better than the cobalt deficient pasture. Stewart (1946) found this to be the case in one of his experiments since the grass on the side of the fence which had been top-dressed with cobalt was usually eaten down while that on the other side of the fence might be five or six inches in length. This would suggest that lambs grazing on cobalt deficient pasture may suffer from loss of appetite and consequently do not eat as much grass as the other lambs grazing on cobalt top-dressed pasture. It would thus appear that loss of appetite, in the lambs grazing cobalt deficient pasture, is responsible for the clinical symptoms of pining. It is possible that cobalt might have a controlling influence on the appetite of lambs. However much more experimental proof was needed to substantiate this theory. It was decided to carry out actual field experiments to study the rôle of cobalt on the appetite of lambs.

Grazing Experiment to Study the Role of Cobalt on the Appetite of Lambs.

Stewart, Mitchell, Stewart and Young (1946) showed by soil and pasture surveys in the district of Criffel Mountain in Kirkcudbrightshire that pining in many farms of this area was due to uncomplicated cobalt deficiency and one of these farms was selected on which to study the effect of cobalt deficiency on the appetite of lambs. This farm was situated on the foothills of the Criffel near Kirkgunzeon. The soil of this field was a well drained brown to dark brown medium loam derived from a glacial drift consisting mainly of local granite but containing a discernible admixture of graywacke, mudstone and sandstone fragments.

Plan of Manuring the Experimental Field (as carried out by Stewart, Mitchell, Stewart and Young in 1945)

A field of 22 acres was divided into two equal areas by an impassable wire fence. A top-dressing of superphosphate at the rate of 1½ cwt. per acre was applied on one half of the field. The other half was also top-dressed with superphosphate at the rate of 1½ cwt. per acre but 2 lbs. of cobalt sulphate was mixed with each 1½ cwt. superphosphate applied. 2 lbs. of cobalt sulphate per acre was used as it was shown to be most economic under Scottish conditions (Stewart, Mitchell, Stewart 1941, Stewart, Mitchell, Stewart 1942.) The top-dressing was applied on April/

April 2nd, 1945. The samples for analysis were taken on July 11th, 1945, 14 weeks after being top-dressed. Stewart et al (1946) presented the following data to show the increase in the cobalt content of the pasture due to cobalt top-dressing. It was shown that the cobalt content of the cobalt deficient pasture was .09 parts per million while the cobalt content of the cobalt treated pasture was .38 parts per million. They also showed that in August 1945 the group of lambs grazing cobalt manured pasture had amean weight of 67.1 lbs. while the group of lambs grazing cobalt deficient pasture had a mean weight of 46.8 lbs. only. This showed that lambs would pine on the cobalt deficient pasture of this field while the group of lambs on the cobalt dressed pasture were quite healthy. This was in agreement with the findings of Stewart, Mitchell and Stewart who reported previously that all pastures with a cobalt content below 10 parts per million are deficient in cobalt.

Plan of the Present Investigation.

As it is rather difficult to measure directly the appetite of lambs on pasture, it was decided to adopt an indirect method depending primarily on the determination of the weight of faeces voided per day by the lambs when grazing the experimental pasture plots.

Watson (unpublished work) devised a technique of measuring the amount of grass eaten by sheep by tethering/

Plate 1

Showing Sheep with Harness.

Plate 2.

Showing Collection of Faeces Sample

tethering experiments. Woodman, Evans and Eden (1937) used the same method but allowed the sheep to graze freely on pasture. They assumed that, if the daily output of faeces by each sheep over a given period is known, and if during this period the digestibility of the herbage is determined by independent digestion trials on other sheep, it is possible to calculate the mean daily grass consumption of sheep over the period of measurement. Assuming the digestibility of the sheep in a metabolism crate to be the same as that of the grazing animals, the intake of dry matter was calculated. Woodman et al (1937) presented data on the faecal excretion and calculated the total dry matter intake of the sheep while grazing on pasture. From the data given it can be shown that the faecal excretion, calculated on a dry matter basis, was proportional to the dry matter intake. If we assume that this is the case for all grazing sheep, and there is no reason to doubt it, then it is possible to compare the appetites of different animals and groups of animals at grass by comparing their faecal excretion. On this basis, the following experiments were carried out.

For measuring the appetite of lambs by this method, it was necessary to collect quantitatively the dung from each sheep while it was grazing the pasture. This was overcome by the use of the type of digestion harness, used by Watson and Woodman, and shown in Plate 1 . The faeces were collected in a special waterproof/

waterproof bag attached to the hindquarters of the animal by a light webbing harness. This bag could be opened and closed by means of a lightning fastener, which passes down the full length of the medium line of the bag. The faeces could thus be removed quite simply without unfastening the harness or detaching the bag. This is illustrated by Plate 2 , which shows the technique of the collection of faeces.

In summer with grazing animals provided with such harness, it is necessary to keep the hindquarters scrupulously clean in order to prevent the attacks of flies and other insects. The wool in the hindquarters was clipped as closely as possible and this part was cleaned daily with soap and water containing lysol. This proved to be a very effective safeguard against the fly danger. The lambs were allowed a few days to get accustomed to the feel of the harness before the actual experimental period began.

Details of the Grazing Experiment During the 1946 Season

A preliminary investigation was carried out in the month of July, 1946. Two groups of lambs of two weeks of age were selected in April. One group containing 15 lambs was placed on the cobalt dressed pasture whilst the other group of 11 lambs was allowed to graze on the cobalt deficient pasture. These lambs were kept on their respective sides of the

Daily Faecal Excretion

Table 1

100% Dry Matter

11.7.46 - 17.7.46

	11.7.46 oz	12.7.46 oz.	13.7.46 oz.	14.7.46 oz.	15.7.46 oz.	16.7.46 oz.	17.7.46 oz.	Total for Week oz.
Lambs Grazing Cobalt Dressed Pasture	12	9	8.5	9.2	10.8	8.8	9.2	67.5
Lambs Grazing Cobalt Deficient Pasture	14.8	7.7	7.9	7.8	8.8	8.8	9.2	65.0

the field continuously till the experiment was carried out. On 10th July, 4 lambs from each group were selected. Each lamb was equipped with harness previously described and allowed to graze on their respective sides of the field. Each day for the seven consecutive days the faeces were collected and weighed. Representative aliquot portions were kept for the determination of dry matter.

Table 1 shows the total daily faecal excretion of the two groups of lambs. It will be seen from Table 1 that the faecal excretion of lambs grazing cobalt manured pasture on 15.7.46 was 10.8 oz. as compared to the corresponding figure of 8.8 oz. of the lambs grazing cobalt deficient pasture. The daily total of faecal excretion of lambs grazing cobalt dressed pasture varied from 8.5 oz. to 12.0 oz. while that of lambs grazing cobalt deficient pasture varied from 7.7 to 14.8 oz. Thus it was noted that there was small variation in the daily total of the faecal excretion in the case of both the groups. The total faecal excretion for the week of the lambs on cobalt manured pasture was slightly higher than that of the other group - 67.5 oz. as compared to 65.0 oz.

The lambs were weighed on 2.7.46 and the whole group of 15 lambs on the cobalt dressed pasture had a mean weight of 60.4 lb. whilst the lambs on cobalt deficient pasture had a mean weight of 63.9 lbs. This/

A small difference in weight between the two groups was to be expected at this time of the year.

Since at the beginning of July, the clinical symptoms of cobalt deficiency are not obvious, the small variation in the total faecal excretion of the individual groups showed that reliable data might be obtained later in summer when the pining is more advanced.

Therefore the investigation was repeated at the beginning of August, the same lambs being used. They had been continuously grazing on their respective sides of the field since the first observations. On this occasion, each lamb was marked individually so that the individual variation could be determined. From the group grazing cobalt dressed pasture were selected Nos. 1, 2, 3 and 4 which was called Group 1 and from those grazing the other half of the field Nos. 5, 6, 7 and 8 form Group 2. They were equipped with harness as before and the faeces were collected daily at 10 a.m. and weighed. The collection was made for seven consecutive days and representative samples were kept for dry matter estimation.

The dry matter values of the faecal excretion of the two groups of lambs are shown in Tables 2 and 3. From the Tables it will be seen that the total excretion of faeces of the Group 1 lambs was 345 oz. while the corresponding figure for the Group 2 lambs was 225 oz. only. The daily excretion of faeces of lamb/

Table 2.

Daily Faecal Excretion

Group 1 Lambs Grazing Cobalt Manured Pasture.

100% Dry Matter
8.8.46 - 14.8.46

Lamb No.	8.8.46 oz.	9.8.46 oz.	10.8.46 oz.	11.8.46 oz.	12.8.46 oz.	13.8.46 oz.	14.8.46 oz.	Total For Week oz.
1	11	11	10	10	9	11	8	70
2	12	13	12	12	11	12	11	83
3	20	21	18	19	18	21	19	136
4	7	8	7	7	8	10	9	56
Total for 4 lambs	50	53	47	48	46	54	47	345

Table 3.

Group 2 Lambs Grazing Cobalt Deficient Pasture

100 % Dry Matt

8.8.46 - 14.8

Lamb No.	8.8.46 oz.	9.8.46 oz.	10.8.46 oz.	11.8.46 oz.	12.8.46 oz.	13.8.46 oz.	14.8.46 oz.	Total For Week oz.
5	5	6	6	6	6	7	6	42
6	11	10	9	10	9	12	12	73
7	9	9	7	8	7	9	9	58
8	8	8	7	8	6	8	7	52
Total for 4 lambs	33	33	29	32	28	36	34	225

lamb No 2 on cobalt dressed pasture varied from 11 oz. to 13 oz. and that of lamb No. 5 on cobalt deficient pasture varied from 5 oz. to 7 oz. which showed that the faecal excretion of the individual lambs remained fairly constant during the experimental period. Comparing Tables 2 and 3 it is found that the total daily excretion of faeces of Group 1 lambs is much higher than that of Group 2, the daily total varying from 46 oz. to 54 oz. in case of lambs grazing cobalt dressed pasture and from 29 oz. to 36 oz. only in case of lambs on cobalt deficient pasture. It is evident therefore from Tables 2 and 3 that the faecal excretion of the lambs grazing cobalt manured pasture was much higher than that of the lambs grazing cobalt deficient pasture. If we assume that the faecal excretion is proportional to the dry matter intake, it would appear that the group of lambs on cobalt dressed pasture was eating much more grass than those on the cobalt deficient pasture.

The average weight of Group 1 lambs was 83 lbs. on 8th of August while the corresponding figure for the Group 2 lambs was only 70 lbs. Thus in August when pining is more advanced, significant difference in appetite and liveweight between the two groups of animals was observed. It is of interest to note that lamb No. 5 on the cobalt deficient pasture whose average daily faecal excretion was only 6 oz. a day was suffering from severe symptoms of pining in August/

August. Lamb Nos. 6, 7 and 8 though not showing the same severe symptoms of pining as lamb No. 5, were apparently not as healthy as the group of lambs on the cobalt dressed pasture.

Details of the Grazing Experiment During the 1947 Season

In view of the success of the grazing experiment in August 1946, it was decided to repeat the experiment in 1947 and to use the same field for the experiment. Although one top-dressing with cobalt at the rate of 2 lbs. of cobalt sulphate per acre is effective for at least 3 years, it was decided to repeat the dressings given to the field in 1945 in order that a definite response would be obtained in the 1947 season. Therefore in March, 1947, the half of the field which had previously been dressed with cobalt, received a top-dressing of cobalt at the rate of 2lbs. of cobalt sulphate plus 1½ cwt. superphosphate per acre while the other half was top-dressed with superphosphate only at the rate of 1½ cwt. per acre. In April 1947 were placed 15 lambs on the cobalt deficient pasture and 14 lambs on the cobalt dressed pasture. All the lambs were kept continuously on their respective sides of the field till the experiment ended in September, 1947. At the beginning of July, 5 lambs were selected at random from each group and numbered so that individual samples could be obtained from day to day. The faeces were collected and weighed daily as described previously and/

Daily Faecal Excretion

Table 4

Group 1 Lambs Grazing Cobalt Manured Pasture

100% Dry Matter
8.7.47 - 14.7.47

Lamb No.	8.7.47 oz.	9.7.47 oz.	10.7.47 oz.	11.7.47 oz.	12.7.47 oz.	13.7.47 oz.	14.7.47 oz.	Total for Week oz.
1	7.4	6.3	6.5	5.0	7.0	6.6	7.1	45.9
2	6.8	7.5	8.5	8.2	8.6	7.9	7.7	55.2
3	5.8	7.0	7.6	6.4	7.6	6.9	7.5	48.8
4	8.3	9.5	9.3	10.6	10.3	11.2	12.8	72.0
5	3.3	6.4	6.4	6.3	6.9	7.2	7.0	43.5
Total for 5 Lambs	31.6	36.7	38.3	36.5	40.4	39.8	42.1	265.4

Table 5

Group 2 Lambs Grazing Cobalt Deficient Pasture

100% Dry Matter
8.7.47 - 14.7.47

Lamb No.	8.7.47 oz.	9.7.47 oz.	10.7.47 oz.	11.7.47 oz.	12.7.47 oz.	13.7.47 oz.	14.7.47 oz.	Total for Week oz.
6	3.8	3.9	3.7	4.6	3.7	5.1	4.3	29.1
7	3.5	3.7	3.1	3.1	2.6	3.6	3.6	23.2
8	3.1	3.4	3.7	3.9	3.9	4.3	4.2	26.5
9	3.7	4.2	4.5	4.1	4.1	4.8	4.7	30.1
10	5.6	6.1	6.4	6.9	5.4	6.3	6.5	43.2
Total for 5 Lambs	19.7	21.3	21.4	22.6	19.7	24.1	23.3	152.1

and dry matter determined in each case. Group 1 lambs grazing cobalt dressed pasture were marked 1, 2, 3, 4 and 5 while the Group 2 lambs on cobalt deficient pasture were marked 6, 7, 8, 9, 10 respectively.

Tables 4 and 5 show the dry matter values of faecal excretion of Group 1 and Group 2 lambs respectively. The total amount of faeces excreted by the Group 1 lambs during week was 265.4 oz. in contrast to 152.1 oz. excreted by Group 2 lambs in the same period. The daily faecal excretion of the individual lamb remained fairly constant. In case of Group 1 lambs, the daily excretion of faeces of lamb No. 2 varied from 6.8 oz. to 8.6 oz. a day and the corresponding figures for lamb 7 of Group 2 varied from 2.6 to 3.7 oz. a day. Of the Group 2 lambs, lamb 10 excreted the largest amount of faeces during the seven days its total being 43.2 oz., whilst of the Group 1 lambs, the largest amount of faeces was excreted by lamb No. 4 its weekly total being 72.0 oz. The daily total of faeces of Group 1 lambs varied from 31.6 to 42.1 oz. while the corresponding figure for Group 2 lambs varied from 19.7 oz. to 24.1 oz. Thus it is evident that the total amount of faeces excreted daily by the lambs grazing the cobalt dressed pasture is much higher than that of the lambs grazing the cobalt dificient pasture

The liveweights of lambs of both the fields are summarised in Table 10.

Daily Faecal Excretion

Table 6

Group 1 Lambs Grazing Cobalt Manured Pasture

100% Dry Matter
10.8.47 - 16.8.47

Lamb No.	10.8.47 oz.	11.8.47 oz.	12.8.47 oz.	13.8.47 oz.	14.8.47 oz.	15.8.47 oz.	16.8.47 oz.	Total for Week oz.
1	11.8	11.7	10.2	13.5	11.0	10.9	13.0	82.1
2	10.1	10.7	9.4	10.1	8.2	10.0	7.6	66.1
3	8.6	8.5	8.4	10.0	9.0	9.5	8.0	62.0
4	14.9	14.7	12.0	12.6	11.2	12.0	12.2	89.6
5	9.2	8.3	9.7	10.3	8.6	9.2	8.6	63.9
Total for 5 Lambs	54.6	53.9	49.7	56.5	48.0	51.6	49.4	363.7

Table 7

Group 2 Lambs Grazing Cobalt Deficient Pasture

100% Dry Matter
10.8.47 - 16.8.47

Lamb No.	10.8.47 oz.	11.8.47 oz.	12.8.47 oz.	13.8.47 oz.	14.8.47 oz.	15.8.47 oz.	16.8.47 oz.	Total for Week oz.
6	2.2	3.4	4.7	4.7	4.4	3.8	3.9	27.1
7	4.4	5.1	4.0	5.3	6.6	5.0	4.9	35.3
8	3.0	2.2	3.1	2.8	3.0	3.2	2.0	19.3
9	6.0	9.3	5.4	5.6	2.7	4.2	5.2	38.4
10	5.5	9.9	5.9	6.9	8.7	6.2	6.7	49.8
Total for 5 Lambs	21.1	29.9	23.1	25.3	25.4	22.4	22.7	169.9

Lamb Liveweights in 1947

Table 10

Group	Date	No. of lambs.	Range of weight lb.	Mean weight lb.
Group of lambs grazing cobalt dressed pasture	6.7.47	14	32-57	45
"	7.8.47	14	52-75	64
"	18.9.47	14	76-101	85
Group of lambs grazing cobalt deficient pasture	6.7.47	15	26-46	37
"	7.8.47	15	32-63	46
"	18.9.47	15	32-85	52

At this date there was not much difference between the liveweights of the lambs between the two groups. On the 6th of July the 14 lambs on the cobalt dressed pasture had an average liveweight of 45 lb. ranging from 32 lbs. to 57 lbs. within the group while the 15 lambs grazing the cobalt deficient pasture weighed 37 lbs. ranging from 26 to 46 lbs.

The experiment was repeated in August on the same animals which had not been off their respective sides of the field since the previous collection in July. Collection of faeces was started on 10th August, 1947. The dry matter figures of faecal excretion of Group 1 and Group 2 lambs are summarised in tables 6 and 7 respectively. The total excretion of faeces for the week by Group 2 lambs was 169.9 oz. only whereas the corresponding figure for Group 1 lambs was 363.7 oz. This/

This large difference between the two groups is highly significant. The largest quantity of faeces was excreted by lamb 10 of group 2 and its total for the week was 49.8 oz. Of the Group 1 lambs the smallest amount of faeces was excreted by lamb 3 but its total was 62.0 oz., which is much higher than even the highest figure in the case of lambs grazing cobalt deficient pasture. Lamb 6 of Group 2 excreted 27.1 oz. of faeces during the seven days while lamb No. 1 during the same period excreted 82.1 oz. which is thrice as much as that of lamb No. 6. The daily total of faecal excretion of Group 1 varied from 48.0 oz. to 54.6 oz. and that of Group 2 from 21.1 oz. to 29.9 oz. In August therefore it is shown that there was a much larger difference in the amount of faeces excreted than in July and this corresponds to a bigger difference in Liveweight. The average weight of lambs on the cobalt treated pasture was 64 lbs. in August and that of those grazing the cobalt deficient pasture was 46 lbs., the weight of the former ranging from 52-75 lbs. and of the latter from 32-63 lbs. as shown in Table 10.

Further data was obtained from a trial in September when the liveweight difference between the lambs was even more marked. The same 5 lambs on each field were used as before but unfortunately lamb No. 3 on cobalt manured pasture started scouring slightly at the beginning of the experiment. It was kept on till 24.9.47 after which it was discarded as it was impossible/

Daily Faecal Excretion

Table 8

Group 1 Lambs Grazing Cobalt Manured Pasture

100% Dry Matter
21.9.47 - 27.9.47

Lamb No.	21.9.47 oz.	22.9.47 oz.	23.9.47 oz.	24.9.47 oz.	25.9.47 oz.	26.9.47 oz.	27.9.47 oz.	Total for Week oz.
1	17.1	14.4	13.3	13.2	13.0	14.3	12.9	98.2
2	11.0	11.8	12.4	11.6	9.8	10.7	9.6	76.9
*3	9.61	11.04	7.79	8.67	-	-	-	-
4	14.7	17.9	16.0	19.0	17.0	17.4	16.2	118.2
5	12.7	12.6	12.6	11.7	11.9	12.3	12.9	86.7
Total for 4 Lambs	55.5	56.7	54.3	55.5	51.7	54.7	51.6	380.0

Table 9

Group 2 Lambs Grazing Cobalt Deficient Pasture

100% Dry Matter
21.9.47 - 27.9.47

Lamb No.	21.9.47 oz.	22.9.47 oz.	23.9.47 oz.	24.9.47 oz.	25.9.47 oz.	26.9.47 oz.	27.9.47 oz.	Total for Week oz.
6	5.4	5.1	4.5	5.2	5.3	6.3	6.2	38.0
7	6.9	6.1	7.1	6.9	6.4	6.8	5.9	46.1
8	3.7	3.3	4.4	3.9	3.8	4.8	4.9	28.8
9	5.3	4.9	5.6	5.4	5.0	4.7	4.2	35.1
10	8.1	7.6	8.6	8.0	7.2	7.0	7.6	54.1
Total for 5 Lambs	29.4	27.0	30.2	29.4	27.7	29.6	28.8	202.1

impossible to collect its excretion.

The dry matter values of faecal excretion of Group 1 and Group 2 lambs for the September trial are summarised in Tables 8 and 9 respectively. The largest amount of faeces was excreted by lamb 4 of Group 1 and its total for the week was 118.2 oz. The highest amount of faeces excreted by a lamb of Group 2 was that by lamb No. 5, its total for the same period being only 54.1 oz. The total amounts of faeces excreted by lamb No. 1 of Group 1 and lamb 6 of Group 2 were 98.2 and 38.0 oz. respectively. In the case of lamb No. 2 on cobalt treated pasture the daily faecal excretion varied from 9.6 oz. to 12.4 oz. while that of lamb No. 8 of the other group varied from 3.3 oz. to 4.9 oz. daily. The total of the faecal excretion for 4 lambs on cobalt manured pasture on 22.9.47 was 56.7 oz. while the total of 5 lambs on the cobalt deficient pasture on the same day was 27.0 oz which is even less than half of the total of the 4 lambs on cobalt manured pasture.

It is interesting to compare these values with the gain in liveweight since the August trial. On 18.9.47 the average weight of the lambs grazing the cobalt dressed pasture had risen from 64 to 85 lbs. whilst those grazing the cobalt deficient pasture had only gained on the average 6 lbs. rising from 46 to 52 lbs. Thus as the liveweight difference between the two groups increased from July to September there was also an increasing difference in the amount of faeces excreted/

excreted.

Discussion

Comparing the experimental findings shown in Tables 1 to 10, it is apparent that with the exception of those of Table 1, the rest of the data showed that the faecal excretion of the group of lambs on cobalt dressed pasture was much higher than that of the lambs on cobalt deficient pasture. We can account for the values in Table 1 by reason of the fact that the clinical symptoms of pining are not usually apparent at the beginning of July and up till then young lambs even in cobalt deficient pasture made normal liveweight gain.

From the findings of Woodman et al (1937),it would appear that the amount of faeces excreted is proportional on a dry matter basis to the amount of pasture ingested. If we assume this to be correct for the present investigation we can show that the lambs on cobalt deficient pasture are not eating as much pasture as those on cobalt manured pasture. From the liveweight observations, it is seen that the lambs on cobalt manured pasture were making the normal liveweight gains approximately 4 lbs. a week. As the lambs on the cobalt deficient pasture were not eating sufficient pasture, they were continually on a low plane of nutrition and as such they failed to make normal growth - in other words they were suffering from/

from malnutrition. The symptoms of cobalt deficiency, wasting and retarded growth, are also those of general malnutrition. Therefore, the low intake of cobalt deficient pasture by the young lambs might be seriously retarding the growth of the lambs as shown by low liveweight gains. It is possible that the low intake of grass in the lambs grazing the cobalt deficient pasture might be due to the low cobalt status of the pasture interfering with the physiological controls of appetite. It was mentioned previously that the cobalt content of the cobalt manured pasture was .38 parts per million as compared to .09 parts per million in the cobalt deficient pasture. Stewart et al (1946) showed that the nutritive value of both the pastures were almost the same excepting the difference in their cobalt content.

The question arises how the appetite could be influenced by cobalt. Little is known on the physiological processes controlling the appetite of lambs but one might suppose that any serious interference with normal rumination might affect the appetite of the animal. Thomson and Gordon (1947) and Ray, Weir, Pope and Phillips (1947) pointed out that cobalt might be concerned primarily with the biological processes in the rumen and suggested that cobalt might act upon some of the micro-organisms of the rumen concerned with the synthesis/

synthesis of B Vitamins.

It is also possible that cobalt may act as an enzyme in one of the processes affecting the break-down of cellulose in the rumen and that if the cellulose is not broken down completely the animal has a disinclination to receive more into its rumen and thus ceases to eat to a normal extent.

At present this must remain a matter of surmise until more data become available but the present investigation clearly demonstrates that one of the chief causes of the poor condition of cobalt deficient animals is due to such animals refusing to eat a normal amount of food and might be another pointer to cobalt playing some physiological rôle in rumination.

Plate 3.

Showing Metabolism Cage.

Metabolism Experiments on Cobalt Deficient Diets

Since the investigations described above showed that cobalt deficient pasture affected in some way or other the usual processes of digestion it was decided to investigate if the digestibility of cobalt deficient diets is depressed owing to the inadequate supply of cobalt. For this purpose use was made of simple metabolism cages and of a diet which was known to produce the clinical symptoms of cobalt deficiency.

Description of Experimental Procedure

Two Scotch Blackface sheep (Nos. 18 and 14) were placed in the metabolism cage as shown in Plate 3. They were allowed to remain there for a few days to adapt themselves to the confined quarters and the feel of the harness, before data was collected. The sheep were weighed before and after each trial. The experimental rations were fed in a preliminary period before the proper feeding period began. The sheep were then placed in the matabolism cage and equipped with the harness already described (See page 8).

The construction of the metabolism cage used in the experiment is shown in Plate 3. The inside of the cage is covered with 'uralite' and a galvanised iron grid is let into the floor. Below this is a lead-lined funnel which connects with a pipe passing through the bottom of the cage. The urine passes down this tube into a glass bottle placed underneath. The feeds are weighed into the boxes which are hung on/

on the side of the cage for providing drinking water to the sheep.

The sheep are provided with a stout leather collar, which is attached to the cage by a length of chain. The dimensions of the cage and the length of the chain are such as to permit of free movement without allowing the animal to turn right round or get its hind legs off the grid by more than a few inches. Thus no loss of urine occurs. The sheep are quite free to lie down and spend most of their time in that position. The harness could be adjusted easily and the faeces are collected daily by opening the waterproof bag, attached to the harness. The sheep are quite contented in the cage, sometimes being continuously there for two months without any apparent effects on health. Special care was taken of their feet as the sheep are apt to become footsore unless the horn is kept well cut down.

Owing to the great importance of taking representative samples in metabolism experiments, the greatest care was taken to ensure that the samples kept for analysis were truly representative in character. The method of sampling the foodstuffs composing the ration is mentioned briefly. The foodstuffs used in the experiments were all ordered in bulk. The whole amount was mixed and sampled for analysis by quartering. It was found by repeated analysis that the composition of the material varied very slightly in bulk. The hay was kept in a cement fodder house and/

and the flaked maize was kept in large closed boxes specially constructed for that purpose. This method of sampling the foodstuffs composing the ration, previously used by (Stewart 1930) was found to be sufficiently accurate to warrant its use in metabolism work so avoiding repeated analysis of the ration.

The amount of faeces was weighed each morning at 9.30 a.m. and the volume of urine measured. One tenth of the daily urine was kept in cold storage. A period sample was obtained by mixing the fourteen daily aliquot samples of each period. One tenth of the daily total of faeces was kept in cold store. Biweekly samples were mixed, the moisture values estimated and the dry matter ground and placed in a Kilner jar for analysis. Thus four biweekly samples of faeces composed each period sample. In this way the digestion coefficients of the ration were estimated for every fourteen day period.

The digestibility of crude protein, Ether extract, crude fibre, total ash, organic matter and Nitrogen free extractives were determined by means of repeated digestibility trials with sheep 18 and sheep 14 on cobalt deficient diets. The digestibility of hay, brought from cobalt deficient areas, was determined and then the digestibility of cobalt deficient diets consisting of flaked maize and hay from cobalt deficient areas, was determined.

Chemical/

Chemical methods of analysis -

The chemical methods of analysis employed for determining digestion coefficients are too well known to be described in detail. Brief descriptions of the methods used are given below.

Nitrogen:- Determined by the usual Kjehldahl's method. The Nitrogen of the faeces has been determined in duplicate on 3 gms. of wet faeces from each biweekly composite faeces as it was thought that Nitrogen might be lost while drying the faeces in the oven. % Crude Protein obtained by multiplying % N_2 by 6.25.

Ether Extract:- By Soxhlet's apparatus using purified Ether as the solvent.

Crude fibre:- By boiling the sample with acid and alkali under standard conditions as in the routine methods followed for determining crude fibre.

Total ash:- Ashing the material in a muffle furnace at a low temperature.

Organic matter:- Obtained by deducting the % total ash from 100.

Nitrogen free extract:- Derived by subtracting the sum of % crude protein, % ether extract, % total ash, % crude fibre from 100.

Composition of Foodstuff used in Experiment.

The composition of hay and flaked maize are summarised in Table 11.

Table 11.

Composition of Foodstuff used in Experiment (Dry Matter Basis)

	Hay Periods (1-4) %	Hay Periods (5-14) %	Hay Periods (15-17) %	Flaked maize Periods (5-14) %	Flaked maize Periods (15-17) %
Crude protein	6.62	5.55	5.32	7.83	7.92
Ether extract	1.32	1.52	1.49	4.43	3.75
Crude fibre	39.07	39.60	41.61	0.79	1.46
Total ash	4.00	6.32	5.24	1.28	1.21
Organic matter	96.00	93.68	94.76	98.72	98.79
Nitrogen free extract	48.99	47.01	46.34	85.67	85.66

Hay - The crude protein content varied from 5.32% to 6.62%: the ether extract from 1.32% to 1.52%: the crude fibre content was slightly higher than that of good quality hay and varied from 39.07% to 41.61%: the ash content varied from 4.00% to 6.32%. The ether extract was just about the same as in ordinary hay. The organic matter was fairly constant varying from 93.68% to 96.00% and Nitrogen free extract varied from 46.34% to 48.99%. The composition of this hay did not vary greatly from that of ordinary hay. The cobalt/

Summary of Digestion Coefficients

Table 12

Sheep 14

Period	Crude protein %	Ether extract %	Crude fibre %	Total ash %	Organic matter %	Nitrogen-free extractives %
1	-	23.25	62.11	29.86	55.18	58.98
2	-	32.95	63.71	29.95	56.80	60.06
3	7.40	12.47	57.65	16.88	50.90	52.47
4	22.78	30.42	61.63	25.53	52.49	52.41
5	44.90	75.50	15.51	23.84	61.44	72.60
6	54.54	75.96	18.80	42.74	73.98	82.38
7	25.21	74.65	1.29	27.69	76,67	84.77
8	47.27	79.91	8.21	45.23	84.40	89.41
9	46.95	76.33	36.75	41.23	74.40	82.42
10	53.26	73.52	27.88	48.11	71.64	81.33
11	31.10	61.87	31.54	55.23	71.36	81.61
12	51.28	60.53	26.21	29.52	74.88	84.49
13	53.85	80.60	19.16	47.87	77.28	85.37
14	52,21	79.05	20.83	45.46	77.07	85.13
15	64.61	79.40	30.71	50.67	80.56	88.48
16	65.05	74.17	22.19	38.51	76.76	86.26
17	53.48	78.71	39.89	46.85	82.69	89.68

Summary of Digestion Coefficients

Table 13

Sheep 18

Period	Crude protein %	Ether extract %	Crude fibre %	Total ash %	Organic matter %	Nitrogen-free extractives. %
1	-	26.09	62.84	27.75	53.67	56.41
2	-	28.11	62.05	19.80	54.88	58.88
3	8.52	14.82	63.44	25.47	53.79	53.32
4	21.77	30.00	61.64	27.99	55.45	56.09
5	42.06	71.04	34.06	20.63	59.53	70.24
6	63.13	85.07	34.82	45.61	73.90	81.88
7	44.74	78.92	12.76	48.00	67.91	77.64
8	48.23	80.12	15.31	39.84	76.92	84.04
9	44.71	80.34	24.61	50.02	78.66	85.11
10	56.21	82.99	13.69	53.43	80.75	85.18
11	58.45	72.69	20.25	57.42	76.21	83.19
12	59.92	79.64	13.12	62.47	76.44	84.10
13	65.88	81.11	9.77	56.49	77.30	84.89
14	66.98	79.95	40.51	61.82	81.18	86.89
15	53.76	75.89	39.03	50.12	76.08	82.43
16	67.91	80.12	62.36	64.32	82.36	81.37
17	52.37	77.06	58.57	47.75	81.28	87.34

cobalt content of the hay was 10 parts per hundred million.

Flaked maize - The crude protein content of flaked maize varied from 7.83% to 7.92%: the ether extract was 4.43% in the first sample and 3.75% in the second sample: the crude fibre content varied from .79% to 1.46%: total ash and organic matter remained fairly constant: the Nitrogen free extract was about 85.66%. Owing to the moderate protein content and richness in carbohydrate, flaked maize was considered to be eminently suited for the present investigation since it was desired to feed a ration containing adequate protein and starch equivalent but with a very low cobalt content.

Discussion of Digestion Coefficients

Summaries of the digestion coefficients of sheep 14 and sheep 18 are contained in Tables 12 and 13 respectively.

Digestion coefficients - Hay only - Periods 1-4.

From Tables 12 and 13, it is at once evident that the sheep did not eat sufficient of the hay to allow of any satisfactory results being obtained. The negative digestibility of the crude protein during periods 1 and 2 in the case of both sheep indicates the very low ingestion of protein. Consideration of the other constituents reveals that in the case of both Nitrogen free extractives and organic matter, the digestion coefficients are of a low order as compared with the normal for good quality hay. It is all the more/

more surprising therefore to find the digestion coefficients of the crude fibre around the 60% mark. The close agreement between the results obtained from the two animals indicates that both animals were digesting the crude fibre component of the hay to even a greater extent than the Nitrogen free extractives. As the amount of hay eaten increased during periods 3 and 4 there was little change in the digestion coefficients except that the digestion of crude protein became positive but the values for both the crude fibre and Nitrogen free extractives remained much the same.

The unsatisfactory and inexplicable nature of the results obtained from a diet of hay only, made it necessary to change the dietary to one which would allow the animals an adequate daily amount of starch equivalent and digestible protein but yet would be cobalt deficient. Use was made of the ration designed by Stewart (1947) who produced all the clinical symptoms of cobalt deficiency in sheep by feeding hay and flaked maize.

Ration used in Experiment - (Periods 5-17)

Stewart (1947) showed that sheep fed on an artificial diet containing 0.068 mg. cobalt per day exhibited the symptoms of cobalt deficiency. The ration consisted of 1100 gms. of flaked maize and 250 gms. of hay and also a mineral supplement which contained most of the cobalt in the ration. In this series of experiments the mineral mixture was not fed.

During/

<u>Table 14.</u>

<u>Sheep 14</u>

Period	Approx. weight	Average weekly consumption				Maintenance requirement per week. (Wood- and Wood).	
		Flaked maize	Hay	Starch equiv.	Dig. prot.	Starch equiv.	Dig. prot.
	lb.	g.	g.	lb.	lb.	lb.	lb.
12	81	3670	933	7.90	.38	7.75	.32

<u>Sheep 18</u>

Period	Approx. weight	Average weekly consumption				Maintenance requirement per week. (Woodman and Wood).	
		Flaked maize	Hay	Starch equiv.	Dig. prot.	Starch equiv.	Dig. prot.
	lb.	g.	g.	lb.	lb.	lb.	lb.
12	80	3517	760	7.51	.42	7.75	.32

During periods 5-17, it was intended to feed the sheep the same ration consisting of 1100gms. of flaked maize and 250 gms. of hay perday but as the animals did not eat that amount daily, the ration had to be cut down to 600 gms. of flaked maize and 150 gms. of hay daily which contained about .030 mg. cobalt. The ration however varied slightly in each period. The amount of food eaten by the sheep supplied them with an adequate amount of starch equivalent and digestible protein, according to Woodman and Wood's data on the maintenance requirement of sheep and is shown in Table 14. Thus the ration was so constituted that it supplied sufficient energy for maintenance purposesbut was cobalt deficient.

Sheep 14 was continually on this cobalt deficient diet during Periods 5-17 while sheep 18 was on cobalt deficient diet during Periods 5-13. During Periods 14-17 sheep 18 was kept on the same diet but was dosed with 41.2 mgs. of cobalt in the form of cobalt chloride solution in each period, 10.3 mgs. of cobalt as cobalt chloride being given twice weekly by mouth. 1 gram of cobalt chloride was dissolved in 600 c.c. of distilled water and kept in a specially cleaned glass bottle. 25 c.c. of this solution,containing 10.30 mgm. of cobalt in the form of cobalt chloride,was given to sheep 18 each Tuesday and Friday during Periods 14-17 so that in each period the animal was receiving an extra dose of 41.2 mgm. of cobalt.

Discussion of Digestion Coefficients.

Cobalt/

Cobalt Deficient Diets Periods 5-17.

The digestion coefficients of sheep 14 and sheep 18 are summarised in Tables 12 and 13 respectively. Franklin (1935) carried out metabolism experiments with the same breed of sheep - Blackface - on the same type of diet consisting of flaked maize and hay. It is interesting to compare the values of digestion coefficients of this investigation and those of Franklin's. It is found that the figures of digestion coefficients of sheep 14 and sheep 18 are in close agreement with those obtained by Franklin.

In case of crude protein Franklin obtained slightly higher figures -- 63.80-68.17% -- which was probably due to his experimental animals consuming more flaked maize. The digestibility of crude protein in the case of sheep 1 4 during periods 5-17 varied from 25.21 to 65.05%. In the case of Period 7 the digestibility of crude protein fell to 25.21% as the animal was not eating sufficient food due to a tendency to scouring. In Period 11, when the sheep 14 ate the maximum amount of hay the digestibility of crude protein was also low. Excepting Periods 7 and 11, the digestibility of crude protein of sheep 14 varied from 44.90 to 65.05%. With sheep 18, the digestibility of crude protein varied from 42.06% to 67.91%. In period 5, the digestibility of crude protein of sheep 18 was lowest probably due to the animal not eating enough flaked maize. There was no appreciable improvement in the digestibility of crude protein when sheep 18 was dosed with cobalt during Period 14-17. The digestibility

of/

of Ether extract of both sheep 14 and sheep 18 were high and was in good agreement with the values obtained by Franklin. It was noticed that addition of cobalt to the diet of sheep 18 did not have any effect on the digestibility of Ether extract. It would appear that the Ether extract of flaked maize is highly digestible as the figures were consistently high with both the sheep.

It is interesting to note that from Periods 5-13 the digestibility of crude fibre of sheep 18 remained very low varying from 9.77% to 34.82%. In Period 10, when only 394.46 gms. of crude fibre was fed, the digestibility of crude fibre was 13.69% while during period 7 when the total amount of fibre fed was 741.54 gms., the corresponding digestibility figure was 12.76%. Thus it would appear thatin cobalt deficient diets, even if the amount of fibre is fed in increasing quantity, the digestibility of crude fibre remains low. The digestibility of crude fibre dropped gradually from 34.06% in Period 5 to 9.77% in Period 13 in case of sheep 18. It would suggest that sheep 18 was unable to digest the crude fibre of the cobalt deficient diet to any appreciable extent. With sheep 14 also, the digestibility of crude fibre during Periods 5-13 was consistently low. Excepting Period 7, it varied from 8.21% to 36.75%. In Period 7 as already mentioned, the animal had a tendency to scouring and so was off its feed and ate only 353.63 gms. of fibre during the whole period, the digestibility of crude fibre being very low. From the Tables it can/

can be easily seen that with both sheep, the digestibility of crude fibre was of a low order. During Periods 14-17, the digestibility of crude fibre of sheep 14 varied from 20.83% to 39.89%.

In period 14 when cobalt was added to the diet of of sheep 18, the digestibility of crude fibre improved to 40.51%. In period 15 also it remained at almost the same figure. In Period 16 the digestibility of crude fibre improved to 62.36% and to 58.57% in Period 17. Thus it would appear that during Periods 14-17, the addition of cobalt to the diet of sheep 18 had a beneficial action on the digestibility of crude fibre.

It is interesting to note that Franklin also obtained low digestibility figures in the case of crude fibre - 12.15% to 36.21% - in his digestibility trials with sheep on a diet consisting of hay and flaked maize and it is quite possible that his ration was also cobalt deficient unless the cobalt content of the hay he fed was unusually high.

No attempt has been made to draw any definite conclusions from the digestion coefficients of total ash. The digestibility of total ash in the case of sheep 18 varied from 20.63% to 64.32% while in the case of sheep 14 it varied from 23.84% to 55.23%.

The digestibility of organic matter in the case of sheep 18 during Period 5 was 59.53%. During Periods 6-13, the digestibility was between 67.91% to 80.75%. The digestibility of organic matter was the highest in Period 10 when 80.75% of organic matter was digested which/

which was probably due to high intake of organic matter. In the case of sheep 14, the digestibility of organic matter was 61.44% in Period 5. The digestibility of organic matter by sheep 14 varied from 71.36% to 84.40% during Periods 6-17. The highest figure of digestibility of organic matter from sheep 14 was obtained in Period 8 when the animal consumed the highest amount of organic matter during the digestion trials.

In Period 14, when sheep 18 was dosed with cobalt for the first time, the digestibility of organic matter showed a slight improvement to 81.18%. In period 15, it was not so high. Again in Period 16 and 17 it improved to 82.36% and 81.28% respectively.

The digestion coefficients of Nitrogen free extract for both the sheep compared very favourably with those obtained by Franklin whereas in the present investigation the figures varied from 70.24% to 89.68%, the figures obtained by Franklin varied from 73.13% to 89.41%.

In connection with the effect of mineral deficiencies on the digestibility of foodstuffs, it is worth mentioning the findings of Woodman and Evans (1930) who concluded that "Malnutrition on "pasturage of subnormal mineral content is due direct-"ly to the failure of the diet to supply the necessary "inorganic materials for constructional purposes and "for maintaining the normal balance of minerals in "the/

"the blood and tissues, and is not, even in part,
"to be ascribed to any indirect effect, such as is
"embodied in the suggestion that the mineral deficiency
"leads to under nutrition of the animal by causing a
"depression of its appetite and its capacity to digest
"the organic constituents of the herbage". Franklin (1935) also supported this view in respect of a deficiency of lime in the diet.

Although this finding is true in the case of major elements, the theory may not hold good in the case of trace elements. It is known that major elements like calcium and phosphorus do not take any active part in the normal digestive processes and as such the deficiency of calcium or phosphorus are not likely to depress the digestibility of the organic constituents. In the case of cobalt however it is quite possible that it is necessary for bacterial or enzyme activity in ruminant digestion. Therefore if the diet is deficient in cobalt, rumen digestion might be altered and the digestibility of the organic constituents might be depressed.

The results of the present investigation suggest that the digestion of crude fibre might be lowered in cobalt deficient diets. Both with sheep 14 and 18 during Periods 5-13, the mean digestibility of crude fibre was in the neighbourhood of 20%. During Periods 14-17, when sheep 18 was dosed with cobalt the digestibility of crude fibre in average was about 50%, although the diet was the same. The average digestion/

digestion coefficient of organic matter of sheep 18 during Periods 5-13 was about 74% which improved to about 80% during Periods 14-17 when it was dosed with cobalt, but this is probably not significant. It would appear that the digestion of crude fibre is improved with the addition of cobalt.

Cobalt Balance - Excretion and Retention of Cobalt in Sheep

During the digestibility trials with sheep on cobalt deficient diets, it was decided to determine the cobalt balance of the sheep. As the sheep were being fed cobalt deficient diets, it was thought that data from cobalt balances might reveal some interesting aspects of cobalt metabolism in the body especially since no such data are available in the literature and the present investigation allowed of the collection of this important information. In view of the fundamental importance of such data for the complete understanding of the physiological rôle of cobalt in the animal body and because of the minuteness of the amounts being assayed, all possible care was taken to ensure that there could be no contamination of the samples from extraneous sources of cobalt.

The method of collecting urine for analysis was as follows. One fifth of each day's urine from sheep 18 and sheep 14 were dried daily in two separate vitreosil basins on a stainless steel waterbath. The period sample was formed by drying 14 days urine in this way. The urine was finally dried in a stainless steel/

Table 15.

Cobalt Content of Faeces (Parts per million - dry matter basis).

Period	Faeces	
	Sheep 14 p.p.m. C_O.	Sheep 18 p.p.m. C_O.
12	0.22	0.30
13	1.20	0.50
14	0.25	17.00
15	0.23	22.00
16	0.31	19.00
17	0.41	25.00

Cobalt Content of the Ash of Urine (Parts per million)

Period	Ash of Urine	
	Sheep 14	Sheep 18
12	0.11	-
13	0.16	0.16
14	0.09	13.00
15	0.22	48.00
16	0.17	39.00
17	0.16	20.00

Cobalt content of the Foodstuffs in Experiment (Parts per million - dry matter basis).

	Hay	Flaked Maize
C_O in p.p.m.	0.10	0.025

Cobalt Balance

Table 16 Sheep 14

Period	Feed in - take mg.	Period cobalt supple- ment mg.	Net Consumption mg.	Excreted in faeces mg.	Excreted in urine mg.	Total excretion mg.	Period balance mg.
12	0.37	-	0.37	0.53	0.008	0.54	-0.17
13	0.31	-	0.31	2.22	0.007	2.23	-1.92
14	0.29	-	0.29	0.44	0.005	0.45	-0.16
15	0.31	-	0.31	0.35	0.013	0.36	-0.05
16	0.28	-	0.28	0.51	0.009	0.52	-0.24
17	0.29	-	0.29	0.52	0.012	0.53	-0.24

Table 17. Sheep 18

Period	Feed in- take mg.	Period cobalt supple- ment mg.	Net Consumption mg.	Excreted in Faeces mg.	Excreted in Urine mg.	Total excretion mg.	Period balance mg.
12	0.33	-	0.33	0.61	-	-	-
13	0.24	-	0.24	0.75	0.004	0.75	-0.51
14	0.27	41.20	41.47	22.80	0.84	23.64	+17.83
15	0.28	41.20	41.48	36.70	2.90	39.60	+1.88
16	0.30	41.20	41.50	23.01	1.44	24.45	+17.05
17	0.34	41.20	41.54	36.18	1.52	37.80	+3.74

Table 18.

Period Totals of Faeces, Urine and Ration

Sheep 14

Period	Faeces 100% Dry Matter g.	Urine c.c	Ration	
			Hay g.	Flaked Maize g.
12	2408.74	6200	1866	7341
13	1856.33	5580	1480	6460
14	1753.63	2830	1440	5980
15	1527.83	2570	1554	6070
16	1630.15	4715	1524	5253
17	1257.73	4340	1567	5391

Sheep 18

Period	Faeces 100% Dry Matter g.	Urine c.c.	Ration	
			Hay g.	Flaked Maize g.
12	2042.45	5405	1521	7035
13	1502.65	3370	1064	5433
14	1341.15	4390	1319	5645
15	1668.32	3100	1412	5410
16	1211.12	4730	1777	4930
17	1446.54	6085	2012	5410

steel oven and weighed till constant weight. The dried urine was ashed at a very low temperature and the percentage ash of urine was determined.

Stainless steel watertroughs specially constructed for cobalt balance experiments were used so that drinking water might not be contaminated. The metabolism cage was kept scrupulously clean so that no rusting of iron fittings could occur. Paper was spread out on the floor to collect any foodstuffs which might be thrown down and the papers were changed frequently so that there was no contamination of the food material from the dust on the ground.

Period samples of food, faeces and ash of urine were kept in specially cleaned glass bottles and despatched to the Macaulay Institute for Soil Research, Aberdeen where they were spectrographically assayed for cobalt by Dr. R. L. Mitchell to whom I am indebted for results shown in Table 15. The cobalt content of the faeces, ash of urine and the hay and flaked maize are given in Table 15.

The cobalt balances of sheep 14 and 18 are summarised in Tables 16 and 17 and the Period Totals of faeces, urine and ration are shown in Table 18.

From Tables 16 and 17, it is found that both sheep 14 and sheep 18 were in negative cobalt balance during Periods 12 and 13. Unfortunately the urine ash of sheep 18 was lost in Period 12, but from the intake value and faeces value, it can be easily seen that the cobalt balance of sheep 18 in Period 12 would have been/

been of the same order as that of Period 13.

It is interesting to note that in Period 14 when sheep 18 was dosed for the first time with 41.2 mgms. of cobalt, it showed a positive balance of +17.83 mgms. with sheep 14 still showing a negative balance of -0.16 mgms. Sheep 18 in Period 15 showed a positive balance of +1.88mgms. only, while sheep 14 was in negative balance of -.05mgm. At the end of Period 15 both Sheep 18 and Sheep 14 were taken out of the metabolism cages and were kept in pens on the usual cobalt deficient diet. Sheep 18 was not dosed with cobalt during this period. After a fortnight's break both the sheep were again put back in the metabolism cages. In Period 16 when the sheep 18 was dosed again with 41.2 mgms. of cobalt, the animal showed a positive balance of +17.05 mgms., sheep 14 still showing a negative balance of -.24 mgm. In Period 17, sheep 18 showed a positive balance of +3.74 mgms. while sheep 14 again had a negative balance of -.24 mgm.

Thus while sheep 14 was in negative balance throughout the experimental Periods 12-17, sheep 18, which had a negative cobalt balance of the same order of sheep 14 during Periods 12 and 13, was in positive cobalt balance during Periods 14-17 when it was dosed with cobalt solution.

In the discussion of cobalt balance, we must take into account the possible ingestion of very minute amounts of cobalt from the drinking water. The average consumption of drinking water was approximately three/

three litres per day or 42 litres per fortnight or Experimental Period. It was found impossible to have the drinking water assayed for cobalt and although only an incredibly small content would be expected, it will be seen from Tables 16 and 17 that if the water contained from 4 to 6 parts per thousand million, the animals on the cobalt deficient diet would have been in cobalt equilibrium. It is unlikely that the drinking water contained quite as much as this in the light of the content of major elements in drinking water. Therefore I think we are justified in assuming that sheep 14 during Periods 12-17 and Sheep 18 during Periods 12 and 13 were in negative balance but it is quite possible that the degree of the negative balance may only have been the amount of cobalt appearing in the urine. It is not considered possible that the degree of the positive cobalt balances of sheep 18 during Periods 14-17, could have been altered significantly if we had been able to give the amount of cobalt in the drinking water for these periods.

Excretion of Cobalt -

From Tables 16 and 17, it can be seen that in the case of sheep 18 during Periods 14-17, when the animal was dosed with 41.2 mgms. of cobalt, the faeces excreted from 22.80-36.70 mgms. of cobalt while the cobalt excreted in urine varied from 0.84-2.90 mgms. only. In Period 13 when the sheep 18 was on cobalt deficient diet, the amount of cobalt excreted in faeces/

faeces was 0.75 mgm. while that of the urine was only .004mgm. With sheep 14 also during Periods 12-17, the amount of cobalt excreted in faeces varied from 0.35 to 2.22 mgms. while that of urine was about .01 mgm. only during these Periods.

Comparing the figures of cobalt excreted in faeces and urine in the case of both sheep 18 and sheep 14, it is seen that the large intestine is the main path of excretion of cobalt in sheep.

From Table 15, it can be seen that cobalt content of faeces of sheep 14 during Periods 12-17 was varying from 1.20 - 0.22 parts per million and that of urine ash varying from 0.09 to 0.22 parts per million. In the case of sheep 18, the cobalt contents of faeces were 0.30 and 0.50 parts per million in Periods 12 and 13 respectively. During Periods 14-17, when sheep 18 was dosed with cobalt, the cobalt content of faeces varied from 17.00 to 25.00 parts per million which indicated the excretion of orally administered cobalt. The cobalt content of urine ash of sheep 18 which was 0.16 parts per million, varied from 13.00 to 48.00 parts per million, during periods 14-17, indicating thereby that a small percentage of administered cobalt is excreted in the urine also.

Although the major portion of administered cobalt is excreted, it is possible that it is at first assimilated in the alimentary tract and then re-excreted.

The cobalt balance experiment has revealed an interesting/

interesting aspect of cobalt metabolism.

From Tables 16 and 17, it can be seen that sheep 14 during Periods 12-17 and sheep 18 during Periods 12 and 13 were receiving about 0.30 mg. of cobalt in each Period and were possibly in negative cobalt balance. Sheep 18 during Periods 14-17, when it is was dosed with 41.2 mgms. of cobalt, was in a positive cobalt balance indicating that it was utilising the extra cobalt that was fed for its metabolic purposes. It has been previously mentioned that the digestion of crude fibre of sheep 18, during Periods 14-17 when it received about 3 mgms. of cobalt a day, increased to a great extent. It might be suggested that crude fibre digestion was influenced by the additional cobalt.

It is also found that sheep kept on cobalt deficient diet, do absorb a certain amount of cobalt if given by mouth as is indicated by the positive balance of sheep 18 during Periods 14-17 when it was dosed with cobalt. From the literature reviewed at the beginning of this thesis it would appear that cobalt stored as a reserve in the liver or other tissues is of little subsequent use in the process of rumination yet in the present experiment quite a large amount was being stored. This would suggest that cobalt might play another rôle in physiology as well as an enzymic one in the rumen.

Calcium Balance

Calcium balances of sheep 14 and 18 are summarised in/

Table 19.

Calcium Balance

Sheep 14

Period	Intake of CaO from food. g.	Excreted in faeces g.	Excreted in urine g.	Total excretion g.	Period balance g.
1	54.64	47.94	.77	48.71	+5.93
2	62.03	70.75	1.01	71.76	-9.73
3	67.74	64.08	1.19	65.27	+2.47
4	68.58	65.68	1.20	66.88	+1.70
5	12.93	24.00	0.65	24.65	-11.72
6	14.90	19.40	0.80	20.20	-5.30
7	13.31	21.39	1.59	22.98	-9.67
8	16.09	13.99	1.53	15.52	+0.57
9	26.82	12.90	0.90	13.80	+13.02
10	31.29	20.26	0.50	20.76	+10.53
11	27.01	12.44	0.30	12.74	+14.27
12	26.00	19.99	1.73	21.72	+4.28
13	21.26	13.18	1.40	14.58	+6.68
14	20.38	11.75	0.48	12.23	+8.15
15	19.43	11.00	0.46	11.46	+7.97
16	18.35	14.34	0.80	15.14	+3.21
17	18.87	14.22	0.74	14.96	+3.91

Table 20

Calcium Balance

Sheep 18

Period	Intake of CaO from food. g.	Excreted in faeces g.	Excreted in urine g.	Total excretion g.	Period balance g.
1	58.60	50.98	1.64	52.62	+5.98
2	60.18	57.88	0.54	58.42	+1.76
3	60.68	55.64	0.49	56.13	+4.55
4	65.80	54.87	0.32	55.19	+10.61
5	11.94	21.90	0.33	22.23	-10.29
6	14.57	19.31	0.45	19.76	-5.19
7	22.08	22.84	0.65	23.49	-1.41
8	19.47	19.33	0.75	20.08	-0.61
9	20.45	14.69	0.74	15.43	+5.02
10	18.80	12.88	0.45	13.33	+5.47
11	18.33	9.07	0.18	9.25	+9.08
12	22.24	12.46	0.38	12.84	+9.40
13	16.07	9.17	0.54	9.71	+6.36
14	18.83	10.19	0.13	10.32	+8.51
15	17.55	11.83	0.25	12.08	+5.47
16	20.21	8.36	0.57	8.93	+11.28
17	22.71	16.64	1.03	17.67	+5.04

in Tables 19 and 20. From the Table 20 it can be seen that sheep 18 was in positive calcium balance during Periods 1-4. Sheep 14 was also in positive balance during the Periods except in Period 2. During Periods 1-4 only hay was fed and as such the intake of calcium was fairly high, probably due to which the animals showed positive calcium balance in most cases. The intake of calcium in case of sheep 18 varied from 58.60-65.80 gms. and that of sheep 14 varied from 54.64 to 68.58 gms. during Periods 1-4.

In Period 5 when the ration was changed to flaked maize and hay both animals showed a sharp negative balance. In succeeding Periods both tended to move towards a state of calcium equilibrium. With sheep 18, the calcium balances in Periods 5, 6, 7 and 8 were -10.29, -5.19, -1.41 and -0.61 respectively. From Periods 9-13, sheep 18 showed a positive calcium balance varying from +5.02 to 9.40 gms. During Periods 14-17, when the sheep 18 was dosed with cobalt, the animal showed a positive calcium balance varying from 5.04 to 11.28 gms.

With sheep 14, the calcium balance was negative during Periods 5-7 and in Period 8, sheep 14 was almost in calcium equilibrium. In Period 13, the animal showed a positive calcium balance of +13.02 due probably to high intake of calcium. In Period 10 when the calcium intake was 27.01 gms., the calcium balance was +14.27 in the case of sheep 18. Thus with large intake of calcium in the cobalt deficient diet/

diet, the animal showed more calcium retention. During Periods 8-17, Sheep 14 showed a positive calcium balance throughout.

During the Periods when the animals were in negative calcium balance, it is quite apparent that the animals must have been drawing on their reserves from their bones. It is also interesting to note the degree of adaptability possessed by the animals.

During Periods 1-4 when the animals were getting an adequate supply of calcium from the hay, they were excreting a large amount. During Periods 5-8, when the diet was changed to flaked maize and hay the animals gradually moved towards calcium equilibrium even though the calcium intake was very low. From Periods 9 onwards both animals showed a positive calcium balance. Franklin (1935) observed that there are at least two forces at work striving to preserve a physiological balance of lime salts in the animal. The one tends to adjust the calcium balances to a state of equilibrium or a positive state even when the food intake is very low, either by more efficient absorption and utilisation of the lime in the food or else by decreased re-excretion through the intestine or in other ways; while the other draws on the reserve supplies in the animal when the deficiency of the blood and other organs becomes serious. The present work confirms this finding.

Comparing the values of calcium excreted in faeces and in urine, it would be noticed that in the case/

case of both the sheep, the amount of calcium excreted in the urine remained very low and the calcium balances varied according to the excretion of calcium in the faeces.

During Periods 14-17. when sheep 18 was dosed with cobalt, the animal showed a positive calcium balance. Although addition of cobalt may not have any direct bearing on the retention of calcium in the body, it had no apparent adverse effect on calcium metabolism in these experiments.

Summary.

This thesis, after a brief review of the literature dealing with the effects of cobalt deficiency in animals and especially the physiological rôle of cobalt describes experiments designed to clarify certain aspects of these problems.

The conclusions arrived at from the present investigation are as follows:-

(a) That lambs grazing cobalt dressed pasture eat more grass than lambs grazing cobalt deficient pasture of equal nutritive value as far as proteins, fats and carbohydrates are concerned. This was established by grazing experiments with groups of lambs equipped with a suitable harness which allowed of the daily measurement of faeces excreted.

(b) That the difference in the amount of nutrients eaten by the two groups would suggest that the clinical symptoms of 'Pining' - the diseased condition associated with cobalt deficiency - were those of malnutrition.

(c) That the digestion of crude fibre by sheep on cobalt deficient diets is of a low order and can be increased by the oral administration of cobalt salts.

(d) That the digestion of the other organic nutrients of the ration is not materially altered by a low cobalt intake.

(e) That a daily diet containing 0.02 mg. of cobalt/

cobalt does not allow of the animal being in a state of positive cobalt balance but that the addition of 3 mg. of cobalt per day changes a negative cobalt balance to a positive one with the animal retaining a large proportion of the 3 mgs. of cobalt.

(f) That the major portion of cobalt ingested is excreted in the faeces and only minute amounts in the urine.

(g) That all the data obtained in the present series of experiments would support the theory that the physiological function of cobalt is connected with rumination.

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Appendix

Digestion Tables

Sheep 14

	Crude protein g.	Ether extract g.	Crude fibre g.	Total Ash g.	Organic matter g.	Nitrogen free extractives g.
Period 1.						
Net Consumption	347.88	69.37	2053.13	210.20	5044.80	2574.42
Voided	385.68	53.24	766.78	147.43	2260.67	1055.86
Digested	-	16.13	1286.35	62.77	2784.13	1518.56
Digestion co-efficients (%)	-	23.25	62.11	29.86	55.18	58.98
Period 2.						
Net Consumption	394.82	78.72	2330.14	238.56	5725.44	2921.76
Voided	407.62	52.78	845.59	167.11	2472.89	1166.88
Digested		25.94	1484.55	71.45	3252.55	1754.88
Digestion co-efficients (%)		32.95	63.71	29.95	56.80	60.06
Period 3.						
Net Consumption	431.16	85.97	2546.73	260.52	6252.48	3190.72
Voided	399.25	75.25	1078.47	216.55	3069.45	1516.49
Digested	31.91	10.72	1468.26	43.97	3183.03	1674.23
Digestion co-efficients (%)	7.40	12.47	57.65	16.88	50.90	52.47
Period 4.						
Net Consumption	436.52	87.04	2576.28	263.76	6330.24	3230.40
Voided	337.08	60.56	988.43	196.41	3007.59	1537.28
Digested	99.44	26.48	1587.85	67.35	3322.65	1693.12
Digestion co-efficients (%)	22.78	30.42	61.63	25.53	52.49	52.41

	Crude Protein g.	Ether extract g.	Crude fibre g.	Total ash g.	Organic matter g.	Nitrogen free extractives g.
Period 5						
Net Consumption	221.86	108.13	446.36	94.71	3054.89	2278.37
Voided	122.25	26.50	377.13	72.13	1177.88	625.13
Digested	99.61	81.63	69.23	22.58	1877,01	1653.24
Digestion co-efficients (%)	44.90	75.50	15.51	23.84	61.44	72.60
Period 6						
Net Consumption	366.19	179.22	466.39	119.40	4877.60	3865.69
Voided	166.45	43.08	378.79	68.37	1269.63	681.31
Digested	199.74	136.14	87.60	51.03	3607.97	3184.38
Digestion co-efficients (%)	54.54	75.96	18.80	42.74	73.98	82.38
Period 7						
Net Consumption	471.73	239.98	353.63	119.55	6133.45	5068.07
Voided	352.82	60.83	349.06	86.46	1430.53	771.84
Digested	118.91	179.15	4.57	33.09	4702.92	4296.23
Digestion co-efficients (%)	25.21	74.65	1.29	27.69	76.67	84.77
Period 8						
Net Consumption	715.52	370.32	364.21	157.45	9198.54	7748.49
Voided	377.31	74.39	334.30	86.23	1435.40	820.45
Digested	338.21	295.93	29.91	71.22	7763.14	6928.04
Digestion co-efficients (%)	47.27	79.91	8.21	45.23	84.40	89.41
Period 9						
Net Consumption	557.39	266.41	883.89	205.87	7530.14	5822.44
Voided	295.69	63.05	559.04	120.98	1926.10	1023.70
Digested	261.70	203.36	324.85	84.89	5604.04	4798.74
Digestion co-efficients (%)	46.95	76.33	36.75	41.23	74.40	82.42

	Crude protein g.	Ether extract g.	Crude fibre g.	Total ash g.	Organic matter g.	Nitrogen free extractives g.
Period 10						
Net Consumption	626.48	297.40	1051.94	238.11	8492.89	6526.82
Voided	292.81	78.75	768.72	123.56	2408.44	1218.65
Digested	333.67	218.65	283.22	114.55	6084.45	5308.17
Digestion co-efficients (%)	53.26	73.52	27.88	48.11	71.64	81.33
Period 11						
Net Consumption	572.10	274.23	885.76	208.33	7715.67	5983.60
Voided	394.19	104.56	606.38	93.27	2209.73	1100.37
Digested	177.91	169.67	279.38	115.06	5505.94	4883.23
Digestion co-efficients (%)	31.10	61.87	31.54	55.23	71.36	81.61
Period 12						
Net Consumption	678.77	334.48	796.93	211.90	8995.11	7185.33
Voided	330.69	132.01	588.04	149.34	2259.16	1124.04
Digested	348.08	202.47	208.89	62.56	6735.95	6061.29
Digestion co-efficients (%)	51.28	60.53	26.21	29.52	74.88	84.49
Period 13						
Net Consumption	587.96	291.88	637.11	176.23	7763.77	6246.83
Voided	271.31	56.61	515.04	91.86	1764.13	913.71
Digested	316.65	235.27	122.07	84.37	5999.64	5333.12
Digestion co-efficients (%)	53.85	80.60	19.16	47.87	77.28	85.37
Period 14						
Net Consumption	548.15	271.25	617.48	167.55	7252.45	5815.55
Voided	261.94	56.83	488.84	91.38	1662.62	864.55
Digested	268.21	214.42	128.64	76.17	5589.83	4951.00
Digestion co-efficients (%)	52.21	79.05	20.83	45.46	77.07	85.13

	Crude Protein g.	Ether extract g.	Crude fibre g.	Total ash g.	Organic matter g.	Nitrogen free extractives g.
Period 15						
Net Consumption	563.41	250.78	735.24	154.87	7469.12	5919.68
Voided	199.38	51.65	509.43	76.40	1451.60	681.95
Digested	364.03	199.13	225.81	78.47	6017.52	5237.73
Digestion co-efficients (%)	64.61	79.40	30.71	50.67	80.56	88.48
Period 16						
Net Consumption	497.12	219.60	710.83	143.42	6633.58	5205.94
Voided	173.75	56.72	553.06	88.18	1541.82	714.92
Digestion	323.37	162.88	157.77	55.24	5091.76	4491.02
Digestion co-efficients (%)	65.05	74.17	22.19	38.51	76.76	86.26
Period 17						
Net Consumption	510.33	225.51	730.74	147.34	6810.55	5344.08
Voided	237.38	48.02	439.20	78.31	1178.69	551.26
Digested	272.95	177.49	291.54	69.03	5631.86	4792.82
Digestion co-efficients (%)	53.48	78.71	39.89	46.85	82.69	89.68

Digestion Tables

Sheep 18

	Crude protein g.	Ether extract g.	Crude fibre g.	Total ash g.	Organic matter g.	Nitrogen free extractives g.
Period 1						
Net Consumption	373.04	74.37	2201.59	225.40	5409.60	2760.59
Voided	429.71	54.98	818.05	162.84	2506.19	1203.45
Digested	-	19.39	1383.54	62.56	2903.41	1557.14
Digestion co-efficients (%)	-	26.09	62.84	27.75	53.67	56.41
Period 2.						
Net Consumption	383.1	76.39	2260.99	231.48	5555.52	2835.05
Voided	427.76	54.92	857.94	185.48	2506.52	1165.91
Digested	-	21.47	1403.05	46.00	3049.00	1669.14
Digestion co-efficients (%)	-	28.11	62.05	19.80	54.88	58.88
Period 3						
Net Consumption	386.28	77.02	2279.73	241.40	5601.60	2858.57
Voided	353.75	65.60	834.28	179.92	2588.08	1334.45
Digested	32.53	11.42	1445.45	61.48	3013.52	1524.12
Digestion co-efficients (%)	8.52	14.82	63.44	25.47	53.79	53.32
Period 4						
Net Consumption	418.85	83.52	2471.96	253.08	6073.92	3099.60
Voided	327.63	58.53	948.13	182.23	2705.77	1335.12
Digested	91.22	24.99	1523.83	70.85	3368.15	1764.48
Digestion co-efficients (%)	21.77	30.00	61.64	27.99	55.45	56.09

	Crude protein g.	Ether extract g.	Crude fibre g.	Total ash g.	Organic matter g.	Nitrogen free extractives g.
Period 5						
Net Consumption	153.23	69.38	435.14	83.24	2186.76	1529.12
Voided	88.78	20.09	268.93	66.07	884.93	455.06
Digested	64.45	49.29	148.21	17.17	1301.83	1074.06
Digestion co-efficients (%)	42.06	71.04	34.06	20.63	59.53	70.24
Period 6						
Net Consumption	293.53	139.60	484.09	110.94	3976.61	3059.40
Voided	108.23	20.84	315.50	60.34	1036.67	554.20
Digested	185.30	118.76	168.59	50.60	2939.94	2505.20
Digestion co-efficients (%)	63.13	85.07	34.82	45.61	73.90	81.88
Period 7						
Net Consumption	427.31	201.91	741.54	166.70	5812.31	4441.48
Voided	236.14	42.55	646.89	86.67	1865.23	993.18
Digested	191.17	159.36	94.65	80.03	3947.08	3448.30
Digestion co-efficients (%)	44.74	78.92	12.76	48.00	67.91	77.64
Period 8						
Net Consumption	574.23	287.06	567.80	164.66	7548.44	6120.34
Voided	297.25	57.07	480.87	99.05	1741.95	976.65
Digested	276.98	229.99	86.93	65.61	5806.49	5143.69
Digestion co-efficients (%)	48.23	80.12	15.31	39.84	76.92	84.04
Period 9						
Net Consumption	584.19	291.05	604.72	171.17	7695.99	6216.87
Voided	323.00	57.20	458.27	85.54	1642.46	922.75
Digested	261.19	233.85	146.45	85.63	6053.53	5294.12
Digestion co-efficients (%)	44.71	80.34	24.61	50.02	78.66	85.11

Sheep 18 - continued

	Crude protein g.	Ether extract g.	Crude fibre g.	Total ash g.	Organic Matter g.	Nitrogen free extractives g.
Period 10						
Net Consumption	601.70	303.22	394.46	163.32	7870.68	6436.58
Voided	263.44	51.55	340.47	76.05	1514.95	953.65
Digested	338.26	251.67	53.99	87.27	6355.73	5482.93
Digestion co-efficients (%)	56.21	82.99	13.69	53.43	80.75	85.18
P eriod 11						
Net Consumption	524.41	261.31	541.69	153.50	6918.37	5581.10
Voided	217.88	71.35	432.03	65.36	1645.64	937.97
Digested	306.53	189.96	109.66	88.14	5272.73	4643.13
Digestion co-efficients (%)	58.45	72.69	20.25	57.42	76.21	83.19
Period 12						
Net Consumption	635.26	316.48	657.90	186.18	8369.82	6760.20
Voided	254.56	64.53	571.56	69.84	1972.16	1074.91
Digested	380.70	251.95	86.34	116.34	6397.66	5685.29
Digestion co-efficients (%)	59.92	79.64	13.12	62.47	76.44	84.10
Period 13						
Net Consumption	484.45	242.73	464.26	136.79	6360.21	5168.77
Voided	165.31	45.84	418.89	59.52	1443.48	780.66
Digested	319.14	196.89	45.37	77.27	4916.73	4388.11
Digestion co-efficients (%)	65.88	81.11	9.77	56.49	77.30	84.89
Period 14						
Net Consumption	515.21	255.45	566.92	155.62	6808.38	5470.81
Voided	170.13	51.23	337.26	59.41	1281.59	712.21
Digested	345.08	204.22	229.66	96.21	5526.79	4758.60
Digestion co-efficients (%)	66.98	79.95	40.51	61.82	81.18	86.98

Sheep 18 - continued

	Crude protein g.	Ether extract g.	Crude fibre g.	Total ash g.	Organic matter g.	Nitrogen free extractives g.
Period 15						
Net Consumption	503.59	227.26	666.72	139.45	6681.56	5288.53
Voided	232.88	54.78	406.49	69.56	1598.44	929.08
Digested	270.71	172.48	260.23	69.89	5083.12	4359.45
Digestion co-efficients (%)	53.76	75.89	39.03	50.12	76.08	82.43
Period 16						
Net Consumption	485.00	211.36	811.81	152.76	6554.23	5046.50
Voided	155.63	42.02	305.54	54.50	1156.51	630.08
Digested	329.37	169.34	506.27	98.26	5397.72	4416.42
Digestion co-efficients (%)	67.91	80.12	62.36	64.32	82.36	81.37
Period 17						
Net Consumption	535.51	232.86	916.18	170.89	7251.11	5566.57
Voided	255.06	52.09	379.55	89.28	1357.72	704.83
Digested	280.45	180.77	536.63	81.61	5893.39	4861.74
Digested co-efficients (%)	52.37	77.06	58.57	47.75	81.28	87.34

Composition of Faeces

(On Basis of Dry Matter)

Sheep 14.

Period	Crude Protein %	Ether Extract %	Crude Fibre %	Total Ash %	Organic Matter %	N-free Extractives %
1	16.01	2.21	31.83	6.12	93.88	43.83
2	15.44	2.00	32.03	6.33	93.67	44.20
3	12.15	2.29	32.82	6.59	93.41	46.15
4	11.76	1.97	32.16	6.13	93.87	47.98
5	11.93	2.12	30.17	5.77	94.23	50.01
6	12.44	3.22	28.31	5.11	94.89	50.92
7	15.29	4.01	23.01	5.70	94.30	50.88
8	14.42	4.84	21.75	5.61	93.39	53.38
9	13.69	3.08	27.31	5.91	94.09	50.01
10	13.52	3.11	30.36	4.88	95.12	48.13
11	17.30	4.54	26.33	4.05	95.95	47.78
12	17.23	5.48	24.41	6.22	93.78	46.66
13	15.02	3.05	27.75	4.95	95.05	49.23
14	14.39	3.24	27.87	5.21	94.79	49.29
15	13.65	3.38	33.34	5.00	95.00	44.63
16	13.32	3.48	33.93	5.41	94.59	43.86
17	11.19	3.82	34.94	6.23	93.77	43.82

Composition of Faeces

(On Basis of Dry Matter)

Sheep 18

Period	Crude Protein %	Ether Extract %	Crude Fibre %	Total Ash %	Organic Matter %	N-free Extractives %
1	16.10	2.06	30.65	6.10	93.90	45.09
2	15.89	2.04	31.87	6.89	93.11	43.31
3	12.78	2.37	30.14	6.50	93.50	48.21
4	12.60	2.03	32.83	6.31	93.69	46.23
5	12.90	2.22	30.14	6.94	93.06	47.80
6	13.32	1.90	28.76	5.50	94.50	50.52
7	12.79	2.18	33.14	4.44	95.56	47.45
8	11.40	3.10	27.07	5.38	94.62	53.05
9	11.82	3.31	26.52	4.95	95.05	53.40
10	10.64	3.24	21.40	4.78	95.22	59.94
11	11.94	4.17	25.25	3.82	96.18	54.82
12	12.79	3.16	27.99	3.42	96.58	52.64
13	13.18	3.05	27.87	3.96	96.04	51.94
14	13.49	3.82	25.15	4.43	95.57	53.11
15	12.47	3.29	24.37	4.17	95.83	55.70
16	14.77	3.47	25.23	4.50	95.50	52.03
17	15.29	3.60	26.23	6.17	93.83	48.71

Period Totals of Faeces, Urine and Ration.

Sheep 14

Period	Faeces 100% Dry Matter g.	Urine g.c.	Ration 100% Dry Matter Hay g.	Flaked Maize g.
1	2409.4	3654	5255	-
2	2639.8	3734	5964	-
3	3286.1	5190	6513	-
4	3203.9	5720	6594	-
5	1249.8	4325	1087	2063
6	1338.4	4465	1100	3897
7	1516.9	5485	784	5469
8	1537.1	6390	748	8608
9	2047.0	8990	2120	5616
10	2532,3	4995	2507	6224
11	2303.1	3025	2121	5803
12	2408.7	6200	1866	7341
13	1856.3	5580	1480	6460
14	1753.6	2830	1440	5980
15	1527.8	2570	1554	6070
16	1630.2	4715	1524	5253
17	1257.7	4340	1567	5391

Period Totals of Faeces, Urine and Ration

Sheep 18

Period	Faeces 100% Dry Matter g.	Urine c.c.	Ration 100% Dry Matter	
			Hay g.	Flaked Miaze g.
1	2668.7	6307	5635	-
2	2692.4	2325	5787	-
3	2768.3	2025	5836	-
4	2887.5	2130	6327	-
5	952.0	1970	1075	1195
6	1097.0	3025	1165	2923
7	1951.7	4320	1789	4190
8	1840.9	6210	1306	6408
9	1727.7	7420	1398	6470
10	1590.9	4460	1200	6834
11	1710.9	4595	1252	5810
12	2042.5	5405	1521	7035
13	1502.7	3370	1064	5433
14	1341.2	4390	1319	5645
15	1668.3	3100	1412	5410
16	1211.1	4730	1777	4930
17	1446.5	6085	2012	5410

Calcium Content of Faeces and Foodstuffs (On Dry Matter Basis) and Urine (per 100 c.c.)

Period	Sheep 18		Sheep 14		Foodstuffs	
	Faeces CaO %	Urine CaO %	Faeces CaO %	Urine CaO %	Hay CaO %	Flaked Maize CaO %
1	1.91	.026	1.99	.021	1.04	-
2	2.15	.023	2.68	.027	"	-
3	2.01	.024	1.95	.023	"	-
4	1.90	.015	2.05	.021	"	-
5	2.30	.017	1.92	.015	1.00	0.10
6	1.76	.015	1.45	.018	"	"
7	1.17	.015	1.41	.029	"	"
8	1.05	.012	.93	.024	"	"
9	.85	.01	.63	.01	"	"
10	.81	.01	.80	.01	"	"
11	.53	.004	.54	.01	"	"
12	.61	.007	.83	.028	"	"
13	.61	.016	.71	.025	"	"
14	.76	.003	.67	.017	"	"
15	.71	.008	.72	.018	0.86	0.10
16	.69	.012	.88	.017	"	"
17	1.15	.017	1.13	.017	"	"