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THE USE OF FAECAL ANALYSES TO ESTIMATE THE PHOSPHORUS INTAKE BY GRAZING SHEEP. I. THE USE OF POOL INSTEAD OF INDIVIDUAL SAMPLES

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ABSTRACT

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Analyses of rectal faeces of sheep for phosphorus, calcium and magnesium may be useful to indicate the mineral status of the herbage being consumed. A number of sheep should be used for each pasture, but this would entail a large number of individual analyses. In this experiment it was shown that a pool analysis of faeces did not differ significantly ($P<0,01$) from the arithmetic mean of the individual samples (20). In 13 replications this was true for phosphorus, calcium and magnesium, whether the sheep were non-pregnant, non-lactating, pregnant, or lactating. From the results it was estimated that 3 faecal pellets from at least 30 sheep should be used to make the pool.

Blood was also taken on 11 occasions for pool and individual (20) analyses. No significant difference was found for plasma inorganic phophate, magnesium and total protein, but on 3 occasions there was a significant difference ($P<0,01$) for plasma calcium.

Résumé

L'UTILISATION D'ANALYSES FAECALES POUR ESTIMER L'ABSORPTION EN PHOSPHORE DE MOUTONS EN PÂTURE. I. L'UTILISATION D'UN ECHANTILLONAGE EN MASSE À LA PLACE D'ÉCHANTILLONS INDIVIDUELS

Les analyses des crottes de moutons afin de déterminer leur teneur en phosphore, calcium et magnesium, peuvent être utiles pour indiquer le taux de ces mineraux dans l'herbage étant consommé par les animaux. Un certain nombre de moutons devrait être utilisé pour chaque pâturage, mais ceci demanderait un grand nombre d'analyses indivuelles. Dans cette expérience, il a été montré qu'une analyse de masse commune de matières faecales ne différait pas significativement ($P<0,01$) de la moyenne arithmétique obtenue d'échantillons individuels (20). Ceci se vérifia vrai pour le phosphore, le calcium et le magnesium dans 13 répétitions, que les moutons soient en état de gestation, de non lactation, en gestation ou en lactation. Il a été estimé à la suite des résultats obtenus que 3 crottes d'au moins 30 moutons devraient être utilisées pour faire une masse commune. Du sang fut aussi pris en 11 occasions pour des analyses communes et individuelles (20). Aucune différence significative ne fut trouvée pour le phosphate inorganique du plasma, le magnesium et la protéine totale, mais en 3 occasions, il y avait une différence significative ($P<0,01$) en ce qui concerne le calcium du plasma.

INTRODUCTION

It has been suggested (Belonje, 1978) that analyses of rectal faecal samples from sheep for phosphorus may be used to indicate the levels of this mineral in the pastures the sheep were grazing. It was also suggested that, to obtain a representative specimen, a number of sheep in the flock should be sampled and that these samples could be pooled before analysis to reduce the number of determinations and arrive at an average result.

In this series of experiments, we set out to determine whether in fact the analysis of a pool sample was the same as the arithmetic mean of the individual samples used to make the pool sample. This was done for both faeces and blood. As calcium and magnesium are intimately related to phosphorus, analyses of these elements were also included. In the first trial faecal samples were taken from 20 non-pregnant, non-lactating sheep in each of 5 flocks, and blood samples from 20 sheep in 3 flocks. This was followed by 5 weekly replications with 20 ewes in late pregnancy and 3 weekly replications with 20 ewes during peak lactation. The results gained were also used to estimate the optimum number of sheep and faecal pellets per sheep to be used to make the pool sample.

MATERIALS AND METHODS

Animals

Preliminary trial: Groups of 20 healthy, non-pregnant, non-lactating sheep selected at random from each of 5 different flocks were used.

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Trial on pregnant ewes: Twenty healthy pregnant Merino ewes were selected. The animals had been artificially inseminated over 3 days after oestrous synchronization. They were sampled during the 16th, 17th, 18th, 19th and 20th week of gestation. When they lambed down, 14 had single lambs, 4 had twins and 2 were not pregnant.

Trial on lactating ewes: Twenty healthy lactating Merino ewes, 17 with single lambs and 3 with twins, were selected. Samples were taken when the age of the lambs averaged 24,3 (± 3,5 SD), 31,3 (± 3,1 SD), and 38,3 (± 3,1 SD) days. These days covered the expected period of peak lactation.

Housing: The pregnant and the lactating ewes were housed in a concrete-floored pen with a roof and uncovered runway.

Feeding: The pregnant and lactating ewes received 22 and 44 kg respectively of a commercial complete pelleted diet based on lucerne, a diet which had been supplied throughout pregnancy. A small grab sample of each day's feed was taken for 7 days. This was repeated for each of the experimental weeks in which the animals were sampled. The pooled feed samples, each about 2 kg, were air-dried, milled finely, mixed well and stored for phosphorus, calcium and magnesium analyses.

The phosphorus, calcium and magnesium levels of the ration fed to the ewes in late gestation and peak lactation are given in Tables 1 & 2 respectively. Water was available at all times.

Body mass of the experimental animals was determined weekly (Tables 1 & 2).

TABLE 1 The phosphorus, calcium and magnesium levels of the ration fed to the ewes in late gestation and the mean (± SD) body mass of the ewes

Gestation days ration sampled	Phosphorus %	Calcium %	Magnesium %	Mean body mass (±SD) at end of week
107–113	0,37	1,07	0,34	52,0±5,85
114–120	0,34	1,05	0,32	50,9±6,10
121–127	0,33	1,08	0,31	52,4±6,45
128–134	0,35	0,90	0,35	53,4±6,83
135–141	0,38	1,12	0,32	54,1±6,92

TABLE 2 The phosphorus, calcium and magnesium levels of the ration fed to the lactating ewes and the mean (±SD) body mass of the ewes

Mean lactation days ration sampled	Phosphorus %	Calcium %	Magnesium %	Mean body mass (±SD) at end of week
18–24	0,38	1,27	0,37	48,8±7,61
25–31	0,37	1,17	0,36	46,5±7,50
32–38	0,38	1,75	0,40	49,1±7,63

Faecal samples: Rectal faeces were taken manually from the non-pregnant, non-lactating sheep. Within each group a pool specimen was made by combining 3 faecal pellets from each of the 20 animals. The individual and pooled specimens were air-dried, milled finely, mixed well and kept for phosphorus, calcium and magnesium analyses. Similar individual and pooled faecal samples were obtained for the groups of pregnant and lactating sheep.

Blood samples: Samples of jugular blood were taken in heparin (100 IU/10 mℓ blood) from 20 non-pregnant, non-lactating sheep at weekly intervals on 3 occasions. About 9 mℓ of blood was kept for individual analyses, while about 1 mℓ from each animal was combined in a pool sample. Plasma was removed after centrifugation for inorganic phosphate determinations which commenced within 30 minutes of bleeding. The remaining plasma was kept frozen for calcium and magnesium determinations. Similar individual and pooled blood samples were taken for the groups of pregnant and lactating sheep.

Processing of samples and analytical methods

Food and faecal samples: These were processed as outlined before (Belonje, 1978) and analysed for phosphorus by the photometric method of Hanson (1950). Calcium and magnesium were analysed by atomic absorption spectrophotometry using a nitrous oxide flame and potassium as an ionization suppressor (Varian Techtron Manual, 1972).

Plasma: Inorganic phosphate was determined by the photometric micromethod of Kallner (1975), calcium and magnesium by atomic absorption as described above, and total protein by the photometric biuret method of Weichselbaum (1946).

Statistical methods

These were calculated as outlined by Snedecor & Cochran (1967) as follows:

Standard deviation (SD)

$$SD = \sqrt{\frac{(x-\bar{x})^2}{n-1}}$$

where n = number of determinations
$\bar{x}$ = mean of the sample
x = each determination.

Standard error (SE)

$$SE = \frac{SD}{\sqrt{n}}$$

Student's t-test

This was used to determine whether there was a difference between the mean of the individual values and the pool value. The form:

$$t_{n-1} = \frac{\bar{x} - xp}{\sqrt{2}\,SE}$$

is obtained by using the same estimate for the standard errors of $\bar{x}$ and xp

where $\bar{x}$ = mean of the individual analyses
xp = the pool analysis
n = number of determinations.

Analysis of variance

This was employed to establish the optimum number of sheep and the number of faecal pellets per sheep to be used for pool sample, using the following formula:

$$\sqrt{\frac{S^2B + nS^2W}{Nn}}$$

where S^2B estimates the "between" component of variance
S^2W estimates the "within" component of variance
N = number of sheep
n = number of faecal pellets per sheep.

The results of the phosphorus analyses on the individual faecal samples taken on the 31st and 38th day of lactation were used for this estimate.

TABLE 3 The phosphorus, calcium and magnesium levels of individual and pooled faecal samples taken at random from 5 different flocks

Faecal analysis	Sample	No. of animals	Pool analyses (P)	Individual analyses (I)		Significance of difference between P and I
				Mean	SE	
Phosphorus %	1	20	0,75	0,73	0,03	Not significant
	2	20	1,19	1,14	0,04	Not significant
	3	20	0,82	0,85	0,02	Not significant
	4	20	1,06	1 04	0,06	Not significant
	5	20	0,86	0,85	0,03	Not significant
Calcium %	1	20	2,32	2,39	0,07	Not significant
	2	20	3,27	3,05	0,10	Not significant
	3	20	2,60	2,80	0,06	P< 0,05
	4	20	2,74	2,71	0,11	Not significant
	5	20	2,26	2,33	0,05	Not significant
Magnesium %	1	20	0,68	0,62	0,02	Not significant
	2	20	0,85	0,86	0,02	Not significant
	3	20	0,73	0,73	0,01	Not significant
	4	20	0,81	0,85	0,03	Not significant
	5	20	0,67	0,67	0,01	Not significant

TABLE 4 The inorganic phosphate, calcium, magnesium and total protein levels of individual and pooled plasma samples taken at random from 3 different flocks

Plasma analysis	Sample	No. of animals	Pool analyses (P)	Individual analyses (I)		Significance of difference between P and I
				Mean	SE	
Inorganic phosphate mg/100 mℓ	1	20	3,65	3,73	0,18	Not significant
	2	20	4,41	4,43	0,24	Not significant
	3	20	4,45	4,41	0,20	Not significant
Calcium mg/100 mℓ	1	20	9,37	9,34	0,14	Not significant
	2	20	10,42	9,42	0,17	P< 0,001
	3	20	9,86	9,27	0,13	P< 0,01
Magnesium mg/100 mℓ	1	20	2,69	2,57	0,03	Not significant
	2	20	2,54	2,56	0,05	Not significant
	3	20	2,64	2,55	0,05	Not significant
Total protein g/100 mℓ	1	20	6,83	6,62	0,08	Not significant
	2	20	6,47	6,26	0,08	Not significant
	3	20	6,33	6,29	0,09	Not significant

Results and Discussion

Trial on non-pregnant, non-lactating sheep taken from grazing flocks

In the trial on non-pregnant, non-lactating sheep taken from grazing flocks, no significant difference was found between the result of the pool analysis for phosphorus and the mean of the individual analyses in respect of either faeces (Table 3) or plasma (Table 4). This was true not only for the 5 replications on faeces but also for the 3 replications on blood plasma.

For some unknown reason the pool analysis (2,60%) for calcium in faeces differed significantly (P<0,05) from the mean (2,80%) of the individual analyses for one group of animals (Table 3). In the plasma analyses significant differences were recorded for 2 groups of animals. They were quite considerable, i.e. 10,42 vs 9,42 mg/100 mℓ (P<0,001) and 9,86 vs 9,27 mg/100 mℓ (P<0,01) (Table 4).

As in the case of phosphorus, the pool analysis for magnesium and total plasma protein was always an accurate reflection of the mean of the individual analyses in both faeces (Table 3) and blood plasma (Table 4).

Trial on pregnant ewes

The results of the trial on pregnant ewes were similar to those of the grazing non-pregnant, non-lactating sheep, in that there was no significant difference between the result of the pool analysis and the mean of the individual analyses for phosphorus, magnesium and total plasma protein in respect of faeces (Table 5) and blood plasma (Table 6). In the case of calcium, there was no significant difference in respect of the faeces, but in one group of animals the result of the plasma pool analysis (10,69 mg/100 mℓ) was significantly different (P<0,01) from the mean (9,43 mg/100 mℓ) of the individual plasma analyses.

Trial on lactating ewes

The results from the trial on ewes at peak lactation were also similar to those of the grazing non-pregnant, non-lactating sheep. No significant difference was found between the result of the pool analysis and the mean of the individual analyses for phosphorus, magnesium and total plasma protein in respect of faeces (Table 7) and blood plasma (Table 8). There was a significant difference (P<0,05) in the respect of

faecal calcium in one group of animals where the pool analysis (2,84%) was greater than the mean (2,68%) of the individual analyses. No significant differences were found between pool analysis and the mean of individual analyses for blood plasma calcium.

TABLE 5 The phosphorus, calcium and magnesium levels of individual and pooled faecal samples from ewes in late gestation

Faecal analysis	Day of gestation	No. of animals	Pool analyses (P)	Individual analyses (I)		Significance of difference between P and I
				Mean	SE	
Phosphorus %	113	20	0,69	0,60	0,04	Not significant
	120	15	0,69	0,68	0,03	Not significant
	127	15	0,62	0,61	0,03	Not significant
	134	20	0,54	0,53	0,02	Not significant
	141	20	0,77	0,77	0,04	Not significant
Calcium %	113	20	3,23	3,03	0,08	Not significant
	120	15	2,98	2,98	0,04	Not significant
	127	15	2,85	2,86	0,05	Not significant
	134	20	2,68	2,61	0,05	Not significant
	141	20	2,97	2,99	0,05	Not significant
Magnesium %	113	20	0,73	0,69	0,02	Not significant
	120	15	0,73	0,73	0,02	Not significant
	127	15	0,66	0,67	0,02	Not significant
	134	20	0,72	0,72	0,02	Not significant
	141	20	0,65	0,68	0,02	Not significant

TABLE 6 The inorganic phosphate, calcium, magnesium and total protein levels of individual and pooled plasma samples from ewes in late gestation

Plasma analysis	Day of gestation	No. of animals	Pool analyses (P)	Individual analyses (I)		Significance of difference between P and I
				Mean	SE	
Inorganic phosphate mg/100 mℓ	112	20	5,81	5,65	0,22	Not significant
	119	20	5,69	5,62	0,24	Not significant
	126	20	4,57	4,61	0,24	Not significant
	133	20	4,35	4,32	0,16	Not significant
	140	20	4,73	4,73	0,23	Not significant
Calcium mg/100 mℓ	112	20	10,69	9,43	0,19	$P<0,001$
	119	20	9,21	8,95	0,11	Not significant
	126	20	8,73	8,83	0,21	Not significant
	133	20	8,85	9,13	0,15	Not significant
	140	20	9,10	9,24	0,13	Not significant
Magnesium mg/100 mℓ	112	20	2,66	2,70	0,04	Not significant
	119	20	2,62	2,52	0,04	Not significant
	126	20	2,69	2,58	0,04	Not significant
	133	20	2,69	2,61	0,04	Not significant
	140	20	2,40	2,44	0,04	Not significant
Total protein g/100 mℓ	112	20	6,00	6,13	0,11	Not significant
	119	20	5,94	6,03	0,10	Not significant
	126	20	6,22	5,99	0,10	Not significant
	133	20	6,29	6,20	0,11	Not significant
	140	20	6,61	6,48	0,09	Not significant

TABLE 7 The phosphorus, calcium and magnesium levels of individual and pooled faecal samples from lactating ewes

Faeces analysis	Mean day of lactation	No. of animals	Pool analyses (P)	Individual analyses (I)		Significance of difference between P and I
				Mean	SE	
Phosphorus %	24	20	0,68	0,71	0,05	Not significant
	31	20	0,54	0,53	0,02	Not significant
	38	20	0,73	0,72	0,03	Not significant
Calcium %	24	20	2,98	3,08	0,08	Not significant
	31	20	2,84	2,68	0,05	$P<0,05$
	38	20	3,21	3,17	0,07	Not significant
Magnesium %	24	20	0,78	0,80	0,02	Not significant
	31	20	0,72	0,68	0,02	Not significant
	38	20	0,86	0,86	0,02	Not significant

TABLE 8 The inorganic phosphate, calcium, magnesium and total protein levels of individual and pooled plasma samples from lactating ewes

Plasma analysis	Mean day of lactation	No. of animals	Pool analyses (P)	Individual analyses (I)		Significance of difference between P and I
				Mean	SE	
Inorganic phosphate mg/100 mℓ.....	23	20	5,11	5,15	0,22	Not significant
	30	20	5,25	5,26	0,24	Not significant
	37	20	4,37	4,30	0,17	Not significant
Calcium mg/100 mℓ.....	23	20	10,66	10,53	0,17	Not significant
	30	20	10,74	10,58	0,13	Not significant
	37	20	10,35	10,38	0,16	Not significant
Magnesium mg/100 mℓ.....	23	20	2,93	2,88	0,07	Not significant
	30	20	2,94	2,95	0,06	Not significant
	37	20	3,07	3,03	0,08	Not significant
Total protein g/100 mℓ.....	23	20	6,31	6,30	0,10	Not significant
	30	20	6,38	6,30	0,07	Not significant
	37	20	6,29	6,20	0,09	Not significant

TABLE 9 The percentage error of the mean calculated for various numbers of sheep and numbers of faecal pellets per sheep to be used for a faecal pool sample

No. of sheep	No. of faecal pellets per sheep				
	1	2	3	4	5
10.....	10,7	9,8	9,4	9,1	9,1
20.....	7,7	6,9	6,7	6,6	6,4
30.....	6,2	5,6	5,4	5,3	5,3
40.....	5,4	4,8	4,6	4,6	4,5
50.....	4,8	4,3	4,2	4,2	4,0

Estimate of the number of animals and number of faecal pellets per animal required to make a pool sample

From Table 9 it is clear that there is little to be gained by taking more than 3 faecal pellets per animal. On the other hand, it is clear that increasing the number of animals reduces the error considerably. About a 5–6% error is considered reasonable, and this means that between 30–40 sheep should be sampled (minimum 30) and that 3 rectal faecal pellets should be taken from each sheep.

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