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FEEDING-INDUCED HYPOCALCAEMIA

STUDIES ON THE UPTAKE OF ^{47}CA FROM THE GASTRO-INTESTINAL TRACT OF SHEEP*

By

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JONES, B. & J. LUTHMAN: *Feeding-induced hypocalcaemia. Studies on the uptake of ^{47}Ca from the gastro-intestinal tract of sheep.* Acta vet. scand. 1978, *19*, 204—214. — It was previously shown that when concentrate was added to the diet of hay-fed sheep, there was a profound decrease of serum calcium. In the present study it was shown that 0.4 kg/day of concentrate was necessary to induce this drop in serum calcium. The same changes occurred in heifers fed 3 kg concentrate per day. Isotope studies showed that the fall in serum calcium was probably not caused by a reduced gastro-intestinal calcium uptake.

concentrate; hypocalcaemia; ^{47}Ca; gastro-intestinal absorption.

In a previous study (*Luthman & Persson* 1977) it was noted that the addition of concentrate to the diet of hay-fed sheep was followed by a disturbance in calcium homeostasis. The final amount of concentrate in this study was 0.5 kg/day. The animals became unable to maintain normal serum calcium levels. After 10—12 days serum calcium decreased to levels which often are associated with paresis. The addition of concentrate to the diet increased the animals' daily calcium intake about 40 %, but yet serum calcium decreased. The result was the same in pregnant as well as in non-pregnant ewes.

The aim of the present investigation was: to study if the same changes occur in cattle, to try to find out the amount of concentrate necessary to induce the observed changes as it ap-

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pears to be of practical importance in sheep farming, and to study if the decrease in serum calcium was associated with a reduced uptake of calcium from the gastro-intestinal tract.

MATERIAL AND METHODS

The investigations were performed as 3 separate experiments.

Experiment 1

This experiment was designed to study the blood calcium levels in sheep given differents amounts of concentrate. Thirty-two ewes, from 2 to 6 years old, were used in this experiment. The animals were taken from pasture at the beginning of September and were then kept indoors and fed only hay for a period of 1 month until the experiment started. All animals were kept in individual pens. The animals were divided into 4 groups, each consisting of 8 ewes. The 4 groups were fed according to the following scheme:

Group 1	1.5 kg hay/day
Group 2	1.5 kg hay/day + 0.2 kg concentrate/day
Group 3	1.5 kg hay/day + 0.4 kg concentrate/day
Group 4	1.5 kg hay/day + 0.6 kg concentrate/day

In Group 3 the concentrate was introduced within the course of 2 days and in Group 4 within the course of 3 days with a daily increase of 0.2 kg. The pelleted concentrate had the following composition: oats 41 %, barley 41 %, soy bean meal 8 %, rape seed meal 3.6 % and cotton seed meal 6.4 %.

Blood was sampled from the jugular vein at the intervals shown on the time scale in Fig. 1.

Analysis of the mineral content of the hay and the concentrate used in the experiment gave the following results:

	Hay	Concentrate
Ca g/kg	4.9	1.5
P g/kg	1.3	4.0
Mg g/kg	1.3	1.6

The values for metabolizable energy (MJ/kg) and digestible protein (g/kg) were calculated from analytical data:

	Hay	Concentrate
Digestible protein	26	138
Metabolizable energy	7.1	11.7

Experiment 2

This experiment was performed to study the gastro-intestinal absorption of intra-ruminally administered ^{47}Ca. Six rams about 7 months old and weighing 26 to 32 kg were used. The animals were fed only hay for a period of 1 month. At the end of this period the animals were given an intra-ruminal injection of 100 μCi ^{47}Ca to study the base line ^{47}Ca metabolism. The radionuclide was in the form of carrier free $^{47}CaCl_2$-solution made isotonic with sodium chloride (The Radiochemical Centre Ltd. Amersham, England). Ten days after the administration of radionuclide the ration was supplemented with concentrate to a final level of 0.6 kg per day. The feeds given were identical to Experiment 1, Group 4. Five days after the introduction of concentrate the animals were given a second intra-ruminal injection of ^{47}Ca to study the metabolism during concentrate supplementation. Blood was taken from the jugular vein during both parts of the experiment at the intervals shown in Table 2. The blood plasma content of ^{47}Ca was determined in 2 ml aliquots using a 3-channel γ-spectrometer. Appropriate standards for calibration were prepared from the same solution as given to the animals. The results were expressed as percentage of given amount of ^{47}Ca per mg total calcium in blood plasma. Furthermore were the exchangeable calcium pool and the turnover calculated from the obtained data.

Experiment 3

This experiment was performed to study the effect of concentrate supplementation on the blood calcium levels in cattle. Twelve heifers, about 1 year old, were used in the experiment. The animals were taken from pasture and kept indoors for a period of 3 weeks before the experiment started. Six of the animals served as controls and were fed hay ad libitum, the 6 others were fed hay ad libitum and 3 kg concentrate per day. The concentrate was introduced within the course of 3 days by giving 1 kg the first day and then 1 kg more each of the next 2 days. Blood was sampled at intervals shown in Fig. 3. The concentrate was of the same composition as in the preceding experiments. The hay given to the heifers was not analysed.

The following serum components were measured: Calcium (fluorimetric determination using a Marius Calcium Titrator from Canalco Europe, Vlaardingen, Netherlands); inorganic phosphorus (*Fiske & Subarrow* 1925); magnesium (Merckotest Magnesium, E. Merck, Darmstadt, Germany) and alkaline phosphatase (Merckotest).

The feed analyses were performed at Statens lantbrukskemiska laboratorium, Uppsala.

Conventional statistical methods were used to test mean differences and in Experiment 2 pair differences.

RESULTS AND DISCUSSION

The results from Experiment 1 are summarized in Fig. 1 and Table 1. As seen from the figure serum calcium began to decrease almost immediately after the concentrate supplementation. Significant changes occurred in Groups 3 and 4. The lowest calcium levels were seen after 5 days of concentrate feeding. A base line level for calcium was calculated from the first 4 values obtained in each group. The base line for Group 3 was 9.72 ± 0.13 mg/100 ml (mean ± s.e.m.), the lowest value in this group was 8.91 ± 0.10 mg/100 ml ($P < 0.001$). The calcium level was significantly reduced already 2 days after the beginning of concentrate feeding (Ca = 9.38 ± 0.13 mg/100 ml, $0.001 < P < 0.01$). After 8 days the calcium level thereafter returned to a level close to the base line, but after 14 days there was a drop to 8.96 ± 0.12 mg/100 ml ($0.001 < P < 0.01$). A similar biphasic curve was previously observed when sheep were fed crushed oats (*Luthman & Persson* 1977).

In Group 4 the base line level was 10.02 ± 0.09 mg/100 ml,

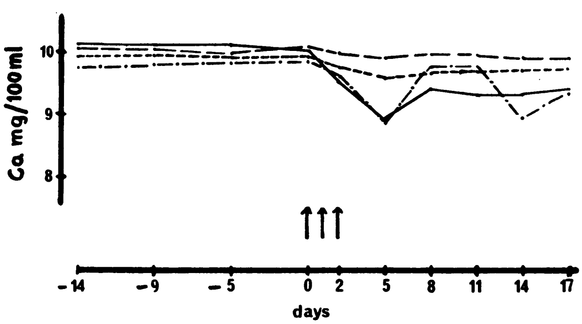

Figure 1. Changes of serum calcium in sheep after concentrate supplementation of a roughage diet. The arrows indicate when the diet was changed. Hay-fed controls — — — —. Hay + 0.2 kg concentrate - - - - - - -. Hay + 0.4 kg concentrate · — · — · —. Hay + 0.6 kg concentrate ————.

Table 1. Changes of serum inorganic phosphorus, magnesium and alkaline phosphatase (AP) after addition of concentrate to the diet of hay-fed sheep. Mean ± s.e.m.

	Day									
	—14	—9	—5	0	2	5	8	11	14	17
					P(mg/100 ml)					
Group 1	4.8 ± 0.3	5.6 ± 0.4	5.8 ± 0.3	5.4 ± 0.3	5.5 ± 0.5	5.6 ± 0.4	5.6 ± 0.4	5.3 ± 0.3	5.3 ± 0.3	4.4 ± 0.4
2	4.7 ± 0.3	5.6 ± 0.2	5.6 ± 0.3	5.2 ± 0.3	5.9 ± 0.2	5.4 ± 0.3	5.4 ± 0.1	5.5 ± 0.3	5.9 ± 0.3	5.5 ± 0.3
3	5.2 ± 0.3	5.0 ± 0.2	5.8 ± 0.3	5.4 ± 0.2	5.5 ± 0.4	5.6 ± 0.4	6.3 ± 0.5	5.9 ± 0.6	5.6 ± 0.2	5.2 ± 0.3
4	4.1 ± 0.1	5.0 ± 0.2	5.4 ± 0.2	5.3 ± 0.3	5.0 ± 0.3	5.8 ± 0.5	6.4 ± 0.4	5.5 ± 0.3	5.4 ± 0.2	4.6 ± 0.3
					Mg(mg/100 ml)					
Group 1	2.5 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.0	2.5 ± 0.1
2	2.6 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.6 ± 0.1
3	2.5 ± 0.1	2.5 ± 0.2	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.1
4	2.7 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.4 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	2.6 ± 0.1
					AP(mu/ml)					
Group 1	34 ± 6	36 ± 6	34 ± 5	27 ± 4	27 ± 4	29 ± 5	28 ± 3	32 ± 6	29 ± 4	28 ± 4
2	52 ± 13	46 ± 12	52 ± 13	48 ± 12	44 ± 11	47 ± 13	38 ± 10	41 ± 9	46 ± 12	44 ± 11
3	43 ± 9	44 ± 10	43 ± 10	39 ± 11	38 ± 9	38 ± 9	38 ± 9	47 ± 11	49 ± 12	51 ± 10
4	26 ± 3	25 ± 2	24 ± 2	20 ± 1	19 ± 1	16 ± 2	24 ± 1	23 ± 3	20 ± 2	28 ± 2

serum calcium was then significantly reduced during the whole observation period. The lowest value 8.96 ± 0.21 mg/100 ml ($P < 0.001$) occurred after 5 days. There were no significant changes in Groups 1 and 2.

The amount of concentrate necessary to induce a significant reduction of serum calcium was about 0.4 kg/day in this experiment. The lowest calcium value observed in a single animal was 7.6 mg/100 ml, in the previous study values down to 4.3 mg/100 ml were observed (*Luthman & Persson*).

Concentrate supplementation increased the calcium intake from 7.4 to 8.0 g/day in Group 3 and from 7.4 to 8.3 g/day in Group 4. The intake of inorg. phosphorus increased from 2.0 to 3.6 g/day in Group 3 and from 2.0 to 4.4 g/day in Group 4. After 8 days of concentrate feeding serum inorg. phosphorus was significantly increased in Groups 3 and 4, but thereafter there was a return to the base line.

In spite of an increase of the magnesium intake from 2.0 to 3.0 g/day in Group 4, the serum level decreased significantly after 2 to 5 days of concentrate feeding.

The results from Experiment 2 appear in Fig. 2 and Table 2. No changes in serum calcium were observed after concentrate supplementation. The serum level was 9.9 ± 0.13 mg/100 ml during the hay feeding period and 10.1 ± 0.21 mg/100 ml when the second ^{47}Ca-injection was given. Gastro-intestinal absorption of ^{47}Ca was observed in all animals already 1 hr. after the administration of the nuclide. There was no apparent difference between the hay feeding and the concentrate feeding experiment in time of absorption. The plasma level of ^{47}Ca increased rapidly and reached a maximum at about 24 hrs. No difference was observed in time to reach this maximum (see Fig. 2). However, 4 of the 6 animals showed a considerably higher concentration of ^{47}Ca in plasma during concentrate feeding. The concentration was 30—80 % higher at maximum concentration than at the corresponding time during hay feeding. At this time the 2 other animals showed a 50 % decrease in the concentration of ^{47}Ca in plasma. As there were no indications of a significant change of the plasma volume in the animals used, these results show that the percentage absorption of calcium was not decreased by concentrate supplementation.

After the maximum was reached the concentration declined with a mean half-time of 30.7 hrs. (range 26.6—41.2 hrs.) during

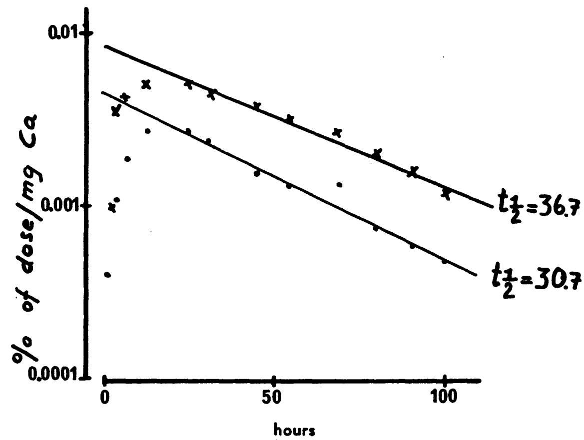

Figure 2. The concentration of ^{47}Ca in blood plasma from a sheep during 1 to 100 hrs. after administration of the radionuclide. The biological half-times have been calculated for the values obtained 24 hrs. and later after the injection. The results from the hay feeding period are marked ● and the results from the concentrate feeding period ×.

hay feeding and with an average half-time of 40.2 hrs. (range 27.9—73.4 hrs.) during concentrate supplementation. The difference was not statistically significant ($P > 0.05$).

The calculated exchangeable pool of calcium decreased in 4 animals from an average of 23.7 g calcium (range 14.0—44.7 g) during hay feeding to 15.7 g (range 10.2—30.3 g) during concentrate supplementation. In the 2 other animals the exchangeable pool increased from 40.7 to 60.3 and from 15.4 to 32.8 g of calcium, respectively, when the diet was changed. At the same time the calculated turnover of calcium decreased from an average of 460.6 mg/hr. (range 338.8—752.4 mg/hr.) to 306.3 mg/hr. (range 212.2—557.9 mg/hr.) in 5 of the sheep. The last sheep showed an increase in turnover from 1059.6 to 1497.0 mg/hr. None of these differences were statistically significant. The number of animals used was small and the obtained results not unequivocal, but there is no indication of a decreased intestinal absorption as 4 of the animals showed an increase in plasma ^{47}Ca radioactivity of 30—80 %, while the calcium supply increased about 12 %. These 4 animals also showed a decrease in the exchangeable calcium pool, and since the biological half-time of plasma ^{47}Ca increased somewhat, the result was a decreased turnover of calcium. It was similarly found by *Hibbs & Conrad*

Table 2. Mean concentration of ^{47}Ca given to 6 sheep, expressed as percentage of dose/mg Ca. Blood samples were taken from 1 to 100 hrs. after the administration of the nuclide at intervals shown in the table.

Time after administration, hrs.	10^{-3} × percentage of dose/mg Ca		Statistical difference
	hay feeding	concentrate feeding	
1	0.44	0.56	
3	1.08	2.30	$0.05 > P > 0.025$
6	1.97	3.03	$0.05 > P > 0.025$
12	2.71	3.74	
24	3.24	3.72	
30	2.45	2.87	
44	2.16	3.39	$0.1 > P > 0.05$
52	1.49	2.32	
68	1.03	1.76	$0.05 > P > 0.025$
76	0.87	1.39	$0.1 > P > 0.05$
92	0.54	1.06	$0.05 > P > 0.025$
100	0.50	0.90	$0.05 > P > 0.025$

(1966) that the calcium absorption increased in dairy cows when grain was added to an all-roughage diet. The present results indicate that the observed differences were caused by a changed bone metabolism due to alterations in the calcium regulating mechanisms. The precise cause behind these observations need further elucidation in future experiments.

The results from Experiment 3 are shown in Fig. 3 and Table 3. Serum calcium was significantly reduced after 3 days of concentrate supplementation ($0.001 < P < 0.01$). The calcium levels at day 10 and day 13 were also significantly lower than during the hay feeding period. Serum inorganic phosphorus was signif-

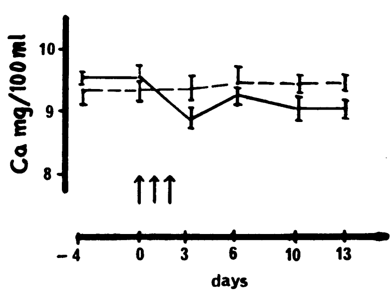

Figure 3. Changes of serum after addition of concentrate (3 kg/day) to the diet of hay-fed heifers. The arrows indicate when the diet was changed. Hay-fed controls — — —. Hay + concentrate ———.

Table 3. Changes of serum inorganic phosphorus and alkaline phosphatase (AP) after addition of concentrate to the diet of hay-fed heifers. Mean ± s.e.m. The diet was changed on day 0.

	Day					
	—4	0	3	6	10	13
			P (mg/100 ml)			
Hay-fed (n = 6)	7.07 ± 0.52	7.00 ± 0.33	6.60 ± 0.33	7.17 ± 0.18	6.50 ± 0.44	5.63 ± 0.65
Hay + concentrate (n = 6)	7.20 ± 0.40	7.12 ± 0.22	9.00 ± 0.43	8.20 ± 0.53	8.67 ± 0.47	8.43 ± 0.32
			AP (mu/ml)			
Hay-fed (n = 6)	42 ± 8	49 ± 14	47 ± 8	44 ± 10	37 ± 11	40 ± 9
Hay + concentrate (n = 6)	42 ± 6	42 ± 7	35 ± 6	38 ± 6	27 ± 4	32 ± 4

icantly elevated ($0.001 < P < 0.01$) at days 3, 10 and 13. It was previously observed that concentrate feeding increased serum inorganic phosphorus significantly in non-pregnant but not in pregnant ewes (*Luthman & Persson*).

Pregnant ewes suffering from spontaneous hypocalcaemia show depressed serum alkaline phosphatase activity (*Jonson et al.* 1973). In the present study alkaline phosphatase activity did not change significantly in any of the sheep groups, but the heifers showed reduced activity of the enzyme after 10 days of concentrate feeding. The decrease was almost significant ($0.01 < P < 0.05$).

The addition of concentrate to the diet of sheep previously fed an all-roughage diet has in the present and in previous works (*Luthman et al.* 1977, *Luthman & Persson*) induced a significant hypocalcaemia in 6 groups of sheep, totally comprising 67 animals. Similarly *Schulz & Kirchner* (1975 a, b, c) reported in a series of papers a dramatic fall of serum calcium in fattening lambs fed pelleted concentrate of various composition. *Bide et al.* (1973) also made the same experience with beef cattle. Unfortunately the sheep in Experiment 2, the group receiving ^{47}Ca, did not show any significant change in serum calcium. The reason for this is not clear. The results did not, however, favour the hypothesis that the decreased calcium level is due to a decrease in gastro-intestinal absorption of calcium after concentrate feeding. The mean biological half-time of ^{47}Ca was somewhat longer during concentrate feeding than during hay feeding.

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SAMMANFATTNING

Utfodringsbetingad hypokalcemi.
Studier av det gastro-intestinala kalciumupptaget hos får.

När kraftfoder börjar utfodras till får som tidigare stått på enbart höutfodring, inträder ibland en drastisk sänkning av serumkalciumkoncentrationen. 0.4 kg/djur och dag visades vara den minsta mängd som ger upphov till en signifikant sänkning av serumkalcium. Hos kvigor kunde en signifikant sänkning av serumkalcium induceras med en giva på 3 kg/dag. Radionuklidstudier visade att orsaken till hypokalcemien sannolikt inte var ett reducerat gastrointestinalt upptag av kalcium.

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