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Calcium metabolism in red deer (*Cervus elaphus*) offered herbages during antlerogenesis: kinetic and stable balance studies

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SUMMARY

Kinetic studies of Ca metabolism, using $^{45}CaCl_2$, were carried out on two mature red deer stags during the period of maximum rate of Ca deposition in the antlers. They were offered green-feed oats to provide energy for maintenance; the diet provided approximately 42 mg Ca/kg W per day.

Ca appeared to be irreversibly lost from the circulation into the antlers and could be treated for kinetic purposes in the same way as loss of Ca from the body in milk. The size of the rapidly exchangeable Ca pool in the body, excluding the antlers, was 0·21 g/kg W, similar to estimates for lactating cattle. Rates of Ca deposition in the antlers of the two stags, calculated from the model of Ca metabolism, were 58·4 and 38·6 mg Ca/kg W per day. Net endogenous loss was 6–7 mg Ca/kg W per day, much lower than estimates available for other ruminant species. Only 25–40% of Ca requirement or 11–24 mg Ca/kg W per day was derived from the diet, suggesting that the availability of Ca in green-feed oats is much lower than current estimates for forages. Bones removed on completion of the experiment showed evidence of considerable skeletal demineralization.

In other stags subjected to stable Ca balances at the same stage of antler growth, while consuming ryegrass–white clover forage, 60–80% of Ca requirement was derived from the diet, with calculated rates of true absorption of 32–46 mg Ca/kg W per day. These rates of Ca absorption are low compared with values observed in other ruminants at times of high Ca demand such as during lactation.

INTRODUCTION

There are no data from which to assess the dietary Ca requirement of red deer stags during antler growth. Calcium deposition in the antlers may exceed 8 g/day (Muir, Sykes & Barrell, 1987). Calculations of requirement based on values for dietary Ca availability and faecal endogenous loss derived in sheep and cattle (Agricultural Research Council, 1980) and knowledge of feed intake of stags during antler growth suggest that most herbages should supply adequate Ca. On the other hand skeletal rarefaction does appear to occur during antler growth (Meister, 1956; Banks *et al.* 1968*a*,*b*; Brown, Cowan & Griel, 1978) which suggests, either that the metabolism of Ca in deer differs from that in sheep and cattle, or that a factorial approach to estimating requirement may be inappropriate in deer during antler growth.

On the other hand estimates used by the Agricultural Research Council (1980) for Ca availability in ruminants are derived predominantly for conserved feeds and grain-based diets. There must be uncertainty as to whether these can be applied directly to green forage diets particularly in view of the findings of osteodystrophies in lambs offered green-feed oats (Ewer & Bartrum, 1948). The deer with its high requirement for Ca during antler growth could provide a good model with which to examine Ca availability in fresh herbages.

This paper describes preliminary kinetic studies of Ca metabolism, using $^{45}CaCl_2$, and stable Ca balances in stags consuming forage diets during the period of rapid antler mineralization.

MATERIALS AND METHODS

Experiment 1

Animals' feeding and management

Two mature red deer stags (Nos 589 and 103) were brought into indoor pens prior to casting of hard antlers. They were offered a commercial pelleted deer diet (NRM Feeds Ltd, Christchurch, New Zealand) for 60 days. They were then adjusted gradually to green-feed oats, until consumption

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rose to 10% above estimated maintenance energy requirement (0·64 MJ ME/kg $W^{0.75}$; Fennessy, Moore & Corson, 1981). After a further 24 days (71 and 79 days, respectively after casting of previous antler remnants) they were transferred to metabolism crates in which faeces and urine were separated by a sloping stainless steel mesh tray. Seven days later consecutive stable Ca balance periods of 10 days (balance 1) and 14 days (balance 2) commenced. The latter balance period was timed to coincide with the phase of most rapid deposition of Ca during antler growth (Muir *et al.* 1987).

The oats were harvested by cutting on two occasions. Daily allowances were weighed into cotton bags and stored at −15 °C. Individual rations were then removed and thawed for 18 h prior to feeding. Cuts 1 and 2 were used during balance trials 1 and 2, respectively.

Faeces were collected daily, weighed and subsamples stored at −20 °C. Urine volume was recorded daily, acidified to pH 3·0 and samples stored at −20 °C. Blood samples were obtained on day 1 of the first balance period and at 4-day intervals thereafter into heparinized tubes, the plasma separated by centrifugation and stored at −20 °C.

Kinetic studies

Three days prior to the second balance period indwelling polyvinyl catheters were placed in each external jugular vein and sewn to the skin of the back. On day 1 of the second balance period 800 μCi ^{45}Ca as $CaCl_2$ (Amersham Australia Pty Ltd) was injected through one catheter. Blood samples were obtained through the contralateral catheter at 0·5 min intervals for the first 2 min and thereafter at 3, 4, 5, 8 and 10 min. Sampling was then carried out at the following intervals: 5 min until 30 min, 10 min until 60 min, 20 min until 2 h, 2 h until 12 h, 4 h until 24 h, 6 h during days 2 to 6 and 12 h until 13·5 days after injection. The catheter was maintained patent by filling it with heparinized saline (50 i.u. sodium heparin per ml). Saline solution and some blood was withdrawn and discarded before 10 ml blood was withdrawn. The time was noted when half the sample had been removed. Plasma was separated by centrifugation within 60 min and stored at −20 °C. Faeces and urine were collected daily, subsampled and stored as described above.

The stags were then killed with sodium pentobarbitone. Antlers were cut off immediately below the coronet, weighed and their length measured. A 3rd rib, tibia and the 3rd lumbar vertebra were removed and these with the antlers were stored at −20 °C.

Chemical analyses

Faeces were freeze dried, ground through a 1 mm sieve, and 5 g was wet ashed in 70 ml nitric–perchloric (72%) acid mixture (6:1; v:v) at 150–200 °C and made up to 20 ml in distilled water.

The left antler was cut into 5 cm long cylinders as described by Muir *et al.* (1987). These and the bones were dried, extracted in petroleum ether and ashed at 550 °C for determination of ash and fat-free organic matter. Ash (1 g) from bones and antler sections was digested as for faeces.

Calcium concentration in faeces, antler and skeletal ash, urine and plasma, was determined by atomic absorption spectrophotometry.

Radio-isotope counting and data analyses

Samples (1 ml) of acid digest and of plasma and urine were mixed with scintillation cocktail (9 ml) which comprised triton X-100 and filtered toluene (1:1; v:v) with 5 g 1, 4-di(5-phenyl-2-oxazolyl) benzene (POPOP) as scintillant. Counts were corrected for quenching using an external standard, and specific correction curves for each type of sample.

Faecal endogenous Ca loss was calculated from the radioactivity excreted in faeces between days 1 and 13·5 divided by the integral of the plasma specific activity curve over time (Braithwaite & Glascock, 1975). Net accumulation of Ca in antlers was calculated by the same method, using total antler activity. This latter calculation made the provisional assumption that ^{45}Ca deposited in antler matrix did not re-enter the body pool.

Rate of absorption of Ca from the alimentary tract (V_a) was the difference between total Ca intake (V_i) and total faecal output (V_F), corrected for faecal endogenous loss (V_f) (Braithwaite & Glascock, 1975),

i.e. $$V_a = V_i - V_F + V_f$$

Model of Ca metabolism

The simulation, analysis and modelling program (SAAM) (Berman & Weiss, 1978), similar to that used for lactating dairy cattle (Ramberg *et al.* 1970), was applied to the data on the assumption that Ca deposited in antlers is lost irreversibly from the system (Fig. 1). Four exponentials, representing four compartments or exchangeable pools, were obtained by manual curve peeling from a semi-logarithmic plot of plasma specific activity (natural logarithms) *v.* time (arithmetic scale). These rate constants were converted to fractional flow rates between compartments and, together with plasma data (in the form of percentage of initial dose) and rate of loss of ^{45}Ca in faeces, urine and net loss in antlers, submitted to the program. An iterative least squares fit produced a steady state solution for rate transfers

Table. 1. *Stable Ca balances of red deer stags offered ryegrass–white clover or oat diets during the period of rapid antler mineralization, and data derived from subsequent kinetic studies for true absorption of Ca and for the proportion of Ca deposited in antlers absorbed from the diet*

	Experiment 1				Experiment 2			
Stable balances...	Oats				Oats	Ryegrass–white clover		
Stag no...	589		103		99	622	4–9	761
Balance trial (BT)	BT1	BT2	BT1	BT2				
Body weight (kg)	170		150		174	158	183	153
Dry-matter intake (kg D.M./day)	2·89	2·40	2·58	2·37	3·26	2·48	2·85	2·72
Rate (mg/kg W per day) of								
Ca intake	64·5	39·2	64·4	43·8	63·3	112·2	111·4	127·1
Faecal Ca	41·8	27·2	50·2	39·3	45·6	79·8	71·5	81·5
Urinary Ca	6·4	2·0	5·9	2·7	2·1	8·0	1·5	14·6
Apparent availability	0·35	0·31	0·22	0·10	0·27	0·28	0·36	0·36
Rate (mg/kg W per day) of:								
Faecal endogenous loss*	6·6	6·6	6·1	6·1	6·3	6·3	6·3	6·3
Accretion of Ca in antlers†	—	50·5	—	41·3	51·3	50·1	40·4	43·2
True absorption of Ca	—	18·5	—	10·6	24·0	38·8	46·2	31·9
Calculated true availability of dietary Ca	—	0·47	—	0·24	0·38	0·35	0·42	0·41
Proportion of Ca requirement derived from diet	—	0·37	—	0·25	0·42	0·60	0·74	0·81

* Calculated from kinetic studies in stags 589 and 103. † Calculated from the equations of Muir *et al.* (1987).

Table 2. *Kinetic model of Ca metabolism in two red deer stags offered green-feed oats during the period of rapid Ca deposition during antler growth*

	Stag	
	589	103
M_1*	0·81	0·92
M_2	2·61	3·12
M_3	9·09	10·1
M_4	23·4	22·3
M_T (total exchangeable Ca = $M_1+M_2+M_3+M_4$)	35·9	36·4
R_{12}†	197·8	103·3
R_{21}	197·8	104·8
R_{23}	38·2	17·8
R_{32}	38·2	19·3
R_{34}	6·47	3·95
R_{43}	6·49	5·44
Ca intake (g/day)	(6·66)	(6·57)
Ca output (g/day)		
Faeces	(4·71)	(5·90)
Antlers	9·93	5·79
Urine	0·24	0·76
Faecal endogenous loss	1·28	1·03
Ca absorption from intestine (g/day)	3·23	1·70
Skeletal Ca accretion (g/day)	0·02	1·49
Skeletal Ca resorption (g/day)	8·24	7·37
Skeletal Ca balance (g/day)	−8·22	−5·88

* M_1–M_4, compartmental masses (g).

† R_{12}, R_{21}, R_{43} etc., flow of Ca from pool M_2 to M_1, from pool M_1 to M_2, from M_3 to M_4 etc. (g/day).

Compartmental masses and flow of Ca between compartments and into antlers, faeces, urine and the skeleton are those estimated by compartmental analysis. Rates of Ca absorption from the diet, total Ca requirement and skeletal Ca resorption were estimated from these and stable balance data.

Table 3. *Composition of bones from the present stags during antler growth. Data are also provided from a stag and sheep with replete skeletons*

		Stag		Replete skeletons	
		589	103	Stag	Sheep
Tibia	A:R	1·60	1·49	1·87	1·95
	SA	34100	47600		
Rib	A:R	1·25	1·29	1·76	1·41
	SA	84300	123600		
Vertebra	A:R	0·99	0·87	1·46	1·38
	SA	100500	150800		

Degree of mineralization (ratio of ash: organic matter) and specific radioactivity (SA) in stags 589 and 103 was determined during the period of maximum rate of Ca deposition and 13 days after injection of 800 μCi ^{45}Ca as $CaCl_2$. Values for replete animals are from a stag which died in mid-winter and for well fed ewes during late pregnancy, from Sykes, Nisbet & Field (1973).

Table 4. *Age, weight and composition of antlers removed from red deer stags at the end of the balance period in Expt 2*

	Stag			
	622	4–9	761	99
Antler age (days after casting)	101	109	113	116
Antler fresh weight (g)	4154	3624	3130	4061
Ca content (g/100 g ash)	36·8	37·3	37·7	37·0
Total Ca content (g)	296	283	273	319
Estimated rate of Ca deposition (g/day)	7·91	7·40	6·61	8·72

Rate of deposition of Ca during the balance period was calculated from the data of Muir *et al.* (1987).

degree of mineralization of bone matrix and vertebra the least. There was an inverse relationship between degree of mineralization and specific activity, with the poorly mineralized vertebra having the highest specific activity. Mean specific activities of Ca in antler were 10–40 times that in skeletal bone.

Experiment 2

Calcium balance data are given in Table 1. Calcium contents (g/kg D.M.) of the ryegrass–white clover and oat herbages were 7·15 and 3·37 and P contents 3·36 and 2·08, respectively. Apparent availability of Ca ranged from 0·27 to 0·36. Urinary Ca, though low in relation to faecal Ca, was extremely variable between animals.

Data on the antlers removed from these stags are given in Table 4. Rate of Ca deposition in the antler during the balance period was calculated from the equations of Muir *et al.* (1987) and was estimated to range from 6·6 to 8·7 g/day.

DISCUSSION

An assessment of the rate of Ca deposition in the antlers during the balance periods is crucial for the interpretation of both the stable balances and the provisional kinetic model of Ca metabolism provided. The most complete description of rate of ash deposition in the antler in relation to antler size and growth rate was given by Muir *et al.* (1987). They showed that weight of ash in the antler could be described by the equation

$$Y = 0{\cdot}000\,120\,X^{2{\cdot}81}\ (r = 0{\cdot}96;\ \text{R.S.D.} = 6{\cdot}7)$$

where Y = proportion of final antler ash weight and X = days from casting of previous antler remnants. This equation and the knowledge that antler ash contains $35{\cdot}8 \pm 0{\cdot}33$ g Ca/100 g ash (Muir *et al.* 1987) was used to calculate rate of Ca deposition in antlers of the present stags using the final antler ash content and time from casting. The values are given in Table 1 and ranged from $40 \pm 3{\cdot}8$ to $51 \pm 4{\cdot}8$ mg Ca/kg W per day.

The values for Ca deposition in antlers of 8·6 and 6·2 g Ca/day calculated for stags 589 and 103, respectively, during balance trial 2 (Expt 1) represent, in each case, 105% of that calculated independently from the ratio of total radioactivity in the antler and the integral of the plasma specific activity curve, namely 8·3 and 5·8 g/day respectively. While slightly different values were obtained from the SAAM model (Table 2) for stag 589 the data confirm the reliability of the equations of Muir *et al.* (1987) for prediction of antler Ca content and daily rate of Ca deposition.

Since the assumption in the model is that antler Ca does not exchange, the agreement of the two estimates suggests, perhaps surprisingly, that Ca, once deposited in antler bone matrix, does not return to the body proper. It seems unlikely, however, that turnover of Ca does not occur within the tissue itself since Belanger, Choquette & Cousineau (1967) described osteocytic osteolysis in trabecular bone of actively growing antlers of male and female reindeer (*Rangifer tarandus*). This process was not quantified and may be negligible compared with net rate of calcium accretion. On the other hand Care *et al.* (1985) provided evidence, in change in specific gravity and in numbers of resorption lacunae, for reduction of resorption of antler bone in a parathyroidectomized stag. It seems plausible that bone mineral, once in an actively growing tissue such as the antler, may be extensively recycled within the tissue but that an antler as a whole operates, in effect, as a sink for Ca.

Movement of Ca into the antlers was therefore treated in a similar way to Ca loss during lactation as an irreversible loss, allowing accretion of Ca in the skeleton and in the antlers to be distinguished and calculation of skeletal Ca resorption in the kinetic model.

The size of the exchangeable Ca pool in the body (M_T), at 0·21 and 0·24 g/kg W in stags 589 and 103 was similar to the value of 0·21 g/kg W obtained in lactating dairy cattle by Ramberg *et al.* (1970). Stephenson & Brown (1984), working with white-tailed deer (*Odocoileus virginianus*) during antler growth, obtained a value of 0·50 g Ca/kg W. This latter estimate did not, however, separate the mass of Ca entering the antler from that entering the exchangeable pool in the body proper.

The model of Ca metabolism developed implies considerable mobilization of skeletal Ca despite high Ca intake. Such a conclusion is supported by the bone data (Table 3). There are few data available on skeletal mineralization in deer from which to judge bone quality. However, a mature healthy stag which died as a result of an accident in late winter provided a tibia, 3rd rib and 3rd lumbar vertebra for comparison. Antler growth had ceased 6 months earlier and the bones could therefore be expected to be well mineralized. By comparison with these and data for well nourished sheep (Sykes, Nisbet & Field, 1973), the bones of the present stags were poorly mineralized (Table 3). The higher specific activity of ^{45}Ca in bone ash in the rib and vertebra (Table 3) further indicates the relative sensitivity of these bones to remodelling during mineral deficiency as has previously been observed by Benzie *et al.* (1956) in sheep.

The rate of faecal endogenous loss (mg Ca/kg W per day), at 6–7, was very low compared with estimates in sheep and cattle of 16 (Agricultural Research Council, 1980). More recently Braithwaite (1982) suggested that endogenous Ca loss in sheep is not constant but a function of feed intake, and

values in excess of 50 mg Ca/kg W per day have recently been observed in young growing lambs (Chrisp, 1986). Application of the equation of Braithwaite (1982) describing the relationship between food intake and endogenous loss of Ca to the present deer gave a value of 11–13 mg/kg W per day, which is double that observed. On the other hand, data from the Ca kinetic studies of Stephenson & Brown (1984) in white-tailed deer can be recalculated to yield an estimate of 6·4 mg/kg W per day which is identical to the present ones. It does appear, therefore, that faecal endogenous loss of Ca in deer may be lower than in other ruminant species.

The rates of true absorption, availability, and the proportion of the Ca requirement provided by the diet were calculated for all the stags in Expts 1 and 2, on the assumption that faecal endogenous loss of Ca was 6·3 mg/kg W per day. These data are given in Table 1. Rates of true absorption, which never exceeded 46 mg/kg W per day, were low compared with estimates for sheep during lactation of 75–115 mg/kg W per day (Braithwaite, 1975; Sykes & Dingwall, 1975; Braithwaite, 1983; Sykes & Geenty, 1986). They were lowest in stags consuming oat diets, ranging from 11 to 24 mg/kg W per day, and these comprised only 25–40% of daily requirement for Ca. In stags consuming ryegrass–white clover, rates of absorption tended to be higher, ranging from 32 to 46 mg/kg W per day, and a much greater proportion of requirement (60–80%) was met from the diet. Interestingly, the proportion of Ca absorbed from each diet (apparent availability) was similar at about 40%. This latter finding may be coincidental for the following reasons. Deposition of Ca in the antlers would depress plasma ionic Ca concentration and lead to increase in parathyroid hormone (PTH) secretion. As a consequence not only would Ca absorption from the intestine be increased but also Ca resorption from the skeleton (Care, Barlet & Abdel-Hafeez, 1980). The proportion of Ca requirement derived from the diet in deer consuming ryegrass–white clover (0·7) may well represent a physiological maximum for the proportion of Ca which can be supplied to the plasma ionic pool from the diet at such high rates of Ca flux to the antlers. This ratio may be largely independent of dietary intake, except that it would be expected to increase with conditions which favour high rates of passive diffusional absorption of Ca. On the other hand very high availability of dietary Ca (> 70%) has generally been observed only when dietary Ca intake has been low and close to requirement (Sykes & Field, 1972; Braithwaite, 1983). Under these circumstances it seems possible that the very low rates of Ca absorption from the green-feed oats in the present studies do reflect a low availability of Ca from those diets. More recently, Chrisp (1986) similarly found very low rates of absorption and availability of the Ca in oats consumed by lactating sheep. Moreover, Ewer & Bartrum (1948) described a high incidence of rickets when lambs were offered green cereal crops, particularly oats, and Grant & O'Hara (1957) demonstrated a rachitogenic factor which they concluded was carotene or vitamin A.

The present data suggest that maximum availability of Ca from oats may be considerably less than 40%, much lower than the general value for ruminants of 68% adopted by the Agricultural Research Council (1980). A more careful examination of Ca supply from fresh forages, for which there are few data, appears therefore to be warranted. Further, the concept that animals will necessarily absorb Ca at the rate consistent with their requirement, assumed in the factorial approach to the estimation of nutrient requirement, must be more carefully scrutinized.

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