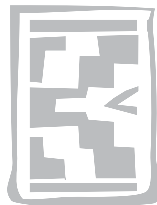

Can osteophagia provide giraffes with phosphorus and calcium?

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ABSTRACT

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The daily requirement for calcium and phosphorus by giraffes to sustain the growth and maintenance of their skeletons is large. The source of sufficient calcium is browse. The source of necessary phosphorus is obscure, but it could be osteophagia, a frequently observed behaviour in giraffes. We have assessed whether bone ingested as a result of osteophagia can be digested in the rumen. Bone samples from cancellous (cervical vertebrae) and dense bones (metacarpal shaft) were immersed in the rumens of five sheep, for a period of up to 30 days, and the effect compared to immersion in distilled water and in artificial saliva for 30 days. Distilled water had no effect on the bones. Dense bone samples were softened by exposure to the saliva and rumen fluid, but did not lose either calcium or phosphorus. In saliva and rumen fluid the cancellous bone samples also softened, and their mass and volume decreased as a result of exposure to saliva, but in neither fluid did they lose significant amounts of calcium and phosphorus. We conclude that although saliva and rumen fluid can soften ingested bones, there is an insignificant digestion of bones in the rumen.

Keywords: Calcium, giraffe, osteophagia, phosphorus

INTRODUCTION

Giraffes, *Giraffa camelopardalis*, (Linnaeus, 1758) require an estimated 20 g calcium (Ca) and 10 g phosphorus (P) per day from birth to 5 years of age for the growth of their unique skeletons, and if they are to maintain a Ca:P ratio in their bones of 2:1 (Mitchell & Skinner 2003; Mitchell, Van Schalkwyk & Skinner 2005). Thereafter, daily requirements in males and females are lower but in females are increased by specific stresses such as lactation (Mitchell *et al*. 2005). Recent analyses of giraffe skeletal biology (Van Schalkwyk, Skinner & Mitchell 2004; Mitchell *et al*. 2005) suggested that by selective browsing giraffes are likely to be able to obtain sufficient Ca for skeletal growth. Sources of sufficient P are more obscure. One possibility is that they obtain P by eating bones. Osteophagia is an often observed behaviour in giraffes (Pattern 1940; Nesbitt-Evans 1970; Western 1971; Wyatt 1971; Leuthold & Leuthold 1972; Hall-Martin 1974; Kok & Opperman 1980; Hampton 2002), and it seems to be reported more frequently in giraffes than almost any other ruminant except for domestic animals on P deficient pasture. Its occurrence is highest in the winter months when the nutrient quality of browse declines (Langman 1978).

Osteophagia has many causes. Boredom, habit and taste contribute, but Theiler, Green & Du Toit (1924) established that osteophagia in cattle (*Bos taurus/indicus*), could be eliminated by supplementary feed-

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ing with P in the form of bone meal. The appetite for bones observed in P deficient cattle is innate, specific, and cued mainly by the smell of bones. It is associated with a decline in the inorganic phosphate fraction of blood plasma and a withdrawal of Ca and P from the reserves in bones (Denton, Blair-West, McKinley & Nelson 1986; Blair-West, Denton, Nelson, McKinley, Radden & Ramshaw 1989; Underwood & Suttle 1999). A giraffe's diet on average has a Ca:P ratio of 7.7:1 (Pellew 1984; Mitchell & Skinner 2003), and if fed to cattle a diet with this Ca:P ratio would result in clinical signs of phosphate deficiency and osteophagia (McDowell 1992; Underwood & Suttle 1999).

For osteophagia to be an effective adaptive behaviour to supply P (and/or Ca), and assuming that ingested bones or bone fragments enter the rumen, then the ingested bones must be small enough to pass through the rumen into the abomasum, or must be able to be digested in the rumen, and P and Ca released in a soluble, absorbable form. Phosphorus can be absorbed from the rumen (Wadhwa & Care 2002). However, removal of Ca and P from bone and their absorption occurs best in a low pH environment. In carnivores, for example, gastric pH is approximately 2 and bones and bone fragments can readily reach the stomach where they are dissolved. In ruminants abomasal pH is somewhat higher. In giraffes it is 3.6 ± 0.1 and is identical to that of five other wild ruminant species (3.6 ± 0.4) (Maloiy, Clemens & Kamau 1982), but nevertheless is sufficiently acidic to dissolve bone and release P from it. However, in ruminants, unlike in monogastric animals, bones cannot directly enter the abomasum. The breakdown of bone is also likely to depend on chewing and possibly rumination, but the time of exposure to these processes is brief and intermittent. Prolonged exposure in the rumen itself is a more likely site of digestion.

We report here, therefore, an investigation into whether Ca and P ingested as bone can be released from bone in the rumen in significant quantities and/or if bone can be reduced to a form that can reach the abomasum and small intestine. As far as could be determined no study on the digestion of bones in the rumen has been done previously. Our study suggests that such digestion is poor.

MATERIALS AND METHODS

Bone samples

Calcium and P extraction from two types of bone was determined: cancellous (porous) and compact (dense) bone, which replicate the range of bone types ingested by giraffes. Bone samples were obtained from giraffe bones used in our previous studies to establish the mineral composition of both types (Van Schalkwyk *et al*. 2004; Mitchell *et al*. 2005). For this study cancellous bone was obtained from third, fourth and fifth cervical vertebrae and compact bone from a single metacarpus shaft.

To standardize surface areas, the bone samples were cut into cubes, the side lengths of which were approximately 1.7 cm. The surface area of each cube was thus about 17.5 cm^3 and their volumes about 5 cm^3 (Fig. 1; Tables 3 and 4).

Measurement of digestion

Digestion was assessed from changes in bone mass, volume and density, and from changes in the Ca and P composition.

Bone mass, volume and density

Initial mass of the bone samples was recorded using a Mettler Toledo Bloc PB 153-S scale (Mettler, Microsep, RSA) to an accuracy of 0.1 g. Volumes of the bone samples were determined by the displacement of water in volumetric flasks, which measured changes in volume to 0.1 mℓ. Density was calculated by dividing dry mass by volume of water displaced, assuming that at 20 °C the density of water is 1 g/mℓ. (Khan, Khan, Khan & Khanam 1997), and was recorded as g/cm^3. Volume, mass, and density measurements were repeated both pre- and post-treatment to determine any significant changes. Post-treatment, all samples were oven dried following Harris (1970) after volume measurements were taken and before mass was determined.

Ca and P analysis of the bones

After initial mass and volume were measured, all the samples of bone were defatted using petroleum ether as described by the Association of Official Analytical Chemists (AOAC), official method 945.16 (Horwitz 2000) at Nutrilab, Department of Animal and Wildlife Science, Faculty of Natural and Agricultural Sciences, University of Pretoria. The lipid free samples were weighed (± 0.001 g) and then oven dried after the method of Harris (1970).

Cancellous bone samples were ground to a powder using a custom-made iron pestle and mortar. For compact bone samples this method produced a coarse powder, which was further ground using a motor-driven mill (Mikro-Feinmühle-Culatti MFC,

Janke IKA®—Labortechnik, 50/60 Hz, 200 W) to particles of approximately 1 mm^3 in size.

Duplicate samples of powdered bone samples, weighing 0.5 ± 0.003 g were oven dried and ashed in a muffle furnace at 550 °C for 4.0 h. The dry ashing technique followed the AOAC official method 999.11 (Horwitz 2000). Samples were left to cool overnight and placed in a desiccator for 30 min prior to determining the ash mass. The ash residue was dissolved in an acid solution, filtered and diluted to a volume of 100 mℓ. Dissolved ash solutions for Ca analysis were diluted 50 times with distilled water and a further ten times with lanthanum chloride ($LaCl_3$, 0.5 %). Solutions for P analysis were diluted 50 times with distilled water.

Phosphorus concentrations were measured using an Auto Analyser II (Techicon™, Bran & Lübbe, Germany) according to the AOAC official method 965.17 (Horwitz 2000). Calcium concentrations were measured in an Atomic Absorption Spectrophotometer (Perkin-Elmer 5100PC, USA) using the AOAC official method 935.13 (Horwitz 2000).

In both cases a difference of less than 10 % between duplicates was accepted. For larger differences analysis of those particular samples was repeated. Calcium and P concentrations measured were converted to mg/g of ash ([volume x dilution x reading] ÷ sample mass), and expressed as a percentage. The mean percentage multiplied by the original mass of the bone sample was used to calculate total Ca (g), total P (g), and total non-Ca + P (g) minerals in each bone sample.

Experimental animals

Five mature, rumen-fistulated Merino sheep wethers were used for the trial (Animal Use and Care Committee, Faculty of Veterinary Science, University of Pretoria approval number V068/04). Sheep were used as experimental animals because their rumen fluid composition is identical to that of wild ruminants including giraffes (Giesecke & Van Gylswyk 1975) (Table 5) and because they exhibit osteophagia (Brothwell 1976; Bazely 1989). The animals were housed at the experimental farm of the Faculty of Biological and Agricultural Science, University of Pretoria, under the supervision of the Department of Animal and Wildlife Science, Faculty of Natural and Agricultural Sciences, University of Pretoria. The sheep were housed individually in cement-floor pens (3 x 2 m), which were covered by a roof, and were fed a ration of good quality teff (*Eragrostis tef*) hay (Table 1). Water was available *ad libitum*. The hay was milled to 1 cm length. The sheep were fed the ration for 16 days prior to the start of the trial. Feed consumption rate was estimated at approximately 2 kg per wether per day, which is above normal consumption rates for ewes on a maintenance diet (Perry, Cullison & Lowery 1999).

The sheep were weighed at the start of the trial and three times afterwards (10, 20 and 30 days). Their body masses ranged from 38.1 kg to 72.0 kg at the start of the trial and they maintained body mass for the duration of the experiment.

Rumen fluid pH (as a marker of rumen health) was measured at each time interval using the model IQ 150 handheld pH/mV/Temperature Meter or model IQ 120 pH meter with a silicon chip sensor (I.Q. Scientific Instruments, Inc., San Diego, USA). Blood samples from all five sheep were taken at the start of the trial and at the respective time intervals to determine plasma Ca and P concentrations. After collection, the blood samples were stored on ice and centrifuged within 1 h. Preparation of blood plasma for inorganic P analysis was done following the procedures described by Little, Robison, Playne & Haydock (1971). After precipitation the solutions were filtered through glass microfibre paper (9.0 cm GF/A Whatman Ltd., England) into acid-cleaned 30 mℓ. McCartney bottles. The remaining plasma in the centrifuged tubes was pipetted into individual, sealable tubes for analysis of inorganic Ca levels. All samples were refrigerated at 5 °C. Calcium and P concentrations in plasma were analysed using the same methods described above for bone sample analysis, except that to minimize interference by P on the spectrophotometer, the plasma samples for Ca analysis were diluted 50 times with lanthanum chloride ($LaCl_3$, 0.1%).

TABLE 1 *Eragrostis* tef hay fed to the sheep throughout the trial. All values are on a DM basis

DM (g/100 g)	GE (MJ/kg)[1]	Moist (g/100 g)	Ash (g/100 g)	CP (g/100 g)[2]	CF (g/100 g)	Ca (g/100 g)	P (g/100 g)	Ca:P
100	18.5	0	4.72	7.16	34.91	0.28	0.32	0.9:1

[1] According to J. van Ryssen (personal communication 2006)
[2] "Dumas" method was used for crude protein analysis (Official method 990.03, Horwitz 2000)

Experimental design

The assumption was made that there would be significant evidence of digestion in the three fluids used, viz. rumen fluid, artificial saliva and distilled water, if the cubed bone samples were immersed in them for 30 days, the artificial saliva and distilled water being used to control for the effects of rumen digestion. Ingested bone is exposed to saliva at the time of ingestion and possibly at intervals during rumination. Saliva has a similar pH and osmolarity to rumen fluid, and contains chemicals similar to those found in rumen fluid, although at different concentrations (Wadhwa & Care 2002).

All the samples used were placed in individual nylon bags (pore size 53 μm; Nutrilab) and kept in distilled water, artificial saliva and rumen fluid via the rumen fistula's for 10, 20 and 30 days. At the appropriate time intervals a number of the bone samples were removed from the respective fluid they were suspended in. These were washed under tap water and oven dried according to Harris' (1970) method for analysis.

Fifteen samples of each bone type were randomly assigned to the three treatments. Five of each type were analysed at each time interval as follows: Fifteen samples of each type were placed in distilled water at pH 5.86–6.38 and 39 °C. Another 15 samples of each type were placed in an artificial saliva solution and incubated at 39 °C. This solution lacked saliva enzymes but contained 9.8 g/ℓ Na HCO_3, 0.57 g/ℓ KCl, 0.47 g/ℓ NaCl, 0.12 g/ℓ $MgSO_4.7H_2O$, 0.04 g/ℓ anhydrous $CaCl_2$ and 3.17 g/ℓ anhydrous Na_2HPO_4, to which 4 mℓ/ℓ hydrochloric acid was added to reduce the pH to rumen pH of 6.5 (McDougall 1948). The last 15 samples of each bone type were suspended in the rumens of the sheep. The nylon bags containing these samples were attached to a 120 g mass so that they would remain submerged in the rumen fluid.

Statistical analysis

A two-tailed Student's t-test was used to compare differences between control samples and test samples. *P*-values < 0.05 were regarded as significant.

RESULTS

Rumen fluid, saliva, distilled water and blood chemistry

Distilled water and rumen pH remained constant over the 30 days of the experiment (Table 2). The pH of saliva increased over time from 6.8 to 7.3. This

TABLE 2 Rumen, saliva, distilled water (DH_2O) and blood chemistry

Fluid type	N	Before treatment	10 days	20 days	30 days
Rumen pH (mean ± SD)	5	6.4 ± 0.2	6.5 ± 0.2	6.5 ± 0.3	6.5 ± 0.3
Saliva pH (mean ± SD)	7	6.8 ± 0.2	7.0 ± 0.2	7.4 ± 0.2	7.3[#]
DH_2O pH (mean ± SD	7	6.5 ± 0.1	6.1 ± 0.4	6.5 ± 0.7	6.4 ± 0.4
Plasma Ca (mmol/ℓ)	5	2.4 ± 0.2	2.4 ± 0.1	2.4 ± 0.1	2.5 ± 0.3
Plasma P (mmol/ℓ)	5	1.4 ± 0.4	1.4 ± 0.3	1.7 ± 0.7	1.5 ± 0.5

[#] 2 measurements only

TABLE 3 Effects of distilled water (DH_2O), artificial saliva, and rumen fluid on metacarpus shaft bone samples

Bone variable	Before treatment[1]	DH_2O after 30 days	Artificial saliva after 30 days	Rumen fluid after 30 days
Mass (g)	9.8 ± 0.9	9.5 ± 1.3	**10.7 ± 0.4**	10.0 ± 1.4
Volume (mℓ)	5.2 ± 0.5	5.0 ± 0.6	5.5 ± 0.4	5.3 ± 0.5
Density (g/cm^3)	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
Total ash (g per sample)	7.0 ± 1.0	6.9 ± 0.9	**7.6 ± 0.3**	7.2 ± 1.1
% Ca	25.6 ± 0.9	25.4 ± 0.5	24.7 ± 1.0	25.6 ± 1.4
% P	11.6 ± 0.2	11.5 ± 0.2	11.7 ± 0.2	11.4 ± 0.5
Total Ca (g per sample)	2.5 ± 0.4	2.5 ± 0.3	2.7 ± 0.1	2.6 ± 0.5
Total P (g per sample)	1.1 ± 0.2	1.1 ± 0.2	1.3 ± 0.1	1.1 ± 0.2
Non-Ca + P (g per sample)	3.3 ± 0.5	3.4 ± 0.4	**3.7 ± 0.1**	3.4 ± 0.5

[1] Forty five bone samples were used to determine pre-treatment means for mass, volume and density, and five for assessment of fluid effects on mass, volume and density at 30 days. Fifteen samples were used to determine means for pre-treatment mineral content and five for the effect of fluids on mineral content at 30 days

Bold = significant (*P* < 0.05) using the t-test, compared to pre-treatment samples

increase was not a result of the presence of bones as the pH of the saliva solution was similar in bone-free and bone-containing saliva. Plasma Ca concentration remained constant at 2.4 ± 0.1 mmol/ℓ (Table 2). Plasma P concentration was more variable, ranging from 1.4 ± 0.3 to 1.7 ± 0.7 mmol/ℓ (Table 2). These Ca and P values are within the normal range for sheep (Hurwitz 1996; Underwood & Suttle 1999).

Bone samples

No significant effects of immersion were measurable after exposure for 10 and 20 days. Some effects were seen after 30 days (Fig. 1; Tables 3 and 4).

Physical appearance

The physical appearance of the two types of bones after 30 days of exposure to the various treatments is shown in Fig. 1A and B. Distilled water had no obvious macroscopic effects. Artificial saliva produced visible erosion of the cervical vertebrae but had no eroding effect on samples derived from the metacarpus. Rumen fluid did not produce visible erosion of bones but did discolour them. Both rumen fluid and artificial saliva caused softening of the cervical vertebrae samples, and cracking of the metacarpus shaft samples.

Chemical and physical analysis

As might have been predicted, the composition of metacarpal bone samples after 30 days of immersion, was unaffected by any of the three fluids (Table 3), except in so far as they softened after immersion in rumen fluid suggesting that their structure was altered. Table 3 shows, however, an apparent significant increase in total ash and non-Ca + P content

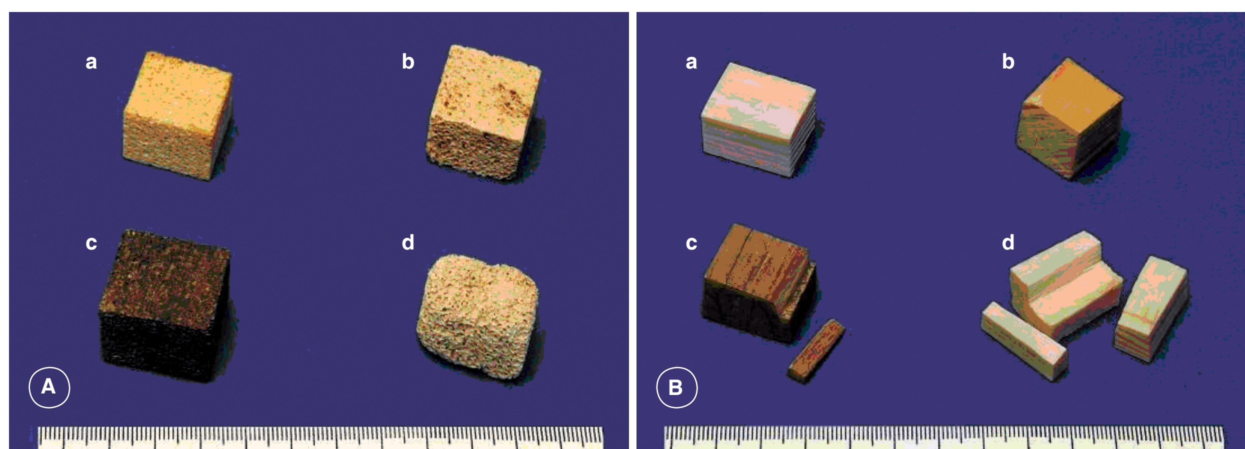

FIG. 1A and B Physical appearance of blocks prepared from cervical vertebrae (A) and metacarpus shaft (B) after 30 days

a = Bone sample prior to immersion in a fluid, b = Bone sample immersed in distilled water for 30 days, c = Bone sample immersed in rumen fluid for 30 days, and d = Bone sample immersed in artificial saliva for 30 days

TABLE 4 The effects of distilled water (DH_2O), artificial saliva, and rumen fluid on cervical vertebrae bone samples

Bone variable	Before treatment[1]	DH_2O after 30 days	Artificial saliva after 30 days	Rumen fluid after 30 days
Mass (g)	5.1 ± 0.7	5.2 ± 0.6	**3.5 ± 0.5**	5.6 ± 0.4
Volume (mℓ)	4.9 ± 0.8	5.4 ± 0.9	**3.5 ± 0.5**	5.7 ± 0.4
Density (g/cm^3)	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
Total ash (g per sample)	2.6 ± 0.4	**3.4 ± 0.5**	3.0 ± 0.4	**3.2 ± 0.2**
% Ca	21.1 ± 0.7	22.0 ± 0.8	**25.5 ± 1.9**	21.8 ± 0.8
% P	9.5 ± 0.6	10.2 ± 0.8	**11.6 ± 1.1**	9.7 ± 0.5
Total Ca (g per sample)	1.0 ± 0.1	1.2 ± 0.2	1.1 ± 0.2	1.2 ± 0.1
Total P (g per sample)	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Non-Ca + P (g per sample)	1.2 ± 0.2	**1.6 ± 0.2**	1.4 ± 0.1	**1.6 ± 0.1**

[1] Forty five bone samples were used to determine pre-treatment means for mass, volume and density, and five for assessment of fluid effects on mass, volume and density at 30 days. Fifteen samples were used to determine means for pre-treatment mineral content and five for the effect of fluids on mineral content at 30 days

Bold = significant (*P* < 0.05) using the t-test, compared to pre-treatment samples

TABLE 5 The composition of rumen fluid in wild ruminants and sheep

Variable	East Africa*	South Africa#	Giraffe◊	Sheep‡
Rumen pH	6.5 ± 0.2	6.5 ± 0.1	6.5 ± 0.2	6.5 ± 0.3
Rumen DM (%)	17.6 ± 3.2	17.0 ± 1.2	13.8	10.1–10.5
Rumen NH_3-N mg.100 mℓ$^{-1}$	18.8 ± 3.7	10.0 ± 6.6	13.6 ± 2.9–24.6 ± 2.1	12.4 ± 5.8–18.2 ± 8.3
Fermentation rate (mℓ gas.gDM^{-1}.h^{-1}	4.7 ± 1.0	4.2 ± 0.5	3.8 ± 0.1	3.6–6.0
Total VFA mmol.ℓ$^{-1}$	156.3 ± 5.3	137.9 ± 11.8	158.3 ± 3.5	113–126
% acetic acid	75.0 ± 1.6	73.8 ± 3.8	75.8	77–81
% propionic acid	15.0 ± 1.7	15.3 ± 2.3	14.2	13–19
% butyric acid	9.2 ± 0.8	9.8 ± 3.6	9.0	2–4

* Species in East Africa were buffalo, eland, waterbuck, oryx, gerenuk, goats, and giraffe (data from Maloiy *et al*. 1982)

\# Species in South Africa were buffalo, wildebeest, oryx, impala, springbok, and kudu (data from Giesecke & Van Gylswyk 1975)

◊ Data from Maloiy *et al*. 1982; Odenyo *et al*. 1999

‡ Data from this study, from Hungate (1966), Church (1979), and Odenyo *et al*. 1999

of metacarpal samples after exposure to artificial saliva. This result can be related to the significantly higher mass of the bones that were analysed. It is not a biological effect.

Cancellous bone samples, on the other hand, were affected by exposure to the fluids, albeit minimally. Distilled water had no effect apart from an anomalous increase in total ash and the non-Ca + P minerals. Artificial saliva solution had several significant effects. The mass and volume of the samples decreased significantly over the 30-day period, confirming the visible effects of saliva shown in Fig. 1A. The percentage of Ca and P in the samples increased significantly over the period, either because the bone samples absorbed Ca and P from the saliva solution or because of a loss of some other component such as protein. This percentage increase in Ca and P did not, however, translate into increased absolute amounts of Ca and P because the mass of the bones decreased. The amount of Ca and P lost from the bones as a result of the change in mass was calculated to be 0.5 g Ca and 0.1 g P over the 30-day period, which are trivial amounts compared to daily physiological requirements. In rumen fluid the total ash content of cancellous bones increased probably because of absorption of minerals other than Ca and P from the rumen fluid: it is the non-Ca plus P fraction of the ash that appeared to increase. Another possibility for this increase is that it represented the consequences of colonization of the bone samples by microbes. We did not analyse this possibility.

DISCUSSION

The giraffe skeleton is unique as it constitutes a greater proportion of its body mass than is the case in other similar sized mammals, and it elongates faster than any other mammalian skeleton. The absolute amounts of Ca and P required by giraffes to support this growth are two to three fold more than the amount required by similar sized mammals such as buffaloes (Van Schalkwyk *et al*. 2004; Mitchell *et al*. 2005). The origin of the Ca needed almost certainly is browse. The origin of sufficient P is less obvious, although Pellew (1984) showed significant selection by giraffe cows for P-rich browse in the wet season of East Africa, and by bulls throughout the year. A possible alternative source of P is osteophagia.

Osteophagia is a well documented phenomenon in African ungulates and occurs in all types of ruminants both domestic and wild, and especially in giraffes (Theiler *et al.* 1924; Nesbitt-Evans 1970; Western 1971; Wyatt 1971; Leuthold & Leuthold 1972; Sutcliffe 1973; Hall-Martin 1974; Sekulic & Estes 1977; Langman 1978; Kok & Opperman 1980; Hampton 2002). It has a distinct geographical distribution that depends largely on the P content of the parent rock on which food plants are growing (Sutcliffe 1973), and factors such as excessive Ca, aluminium or iron, which can reduce the availability of P to plants (Sutcliffe 1973). The craving for bones can be attributed primarily to a P deficiency (Theiler *et al*. 1924; Denton 1982; Denton *et al*. 1986), but its function may be related more to maintaining a proper Ca to P ratio than simply increasing the intake of one of the two (Barrette1985). It also has a distinct seasonal occurrence being more common in winter than summer (Langman 1978). The fact that it is more frequently reported in giraffes than other wild ruminants, suggests that the unique P demands of the giraffe skeleton is the cause.

If osteophagia has evolved as an adaptation for providing minerals and specifically P, it could be ex-

pected to be both directed and selective. Moreover, ingested bones or bone fragments too large to enter the lower gastrointestinal tract (abomasum and duodenum) directly should be able to be digested in the upper digestive tract (reticulo-rumen). However there is no evidence that giraffes select, for example, cancellous bone, which is easier to crush, over dense bone, which even with sophisticated machinery is difficult to reduce to powder or small particles. The many observations made of osteophagia in giraffes show that the bones selected range from fresh to weathered, and from dense nonporous bones to bones that are highly porous and often brittle. Furthermore giraffes, and ruminants in general, do not have mouth parts designed for crushing and grinding bone. Their molar teeth are adapted for grinding herbage, although Sutcliffe (1973) has reported that deer chew bones in a "cigar-like manner".

Bones are a potentially large source of minerals and consist of approximately 460 g mineral per kg, 360 g protein per kg, and 180 g fat per kg (McDonald, Edwards, Greenhalg & Morgan 2002). Calcium and P are the two most abundant mineral elements constituting about 36 % and 17 %, respectively, of the mineral component of the bones we used and of other mature bone (Underwood & Suttle 1999). Thus, if bones were digested in the rumen and were reduced to a size that allows them to pass through the reticulo-omasal orifice to enter the abomasum and small intestine where further digestion and absorption is more certain, then osteophagia would be a highly advantageous behaviour, especially if the rumen is itself adapted to digest bone.

Our results show, however, that there was little digestion of bone in either artificial saliva or the rumen at least in the first 30 days of immersion. Insignificant amounts of Ca and P were removed. Both types of bone softened in the rumen but this digestion was not associated with significant loss of Ca or P. Immersion in artificial saliva resulted in some digestion. In saliva cancellous bone not only softened, its mass and volume decreased, and Ca and P were also removed in proportion to the decline in mass (bone density remained constant), although the absolute amounts removed are miniscule compared to daily requirements. These effects were produced despite the absence of the digestive enzymes normally found in saliva. The elution of minerals by saliva could simply be an effect of pH, but this is unlikely. At the start of our trials the pH of distilled water, saliva and rumen fluid was acidic and similar, but only saliva had the effects reported here. Moreover the pH of the saliva solution became more alkaline with time (Table 2).

The softening of cancellous bone and dense bone, taken together with our observation that the samples became softer the longer they were in the fluids, suggests that immersion could facilitate mechanical digestion during rumination. The possibility exists therefore, that if giraffe saliva contains digestive enzymes and if it is of similar chemical composition to the artificial saliva solution used in this investigation, then through the mechanical effect of chewing, and the chemical action of saliva, minerals may be released from bones, especially cancellous bones. Potentially more important consequences of this effect are that re-chewed, softened, bones could be more susceptible to rumen digestion and that bones could be reduced to the size that could pass through the rumen without further digestion to enter the lower gastrointestinal tract. They also may be more susceptible to acid digestion in the abomasum. We are doubtful though if these are likely scenarios. Prolonged and continuous exposure to saliva (10 or more days) obviously does not occur.

The most plausible reasons for the lack of effect found in this study are that the bones were not exposed for a sufficiently long time for digestion to occur, or that proteolytic activity in the microbial population in the rumens of the sheep was low, and/or that the rumen fluid of sheep differs from that of giraffes and other ruminants. The possibility that a longer time is required for digestion seems unlikely but cannot be excluded by the data obtained. Minerals are required daily, after 30 days the amount of mineral released was trivial, and the degree of bone digestion was small. We are not convinced that longer exposure would have altered any of these findings significantly. Reduced proteolysis is a more likely reason. Proteolysis would release Ca and P from bone. The diet provided was relatively low in protein (7 % of DM) and so proteolysis might have been lower than it could have been: low protein hay is associated with low rumen ammonia-N production which is a marker of proteolytic activity (Van Gylswyk 1970). However, this diet accurately mimics the composition of ingested feed during the winter by giraffes. Thus even if this is the reason for the lack of digestion that was found, it is likely that a similar low rate of digestion will occur in the giraffe rumen in winter.

The composition of rumen fluid in sheep has been studied in detail by Church (1979) and Hungate (1966). Giesecke & Van Gylswyk (1975), Maloiy *et*

al. (1982) and Odenyo, McSweeney, Palmer, Negassa & Osuji (1999) have analysed the rumen contents of ten wild ruminant species including giraffes, and in free-ranging sheep and goats in East Africa (Maloiy *et al.* 1982; Odenyo *et al.* 1999) and South Africa (Giesecke & Van Gylswyk 1975). A summary of these data is shown in Table 5. These species include selective foragers (giraffe and gerenuk), bulk or roughage feeders (buffalo, waterbuck, wildebeest, oryx, sheep) and intermediate feeders (eland, kudu, impala and springbok). As Table 5 shows, the composition of rumen fluid varies little between species. The only report that is not consistent with these data is that of Jones, Meyer, Bechaz, Stoltz, Palmer & Van der Merwe (2001) who found that the rumen pH of browsers, including giraffes was lower (5.8 ± 0.1) than that of grazers (6.7 ± 0.1). They could find no consequences of the lower pH, however, and our data show that pH itself has little effect on the digestion of bones. Indeed, Jones *et al*. (2001) showed that at the higher pH nitrogen and dry matter digestion were greater than they were at the lower pH.

The general conclusion is that there is little difference in rumen fluids between African ruminants with different morphological adaptations of the gut, or between wild and domestic ruminants, or between those of different size, or because of locality (Giesecke & Van Gylswyk 1975; Gordon & Illius 1994; Robbins, Spalinger & Van Hoven 1995). We conclude that differences between rumen fluids are insignificant, do not account for the lack of bone digestion in our study, and that differences in rumen digestion between species, if it exists, is unlikely to be large.

The data presented here shows, therefore, that although osteophagia is a potentially large source of Ca and P for ruminants, there was little digestion of bones in the rumen, at least in the model and for the time period used here. It is possible that if large quantities of very weathered bones are incubated in the rumen for a long time after repeated exposure to saliva by rumination, that digestion may occur. Our data suggests, however, that even this scenario is unlikely to provide sufficient P for skeletal growth. If P (and Ca) is to be obtained from bone or bone fragments then they must reach the abomasum.

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