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Lactation growth influences mineral composition of first antler in Iberian red deer *Cervus elaphus hispanicus*

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Deer antlers are bony fighting structures which are unique in that they are both easily accessible for analysis and that they are grown every year; thus, they make up good models for the study of bones. Previous studies have shown that antler bone composition is related to the external quality (antler size and weight) and the mechanical quality of the antlers, and that it reflects mineral nutrition and early growth. Because one of the main nutritional factors influencing early growth is maternal milk production and composition, and because lactation plays an important role in post-weaning growth, we set out to examine whether milk yield and composition are correlated with the mineral composition of spike antlers of 22 yearling Iberian red deer *Cervus elaphus hispanicus*. Total milk protein yield was positively associated with ash, Ca and P content in antler, inversely with K content, but no relationship was found for Na, Mg, Fe or Zn. This association was evidently exerted through an increase in calf growth during lactation, because in the model, the inclusion of calf weight gain up to week 18 (approximately the age at weaning) rendered milk production and composition non-significant. However, this correlation was not observed for the minor minerals Na, Mg, Fe and Zn. Gains during lactation, but not between lactation and antler growth, influenced the composition of major minerals. Manipulating milk quality could not only affect general calf growth, but also antler quality and very likely the quality of other bones, as well as mechanical performance, which is linked to ash or Ca content.

Key words: Cervus elaphus hispanicus, lactation, mineral composition, red deer, spike antler, stag

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Research reviews in animal science have highlighted how neglected the study of bone biology has been despite its importance for animal growth and animal science (Loveridge 1999). This is also true for game biology and ecology, where the study of antlers might be an invaluable source of information. Antlers constitute a unique model for basic research in bone biology and animal science, because they are the only animal bone that is accessible without the intrusiveness of surgical procedures and the potentially adverse effects of such procedures, because antlers grow and are cast every year. They are costly sexual secondary characters (Harvey & Bradbury 1991) which, for their growth, require a partial demineralisation of the skeleton because the diet cannot supply the enormous amount of minerals required for their rapid growth (Meister 1956, Muir et al. 1987), which can reach 2-4 cm per day (Goss 1983). For this reason, it has been suggested that antler size might not only be an index of male quality, but also of habitat or of diet quality. Indeed, antler size is inversely related to conditions that exert stress on the physiology of the animal. One of the most important factors is likely to be nutrition. Nutrition measured directly (in white-tailed deer *Odocoileus virginianus*; Ullrey 1983) or indirectly through weather effects on plant productivity (in red deer *Cervus elaphus*; Schmidt et al. 2001; in reindeer *Rangifer tarandus*; Weladji et al. 2005) affects various parameters, from spike length to relative antler length and number of tines in adult deer. A promising area of interest regarding the effects of nutrition on antlers concerns antler composition and its associated mechanical properties (Landete-Castillejos 2007a). In fact, chemical composition trends along the main growth axis of antlers reflect mineral constraints in the habitat (Landete-Castillejos et al. 2007b), and differences in mean antler mineral composition between deer populations also reflect the ratio of the same minerals in diets available to both populations (J.A. Estevez, T. Landete-Castillejos, A. Martínez, A.J. Garcia, F. Ceacero, E. Gaspar-Lopez, A. Calatayud & L. Gallego, unpubl. data). As a source of nutrition, milk might be particularly important for calf growth and subsequent effects on antlers because, even when the calf is ingesting solid food in addition to milk, solid food seems to be used for body maintenance whereas milk energy and protein seem to be used mainly for growth (White 1992). As far as lactation as a source of nutritional effects on antlers is concerned, recent research has found that milk protein content can explain up to a third of the variability in spike length and weight of Iberian red deer *Cervus elaphus hispanicus* (Gómez et al. 2006). Furthermore, the variability in composition of only five minerals can predict 75% of antler weight or length (Landete-Castillejos et al. 2007c). As lactation is one of the best known maternal effects which in general are known to later affect adult characteristics (Bernardo 1996), it is also very likely that it might influence adult body size. Thus, lactation affects growth of Iberian red deer calves at least up to two years of age (Gómez et al. 2006). In fact, the relationship between skeleton size and antler weight is one of the longest-known allometric relationships in animals (Huxley 1931). Overall, it is therefore not surprising that lactation effects on early antlers and growth trajectory of the calf might influence antler characteristics in adult deer, including composition. Also, because lactation is greatly influenced by the level of nutrition of the environment, and food restriction has a greater impact on calves than on their mothers (Landete-Castillejos et al. 2003), ecological conditions affecting habitat quality might exert a considerable influence through lactation. Because antler mineral profile and mechanical properties are associated in more ways than just the effects of Ca and P, such effects might influence both antler and bone quality, thus probably also affecting the risk of fractures and deaths.

The aim of our study was to assess whether major lactation variables (i.e. milk production and composition in terms of protein, fat and lactose) influence antler composition of first (spike) antlers. To assess the relative importance of growth caused by lactation (the fastest growth in the life of a mammal) and growth after weaning (slower than during lactation, but closer to the period of antler growth), gains during and after lactation were also included in the same model in a subsequent step.

Material and methods

Animals

We used 22 yearling Iberian red deer males and their mothers for our study. The animals were kept at the University experimental farm (Albacete, Spain, 38°57'10"N, 1°47'00"W, 690 m a.s.l.) in a 10,000 m^2 open door enclosure on an irrigated pasture including tall fescue *Festuca arundinacea* (50%), cocksfoot *Dactylis glomerata* (30%), alfalfa *Medicago sativa* (15%) and white clover *Trifolium repens* (5%). As suggested by Brelurut et al. (1990), the feed of the mothers was supplemented with concentrate feed (16% of crude protein) composed of leguminous plants and cereals provided *ad libitum* all year round. In the same way, water was available all year round and cereal straw was provided *ad libitum* in winter.

Experimental design and assays

To assess the relation between milk production and composition and antler mineral composition, hinds were milked in weeks two, three, four and six, and then every four weeks up to week 18 after the birth of their calves. Milking frequency was reduced to the minimum considered essential to prevent stress and potential damaging effects of the anaesthesia, which was carried out using a low-dose combination of xylazine (0.5 mg/kg body mass) and ketamine (1 mg/kg) intravenously injected. Before each milking, hinds were isolated from their calves for six hours (set up at 10:00 to 16:00). Before this isolation period, milking to standardise udder reserves was not performed for ethical reasons fully explained in Landete-Castillejos et al. (2000a). Milking was carried out with a milking machine set to a 50/50 massage/milking ratio and 44 kPa of vacuum. After one to two minutes of machine milking depending on udder capacity, the hinds were milked by hand to collect the remaining milk. Daily milk production was estimated to be four times the volume collected at each milking. Two 30-ml samples of milk (replicates) were collected for chemical analysis to assess protein, fat and lactose percentages. These samples were analysed separately (not pooled) in an automatic milk analyser as described in Landete-Castillejos et al. (2000b). Total milk yield was computed by multiplying the midpoint between the amount collected in consecutive trials by the period between them and then adding results for all trials. Total protein yield (TPY), total fat yield (TFY) and total lactose yield (TLY) were computed by multiplying the production between trials by the average composition of each nutrient.

Samples were obtained after antler casting. Antlers were filed with a metal file made of Fe, C, Si, Mn, Cr, Mo, V and W, with a hardness of 63-65 HRC, and wearing away less than 0.1 mg in the whole test, to obtain 0.2 grams of cortical bone per sample (one from the base and one from the tip of the tine). Mineral analysis was performed by drying antler samples at 102°C for three hours, and then at 130°C for 24 hours. After this period, they were incinerated at 520°C for 12 hours. Ash was then dissolved with 10 ml of 3N HCl and then heated until the dilution emitted white smoke. After cooling, samples were filtered with paper ALBET REF.1300 in a 50 ml volumetric flask and the process was repeated in the capsule that contained the ash to dissolve any remains. The volume was completed with Ultrapure water so that a 1/1000 dilution resulted for Ca analysis, 1/200 for Na, Mg and K, and 1/10 for Fe and Zn to adjust concentrations to calibration lines.

Ash samples were analysed using an atomic absorption spectrophotometer (Perkin-Elmer 2280). The concentrations of Ca, Mg, Fe and Zn were analysed with atomic absorption spectrophotometry, while K and Na were analysed using atomic emission (using the same equipment without the hollow cathode lamp used above). In the case of Ca, 0.2% lanthanum trichloride was used to prevent interference from other elements. The spectrum lines for Ca, Mg, Na, K, Zn and Fe were 422.7, 285.2, 589.0, 766.5, 213.9 and 248.3, respectively. Absorbance was measured at 2-second intervals. Each datum was the mean of five measures recorded at the interval mentioned above, after having checked that their coefficient of variation was $<2\%$. For P determination, UV visible spectrophotometry was used according to the colorimetric method, analysing it as phosphomolybdic acid according to Osborne & Voogt (1978). The equipment used was a

Shimadzu UV 1230 spectrophotometer at 650 nm wave length.

To assess the precision of the method used to determine mineral concentration, we analysed five samples of skim milk powder supplied by the Community Bureau of Reference (BCR-63).

Calves were weighed weekly using a 10-g precision electronic balance during lactation. This study complies with current Spanish and European legislation on welfare and other guidelines on ethical use of animals for experiment (ASAB 2005).

Table 1. Mean values of variables related to milk production and composition and calf growth of 22 male calves of Iberian red deer born in the years of 2000 (N = 13) and 2001 (N = 9). The acronyms stand for: CBW = calf birth weight, CWG18 = calf weight gain at week 18, CGL = calf weight gain after lactation and up to one year of age, TMY = total milk yield, TPY = total protein yield, TFY = total fat yield, TLY = total lactose yield, P = percentage of milk protein, F = percentage of milk fat, and L = percentage of milk lactose.

Variable	Mean	SEM
CBW (in kg)	8.5	0.3
CWG18 (in kg)	32.6	1.4
CGL (in kg)	37.9	1.2
TMY (in kg)	196.5	16.4
TPY (in kg)	12.5	1.1
TFY (in kg)	18.1	1.8
TLY (in kg)	11.2	0.9
P (in %)	6.4	0.1
F (in %)	9.3	0.2
L (in %)	5.6	0.0

Statistical analysis

Pearson correlations (bivariate) tested relationships between variables related to lactation stage and the different minerals of the first antler using the mean of the two measures (one datum per individual).

General Linear Models (GLM; Statgraphics, Manugistics, Rockville MD) examined the association between calf birth weight, total milk production and that of its components (protein, lactose and fat), male weight gain up to week 18 (approximately the weaning age in standard conditions) and male weight gain after week 18 and up to one year of age, with mineral composition of spike antlers (calculated as the mean between content at antler tip and base for each individual). To examine these associations in more detail, models were analysed separately for each mineral. These analyses were carried out in two steps: the first one tested variables directly related to milk production (i.e. milk, protein, fat and lactose production and milk protein, fat and lactose percentage). In the second step, calf weight gain during lactation and calf weight gain after week 18 and up to one year of age (a period closer to the growth of the antlers) was also included. The quadratic term of milk composition factors and of calf weight gain was also tested to assess non-linear effects (i.e. that for instance total protein yield correlated with Ca content below a certain optimum Ca percentage in antler). To prevent multicollinearity problems between related variables (protein production vs milk production), variance inflation factors were examined to discard variables with variance inflation factor > 10 (Neter et al. 1996). The models were calculated by backward stepwise selection until a model including only significant factors was achieved.

Table 2. Correlation coefficients between production of milk nutrients (TMY, TPY, TFY and TLY), calf weight variables and antler mineral composition variables of 22 male calves of Iberian red deer born in the years of 2000 (N = 13) and 2001 (N = 9). Significant correlations at P < 0.1, 0.05, 0.01 and 0.001 are indicated by †, *, ** and ***, respectively. See Table 1 for description of acronyms.

	Ash	Ca	P	Na	Mg	K	Fe	Zn
TMY	0.49*	0.39†	0.45*	-	-	-0.45*	-	-
TPY	0.50*	0.41†	0.47*	-	-	-0.45*	-	-
TFY	0.52*	0.41†	0.44*	-	-	-0.50*	-	-
TLY	0.48*	0.37†	0.44*	-	-	-0.44*	-	-
CBW	-	-	-	-	-	-	-	-
CWG18	0.58**	0.56**	0.67***	-	-	-0.67***	-	-
CGL	-	-	-	-	-	-	-	-
Ca	0.96***							
P	0.85***	0.85***						
Na	-0.61**	-0.58**	-0.42*					
Mg	0.71***	0.67**	0.47*	-0.51*				
K	-0.64**	-0.61**	-0.67**	-	-			
Fe	-	-	-	-	-	-		
Zn	-	-	-	-	-	-	0.41†	

Table 3. General Linear Models showing lactation variable effects on antler composition in one year old deer (N = 22). Lactation variables included calf birth weight, total milk production and that of its components (protein, lactose and fat) and male weight gain up to week 18 affecting the composition of the different minerals of the first antler in Iberian red deer. The results show only the significant variables in the final model resulting from analysing data in two ways: A) including milk variables and calf birth weight but not calf gains, and B) introducing these variables, calf gains during lactation and also calf gains from weaning (at the age of 18 weeks) up to one year of life. Significant differences at $P < 0.05$, 0.01 and 0.001 are indicated by *, ** and ***, respectively. Na, Mg, Fe and Zn were not affected by milk production or by its nutrients. See Table 1 for description of acronyms. The superscript at $F_{1,20}$ and R^2 shows the actual probability.

A) Models including milk variables and calf birth weight but not calf gains				
Dependent variable	Intercept	TPY	$F_{1,20}$	R^2
Ash (%)	48.1 ± 2.1	0.41 ± 0.16	6.52*	24.6*
Ca (%)	15.20 ± 0.73	0.109 ± 0.054	$4.02^{0.059}$	$16.8^{0.059}$
P (%)	6.85 ± 0.38	0.065 ± 0.028	5.51*	21.6*
K (mg/kg)	880 ± 120	-20.5 ± 9.1	5.03*	20.1*
B) Model including previous variables CWG18 and CGL in the analysis				
Dependent variable	Intercept	CWG 18	$F_{1,20}$	R^2
Ash (%)	41.2 ± 3.8	0.40 ± 0.10	10.36**	34.1**
Ca (%)	12.80 ± 1.27	0.115 ± 0.038	9.10**	31.3**
P (%)	5.28 ± 0.60	0.073 ± 0.018	16.64***	45.4***
K (mg/kg)	1390 ± 200	-23.6 ± 5.9	15.83***	44.2***

Results

The composition of the mature hardened first antler in the Iberian red deer is on average (dry weight values, mean ± SE) 53.2 ± 0.9% ash with a Ca/P weight ratio of 2.2 ± 0.2, 165.7 ± 2.9 mg/g Ca, 76.7 ± 1.5 mg/g P, 7.7 ± 0.1 mg/g Na, 4.7 ± 0.1 mg/g Mg, 619.9 ± 59.9 μg/g K, 93.6 ± 13.0 μg/g Fe and 86.8 ± 2.7 μg/g Zn. This means that about 95% of the ash of this first antler is composed of Ca and P (65 and 30%, respectively). Na represents 3%, Mg 2% and K, Fe and Zn together 0.27%.

The mean values of the variables associated with milk production and composition and calf growth included in this study are presented in Table 1.

These variables related to the lactation period correlated positively with the minerals present in a greater proportion in this first antler (ash, Ca and P) and negatively with the K content (Table 2).

In this sense, lactation was strongly associated with the concentration of ash, Ca, P and K in the first mature antler, but not with that of Na, Mg, Fe or Zn. Results of the GLM showed that total protein yield was the only variable significantly affecting models for ash, Ca, P and K (Table 3) when calf gains were not included. The relationship of total protein yield was positive with ash, Ca and P, but inverse in the case of K content. Total protein yield rendered non-significant similar milk lactation variables such as total milk yield, total fat yield and total lactose yield.

The association of milk production and that of its nutrients with the antler mineral content appeared to be exerted through weight gained during the first 18 weeks of life as this measurement absorbed the variance explained by lactation variables and rendered them no longer significant. Thus, calf weight gain up to week 18 was the only significant variable explaining the mean percentage of ash, Ca, P and K in this first antler, and amounted to 34, 31, 45 and 44% of the variance explained by the model for these variables, respectively. Surprisingly, growth during lactation was the only growth period affecting antler composition, even when calf gains after lactation and up to one year of age was included in the model.

No effects of lactation or calf growth up to weaning or after weaning and up to one year of age were found for Na, Mg, Fe or Zn. Similarly, no threshold effect was found for any model as quadratic components of lactation variables or calf growth were in no case significant.

Discussion

Our results suggest that milk production and composition are associated with the mineral content of the first antler. Both the correlations and the GLM reveal that a greater milk supply, particularly protein, was associated with antler ash, Ca, P and K, and this suggests that milk protein yield correlates with skeletal size and growth, which in turn correlates with antler mineral composition.

The influence of lactation on antler composition seems to be exerted via calf weight gain during lactation. This is suggested by the fact that the variability explained by lactation variables was absorbed by calf weight gains during lactation once this is included in the model, and also that both affected the same minerals (ash, Ca, P and K) and no others. Such a positive effect of better milk provisioning on antler ash, Ca and P might be important because these are the major minerals of antlers, and mineral content is responsible for mechanical properties of any bone (Currey 1979, 1984, 2004). An interesting finding is that, with respect to antlers, lactation was the only important growth period for calf weight gains even after we included post-lactation growth up to one year of age (which is of course closer to the actual start of antler growth). This suggests that growth after lactation is not as relevant to antler composition and might imply that lactation sets a body growth trajectory that is little modified after weaning. Our study with its subsequent *ad libitum* food conditions should have provided the maximum opportunity for later growth to be compensatory, so the absence of such compensation in our data suggests that lactation effects in harsher conditions (as might be expected in the wild), might exert an even greater influence in antler composition and mechanical quality than in our captive setting.

This connection of lactation with antler growth despite a delay of six months is not unique. It has been shown that the conditions under which individuals are born may have important long-term consequences for the life history of the cohort (Albon et al. 1992, Forchhammer et al. 2001, Gaillard et al. 2003). Results in the Iberian red deer show that the effects of lactation are very important because they result in weight differences at least up to two years of age (Landete-Castillejos et al. 2001, Gómez et al. 2006).

Lactation and other sources of nutrition have also been shown to influence antler size and weight. Milk composition affects morphologic characteristics of the first antler such as its length or width (Gómez et al. 2006). Because an earlier study showed that the chemical composition is related to the quality of the antlers based on external characteristics (Landete-Castillejos et al. 2007c), it is thus not surprising that milk can influence antler chemical composition. In red deer, antler length is usually used as quality index of individuals, because yearling antler length is a largely nutrition-mediated phenotypic character (Schmidt et al. 2001). The results of Schmidt et al. (2001) showed that antler length increases with birth weight and decreases with increasing birth date delay, reflecting the persistent influence of conditions in early life.

These effects should be seen as an important particular case of reported negative effects of reduced food availability on antler growth or length (Scribner et al. 1989, Schmidt et al. 2001), as well as horn growth (Festa-Bianchet et al. 2004), and they could all have subsequent cumulative effects in the male (Asleson et al. 1997).

How could milk nutrition influence the chemical composition of antlers? The answer is suggested when calf weight gain during lactation absorbed the variability explained by milk provisioning variables. Yearly growth and replacement of antlers create a huge nutritional demand on male deer (French et al. 1956, Ullrey 1983, Asleson et al. 1997) that is met by bone resorption from several parts of the skeleton (Meister 1956, Arnold 1960, Brown 1990, Baxter et al. 1999). Thus, a greater milk production (particularly that of protein) would result in larger body size and higher growth trajectory (Landete-Castillejos et al. 2001, Gómez et al. 2006), and this would mean larger skeletons from which greater amounts of minerals could be mobilised to increase size and mineral content of antlers. What is very interesting is that such greater milk production is connected with bone chemical composition and not only with antler size. However, other sources of nutrition appear to affect antler composition and possibly that of other bones. Thus, differences in antler Na, Mg and K content between deer with different planes of nutrition seem to be caused by a constraint in the availability of these minerals in natural habitats (Landete-Castillejos et al. 2007b).

Other evidence suggesting the importance of nutrition to antler composition is that the cessation of supplemental feeding affects bone Ca and P in mature red deer, and total mineralisation declines even in areas where availability of natural nutrients is so good that no change in antler morphology, growth rate or body weight is observed (Groot-Bruinderink et al. 2000).

Why was no association found for Na, Mg, Fe or Zn? Our data do not allow us to draw definite conclusions, but all of these elements are found in small amounts in the antler ($<3\%$). Some of them, such as Fe, are not mainly stored in the skeleton, but in the liver and other organs (McDowell 2003), which are more variable as a reserve than the skeleton. Thus, daily intake, even at low amounts in the diet, is more likely to affect small circulating levels of minerals than large amounts of Ca and P needed to grow antlers. However, the sample size in our study may have failed to detect lactation effects on minor minerals that might be discerned with larger sample sizes.

A particularly striking effect is the negative association of milk composition and calf gains with K content in antlers. Potassium is the third most important element in the body (McDowell 2003), after Ca and P, and it is thus not surprising that lactation could be associated with the body stores of K in the calf. However, evidence of its important influence in other studies does not point to its abundance in the body, but rather its role in Ca metabolism (Rafferty et al. 2005). It is suggested that K intake is raised because it reduces Ca urinary losses as calcium is mobilised out of the skeleton to grow antlers (Landete-Castillejos et al. 2007b). Thus, it is not surprising that it showed an inverse relationship with milk provisioning. Individuals with lower milk intake would grow lighter skeletons, and hence would have a lower Ca store for growing antlers. They would thus need to be more efficient in using the smaller amount of Ca available, and it would not be surprising that they increase K intake to reduce urinary losses of Ca at the lowest level possible.

In conclusion, lactation seems to be important not only for calf growth, but also to grow antlers of high quality in terms of chemical composition. This effect is likely exerted by setting up a growth trajectory during lactation that is more important than post-weaning growth even in *ad libitum* conditions such as those in our setting.

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References

Albon, S.D., Clutton-Brock, T.H. & Langvatn, R. 1992: Cohort variation in reproduction and survival: implications for population demography. - In: Brown, R.D. (Ed.); The Biology of Deer. Springer-Verlag, New York, pp. 15-21.

ASAB 2005: Guidelines for the treatment of animals in behavioural research and teaching. - Animal Behaviour 69: i-vi.

Asleson, M.A., Hellgren, E.C. & Varner, L.W. 1997: Effects of seasonal protein restriction on antlerogenesis and body mass in adult male white-tailed deer. - Journal of Wildlife Management 61: 1098-1107.

Baxter, B.J., Andrews, R.N. & Barrell, G.K. 1999: Bone turnover associated with antler growth in red deer (Cervus elaphus). - Anatomical Record 256: 14-19.

Bernardo, J. 1996: Maternal effects in animal ecology. - American Zoology 36: 83-105.

Brelurut, A., Pingard, A. & Thériez, M. 1990: Le cerf et son élevage. - INRA, Paris, 143 pp. (In French).

Brown, R.D. 1990: Nutrition and antler development. - In: Bubenik, G.A. & Bubenik, A.B. (Eds.); Horns, Pronghorns and Antlers: Evolution, Morphology and Social Significance. Springer-Verlag, New York, pp. 426-441.

Currey, J.D. 1979: Mechanical properties of bone tissues with greatly differing functions. - Journal of Biomechanics 12: 313-319.

Currey, J.D. 1984: Comparative mechanical properties and histology of bone. - American Zoology 24: 5-12.

Currey, J.D. 2004: Tensile yield in compact bone is determined by strain, post yield behaviour by mineral content. - Journal of Biomechanics 37: 549-556.

Festa-Bianchet, M., Coltman, D.W., Turelli, L. & Jorgenson, J.T. 2004: Relative allocation to horn and body growth in bighorn rams varies with resource availability. - Behaviour and Ecology 15: 305-312.

French, C.E., McEwan, L.C., Magruder, N.D., Ingram, R.H. & Swift, R.W. 1956: Nutrient requirements for the growth and antler development in the white-tailed deer. - Journal of Wildlife Management 20: 221-232.

Forchhammer, M.C., Clutton-Brock, T.H., Lindström, J. & Albon, S.D. 2001: Climate and population density induce long-term cohort variation in a northern ungulate. - Journal of Animal Ecology 70: 721-729.

Gaillard, J-M., Loison, A., Toïgo, C., Delorme, D. & Van Laere, G. 2003: Cohort effects and deer population dynamics. - Ecoscience 10: 312-320.

Gómez, J.A., Landete-Castillejos, T., García, A.J. & Gallego, L. 2006: Importance of growth during lactation on body size and antler development in the Iberian red deer (Cervus elaphus hispanicus). - Livestock Science 105: 27-34.

Goss, R.J. 1983: Deer antlers: regeneration, function and evolution. - Academic Press, New York, 336 pp.

Groot-Bruinderink, W.T.A., Lammertsma, D.R. & Hazebroek, E. 2000: Effects of cessation of supplemental feeding on mineral status of red deer Cervus elaphus and wild boar Sus scrofa in the Netherlands. - Acta Theriologica 45: 71-85.

Harvey, P.H. & Bradbury, J.W. 1991: Sexual Selection. - In: Krebs, J.R. & Davies, N.B. (Eds.); Behavioural Ecology: an evolutionary approach. Blackwell Scientific, Oxford, pp. 203-233.

Huxley, J. 1931: The relative size of antlers of deer. - Proceedings of the Zoological Society (London) 1931: 819-864.

Landete-Castillejos, T., García, A.J. & Gallego, L. 2001: Calf growth in captive Iberian red deer (Cervus elaphus hispanicus): Effect of birth date and hind milk production and composition. - Journal of Animal Science 79: 1085-1092.

Landete-Castillejos, T., García, A.J., Garde, J. & Gallego, L. 2000a: Milk intake and yield curves and allosuckling in captive Iberian red deer (Cervus elaphus hispanicus). - Animal Behaviour 60: 679-687.

Landete-Castillejos, T., García, A.J., Molina, P., Vergara, H., Garde, J. & Gallego, L. 2000b: Milk production and composition in captive Iberian red deer (Cervus elaphus hispanicus): effect of birth date. - Journal of Animal Science 78: 2771-2777.

Landete-Castillejos, T., García, A., Gómez, J.A. & Gallego, L. 2003: Lactation under food constraints in Iberian red deer Cervus elaphus hispanicus. - Wildlife Biology 9: 131-139.

Landete-Castillejos, T., Currey, J.D., Estevez, J.A., Gaspar-López, E., García, A. & Gallego, L. 2007a: Influence of physiological effort of growth and chemical composition on antler bone mechanical properties. - Bone 41: 794-803.

Landete-Castillejos, T., Estevez, J.A., Martínez, A., Ceacero, F., García, A.J. & Gallego, L. 2007b: Does chemical composition of antler bone reflect the physiological effort made to grow it? - Bone 40: 1095-1102.

Landete-Castillejos, T., García, A.J. & Gallego, L. 2007c: Body weight, early growth and antler size influence antler bone mineral composition of Iberian Red deer (Cervus elaphus hispanicus). - Bone 40: 230-235.

Loveridge, N. 1999: Bone: More than a stick. - Journal of Animal Science 77: 190-196.

McDowell, L.R. 2003: Minerals in animal and human nutrition. - Elsevier, Amsterdam, 644 pp.

Meister, W. 1956: Changes in biological structure of the long bones of white-tailed deer during the growth of antlers. - Anatomical Record 124: 709-721.

Muir, P.D., Sykes, A.R. & Barrell, G.K. 1987: Calcium metabolism in red deer (Cervus elaphus) offered herbages during antlerogenesis: kinetic and stable balance studies. - Journal of Agriculture Science 109: 357-364.

Neter, J., Kutner, M.H., Nachtsheim, C.J. & Wasserman, W. 1996: Applied linear statistical models. 4th Edition. - Chicago, pp. 385-392.

Osborne, D.R. & Voogt, P. 1978: The Analysis of Nutrients in Foods. - Academic Press, London, 251 pp.

Rafferty, K., Davies, K.M. & Heaney, R.P. 2005: Potassium intake and the calcium economy. - Journal of the American College of Nutrition 24: 99-106.

Schmidt, K.T., Stien, A., Albon, S.D. & Guinness, F.E. 2001: Antler length of yearling red deer is determined by population density, weather and early life-history. - Oecologia 127: 191-197.

Scribner, K.T., Smith, M.H. & Johns, P.E. 1989: Environmental and genetic components of temporal variability in white-tailed deer antler growth. - Journal of Mammalogy 70: 284-291.

Ullrey, D.E. 1983: Nutrition and antler development in white-tailed deer. - In: Brown, R.D. (Ed.); Antler Development in Cervidae. Caesar Kleberg Wildlife Research Institute, Kingsville, Texas, pp. 49-59.

Weladji, R.B., Holand, Ø., Steinheim, G., Colman, J.E., Gjøstein, H. & Kosmo, A. 2005: Sexual dimorphism and intercohort variation in reindeer calf antler length is associated with density and weather. - Oecologia 145: 549-555.

White, R.G. 1992: Nutrition in relation to season, lactation, and growth of north temperate deer. - In: Brown, R.D. (Ed.); The biology of deer. Springer-Verlag, New York, pp. 407-418.