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Smaller does not mean worse: variation of roe deer antlers from two distant populations in their mechanical and structural properties and mineral profile

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Abstract

Antler size, structure, composition and mechanics have been shown to reflect nutrition, climate and body effects in red deer, but studies have only assessed effects on size in the roe deer (*Capreolus capreolus*). Roe deer show little sexual dimorphism, lower inter-male fighting and could form groups during part of the year but does not form harems, in contrast to red deer. Thus, it is interesting to assess how nutrition and habitat affects investment in antlers as compared to red deer. Antlers were collected from adult males of two game estates differing in location, climate and management: 13 from the south-east of Spain (mild winter, hot summer, dry habitat and rich supplementary feeding), and 10 specimens came from central-southern part of the Czech Republic (snowy winter, mild summer, humid habitat and limited supplementary feeding). After measuring whole-antler parameters, a destructive sampling was performed to obtain a full-transversal section and cortical bone samples from two sampling position along the main beam. Then bone structure, mechanical properties (three-point bending test, impact test) and the mineral profile were studied. Roe deer from Spain had heavier and longer antlers than Czech roe deer. Their bone material had a higher mechanical quality, although Czech roe deer compensated by developing antlers with thicker walls. Mineral composition also differs, particularly by greater contents in Czech antlers in 3 minerals associated with nutrient stress: Fe, K and Zn. We concluded that the differences found between populations may be caused by differences in habitat quality and diet, in a similar way as reported for red deer, despite interspecific differences. Our study suggests that habitat affects antler parameters and, as previous results in red deer, suggests that improving diet quality may affect size, composition and mechanical quality of antler material. Certainly, antlers of roe deer provide information useful for population management.

Introduction

The European roe deer (*Capreolus capreolus*) is the most abundant ungulate species in Europe (Linnell & Zachos, 2011). Commonly, roe deer is treated as a monotypic species in Europe with numerous variations that have been considered subspecies by some authors (Sempéré *et al.*, 1996; Aragon *et al.*, 1998; Royo *et al.*, 2007). Some studies have investigated the factors that determine its distribution: vegetation cover (Acevedo *et al.*, 2005), food availability (Plard *et al.*, 2014), climate (Cagnacci *et al.*, 2011) or population density (Pettorelli *et al.*, 2001). Other studies have described the patterns of age-specific antler development (Stubbe, 1966) or the timing and physiology of antler cycle (Sempéré, 1990), some others have investigated roe deer cranial morphology and habitat use (Milošević-Zlatanović *et al.*, 2016) or biometric differences of skulls that could reflect morphological adaptations to different habitats (Fandos & Reig, 1993). Other authors have studied deer behaviour assessing the role of antler in reproductive activity (Clutton-Brock, 1982; Geist, 1998; Hoem *et al.*, 2007) and agonistic behaviour patterns of roe deer (Kurt, 1968), and the genetic components in antler dimensions (Geist, 1966; Hartl, Apollonio & Mattioli, 1995).

One of the main reasons for studying antlers is their rapid growth and annual regeneration which make them a good model for studying bone tissue and the possible factors in the bone growth process (Kierdorf *et al.*, 2013; Landete-Castillejos *et al.*, 2019). Generally, these factors, apart from the genetic characteristics, are populational density (Santiago-Moreno *et al.*, 2001), climate (Mysterud *et al.*, 2005; Landete-Castillejos *et al.*, 2010) and the food quality (Brown, 1990). The latter two interact with first one to realize full genetic potential and influence the animal's weight, which in turn influence the antler growth (bigger antlers are grown by heavier animals: Huxley, 1931; Vogt, 1936; Geist, 1986; Gómez *et al.*, 2006, Gómez *et al.*, 2012; Ramanzin & Sturaro, 2014). Moreover, the antler mass in *C. capreolus* represents approximately up to 23% of the dry skeleton and, due to its small body size, antler investment (antler mass relative to body or skeleton mass) may be larger than in heavier deer species according to Ceacero (2016). This author hypothesized that larger species are subjected to greater physiological constraints than smaller ones, and these are mainly linked to skeleton size, although the quadratic relationship between antler mass vs. body mass of this paper appear to be due to very few species, and the rest follow a linear relationship showing a similar investment in antlers. Other authors have indicated that the investment in antlers in roe deer is moderate and related to little sexual dimorphism; whereas, in red deer, it is greater and related to greater sexual dimorphism in body weights (Geist, 1998). Furthermore, roe deer body mass and, to a lesser extent, antler length influenced the male breeding success (Vanpé *et al.*, 2010).

In addition, secondary sexual traits, such as antlers, are signals of male quality for reproduction and are expected to be costly to produce, particularly for males in poor condition (Vanpé *et al.*, 2007; Ciuti & Apollonio, 2011). Thus, well-developed antlers could be an indicator of a male with good nutrition (Vogt, 1936; Geist, 1986; Landete-Castillejos *et al.*, 2007b, 2007c, 2012) or an indicator of allocation of resources in adulthood (Lemaître *et al.*, 2018). Therefore, antlers can be considered as a valuable tool for studying the performance of a population, and so far, the hypothesis that antlers constitute quality indicators has received specific attention in the past (Ueckermann, 1951; Pélabon & van Breukelen, 1998; Vanpé *et al.*, 2010; for other species: Solberg & Saether, 1993; Landete-Castillejos *et al.*, 2007c, 2013). However, few studies have focused on the nutrition and ecological effects in cervid antler composition, mechanical properties and histology (in Landete-Castillejos *et al.*, 2019), and even fewer of these have assessed roe deer (Kierdorf & Kierdorf, 2005).

Generally, roe antler growth cycle starts in October–November, when the antlers of the previous year are cast. The antlers grow protected by an epithelial tissue (*velvet*) until March. Then, the loss of the external velvet (*shedding*) takes place (March–May). Naturally, the animal remains with clean antlers during the mating season (Sempéré, 1990).

Since a qualitative and quantitative analysis of antler bone of roe deer is lacking, and there are no studies comparing these features in populations grown in different habitats characteristics, this study has three aims: (1) to get an initial morphometric characterization of roe deer antlers; (2) to compare the antler bone structure characteristics, mechanical properties and mineral profile of two populations greatly differing in environmental conditions (mostly distinct diet and vegetation availability); and (3) to discuss parameters and trends in roe deer antlers with those reported in antlers of red deer.

Material and methods

Two groups of roe deer antlers of adult animals (more than three years old) were collected: 10 pairs from Czech Republic and 13 individual antlers from Spain. The Czech antlers were collected from an open game estate around Vysoký Chlumec (Czech Republic; 600 m altitude); each pair of antlers was obtained from hunted animals. Animals were born and grown inside the area, where no mineral supplementation during winter was used, only meadow hay and oat. Habitat was a mosaic structure of mixed coniferous and broad-leaved forests, crop fields and meadows, where other cervid were present (fallow deer). The climate conditions were characterized by a temperate continental climate, with warm summers and cold and snowy winters. The Spanish antlers were collected inside a game estate located in the south-east (near Caravaca de la Cruz, Spain, 685 m altitude); animals lived on soil sown with selected herbs; in addition, animals had access, throughout the year, to additional food such as pellets, barley and oats. The climate conditions were characterized by a Mediterranean-semi-arid climate with irregular rainfall. The daily thermal amplitude is moderate, but compared to other regions of Europe, the annual range of temperatures is very narrow. The rainfall occurs mainly in spring and especially autumn (but there is never snow or ice), separated by a summer of severe drought.

For each roe deer, if possible, we selected the right antler, for the destructive analysis described below. Antler bone samples were extracted from two levels along the main vertical axis, in order to assess whether the properties of the bone tissue change at different stages of growth, thus reflecting the physiological effort made in growing the antler, as it happens in red deer (Landete-Castillejos *et al.*, 2007b, 2007c; Estevez *et al.*, 2008). The methodology to process these samples followed that previously used in studies on red deer (Landete-Castillejos *et al.*, 2010), adapted to the smaller size of the roe deer antlers. Thus, the sampling positions were as follows: *position 1* (directly above the burr) and *position 2* (below the distal bifurcation or 5 cm under the tip), see Fig. 1. From each position a 1 cm-slice (complete transverse cross-section), if possible, and a cylinder of about 5–6 cm were extracted.

Firstly, to obtain raw bone bars, a circular low-speed saw was used. Then, the surfaces of bars were abraded using a semiautomatic equipment for polishing (MetaServ® 250 Double, Buehler-Illinois Tool Works Inc., Lake Bluff, USA) to get the right size; the width and depth of each sample were recorded to the nearest 0.01 mm using a digital calliper prior to testing. Heating of the bone was controlled by keeping the specimens wet during machining and sanding. The final size was 4.5 × 2.5 mm and a variable length allowing a gauge length of 40 mm (a length at which the shear effects are reduced thus minimizing errors in the estimate of the *Young's Modulus*; Landete-Castillejos *et al.*, 2010).

Subsequently, to study the bone tissue structure, the transverse cross-sections were used. However, sometimes antler length was too short to get slices. In these cases, we estimated

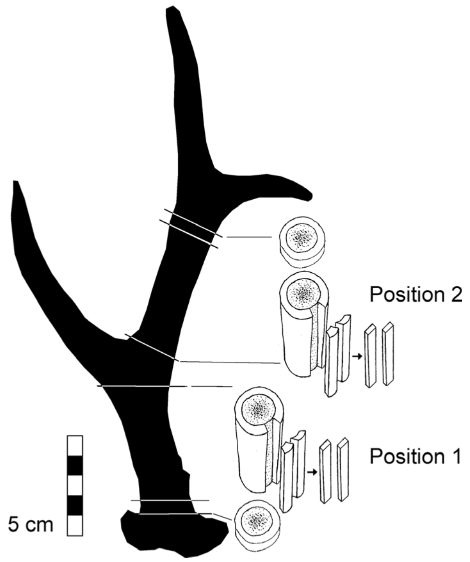

Figure 1 Sampling technique for adult deer antlers of *Capreolus capreolus*: the antler bone was cut at two levels along the vertical axis, and in each sampling, position was obtained a complete transverse cross section if possible (to study the structural characteristics) and a cylinder from which two cortical bone bars were extracted to study the mechanical properties and the mineral profile.

structural properties from transverse sections directly from the basal face of the cylinders of the *position 1* and upper face of the cylinders in *position 2* before the cylinders were cut to obtain the bars. Thus, the complete cross sections were scanned on a flatbed scanner (ScanJet 4370 Photo Scanner, HP Inc., Palo Alto, USA) at 600 dpi. Each image was processed with an image analysis software (ImageJ); the cortical bone width was measured at six equally spaced points around the perimeter to obtain the average cortical width (*Ct.B.Wi* in cm) and the ratio between cortical bone width and total diameter of the section (*Ct.B.Wi%* in percentage). Then, we measured the total area of the section, and the area of cortical and cancellous bone tissues (to calculate the ratio between cortical and total area, *Ct.B.Ar*).

Specimens for mechanical testing were first subjected to a procedure to standardize humidity content as described in Cappelli *et al.* (2015). This way, the bone bars had homogeneous humidity content when they were tested. Three-point bending test with the periosteal side in tension was carried out in a Zwick/Roell 500N machine and analysed with the software testXpert II (Zwick GmbH & Co, Ulm, Germany). Speed of the machine head was 32mm/min, and the distance between supports was 40 mm (machine compliance was found to be negligible at this gauge length and sample depth, which has an aspect ratio length to depth (AR) of 16; Currey *et al.*, 2009a; Spatz, O'Leary & Vincent, 1996).

In this first test, the following mechanical features were observed: Young's Modulus of elasticity (*E*), an estimate of stiffness; Bending strength (*BS*), calculated from the maximum stress at the greatest load borne; and Work to peak force (*W*), the total work under the load-deformation curve up to the maximum load borne, divided by the cross-sectional area (Currey *et al.*, 2009b). Moreover, the influence of structural stiffness of the antlers was calculated in terms of *E* x *I*, where *I* is the second moment of area calculated on the transverse section (Currey, 2002; Burr & Turner, 2003), as an *annulus* or ring-shaped object: $I = \pi/4\ (B.Dm^4\text{-}C.Dm^4)$; where *B.Dm* is the total bone diameter of the section and *C.Dm* is the cancellous bone diameter (Burr & Turner, 2003).

The second mechanical test (Charpy test) was performed using an oscillating pendulum which broke an un-notched sample with the periosteal side in tension. The loss of kinetic energy of the pendulum was measured, and this was the energy required to break the sample (Landete-Castillejos *et al.*, 2010). This energy was normalized by dividing it by the cross-sectional area of the specimen, producing the impact work (*U*). Tests were carried out in a CEAST-IMPACTOR II testing machine (CEAST S.p.A., Pianezza, Italy).

The fragments resulting after the mechanical tests were polished to achieve a regular shape and were used to study the antler bone density and the ash content. For such purpose, two fragments for each sampling position were placed in a controlled heating chamber (Memmert UN110, Memmert GmbH, Schwabach, Germany) for 72 h at 60°C to dry them out fully. Afterwards, in order to calculate the cortical bone density (*Ct.B.Dn*), one fragment was weighed with a precision balance ($\pm$0.01 g) and measured with a precision scale ($\pm$0.01 mm); *Ct.B.Dn* was calculated dividing the weight by the volume. The same fragment was used to assess the antler mineral composition. Thus, samples were digested with HNO_3-HCl and diluted with ultrapure deionized water. Then, total concentrations of minerals (Ca, P, Mg, Na, S, K, B, Cu, Fe, Mn, Sr, Zn, Al, Bi, Li, Cr) were quantified with optical emission spectroscopy (ICP-OES) using a Perkin-Elmer Optima 5300 DV (Shelton, CT, USA); for full details see Landete-Castillejos *et al.* (2010). The second fragment was used to measure ash content, by weighing the dried samples with a precision balance ($\pm$0.01 g) to get the dry weight, and, subsequently, they were placed in a muffle furnace (HTC 1400, Carbolite-Gero Ltd, Derbyshire, UK) for 6 h at 480°C. Ash content was calculated as the value of ashes thus obtained divided by the dry weight.

In the statistical analysis, the first step was to obtain a comparative analysis of the morphological measurements, structural and mechanical properties and mineral profile of the two groups of roe deer antlers, using a one-way ANOVA on the mean value per antler. Later, we performed a GLM analysis on the antler characteristics to assess the influence of two factors: *origin* (geographical origin of antlers), and *sampling position* (*position 1* vs. *position 2*); as well as the interaction between these factors. In addition, the mean values for each sampling position, separately for each group of antlers, were compared using one-way ANOVAs. These values often show the variation between base and top part of the antler. Furthermore, a Pearson correlation analysis was performed to study the possible relationships between the observed mechanical and structural properties. The characteristics subjected to analyses were as follows: 1) the morphological measurements (antler beam length, first tine length – *front tine*, second tine length – *back tine*, burr circumference, antler weight); 2) intrinsic mechanical properties (Young's modulus of elasticity, *E*; bending strength, *BS*; work to peak force, *W*; and impact energy, *U*) and structural stiffness calculate as *E* x *I*; 3) structural and physical characteristics (average cortical tissue width expressed in cm, *Ct.B.Wi*; the same expressed as percentage of total diameter of the section, *Ct.B.Wi%*; ratio cortical to total antler section area of the bone slice in proportion, *Ct.B.Ar*; density of the cortical bone, *Ct.B.Dn*); 4) mineral composition and ash content in percentage. All analyses were carried out with SPSS version 22 (SPSS Inc., Chicago, IL, USA).

Results

The two studied populations have slightly different characteristics in the morphology of their antlers. Roe deer in the Spanish population showed antlers with a longer length and greater weight (see Fig. 2). Almost all the antlers studied had three well-developed tines with larger tines in the Spanish group (6.7 ± 0.4 and 4.4 ± 0.4 vs. Czech group: 4.2 ± 0.7 and 2.7 ± 0.4, for first and second tine length respectively). The mechanical and structural characteristics do not show differences in the same direction: Czech roe deer showed higher values for *E* and *BS*, but lower for *W* and *U* (10.92 ± 0.76 vs. 17.97 ± 1.34 for Czech and Spanish groups respectively). The *E x I* was significantly higher for roe deer in the Spanish population; whereas, the *Ct.B.Wi%* and *Ct.B.Ar* were higher in antlers of the Czech population, only *Ct.B.Ar* difference achieved significance. Bone mineral content showed significant differences between the two population: antlers in the Czech population showed higher values in respect of Spanish roe deer, for Ca, K, P, Cr, Li, Sr and Zn (+11%, +15%, +16%, +35%, +39%, +19% and + 16%, respectively), whereas antlers in the Spanish population showed higher values for Na (+13%), Mn (+19%), Tl (+84%) and for the *Ca/P ratio* (+24%), see Table 1 for full results.

The GLM analyses, assessing jointly origin and antler position effects, confirmed that antler features differed between the two origins, but also that position affected mechanical properties (*E*, *BS* and *W*), the $E \times I$ ($R^2 = 0.81$) and *Ct.B.Wi* ($R^2 = 0.45$). Furthermore, origin of antlers affected almost all minerals, but only on the Zinc content showed also an effect due to the sampling position ($R^2 = 0.36$, $P = 0.035$), see Table 2 for details. Pearson's correlation coefficients showed that Spanish group had stronger relationship between mechanical and structural properties of antler, especially for *E* and *BS* (Table 3).

Antlers characteristics and the mineral profile, according to the sampling positions, showed variable trends with few significant results. In Czech roe deer antlers, only *U* showed a decreasing tendency (*position 1*: 13.06 ± 0.83 kJ/m^2 and *position 2*: 10.98 ± 0.62 kJ/m^2, but marginally significant $P = 0.065$). Structural characteristics showed significant values only for *Ct.B.Wi* (*position 1*:0.51 ± 0.03 and *position 2*:0.30 ± 0.02 cm, $P < 0.001$) and *Ct.B.Dn* (*position 1*: 1.71 ± 0.01 and *position 2*: 1.59 ± 0.05, kg/dm^3, $P = 0.045$), whereas the ash content showed marginally significant results (58.93 ± 0.43 vs. 55.76 ± 1.72 %, *position 1* and *position 2* respectively, $P = 0.091$). For the mineral profile, only Zn increased significantly (53.83 ± 2.78 vs. 61.79 ± 1.87 mg/kg, *position 1* and *position 2* respectively, $P = 0.029$). Spanish roe deer antlers showed the same pattern for mechanical properties and structural characteristics. In *position 1*, we observed higher values only for *BS* (304.9 ± 8.5 vs. 268.3 ± 15.7 MPa, $P = 0.05$), *W* (39.4 ± 2.5 vs. 26.4 ± 2.7 kJ/m^2, $P = 0.002$) and *Ct.B.Wi* (0.55 ± 0.04 vs. 0.41 ± 0.03 cm, $P = 0.007$). In contrast, the mineral profile did not show significant differences. The full list of non-significant results is not shown in tables for reasons of conciseness.

Discussion

This is the first study analysing the mineral profile and the mechanical and structural characteristics of roe deer antlers, comparing at the same time these features in two populations living in different semi-natural environments; the results obtained show that the two roe deer populations have significant differences in morphometric measurements (in the Spanish group: +62.7%, for first tine and + 63.8%, for second tine), as well as in certain structural characteristics (Ct.B.Ar was higher in Czech antler: +19%) and mechanical properties (Czech antler have higher values for *E*:+11% and *BS*:+14%, but lower values for *U*: −65%), and in the bone mineral profile. Generally, cortical bone tissue shows lower values in antler tip for density, cortical width and mechanical properties, but not for the chemical composition (indicating almost no clear trends in physiological constraints for the completion of the mineralization phase of bone tissue). However, the two populations have different mechanical properties of bone material: roe deer antlers from Spanish population have higher *W* and *U*, while those from Czech population compensate the risk of fracture developing larger cortical bone area although having higher *E*.

One of the most interesting results of our analysis shows how most antler properties vary in a pattern in which antler of animals with an improved nutrition have some better bone characteristics. This pattern is also recognized in the external structure. The greatest difference was the antler weight of Spanish deer (118%) compared to Czech individuals with lower availability of food resources. It is not surprising that quality of roe deer antlers is mostly indicated by their weight, because the differences in tine length were almost half that in weight (64% and 63% greater for Spanish vs. Czech antlers for second and first tine, respectively). The total length of the antler was 25% greater for roe deer with better diet. These trends could support the proposed hypothesis that a richer diet is also reflected in the development of antlers, since the Spanish animals were kept with food supplements all year long and they were able to feed on a continuous presence of natural vegetation, whereas the Czech set had poor vegetation availability during the winter period (when the roe deer antlers grow, Sempéré, 1990). This positive effect on antler size is well known since the Thirties of the 20th century (Vogt, 1936; Geist, 1986).

How such effect of better nutrition translates into differences in the rest of antler characteristics? The Spanish population,

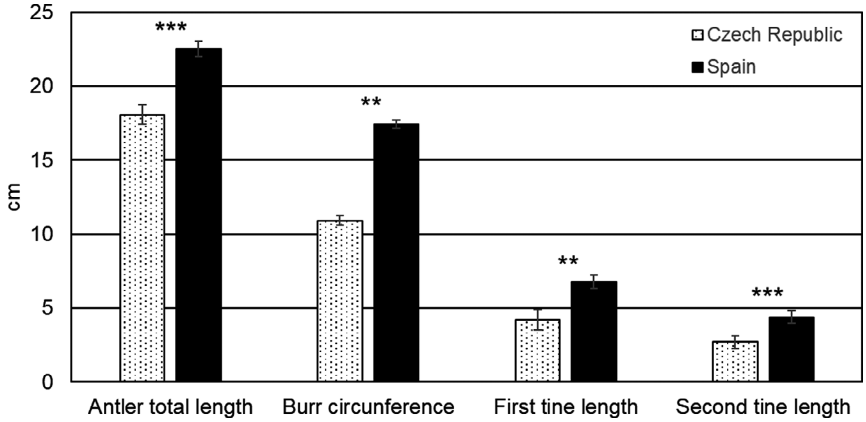

Figure 2 Descriptive statistics of antler morphological characteristics of *Capreolus capreolus*. Mean for antlers from the two roe deer groups: Spanish group (black coloured; n = 13) and Czech group (dotted; n = 10). Probability calculated using ANOVA; levels $P < 0.01$ and $P < 0.001$ are indicated by ** and ***, respectively.

Table 1 Morphological and structural characteristics, mechanical properties and mineral profile for adult roe deer antlers from Spain (n = 13) and Czech Republic (n = 10). The P-value corresponds to one-way ANOVA on the mean (±SE) per antler of the two positions examined. Dashes indicate coefficients that were not significant

Variables	Czech Republic	Spain	P
Burr circumference (cm)	10.90 ± 0.31	17.41 ± 0.28	<0.001
First tine length (cm)	4.17 ± 0.69	6.75 ± 0.45	0.003
Second tine length (cm)	2.68 ± 0.42	4.39 ± 0.42	0.010
Antler beam length (cm)	18.06 ± 0.65	22.52 ± 0.50	<0.001
Antler weight (g)	52.71 ± 2.11	114.88 ± 3.71	<0.001
Young's modulus of elasticity (E), GPa	27.91 ± 1.00	24.88 ± 0.7	0.019
Bending Strength (BS), MPa	333.23 ± 6.35	286.63 ± 8.78	<0.001
Work to peak force (W), kJ/m^2	26.37 ± 2.28	31.85 ± 1.71	0.064
Impact work (U), kJ/m^2	10.92 ± 0.76	17.97 ± 1.34	<0.001
$E \times I$	10.39 ± 1.76	28.06 ± 1.93	<0.001
Ct.B.Wi (cm)	0.40 ± 0.02	0.48 ± 0.02	0.024
Ct.B.Wi% (%)	0.54 ± 0.04	0.45 ± 0.03	-
Ct.B.Ar (%)	0.84 ± 0.01	0.68 ± 0.03	<0.001
Ct.B.Dn (kg/dm^3)	1.62 ± 0.04	1.68 ± 0.02	0.002
Ashes (%)	57.35 ± 0.92	56.90 ± 0.79	-
Ca (g/100g)	17.10 ± 0.29	15.46 ± 0.49	0.009
K (g/100g)	0.0250 ± 0.0005	0.0212 ± 0.0008	0.002
Mg (g/100g)	0.296 ± 0.008	0.347 ± 0.020	0.055
Na (g/100g)	0.472 ± 0.006	0.533 ± 0.013	0.001
P (g/100g)	11.27 ± 0.21	9.44 ± 0.61	0.019
S (g/100g)	0.265 ± 0.003	0.250 ± 0.009	-
Al (mg/kg)	17.17 ± 0.52	17.35 ± 1.37	-
Cu (mg/kg)	-	0.365 ± 0.040	-
Cr (mg/kg)	0.76 ± 0.02	0.49 ± 0.01	<0.001
Fe (mg/kg)	18.91 ± 2.87	16.87 ± 2.43	-
Li (mg/kg)	3.31 ± 0.15	2.03 ± 0.05	<0.001
Mn (mg/kg)	19.50 ± 0.52	23.18 ± 0.89	0.003
Sr (mg/kg)	172.88 ± 11.37	140.18 ± 20.42	-
Tl (mg/kg)	27.87 ± 0.72	51.20 ± 2.78	<0.001
Zn (mg/kg)	57.81 ± 2.17	48.41 ± 1.78	0.003
Ca/P	1.52 ± 0.01	1.88 ± 0.02	<0.001

with supplemented nutrition throughout the year, had a 64% increase in impact energy (U) with respect to the Czech one. The effect is more subtle in the W, which is 21% higher in the larger Spanish antlers. A similar environmental effect on U compared to W was found in red deer in the same population in years differing in the quality of the food (Landete-Castillejos

Table 2 GLMs analyses showing the influence of antler position and population origin, on the composition, structure and mechanical properties of antlers in roe deer. The coefficient β (± SE) is related to the difference of the value observed in position 1 with respect to the position 2, and for the origin is related to the difference of the value observed in Czech antlers in respect of the Spanish antlers. Dashes indicate coefficients that were not significant

			Factors in the model					
			Position		Origin		Position*Origin	
Variables	R^2	Intercept ± SE	β ± SE	Sig.	β ± SE	Sig.	β ± SE	Sig.
Young's modulus of elasticity (*E*), GPa	0.22	23.78 ± 0.88	2.19 ± 1.07	0.046	3.03 ± 1.208	0.007	-	-
Bending Strength (*BS*), MPa	0.28	273.36 ± 10.20	26.54 ± 12.34	0.037	42.19 ± 12.49	0.002	-	-
Work to peak force (*W*), kJ/m^2	0.33	26.37 ± 2.45	13.003 ± 3.472	0.008	−0.86 ± 3.72	0.018	−11.29 ± 5.27	0.038
Impact work (*U*), kJ/m^2	0.37	17.97 ± 0.91	-	-	−7.05 ± 1.38	<0.001	-	-
$E \times I$	0.81	3.47 ± 2.79	10.74 ± 4.06	<0.001	7.95 ± 3.78	<0.001	24.39 ± 5.38	<0.001
Ct.B.Wi (cm)	0.45	0.40 ± 0.03	0.17 ± 0.03	<0.001	−0.08 ± 0.03	0.019	-	-
Ct.B.Wi% (%)	-	-	-	-	-	-	-	-
Ct.B.Ar (%)	0.26	0.68 ± 0.02	-	-	0.14 ± 0.04	<0.001	-	-
Ct.B.Dn (kg/dm^3)	-	-	-	-	-	-	-	-
Ashes (%)	0.15	56.90 ± 0.39	-	-	1.71 ± 0.62	0.009	-	-
Ca (g/100g)	0.23	15.46 ± 0.36	-	-	1.64 ± 0.50	0.002	-	-
K (g/100g)	0.34	0.021 ± 0.001	-	-	0.004 ± 0.001	<0.001	-	-
Mg (g/100g)	0.14	0.34 ± 0.11	-	-	−0.04 ± 0.02	0.011	-	-
Na (g/100g)	0.31	0.53 ± 0.09	-	-	−0.06 ± 0.01	<0.001	-	-
P (g/100g)	0.24	9.34 ± 0.35	-	-	1.93 ± 0.52	0.001	-	-
S (g/100g)	-	-	-	-	-	-	-	-
Al (mg/kg)	-	-	-	-	-	-	-	-
Cr (mg/kg)	0.74	0.49 ± 0.02	-	-	0.27 ± 0.03	<0.001	-	-
Fe (mg/kg)	-	-	-	-	-	-	-	-
Li (mg/kg)	0.78	2.03 ± 0.07	-	-	1.28 ± 0.10	<0.001	-	-
Mn (mg/kg)	0.30	23.14 ± 0.56	-	-	−3.63 ± 0.84	<0.001	-	-
Sr (mg/kg)	-	-	-	-	-	-	-	-
Tl (mg/kg)	0.78	51.20 ± 1.49	-	-	−23.33 ± 2.06	<0.001	-	-
Zn (mg/kg)	0.36	50.60 ± 1.90	−4.89 ± 2.24	0.035	9.65 ± 2.26	<0.001	-	-
Ca/P	0.86	1.88 ± 0.01	-	-	−0.36 ± 0.02	<0.001	-	-

et al., 2010). Greater resistance to impact or fracture is often achieved by increasing the protein content (which means a reduced ash content), which reduces the stiffness or *E*. In our results, populations did not differ in ash content, but the bulk of such content, Ca and P content, was significantly lower in the Spanish larger antlers by 11% and 16%, respectively. The effect of such reduction was likely a reduction of 11% of *E* and 14% for *BS* in antlers of the population with better diet. However, the roe deer with worse diet appeared to compensate by developing a greater percentage of width occupied by cortical bone (*Ct.B.Wi%,* 16% more than better fed deer), and a similar trend was achieved in cortical bone area (*Ct.B.Ar,* +19%). Nevertheless, they appeared to be smaller in overall diameter (*Ct.B.Wi* in cm, −20%), which increases the risk of fracture (Davison *et al.*, 2006). Because bone strength is determined by both its material and structural properties, and increases with increasing width of the cortical tissue and diameter of the bone (Currey, 2002; Davison *et al.*, 2006), this greater percentage of diameter invested in achieving a greater cortical bone may be an attempt to compensate for both the lower mechanical performance of the bone material and the reduced overall diameter. For the differences in values observed in the sampling *position 1* and *position* 2, the bone mechanical and structural properties, on both antler groups, showed slightly negative or almost null trends. In the *position 2*, the cortical bone was characterized by less *Ct.B.Dn*, less average width and lower mechanical features (only *W, BS* in Spanish antlers, and *U* in Czech antlers). Probably, the differences are minimal due to the small dimensions of antlers. Ceacero (2016), hypothesized that the antler mass reflected a greater physiological constraint for larger sized species, although, as mentioned earlier, if two species are discarded, the trend is linear and therefore shows a similar investment in terms of body mass. Geist (1998), in contrast, indicated that the investment in antlers in roe deer is lower than in red deer because the former shows little sexual dimorphism and red deer show a much greater one. According to this, the greater effort in red deer would result in physiological exhaustion and, thus, in antler properties reflecting much worse quality in top parts compared to lower ones, and roe deer should not show this exhaustion, and this is coherent with our results.

Is the effect of nutrition in roe deer antlers consistent with studies in other deer species? The study of Landete-Castillejos *et al.* (2007c), assessing antlers from red deer with different diet showed that antlers developed under a better diet had bone material with a greater *W*, greater per cent of protein and greater average antler perimeter. In a subsequent study (Landete-Castillejos *et al.*, 2013), assessing antlers from public

Table 3 Pearson's correlation coefficients between mechanical and structural variables for adult roe deer antlers from Spain (n = 13) and Czech Republic (n = 10). Probability at levels $P < 0.05$, $P < 0.01$ and $P < 0.001$ are indicated, respectively, by *, ** and ***

Variables	E (GPa)	BS (MPa)	W (kJ/m^2)	U (kJ/m^2)	Ct.B.Dn (Kg/dm^3)	Ashes (%)	Ct.B.Wi (cm)	Ct.B.Wi% (%)
Spain antler set								
BS (MPa)	0.835**							
W (kJ/m^2)	0.066	0.434						
U (kJ/m^2)	−0.538	−0.736**	−0.714**					
Ct.B.Dn (kg/dm^3)	0.346	0.104	−0.198	0.066				
Ashes (%)	0.725**	0.714**	0.313	−0.571*	0.374			
Ct.B.Wi (cm)	0.495	0.495	−0.148	−0.319	0.209	0.275		
Ct.B.Wi% (%)	0.473	0.473	0.033	−0.412	−0.066	-	0.802**	
Ct.B.Ar (%)	0.582*	0.648*	0.137	−0.451	0.121	0.214	0.813**	0.923**
Czech antler set								
BS (MPa)	0.745*							
W (kJ/m^2)	−0.030	0.224						
U (kJ/m^2)	0.442	0.745*	0.539					
Ct.B.Dn (kg/dm^3)	0.721*	0.564	−0.139	0.406				
Ashes (%)	0.103	−0.127	−0.600	−0.176	0.188			
Ct.B.Wi (cm)	0.479	0.285	−0.539	−0.055	0.467	0.382		
Ct.B.Wi%(%)	0.236	0.067	−0.770**	−0.285	0.358	0.588	0.745*	
Ct.B.Ar (%)	0.055	0.212	−0.491	−0.030	0.236	0.394	0.588	0.770**

Bold values underline significant coefficients.

game estates (with no supplementary food) vs. private managed estate (including food supplements), antlers of deer with supplementary food were longer with an increased W at the cost of a reduced stiffness E, as in the present study with roe deer. Obviously, when comparing the characteristics of the deer antlers, we must also consider that their diversity is regarded as a multiple solution to the same problem, fighting other males and develop them according to quantity and quality of food exploited by each species (Geist, 1966). Geist (1998) indicated that antler size is related to sexual dimorphism, and Plard, Bonenfant & Gaillard (2011) found that highly polygynous species have relatively longer antlers than less polygynous ones but the difference in the effect of breeding group size on relative antler length is weak. Thus, roe deer has a *low-risk low-gain* strategy regarding frequency of mating with females, and their success is related to multi-year tenure of a resource territory (Linnell & Andersen, 1998; Vanpé *et al.*, 2010). Thus, most of the male–male interactions ended with low levels of escalation, and the level of fighting escalation is influenced by the presence/absence of a territorial male (from 41% to 15%) or a similar body size (Hoem *et al.*, 2007).

On the other hand, some authors performing comparative studies have concluded that also the habitat-specific changes in male fighting styles are likely to have contributed to affect the size of male weapons (Emlen, 2008).

Concluding all the above, red deer would have larger antlers because they have a larger body size, greater sexual dimorphism, a mating system in which one male fights more intensely because he can gather a large harem. Roe deer are smaller, show little sexual dimorphism, do not form harems, and therefore male–male competition is less intense. In large Cervid species adapted to relatively open habitats like red deer, antlers are not only effective weapons but also showy ornamental organs used to signal their strength and fighting ability, threaten other males and attract females (Clutton-Brock, 1982). For roe deer, originally adapted to dense cover (bushy areas, compact forests), antlers are less conspicuous, smaller and less branched. All this results in a more intense fighting in red deer, likely with greater pushing forces than in roe deer, and this would result in roe deer having smaller antlers and less branched.

What about the mineral profile of the antler? Making comparisons for the antler's mineral profile with other deer species is not a simple procedure, but certain metabolic processes in the growth of antlers could be similar (Kierdorf *et al.*, 2013). Moreover, in roe bucks, like other Cervids, the minerals necessary for the formation of the antlers come also from a mobilization of mineral components from skeletal bone material (Baxter, Andrews & Barrell, 1999; Fandos & Burón, 2015), and a reversible cyclic osteopenia occurs in internal bone during the antler cycle (Brockstedt-Rasmussen *et al.*, 1987). For two main minerals of the bone tissue, Ca and P (Wilson, 1995; Currey, 2002), the values observed differed from those of other Cervids. Ca is slightly lower, whereas P is quite similar according to the species (in *C. elaphus*: 21.0 and 10.1, Landete-Castillejos *et al.*, 2010; in *A. porcinus*: 22.5 and 12.2, *A. axis*: 23.4 and 10.8, *C. duvaceli*: 23.4 and 12.6, Pathak *et al.*, 2001; respectively). In this study, the *Ca/P ratio* is considerably lower for the Czech roe deer population compared to Spanish one, the reason could be the high value of P in this group of animals (+19.4%), which may be due to possible replacements of P in hydroxyapatite crystals (HPO_4^{2-} -containing apatites have been called Ca-deficient apatites; Dorozhkin, 2009). Furthermore, the value for *Ca/P ratio* is much lower compared to other species (2.21 in red deer; Kierdorf *et al.*, 2014). Nevertheless, results regarding particularly Ca content should be carefully considered in future studies of roe antler mineral profile, to confirm the effect or point our population as an abnormality. For Na, the values observed in Spanish roe deer are almost similar to other species kept captive (0.56 in *E. davidianus*, 0.55 in *C. canadensis*; Ceacero *et al.*, 2015). Whereas, Czech roe deer had a trend similar to that observed in red deer under poorer diet (Landete-Castillejos *et al.*, 2007b, 2007c, 2013), with a lower content of Na in their antlers than those of roe deer with a better diet. In our study, the content of Mn was lower when diet was poorer, as in the study of Landete-Castillejos *et al.* (2010). However, in the study by Landete-Castillejos *et al.* (2013) both private offered supplementary food and farmed red deer had a lower content in Mn (a lower content in Mn has been previously found in farmed deer compared to wild ones: Hyvarinen *et al.*, 1977). Regarding Cu, it was found only for the Spanish roe deer but not in the Czech one; this difference may be attributable to the large range of factors influencing tissue Cu concentrations (Wilson & Grace, 2002). In general, there seem to be three minerals that usually indicate that antlers have grown under a physiologically stressful situation: Fe, K and Zn. In red deer, Fe was found higher in public as compared to private management with food supplements (Landete-Castillejos *et al.*, 2013), as it were compared free ranging deer to farmed deer (Landete-Castillejos *et al.*, 2007c). K was higher in red deer with poorer diet (10% higher in public vs. private management with food supplements, Landete-Castillejos *et al.*, 2013). The last mineral is Zn, linked to the enzyme mineralizing the antler, alkaline phosphatase, which shows the degree of mineralization of the antler (i.e. it is present when the deer has not been able to complete the mineralization of last parts of the antler: Landete-Castillejos *et al.*, 2012). In the present study, as shown in Table 1, all three were higher in antlers grown from roe deer in the Czech population, which suggest that they were under a greater nutrition stress than roe deer of the Spanish population.

Although our results are derived from a small sample of individuals, they show also in roe deer that the study of the antler characteristics can be useful to assess a better management practice. Indeed, a rich feed for roe deer likely resulted in relatively larger and heavier antlers, but also made the bone material more resistant to impact or fracture, and finally, the antlers grown with a better diet were formed with a lower physiological exhaustion, observing the 3 minerals related to nutritional stress (K, Zn and Fe). In conclusion, differences in the quality of the diet likely affected the external characteristics of roe deer antlers, their internal structure, mechanical properties and mineral content of the bone showing a number of effects similar to what has been published for red deer also differing in the quality of their diet. Despite interspecific differences, the present study shows general trends that may depend upon the cervid physiology mechanisms for growing antlers, and for these and probably other deer species, our results confirm that assessing antlers is a highly valuable tool for population diagnostic and management.

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Conflict of interest

The authors declare that they have no financial interests and no conflict of interest.

Author contributions

JC involved in sampling and execution of analysis, data analysis and drafting of the manuscript; FC involved in study design, antler and sampling collection and critical revision; TLC involved in critical revision; LG involved in critical revision; AG involved in antler and sampling collection and critical revision.

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Ethical approval

All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

Data availability statement

The data sets are available from the corresponding author on request.

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