REQUIREMENTS AND ROLE OF SELECTED MICRO AND MACRO ELEMENTS IN NUTRITION OF CERVIDS (*CERVIDAE*) - REVIEW

TAJCHMAN, K.[1] – STEINER-BOGDASZEWSKA, Ż.[2] – ŻÓŁKIEWSKI, P.[3*]

[1]*University of Life Sciences in Lublin, Department of Ethology and Animal Welfare Akademicka 13, 20-950 Lublin, Poland*

[2]*Institute of Parasitology of the Polish Academy of Sciences Research Station in Kosewo Górne, Poland*

[3]*University of Life Sciences in Lublin, Institute of Animal Breeding and Biodiversity Protection Akademicka 13, 20-950 Lublin, Poland*

*Corresponding author
e-mail: pawel.zolkiewski@up.lublin.pl; phone: +48-814-456-848

Abstract. Cervidae are characterised by specific requirements for nutrients due to the structure of their gastrointestinal tract. The period of development of male antlers and lactation in females is associated with increased demand for protein, energy, and minerals. The paper presents the importance of Ca and P in cervid nutrition and the effect of these minerals on the health and ontogenic quality of these animals. The requirements for these macronutrients in relation to the age and sex of cervids as well as food availability are presented in the study. Periods in animals' life that require particular attention especially in farm breeding and methods for balancing dietary doses are indicated. Additionally, the relationships governing the availability of Ca and P in the environment and the effects of deficiency and excess of these minerals in the deer diet are discussed.
Keywords: *Cervidae, availability of mineral components, nutrition, demand, calcium, phosphorus*

Introduction

Nutrition exerts an impact on the behavioural, morphological, and biochemical functions of each animal organism, thereby influencing the dynamics of the entire species population. Herbivorous animals consume food with a completely different chemical composition than that of their own tissues, in contrast to carnivores, which digest material with features similar to the characteristics of their bodies (Barboza et al., 2009).

The population dynamics is closely related to available food and environmental requirements of individuals. The nutrient richness in the environment is reflected in the spatial and seasonal distribution of the population. Environmental factors that are important for each organism include e.g. light, temperature, soil richness in nutrients that are essential to plants, food availability, shelter, and the presence of predators (Barboza et al., 2009).

The availability of mineral components depends on the geology and hydrology of animals' habitats. The mineral content highly varies in plant and animal tissues and each element can serve several functions. Macronutrients (Na, K, Ca, P, Mg, S) are commonly present in tissues at high levels, whereas microelements (Mn, Cu, Fe, Zn, I, Se) are contained in trace and irregular amounts (Barboza et al., 2009).

Physiological processes and biochemical transformations support animal's health, and appropriate nutrition has a substantial impact on physiological activities, e.g. growth, pregnancy, lactation, or development of antlers. Improvement of the habitat to meet the nutritional requirements of *Cervidae* is an important factor in proper management of healthy populations of these animals. Nutrition is one of the three main determinants of antler growth and animal body size. Age and genetics are the other two factors (Landete-Castillejos et al., 2013; Hewitt, 2011).

Organism growth and development is usually limited by a deficient component, which is impossible to replace by another environmental factor, as specified in Liebig's law: *an excess of factors is usually less harmful to the organism than deficiency* (Odum, 1971). However, humans have no huge impact on the composition of wild-living animals' diet, whereas the optimal fodder composition can be provided to cervids in farm breeding systems. The "foraging" theory proposes that animals should have an opportunity to assess the nutrient content in the consumed food and to adjust their diet to meet their current needs (Ceacero et al., 2010).

The paper demonstrated the impact and importance of calcium and phosphorus in cervids nutrition in relation to the age, sex, and environmental conditions.

Nutrient requirements in cervids

Cervidae are characterised by specific requirements for nutrients due to the structure of their gastrointestinal tract, as they are able to digest cellulose-containing plant material e.g. woody stems, grass stalks, bark, or the skin of some berries. The highest nutrient demand in cervids is associated with the growth of male's antlers and lactation in females. Each deer species is characterised by an individual dynamics of growth and antler development depending on the animal's age (Ceacero et al., 2015; Estévez et al., 2009; Landete-Castillejos et al., 2007b; Szczepański et al., 2006).

The antler is mainly composed of protein - 45%, calcium - 22%, and phosphorus - 11%. A diet rich in protein at a level of 13-16% ensures optimal development of this part of the body. To ensure normal function of the rumen, the red deer should ingest a minimum of 6-7% of crude protein in the diet (Perkins, 1991). The daily Ca and P demand has been established at 0.09-0.64% and 0.14-0.56%, respectively (*Table 1*). This range is relatively wide given the possibility of resorption of mineral compounds from long bones, which has been detected during antler growth in the Iberian deer (*Cervus elaphus hispanicus*). Detailed analyses have shown that a daily intake of 25-40% of the Ca level required for antler mineralisation causes transient demineralisation of the skeleton (Landete-Castillejos et al., 2007b).

The Ca requirement in cervids is also related to animal's age and sex. Young males require a relatively large amount of protein, even up to 22%, and lower levels of calcium - 0.45% and phosphorus - 0.28%. Different nutritional requirements are exhibited by pregnant and lactating females. The demand for mineral components in hinds in this period is similar to that noted in stags during antler development, and the required protein amount may be even higher, i.e. 22-24% DW (USDA-NRCS, 1999).

Perkins (1991) has shown that the optimal mineral composition should include 0.64% Ca and 0.56% P. The most optimal calcium-to-phosphorus ratio should be 12:58-59 (*Table 1*). Higher amounts can be harmful. Proper mineral supplementation of cervid diet may prove beneficial in some conditions; however, in most cases, it is recommended to provide properly balanced fodder for farmed animals.

Table 1. *Recommended Ca and P level in deer diets and their content and proportion in the bones and antlers of wild individuals*

Recommended level in deer diets	Ca	P
range (Landete-Castillejos et al., 2007a)	0.9-6.4 g/day	1.4-5.6 g/day
minimum for 60 kg of animal (NRC, 2007)	2.7 g/day	2.2 g/day
transport with blood during intensive growth of antlers (Landete-Castillejos et al., 2007a)	8.4 g/day	2.4 g/day
Depending on age and gender		
nurseling with mother milk (Ullrey, 1982)	0.5-1.0 g/day	0.25-0.5 g/day
young males (USDA-NRCS, 1999)	4.5 g/day	2.8 g/day
fawns <1 year (Perkins, 1991)	6.0 g/day	4.0 g/day
adults >1 year(Perkins, 1991)	4.0-7.5a g/day	3.0-4.5a g/day
Depending on the growth phase Ca+P (Chen et al. 2008)		
skeleton	34 g/day	
antlers	100 g/day	
Mineral content		
antler (Nowicka et al., 2006)	133 mg/g	83.58 mg/g
bones of the skull (Nowicka et al., 2006)	123 mg/g	83.58 mg/g
3 kg antler (Gomez et al., 2012)	536 g	-
absorbed in the last period of antler growth (Gomez et al., 2012)	348 g	-
completely formed ossified antlers (Landete-Castillejos et al., 2007a)	400 g	200 g
Ca: P proportions		
food dose (Perkins, 1991)	0.2 (12:58-59)	
bones 10 days old animals (Nowicka et al., 2006)	1.31	
bones 50 days old animals (Nowicka et al., 2006)	2.04	
antler (Nowicka et al., 2006)	2.0	

(a) Feed higher energy levels during periods of climatic, physiological stress, lactation or active antler growth

It has been evidenced by researchers that 2-year-old males of the black-tailed mule deer (*Odocolieus hemionus*) receiving fodder with 8% protein content developed antlers characterised by half the weight of those developed by individuals at the same age fed with 16% of protein in the diet. Four-year analyses revealed that antlers in the former group were larger but those in the latter group were by 20 cm longer (Hewitt, 2011).

The effect of supplementation in farm feeding of the Iberian deer (*Cervus elaphus hispanicus*) has been investigated (Olguin et al., 2013). For three post-weaning years, the animals received an increased amount of minerals: Ca, P, Mg, K, Na, S, Cu, Fe, Mn, Se, Zn, B, and Sr in the supplementary diet. The increased availability of microelements

in the feed was reflected in the chemical composition and mechanical properties of bones. There was a significant difference in biometric parameters between the control group and animals receiving the enriched diet. The mean body weight of the control animals was 83.6 kg $\pm$ 1.6, whereas the weight in the supplemented group was higher, i.e. 90.1 kg $\pm$ 1.4. Similarly, the kidney fat index KFI differed between the groups and was 65.42 kg $\pm$ 9.9%, and 131.26 kg $\pm$ 13.7% respectively. In turn, there were no differences between the chest circumference and body length as well as femur length, cortical thickness, or mechanical properties (Olguin et al., 2013). These results suggest that supplementation with mineral compounds has a positive impact on the weight and form of antlers and animal fitness.

Approximately 80-90% of calcium and phosphorus in the organism of the white-tailed mule deer (*Odocolieus virginianius*) is stored in its skeletal system. Males fed a low-mineral diet often exhibit delayed antler growth and velvet shedding. In normal conditions, calcium and phosphorus are indirectly available from soil and vegetation; yet, the content of the latter microelement in acid soils is low. The calcium demand is especially high; it is supplied with feed only in 25-40% and the other part is acquired by cervids from the pool of the element stored in their bones (Muir et al., 1987).

Both calcium and phosphorus are required for the animal growth and play an important role in the reproduction process. Cervids can accumulate minerals in the skeleton until e.g. the antler growth period, when the ingested food cannot meet the demand for these elements. Transient osteoporosis is usually observed in this period, i.e. uptake and utilisation of some minerals from the skeletal system in the process of antler mineralisation. After this period, animals try to acquire these deficient nutrients through their diets (Hewitt, 2011).

The content of calcium, magnesium, and phosphorus in the antlers and cranial bones of the red deer *Cervus elaphus* inhabiting three areas in northern Poland has been investigated (Piotrowska, 2005). The levels of the main inorganic components in two adjacent bone tissues were compared. It was found that the Ca content was higher in the antlers (mean 133.96 mg/g) than in the cranial bones (mean 123.79 mg/g). In turn, the P content was slightly higher in the cranial bones than in the antlers (84.62 mg/g and 83.58 mg/g, respectively). This suggests that the Ca/P proportions differ between these tissues. There were no significant differences in the magnesium content (5.23 mg/g : 5.46 mg/g). The Ca/P weight ratio in the bones has been found to differ between *Cervidae* species and depend on the age of the animal. In young animals, it ranged from 1.31 (10 days) to 2.04 (50 days). As shown by Piotrowska (2005), the Ca/P ratio in the deer antler was 2.00 (*Table 1*). It can therefore be concluded that P is the most stable component of bones and antlers. The Mg content in bones and antlers declines with age, whereas the level of Ca fluctuates significantly and is higher in antlers than in cranial bones (Nowicka et al., 2006).

The quality of nutrition obtained at the youngest age is important in cervids. Calves of the Iberian deer (*Cevrus elaphus hispanicus*) reared on farms and fed with milk in the first weeks of life have been examined. The energy and protein supplied with this food is used primarily for the growth of young animals (García et al., 1999; Vergara et al., 2003). However, it has been shown that the first stage of cervid nutrition can exert an effect on antler size and weight. Mothers' lactation influences calves for the first two years of life. The correlation between the skeleton size and the antler weight is one of the longest known allometric relationships in animals. The rate of growth and the size of the first antlers developed by young males in the second year of life are dependent on

the quality of food provided by their mothers as well as the habitat and ecological conditions provided to the mothers (Gómez et al., 2008). The role of hinds is also to teach their offspring to ingest and select appropriate food. Obviously, young animals take up the same minerals as those contained in the food consumed by their mothers in the food selection process (Ceacero et al., 2010a; Janiszewski et al., 2018). The choice of food is also determined by physiological effort, age, sex, and physiological condition of the animal (Ceacero et al., 2010b).

Despite the six-month period between the processes, the relationship and impact of hind lactation on antler growth in young Iberian deer (*Cevrus elaphus hispanicus*) males can have significant long-term consequences in the life of cervids (Gómez, 2004; Landete-Castillejos et al., 2000). It has been demonstrated that the milk amounts and composition are correlated with the mineral content in the antlers and skeleton of the animal. Moreover, high protein content in milk may result in development of 1/3-fold larger and heavier antlers by the Iberian deer (*Cervus elaphus hispanicus*) due to the availability and integrity of milk proteins (*e.g.* casein) associated with the presence of calcium and phosphates in milk. This explains e.g. the decline in the growth rate in post-weaned calves, which is associated with consumption of less nutritious food (Gómez, 2004). Ingestion of the mild milk is more beneficial to animals than consumption of fodder from arable land, which is usually homogeneous in its botanical composition (Stachowicz, 2010). These observations have been confirmed by analyses of the macronutrient concentration in the plasma of European deer (*Cervus elaphus*) calves. Relatively high plasma levels of calcium, phosphorus, and magnesium were detected in the first two months of animal life; this was probably an effect of mother' milk ingestion (Kuba, 2014). As reported by Ullrey (1982), the development of antlers requires a level of 0.5-1 g of calcium and 0.25-0.5 g of phosphorus in milk (*Table 1*). Rapid body gains were observed up to 18 weeks of age, i.e. when the animals were fed with milk, and lower gains were noted after this period until the end of the first year of life. It has been shown in the Iberian deer (*Cervus elaphus hispanicus*) that the composition of milk has an impact on the morphological traits of the first antler, primarily on its length. The length of the antler was measured from 2.5 cm to the end of the growth period. Between weeks 2 and 8, the length gain was inconsiderable, i.e. from 0.5-3.0 cm to 1-19.0 cm. The highest increase in the length was observed between weeks 8 and 20, i.e. 10.0-55.0 cm. The final period of the growth between weeks 20 and 28 was characterised by a 21.5-55.5 cm gain. In comparison with wild-living animals, in which the length of the first antler can be highly variable and range from 15 cm to 60 cm, this value in a majority of individuals receiving adequate nutrition is stable and reaches an average length of 38.3 cm (Gaspar-Lopez et al., 2008).

The length of the first antler in deer is an index trait of the ontogenic quality and a phenotypic trait reflecting the quality of nutrition (Gaspar-Lopez et al., 2008). It has also been demonstrated that the length of antlers declines with a delay in the time of birth, which may indicate a high impact of trophic conditions in the first period of life and early mating of females. An appropriate composition of mother's milk can contribute to the development of larger antlers and earlier initiation of their development (Gaspar-Lopez et al., 2008). These results suggest a need for creation of appropriate living conditions immediately after birth and proper nutritional balance for lactating hinds in the breeding practice. Higher production of milk by females contributes to a larger body size and faster growth of their calves, including the skeletons, from which larger amounts of minerals can be activated to intensify the

increase in the antler size and weight. Interestingly, not only antler growth but also milk production is linked with the bone mineral composition (Gómez et al., 2008; Landete-Castillejos et al., 2000).

A negative correlation of the milk composition with the antler size and potassium content has been found. Potassium is the third most important element in the organism after calcium and phosphorus and, surprisingly, calf feeding by females may be associated with potassium stored in the organism. The total milk protein yield has been found to correlate positively with the content of Ca and P in the organism and reversely with the K content. A major role is attributed to potassium, as this component limits losses of calcium excreted in urine, and activation of Ca from the skeleton enhances antler growth (Landete-Castillejos et al., 2007a,b). Clinical human studies have shown that K supplementation reduces urinary excretion of Ca. It has also been shown that K limits absorption of Ca in the intestine. Therefore, in order to improve the efficiency of Ca utilisation, deer should be provided with increased K intake during antlerogenesis (Landete-Castillejos et al., 2007a).

As previously noted, the lactation period plays a very important role in the process of skeleton and antler growth. This has been confirmed by investigations of the mandible length in cervids. It has been proven that its growth has the higher and fastest rate between the birth and the first year of life, whereas the subsequent 4 years are characterised by a lower growth rate (Zannèse et al., 2006). The first antler grows with an average rate of 1.95 ± 0.05 cm per week and is the highest at week 14 (Gaspar-Lopez et al., 2008). The conditions provided to a young male in the early months of life have important effects on the final size and fitness of the male.

The average percent mineral content in the first antler of the Iberian deer (*Cervus elaphus hispanicus*) was shown to be 34% Ca, 31% P, and 44% K. It has also been proved that such elements as Na, Mg, Fe, and Zn have no impact on the ontogenic development of calves. Even the best post-weaning nutrition cannot compensate the nutrition obtained during lactation. The gain during lactation was 25% and 24% for Ca and total ash, respectively (Landete-Castillejos et al., 2007a).

A strong correlation of the amount and composition of milk with the mineral content in the first antler has been found (Gómez et al. 2008; Clutton-Brock et al., 1982). Animal growth in the post-lactation period is less relevant for antler development, which may suggest that the period of ingestion of mother's milk is the most important. The quality of lactation in less favourable environmental conditions (wild-living animals) may have an even greater impact on the composition and quality of the skeleton and antlers. Moreover, it has been demonstrated that only five minerals had the greatest effect on the length of beams and weight of antlers in the Iberian deer (Gómez et al., 2008).

Antlers and mineral metabolism

Antlers are secondary sex feature present in cervid males (with the exception of reindeer). The size of the antler is important in establishment of the hierarchy and position of the male and, consequently, ensures access to females (Gómez et al., 2012; Landete-Castillejos et al., 2001).

Antlers can be a good model for investigations of bone biology; they are easily available, as they are shed by animals every year. Antlers grow fast and require substantial mineral transfer from the skeleton. The rapid growth does not allow natural bone restoration (internal and external bone remodelling) and can thus conceal the bone

"archaeology". Nutrition has an impact on bone traits, i.e. weight, density, microstructure, and some mechanical properties. In skeletal bones, unfavourable feeding periods are often compensated later by bone resorption. Adaptive bone tissue remodelling through continuous biomineralisation takes place throughout the life, in contrast to antlers, which are shed every year (Landete-Castillejos et al., 2010).

The process of antler formation can be a useful model for investigations of mineral metabolism and bone diseases. It can also be a bioindicator of environmental contamination with chemical compounds, including radioactive substances. During antler growth, the calcium content in the skeleton is reduced, especially in long bones but not in cranial bones. Such reversible osteoporosis has been detected during the antler mineralisation period in *Cervus elaphus*, *Capreolus capreolus*, and *Dama dama*. Organic matter, mainly protein, accounts for approximately 44% of the antler composition, whereas 56% is inorganic matter. The inorganic portion consists of 48% of calcium phosphate, 5% of calcium carbonate, 2 % of magnesium carbonate, and other mineral compounds (Nowicka et al., 2006).

The structure of antlers, which are subjected to heavy loads during impact, allows them to counteract the pressure of the opponent's antler without breaking. The composition and structure of antlers resemble mammalian long bones, but they contain no internal fluids such as bone marrow. Intensive antler growth (*antlerogenesis*) requires a supply of a large amount of calcium and phosphorus within a very short time. This demand in the red deer (*Cervus elaphus*) is approximately 100 g/day, while only ca. 34 g/day is sufficient for the proper growth of skeletal bones (*Table 1*). The animal is not able to acquire such a large amount of minerals with natural food. It has been demonstrated that these components originate from the skeleton, in particular from long bones, e.g. ribs or limbs (metacarpal, antebrachial, or metatarsal and humeral bones), which are the richest sources of these mineral compounds (Chen et al., 2008).

Analyses of the internal antler structure have shown that the central part has a specific "core" with a trabecular (spongy) structure surrounded by longitudinal compact tissue. The trabecular part of the antler is anisotropic, i.e. it has channels aligned parallel to the longitudinal axis of the antler. The core is surrounded by a layer of compact bone tissue composed of bone cells arranged concentrically around Haversian canals in the form of bone laminae, i.e. layers of compact plates. In turn, the Haversian canals comprise blood vessels, which nourish tissues during antler growth, and nerve fibres. Concentric rings that form osteons contain collagen fibrils with a mineral hydroxyapatite structure. Hydroxyapatite is composed of calcium hydroxyphosphate $Ca_{10}(PO_4)_6(OH)_2$ and serves as a mineral scaffolding of connective tissue, which is responsible for mechanical bone strength (Chen et al., 2008).

Collagen fibrils together with hydroxyapatite make up laminates, i.e. composites of two materials with different chemical, physical, and technological properties. The reinforcing components are arranged in layers (hydroxyapatite) with collagen fibres serving a role of a binder between the layers. This composition exhibits good strength along the fibres but very weak strength perpendicular to their layers (Chen et al., 2008).

Mechanical tests carried out on bones and antlers have contributed to determination of the characteristics of the degree of their elasticity. It was found that an increase in the mineral content in antlers was accompanied by their higher elasticity and lower strength (Currey et al., 2009). *Alces alces* antlers appeared to have a higher modulus of elasticity (11.6 GPa) than in the case of *Cervus elaphus* antlers (6.8 GPa). This is attributed to the difference in the behaviour of fighting stags and the different antler structure. Elk

antlers are blade-shaped and have relatively small outgrowths at the margins, whereas deer develop long, laterally branched beams. Consequently, it is impossible for the antlers of elk males fighting with each other to be interlocked, but they are subjected to greater bending moments; therefore, they are characterised by a higher modulus of elasticity than that in deer antlers (Chen et al., 2008).

The antler tensile strength has been analysed by application of a tensile load longitudinally and transversely to the beam axis. The transverse tensile strength was estimated at 20.3-6 MPa, while the longitudinal strength was 115.4-16.6 MPa. Comparison of this trait in different *Cervidae* species has demonstrated a higher value in the deer (158 MPa) than in the reindeer (95 MPa). In comparison, this parameter in the cattle femur had a value of 148 MPa, which indicates similarity of the compact bone structure both in antlers and in long bones. Longitudinal strength is greater than transverse strength due to the anisotropic orientation (arrangement) of collagen fibre mineralisation. Collagen fibres are arranged approximately along the longitudinal axis, which prevents fractures (Chen et al., 2008).

The quality of antlers in wild-living and farmed deer was compared. Animals supplied with a diet characterised by an approximately 10 % lower protein content and 3-6-fold lower levels of several mineral compounds developed considerably weaker antlers than farmed animals provided with a 16% protein level in the diet. Both groups of animals lived in a similar area of the same region, i.e. the wild-living deer inhabited an area located approximately 70 km away from the farm. The histological structure of the porous layer and its determinants were compared in the antlers from both groups. It was demonstrated that, in comparison with the antlers of the farmed animals, the porosity of the antlers of the wild-living deer males was five-fold higher than in the cortical layer and 25% lower in the trabecular bone layer, which is highly relevant. Additionally, there was a general increase in the porosity of the cortical layer of antlers in older individuals and a positive correlation between this trait and the social status of the male in the herd. Porosity is a result of incomplete osteon formation during primary osteogenesis. The incomplete formation affects the mechanical properties of bone tissue, including antlers. It has been shown that porosity is associated with poorer nutrition and, hence, deficiency of micro- and macroelements leading to incomplete filling of osteons. Examinations of antlers of wild-living Cervidae can detect problems with proper mineralisation related to calcium-poor diet. Moreover, it has been demonstrated than porosity is negatively correlated with the cortical layer thickness. Wild-living individuals were characterised by a 2-mm thinner cortical layer in their antlers. The proportion of the cortical layer in the antlers exerts a substantial effect on their mechanical properties, as this is a "measure" of the so-called voids in the bone formations (densitometry). Incompletely filled osteons have a thicker osteoid seam containing higher amounts of Zn. Zinc is the main determinant of the mechanical properties of the porous parts of the antler. A major role is also played by potassium, which is a scaffolding material around osteons. The level of zinc and potassium rises with increasing porosity. This is caused by incomplete mineralisation of osteons with calcium and phosphorus and a larger size of the trabecular layer. Both features indicate an impossibility of complete osteon mineralisation and are associated with physiological effort. Antler porosity increases from the base towards the apices of tines and is substantially greater in male deer consuming a poorer diet (Landete-Castillejos et al., 2012; Pawłowska et al., 2014).

It has been found that a 3-kg antler contains 536 g of calcium, 348 g of which is absorbed in the final period of antler mineralisation (*Table 1*) (Gómez et al., 2012). Landete-Castillejos et al. (2007b) proved so total antler formation requires a displacement of about 400 g of Ca and 200 g of P from the skeleton, which means a transport of even 8.4 g of Ca and 2.4 g of P in every day of most intensive growth (*Table 1*). This demonstrates the scale of demand on these minerals and explains the decrease in concentrations of calcium ions and inorganic phosphorus. A decrease in magnesium is related to formation of magnesium phosphate, which contains about 3% of total bone composition and is contributed to creation of less frequent forms of apatite (Landete-Castillejos et al., 2007b; Kuba, 2014). Moreover Mg can be incorporated into the bone directly, which in fact impairs the structure of the bone, but may be essential in case of large calcium deficiency. However interesting is the fact of small increase in Mg concentration in the last month of study. Landete-Castillejos et al. (2007c) showed a similar tendency in magnesium content in antler tissue in the final period of mineralization in farm animals. Such excessive investment of mineral compounds in the development of antlers largely weakens the animal skeleton and can cause fractures and, consequently, even death. It has been shown that even small changes in the composition of deer's diet, such as enrichment with mineral additives, results in lower demineralisation of long bones and thus greater resistance to damage. It should be underlined that the investment in antler formation in young animals is relatively modest, but it increases with males' age. The amount of resources invested in the development of antlers can have serious consequences for social behaviour and reproductive success but is also associated with the risk of greater susceptibility to fractures (Gómez et al., 2012). All these facts and observations explain how important is the role of marco and microelements in animal homeostasis.

Nutritional requirements and food availability

Estévez et al. (2010) assessed the seasonal differences in the content of minerals in plants that are frequently consumed by wild-living deer (*Cervus elaphus hispanicus*) in south-eastern Spain. The investigations demonstrated that the concentrations of K, Mg, Mn, Na, P, Cu, and Zn were usually low in plants consumed by deer. However, no distinct seasonal trend was reported in the distribution of mineral compounds in plants.

Consumption in young animals is correlated with rapid growth, which suggests that it depends on the demand (Ceacero et al., 2009; Janiszewski and Szczepański, 2002). Chemical analyses of soils and grasses suggest that the largest mineral deficiencies occur in the dry season, which prompts herbivores to migrate (Kreulen, 1975; McNaughton, 1988, 1990). A disease called grass tetany has long been known. The presence of tannins in the fodder can also be an important factor in the choice of a particular type of food. Tannins reduce voluntary intake as well as digestibility and mineral absorption (Roy and Mukherji, 1979; Van Hoven, 1984). Campbell and Hewitt (2004) have reported that deer use minerals to detoxify secondary plant compounds. Therefore, mineral availability can determine food selectivity as well as migration and use of natural habitats by herbivores. Another problem is posed by chemical compounds that can interfere with digestion in ruminants, e.g. carbonates, which alter the DW intake and milk production (Clark et al., 1989).

Deer (*Cervus elaphus hispanicus)* from areas located in the Mediterranean climate with soils comprising limestone fragments and characterised by the presence of natural

pastures, shrubs, and forest areas have been examined (Estévez et al., 2010). The forests were dominated by three species *Querqus ilex*, and *Pinus halepensis*, and *P. pinea*, and natural clearings were characterised by the presence of rosemary *(Rosmarinus officinalis)*, lavender (*Lavandula sp.*), thyme (*Thymus sp.*), and juniper (*Juniperus sp.*). There were low concentrations of Na (*Querqus ilex*-0.1 g/kg DW, *Oak acorns*-0.0 g/kg DW, *Brachypodium* sp.-0.1 g/kg DW, *Asphodelus sp.*-0.1 g/kg DW), Mg (*Querqus ilex*-1.1 g/kg DW, *Oak acorns*-0.2 g/kg DW, *Brachypodium* sp.-0.4 g/kg DW, *Asphodelus sp.*-1.2 g/kg DW), and P (*Querqus ilex*-0.5 g/kg DW, *Oak acorns*-0.1 g/kg DW, *Brachypodium* sp.-0.3 g/kg DW, *Asphodelus sp.* - 0.9 g/kg DW). There were also alarming contents of such minerals as K (*Querqus ilex*-3.5 g/kg DW, *Oak acorns*-1.2 g/kg DW, *Brachypodium* sp.-3.8 g/kg DW, *Asphodelus sp.*-12.3 g/kg DW), Zn, Mn, and Cu. In acorns, the content of all analysed mineral compounds was unsatisfactory except for Mo and Cu. The intake of Mg, Na, P, Cu, and Zn was below the optimal level (Mg (g/kg) -1.5–1.9, Na (g/kg)- 0.6–0.7, P (g/kg)- 2.5–2.9, Cu (mg/kg)- 4.0–14.9, Zn (mg/kg) -25.0–34.9, and Ca (g/kg) - 3.0–4.9). The intake of K (*Querqus ilex*-3.8 g/kg DW, *Oak acorns*-4.9 g/kg DW, *Brachypodium* sp.-3.1 g/kg DW, *Asphodelus sp.*-5.6 g/kg DW) and Mn (*Querqus ilex*-25 g/kg DW, *Oak acorns* -3.9 g/kg DW, *Brachypodium* sp. -17.4 g/kg DW, and *Asphodelus sp.*-5.6 g/kg DW) was also below the norm in the summer and autumn periods. Lower intake of Ca was noted only in autumn (*Querqus ilex*- 7.8 g/kg DW, *Oak acorns*-1.5 g/kg DW, *Brachypodium* sp.-3.4 g/kg DW, *Asphodelus sp.* -0.0 g/kg DW). However, it should be remembered that the animals probably did not suffer from Ca deficiency due to the quality of soils in the area (Estévez et al., 2010).

In comparison with the values in diets published for other ungulates, the intake of Fe, Mo, Co, and Se was sufficient, but the amounts of Mg, Na, P, Cu, and Zn were below the optimal level throughout the year. Moreover, low quantities of K and Mn were available in the summer and autumn periods. Minerals, especially Na and Ca, are important especially for lactating females. Deficiency of these elements can cause slow growth and higher mortality of neonatal animals (Power et al., 1999).

Antlers develop in spring and summer. The process starts in March, with a peak in July and the end in late August. Although the Na demand is higher during the antler growth period than during pregnancy and lactation (Hellgren and Pitts, 1997), supplementation with Na and other minerals improves rumen buffering and simultaneously increases mineral consumption (Beede and Collier, 1986). Additionally, increased demand for Na is observed during the summer thermal stress, as the element is excreted with sweat (Kadzere et al., 2002).

A high intake of Ca, P, and I is disadvantageous, as it reduces Mn absorption (McDowell, 2003). Cu deficiency may occur not only at a level of 5 mg/kg Cu in the feed but also at Mo content above 3-5 mg/kg and Fe above 200 mg/kg (Berger, 1987). A similar effect on Mg absorption is exerted by high intakes of K (grass tetany), as an increased dietary dose of K inhibits Mg absorption (Ram et al., 1998).

Most mineral requirements in deer are established with the use of standard specification for livestock (*e.g.* domestic cattle) or intensive deer breeding (Blaxter et al., 1988), ignoring spatial and temporal factors (*e.g.* climate) with deviations in terms of the quality and abundance of feed available in most natural habitats (Arnold et al., 2004).

Designing an appropriate mineral supplement meeting the nutritional requirements of *Cervidae* is necessary, given the seasonal increase in the organism requirements,

especially during the growth, reproduction, or parturition periods. In particular, in areas where *Q. ilex* and *Brachypodium* and *Asphodelus spp*. grasses are the main diet for deer, supplementation with additional amounts of Na, K, Mg, P, Cu, Se, and Zn is indispensable. Supplementation with mineral compounds is recommended especially in spring and summer, when the physiological activity of animals is high. Later, with a shorter day, the appetite is reduced and the organism prepares for the wintertime.

Most breeders on deer nutrition have focused mainly on the content and quality of proteins and energy compounds in feed. However, the role of macronutrients, including calcium, phosphorus, and magnesium, should not be ignored. Magnesium is required for maintenance of the proper structure of calcium phosphate crystals forming the antler. Moreover, it is indispensable in the case of large calcium deficiencies, since this divalent cation can be incorporated in phosphate salts and create rare forms of apatite constituting part of bone mass (Landete-Castillejos et al., 2007a,b,c). This phenomenon is most often observed when the calcium demand cannot be compensated from the skeletal system. Magnesium is probably the basic building material at the time of the critical demand for calcium (Kuba, 2014).

Properly managed meadows and pastures in deer breeding areas and an early onset of the vegetation period can provide a sufficient source of macroelements in spring and summer, when herbs and grass are the basic components of the diet (Nazaruk et al., 2009; Borys et al., 2012). However, this is a rare phenomenon in the highly variable climate of Poland. It should also be mentioned that a pasture exploited in winter and early spring is not characterised by a high nutritional value and digestibility, and the selection of supplementary feed is often prompted by the price and availability on the market (Borys et al., 2012).

Farmed animals have no possibility to move and instinctively search for better-quality fodder, as in the case of wild-living animals. In the natural environment, they instinctively seek sources of inorganic minerals; for instance, in winter they can often be encountered along salted roads, where they try to supplement sodium. Extreme cases include deliberate killing of birds, whose bones and feathers are a rich source of calcium, phosphorus, magnesium, and zinc (Ceacero, 2010).

The nutritional quality of available fodder in the environment may vary depending on the phenological phase of plants and the vegetation classes, aspect, and density structure. The concentrations of mineral compounds in the vegetation have been found to vary with no predictable trend and to exceed allowable levels specified for fodder species (Alldredge et al., 2002).

The quality of fodder occurring naturally in Idaho and Oregon has been investigated. Approximately 36% of the area was covered by grass and shrub vegetation (*Poa pratensis L., Carex geyeri Boott, Anaphalis margaritacea L., Epilobium angustifoliums L., Senecio triangularis Hooker, Pentstemon spp Mitch., Calamagrostis rubescens Buck., Fragaria vesca L., Trifolium repens L., Acer glabrum Torrey, Ceanothus sanguineus Pursh, Amelanchier alnifolia Nuttall, Alnus sinuata Rydberg*) (Alldredge et al., 2002). Coniferous tree communities accounted for ca. 21% of the surface area (most frequent species - *Pseudotsuga menziesii Franco,* less frequent species - *Rubus parviflorus Nuttall, Salix scouleriana Barratt, Vaccinium membranaceum Douglas, V. globulare Rydberg, Menziesia ferruginia Smith, Coptis occidentalis Nuttall, Linneaea borealis L., Smilacina stellata L.,* and *Gallium triflorum Michx*). The concentration of Ca and P in the plants was variable, i.e. clover -9.683-13.780 µg/g (Ca), 3.267-5.633 µg/g (P), goldthread - 2.467-13.750 µg/g (Ca), 2.375-3.817 µg/g (P), elk sedge - 5.608-

11.367 µg/g (Ca), 1.260-4.525 µg/g (P), bluegrass - 2.983-10.217 µg/g (Ca), 970-5.258 µg/g (P), redstem - 7.167-13.200 µg/g (Ca), 1.273-3.250 µg/g (P), serviceberry- 3.450-8.950 µg/g (Ca), 1.633-2.733 µg/g (P), and scouler willow - 12.000-14.283 µg/g (Ca), 1.767-3.000 µg/g (P) (Alldredge et al., 2002). The high degree of variability of the concentration of mineral compounds in the plants was predictable. The differences among the plant species are typical due to the different components forming their cell walls. Other investigations have demonstrated a high level of variation in the concentrations of mineral compounds associated with the type of soil, precipitation rates, and weather conditions. Especially in spring, weather conditions may have the most important influence on initiation of plant growth, which in turn determines the subsequent stages of plant phenology throughout the growing season. Better growing conditions can promote absorption of higher levels of minerals that are able to meet the biological requirements of animals. Deer feed on forest and fodder species; thus, they compensate for the deficiencies in the concentrations of mineral compounds (Alldredge et al., 2002; Estévez et al., 2010; Janiszewski and Szczepański, 2001 a,b).

The level of crude protein was found to decline in all species between May and November. Its level was high (ca. 24%) throughout the vegetation season only in the shrubs and clovers. The shrubs met the deer's protein dietary requirements in all seasons except for November, while the grass vegetation was sufficient only in May. Already in August, the level of crude protein in the grasses was significantly lower than that required by the deer (*Cervus elaphus*). The amount of digestible energy decreased from May to November and increased from August to November in some plant species. The lowest level of digestible energy was observed in all plant species in November. From mid-June to mid-August, the digestible energy content in the grasses decreased below the amount contained in the shrubs (Alldredge et al., 2002).

The content of nutrients in grass vegetation decreases in the second half of summer, which may suggest that deer migrate from grasslands depending on the season of the year. Nutrition is the major determinant of deer distribution, productivity, and population size. In autumn, shrubs provide more digestible energy. In turn, the content of nutrients in wintertime declines below the nutritional requirements even in silage. Nutritional deficiencies exert a serious effect on reproduction performance and can lead to low birth weight and, hence, a low probability of calf survival. Poor feed also results in delayed ovulation, ovarian resorption, and fertilisation failure. In the case of caribou and deer, only 70% of females were fertilised in the area of Idaho and 55% in the western part of Oregon. Such a low fertility rate is probably caused by inadequate feeding in summer and autumn. Low levels of digestible energy can inhibit reproduction and cause slow growth of calves in summer and autumn as well as increased mortality in winter (Alldredge et al., 2002) and to increase the vulnerability of parasitic infections (Burliński et al., 2011).

Summary

Commercial feeds produced currently cover the animals' mineral requirements in approximately 40%. The deficit of mineral compounds in fodder plants and deficiencies of bioelements in animals are a result of abundant fertilisation and chemisation. The most common shortages are detected in the case of calcium and phosphorus as well as magnesium, sodium, selenium, copper, and zinc. Calcium is the basic component of bones and is present in all animal tissues and body fluids. The deficiency of this mineral

is most pronounced in young animals and lead to development of rickets. In terms of the content, phosphorus is the second bone component after calcium; its deficiency causes inhibition of growth and reduction of animal productivity and fertility. In the case of *Cervidae*, these two components are highly important, since these animals produce new antlers every year.

Special attention to nutrition is paid in the case of farmed animals produced for high meat or milk yield. Besides meeting the physiological requirements, minerals and vitamins are removed with secretions and excretions and the higher the productivity, the higher the mineral loss. Inadequate concentrations of calcium, magnesium, and phosphorus lead to diseases of the nervous and locomotor systems and affect fertility. A reduced calcium concentration in the organism reduces muscle contractility, which can lead to dislocation of the abomasum, reduction of proventricular peristalsis, mastitis (teat canal incompetence), and post-partum uteritis (involutional disorder). Calcium deficiency can also cause ketosis, as its reduced concentration in the blood contributes to loss of appetite, which leads to a negative energy balance and triggers enhanced fat mobilisation. Calcium deficiency manifests itself by a clinical form of postpartum paralysis (recumbent or lateral position, impaired consciousness from apathy to coma, loss of appetite and thirst, reduction or loss of proventricular peristalsis, impaired defecation and urination, and loss of lactation), peak lactation hypocalcaemia, and subclinical hypocalcaemia reducing the overall productivity of the herd. Calcium deficiency in the blood leads to elevation of the parathyroid hormone. It has a beneficial effect on calcium metabolism but does not promote phosphorus accumulation in kidneys, which exerts an adverse impact on the content of inorganic phosphorus in blood and may lead to development of clinical forms of phosphorus deficiency. Hypophosphataemia contributes to the development of osteopathic changes. In cows, it is manifested by difficulty in standing up, lameness, pain of extremities as well as loss of appetite and reduction of milk production during the progression of the disease (Lutnicki et al., 2015).

In addition to the meat production of lactation period in females, Cervidae are especially susceptible to hypocalcaemia or hypophosphataemia due to the annual increased demand for Ca and P during antler development. These two periods as special, as they can be associated with development of osteoporosis of the skeletal system and, hence, weakening of the entire body scaffolding in deer.

Breeders of ruminants who focus on providing their animals with proper nutrition usually pay attention to three nutritional components: energy, protein, and fibre. It is known that both deficiency and excess of these elements can lead to abnormal growth and development of young animals, low daily gains, reduction of milk yield, and a number of metabolic diseases and reproduction disorders. A properly balanced dietary ration should include another element, i.e. a proper level of mineral-vitamin compounds. Many vital functions depend on adequate dosage of macro- and microelements meeting animals' requirements. Minerals constitute part of hormones, activate enzymes, regulate osmotic pressure and pH, and serve a function of a building material. Depending on the animal performance and type of breeding of such livestock as cattle, horses, sheep, or goats, special attention should be paid to the increased mineral nutrient demand of these animals. Despite the knowledge of the significant role of macro- and micronutrients in animal nutrition, the nutritional needs for these components in the diet are usually misbalanced. It should be borne in mind that both their insufficient and excess quantities and the synergy with other minerals or vitamins

can cause serious breeding problems. The difficulty in adequate mineral supplementation in ruminants is related to the fact that roughage is the major source of their food. The content of individual minerals in both roughage and fodder produced on farms largely fluctuates depending on the mineral composition of the soil, fertilisation level, weather conditions, or harvest time. Therefore, their level often does not fully meet the demand, especially in periods characterised by increased demand for these components. To sum up, farm nutrition should be based on appropriate mineral-vitamin mixtures. In the case of *Cervidae*, the blends should contain increased contents of calcium and phosphorus, as these minerals are the main building material of antlers. Furthermore, the doses should be adjusted to the age, sex, and physiological status of the animals.

Seifi et al. (2010) have underlined a significant positive effect of anionic diet in prevention of mineral deficiencies and metabolic diseases. This type of diet can contribute to reduction of calcium amounts supplied with feed, thereby improving absorption of this element. To calculate properly and apply the dietary cation-anion difference DCAD, the feed has to be subjected to chemical analysis and the dietary ration should be balanced in terms of the mineral content. The current comprehensive programs that have been developed for livestock (e.g. dairy cattle) should be adapted for farmed deer as well.

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