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ANALYSIS OF CHANGES IN THE CONCENTRATION OF Ca, P AND Mg IN BLOOD SERUM OF RED DEER (*CERVUS ELAPHUS*) IMMATURE MALES IN FARM BREEDING

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Abstract. The aim of this work was to demonstrate the periods of the increased demand on Ca, P and Mg in immature males of red deer. In blood serum concentrations of Ca^{2+}, inorganic P and Mg^{2+} were evaluated using a spectrophotometric method. In the first autumn of life the highest concentrations were observed at the levels of: Ca – 2.858 $\pm$ 0.62 mmol $\cdot$ l^{-1}; P – 2.617 $\pm$ 0.25 mmol $\cdot$ l^{-1} and Mg – 0.878 $\pm$ 0.09 mmol $\cdot$ l^{-1}. In October the lowest concentration of Mg was noticed, reaching 0.584 $\pm$ 0.18 mmol $\cdot$ l^{-1}, while in case of Ca and P the lowest concentrations occurred in second November of life, with the values of 1.406 $\pm$ 0.19 mmol $\cdot$ l^{-1} and 1.596 $\pm$ 0.28 mmol $\cdot$ l^{-1} respectively. The results show the validity of mineral supplementation in red deer males in the period of entering puberty, mainly because of Ca–P–Mg expenses related to skeleton growth and development of first antler. Application of mineral supplements in summer should reduce the risk of decrease in skeleton growth rate caused by antler mineralization in autumn.

Key words: farming, macroelements, males, red deer, season

INTRODUCTION

In recent years in Poland a rising interest in red deer farming is observed. This tendency is an effect of high demand on meat of these animals, which is characterized by desirable nutritional values and usually is produced with modern,

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ecological methods [Borys et al. 2012]. In our country red deer farm breeding, focused on production of meat and leather, is regulated since 2001. However still many farmers have problems with proper nutrition of these animals. Therefore, to increase the profitability of farming, the studies resulting in deepening the knowledge about red deer nutritional demands are required.

Red deer is classified as an opportunistic ruminant, between sprout-eaters and herb-eaters, and thereby this species is able to cope with the problem of changing feed quality over a year, selecting the food instinctively [Dmuchowski 2004]. Still the diversity in diet may be an obstacle because different types of fodder are unequal in energetic value and composition (proteins, vitamins, minerals). For this reason the farmer is responsible for selecting the fodder which responds the nutritional requirements of an animal in changing time of the year [van Soest 1994].

Spring and summer are very demanding periods for cervids. In this time males grow their antlers, females gestate and prepare for lactation and the newborn calves begin their growth. All this effects in large nutritional demands in this part of the year. Later, shortening of day length causes decrease in appetite and preparation of the organism to winter. In this time the smallest energetic effort is beneficial [Gaspar-López et al. 2008]. The issue of proper feeding is important especially in calves, in which survival of the first winter depends on achieving a sufficient body weight [Fenessy et al. 1991]. The studies showed that in young animals early growth is particularly relevant in males – it affects on body weight in adult life but also determines selected features of the antler (mass, length), prejudging the future reproductive success [Gómez et al. 2006, 2008, Landete-Castillejos et al. 2007 a]. Gallego et al. [2009] showed differences in composition of hind's milk in relation to the sex of her calf. Thus the most intensive growth covers the period of lactation [Gómez et al. 2004]. Usually in red deer this period lasts from 14 to 18 weeks [Arman et al. 1974, Clutton-Brock et al. 1982], however elongation of this time to 34 weeks may effect in increase of calves' body weight even up to 25% [Landete-Castillejos et al. 2001]. These facts demonstrate the scale of deterioration in quality of food, which a young animal has to bear while switching to solid forage. It also shows the importance of its correct composition. Determination which nutrients are most relevant in following seasons is crucial to ensure the proper conditions for calves' development in farm breeding [Manly et al. 2002].

Until now most of the studies on nutrition in cervids focused mainly on content and quality of proteins and energetic compounds. However the role of macroelements including calcium, phosphorus and magnesium cannot be omitted. In red deer these elements play a crucial role not only in skeleton building (in males and females) but also in the processes of growing and mineralizing the antler (only in males). Calcium and phosphorus are the basic structure element [Landete-

Castillejos et al. 2007 b]. Especially big demand is observed for calcium, which is supplied with forage only in 25–40%. The rest origins from the pool deposited in bones [Muir et al. 1987]. Magnesium in necessary for maintaining the proper structure of calcium phosphate crystals forming the antler [Landete-Castillejos et al. 2012]. Therefore the demand on macroelements supplied with forage is higher in males than in females [Ceacero et al. 2009] and a proper content of these is necessary for balanced calves development. Usually studies on Ca, P and Mg in this species concerned the question of antler production in adult males (as a subject of trade or a trophy), but did not mention the demands of young animals. Therefore the aim of this study was to demonstrate the periods of the increased demand on Ca, P and Mg in immature males of red deer.

MATERIAL AND METHODS

The study was performed in 8 immature males of red deer (*Cervus elaphus elaphus*), in the age of 6 months in the beginning of the experiment. Blood samples (10 ml) were collected from the jugular vein. Serum samples obtained after centrifugation (10 min, 4000 rpm) were stored in –20°C until the laboratory analyzes. Samples were collected monthly since November 2011.

The animals were bred in a farm located in Lubuskie voivodeship. The farm covers the surface of 300 ha of lowlands near the Noteć river and Osiek lake. In the extensive breeding system about 1000 deer are farmed, grouped in 1–2 ha casements, with an access to fresh water. In summer animals retrieve food only from the pasture and in winter supplementary feeding with haylage, vegetables and cereals is applied. No mineral supplementation was used in examined animals.

In all samples the concentrations of calcium ions, inorganic phosphorus and magnesium ions were evaluated with a spectrophotometric method, using Alpha Diagnostics kits (Alpha Diagnostics, Poland) and the Epoll-20 unit (Poll LTD, Warsaw, Poland). The results were analyzed with Statistica 10 software and the ANOVA test ($P < 0.05$).

The experiment obtained the acceptance of Local Ethical Committee in Faculty of Biotechnology and Animal Husbandry, West Pomeranian University of Technology, Szczecin (number of agreement: 29/2012).

RESULTS AND DISCUSSION

As a result of this study in examined animals four periods of different concentrations of macroelements can be established: a) high concentrations with decreasing tendency (November and December), b) low, but stable concentrations (winter-spring period), c) rise of concentrations (July to September) and d) rapid

decrease of concentrations (second autumn of life). The values and trend lines of concentrations for each macroelement are shown in Figure 1. The highest concentrations of calcium, phosphorus and magnesium were observed in November in the first year of study, reaching Ca: 2.858 $\pm$ 0.62 mmol $\cdot$ l^{-1}; P: 2.617 $\pm$ 0.25 mmol $\cdot$ l^{-1}; Mg: 0.878 $\pm$ 0.09 mmol $\cdot$ l^{-1}. In the next month a small decrease was noticeable, but the values were still among the highest observed during the experiment. High concentrations of macroelements in the first two months of the study are probably an effect of mother milk feeding. Because of strongly pronounced seasonality in our climatic zone, calves of red deer are usually born at the turn of May and June [Asher 2011, Karpowicz 2012]. In the first several months mother's milk is a basic compound of calves' diet [Goldman 2002]. Therefore the turn of October and November is a period in which concentrations of calcium, phosphorus and magnesium stand on a high level.

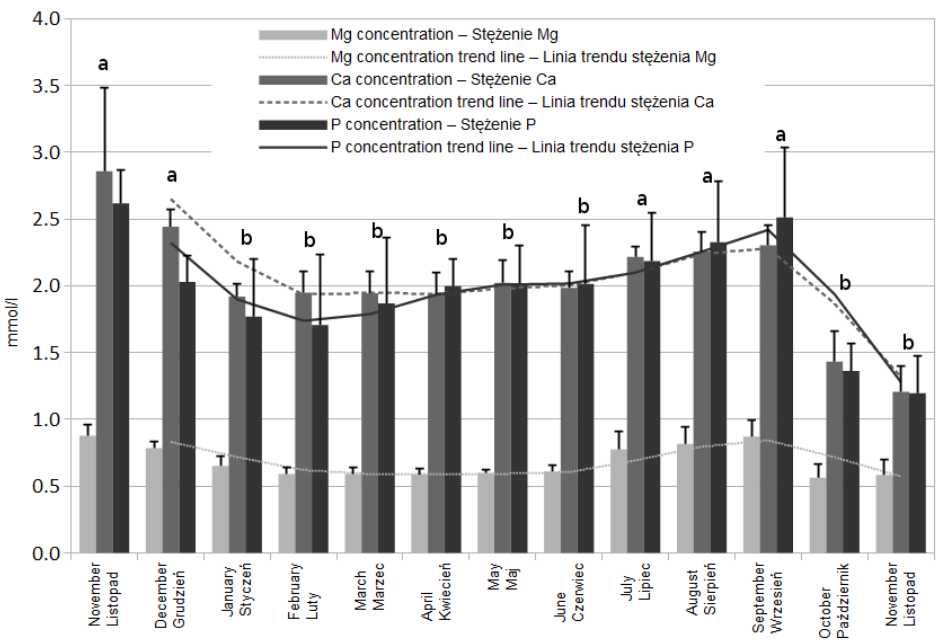

Fig. 1. Concentrations of Ca, P and Mg with trend lines demonstrating changing tendencies in following months of the experiment. Months labeled with different characters differ significantly for concentrations of all three examined macroelements ($P < 0.05$)

Rys. 1. Stężenia Ca, P i Mg oraz linie trendów obrazujące tendencje zmian tych stężeń w kolejnych miesiącach doświadczenia. Miesiące oznaczone różnymi literami różnią się istotnie pod względem stężenia wszystkich badanych makroelementów ($P < 0,05$)

Following months cover the period of winter and spring (January–June). In the samples from this time, concentrations of all macroelements remained on lower levels than in the beginning of the experiment and did not exhibit considerable fluctuations. The average concentration for whole period was at the levels of 1.960 ± 0.15 mmol $\cdot$ l^{-1} in calcium, of 1.836 ± 0.40 mmol $\cdot$ l^{-1} in phosphorus and of 0.606 ± 0.05 mmol $\cdot$ l^{-1} in magnesium. A decrease in concentrations of examined elements is connected with the end of lactation in hinds and a compulsion to switch into less nutritious plant food. In this period a diversification in usage of nutritional compounds from both sources is noticeable [White 1992]. Ruminal digestion of solid food satisfies the current needs of an organism while nutrients from milk translate into the intensive growth of young animal. The growth depends mainly on availability of proteins in mother's milk, and the integrity of them (e.g. caseins) is maintained thanks to presence of calcium and phosphates [Holt 1997]. This explains the decrease in growth rate after weaning [Gómez 2004]. The lowest concentrations result also from the changes in ingestive behavior, which depends on the season [Thèriez 1988]. The highest appetite is observed in summer while the lowest is characteristic for winter [Kay 1979]. These differences are caused by dependent on photoperiod changes in the concentration of melatonin and this effect is more evident in males [Heydon et al. 1993]. It should also be mentioned that the pasture forage consumed in winter and in early spring is not characterized by high nutritional values and digestibility and the selection of food intended for supplementary feeding is often based on price and a good availability on market.

Subsequent growth in concentrations of calcium, phosphorus and magnesium was observed in July, wherein the tendency remained increasing until September. This suggests a positive impact of plant vegetation on availability of nutrients in red deer. A possibility to consume mellow roughage is more beneficial for animals than consumption of forage from arable land, which usually is not varied in botanical composition [Stachowicz 2010]. Properly handled meadows and pastures are a sufficient source of macroelements in spring-summer period, when herbs and grass are the basic compound of diet [Nazaruk et al. 2009, Borys et al. 2012]. In early spring in second year of life young animals enter the period of puberty (in the age of 16 to 18 months) which strongly affects their nutritional demands [Karpowicz 2012]. Therefore early beginning of plant vegetation period helps young animals to recover after winter more quickly and to collect energetic reserves necessary for further development and reaching puberty [Pettorelli et al. 2005]. In proper environmental conditions, ensuring a good condition of animals, young deer may enter the puberty even up to two months earlier (in the age of 14 to 16 months) [Asher and Cox 2013]. On the other hand unfavorable conditions may delay that moment even to third or fourth autumn after birth. This border is fluent

because the puberty begins when animals reach 65–70% of target weight, which is related to availability and quality of fodder [Asher et al. 2011, Cilulko 2011]. In this study no significant growth in concentrations of Ca, P and Mg was observed in spring and no delay in entering puberty was noticed. This suggests that the length of vegetation period was sufficient to bear the cost of mineral expenses related with growth of first antler, which usually is an exhausting process for the organism of red deer male [Illius and O'Connor 2000].

At the turn of September and October a rapid decrease in concentrations of studied macroelements was observed, which in case of calcium and phosphorus lasted until the end of the experiment (November 2012). In this month the lowest concentrations were noticed (Ca: 1.406 ± 0.19 mmol $\cdot$ l^{-1}; P: 1.596 ± 0.28 mmol $\cdot$ l^{-1}). In case of magnesium the lowest concentration was observed in October (0.584 ± 0.18 mmol $\cdot$ l^{-1}) and in the following month a small growth in the concentration occurred. These low concentrations are the consequence of antler mineralization process. In adult red deer males in the age of 3–5 years old, antler cleaning and mineralization occurs usually at the turn of August and September [Muir 1985, Gaspar-López et al. 2010]. However observations made by hunters and farmers indicate that in males which build antler first time, mineralization begins about one month later – at the turn of September and October. This observation is confirmed by the fact, that mineralization is an effect of increase in testosterone level, which in the adult males may be observed from August, a month earlier than in males that are just entering the puberty [Bubenik 2006].

A crucial role in antler mineralization is played by the minerals that are studied in this paper [Landete-Castillejos et al. 2012]. This process is such a big effort for the organism because of the fact that the diet is insufficient to cover the large demand on Ca and P. Therefore in the time of antler growth a gradual skeleton demineralization is typical, in which these elements are mobilized and transferred from bones to growing antler [Klevezal 1996, Ceacero 2010], which is built in 55–65% from a hexagonal form calcium phosphate, called hydroxyapatite [Estévez et al. 2009]. 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. 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. 2007 b]. 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.

[2007 c] showed a similar tendency in magnesium content in antler tissue in the final period of mineralization in farm animals. Moreover, an increase in amount of magnesium is correlated with a decrease in content of calcium. Due to the inability to cover the demand on macroelements through the feed and temporal skeleton osteoporosis, in well-nourished farm animals deficiency in calcium may be compensated by magnesium deriving from the forage. Because of the ability to act as a divalent cation and a possibility to form salts with phosphates, magnesium may probably be used as a basic structural material in the moment of critical demand, which explains an increase of this element in the blood of examined animals.

No information about changes in concentrations of calcium, phosphorus and magnesium in blood serum of immature calves in particular months or seasons is present in the available literature. Peinado et al. [1999] showed concentration of Ca equal 2.240 ± 0.08 mmol $\cdot\, 1^{-1}$ and of P equal 3.870 ± 0.59 mmol $\cdot\, 1^{-1}$ in 4 year old males of red deer. These results are in fact comparable to the values obtained in this study, however it should be noted the differences in the age of examined animals as well as lack of information about the period of year in which the study was performed. For this reason the comparisons that are possible to be made at the currently published data can only be indicative.

Natural need to satisfy mineral requirement in wild ungulates may be that strong, that animals instinctively search for sources of minerals in unexpected locations. For example in winter animals can often be met near the salted road, where they attempt to provide sodium.

Extreme cases involve even intentional killing of birds, whose bones and feathers and a rich source of calcium, phosphorus, magnesium and zinc [Ceacero 2010]. All these facts and observations explain how important is the role of marco- and microelements in animal homeostasis.

CONCLUSIONS

1. Between all feeds the calves are fed, mother's milk ensures the highest availability for Ca, P and Mg.
2. Supplementary feeding with vegetables and cereals in winter ensures low but stable level of examined macroelements.
3. Antler mineralization is the period of biggest effort in mineral metabolism for the organism of a male.
4. Supplementation the fodder with Ca, P and Mg from June to September should mitigate drastic decreases in levels of these minerals in blood serum during the antler fraying period. Thereby this period would not contribute to the risk of retardation in skeletal growth, caused by mineral expenses.

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ANALIZA ZMIAN STĘŻEŃ Ca, P I Mg W SUROWICY NIEDOJRZAŁYCH PŁCIOWO SAMCÓW JELENIA SZLACHETNEGO (*CERVUS ELAPHUS*) UTRZYMYWANYCH W CHOWIE FERMOWYM

Streszczenie. Celem pracy było wykazanie okresów wzmożonego zapotrzebowania na Ca, P i Mg w ciągu roku, u niedojrzałych płciowo samców jelenia szlachetnego. W surowicy oznaczono stężenia Ca^{2+}, fosforu nieorganicznego oraz Mg^{2+} z wykorzystaniem metody spektrofotometrycznej. W trakcie życia podczas pierwszej jesieni zaobserwowano najwyższe stężenia na poziomie: Ca – 2.858 ± 0.62 mmol $\cdot l^{-1}$; P – 2.617 ± 0.25 mmol $\cdot l^{-1}$ and Mg – 0.878 ± 0.09 mmol $\cdot l^{-1}$. W październiku zaobserwowano najniższe stężenie Mg, wynoszące 0.584 ± 0.18 mmol $\cdot l^{-1}$, podczas gdy w przypadku Ca i P najniższe stężenia wystąpiły w czasie drugiego listopada życia, wynoszące odpowiednio 1.406 ± 0.19 mmol $\cdot l^{-1}$ i 1.596 ± 0.28 mmol $\cdot l^{-1}$. Uzyskane wyniki wskazują na zasadność stosowania suplementacji mineralnej u samców jelenia szlachetnego wchodzących w okres dojrzałości płciowej, głównie ze względu na znaczne wydatki w gospodarce wapniowo-fosforowo-magnezowej. Jest to związane ze wzrostem szkieletu i wytwarzaniem pierwszego w życiu poroża. Zastosowanie suplementacji mineralnej w lecie powinno ograniczyć ryzyko spowolnienia wzrostu szkieletu spowodowane mineralizacją poroża zimą.

Słowa kluczowe: hodowla, jeleń szlachetny, makroelementy, samce, sezon

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