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ORIGINAL ARTICLE

Effect of Ca and P supplementation on the haematological parameters and content of selected minerals in the blood of young farmed fallow deer males (*Dama dama*)

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Abstract

The aim of the study was to assess the effect of supplementation of feed rations with increased calcium and phosphorus doses on the haematological parameters and plasma zinc (Zn), phosphorus (P), magnesium (Mg), copper (Cu), calcium (Ca), and iron (Fe) content as well as the body weight and the growth and development of the first antler in farmed fallow deer (*Dama dama* Linnaeus, 1758). The mean level of erythrocytes (RBC), haemoglobin (HGB), and haematocrit (HCT) was increased in the Ca- and P-supplemented group after the treatment period. The change was statistically significant ($p < 0.05$) in the case of RBC and HCT. The other haematological parameters (mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelet count (PLT)) were reduced. An increase in the Zn content was observed in the plasma of slaughtered animals. The concentration of other minerals (P, Mg, and Cu only in group II receiving a higher level of Ca and P in the feeding dose; Ca and Fe only in group I supplemented with a lower content of Ca and P in a nutritional dose) in blood plasma decreased slightly after the supplementation period and declined further after the slaughter. Noteworthy, there was a significant increase in the plasma Cu and Fe levels in group I in group II, respectively, in the post-supplementation period. No significant differences were observed in the body weight between the groups, but there was a beneficial effect of the higher Ca and P dose in the feed ration for the farmed fallow deer on the length of the first antler ($p < 0.05$). The antlers of animals in group II were on average 2.3 cm longer than in group I.

Keywords *Dama dama* · Supplementation · Haematology · Minerals · Farming

Introduction

Cervids undergo spectacular physiological changes during the year. The antler cycle begins in spring with the cast of the old set. Afterwards, a new antler grows and is cleaned from velvet at the end of summer (Suttie et al. 1991). However, it has been evidenced that the first stage of cervid nutrition can exert an effect on antler size and weight. The correlation between the skeleton size and the antler weight is one of the longest known allometric dependencies occurring in animals (Zannèse et al. 2006). The growth rate and size of the first antlers developed by young males in the second year of life depend on the quality of food provided by their mothers and by the habitat and ecological conditions existing during the winter period (García et al. 1999; Gómez et al. 2008; Janiszewski et al. 2008). Bartoš (1980) as well as Bartoš and Bubenik (2011) have shown that behavioural aspects, e.g. dominance in group and social structures or other stress factors, can also be a cause of formation of larger antlers in deer.

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The length of the first antler in deer is an index trait of the ontogenic quality and a phenotypic trait that reflects the quality of nutrition (Gaspar-López et al. 2008). The first antler grows with an average rate of 1.95 ± 0.05 cm per week, which is the highest at week 14 (Gaspar-López et al. 2008). The conditions provided to a young male in the early months of life exert important effects on the final size and fitness of the adult male. Intensive antler growth (antlerogenesis) requires a supply of a large amount of calcium and phosphorus in a very short time. This demand for the red deer is ca. 100 g/day, while only ca. 34 g/day is sufficient for efficient growth of skeletal bones (*Cervus elaphus* Linnaeus, 1758). These animals are not able to acquire such an amount of mineral ingredients with natural nutrition (Chen et al. 2008).

The average percent mineral content in the first antler of the Iberian deer (*Cervus elaphus hispanicus* Hilzheimer, 1909) was shown to be 34% Ca, 31% P, and 44% K. Even the best post-weaning nutrition cannot compensate for the nutrition obtained during lactation (Landete-Castillejos et al. 2007a). 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 (Gómez et al. 2012). Landete-Castillejos et al. (2007b) proved that total antler formation requires displacement of approx. 400 g of Ca and 200 g of P from the skeleton, which means transport of even 8.4 g of Ca and 2.4 g of P on each day of the most intensive growth. This demonstrates the scale of the demand for these minerals (Landete-Castillejos et al. 2007b; Kuba 2014). Calcium is the basic component of bones and is present in all animal tissues and body fluids. 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. These two components are highly important in Cervidae, since these animals produce new antlers every year. The period of antler growth is a time of a rich blood supply, which is a carrier of nutrients, e.g. minerals required for development of antlers (Landete-Castillejos et al. 2007b)

In winter, farm fawns are usually fed with farm feed, which can be supplemented with mineral additives. In turn, they spend summer in pastures. As in domesticated ruminants, this change in feeding can be related with a period of adaptation of rumen microflora and microfauna. This period is frequently associated with diarrhoea, which may cause changes in the haematological profile (Kováč et al. 1997). Moreover blood is a very sensitive indicator of metabolic changes in both the physiological and pathological status of animals (Weiss and Wardrop 2010). In such situations, the haematological indices facilitate immediate detection of emerging potential anomalies or changes in the organism (Neumefister et al. 2001). The influence of many intra- and extrasystemic factors may lead to disruption of the homeostatic balance in the organism, which can be reflected in distinct changes in the values of individual blood components. The aim of the study was to determine the effect of supplementation with increased doses of Ca (calcium) and P (phosphorus) in the diet on the haematological parameters and plasma content of Zn (zinc), P (phosphorus), Mg (magnesium), Cu (copper), Ca (calcium), and Fe (iron). The impact of the supplementation on the body weight as well as growth and development of the first antler in farmed fallow deer was examined in the study.

Materials and methods

Experimental design

The research was carried out at the Research Station of the Institute of Parasitology, Polish Academy of Sciences, Kosewo Górne (Region of Warmia and Mazury; Poland; 53°48’ N; 21°23’ E) in 2016–2017 (December–August). The study involved fawns born in the same year (the same age). In order to provide dose adjustment for males, the animals had to be placed in different enclosures, thus modifying the social environment. Both research groups had the same social structures and were influenced by the same stress factors. It has been demonstrated that fallow deer live in unisexual groups for most of the year (Braza et al. 1990). All animals were adapted to routine management and maintained in good health and body condition during the experiment. Handling procedures and sampling frequency were designed to reduce stress and health risk for the animals. During the experiment, no negative behaviour and anomalies were observed. No record of individual intake of feed was attempted at any stage. The 24 male fawns in the first year of life were divided into two equal groups ($n = 12$) receiving different nutrition for 5 months (December–April):

– group I - standard nutrition supplemented with a complementary mineral feed mixture for fawns “Cielak B Plus©” from LNB (Cargill, Poland). The supplement constituted 2.5% of the standard farm nutrition - 6.5 g (Table 1),
– group II - standard nutrition supplemented with a complementary mineral feed mixture for fawns “Cielak B Plus©” from LNB (Cargill, Poland). The supplement constituted 2.5% of the standard farm nutrition - 6.5 g; it was additionally enriched with monocalcium phosphate (0.9 g) to increase the Ca and P level up to 25% and 13%, respectively (Table 1).

The demand for Ca and P in cervids (animals exceeding 60 kg of body weight) is 2.7 g per day and 2.2 g per day, respectively (NRC 2007). These recommendations are indispensable for animal survival. The antler mineralisation process is such a big effort for the organism (Landete-Castillejos et al. 2012) that the typical diet may be insufficient to cover

Table 1 Composition of the dietary supplement provided to farmed fallow deer fawns

Premix composition	Components	Group I	Group II
		Content (in 1 kg)	
Macronutrients (%)	Calcium (Ca)	**18.3**	**25**
	Phosphorus (P)	**7.2**	**13**
	Sodium (Na)	8	8
	Magnesium (Mg)	4.3	4.3
Micronutrients (mg)	Manganese (Mn)	2000	2000
	Zinc (Zn)	3750	3750
	Iron (Fe)	2900	2900
	Copper (Cu)	375	375
	Cobalt (Co)	12.5	12.5
	Iodine (I)	50	50
	Selenium (Se)	12.5	12.5
Vitamins	A (j.m.)	1000000	1000000
	D3 (j.m.)	200000	200000
	E (mg)	1500	1500
	K3 (mg)	40	40
	B1 (mg)	30	30
	B2 (mg)	140	140
	B6 (mg)	30	30
	B12 (mcg)	600	600
	Folic acid (mg)	20	20
	Pantothenic acid (mg)	300	300
	Niacin (mg)	600	600
	Biotin (mcg)	600	600
	Choline chloride (mg)	2000	2000

Bold was used to emphasize that you was examined impact Ca and P supplementation depending on the amount of these minerals in the dose

the large demand for Ca and P at this time. At the beginning of the experiment, the animals from both groups were at the age of 6 months and weighed on average by half less (29.3 kg); therefore, the supplementation was adjusted. The first experimental group (I) received 1.2 g of Ca per day and 0.47 g of P per day, while the second experimental group (II) received 1.8 g of Ca per day and 0.9 g of P per day as well as standard farm nutrition. To increase the concentration of Ca and P in the nutritional dose, the animals were administered "Cielak B Plus" from LNB (Cargill, Poland) and monocalcium phosphate.

Measurements

The animals spent the winter (December–April) in a shelter situated in the wintering ground divided into two identical rooms, where the amount of feed taken was controlled. The walls of the shelter were made of horizontal wooden boards and provided protection against the adverse impact of weather conditions. In the shelter, there was a feeding rack for haylage, water containers, and feeding troughs for concentrated feed. The concentrate was provided once daily. The animals had free access to water over the entire wintering period. After the winter period, the animals stayed on the available pasture without supplementation (May–August). In European countries (e.g. the Czech Republic, Denmark, France, and Great Britain), it is common practice that weaned red or fallow deer calves are wintered indoors from September to the following spring in order to protect them from aversive weather conditions, reduce expenses for winter feeding, and encourage taming of the animals (Bartoš and Šiler 1993). The animals were provided with adequate space during the winter and grazing periods as recommended in DEFRA (2018), FEDFA (2018), and Mattiello (2009). Body weight was measured with the use of a set of MP 800 sensors coupled with a Tru-test DR 3000 weight reader. As declared by the manufacturer, the accuracy of this set is ± 1% and the minimum resolution is 100 g. To obtain additional information about the ontogeny of the young fallow deer males, the growing antlers with the pedicle (at the skull base) were measured when the development was completed. The measurements were carried out using a tape measure at the age of 14 months (17 August 2017) and the mean values of the measurement of both beams were noted.

Sampling

Blood was sampled from *vena jugularis externa* always at the same time (from 1 to 3 h after dawn) to avoid variations associated with circadian rhythms before the supplementation period (28 December 2016), after the supplementation period (20 April 2017), and during slaughter (17 September 2017). There was no need for sedation and it was carried out as in research Langridge (1992), García et al. (2002), and Gaspar-López et al. (2011). For haematological analyses, 5-ml volumes of blood were collected into vacuum tubes containing an anticoagulant agent (EDTA). The samples were chilled (4–8 °C) for 15 min after collection and analysed 2 or 3 h later. The analyses were carried out with the use of an automatic haematological analyser Mythic 18**.** The device was calibrated each time before the analysis of the samples. The blood was analysed to determine the levels of leukocytes (WBC), erythrocytes (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelets (PLT).

The blood samples (5 ml) were mixed with heparin as an anticoagulant. Plasma for analysis of the biochemical parameters was obtained by centrifugation of whole blood at 3000 rpm for 10 min in a laboratory centrifuge (MPW-350R, MPW Medical Instruments, Warsaw, Poland) at a temperature of 4 °C. After centrifugation, plasma Zn, P, Mg, Cu, Ca, Fe levels were determined using reagent kits (BioMaxima, Lublin, Poland) according to the manufacturer's protocols and a random access biochemical analyser Metrolab 2300 GL

(Metrolab SA, Buenos Aires, Argentina). Haematological parameters were determined in the first two terms (before and after the supplementation, i.e. at the beginning of antler growth in April), since slaughter exerts adverse effects and the results may not be comparable to those obtained in the other periods (Marco and Lavín 1999; Kocan et al. 1981). The plasma mineral content was determined in the three terms (before the supplementation, after the supplementation at the beginning of antler growth in April, and post slaughter - after ossification of antlers). The article is part of the research of a large scientific project consisting of several studies.

Statistical analysis

The results were subjected to statistical analysis. The analysed values were presented as a mean value and standard deviation in the case of measurable parameters and as cardinality and percentage in the case of non-measurable variables.

The normality of the distribution of variables in the analysed groups was verified with the Shapiro-Wilk test. The differences between the two groups were assessed with the Student's *t* test or with the Mann-Whitney test when the former could not be applied. The comparison of the haematological results and plasma mineral concentrations before and after the supplementation as well as before the supplementation and post slaughter was performed with a Student's *t* test for dependent variables or a paired-samples Wilcoxon rank test when the conditions for application of the former test were not met. The comparison of the weight results obtained in the subsequent measurements was carried out with a Friedman ANOVA analysis, and pairwise comparisons were performed with a paired-samples Wilcoxon rank test with Bonferroni correction. A significance level of $p < 0.05$ was assumed, which indicated the presence of statistically significant differences or correlations. The database was compiled and statistical analyses were carried out in the Statistica 9.1 software (StatSoft, Polska).

Results

In the study, many interesting relationships were observed between the haematological parameters and the content of selected minerals supplemented in the nutritional dose. There was a significant increase in RBC in the study groups in the post-supplementation period: from 11.035×10^6 μl^{-1} to 12.954×10^6 μl^{-1} in group I and from 10.355×10^6 μl^{-1} to 12.942×10^6 μl^{-1} in group II receiving the higher Ca and P dose in the diet. Similarly, the increase in HCT was higher in group II, i.e. from 37.983% to 47.200%, than in group I (from 40.481% to 48.127%). In turn, the MCHC value was reduced from 40.509 g dl^{-1} to 38.718 g dl^{-1} in group I and from 42.825 g dl^{-1} to 39.725 g dl^{-1} in group II. There were significant differences in the blood haematological parameters, i.e. in the level of RBC, HCT, and MCHC ($p < 0.05$), within both groups and between the groups before the winter supplementation. There were no significant differences at the end of this period (Table 2). In contrast, the content of RBC and HCT changed significantly ($p < 0.05$) in the post-supplementation period (Table 3). Furthermore, significant differences in the level of RBC, HGB, HCT, and MCHC were noted between both study groups before and after the supplementation. Additionally, there was a significant decline in PLT after the supplementation in group I (from 457.182×10^3 μl^{-1} to 378.545×10^3 μl^{-1}, $p = 0.033$). In group II, there was a significant change in the WBC value (from 4.808×10^3 μl^{-1} to 4.214×10^3 μl^{-1}, $p < 0.001$) and MCH (from 15.875 pg to 14.458 pg, $p = 0.014$) (Table 4).

The plasma mineral content differed significantly between the study groups in the pre-supplementation period in the case of Zn (35.821 μmol L^{-1} in group I and 45.600 μmol L^{-1} in group II, $p = 0.001$), Mg (0.976 mmol L^{-1} in group I and 0.825 mmol L^{-1} in group II, $p < 0.001$), Cu (14.694 μmol L^{-1} in group I and 13.964 μmol L^{-1} in group II, $p = 0.018$), and Fe (41.695 μmol L^{-1} in group I and 32.005 μmol L^{-1} in group II, $p = 0.003$). After the supplementation period, significant differences were observed only in the case of Cu (20.780 umol L^{-1} in group I and 11.953 umol L^{-1} in group II, $p = 0.035$). In turn, there were differences in the levels of Zn (57.783 μmol L^{-1} in group I and 49.650 μmol L^{-1} in group II, $p = 0.001$) and P (1.640 mmol L^{-1} in group I and 1.838 mmol L^{-1} in group II, $p = 0.038$) in the post-slaughter period following the summer grazing period. Additionally, the plasma Zn content was increased in the post-slaughter period (by 21.9–22.1 μmol L^{-1} in group I and by only 4.05–14.5 μmol L^{-1} in group II). The content of other minerals (P, Mg, and Cu only in group II; Ca and Fe only in group I) in the blood plasma in the post-supplementation period decreased slightly and was even lower after the slaughter. Noteworthy, there was a significant increase in the plasma levels of Cu (from 14.694 μmol L^{-1} to 20.780 μmol L^{-1}) in group I and Fe (from 32.005 μmol L^{-1} to 34.475 μmol L^{-1}) in group II after the supplementation period (Table 5). Both groups showed significant changes in the P and Cu content in the post-supplementation period and in the Zn, Mg, and Ca levels after the slaughter (Table 6).

Significant changes in the plasma P and Mg content were demonstrated in both groups in the pre- and post-supplementation periods and in the post-slaughter period. Additionally, there was a significant change in the plasma Zn and Cu levels in group I between the post-supplementation and post-slaughter periods. The content of Ca and Fe in the plasma changed significantly in group I in the pre- and post-supplementation periods as well as the post-supplementation and post-slaughter periods. The group receiving the higher dose of Ca and P in the feed ration (II) exhibited a significant change in the plasma Zn level after the supplementation (Table 7).

Table 2 Comparison of haematological parameters in farmed fallow deer before the beginning of the supplementation to the end of the supplementation

Haematological parameters	Group	Before supplementation				After supplementation			
		M	SD	$t^a/U^b/Z^c$	p	M	SD	$t^a/U^b/Z^c$	p
WBC ($10^3\ \mu l^{-1}$)	I II	4.749 4.808	0.755 0.705	−0.077[c]	0.938	4.417 4.214	0.532 0.639	1.165[a]	0.250
RBC ($10^6\ \mu l^{-1}$)	I II	11.035 10.355	0.899 1.377	2.234[c]	0.025*	12.954 12.942	0.535 0.579	-0.561[c]	0.574
HGB (g dl^{-1})	I II	16.363 16.133	0.978 0.745	1.667[c]	0.095	18.609 18.708	0.733 0.569	-1.356[c]	0.174
HCT (%)	I II	40.481 37.983	2.345 3.602	2.938[c]	0.003*	48.127 47.200	2.845 2.979	-0.869[c]	0.384
MCV (fl)	I II	36.790 36.916	1.392 1.942	0.429[c]	0.667	37.100 36.433	1.066 1.077	1.535[c]	0.124
MCH (pg)	I II	14.900 15.875	1.418 2.494	-1.136[c]	0.255	14.354 14.458	0.181 0.258	-1.561[a]	0.126
MCHC (g dl^{-1})	I II	40.509 42.825	2.439 4.499	−2.502[c]	0.012*	38.718 39.725	1.000 1.582	−1.753[c]	0.079
PLT ($10^3\ \mu l^{-1}$)	I II	457.181 445.416	81.158 175.665	0.077[c]	0.938	378.545 376.250	118.994 156.313	52.000[b]	0.627

For abbreviations WBC, RBC, HGB, HV-CT, MCV, MCH, MCHC and PLT (see material and methods); [a] the Student's *t* test result, [b], [c] Mann-Whitney test results, M - mean, SD - standard deviation, *values of correlation coefficients are statistically significant at $p < 0.05$

The determination of the effects of the supplementation on blood parameters was accompanied by an analysis of its impact on the body weight and the length of the first antler in the farmed fallow deer. There were no significant differences in the body weight of the animals between the groups, whereas the increased Ca and P content in the feed ration was shown to exert a significant positive effect on the first antler length ($p < 0.05$). The animals from group II developed on average 2.3 cm longer antlers than the individuals in group I (Online Resource: Table A1). Additionally, there were significant correlations in the case of the body weight in both groups of the farmed

Table 3 Analysis of changes in haematological parameters of farmed fallow deer after the supplementation period

Haematological parameters	Group	Change after supplementation			
		M	SD	$t^a/U^b/Z^c$	p
WBC ($10^3\ \mu l^{-1}$)	I II	−0.331 −0.594	0.655 0.582	1.309[c]	0.190
RBC ($10^6\ \mu l^{-1}$)	I II	1.919 2.587	0.941 1.032	−2.277[c]	0.022*
HGB (g dl^{-1})	I II	2.245 2.575	0.795 0.961	−1.487[c]	0.136
HCT (%)	I II	7.645 9.216	2.720 2.309	-1.837[c]	0.066
MCV (fl)	I II	0.309 −0.483	2.014 2.837	0.738[c]	0.460
MCH (pg)	I II	−0.545 −1.416	1.477 2.381	1.182[c]	0.237
MCHC (g dl^{-1})	I II	−1.790 −3.100	2.279 3.244	1.795[c]	0.072
PLT ($10^3\ \mu l^{-1}$)	I II	−78.636 −69.166	140.014 243.356	1.328[a]	0.199

For abbreviations WBC, RBC, HGB, HV-CT, MCV, MCH, MCHC and PLT (see material and methods); [a] the Student's *t* test result, [b], [c] Mann-Whitney test results, M - mean, SD - standard deviation, *values of correlation coefficients are statistically significant at $p < 0.05$

Table 4 Comparison of haematological parameters in farmed fallow deer between group I and II before the beginning of the supplementation to the end of the supplementation

Haematological parameters	Group I						Group II					
	Before supplementation		After supplementation		Before-after		Before supplementation		After supplementation		Before-after	
	M	SD	M	SD	Z[c]	*p*	M	SD	M	SD	Z[c]	*p*
WBC (10^3 μl^{-1})	4.749	0.756	4.417	0.532	1.445	0.149	4.808	0.705	4.214	0.639	3.543	<0.001*
RBC (10^6 μl^{-1})	11.035	0.899	12.954	0.535	4.107	<0.001*	10.355	1.378	12.942	0.579	4.286	<0.001*
HGB (g dl^{-1})	16.363	0.978	18.609	0.733	4.107	<0.001*	16.133	0.745	18.708	0.569	4.286	<0.001*
HCT (%)	40.482	2.345	48.127	2.846	4.107	<0.001*	37.983	3.602	47.200	2.979	4.286	<0.001*
MCV (fl)	36.791	1.393	37.100	1.066	2.390	0.017*	36.917	1.942	36.433	1.077	0.286	0.775
MCH (pg)	14.900	1.419	14.354	0.182	1.415	0.157	15.875	2.495	14.458	0.259	2.451	0.014*
MCHC (g dl^{-1})	40.509	2.439	38.718	1.001	3.230	0.001*	42.825	4.499	39.725	1.582	3.879	<0.001*
PLT (10^3 μl^{-1})	457.182	81.158	378.545	118.994	2.126	0.033*	445.416	175.665	376.250	156.313	1.686	0.092

For abbreviations WBC, RBC, HGB, HV-CT, MCV, MCH, MCHC and PLT (see material and methods); [c] Mann-Whitney test results, M - mean, SD - standard deviation, *values of correlation coefficients are statistically significant at $p < 0.05$

fallow deer in almost all the terms of weighing, except for term 2 and 3 (Online Resource: Table A2, A3).

Discussion

There are few studies on the effect of supplementation on haematological parameters and mineral concentration in the plasma of farmed fallow deer. This type of research is important for breeders since this species is the most commonly bred Cervidae in Poland (FEDFA 2018). Commercial deer breeding in Poland began in 2002. As demonstrated by data presented in 2014 by the Polish Union of Deer Breeders at the FEDEFA congress, it can be assumed that there are 6000–9000 red deer and 25000–30000 fallow deer in Poland (Janiszewski et al. 2014).

Table 5 Comparison of the content of Zn, P, Mg, Cu, Ca, and Fe in the plasma of farmed fallow deer before the beginning of the supplementation to the end of the supplementation and in the post-slaughter period

The content of minerals in plasma	Group	Before supplementation				After supplementation				Post-slaughter			
		M	SD	t[a]/U[b]/Z[c]	*p*	M	SD	t[a]/U[b]/Z[c]	*p*	M	SD	t[a]/U[b]/Z[c]	*p*
Zn (μmol L^{-1})	I	35.821	8.604	96.000[b]	0.001*	35.704	8.727	54.000[b]	0.722	57.783	1.814	20.000[b]	0.001*
	II	45.600	10.892			35.186	10.831			49.650	9.894		
P (mmol L^{-1})	I	2.775	0.370	152.000[b]	0.107	2.142	0.234	0.621[a]	0.542	1.640	0.228	36.000[b]	0.038*
	II	2.618	0.311			2.146	0.478			1.838	0.227		
Mg (mmol L^{-1})	I	0.976	0.152	56.000[b]	<0.001*	0.726	0.090	32.000[b]	0.069	0.558	0.086	52.000[b]	0.265
	II	0.825	0.067			0.696	0.124			0.560	0.020		
Cu (umol L^{-1})	I	14.694	1.851	124.000[b]	0.018*	20.780	14.402	28.000[b]	0.035*	4.833	0.566	40.000[b]	0.068
	II	13.964	6.397			11.953	2.160			4.388	0.570		
Ca (mmol L^{-1})	I	3.481	0.690	160.000[b]	0.159	2.966	0.278	36.000[b]	0.122	2.728	1.075	48.000[b]	0.178
	II	3.021	0.307			2.776	0.234			2.766	0.538		
Fe (μmol L^{-1})	I	41.695	12.468	104.000[b]	0.003*	39.404	7.345	1.165[a]	0.250	27.070	3.793	60.000[b]	0.513
	II	32.005	4.027			34.475	7.893			34.660	12.805		

For abbreviations Zn, P, Mg, Cu, Ca and Fe (see introduction); [a] the Student's *t* test result, [b], [c] Mann-Whitney test results, M - mean, SD - standard deviation, *values of correlation coefficients are statistically significant at $p < 0.05$

Table 6 Analysis of changes in the Zn, P, Mg, Cu, Ca, and Fe content in the plasma of farmed fallow deer in the pre-supplementation and post-slaughter periods

The content of minerals in plasma	Group	Change after supplementation				Change post-slaughter			
		M	SD	$t^a/U^b/Z^c$	p	M	SD	$t^a/U^b/Z^c$	p
Zn	I	−1.414	10.718	56.000[b]	0.821	20.562	5.514	4.000[b]	<0.001*
(uμmol L^{-1})	II	−9.862	17.521			−1.688	9.843		
P	I	−0.500	0.548	8.000[b]	<0.001*	−1.055	0.496	38.000[b]	0.051
(mmol L^{-1})	II	−0.506	0.271			−0.742	0.418		
Mg	I	−0.334	0.196	52.000[b]	0.627	−0.475	0.179	14.000[b]	0.000*
(mmol L^{-1})	II	−0.091	0.087			−0.222	0.072		
Cu	I	6.608	15.857	−4.239[a]	0.001*	−9.108	1.423	52.000[b]	0.265
(μmol L^{-1})	II	1.375	1.570			−10.478	8.099		
Ca	I	−1.048	0.598	44.000[b]	0.313	−1.090	0.960	32.000[b]	0.021*
(mmol L^{-1})	II	−0.191	0.245			−0.365	0.910		
Fe	I	6.864	7.633	1.309[c]	0.190	−6.995	5.756	52.000[b]	0.265
(μmol L^{-1})	II	3.851	8.520			1.890	16.387		

For abbreviations Zn, P, Mg, Cu, Ca and Fe (see introduction); [a] the Student's *t* test result, [b], [c] Mann-Whitney test results, M - mean, SD - standard deviation, *values of correlation coefficients are statistically significant at $p < 0.05$

In the present experiments, a decline in the WBC count was observed in both groups in the spring (after the supplementation period). In group II supplemented with the increased amounts of Ca and P, this parameter had higher values, i.e. $0.6 \times 10^3\ \mu l^{-1}$, than in group I ($0.3 \times 10^3\ \mu l^{-1}$). This may indicate a higher rate of antler growth in the fallow deer, which is in agreement with the findings reported by Gaspar-López et al. (2011), who showed that the size of antlers was negatively correlated with the WBC count in cervids' blood. As shown by their results, larger antler sizes were found in animals that had lower values of the WBC count, MCHM, and MPV (Gaspar-López et al. 2011; Weiss and Wardrop 2010). The WBC and antler score were negatively correlated. This negative relationship between the leukocyte count and antler size may be explained by the theory proposed by Coop and Kyriazakis (1999) that the requirements of the immune system are only covered after the needs of maintenance of body protein or reproduction. Another possible explanation is the weaker condition of the first defence line in animals with smaller antlers, as suggested by Landete-Castillejos et al. (2002). This would be associated with a higher expense in the immune system, reducing the amount of resources available for antler growth (Landete-Castillejos et al. 2007b).

The mean leukocyte count was slightly higher than that reported by Mohri et al. (2000) in Persian fallow deer (*Dama mesopotamica* Brooke, 1875) and Chapman and Chapman (1982) in fallow deer (*D. dama*), but it was the same as the result presented by English and Lepherd (1981), Zomborsky et al. (1997), and Kováč et al. (1997). The differences may have been related to the method applied for immobilisation of the animals during blood collection. The use of physical methods, as in the case of farm fallow deer, may result in elevated leukocyte counts, in comparison with chemical methods. As shown by Tomkins et al. (1991), the diet may be another cause of reduced WBC and elevated RBC counts. In the present study, there was an increase in the mean number of erythrocytes in the post-supplementation period; a higher increase, i.e. by $2.6 \times 10^6\ \mu l^{-1}$, was observed in group II. Increased blood platelet counts are observed in cervids in spring (Gaspar-López et al. 2011); however, the value of this parameter measured in the experimental animals in April was reduced by $78.6 \times 10^3\ \mu l^{-1}$ in group I and by $69.2 \times 10^3\ \mu l^{-1}$ in group II. The increase in the haematological parameters as well as haemoglobin and haematocrit may support the positive effect of supplementation with an increased amount of Ca and P in the diet.

The mean number of erythrocytes in the fallow deer blood was similar to the value reported by Mohri et al. (2000) as well as Chapman and Chapman (1982). Similarly, the level of haemoglobin was similar to that shown by Mohri et al. (2000), Barić Rafaj et al. (2011), Chapple et al. (1991), and Cross et al. (1994) and slightly higher than that reported by Rosef et al. (2004). The value of haematocrit was higher in the farmed fallow deer in the post-supplementation period and similar in the pre-supplementation period to the values reported by Mohri et al. (2000), Barić Rafaj et al. (2011), Chapple et al. (1991), and Cross et al. (1994). The MCV parameter was slightly lower in the farmed fallow deer than that shown by Cross et al. (1994) and Rosef et al. (2004) and higher than the value demonstrated by Chapple et al. (1991). The MCH value was lower than in Persian fallow deer (Mohri et al. 2000), similar to that reported by Barić Rafaj et al. (2011) and Cross et al. (1994), and higher than in investigations conducted by Chapple et al. (1991). The MCHC parameter exhibited higher values than those presented by Mohri et al. (2000) or those determined in other cervid species by Audigé (1992),

Table 7 Comparison of the content of Zn, P, Mg, Cu, Ca, and Fe in the plasma of farmed fallow deer between group I and II before the beginning of the supplementation to the end of the supplementation and in the post-slaughter period

Group I

The content of minerals in plasma	Before supplementation		After supplementation		Before-after	Before supplementation		Post-slaughter		Before-post-slaughter
	M	SD	M	SD	Z^c /p	M	SD	M	SD	Z^c /p
Zn (μmol L^{-1})	37.118	6.121	35.704	8.728	0.153 NS	37.221	5.542	57.783	1.815	3.059 0.002*
P (mmol L^{-1})	2.642	0.418	2.142	0.234	2.497 0.012*	2.695	0.398	1.640	0.228	3.059 0.002*
Mg (mmol L^{-1})	1.060	0.162	0.726	0.091	2.803 0.005*	1.033	0.159	0.558	0.086	3.059 0.002*
Cu (μmol L^{-1})	14.172	1.644	20.780	14.402	0.968 NS	13.941	1.581	4.834	0.566	3.059 0.002*
Ca (mmol L^{-1})	4.014	0.434	2.966	0.278	2.803 0.005*	3.818	0.602	2.728	1.076	2.824 0.004*
Fe (μmol L^{-1})	32.540	2.788	39.404	7.345	2.089 0.037*	34.065	4.364	27.070	3.793	3.059 0.002*

Group II

The content of minerals in plasma	Before supplementation		After supplementation		Before-after	Before supplementation		Post-slaughter		Before-post-slaughter
	M	SD	M	SD	Z^c /p	M	SD	M	SD	Z^c /p
Zn (μmol L^{-1})	45.049	11.590	35.187	10.831	1.961 0.049*	51.338	4.084	49.650	9.895	0.784 NS
P (mmol L^{-1})	2.653	0.314	2.147	0.478	3.059 0.002*	2.580	0.241	1.838	0.228	3.059 0.002*
Mg (mmol L^{-1})	0.788	0.072	0.697	0.124	2.824 0.005*	0.782	0.065	0.560	0.020	3.059 0.002*
Cu (μmol L^{-1})	10.578	1.249	11.953	2.160	2.197 0.028*	14.866	7.902	4.389	0.570	3.059 0.002*
Ca (mmol L^{-1})	2.968	0.103	2.777	0.234	2.275 0.023*	3.132	0.404	2.767	0.538	0.470 NS
Fe (μmol L^{-1})	30.623	2.539	34.475	7.894	0.471 NS	32.770	4.817	34.660	12.804	0.470 NS

For abbreviations Zn, P, Mg, Cu, Ca and Fe (see introduction); [c] Mann-Whitney test results, M - mean, SD - standard deviation, *values of correlation coefficients are statistically significant at $p < 0.05$

Barić Rafaj et al. (2011), Chapple et al. (1991), and Cross et al. (1994). The mean number of platelets was higher than the value reported from Persian fallow deer (Mohri et al. 2000) or shown by Barić Rafaj et al. (2011); yet, it was in the range reported by Cross et al. (1994). These seasonal changes in the haematological parameters may have been associated with the changes in the diet, as demonstrated by DelGuidice et al. (1992). Nevertheless, it should be borne in mind that haematological parameters usually have higher values in young animals up to 18 months of age (Weiss and Wardrop 2010).

Besides haematological parameters, the content of some minerals in the blood plasma was analysed. The highest concentration of most minerals (P, Mg, Cu only in group II, Ca and Fe only in group I) was detected in the first period, i.e. before the supplementation was included in the youngest animals. This can be explained by the high activity of osteoblasts in calves in bone tissue remodelling (Rosef et al. 2004; Kučer et al. 2013). Additionally, the slight decrease in the plasma mineral content in the post-supplementation period may have been caused by the change in the diet and the onset of the antler development period. However, in contrast to the present research, Graham et al. (1962) as well as Morris and Bubenik (1983) showed that the concentration of minerals in the plasma remained stable during antler growth.

The increase in the Zn concentration in the plasma of the farmed fallow deer observed in the post-slaughter period, i.e. after cleaning off the velvet and ossification of antlers, may have been related to the approaching mating season. Zn is responsible for normal testosterone concentrations and proper function of the immune system (Weiss and Wardrop 2010; Bartoskewitz et al. 2007). After antler growth and mating periods, the immune system of cervids can return to homeostasis, hence the increased uptake of this mineral in the blood (Kun et al. 2015). This may also result from the supplementation, as in the investigations conducted by Suresh et al. (2013).

Scharfe et al. (1998) and Chapple et al. (1991) found a P concentration of 2.40 mmol L^{-1} in neonatal fallow deer. A similar concentration of this mineral was determined in the plasma of the farmed fawns in the pre-supplementation period. The value declined in the subsequent terms with the age of animals. There were no significant differences between the study groups despite the different P content in the diet. In other studies, the mean concentration of P at unrestricted access was 3.20 mmol L^{-1} in a population of young red deer and 1.48 mmol L^{-1} in adult animals. The decline with age is understandable, as the P demand is higher during early growth (Kučer et al. 2013).

It is known that the Mg concentration decreases with age due to stress susceptibility, defective membrane functions, and disruption of intracellular calcium metabolism, inflammation, cardiovascular diseases, including atherosclerosis and ischemic injury, diabetes, fibrosis, immune dysfunction, and other diseases associated with aging (Rayssiguier et al. 1993). The Mg concentration in the farmed fallow deer was similar to that reported by Kučer et al. (2013), and a significant decline in this parameter was noted after the grazing period without supplementation.

Cu deficiency is common in farmed red deer livestock. Padilla et al. (2000) and Bao et al. (2010), however, detected low serum Cu (9.86 μmol L^{-1}) values in young red deer kept on pasture. Values lower than 8 μmol L^{-1} are below the critical level for Cu deficiency (Mackintosh et al. 1987). Probably, the distinct decline in the plasma Cu content noted in the post-slaughter period in the farmed fallow deer was caused by the preceding pasture period, which is sometimes characterised by deficiencies of this mineral in pasture-kept animals. In turn, the study conducted by Rosef et al. (2004) showed a mean Cu value of 13.0 μmol L^{-1}. A similar level of this element was noted in the farmed fallow deer in the pre-supplementation period before the winter.

The mean values of Ca, Mg, and P in the fallow deer blood were higher than the concentrations described by Marco and Lavín (1999) and Rosef et al. (2004). This confirms the positive effect of the supplementation applied during the winter period. However, the Ca to Mg ratio is important in the absorption, utilisation, and excretion of these minerals (Somer 1995). Magnesium and calcium act in conjunction; for instance, they are involved in the regulation of nerve and muscle tone. A high Ca2+/Mg2+ ratio also predisposes to arterial spasms and increases catecholamine release (Sheehan and Seelig 1984). Probably, the changes in the Fe concentration in the blood of the group I fallow deer were induced by an unfavourable Ca/Mg ratio in the diet. Evidently, the group II animals received a better-balanced and more beneficial diet containing an increased content of Ca and P in the feed ration. At a proper and constant level of nutrition, the concentration of Ca and P in the blood does not change despite the period of antler growth (NRC 2007; Gaspar-López et al. 2011).

Proper nutrition is especially important in Cervidae calves, whose survival of the first winter is determined by achievement of normal body weight (Fennessy et al. 1991). A comparison of the effect of supplementation of feed for farmed Iberian red deer (*C. elaphus hispanicus*) demonstrated a significant difference in biometric parameters between the control group and the group of animals receiving enriched diets (Olguin et al. 2013). The present study showed no effect of the supplementation on the body weight in the analysed fallow deer. In other studies of farmed fallow deer, fawns that spent winter with does were characterised by an average 5.43% decline in body weight, in comparison with fawns wintered under a shelter - 10.24% (Janiszewski et al. 2008). Appropriate conditions provided to animals during the first winter are essential, as confirmed by the above-mentioned experiments and the present study, which demonstrated a positive effect of the supplementation on the body weight and antler length. Moreover, the final antler size was negatively related to the leukocyte count and the body condition had a more important effect on the final antler size, which was bigger than that exerted by the body weight, as also confirmed by Gaspar-López et al. (2011). However, one cannot forget that the size of antlers can also be influenced by animal behaviour and their social structure (Bartoš 1980; Bartoš and Bubenik 2011).

The increased Ca and P supply contributed to elevation of the plasma Zn content and improved some haematological indices, e.g. RBC, HGB, and HCT. Moreover, the increased doses of Ca and P in the diet exerted a positive effect on the concentration of minerals in the blood of cervids. Both animal groups exhibited a significant increase in the plasma Cu and Fe levels in group I in the post-supplementation period. Thus, the supplementation of the fawn nutrition had a positive effect on animal fitness and antler growth.

Compliance with ethical standards

Ethical statement This study was carried out in strict accordance with the Polish legislation for the use of animals in research (Act of 15 January 2015 on the protection of animals used for scientific or educational purposes). The protocol was approved by the Local Ethics Committee 0069, Resolution No. 42/2016 at the University of Warmia and Mazury in Olsztyn. Animals were restrained using a cushioned crush specifically designed to restrain deer movements - a handling box (2 m × 2 m × 0.6 m) only for a few minutes. At that time, the fallow deer were weighed, the blood was collected, and the antlers were measured.

Conflict of interest No potential conflicts of interest were reported by the authors.

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