IMPACT OF DAY LENGTH AND TOTAL PROTEIN CONTENT IN THE DIET OF FARMED FALLOW DEER (*DAMA DAMA*) ON THEIR PLASMA MINERAL LEVEL AND HAEMATOLOGICAL INDICES

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Abstract. Blood is the main carrier of nutrients and the first indicator of changes occurring in an organism. Hence, the aim of the study was to demonstrate the effect of the day length and the total protein content in the diet for farmed fallow deer (*Dama dama*) on the selected mineral contents of the plasma and blood haematology. The investigation of farmed fallow deer revealed an increase in the level of red blood cells (RBC) and haemoglobin (HGB) induced by altered light conditions, which may the evidence stimulation of erythropoiesis by an increased concentration of androgens. The level of white blood cells (WBC) changed significantly in the control group, which was kept in the least favourable conditions, as indicated by leukocytosis. In the present study, animals exposed to a changed day length exhibited a significant increase in the plasma concentrations of most minerals (phosphorus - P, magnesium - Mg, copper - Cu, calcium - Ca, iron - Fe) after the winter period in the first year of studies, which indicates accelerated development of antlers in farmed fallow deer. However, there was no equally intensive increase in the plasma mineral content (except for Ca) in the same group of animals in the second year of the study. This probably resulted from the administration of adequate nutrition, which prevented changes in the blood mineral concentration despite the antler growth.
Keywords: *Dama dama, wintering conditions, photoperiodism, nutrition, haematology, minerals*

Introduction

Blood is a living tissue composed of different cells which are suspended in the liquid part of the blood called plasma. Its components form a highly developed defense and transport system on which animal body health highly depend. It is, among others, a transporter of various compounds that provides cells with oxygen, nutrients, removes carbon dioxide and other unnecessary or harmful by-products of metabolism, but is also a carrier of hormones, chemical messenger substances, and thus participates in the transmission of information throughout the body. In addition, blood is involved in the

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regulation of body temperature, performs defense functions, protects against pathogens, cures the body - thanks to its coagulation properties (Weiss and Wardrop, 2010).

All this makes so blood is the main and the first indicator of changes occurring in the organism (Weiss and Wardrop, 2010). All factors that can affect an animal are reflected by basic blood tests. Adequate nutrition and enhanced light conditions are often used in farm breeding. The impact of the photoperiod and an appropriate composition of the feed ration have been extensively investigated in cervids, but insufficient attention has been devoted to the closely related fallow deer (*Dama dama*). It is difficult to find literature with data on the influence of an altered day length on the basic haematological parameters in European fallow deer. The day length is extremely important in the life of cervids (Jaczewski, 1954; Pollock, 1975) especially in their hormonal economy (Asher et al., 1989; Suttie et al., 1984; Sempere et al., 1992; Elliott et al., 1996), which may affect the basic haematological parameters.

One of the most important stages in the life of deer is the growth of the antlers regulated by among other things, the hormonal economy which is affected by the daily photoperiod as demonstrated by Jaczewski (1954). Goss (1969) subsequently demonstrated that acceleration of antler cycles can be induced by increasing the frequency of annual daylight cycles with up to four complete antler growth cycles being achieved in 12 months. Changes in food intake, behaviour and plasma hormone concentrations of red deer stags were also fully entrained to a 6-monthly photoperiod cycle (Goss, 1969).

Growing Cervids require an additional 14–20% protein (Ullrey et al., 1967) especially during the growth of antlers. However, Dryden (2016) showed antler growth in adult stags is little affected by diet protein concentrations over 7%, but supplements of protected protein or methionine may improve antler growth. Richardson et al. (2008) research has shown growing antlers consist almost entirely of protein (collagen) and typically consist of 35–45% protein once they harden or "mineralize." While antlers are growing, bucks require a diet of 13–16% protein for optimum development, along with other required nutrients. Only 6–10% protein is required for maintenance of adult deer (Richardson et al., 2008). Antler development in deer results from the collective contribution of nutrition, genetics and age (Ullrey, 1982). It was shown that, white-tailed deer require a diet containing 13 to 16% crude protein (including adequate levels of carbohydrates, fats, minerals and vitamins) in order to express their genetic potential for antler development. In the other studies showed wild cervids require roughly 5% crude protein of dry matter intake for maintenance, but up to 25% for reproduction (Dryden, 2011; Felton et al., 2018). Natural forage for northern cervids typically contains 5–15% crude protein, with rare extremes of 25–30% (Marell et al., 2006; Wam et al., 2016). The objective of protein diet and supplementation is therefore to provide additional nutrients to the deer's natural diet during times when native forage is thought to be inadequate in quantity or quality (especially winter months in the temperate climate zone). Moreover Felton et al. (2018) revealed a lack of evidence that cervids more strongly select for protein in summer than they do in winter. In addition, under farm conditions, the amount of feed consumed by cervids can be controlled only in winter (outside the pasture period).

It has been proven that changes in animal nutrition affect the basic haematological parameters and the concentration of minerals in the blood (Kováč et al., 1997). Also blood is a very sensitive indicator of metabolic changes in both the physiological and pathological status of animals (Weiss and Wardrop, 2010). Thus, the aim of the study

was to demonstrate the effect of the day length and total protein content in the diet for farmed fallow deer on the plasma level of selected minerals and haematological blood indices.

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; N: 53°48'; E: 21°23'). All analyses were performed with the consent of the Local Ethics Committee 0069, Resolution No. 42/2016. The study involved 36 male stags in the 3rd-6th year of life divided on an analogous basis into three equal groups (n = 12) receiving different nutrition and provided with different daily photoperiods in winter months (from January to the end of March 2016 and from December 2016 to the end of March 2017):

Experimental group I: Standard farm nutrition with a total protein level of 16% (each animal ingested on average 600 g of the mixture per day with the following composition: 70% of crushed oats in 15% of universal rapeseed concentrate (producer: Eko-pasz, Mońki, Poland) containing 33% of crude protein and in 15% of universal soybean concentrate (producer: Eko-pasz, Mońki, Poland) with 45% crude protein content). The diet included hay and grass silage provided *ad libitum* and a mineral feed mixture Opas Ekstra 7669 from LNB (producer: Cargill, Polska) (*Table 1*). The supplement constituted 2.5% of the standard farm nutrition - 15 g. The experimental group I animals were kept on a run between 7:00 and 15:00 and spent the rest of the day inside the shelter; they were exposed to the natural length of the daylight.

Table 1. Composition of the diet supplement provided to all groups farmed fallow deer

Premix composition	Components	Content (in 1 kg)
Macronutrients (%)	Calcium (Ca)	21
	Phosphorus (P)	3
	Sodium (Na)	10
	Magnesium (Mg)	4
Micronutrients (mg)	Manganese (Mn)	3 000
	Zinc (Zn)	6 000
	Iron (Fe)	4 000
	Copper (Cu)	1 000
	Cobalt (Co)	25
	Iodine (I)	100
	Selenium (Se)	25
Vitamins	A (j.m.)	500 000
	D3 (j.m.)	100 000
	E (mg)	1 000
	B1 (mg)	4 000

Experimental group II: Nutrition was identical as in the experimental group I. Group II was subjected to changed photoperiod conditions, i.e. the day length was artificially prolonged in relation to natural conditions. The experimental group II animals were kept on a run adjacent to the shelter between 7:00 and 15:00 and spent the rest of the day inside. The shelter was equipped with electric LED lamps with a nominal power of 18 W and a declared light stream of 1850 lumens. The emitted light was cold white (colour temperature 6000 K). Light intensity in the shelter and outside was measured using an AB-8809A luxometer (producer: Abatronic, Radom, Poland).

In accordance with the adopted assumptions, the day length was extended by illumination of the shelter from January to the end of March 2016 and from December to the end of March 2017. Unfortunately, the same length of the artificial illumination plan was not used in both years, because in the first one the work associated with the breeding treatment performed annually was delayed by a month. The light in the shelter was turned on and off automatically. The comparison of the length of the natural daylight and the applied photoperiod regime are presented in *Figure 1*.
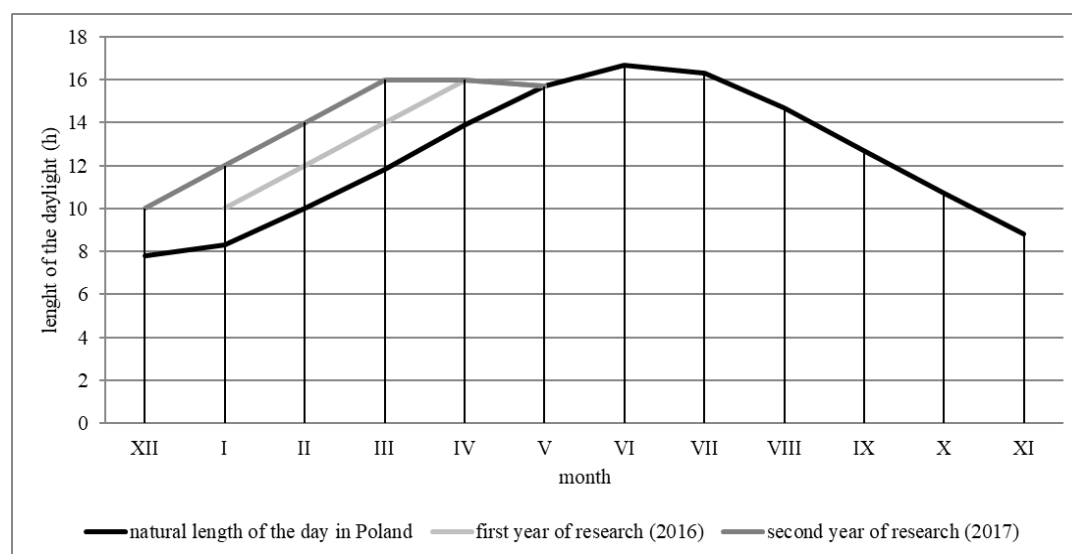

Figure 1. Comparison of the natural day length in Poland and the photoperiod regime in 2016 and 2017

Control group III: Standard farm nutrition with a total protein content of 10%. The diet included hay and grass silage provided *ad libitum* and a mineral feed mixture Opas Ekstra 7669 from LNB (producer: Cargill, Poland) (*Table 1*). The supplement constituted 2.5% of the standard farm nutrition - 15 g. The group was kept on a run throughout the day and exposed to the natural length of the daylight.

Observations and measurements

The animals from groups I and II spent the winter (December - March) under a shelter situated in the wintering ground divided into two identical rooms so that it was possible to control the amount of consumed feed. The walls of the shelter were constructed of horizontal wooden boards and provided protection against the adverse impact of weather conditions. Under the shelter, there was a feeding rack for haylage, water containers, and feeding troughs for concentrated feed. The concentrate was

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provided once daily. The animals had free access to water over the entire winter period. After the winter period, the animals stayed on the available pasture without supplementation (April - October) (*Fig. 2*). Body weight was measured with the use of a set of MP 800 sensors coupled with a Tru-Test DR 3000 weight reader LNB (producer: Tru-Test Group, Auckland, New Zealand). As declared by the manufacturer, the accuracy of this set is +/- 1% and the minimum resolution is 100 g.

Figure 2. *Farm fallow deer during the pasture period*

In accordance with the routine zootechnical practice adopted on the farm, antlers were cut at a height of 1 cm above the rose, which is the minimum height in technical terms. This treatment not only does not affect the welfare of experimental animals but also enhances their safety during the mating season, as a high density of relatively large groups of males on farms with limited space increases the risk of serious injuries and even deaths. After removal of dried velvet and mineral impurities and after final drying (in natural conditions) for 30 days, the antlers were weighed (each beam separately) on an electronic balance WLC F1/K with an accuracy of +/- 0.5 g (producer: Radwag, Poland).

Sampling

Samples were collected while the fallow deer were standing inside a small handling box (2 m × 2 m × 0.6 m) (*Fig. 3*) with no need of sedation. Blood samples were collected from *vena jugularis externa* always at the same time (from 1 to 3 h after dawn) to avoid variations associated with circadian rhythms as in research of Gáspar-López et al. (2011). For haematological analyses, 5-ml blood samples were collected into vacuum tubes containing an anticoagulant agent (EDTA) and into tubes with heparin as an anticoagulant. The samples were chilled (4-8 °C) within 15 min after collection. Haematological analysis was carried out within 2 or 3 h after extraction with the use of an automated haematological analyzer Mythic 18 (producer: Orphee S.A.,

Switzerlan). The device was calibrated each time before the analysis of the samples. The blood was analysed for the content of white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), and haematocrit (HCT).

Figure 3. Farm fallow deer in the handling box

Concurrently, 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 (producer: MPW Medical Instruments, Warsaw, Poland) at a temperature of 4 °C. After centrifugation, plasma zinc (Zn), phosphor (P), magnesium (Mg), copper (Cu), calcium (Ca), iron (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 (producer: Metrolab SA, Buenos Aires, Argentina).

The haematological parameters and the plasma content of selected minerals were determined in two terms: before and after the winter in 2016 and 2017, because in these times all animals were kept in the handling box and where weighed. After the procedure, they are moved to a suitable room (before winter) depending on the group to which they belong or to the pasture (after winter). The article is part of a cycle of research of farmed fallow deer. These animals have participated too in study in which we examined some hematological parameters (Tajchman, 2019).

Statistical analysis

The results were analysed statistically. The 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.

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The normality of the distribution of variables in the analysed groups was verified with the Shapiro-Wilk test. The differences between the two measurement terms were assessed with the Student's t-test or with the Mann-Whitney test for dependent samples and with the paired-samples Wilcoxon test when the conditions for application of the former test were not met. The differences between the three experimental groups were evaluated with the ANOVA analysis of variance and the post-hoc RIR Tukey test. When the conditions for application of the former analysis were not fulfilled, the Kruskal-Wallis test was used. In order to analyze the interaction of the effect of various factors (diet, lighting, time/year), a mixed multivariate ANOVA analysis of variance was performed. In the case of sphericity disorders (tested by the Meuchly test), the Greenhouse-Geisser correction was applied to the F statistics. 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

The investigations consisted in comparison of the haematological parameters and mineral concentration in the blood plasma of the farmed fallow deer assessed before the winter period in the first year (2016) of the study and before the winter period in the second year (2017). There was a significant decline from 5.288 to 3.638 $\times$ 10^9 L^{-1} ($P = 0.041$) in WBC in group I and on average from 14.397 to 11.564 $\times$ 10^{12} L^{-1} ($P < 0.05$) in RCB in all experimental groups (*Table 2*). In turn, the Zn content increased significantly, on average by 19.11 µmol L^{-1} ($P < 0.05$), in all groups. The highest increase, i.e. by 30.559 umol L^{-1} was noted in group I. A significant increase by 0.267 ($P = 0.034$) in the plasma P content was noted in group I, while the level of Mg increased by 0.174 mmol L^{-1} ($P = 0.0015$) in group II. The plasma Ca content increased significantly in all study groups assessed in the pre-wintering period in 2016 and 2107, with the highest increase by 0.685 ($P < 0.05$) observed in the control group. The Fe concentration also increased significantly in all groups, and the highest increase was noted in group II, i.e. by 13.046 ($P = 0.004$) (*Table 2*).

A statistical analysis of differences between the haematological parameters and the plasma mineral content recorded after the winter period in the first (2016) and second (2017) years of the study was carried out. The data showed a significant increase by 0.657 $\times$ 10^9 L^{-1} ($P = 0.049$) in WBC only in the control group and a significant reduction of RBC, on average by 2.404 $\times$ 10^{12} L^{-1} ($P < 0.05$), in all groups (*Table 3*). The plasma Zn concentration increased in group I and in the control, with a higher value in the control group (III), i.e. by 16.970 umol L^{-1} ($P < 0.05$). The plasma P concentration declined on average by 0.875 mmol L^{-1} ($P < 0.05$) after the winter period. There was a significant reduction on the Mg level (by 0.145 mmol L^{-1}) in group II. The levels of Ca and Fe in group I significantly increased by 0.381 mmol L^{-1} and 16.024 umol L^{-1}, respectively (*Table 3*).

We also compared the haematological parameters and plasma mineral content recorded before and after the wintering periods in 2016 and 2017. In the first year of the research, RBC and HCT significantly decreased by 1.031 $\times$ 10^{12} L^{-1} and 2.239% (P < 0.05), respectively, after the winter period. In group I, a higher level of only Cu (by 5.168 umol L^{-1}) was observed. With the exception of Zn, the plasma concentrations of all minerals increased significantly in group II. There was an increase in the level of P from 2.448 to

2.812 mmol L^{-1}, Mg from 0.682 to 0.880 mmol L^{-1}, Cu from 12.438 to 16.437 umol L^{-1}, Ca from 2.301 to 2.596 mmol L^{-1}, and Fe from 32.386 to 36.106 umol L^{-1} (P < 0.05). A significant increase in the control group was only noted in the case of P, Mg, and Cu. The content of P increased from 1.988 to 2.442 mmol L^{-1}, Mg from 0.641 to 0.747 mmol L^{-1}, and Cu from 12.277 to 15.754 μmol L^{-1} (P < 0.05) (*Table 4*).

In the second year of the investigations, there was a significant decrease (by 0.61 × 10^9 L^{-1}) in WBC in the control group (III) after the winter period, whereas RBC and HGB significantly increased by 0.896 × 10^{12} L^{-1} and 1.200 g L^{-1}, respectively, (*P* < 0.05). The plasma Zn and P concentration in group I decreased after the winter period from 49.979 to 29.697 umol L^{-1} and from 2.802 to 1.629 mmol L^{-1}, respectively (*P* < 0.05). In turn, the Mg and Cu content increased from 0.675 to 0.766 mmol L^{-1} and from 11.867 to 15.063 umol L^{-1}, respectively (*P* < 0.05). In the other groups (II and III), the content of Ca declined statistically significantly after the winter period, i.e. by 0.349 mmol L^{-1} (*Table 5*).

Additionally, the antler and body weight was compared in the study groups. In all groups, the weight of the animals' antlers increased significantly between 2016 and 2107. The increase was substantially higher in group I (from 941.176 to 1558.235 g) and III (from 1064.706 to 1450.588 g) than in group II (from 1062.437 to 1185.000 g) (*P* < 0.05). The body weight of the fallow deer before and after the winter period significantly increased in all groups as well (*Table 6*). Furthermore, the cycle of antler growth, velvet loss, and antler shedding was accelerated by on average 3 weeks in group II.

The changes in the haematological parameters and plasma mineral content in the farmed fallow deer were analysed statistically. A statistically significant difference was found in the magnitude of the change in the Zn level between the groups analysed in the pre-wintering period in the first and second year of the study (*F* = 11.653, *P* = 0.003). Statistically significant differences were found between groups I and II (a mean increase by 30.559 umol L^{-1} in group I and by 12.609 umol L^{-1} in group II) and between groups I and III (a mean increase by 12.609 umol L^{-1} in group I and by 14.185 umol L^{-1} in group III) (*Table 7*).

There was a statistically significant difference in the magnitude of the change in the Ca content between the analysed groups (*H* = 8.343, *P* = 0.015) before the wintering period in 2016 and 2017. Statistically significant differences were found for groups I and II (a mean increase by 0.260 mmol L^{-1} in group I and by 0.661 mmol L^{-1} in group II) and between groups I and III (a mean increase by 0.260 mmol L^{-1} in group I and by 0.684 mmol L^{-1} in group III) (*Table 7*).

After the wintering period in the first and second year of the study, there was a statistically significant difference in the magnitude of the change in the levels of Mg (*F* = 5.001, *P* = 0.013) and Ca (*F* = 4.041, *P* = 0.027) between the analysed groups. Statistically significant differences were found between groups I and II. On average, the Mg content increased by 0.114 mmol L^{-1} in group I and declined by 0.144 mmol L^{-1} in group II. Similarly, the content of Ca increased on average by 0.382 mmol L^{-1} in group I and decreased by 0.143 mmol L^{-1} in group II (*Table 7*). There was a statistically significant difference in the magnitude of the changes in the antler weight between the analysed groups (*H* = 18.544, *P* = 0.0001). Statistically significant differences were found between groups I and II (a mean increase in the weight by 617.059 g in group I and by 122.563 g in group II) (*Table 7*).

Statistically significant differences were also found between the measurement terms in the study years. Before and after the wintering periods in 2016, there were

differences in the magnitude of changes in the levels of Ca ($F = 4.464$, $P = 0.017$) and Fe ($F = 3.727$, $P = 0.032$). Statistically significant differences were noted between groups I and II. On average, the Ca content decreased by Ca o 0.144 mmol L^{-1} in group I and increased by 0.296 mmol L^{-1} in group II. Similarly, the Fa content increased on average by 2.989 umol L^{-1} in group I and increased by 3.720 umol L^{-1} in group II (*Table 8*). Before and after the wintering period in 2017, there were significant differences in the magnitude of the changes in the Zn content only ($F = 4.199$, $P = 0.024$). Statistically significant differences were found between groups I and III. On average, the Zn content decreased by 20.283 umol L^{-1} in group I and decrease by 0.998 umol L^{-1} in group III (*Table 8*).

The research also analyzed the effect of out-object (natural and artificial light length and 10% and 16% protein in the diet) and intra-object (change of results over time). No statistically significant effect of time as well as the effect of protein alone and light on WBC was observed. However, a significant effect of time and protein interaction was noted ($F (3, 60) = 3.864$, $P = 0.038$, $eta^2 = 0.162$) and the effect of time and light interaction ($F (3, 60) = 4.089$, $P = 0.032$, $eta^2 = 0.170$) (*Table 9*).

In the case of RBC, only the effect of time ($F (3, 60) = 26.913$, $P < 0.001$, $eta^2 = 0.574$) was significant, the difference was between the measurements from 2016 and 2017: 1 - 3, 1 - 4, 2 - 3, 2 - 4 (*Table 9*).

No significant effect of time, protein and light, and interaction between them on HGB and HCT was observed (*Table 9*).

In the case of Zn, a significant effect of time alone was demonstrated ($F (3, 60) = 32.972$, $P < 0.001$, $eta^2 = 0.507$), the significant difference was met between the measurements from 2016 and 2017: 1 - 3, 1 - 4, 2 - 3, 2 - 4. A significant effect of time and protein interaction ($F (3, 60) = 5.374$, $P = 0.008$, $eta^2 = 0.144$) and time and light interaction ($F (3, 60) = 4.793$, $P = 0.013$, $eta^2 = 0.013$) were also met for Zn concentration in fallow deer plasma. However, the time effect itself was the strongest ($eta^2 = 0.507$) (*Table 9*).

No significant effect of time, protein and light as well as the relationship between them on the P plasma concentration of animals was observed (*Table 9*).

The plasma Mg content was influenced by the time effect ($F (3, 60) = 5.625$, $P = 0.001$, $eta^2 = 0.149$), a difference was dependent between measurements from 2016 and 2016 and 2017: 1 - 2, 1 - 3, 1 - 4. A significant effect of time and light was also shown ($F (3, 60) = 2.816$, $P = 0.043$, $eta^2 = 0.081$). The time effect was stronger ($eta^2 = 0.149$) (*Table 9*).

The plasma Cu content was affected only by the time effect ($F (3, 60) = 5.551$, $P = 0.005$, $eta^2 = 0.148$), a statistically significant difference was between measurements 1 - 2, 1 - 4 (*Table 9*).

In the case of Ca, a significant effect of time alone was demonstrated ($F (3, 60) = 20.736$, $P < 0.001$, $eta^2 = 0.393$), the significant difference was between the measurements from 2016 and 2017 and from 2017 alone: 1 - 3, 1 - 4, 2 - 3, 3 - 4. In addition, a significant effect of time and light interaction ($F (3, 60) = 4.923$, $P = 0.003$, $eta^2 = 0.133$) on the fallow deer plasma Ca content was also demonstrated. The time effect itself was again stronger in this case (*Table 9*).

Demonstrated a significant effect of time alone on plasma Fe concentration ($F (3, 60) = 12.506$, $P < 0.001$, $eta^2 = 0.281$), a significant difference was between the measurements: 1 - 3, 1 - 4, 2 - 3, 2 - 4 (*Table 9*).

Table 2. *Comparison of some haematological parameters and mineral concentration in the plasma of farmed fallow deer before the winter period in 2016 and 2017*

Analyzed variable	Group I						Group II						Group III					
	Before wintering 2016		Before wintering 2017		2016-2017		Before wintering 2016		Before wintering 2017		2016-2017		Before wintering 2016		Before wintering 2017		2016-2017	
	M	SD	M	SD	t^a/Z^b	P	M	SD	M	SD	t^a/Z^b	P	M	SD	M	SD	t^a/Z^b	P
Haematological parameters																		
WBC (10^9 L^{-1})	5.288	2.772	3.638	0.753	2.039^b	0.041*	3.380	1.010	3.874	0.607	-1.308^a	0.232	3.807	0.625	4.442	0.876	-2.145^a	0.085
RBC (10^{12} L^{-1})	15.008	0.878	11.916	0.826	14.909^a	< 0.005*	14.050	0.872	11.140	1.348	8.429^a	< 0.005*	14.133	1.015	11.637	1.127	10.485^a	< 0.005*
HGB (g L^{-1})	17.700	0.773	17.658	0.867	0.275^b	0.784	17.887	0.356	16.925	1.801	1.581^a	0.158	17.850	0.493	17.450	1.508	0.737^a	0.494
HCT (%)	49.750	2.385	48.500	2.938	1.077^a	0.304	50.275	2.111	47.650	5.261	1.451^a	0.190	48.633	1.311	48.400	5.230	0.123^a	0.907
Content of minerals in plasma																		
Zn (umol L^{-1})	19.420	2.811	49.979	11.053	3.059^b	0.002*	22.237	4.501	34.846	11.800	-3.534^a	0.004*	22.258	4.510	36.442	10.661	-3.949^a	0.002*
P (mmol L^{-1})	2.535	0.338	2.802	3.154	2.118^b	0.034*	2.507	0.424	2.165	0.529	1.707^a	0.116	1.864	0.852	5.631	11.956	0.978^b	0.328
Mg (mmol L^{-1})	0.594	0.225	0.675	0.064	-1.101^a	0.294	0.680	0.063	0.854	0.206	2.432^b	0.015*	0.647	0.094	0.787	0.262	1.529^b	0.126
Cu (umol L^{-1})	11.231	1.762	11.867	3.439	0.314^b	0.754	12.269	2.516	13.991	2.629	-1.860^a	0.089	12.344	2.508	13.352	3.862	-0.925^a	0.375
Ca (mmol L^{-1})	2.576	0.218	2.836	0.425	2.628^b	0.008*	2.234	0.134	2.895	0.326	-6.805^a	< 0.005*	2.213	0.250	2.898	0.411	-5.090^a	< 0.005*
Fe (umol L^{-1})	31.903	3.522	38.552	7.983	-2.672^b	0.0217*	32.835	4.274	45.881	12.144	-3.619^a	0.004*	29.863	5.638	36.689	6.201	-3.523^a	0.004*

a – the Student's *t*-test result, b – Mann-Whitney test results, M-mean, SD- standard deviation, *values statistically significant *P* < 0.05

Table 3. *Comparison of some haematological parameters and mineral concentration in the plasma of farmed fallow deer after the winter period in 2016 and 2017*

Analyzed variable	Group I						Group II						Group III					
	After wintering 2016		After wintering 2017		2016-2017		After wintering 2016		After wintering 2017		2016-2017		After wintering 2016		After wintering 2017		2016-2017	
	M	SD	M	SD	t^a/Z^b	P	M	SD	M	SD	t^a/Z^b	P	M	SD	M	SD	t^a/Z^b	P
Haematological parameters																		
WBC (10^9 L^{-1})	3.868	0.769	3.912	0.781	-0.176^a	0.864	4.053	0.918	3.761	0.816	0.917^a	0.379	3.904	0.885	4.561	0.733	-2.211^a	0.049*
RBC (10^{12} L^{-1})	14.140	0.899	12.375	0.242	6.144^a	< 0.005 *	14.675	3.135	12.060	0.559	2.667^b	0.007*	15.042	2.059	12.209	0.496	3.059^b	0.002*
HGB (g L^{-1})	17.640	1.265	18.300	0.558	-2.199^a	0.055	18.425	1.835	18.067	0.943	0.039^b	0.969	18.300	1.467	18.192	0.640	0.470^b	0.638
HCT (%)	47.860	3.739	47.740	2.737	0.127^a	0.902	49.333	8.564	47.200	2.676	0.789^a	0.446	50.975	6.039	47.133	2.319	1.647^b	0.099
Content of minerals in plasma																		
Zn (umol L^{-1})	19.455	3.286	28.737	11.581	-3116^a	0.008*	21.614	2.725	32.013	16.466	-1.914^a	0.085	18.475	4.549	35.445	11.924	-4.674^a	< 0.005 *
P (mmol L^{-1})	2.603	0.520	1.609	0.558	4.652^a	< 0.005 *	2.834	0.296	1.777	0.369	9.862^a	< 0.005 *	2.400	0.328	1.821	0.355	3.760^a	0.003*
Mg (mmol L^{-1})	0.656	0.168	0.770	0.111	-1.814^a	0.094	0.885	0.099	0.740	0.160	2.856^a	0.017*	0.735	0.139	0.746	0.125	-0.192^a	0.852
Cu (umol L^{-1})	16.888	8.093	14.718	4.032	0.834^a	0.420	16.273	1.563	15.318	7.690	0.403^a	0.695	15.305	3.712	16.192	7.361	-0.356^a	0.729
Ca (mmol L^{-1})	2.391	0.344	2.772	0.441	-3.562^a	0.0048	2.616	0.242	2.474	0.443	0.809^a	0.437	2.415	0.378	2.625	0.239	-1.889^a	0.085
Fe (umol L^{-1})	29.747	4.733	45.773	13.085	-4.819^a	< 0.005 *	37.888	4.956	54.575	32.823	-1.671^a	0.125	31.997	7.776	46.989	25.171	-1.900^a	0.083

a – the Student's *t*-test result, b – Mann-Whitney test results, M-mean, SD- standard deviation, *values statistically significant *P* < 0.05

Table 4. *Comparison of some haematological parameters and mineral concentration in the plasma of farmed fallow deer before and after the winter period in 2016*

Analyzed variable	Group I						Group II						Group III					
	Before wintering 2016		After wintering 2016		Before-after		Before wintering 2016		After wintering 2016		Before-after		Before wintering 2016		After wintering 2016		Before-after	
	M	SD	M	SD	t^a/Z^b	*P*	M	SD	M	SD	t^a/Z^b	*P*	M	SD	M	SD	t^a/Z^b	*P*
Haematological parameters																		
WBC (10^9 L^{-1})	4.841	2.535	3.809	0.689	0.804^b	0.422	3.240	0.879	3.761	0.599	-1.707^a	0.119	3.564	0.775	3.764	0.898	-1.068^a	0.316
RBC (10^{12} L^{-1})	15.054	0.855	14.023	0.891	3.640^a	0.414*	14.218	0.785	14.518	2.749	0.000^b	1.000	14.322	0.960	15.789	2.996	0.533^b	0.594
HGB (g L^{-1})	17.585	0.779	17.515	1.152	0.278^a	0.786	17.873	0.307	18.291	1.566	0.509^b	0.610	17.911	0.521	18.955	2.186	0.889^b	0.374
HCT (%)	49.600	2.475	47.361	3.381	2.359^a	0.036*	50.009	1.844	49.627	7.37	0.157^a	0.878	49.056	1.859	53.267	8.841	0.886^b	0.374
Content of minerals in plasma																		
Zn (umol L^{-1})	19.680	2.720	18.348	3.655	1.126^a	0.277	27.563	4.257	21.707	3.822	-0.096^a	0.925	21.234	4.597	18.911	4.924	1.706^b	0.088
P (mmol L^{-1})	2.471	0.365	2.496	0.528	-0.170^a	0.867	2.448	0.493	2.812	0.326	-2.475^a	0.026*	1.988	0.742	2.442	0.351	2.499^b	0.012*
Mg (mmol L^{-1})	0.569	0.228	0.636	0.168	-0.865^a	0.399	0.682	0.0567	0.880	0.106	-6.736^a	< 0.005*	0.641	0.084	0.747	0.135	2.508^b	0.012*
Cu (umol L^{-1})	10.884	1.780	16.052	7.694	-2.485^b	0.013*	12.438	2.282	16.437	2.287	-4.913^a	< 0.005*	12.277	2.286	15.754	3.516	-3.459^a	0.003*
Ca (mmol L^{-1})	2.506	0.244	2.362	0.516	1.107^a	0.284	2.301	0.182	2.596	0.253	-3.339^a	0.004*	2.222	0.236	2.406	0.364	-1.819^a	0.088
Fe (umol L^{-1})	31.691	3.716	28.701	4.848	1.902^a	0.075	32.386	4.412	36.106	5.837	-2.327^a	0.034*	29.413	5.785	32.178	8.324	-1.136^a	0.274

a – the Student's *t*-test result, b – Mann-Whitney test results, M-mean, SD- standard deviation, *values statistically significant *P* < 0.05

Table 5. *Comparison of some haematological parameters and mineral concentration in the plasma of farmed fallow deer before and after the winter period in 2017*

Analyzed variable	Group I						Group II						Group III					
	Before wintering 2017		After wintering 2017		Before-after		Before wintering 2017		After wintering 2017		Before-after		Before wintering 2017		After wintering 2017		Before-after	
	M	SD	M	SD	t^a/Z^b	*P*	M	SD	M	SD	t^a/Z^b	*P*	M	SD	M	SD	t^a/Z^b	*P*
Haematological parameters																		
WBC (10^9 L^{-1})	3.638	0.753	4.042	0.745	-1.281^a	0.226	3.962	0.655	3.761	0.816	0.353b	0.724	3.951	0.917	4.561	0.733	-2.211^a	0.049*
RBC (10^{12} L^{-1})	11.916	0.826	12.374	0.359	1.490^b	0.136	11.164	1.181	12.060	0.559	-3.442^a	0.005*	11.943	1.189	12.209	0.496	-1.103^a	0.293
HGB (g L^{-1})	17.658	0.867	18.350	0.620	1.579^b	0.114	16.867	1.598	18.067	0.943	-3.035^a	0.011*	17.633	1.536	18.192	0.640	-1.582^a	0.142
HCT (%)	48.500	2.938	48.050	2.800	0.549^b	0.583	47.391	4.631	47.200	2.675	0.177^a	0.862	49.058	4.744	47.133	2.329	1.858^a	0.090
Content of minerals in plasma																		
Zn (umol L^{-1})	49.979	11.053	29.697	11.542	6.867^a	< 0.005 *	35.599	12.069	32.013	16.467	0.522^a	0.613	36.442	10.661	35.444	11.924	0.191^a	0.852
P (mmol L^{-1})	2.802	3.154	1.629	0.578	2.118^b	0.034*	2.128	0.539	1.777	0.369	2.094^a	0.063	5.631	11.956	1.812	0.371	1.689^b	0.091
Mg (mmol L^{-1})	0.675	0.064	0.766	0.115	-2.547^a	0.027*	0.873	0.204	0.740	0.160	1.274^b	0.202	0.786	0.262	0.746	0.124	0.157^b	0.875
Cu (umol L^{-1})	11.867	3.439	15.063	4.008	-2.309^a	0.041*	14.022	2.756	15.318	7.690	-0.493^a	0.632	13.352	3.862	16.192	7.361	-1.180^a	0.263
Ca (mmol L^{-1})	2.836	0.425	2.811	0.437	0.156^a	0.879	2.899	0.341	2.473	0.443	3.104^a	0.011*	2.898	0.411	2.625	0.239	2.400^a	0.035*
Fe (umol L^{-1})	38.552	7.983	47.158	12.632	-1.692^a	0.119	46.684	12.399	54.575	32.824	0.000^b	1.000	36.689	6.201	46.989	25.171	-1.349^a	0.204

a – the Student's *t*-test result, b – Mann-Whitney test results, M-mean, SD- standard deviation,*values statistically significant *P* < 0.05

Table 6. *Comparison of the antler and body weight before and after the winter period in 2016 and 2017 (calculations are given only for factors that were statistically significant)*

	Group I						Group II						Group III					
	2016		2017				2016		2017				2016		2017			
	M	SD	M	SD	t^a/Z^b	P	M	SD	M	SD	t^a/Z^b	P	M	SD	M	SD	t^a/Z^b	P
Weight of the antler (g)																		
	941.176	418.418	1558.235	300.317	3.621^b	< 0.005*	1062.437	461.535	1185.000	327.088	-2.490^a	0.025*	1064.706	492.368	1450.588	506.439	-6.346^a	< 0.005*
Body weight before wintering (October) (kg)																		
	72.588	11.847	79.824	9.105	-3.289^a	0.004*	70.125	6.459	78.250	6.618	-6.889^a	< 0.005*	72.705	10.110	84.353	6.509	2.769^b	0.005*
Body weight after wintering (April/May) (kg)																		
	57.235	10.047	66.941	6.932	-8.349^a	< 0.005*	59.500	10.857	69.125	5.488	3.181^b	0.001*	57.000	8.374	63.705	5.643	-6.638^a	< 0.005*

a – the Student's *t*-test result, b – Mann-Whitney test results, M-mean, SD- standard deviation, *values statistically significant *P* < 0.05

Table 7. *Statistical analysis of the magnitude of changes in the haematological parameters, plasma mineral levels, and body and antler weight in the fallow deer before wintering period in 2016 and 2017 and after the winter periods in 2016 and 2017 (calculations are given only for factors that were statistically significant)*

Compare terms	Analyzed variable	Group I		Group II		Group III		F^a/H^b	P	Intergroup differences
		M	SD	M	SD	M	SD			
Content of minerals in plasma										
Before wintering 2016-2017	Zn (umol L^{-1})	30,559	10,787	12,609	12,360	14,185	12,441	11.653^b	0.003*	I-II, I-III
	Ca (mmol L^{-1})	0,260	0,349	0,661	0,336	0,684	0,466	8.343^b	0.015*	I-II, I-III
After wintering 2016-2017	Mg (mmol L^{-1})	0.114	0.226	-0.144	0.168	0.011	0.196	5.001^a	0.013*	I-II
	Ca (mmol L^{-1})	0.382	0.387	-0.143	0.585	0.209	0.385	4.041^a	0.027*	I-II
After wintering 2016-2017	**Mass of antler and body weight**									
	mass of antler (g)	617.059	281.930	122.563	196.885	385.882	440.000	18.544^c	0.0001*	I-II

a - ANOVA variance analysis, b - Kruskal-Wallis test, M-mean, SD- standard deviation, *values statistically significant *P* < 0.05

Table 8. *Statistical analysis of the magnitude of changes in the haematological parameters, plasma mineral levels in the fallow deer before and after the winter periods in 2016 and before and after the winter periods in 2017*

Compare terms	Analyzed variable	Group I		Group II		Group III		F^a/H^b	P	Intergroup differences
		M	SD	M	SD	M	SD			
	Content of minerals in plasma									
Before - after wintering 2016	Ca (mmol L^{-1})	-0,144	0,537	0,296	0,354	0,184	0,404	4.464^a	0.017*	I-II
	Fe (umol L^{-1})	-2,989	6,479	3,720	6,394	2,764	9,731	3.727^a	0.032*	I-II
Before - after wintering 2017	Zn (umol L^{-1})	-20,283	10,231	-3,586	22,799	-0,998	18,051	4.199^a	0.024*	I-III

a - ANOVA variance analysis, b - Kruskal-Wallis test, M-mean, SD- standard deviation, *values statistically significant $P < 0.05$

Table 9. *The effect of the influence of time, protein level in the diet, light day length and interaction between the analyzed variables on selected haematological parameters and the concentration of minerals in plasma of fallow deer (calculations are given only for factors that were statistically significant)*

Analyzed variables			Estimated marginal averages				$F(3, 60)$	P	eta^2
			(1) Before wintering	(2) After wintering	(3) Before wintering	(4) After wintering			
			2016		2017				
	Haematological parameters								
WBC (10^9 L^{-1})	Protein	10%	3.333	3.833	4.000	4.000	3.864	0.038*	0.162
		16%	3.826	3.340	3.250	3.396			
	Daylight	Natural	4.056	3.694	3.500	3.833	4.089	0.032*	0.170
		Artificial elongated	2.875	3.125	3.500	3.125			

RBC (10^{12} L^{-1})	Time		14.439	14.741	11.586	12.156	26.913	< 0.001*, Intergroup differences: 1-3, 1-4, 2-3, 2-4	0.574

Content of minerals in plasma

Zn (umol L^{-1})	Time		20.970	20.068	40.674	32.385	32.972	< 0.001*, Intergroup differences: 1-3, 1-4, 1-3, 1-4	0.507
	Protein	10%	22.257	18.476	36.442	35.445	5.374	0.008*	0.144
		16%	20.326	20.864	42.789	30.855			
	Daylight	Natural	20.839	19.195	43.211	32.571	4.793	0.013*	0.130
		Artificial elongated	21.232	21.814	35.599	32.013			
Mg (mmol L^{-1})	Time		0.637	0.761	0.778	0.751	5.625	0.001, Intergroup differences: 1-2, 1-3, 1-4	0.149
	Daylight	Natural	0.621	0.700	0.731	0.756	2.816	0.043	0.081
		Artificial elongated	0.668	0.885	0.874	0.740			
Cu (umol L^{-1})	Time		11.754	16.309	13.080	15.524	5.551	0.005*, Intergroup differences: 1-2, 1-4	0.148
Ca (mmol L^{-1})	Time		2.339	2.477	2.877	2.636	20.736	< 0.001, Intergroup differences: 1-3, 1-4, 2-3,3-4	0.393
	Daylight	Natural	2.395	2.408	2.867	2.718	4.923	0.003	0.133
		Artificial elongated	2.227	2.616	2.899	2.474			
Fe (umol L^{-1})	Time		31.495	33.359	40.641	49.574	12.506	< 0.001*, Intergroup differences: 1-3, 1-4, 2-3, 2-4	0.281

F- ANOVA variance analysis, *P* - statistically significant values, *correct epsilon Greenhouse-Geisser

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Discussion

There are only few studies on the effect of nutrition and altered day length on haematological parameters and plasma mineral concentrations in farmed fallow deer. Studies on various temperate cervid species conducted with the use of artificial manipulations of the daily photoperiod have shown that light has a significant impact on cervids (Jaczewski, 1954; French et al., 1960; Goss, 1969; Pollock, 1975; Simpson et al., 1983/84; Snyder et al., 1983).

Changing breeding conditions exert an effect on animal physiology, as evidenced by the results of the present study. The level of WBC in the farmed fallow deer usually had lower values in all groups (except for group I before the winter period in 2016 when the values were similar) than values reported in investigations of farmed deer (Gaspar-López et al., 2011) in the respective months. It was shown in the fallow deer that the WBC count changed significantly in the control group, especially in 2017. This group was provided with the least favourable conditions, as reflected in the elevated plasma WBC levels. In the other research groups, the WBC count did not change significantly after the wintering period, as shown by Landete-Castillejos et al. (2002) or Landete-Castillejos et al. (2007), although that was the beginning of the antler development period. Another factor contributing to the slight reduction of WBC and the increase in RBC can be ascribed to the diet, as shown in the present study and investigations reported by Tomkins et al. (1991). The level of leukocytes varies seasonally and may constitute an adaptation to anticipated immune challenges by variation of the use of day length by animals (Nelson, 2004). Unfortunately, the present investigations do not confirm this finding. The RBC level in the farmed fallow deer was usually higher (especially in 2016); it was similar to the values noted in April by Gaspar-López et al. (2011) only after the winter period in 2017. Similarly, the HGB level was higher in the farmed fallow deer than those reported in investigations of farmed deer (Gaspar-López et al., 2011). HCT was higher in the farmed fallow deer, with the exception of group I, in which the value was similar to that reported by Gaspar-López et al. (2011) only after the winter period. The investigations conducted by Gaspar-López et al. (2011) demonstrated characteristic fluctuations in the RCB, HGB and HCT levels, which were substantially lower in November-December than in March-April. As suggested by DelGiudice et al. (1992), changes in nutrition may be a cause of seasonal fluctuations, which could explain the differences related to the altered light conditions in the present study. However, Gaspar-López et al. (2011) suggested another cause of these changes. The high testosterone level during the mating period (Gaspar-López et al., 2010) may be associated with high levels of the haematological parameters described in this study. Available literature provides evidence for stimulation of erythropoiesis by androgens (Gordon et al., 1970). Contrasting data were presented by Bubenik (2006), who suggested that antlers grow during a period of low concentrations of reproductive hormones. Interestingly, the RCB and HGB levels in the present study increased under the impact of the altered light conditions in the second year of the study, which may confirm stimulation of the erythropoiesis process probably by the increased estradiol content during this time. The investigations conducted by Bubenik et al. (1979) demonstrate an increase in the concentration of this hormone in spring and autumn in male white-tailed deer (*Odocoileus virginianus*). Estradiol blocks of the activity of testes and its high levels in spring prevent premature stimulation of spermatogenesis in

a period that is unsuitable for deer reproduction. This was confirmed by Bubenik et al. (2005) by analysis of blood collected from growing antlers and velvet, where this hormone was present in high concentrations.

The plasma concentration of Ca, P, and Mg in the farmed fallow deer in the analysed months was higher than or similar to the values reported by Kuba (2014). In the present study, the animal group exposed to the altered day length regime exhibited a significant increase in the plasma concentration of most minerals (P, Mg, Cu, Ca, Fe) after the winter period in 2016, which indicates accelerated development of antlers in the farmed fallow deer. Only the Cu content increased in group I and higher Mg and Cu levels were observed in group III. However, as early as in 2017, such an intensive increase in the mineral content (except for Ca) was not noted in the plasma of the group exposed to the greater day length. This was probably associated with the proper and constant level of nutrition, which prevented changes in the blood mineral concentration despite the period of antler growth (Gaspar-López et al., 2011). Interestingly, group I only, which was supplemented with a higher amount of protein in the diet, exhibited lower Zn and P content and a higher Mg and Cu level in the plasma after the winter period in 2017.

Additionally, high Zn concentrations in the plasma of the farmed fallow deer were observed before the winter period in all groups in 2017. This was probably related to the fact that the animals were subjected to the experimental conditions a month earlier than in 2016. Zn is responsible for the normal testosterone concentration and proper function of the immune system (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 be a result of supplementation, as shown by Suresh et al. (2013).

The concentration of P and Mg in the plasma of the farmed fallow deer was in the range reported by Kučer et al. (2013). The Cu content in the fallow deer plasma significantly increased after the winter period in all groups in 2016 and only in group I in 2017. This indicates a high demand for Cu after the pasture period as well as a need for adequate supplementation and the necessity to provide the animals with appropriate conditions in winter to compensate for nutritional deficiencies caused by the decline in the nutritional value of pastures in late autumn (Padilla et al., 2000; Bao et al., 2010).

The fallow deer body weight in the pre-wintering period was evidently higher than after the winter, which was reported in investigations of other cervid species as well. Increased liveweight gain during summer in comparison to winter has been recorded in all arctic, boreal and temperate deer so far studied (McEwan, 1968; Bandy et al., 1970; Pollock, 1975; Asher, 1993). The deer voluntarily reduce their appetite during winter (McEwan, 1968). The cycle of voluntary food intake and growth are known to be under photoperiodic control (Suttie and Simpson, 1985). Whereas for reproductive seasonality the short days of autumn are thought to provide a stimulatory cue for reproduction in temperate deer.

The body weight increased on average by 9.6 kg in the groups with higher protein content in the diet and with the modified day length and by only 6.7 kg in the control group (III). Appropriate conditions provided to animals during winter are essential, as confirmed by experiments conducted by Olguin et al. (2013), Gaspar-López et al. (2011), Janiszewski et al. (2008) and in the present study.

Similarly, the antler weight depended on the conditions provided to the animals during the winter period. However, the greatest effect was exerted by the protein content in the diet, adequate supplementation, and milder wintering conditions. In turn,

the extended day length accelerated development of antlers and velvet loss; hence, the antlers in this group exhibited the lowest weight. This phenomenon was confirmed by Suttie et al. (1991) as well as Suttie and Webster (1995).

Conclusion

In conclusion, an appropriate level of total protein and supplementation of the stag nutrition had a positive effect on animal fitness and antler growth. Time had the greatest effect on selected haematological parameters and mineral content in farm fallow deer plasma. The differences were mainly observed between the 2016 and 2017 measurements for RBC, Zn, Mg, Cu, Ca and Fe. In contrast, the equations between the measurements were in the first year of testing only in the case of Mg concentration, while in the second year in the case of Ca. Generally, prolonged repeated of breeding treatments give a greater effect. The effect of time and protein interaction had on WBC and Zn, while the effect of time and light interaction on WBC, Zn, Mg, Ca. Increased protein supply, separately, in the fallow deer diet causes an increase in the concentration of P, Mg, Cu and Ca in their plasma, while the extension of the light day has a positive effect on the content of RCB, HGB, P, Mg, Cu and Ca. On the other hand, the reduction in the quality of wintering conditions for animals was marked by an increase in WBC (*Table 10*). Therefore it can be assumed that the results of the present study have not only a theoretical but also application value.

Table 10. Summary of results obtained

Analyzed variable	Group I Total protein content of 16%, natural length of the daylight		Group II Total protein content of 16%, artificially extended daylight		Group III Total protein content of 10%, natural length of the daylight	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**
WBC	≈	≈	≈	≈	≈	▲
RBC	▼	≈	≈	▲	≈	≈
HGB	≈	≈	≈	▲	≈	≈
HCT	▼	≈	≈	≈	≈	≈
Zn	≈	▼	≈	≈	≈	≈
P	≈	▼	▲	≈	▲	≈
Mg	≈	▲	▲	≈	▲	≈
Cu	▲	▲	▲	≈	▲	≈
Ca	≈	≈	▲	▼	≈	▼
Fe	≈	≈	≈	≈	≈	≈
Body weight	▲		▲		▲	
Mass of antler	▲		▲		▲	

▲- significant increase, ▼- significant decrease, ≈- no significant change

In future research, it is planned to take blood from deer more often and determine hematology and mineral levels to capture more accurate changes over time. In addition, an identical artificial lighting plan should be used. In addition, as recommended by other researchers (Felton et al., 2018) to assess digestibility protein and energy both in

vivo and in vitro. Estimations of protein- and energy-digestibility are problematic to extrapolate to the population level, because of complex interactions between diet and individual, and many of the studies were compiled at the population level. Furthermore, in the case of research into small ruminants, a constant supply of drinking water should be ensured in pasture management (Fischer et al., 2017), which would also be worth to check in moderate climate conditions.

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