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Selected haematological indices in farmed male fallow deer (*Dama dama*) depending on the different conditions during the wintering period

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Abstract: Fallow deer (*Dama dama*) are the most common breeding species among farmed cervids in Poland. Monitoring the animals' health, nutritional status, and welfare are highly important aspects in their breeding. Haematological variables are important indicators for comparing the physiological status of the animals and for monitoring the changes in the organism related to the adaptation to the breeding conditions. The aim of this study was to assess the impact of the day's length and the total protein content in the diet for the farmed fallow deer on the selected haematological variables. The study demonstrated a significant decline in the mean corpuscular volume (MCV) and an increase of the mean corpuscular haemoglobin concentration (MCHC) ($P < 0.05$) in all the animals after the winter period. However, the animal group exposed to prolonged daylight exhibited a significant increase in the platelet distribution width (PDW) and platelet large cell ratio (P-LCR) ($P < 0.05$). In turn, the group receiving a lower amount of protein in the diet was characterised by a significant reduction in the platelet count (PLT) ($P < 0.05$). Thus, the length of daylight and the protein content of the diet for fallow deer exert a significant impact on several haematological characteristics, which may serve as indicators of an animal's nutritional status and welfare.

Keywords: *Dama dama*; haematology; daylight length; total protein

The accurate assessment of the health and nutritional status of wild, semi-captive, or domesticated animals is important for their welfare. Varied nutritional regimes and prolongation of the day's length, i.e., daylight exposure, are often used in deer farming. The impact of photoperiodism as well as the appropriate composition of the feed ration has been extensively studied in cervids (Jaczewski 1954; French et al. 1960; Goss 1969a; Goss 1969b; Goss 1976; Goss 1977; Goss 1980; Goss and Rosen 1973; Blaxter et al. 1974; Pollock 1975; Budde 1983; Simpson et al. 1983/84; Webster and Barrell 1985). However, little attention has been devoted to the closely related fallow deer (*Dama dama*).

This species is bred on Polish farms the most often (FEDFA 2018); therefore, such investigations may be very useful to the breeders. Furthermore, there are not any literature data available on the effect of prolonged daylight on the haematological indices. The influence of many intra- and extra-systemic factors may lead to the disruption of the homeostatic balance in the organism, which can be reflected in distinct changes in the values of the individual blood components. In such situations, the haematological indices facilitate the immediate detection of potential anomalies or changes emerging in the organism, which can cause dangerous diseases in the future if unnoticed at the proper

time (Neumeister et al. 2001). Blood is a very sensitive indicator of the metabolic changes in both the physiological and pathological status of animals (Weiss and Wardrop 2010).

Besides the day's length, a strong effect is exerted by the appropriate nutrition and wintering conditions on the deer (Janiszewski et al. 2008). Protein is the most important component of animal tissues and a continuous supply thereof is required throughout the life (Huapeng et al. 1997). Growing deer require 14–20% of protein, with buck fawns requiring slightly more than doe fawns (Ullrey et al. 1967). Growing antlers consist almost entirely of protein (collagen) and typically comprise 35–45% of protein once they harden or “mineralise”. When the antlers are growing, bucks require a diet with 13–16% of protein for the optimum development, along with the other required nutrients. On the other hand, only 6–10% of protein is required for the maintenance of adult deer with grown antlers (Brown 1996; Richardson et al. 2008).

The research hypothesis is that artificially extended daylight and various amounts of the total protein in the diet may affect several haematological indicators and do not adversely influence animal welfare.

The aim of the study was to indicate which haematological variables are affected by the changing conditions of fallow deer farming.

MATERIAL AND METHODS

Experimental design. The research was carried out at the Research Station of the Institute of Parasitology, the 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 fallow deer stags aged 3–6 years divided on an analogous basis into three equal groups ($n = 12$). The three animal groups received different nutritional regimes and were kept at different daily photoperiods in the winter months (from December, 2016 to the end of March, 2017):

Group 1 – 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,

Table 1. Composition of the diet supplement

Premix composition	Components	Group 1, 2, 3
		Content (in 1 kg)
Macronutrients (%)	Calcium (Ca)	21
	Phosphorus (P)	3
	Sodium (Na)	10
	Magnesium (Mg)	4
	Manganese (Mn)	3000
Micronutrients (mg)	Zinc (Zn)	6000
	Iron (Fe)	4000
	Copper (Cu)	1000
	Cobalt (Co)	25
	Iodine (I)	100
	Selenium (Se)	25
	Vitamins	A (IU)
D3 (IU)		100 000
E (mg)		1000
B1 (mg)		4000

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 (Cargill, Polska). The supplement constituted 2.5% of the standard farm nutrition – 15 g. The animals of Group 1 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 daylight length.

Group 2 – the nutrition was identical as in Group 1, but Group 2 was subjected to changed photoperiod conditions, i.e., the day's length was artificially prolonged in relation to the natural conditions. The animals of Group 2 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). The light intensity in the shelter and outside was measured using an Abatronic AB-8809A luxometer.

In accordance with the adopted assumptions, the day's length was extended by the illumination of the shelter from December, 2016 to the end of

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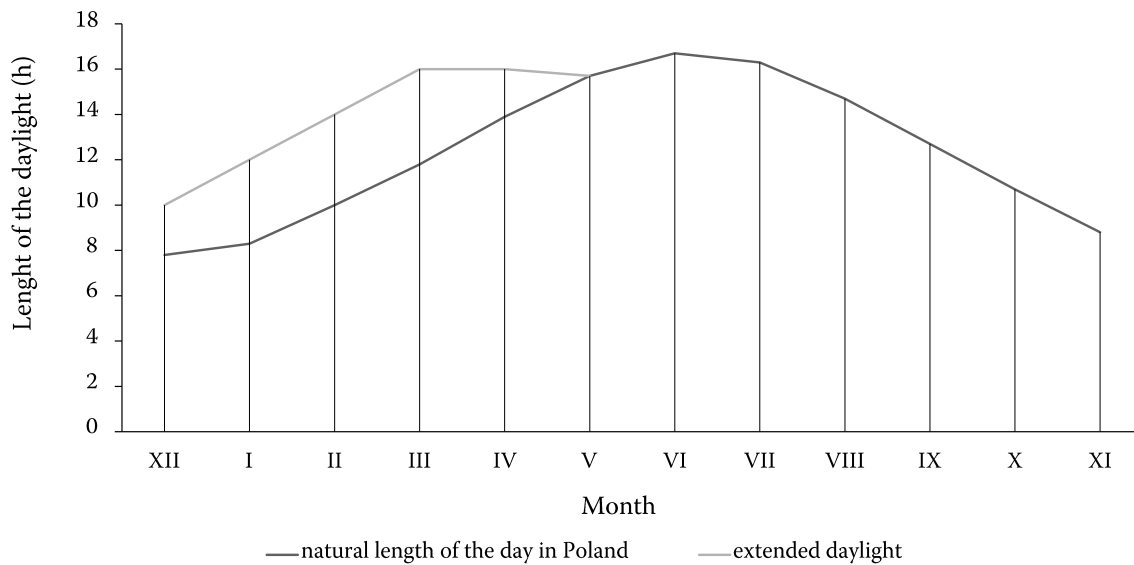


Figure 1. Comparison of the natural day length in Poland and the photoperiod regime in Group 2

March, 2017. 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 is presented in Figure 1.

Group 3 (control) – 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 (Cargill, Polska). The supplement constituted of 2.5% of the standard farm nutrition – 15 g. Group 3 was kept on a run throughout the day and exposed to the natural daylight length.

Sampling. Blood samples were collected while the fallow deer were standing inside a small handling box (2 m × 2 m × 0.6 m) with no need of sedation. The samples were taken from the *vena jugularis* externa always at the same time (from 1 to 3 hours after dawn) to avoid variations associated with circadian rhythms. For the haematological analyses, 5-ml blood samples were collected into vacuum tubes containing an anticoagulant agent (EDTA). The samples were chilled (4–8 °C) within 15 min after collection. The haematological analysis was carried out within 2 or 3 h after extraction with the use of an automated veterinary haematological analyser Exigo BM800 (Boule Medical AB, Stockholm, Sweden). The device was calibrated each time before the analysis of the samples. The following indicators were determined in the blood: the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH), the mean corpuscular haemoglobin concentration (MCHC),

the red blood cell distribution width coefficient of variation (RDW-CV), the platelets (PLT), the mean platelet volume (MPV), the platelet distribution width (PDW), the *platelet large cell ratio* (P-LCR), and the plateletcrit (PCT). The selected haematological variables were determined in two terms: before (December, 2016) and after the winter (April, 2017).

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

The normality of the distribution of the variables in the analysed groups was verified using the Shapiro-Wilk test. The differences between the two measurement terms were assessed using the Student's *t*-test for the dependent samples and by the Wilcoxon test with the paired-samples when the conditions for application of the former test were not met. The differences between the three experimental groups were evaluated with ANOVA (the analysis of variance) and the post-hoc RIR Tukey test. When the conditions for the application of the former analysis were not fulfilled, the Kruskal-Wallis test was used. The significance level (probability) of $P < 0.05$ 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).

Table 2. Comparison of the selected haematological indicators of the farmed fallow deer before and after the winter period

Haematological indicators	Group 1				Group 2				Group 3					
	before wintering		after wintering		before wintering		after wintering		before wintering		after wintering			
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD		
MCV (fl)	40.8	2.7	38.8	2.1	42.5	2.5	39.2	2.1	41.1	0.99	38.6	1.2	10.8 ^a	< 0.05 [*]
MCH (pg)	14.9	0.62	14.8	0.44	15.1	0.67	14.9	0.44	14.8	0.39	14.9	0.28	-1.3 ^a	0.21
MCHC (g/l)	364	11.9	382	13.9	356	0.02 [*]	383	13.3	359	9.2	386	12.7	-70.9 ^a	< 0.05 [*]
RDW-CV (%)	28.3	1.9	27.8	2.2	27.1	1.5	26.8	1.6	28.1	1.3	28.2	0.89	-0.05 ^a	0.96
PLT (10 ³ /ul)	359	135	318	64.2	403	0.21	355	127	403	55.8	360	53.5	2.9 ^a	0.01 [*]
MPV (fl)	6.2	0.4	6.3	0.29	6.1	0.226	6.2	0.27	6.3	0.2	6.3	0.23	0.00 ^a	1.0
PDW (fl)	7.1	0.81	7.2	0.66	7.1	0.59	7.3	0.89	7.6	0.73	7.7	1.2	-0.49 ^a	0.63
P-LCR (%)	3.7	2.2	3.7	0.88	3.5	0.77	3.81	0.65	4.2	1.2	4.2	1.4	-0.06 ^a	0.95
PCT (%)	0.24	0.05	0.24	0.04	0.26	0.05	0.24	0.05	0.25	0.04	0.25	0.03	0.57 ^a	0.58

M = mean; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; MVP = mean platelet volume; P-LCR = platelet large cell ratio; PCT = plateletcrit; PDW = platelet distribution width; PLT = platelets; RDW-CV = red blood cell distribution width coefficient of variation; SD = standard deviation

^at Student's t-test; ^bZ Mann-Whitney test; *P < 0.05 the values of probability according to which the results are statistically significant

RESULTS

The haematological indices were analysed statistically before and after the winter period in 2017. The data demonstrate a significant decline in the MCV in all the analysed groups. The greatest decrease in the MCV value was noted in Group 2, from 42.5 fl to 39.2 fl. The value was lower by 2.0 fl in Group 1 (from 40.8 fl to 38.8 fl) and by 2.5 fl in Group 3 (from 41.1 fl to 38.6 fl) (P < 0.05). In turn, there was a significant increase in the MCHC in all the groups: from 364 g/l to 382 g/l in Group 1, from 356 g/l to 383 g/l in Group 2, and from 359 g/l to 386 g/l in Group 3 (P < 0.05). Only Group 3 was characterised by a significant reduction in the PLT count from 403 × 10³/ul to 360 × 10³/ul (P = 0.01). In Group 2, exposed to the prolonged length of day, there was a significant increase in the PDW (from 7.0 fl to 7.3fl, P < 0.05) and the P-LCR (from 3.5% to 3.8%, P < 0.05) (Table 2). The MCH was characterised by very small changes in all the groups. The RDW-CV slightly decreased in Groups 1 and 2 after the wintering period. The other indices (MPV, PDW, P-LCR, PCT) did not change in any of the groups (Table 2). Moreover, after the wintering period, a statistically significant difference was noted in the MCV (F = 4.3, P = 0.02) between Groups 1 and 2. On average, the MCV value declined by 2.0 fl in Group 1 and by 3.4 fl in Group 2 (Table 3).

DISCUSSION

This study conducted on farmed fallow deer demonstrated a decrease in the MCV value after the winter period. This result was lower than the values presented in a study of Persian fallow deer (Mohri et al. 2000) and data described by Cross et al. (1994) and similar (especially after the winter) to the values presented by Rosef et al. (2004). The values obtained before the wintering period were in the range specified by Baric Rafaj et al. (2011). The level of the MCH in the present study was lower than the data provided by Mohri et al. (2000) and Cross et al. (1994), consistent with the range described by Baric Rafaj et al. (2011), and higher than in Chapple et al. (1991) and Gupta et al. (2007). Zomborszky et al. (1997) also showed a significantly lower MCV in fawns than in adult farmed fallow deer in the winter period.

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Table 3. Statistical analysis of the magnitude of the changes in the haematological indicators in the fallow deer before and after the winter period

Haematological indicators	Group 1		Group 2		Group 3		F^a/H^b	P	Intergroup differences
	M	SD	M	SD	M	SD			
MCV (fl)	-2.1	0.94	-3.4	1.58	-2.5	0.79	4.3 ^a	0.02*	I-II
MCH (pg)	-0.01	0.37	-0.15	0.56	0.13	0.35	1.3 ^a	0.29	-
MCHC (g/l)	18.2	9.9	27.0	10.2	26.7	13.1	24.4 ^a	0.10	-
RDW-CV (%)	-0.53	1.79	-0.33	2.3	0.02	1.2	0.27 ^a	0.76	-
PLT (10 ³ /ul)	-40.9	106	-48.1	135	-42.8	50.8	0.02 ^b	0.99	-
MPV (fl)	0.10	0.57	0.12	0.36	0.00	0.29	0.26 ^a	0.77	-
PDW (fl)	0.13	1.09	0.28	1.11	0.16	1.1	0.48 ^b	0.79	-
P-LCR (%)	-0.03	2.5	0.33	0.91	0.03	1.4	1.38 ^b	0.51	-
PCT (%)	-0.01	0.06	-0.02	0.07	-0.01	0.04	0.35 ^b	0.84	-

M = mean; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; MVP = mean platelet volume; P-LCR = platelet large cell ratio; PCT = plateletcrit; PDW = platelet distribution width; PLT = platelets; RDW-CV = red blood cell distribution width coefficient of variation; SD = standard deviation

^aANOVA (the analysis of variance); ^bKruskal-Wallis test; * $P < 0.05$ the values of probability according to which the results are statistically significant

The MCHC value was lower than in the investigations of Persian fallow deer (Mohri et al. 2000), higher than the data presented by Cross et al. (1994) and Gupta et al. (2007), and similar (especially after the winter season) to the values reported by Rosef et al. (2004). It was in the range described by Baric Rafaj et al. (2011) before the wintering period and slightly higher after the winter period.

The MCHC increased in all the groups after the winter period. It is known that the red blood cell markers (MCV and MCHC) are mainly used to determine the type of anaemia (Begemann 1985; Neumfister et al. 2001; Weiss and Wardrop 2010). Szczeklik (2005) demonstrated that the MCHC increases in certain hereditary defects of the erythrocyte structure and more often accompanies severe prolonged dehydration of the organism in humans. This phenomenon was most likely caused by the intake of winter feed, which contains less water in its composition than the natural food of cervids, i.e., herbaceous plants or shoots of trees and shrubs. However, the fallow deer in the experiment had *ad libitum* water access. The change in feeding from winter to spring is related to the period of adaptation of the rumen microflora and microfauna, as in domesticated ruminants. This period is frequently associated with diarrhoea, which may cause changes in the haematological profile (Kovac et al. 1997). However, the red blood cells of red deer and fallow

deer utilise some nutrients more efficiently than other species of ruminants (Agar and Godwin 1992).

The mean PLT count was similar to that reported by Mohri et al. (2000), higher than that demonstrated by Cross et al. (1994) and Rosef et al. (2004), and lower than in the investigations conducted by Gaspar-Lopez et al. (2011). It was also in the range reported by Baric Rafaj et al. (2011). The MPV and PCT values were consistent with those presented by Baric Rafaj et al. (2011). These seasonal changes in the haematological indices may have been related to the changes in the diet, as evidenced in the study conducted by DelGiudice et al. (1992).

The PLT count was reduced in the animals bred in the least favourable conditions (Group 3); however, the platelet indices were not altered, which indicates the absence of infection. Although the platelets count may be altered in viral and parasitic infections, they cannot be identified as indicators of an infection, especially in the case of a healthy deer population.

Group 2 exposed to the prolonged daylight exhibited a significant increase in the PDW and P-LCR after the winter period, although the mean PLT count did not increase and the MPV and PCT did not undergo significant changes. The animals in this group developed antlers three weeks earlier, which was caused by the extension of the day's length resulting in changes in their hormonal me-

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tabolism (Bubenik et al. 1979; Bubenik et al. 2005; Bubenik 2006), possibly influencing the haematological parameters mentioned above.

Platelets contribute to angiogenesis as well as tissue and cell remodelling. Platelets release > 300 proteins and small molecules from their granules (chemokines, cytokines like interleukin-1 β , CD40 ligands, β -thromboglobulin, growth factors, etc.), which can influence the function of the vascular wall and circulating immune cells (Budak et al. 2016). This may explain the significant increase in the PDW and P-LCR in Group 2, in which the animals developed antlers three weeks earlier. However, in the description of the platelet indices, it should be mentioned that the current insufficient harmonisation should be regarded as a serious limitation for the comparability of the platelet indices obtained with different haematological analysers, even in human medicine (Budak et al. 2016).

The obtained results confirm the assumed research hypothesis. The day's length (in Group 2) had a negative effect on the MCV, but a positive impact on the PDW and P-LCR. In the group supplemented with the lower protein (Group 3) amount in the forage, the seasonal change causes a lower MCV, a higher MCHC, and a decrease in the total platelet count in the circulation. Artificially extended daylight and proper nutrition during wintering have a beneficial effect on the health and fitness of farmed fallow deer. Also, it is very important in practice and for the animal's welfare that the animals have constant access to drinking water during this period.

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