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Influence of dietary antioxidant supplement to blue fox diet (*Alopes lagopus*) on chosen blood hematological parameters

Wpływ dodatku przeciwutleniacza do karmy lisów polarnych (*Alopex lagopus*) na wybrane wskaźniki hematologiczne krwi

Summary. The studies carried out during the monitoring stage covered adult female blue foxes from the A, B, C farms. The second stage of the experiment included the animals from farm C. The adult females with offspring at an equal sex ratio constituted the experimental and control groups. The carnivorous fur animals received an antioxidant supplemented-diet to protect its components. The effect of dietary antioxidant additive to the blue fox (*Alopex lagopus*) feed on the chosen blood hematological indices (RBC, HGB, PCV, WBC, PLT, MCV, MCH, MCHC) was established.

The dietary antioxidant dose employed (Rendox) did not negatively influence the animal health status. This supplement did not induce any significant changes in the blood hematological indices in female blue foxes and their offspring.

Key words: blue fox, antioxidant, hematological indices

INTRODUCTION

Nutrition plays a key role in the health, well being, proper functioning and overall productivity of animals. Deficiency of basic dietary nutrients, their poor quality or inappropriate balance cause substantial breeding-related losses, especially in fur animals. Therefore, good nutrition management of animals includes not only a collection of good quality feedstuffs but proper storage conditions as well as sufficient feed protection against disadvantageous environmental factors. For these reasons, animal diets are supplemented by some natural substances and synthetic ones – antioxidants [Sławoń *et al*. 2000] that reduce the formation of oxidizing free radicals [Maddock *et al*. 2003]. Re-

search on fur animal rearing issues aims to set the maximum daily intake of antioxidants accepted as feed additives [Bartosz 2004, Bis-Wencel *et al*. 2006]. Besides, the comprehensive hematological, biochemical studies including oxidative state markers are conducted to characterize the antioxidant impact on the organism.

The objective of the research was to determine the influence of a chosen antioxidant supplement to the Arctic fox (*Alopex lagopus*) diet on some blood hematological parameters.

MATERIAL AND METHODS

The studies were conducted in the years 2004 and 2005. They comprised II research stages. In stage I, monitoring included three breeding farms of Arctic fox (*Alopex lagpous*) located in three provinces: Wielkopolskie (farm A), Pomorskie (farm B) and Podkarpackie (farm C). Throughout the study period, the basic herds at these farms amounted to 400, 200 and 100 foxes respectively with a similar management system. The animals were fed the same diet (Tab. 1) during the research period.

Twenty polar fox females were selected for the monitoring study at each object. At this investigation stage, blood from vena saphena was collected four times, i.e. at each breeding season.

The full blood samples were examined to determine the count of red blood cells (RBC), white blood cells (WBC), hemoglobin concentration (HGB), hematocrit value (PCV), mean corpuscular values (MCV, MCH, MCHC) and blood platelets (PLT). The morphological analyses were performed using a hematology analyzer MS 9 Melet Schlosing.

On the grounds of the obtained research results, farm C was selected for the further investigation (II research stage). The study covered adult Arctic fox females and their offspring. The experimental group (group D) comprised adult dams (20 animals) and their offspring at an equal sex ratio (10 animals each), whereas the control group (group K) consisted of adult females (20 animals) and their offspring at an equal sex ratio (10 animals each).

The animals from groups K and D (both adults and juveniles) were fed the diet of the same composition and energy level (Tab. 1). Female feed ration size at the premating period was established individually, subject to animal body condition. To enrich the diet composition with minerals and vitamins so that animal developmental stage needs could be met, vitamin-mineral premix Guyofox with an abundant supply of vitamin E, B1 and Fe was added to feedstuffs. During the study period in both treatment groups preservative sodium pyrosulfite (E2223) was applied in an amount making up 0.2–0.3% per ton of ready feed in the spring-summer season and 0.1–0.15% in autumn-winter period. The animals from the experimental group (group D) obtained the preservative-supplemented feed during the research period (just like group K) with additional antioxidant content – Rendox at a dose of 200–250 ml per tone of meat-poultry feedstuff. The animals from both groups were fed ad libitum and had free access to water. Throughout the investigation period, the animals were provided with routine zootechnical-veterinary care, their health state checked through clinical examinations, regular immunization and disinfestations procedures.

Table 1. Nutritional value of dietary units for blue foxes standardized for all the objects under study
Tabela 1. Wartość pokarmowa dawki żywieniowej lisów polarnych ujednolicona dla wszystkich badanych obiektów

Feed Pasza	Raw material % in dose Udział surowca w dawce, %			
	1 XII–1 V	2 V–15 VII	16 VII–30 IX	1 X – slaughter 1 X – do uboju
Plaice, cod – 10% (post filleting offals) Flądra, dorsz – 10% (odpadki pofiletowe)	47	30	5	55
Greaves Skwarki	5	5	-	-
Poultry offals mixed Odpady drobiowe mieszane	13	30	55	-
Soybean-fish meal or meat-bone meal (50%) Mączka sojowo-rybna lub mięsno-kostna (50%)	6	5	10	11
Animal fat Tłuszcz zwierzęcy	-	-	2	4
Cereals (wheat-dry ground grain) Zboże (pszenica – śruta sucha)	10	11	12	13
Wheat bran Otręby pszenne	1	1	1	1
Water Woda	18	18	15	16
EM kcal/kg EM kcal/kg	1220	1360	1700	1830
EM kcal/kg % in dose of Procent udziału EM kcal/kg w dawce – protein, białka – fat, tłuszczu – carbohydrates, węglowodanów	 50.0 33.4 16.6	 43.0 40.8 16.2	 31.0 54.6 14.4	 28.2 57.3 14.4

In research stage II, blood was collected from the foot vein (vena saphena) at the following periods:

Adult females (group K, D) – 4 times each breeding season

Juvenile foxes (group K, D) – 3 times from weaning to slaughter

The hematological parameter determinations were performed just like in research stage I.

The obtained research results were analyzed statistically, calculating the arithmetic means ($\bar{x}$) and standard deviation (SD). Statistical significance for the investigated parameters were calculated using variance analysis for double and triple cross classification at weight restrictions. There were assumed two significances levels, i.e. $P \leq 0.01$ and $P \leq 0.05$; the numbers denoted with the same letters differed in a statistically significant way.

RESULTS AND DISCUSSION

Blood morphological composition is a species-characteristic feature that may vary within a species subject to the metabolism rate. It is related to animal utilization way, performance, nutritional conditions and management [Stankiewicz 1973, Winnicka 2004].

Average values of morphological parameters determined in Arctic fox females at monitoring research stage I are presented in Table 2.

Red blood cell count established in the animals from all the objects was similar and ranged within 9.55-9.87×10^{12} (Tab. 2). The data were shown to exceed the values reported by Winnicka [2004], Berestov *et al*. [1989], Meyer and Harvey [1998] but congruent with those presented by Gliński and Kostro [2002].

The mean count of white blood cells (WBC) in females from farms A, B, C was similar (Tab. 2), in accord with those mentioned by Winnicka [2004], Meyer and Harvey [1998], Gliński and Kostro [2002]. The most alike WBC data was recorded in the dams from farm C. In Canidae, a phenomenon called digestive leukocytosis occurs approximately an hour after eating [Janicki 2001, Winnicka 2004], but this correlation was ruled out in the present study as the research material for analyses was collected before animal feeding.

The levels of hemoglobin and hematocrit in all the objects under investigation appeared as very stable (Tab. 2) and agreeing with the values reported by Winnicka [2004], Gliński and Kostro [2002]. As for hemoglobin, the data presented by Berestov *et al*. [1989] was exceeded slightly those in the present research, while the hematocrit values were assessed as most stable in the females from farm C.

The indirect blood indices also defined as blood cell parameters, i.e. mean corpuscular values (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) aid in determination of anemia type [Winnicka 2004].

In the present investigation, the mean corpuscular value (MCV)in the foxes from all the monitored farms maintained below the values reported by Winnicka [2004], Meyer and Harvey [1998] (Tab. 2). The analyzed material collected from the animals from each farm showed slight variation of the MCV level but the most stabile data was recorded for the farm C vixens. The mean corpuscular hemoglobin (MCH) differed significantly in all the individuals (Tab. 2) and the resulting values surpassed the reference ones presented by Berestov *et al*. [1989], Meyer and Harvey [1998] but were congruent with the norms given by Winnicka [2004]. The mean corpuscular hemoglobin concentration (MCHC) was the lowest in the animals at farm C (33.58 g/dl) and differed significantly from the data obtained from the females from farm A (34.49 g/dl) (Tab. 2). The most stable MCHC values were recorded for the individuals at farm B as all the resulting values were found within the reference value range reported by Winnicka [2004].

Mean count of blood platelets (PLT) in the vixens from all the research objects ranged from 504.41×10^9/l (farm A) up to 536.35×10^9/l (farm B) (Tab. 2). All the values were in accord with the reference values mentioned by Winnicka [2004]. However, the mean platelet count was based on the data provided by analyses on animals from farm C (527.82×10^9/l).

Table 2. Means of hematological parameters in female blue foxes at experimental stage I
Tabela 2. Średnie wartości parametrów hematologicznych samic lisów polarnych w I etapie badań

Parameter Wskaźnik	Farm A ferma A		Farm B ferma B		Farm C ferma C	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Red blood cell count (RBC), 10^{12}/l Liczba czerwonych krwinek	9.55	0.26	9.60	0.19	9.87	0.10
White blood cell count (WBC), 10^{9}/l Liczba białych krwinek	9.28	1.04	9.26	0.64	9.12	1.03
Hemoglobin level (HGB), mmol/l Poziom hemoglobiny	11.20	0.17	11.28	0.56	11.02	0.44
Hematocrit (HCT), l/l Hematokryt	0.53	0.01	0.52	0.02	0.53	0.00
Mean corpuscular volume (MCV), fl Średnia objętość krwinki czerwonej	54.77	1.75	54.21	1.34	55.18	0.58
Mean corpuscular hemoglobin (MCH), pg Średnia masa hemoglobiny	19.06**A**	0.30	18.93**A**	0.40	20.21**A**	3.74
Mean corpuscular hemoglobin concentration (MCHC), g/dl Średnie stężenie hemoglobiny w krwince czerwonej	34.49**a**	0.55	34.19	0.17	33.58**a**	0.86
Platelet count (PLT), 10^{9}/l Liczba płytek krwi	504.41	40.13	536.35	15.40	527.82	43.96

$\bar{x}$ – arithmetic mean, średnia
SD – standard deviation, odchylenie standardowe
A – differ significantly at p p ≤ 0.01, różnią się istotnie statystycznie przy p ≤ 0,01
a – differ significantly at p p ≤ 0.05, różnią się istotnie statystycznie przy p ≤ 0,05

The results of hematological evaluation of Arctic fox females at research stage II are summarized in Table 3.

The mean red blood cell count in group K amounted to 10.37×10^{12}/l, while in group D – 9.58×10^{12}/l. The data exceeded the reference values presented by Winnicka [2004], Berestov *et al*. [1989], Meyer and Harvey [1998]. The resulting research data from the control group surpassed the values determined for dogs by Czubek [2002].

White blood cell count in the control group (8.92×10^{9}/l) and experimental group (7.29×10^{9}/l) differed significantly at P ≤ 0.05 (Tab. 3). All the values were congruent with those given by Winnicka [2004], Meyer and Harvey [1998]. The results obtained for the foxes in the present study were similar to those established for dogs [Czubek 2002] and foxes in the first experimental year Nowakowicz-Dębek [2006].

Hemoglobin levels in the vixens from both groups were very close to each other (Tab. 3). The values exceeded the concentration rate reported by Berestov *et al*. [1989] but remained near the upper limits of the reference data mentioned by Winnicka [2004]. Lower values of this parameter for adult polar foxes but of similar fluctuations in the annual cycle were reported by Nowakowicz-Dębek [2006], whereas the similar ones by Czubek [2002] for dogs.

Table 3. Means of hematological parameters in female blue foxes at experimental stage II
Tabela 3. Średnie wartości parametrów hematologicznych samic lisów polarnych w II etapie badań

Parameter Wskaźnik	Group K Grupa K		Group D Grupa D	
	$\bar{x}$	SD	$\bar{x}$	SD
Red blood cell count (RBC), 10^{12}/l Liczba czerwonych krwinek	10.37	0.56	9.58	0.62
White blood cell count (WBC), 10^{9}/l Liczba białych krwinek	8.92**a**	1.62	7.29**a**	2.24
Hemoglobin level (HGB), mmol/l Poziom hemoglobiny	11.17	0.47	11.14	0.65
Hematocrit (HCT), l/l Hematokryt	0.54	0.04	0.54	0.03
Mean corpuscular volume (MCV), fl Średnia objętość krwinki czerwonej	51.89**b**	1.64	53.27**b**	1.70
Mean corpuscular hemoglobin (MCH), pg Średnia masa hemoglobiny	17.91	0.86	18.00	0.65
Mean corpuscular hemoglobin concentration (MCHC), g/dl Średnie stężenie hemoglobiny w krwince czerwonej	33.66	1.57	33.78	2.29
Platelet count (PLT), 10^{9}/l Liczba płytek krwi	530.89	39.91	462.96	110.80

a, b – differ significantly at $p \leq 0.05$
a, b – różnią się istotnie statystycznie przy $p \leq 0{,}05$

Hematocrit value for the vixens from group K and D (Tab. 3) remained near the top limits presented by Winnicka [2004]. In each blood collection, there were observed characteristic fluctuations of hematocrit value related to hemoglobin concentration. Similar distribution of this parameter in the annual cycle, yet of lower values, was noted in the control group by Nowakowicz-Dębek [2006].

Out of blood cell parameters, the statistically significant differences at $p \leq 0.05$ were determined only for mean corpuscular value (MCV) (Tab. 3). The mean values were found within the ranges of values presented by Winnicka [2004], Meyer and Harvey [1998].

Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) appeared to agree with the values mentioned by Winnicka [2004] and Berestov *et al.* [1989]. The indirect blood cell parameters obtained by Nowakowicz-Dębek [2006] did not differ markedly from the present research results.

Mean blood platelet count was higher in the control group (Tab. 3) and the resulting data was found within the broad range of values presented by Winnicka [2004]. A similar mean level of blood platelets recorded in the present experiment in the animals from the experimental group was obtained by Nowakowicz-Dębek in the control group [2006].

The results of hematological analyses in the II stage of research series including the juvenile Arctic foxes are presented in Table 4.

Red blood cell count in the juveniles in both studied groups had similar values, i.e. K – 10.16–10.85×10^{12}/l and D – 8.97–10.55×10^{12}/l (Tab. 4). These values surpassed the reference ones for juvenile polar foxes according to Berestov *et al*. [1989] but were consistent with the values mentioned by Gliński and Kostro [2002].

Table 4. Means of hematological parameters in young blue foxes at experimental stage II
Tabela 4. Średnie wartości parametrów hematologicznych młodych lisów polarnych w II etapie badań

Parameter Wskaźnik	Group K Grupa K				Group D Grupa D			
	female samice		male samce		female samice		male samce	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Red blood cell count (RBC) 10^{12}/l Liczba czerwonych krwinek	**10.85**	1.25	**10.16**	0.49	**10.55**	1.09	**9.97**	1.05
White blood cell count (WBC), 10^9/l Liczba białych krwinek	**8.26A**	0.21	**7.22A**	0.26	**7.61B**	0.18	**8.77B**	1.11
Hemoglobin level (HGB), mmol/l Poziom hemoglobiny	**11.23**	0.77	**11.28**	0.34	**11.06**	0.72	**10.75**	0.90
Hematocrit (HCT), l/l Hematokryt	**0.55**	0.06	**0.54**	0.03	**0.53**	0.04	**0.52**	0.06
Mean corpuscular volume (MCV), fl Średnia objętość krwinki czerwonej	**5.,60**	0.98	**55.06**	0.94	**54.84**	0.68	**55.57**	0.13
Mean corpuscular hemoglobin (MCH), pg Średnia masa hemoglobiny	**17.97**	0.31	**18.29**	0.22	**17.88**	0.16	**17.91**	0.47
Mean corpuscular hemoglobin concentration (MCHC), g/dl Średnie stężenie hemoglobiny w krwince czerwonej	**32.73**	0.94	**32.71**	0.40	**32.73**	0.50	**32.29**	0.93
Platelet count (PLT), 10^9/l Liczba płytek krwi	**609.00AB**	71.37	**570.87AC**	42.85	**524.03CD**	45.75	**616.87BD**	43.94

A, B – differ significantly at $p \leq 0.01$
A, B – różnią się istotnie statystycznie przy $p \leq 0{,}01$

The studies conducted by Lorek *et al*. [2000] with a concentrate mixture inclusion (feed produced from rye, soybean meals and animal meals) to the foxes`diet revealed that the experimental factor changed feed ration proportions but did not induce any significant changes in the hematological parameters. According to the Authors, a rise of RBC, MCV, MCH, MCHC and HCT value determined in IV nutritional period gave evidence of the proper course of erythropoesis process and the hemopoietic system efficiency, which has been confirmed in this research to some degree.

The number of leukocytes in young organisms in the groups under study was differentiated (Tab. 4). There were noted statistically significant differences between sexes in groups K and D at $P \leq 0.01$.The obtained WBC values averaged between $3.8–11.14 \times 10^9$/l as defined by Gliński and Kostro [2002].

Hemoglobin concentration in both treatment groups remained at a similar level (Tab. 4). The results exceeded those reported by Berestov *et al*. [1989] for juvenile foxes; however, were congruent with the values presented by Gliński and Kostro [2002].

Hematocrit value in young foxes from the control group somewhat surpassed the reference values mentioned by Gliński and Kostro [2002] (Tab. 4). The consecutive

blood collections showed a slight increase of this parameter in both groups – control and experimental ones.

Mean values of blood cell parameters in young foxes are comprised in Table 4. Mean values of MCV, MCH and MCHC in both studied groups were relatively the same. Mean corpuscular value (MCV) in the juveniles was shown to increase in the consecutive samplings in all the groups.

The number of blood platelets in both experimental groups differed significantly at $P \leq 0.01$ within sex (Tab. 4). The hematological analysis in the present studies slightly exceeds, in both females and males, the count of RBC, PLT, the levels of HGB, HCT, MCH compared to the values obtained by Nowakowicz-Dębek [2006]. For the other parameters, the resulting values were lower.

CONCLUSIONS

1. Monitoring the metabolism of the animals from farm A, B, C revealed the most similar blood hematological parameters in the foxes from the object C.

2. Antioxidant supplement did not significantly change the hematological parameters in polar fox females and their offspring.

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Streszczenie. Badania przeprowadzone w etapie monitoringowym objęły dorosłe samice lisów polarnych z ferm A, B, C. W etapie II do doświadczenia wytypowano zwierzęta z fermy C. Grupę doświadczalną i kontrolną stanowiły samice dorosłe i ich potomstwo z równym udziałem obu płci. Do karmy podawanej mięsożernym zwierzętom futerkowym wprowadzono syntetyczny przeciwutleniacz, by zabezpieczyć jej komponenty. Określono wpływ dodatku wybranego przeciwutleniacza do karmy lisów polarnych (*Alopex lagopus*) na wybrane wskaźniki hematologiczne krwi (RBC, WBC, HGB, PCV, MCV, MCH, MCHC, PLT).

Zastosowana dawka przeciwutleniacza (Rendox) wpłynęła niekorzystnie na stan zdrowia zwierząt. Dodatek przeciwutleniacza nie wpłynął istotnie na zmianę wskaźników hematologicznych u samic lisów polarnych oraz ich potomstwa.

Słowa kluczowe: lisy polarne, przeciwutleniacz, wskaźniki hematologiczne