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Effect of synthetic antioxidant on biochemical indices in blood of arctic fox (*Alopex lagopus*)

Wpływ syntetycznego przeciwutleniacza na wskaźniki biochemiczne krwi lisów polarnych (*Alopex lagopus*)

Summary. The investigations conducted during the monitoring stage included adult female blue foxes from the A, B, C farms, while II experimental stage covered the animals from farm C. The experimental and control groups were made up by adult females with their offspring at an equal sex ratio. The diet supplied to carnivorous fur animals was supplemented with a synthetic antioxidant (Rendox^{TM} Liquid) in order to protect feed components. There was determined the effect of that antioxidant additive to blue fox (*Alopex lagopus*) diet on some blood biochemical indices (AST, ALT, ALP, LDH and content of cholesterol, urea, creatinine, glucose, bilirubin, uric acid – some of them being recognized as non-enzymatic defense mechanisms and scavengers of reactive oxygen species).

The antioxidant dose applied to animal feed did not have any disadvantageous effect on the animal health state. The female blue foxes and their offspring fed the feed supplemented with the antioxidant did not demonstrate any significant changes in the blood biochemical indices.

Key words: blue fox, antioxidant, biochemical parameter

INTRODUCTION

The latest nutritional trends, including animal welfare issues, have aimed at animal performance improvement through modeling of the digestive processes. These efforts focus on the enhancement of nutrient utilization and eradication of pathogenic agents. The fur animal nutrition is based on rejected slaughtered animals, mainly unfit for human consumption portions of beef, porcine and equine carcasses, fish, poultry and slaughter offals. It is undoubtedly the simplest and least expensive method for animal by-products feed disposal. However, numerous concerns have been expressed over the specific nature of such a nutritional strategy. The major course for feedstuff quality dete-

rioration due to lipid metabolism proves to be oxidation process [Lorek and Gugołek 2001, Bartosz 2004], which not only promotes feed spoilage but animal tissue damage as well. Limiting the oxidation process can be obtained through efficient use of preparations called antioxidants. The preparations implemented commonly in farm management practices include Rendox, Endox, Loxidan and vitamin E as well as selenium – a natural antioxidant [Lorek and Gugołek 2001, Kopczewski *et al*. 2002]. It is worth of notice that antioxidants as substances of high chemical activity do affect the health condition of animals fed an antioxidant supplemented diet.

The objective of the present study was to determine the influence of a dietary synthetic antioxidant additive to Arctic fox (*Alopex lagopus*) feed on some blood biochemical indices.

MATERIAL AND METHODS

The studies were conducted in the years 2004 and 2005. They comprised two stages. In I research stage, monitoring covered three breeding farms of Arctic foxes (*Alopex lagopus*) located in the following provinces: Wielkopolskie (farm A), Pomorskie (farm B) and Podkarpackie (farm C). During the research period, the basic herds of 400, 200 and 100 foxes, respectively were housed in the farms, with a similar management system.

For research purposes, the animal nutrition was uniform (Tab. 1). From each experimental object, 20 polar fox females were selected to undergo the monitoring evaluation. At this research stage, blood from the small saphenous vein (vena saphena parva) was taken four times (each breeding season).

Blood serum was analyzed to establish the following metabolic profile parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) and the levels of cholesterol, urea, creatinine, glucose, bilirubin, uric acid. Some of these indices are recognized as non-enzymatic defensive mechanisms and scavengers of reactive oxygen species (ROS).

On the grounds of the study results (II stage), farm C characterized by the middle parameters was selected for the further investigations.

The experimental group (group D) comprised adult dams (20 units) and their offspring at an equal sex ratio (10 units each), while control group (K) – adult females (20 units) and their offspring at an equal sex ratio (10 units each).

The animals from groups K and D (both adult and juvenile) were fed the diet of the same composition and energy level (Tab. 1). Energy value of 1kg feed ranged from 1220 up to 1830 cal/EM, subject to animal growth and development stage, including the content of protein energy 28.2–50%, fat energy 33.4–57,3% and sugar energy 14.4–16.6%. A feed ration of females at the pre-mating season was formulated individually and it was dependent on animal body condition. To supplement feed composition with minerals and vitamins according to animal development stage needs, vitamin-mineral premix GuyoFox, rich in E, B1 vitamins and Fe was added to the diet. Throughout the research period, both experimental groups received a preservative – sodium pyrosulfite (E-223) at the rate of 0.2–0.3% per 1 ton ready feed in spring-summer season and 0.1–0.15% per 1 ton ready feed in autumn-winter period. The animals from the experimental group (group D) had the diet supplemented with a preservative (like the control)

over the research period and additionally, antioxidant Rendox^{TM} Liquid at the dose of 200–250 ml per 1 ton of meat-poultry feedstuff cold-stored. The animals from both groups investigated were fed ad libitum and had free access to water.

During the experimental period, the animals were provided with the standard zootechnical-veterinary services, health condition checked through clinical examinations, regular vaccination and disinfestation practices.

Table 1. Nutritional value of dietary units for blue foxes standardized for all 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

Blood from the small saphenous vein (vena saphena parva) was collected at the following periods:

– adult females – four times each breeding season,

– juvenile foxes – three times, from weaning to slaughter.

Blood serum was examined to determine the metabolic profile parameters. There was established the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) and concentrations of cholesterol, urea, creatinie, glucose, bilirubin and uric acid. The biochemical analyses were performed by the spectrophotometric method with Cormay monotests on Cormay Plus device.

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

RESULTS AND DISCUSSION

Enzymologic diagnostics includes indicator, excretory and secretory enzymes. Enzymes are synthetized inside cells and operate in the intracellular space. In normal plasma, a low enzyme content is detected. Their concentration is markedly affected by, among others, biosynthesis and secretory enzyme production, indicator enzyme release to blood or catabolism and excretion of enzymatic proteins in urine. A measure of enzyme content in blood serum proves to be its activity. Differentiated enzymatic activity is attributed to a change in cell membrane permeability, enhanced cell breakdown induced by pathological processes or compromised enzyme biosynthesis, especially secretory ones, due to parenchymal organ injury [Tomaszewski 2001]. Mean values of biochemical parameters of Arctic fox females reported in I research stage in three farms are summarized in Table 2.

The indicator enzymes include, among others, aminotransferses: alanine and aspartate, lactic dehydrogenase or alkaline phosphatase which is a secretory enzyme as well [Tomaszewski 2001].

The activity of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) appeared to be the lowest in the animals for farm C and was ALP – 87.35 U/l, AST – 33.45 U/l and ALT – 74.32 U/l, respectively (Tab. 2). All the ALP and AST values were within the reference range reported by Winnicka [2004], whereas the ALT activity exceeded these values. The most uniform values of the enzyme activity were noted in polar fox females in farm C.

The highest activity of lactic dehydrogenase was established in the foxes from farm C, where it reached 310.83 U/l (Tab. 2). The successive samplings showed the increase of this enzyme activity in all the monitored research objects.

Bilirubin plays a role as an antioxidant [Malinowska 1999] and being albumin-bound; it protects linoleic acid from oxidation. Glucuronate conjugated bilirubin may react with peroxide radicals and act as efficient scavenger of singlet oxygen [Asad *et al.* 2001, Dudnik and Khrapova 1998].

Table 2. Means of biochemical parameters in female blue foxes at I experimental stage
Tabela 2. Średnie wartości wskaźników biochemicznych 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
Alkaline phosphatase, (ALP) U/l Fosfataza zasadowa	95.50	16.23	96.36	10.79	87.36	2.55
Aspartate aminotransferase, (AST) U/l Aminotransferaza asparaginianowa	37.43	2.58	35.47	2.36	33.45	1.33
Alanine transferase, (ALT) U/l Aminotransferaza alaninowa	78.11	16.41	81.71	12.92	74.32	9.86
Lactate dehydrogenase, (LDH) U/l Dehydrogenaza mleczanowa	288.41	106.86	308.78	56.59	310.83	38.49
Bilirubin, µmol/l Bilirubina	5.37	0.45	5.90	2.35	6.22	1.73
Urea, mmol/l Mocznik	6.58	1.44	6.57	1.29	6.82	0.86
Uric acid, mmol/l Kwas moczowy	0.10**a**	0.02	0.11**b**	0.01	0.08**ab**	0.01
Creatinine, µmol/l Kreatynina	74.45	11.74	75.70	4.32	76.17	7.44
Cholesterol, mmol/l Cholesterol	6.20	1.46	5.82	0.09	5.47	0.59
Glucose, mmol/l Glukoza	4.45**ab**	0.84	5.05**a**	1.18	5.29**b**	0.80

$\bar{x}$ – arithmetic means, średnia
SD – standard deviation, odchylenie standardowe
a, b – differ significantly at $p \leq 0.05$, różnią się istotnie statystycznie przy $p \leq 0,05$

The foxes from farm C showed some higher bilirubin levels (Tab. 2). The analytic data of this parameter recorded in all the objects slightly exceeded the values mentioned by Meyer and Harvey [1998].

Urea, uric acid and creatinine are protein metabolites and their levels in blood reflect the status of kidney function. Creatinine content rise indicates kidney insufficiency and is proportional to a decline of glomerular filtration rate [Tomaszewski 2001].

The levels of urea and creatinine was similar in the animals from all the farms under study [Tab. 2]. All the data proved congruent with the reference values for dogs.

The fox blood analysis revealed that creatinine concentration in dams in all the farms was consistent with the values recommended by Winnicka [2004], Meyer and Harvey [1998] and was A – 74.45 µmol/l, B – 75.70 µmol/l, C – 76.17 µmol/l (Tab. 2). Analyzing the subsequent samplings, there was shown an upward tendency of this parameter in all the objects.

Uric acid, like creatinine, is regarded a low-molecular weight compound with antioxidant properties [Bartosz 2004]. The lowest contents of uric acid were recorded in the dams from farm C, the values were statistically significant at $p \leq 0.05$ (Tab. 2).

Table 3. Means of biochemical indices of female blue foxes at II experimental stage
Tabela 3. Średnie wartości wskaźników biochemicznych samic lisów polarnych w II etapie badań

Parameter Wskaźnik	Group K Grupa K		Group D Grupa D	
	$\bar{x}$	SD	$\bar{x}$	SD
Alkaline phosphatase, (ALP) U/l Fosfataza zasadowa	39.02	6.03	40.95	5.30
Aspartate aminotransferase, (AST) U/l Aminotransferaza asparaginianowa	40.38	2.97	39.93	6.08
Alanine transferase, (ALT) U/l Aminotransferaza alaninowa	57.55	14.63	59.83	23.60
Lactate dehydrogenase, (LDH) U/l Dehydrogenaza mleczanowa	332.45**a**	87.54	270.25**a**	49.09
Bilirubin, µmol/l Bilirubina	2.85	0.95	3.13	1.15
Urea, mmol/l Mocznik	6.93	1.33	6.73	1.70
Uric acid, mmol/l Kwas moczowy	0.17	0.02	0.17	0.02
Creatinine, µmol/l Kreatynina	61.29	6.86	61.46	12.94
Cholesterol, mmol/l Cholesterol	5.94	0.44	5.84	0.57
Glucose, mmol/l Glukoza	5.70	0.32	6.06	0.30

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

Cholesterol is one of numerous structural membrane components which inhibits lipid peroxidation [Bartosz 2004]. This parameter level was similar in the monitored objects (Tab. 2). Glucose concentration is reflective of the overall systemic sugar metabolism and remains under tight hormonal control. Besides, it is considered a key scavenger of reactive oxygen species [Bartosz 2004]. This index value varied significantly at $p \leq 0.05$ (Tab. 2). The obtained study levels ranged within the reference values.

The monitoring-based selection of animals for the further research stage (II) was performed. The research results of blood biochemical indices of Arctic fox females are summarized in Table 3.

The activity of alkaline phosphatase (ALP) and alanine aminotransferase (ALT) proved higher in the control group (Tab. 3). The obtained ALP results for both groups under investigation were found within the reference range given by Winnicka [2004] and Meyer and Harvey [1998]. The ALT activity in groups K and D surpassed the reference values reported by Winnicka [2004] but agreed with the indices presented by Meyer and Harvey [1998]. The present research results revealed a downward tendency of ALP in the annual cycle, contrary to that indicated by Nowakowicz-Dębek [2006]. However, the values noted in the present study in both groups were lower and thus consistent with the results obtained on dogs by Czubek [2002].

Table 4. Means of biochemical indices in young blue foxes at II experimental stage
Tabela 4. Średnie wartości wskaźników biochemicznych młodych lisów polarnych w II etapie badań

Parameter Wskaźnik	Group K Grupa K				Goup D Grupa D			
	female samice		male samce		female samice		male samce	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Alkaline phosphatase, (ALP) U/l Fosfataza zasadowa	**70.73a**	18.37	**80.17b**	11.19	**76.87ab**	17.26	**79.45**	22.63
Aspartate aminotransferase, (ALP) U/l Aminotransferaza asparaginianowa	**48.30c**	8.90	**46.09d**	1.39	**59.14c**	16.02	**57.01d**	13.79
Alanine transferase, (ALP) U/l Aminotransferaza alaninowa	**59.73e**	20.27	**64.93**	21.30	**48.21e**	3.52	**59.82**	7.39
Lactate dehydrogenase, (ALP) U/l Dehydrogenaza mleczanowa	**406.95**	38.87	**477.20**	65.07	**378.95**	41.16	**409.21**	74.01
Bilirubin, mmol/l Bilirubina	**2.61**	0.14	**2.74**	0.24	**3.42**	0.24	**3.34**	0.35
Urea, mmol/l Mocznik	**7.72AB**	0.38	**8.11AC**	0.18	**5.47CD**	0.28	**7.95BD**	1.60
Uric acid, mmol/l Kwas moczowy	**0.15**	0.04	**0.14**	0.01	**0.13**	0.00	**0.15**	0.05
Creatinine, mmol/l Kreatynina	**59.07**	3.98	**57.73**	6.51	**56.99**	6.35	**54.93**	2.75
Cholesterol, mmol/l Cholesterol	**7.54**	0.59	**7.48**	0.07	**6.84**	0.40	**7.38**	0.36
Glucose, mmol/l Glukoza	**5.21**	0.28	**5.57**	0.54	**5.26**	0.27	**5.31**	0.61

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

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

Throughout the experimental period the LDH activity in polar fox females was significantly higher ($P \leq 0.05$) in control animals (332.45 U/l) (Tab. 3). This enzyme activity appears as congruent with the reference values determined by Winnicka [2004] but exceeded the data presented by Berestov *et al.* [1989]. Comparing the results from the control groups in the present study and Nowakowicz-Dębek`s researches, the higher LDH values were observed in the present investigation. The activity of the parameter, especially in first experimental year, was similar to that characterizing a fox group with the antioxidant supplement.

Determination of a serum bilirubin level may not be a particularly sensitive indicator of liver function or prognosis but it is commonly established and necessary [Berkow 1995]. Bilirubin content during this research stage was higher in the experimental group females and amounted to 3.13 μmol/l (Tab. 3). All the levels were consistent with the reference values for dogs and comparable with those reported by Nowakowicz-Dębek [2006].

The high urea concentration may point to elevated protein catabolic process rate, whereas a lowered level may indicate protein deficit, acute liver parenchyma damage

[Tomaszewski 2001]. Another kidney profile index – uric acid – is believed to be the superior agent affecting the overall plasma antioxidant capacity as well as free radical scavenger [Squadrito *et al*. 2000]. While an increased creatinine level is associated with kidney failure and glomerular filtration rate decrease, a decline is observed in muscle dystrophy [Malinowska 1999].

Mean values of urea, uric acid, creatinine and cholesterol were similar in groups K and D (Tab. 3).

Creatinine concentration was shown to increase in the succeeding samplings. The obtained data were lower compared to the reference values for dogs, but almost twofold higher as compared to the studies by Nowakowicz-Dębek [2006].

Cholesterol levels in each sampling demonstrated only slight and insignificant fluctuations.

Glucose concentration in body fluids is paramount to supporting the activity of tissues, especially the nervous one, as glucose is essential tissue fuel [Tomaszewski 2001, Malinowska 1999].

Slightly higher glucose levels were recorded in the dams from the experimental group (6.06 mmol/l) (Tab. 3), but the values did not surpass the reference range mentioned by Winnicka [2004].

The results of biochemical analyses performed during II research stage involving juvenile Arctic foxes are presented in Table 4.

Alkaline phosphatase activity in juveniles was characterized by the following values in the groups: K – 70.73–80.17 U/l, D – 76.87–79.45 U/l (Tab. 4). Differences in the ALP activity were determined between the females and males in each group and they were statistically significant at $p \leq 0.05$. The successive samplings reflected a marked decline in this enzyme activity in both groups and the statistically significant differences referred to a sex factor in group K.

Activity of aspartate aminotransferase was demonstrated to be higher in the experimental group, namely for the females – 59.14 U/l and males – 57.01 U/l (Tab. 4). The analysis confirmed the statistical differences between groups K and D at $p \leq 0.05$. The analyzed values, however, exceeded the reference values.

Average activity of alanine aminotransferase (ALT) in juvenile foxes at this experimental stage was notably higher in control animals (Tab. 4). The statistical analysis revealed some significant differences for the females from the studied groups ($p \leq 0.05$). The data surpassed the reference range.

The function of the LDH is to catalyze the reversible reaction of L-lactate oxidation or the reduction of pyruvate. A considerable rise of its activity is seen with megaloblastic anemia, leukemia, kidney damage etc. Physiologically higher activity is observed in young organisms [Berkow 1995, Tomaszewski 2001]. The activity of lactic dehydrogenase (LDH) in the studied period was markedly elevated in the control group but not statistically significant (Tab. 4). The values surpassed the limits established by Berestov *et. al*. [1989].

Bilirubin level in the juveniles was higher in the experimental group (females – 3.42 μmol/l, males – 3.34 μmol/l) (Tab. 4). As for urea, the statistically significant differences were noted for females and males from group K and D, as well as between them, at $p \leq 0.01$ (Tab. 4). The reference values for this index (3.32–7.47 mmol/l) were exceeded, mainly in the control.

Uric acid concentration in both groups was very similar in both groups (Tab. 4). All the data agreed with the reference values.

Throughout the experimental period creatinine content was a little raised in the control group (Tab. 4). The values were congruent with those recommended by Meyer and Harvey [1998] but under the range presented by Winnicka [2004].

Referring to the studies of Nowakowicz-Dębek [2006], a higher level of the studied biochemical parameters, except for creatinine and urea, was determined.

The levels of cholesterol and glucose in both experimental groups did not differ significantly (Tab. 4). At each sampling, cholesterol content was quite even and agreed with the reference values given by Winnicka [2004] but exceeded those presented by Meyer and Harvey [1998]. Glucose level differed significantly in I sampling in the control group at $p \leq 0.05$.

All the research results of biochemical indices obtained in the present study, except for AST, ALT, urea and cholesterol (juveniles) were found within the reference range for dogs presented by Winnicka [2004] and Meyer and Harvey [1998].

CONCLUSIONS

1. Metabolic profile indices in foxes from the investigated farms (A, B, C) were similar but most equal in the animals from the object C.

2. Synthetic antioxidant supplement did not have a significant impact on the development of the biochemical parameters in Arctic 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 (Rendox^{TM} Liquid), by zabezpieczyć jej komponenty. Określono wpływ dodatku tego przeciwutleniacza do karmy lisów polarnych (*Alopex lagopus*) na wybrane wskaźniki biochemiczne krwi (AST, ALT, ALP, LDH oraz poziom cholesterolu, mocznika, kreatyniny, glukozy, bilirubiny, kwasu moczowego), z których to część uznawana jest za nieenzymatyczne mechanizmy obronne i zmiatacze reaktywnych form tlenu.

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

Słowa kluczowe: lisy polarne, przeciwutleniacz, wskaźniki biochemiczne