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# Influence of feeding complete dry diets mixed with water on production traits and health status of blue foxes (*Alopex lagopus*)

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An experiment was performed on 120 growing blue foxes (*Alopex lagopus*), 60 animals per group. Control group (C) was offered traditional diets prepared from thawed components while the experimental (E) was offered a pulp obtained from mixing dry pulverized components (animal meals, ground wheat, fat, witamin-mineral mix), with water 5 h prior to feeding. The body weight of foxes was recorded, and fur quality assessed based on live measurements and pelt quality evaluation. The diets were monitored for microbial contamination. The effects of diets on the overall health of animals were estimated based on blood biochemical and morphological parameters as well as anatomopathological changes of the digestive tract and internal organs. It was found that the risk of microbial contamination was lower in experimental diets, compared to conventional ones. Experimental feeding had no influence on the final body weights of foxes, but had positively affected fur quality (P<0.01). No negative impact of experimental diets on the health status of animals was observed.

KEY WORDS: animal meal / feeding / fox / health status / microbiology / production traits

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Fur-bearing carnivores, in particular mink and sporadically foxes, are fed not only conventional diets, but also complete dry feed in the form of pellets, the main components of which are animal meals. These diets are practically applied for feeding pets. Hides obtained from foxes fed pelleted feed are shorter, but characterized by good quality [Weiss 1987, Lorek et al. 1999]. The smaller size of hides may be a consequence of water unbalance, as confirmed by the consistency of faeces. Feeding systems should be evaluated based on production results, but also in view of their impact on the health status of animals. The values of blood biochemical and morphological indicators provide reliable information on the function of the animal organism. Changes in the activity of certain blood enzymes may be caused by nutritional, health-related or growth-related factors [Kopczewski et al. 2002, Lorek et al. 2002, Nenonen et al. 2004]. Histopathological analysis is another valuable source of information about the effect of the diet on the functions of internal organs. It enables to diagnose subclinical statuses that cannot be determined during the relatively short production cycle of fur animals. Feed hygiene also plays an important role since an increase in total bacterial counts in feed adversely affects the health of animals, though clinical symptoms are sometimes difficult to observe. A correlation has been reported between bacterial contamination of feed and a serious health risk for animals [Kopczewski et al. 2000].

The aim of the present study was to determine, based on production and health parameters, whether blue foxes can be fed complete dry, non pelleted diets mixed with water before feeding.

#### Material and methods

The experiment was conducted on 120 blue foxes aged 8 weeks, divided into two equal groups: control -C and experimental -E, identical in terms of origin and sex. Two animals of the same sex were kept in standard cage according to common furfarming practice. The experimental factor was nutrition system (Tab. 1). C group was fed traditional (C1, C2), while group E – experimental diets (E1, E2) – based on dry and powdery components mixed with water 5 hours before feeding to obtain a consistency of a pulp. All diets met the nutrient requirements of growing foxes. Diets C1 and E1 were used during July, August and September while C2 and E2 during October and November [NRC 1982]. The rations for both groups differed in composition, but their energy and nutritive value as expressed in per cent of metabolizable energy from protein, fat and carbohydrates were comparable. Animals were fed and watered ad *libitum*. A microbiological analysis of the diets was performed at 30-day intervals, in two replications: of "A" samples taken before feeding, about two o'clock p.m., and "B" samples taken from feeders the next morning, about 8 o'clock a.m. Immediately after collection, bulk samples (approx. 1 kg) were transported in an isothermal container to the laboratory, where three 10 g samples were taken from different places, suspended in 90 ml of sterile physiological saline and shaken for 30 min. Serial 10-fold dilutions (10<sup>-1</sup> to 10<sup>-6</sup>) were prepared. Samples of each dilution were inoculated into

Itom	Diet			
Item	C1	C2	E1	E2
Hard poultry offal	23.7	25.6	-	-
Poultry by-products	19.8	17.0	-	-
Soft poultry offal	17.2	19.2	-	-
Various poultry offal products	17.0	15.3	-	-
Meat by-products	2.3	2.0	-	-
Ground wheat	18.7	19.6	33.0	36.0
Green forage, vegetables	1.3	1.3	-	-
Poultry meal	-	-	38.0	30.0
Blood and feather meal	-	-	10.0	15.0
Fish meal	-	-	5.0	3.0
Poultry fat/soybean oil	-	-	10.0	12.0
Dried whey	-	-	2.0	2.0
Lucerne meal	-	-	2.0	2.0
Vitamin-mineral preparation	+	+	+	+
Percent of energy from				
protein	35	31	34	31
fat	46	48	46	48
carbohydrates	19	21	20	21
Dry matter	29.88	32.22	34.02	35.02
MĚ (MJ/kg)	5.368	5.910	6.300	6.537

Table 1. Composition (%) and nutritive value of diets

The water content of diets C1 and C2 was 25% of their total weight (including water used for steam treatment and for mixing with feed prior to feeding). The water content of diets E1 and E2 was 60% of the total weight of dry feed.

three plates. The following standard methods were used: standard agar to determine total microbial counts, Levin, MacConkey and SS media to isolate bacteria of the family Enterobacteriaceae, Chapman medium to isolate Staphylococci, blood agar to isolate Streptococci, Saboutaud-chloramphenicol agar to isolate fungi and Wrzosek broth to determine anaerobic spore-forming bacilli [Polish Standard 1994, Kendzia, Komiar 1980]. In the last case samples were heated at 80°C for 30 min. prior to inoculation. After incubation, the colonies were counted per plate and microbes were identified using microscopic preparations made for different colonies to follow the biochemical analysis. Foxes were weighed at the beginning and at the completion of the experiment, always prior to feeding, accurate to 0.1 kg. Towards the end of the furring period the body conformation of foxes was evaluated based on the Polish Standard [Standards for Arctic Foxes 1999]. Blood samples (approx. 5 ml) for morphological and biochemical analyses were collected from the foot vein of 10 males and 10 females aged 24 weeks, selected randomly of each group. Red blood cell count (RBC), mean corpuscular volume (MCV), red blood cell distribution width (RDW), white blood cell count (WBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined by standard methods [Winnicka 2004].

Then 15 males and 15 females selected randomly from each group were pelted in accordance with normal farming practice. Hide length and fur quality were determined (grade estimates) in accordance with the International Trading System. For comparison, particular grades were denoted by numbers: Saga – 1, B – 2, C – 3.

Histopathological examinations were carried out on the carcasses of 5 males and 5 females of each group. Sections of the stomach, duodenum, large intestine, liver, pancreas and kidneys were fixed by immersing in 10% neutralized formalin, and paraffin-embedded. Microtome sections were stained with hematoxylin-eosin and, if necessary, by the P.A.S. procedure (McManus), and silver-impregnated.

Numerical data were processed statistically by analysis of variance in a one-factor orthogonal design combination [Statistica PL 2007].

### **Results and discussion**

The total counts of bacteria, yeasts and molds occurred substantially lower in experimental diets, both in samples A and B (Tab. 2). Bacteria of the family *Enterobacteriaceae* were more abundant in conventional diets. *Staphylococci* and anaerobic spore-forming bacilli were isolated from both types of diets. The analysis of the leftovers (samples B) showed that microbial counts increased at a slower rate in experimental diets.

		Diet/Sample collection			
Item	C1	C1,C2		E1,E2	
	А	В	А	В	
Total bacterial count Total counts of yeasts and moulds <i>Enterobacteriaceae</i> <i>Staphylococci</i>	$\begin{array}{c} 4.53 \times 10^5 \\ 4.32 \times 10^2 \\ 1.10 \times 10^5 \\ 1.10 \times 10^4 \end{array}$	$\begin{array}{c} 6.25 \times 10^5 \\ 7.80 \times 10^2 \\ 2.05 \times 10^5 \\ 2.90 \times 10^4 \end{array}$	$\begin{array}{c} 1.67 \times 10^{5} \\ 2.50 \times 10^{2} \\ 4.70 \times 10^{4} \\ 5.75 \times 10^{3} \end{array}$	$\begin{array}{c} 1.98 \times 10^{5} \\ 4.00 \times 10^{2} \\ 7.80 \times 10^{4} \\ 1.20 \times 10^{4} \end{array}$	
Anaerobic spore-forming bacilli per 0.001 g of diet	+	+	+	+	

Table 2. Microbial contamination of diets

The family *Enterobacteriaceae* includes numerous pathogenic bacteria, e.g. members of the *Salmonella* genus, posing a serious health hazard for foxes [Dietz *et al.* 2006]. Kopczewski *et al.* [2000] reported a correlation between the cultures of microbes isolated from feed and from internal organs of foxes, which confirms that the health of animals is related to the microbiological contamination of feed. The following microbes were isolated most frequently by the authors mentioned: *E. coli*, anaerobes of the genus *Clostridium, Salmonella enteritidis* and *Staphylococci*. According to Śmielewska-Łoś and Klimentowski [1996] the bacteria encountered

most frequently in raw diets components of animal origin are members of the genus *Salmonella, E. coli*, tubercle bacilli and anaerobes. In that study *E. coli, Staphyloccocus aureus, Streptoccocus pyogenes* and *viridans*, members of the genera *Enterococcus* and *Klebsiella* were isolated most frequently from the tissues of dead fox pups.

Table 3 presents some performance traits of foxes. The final body weights of control and experimental group (9.52 and 9.79 kg, respectively) were comparable and may be considered typical of this species. An evaluation of the body conformation of foxes showed no statistical differences in body size and constitution, color type and color purity. However, fur quality and total scores were highly significantly higher in group E than in C. The quality of skins in group E was comparable to Saga – 1.

Trait		Group		
		С	Е	
Body weight (kg)	initial final	2.70±0.37 9.52±1.11	2.70±0.36 9.79±1.22	
Body conformation (points)	trunk length (cm) body size and constitution colour type colour purity fur quality total score	$\begin{array}{c} 64.42{\pm}2.02\\ 5.40{\pm}1.01\\ 3.00{\pm}0.00\\ 3.00{\pm}0.00\\ 4.70^{xx}{\pm}1.14\\ 16.10^{xx}{\pm}1.74 \end{array}$	65.64±2.65 5.92±0.39 3.00±0.00 3.00±0.00 6.66 <sup>xx</sup> ±0.82 18.58 <sup>xx</sup> ±1.08	
Hide characteristic	length (cm) quality	111.82±3.76 1.96 <sup>xx</sup> ±0.63	111.43±4.55 1.53 <sup>xx</sup> ±0.50	

Table 3. Means and standard deviations (SD) for performance traits of foxes

<sup>xx</sup>Within rows means bearing the same superscripts are significantly different at  $P \le 0.01$ .

Similar final body weight of foxes was obtained by Lorek *et al.* [2002], and Skrede and Ahlstrom [2004]. Weiss [1987] and Lorek *et al.* [1999, 2002] fed foxes with pelleted feed containing animal meals comparable to those used for the present E group and found that feeding system resulted in lower daily gain and final body weight, which could be related to water unbalance or to the fact that pellets might have been falling through the wire mesh.

Lorek *et al.* [2002] reported better quality of hides obtained from foxes fed pellets. This suggests that feed containing animal meals, served dry or mixed with water (own investigations), has a positive effect on fur quality. This probably results from the fact that such feed is well-balanced and can be offered regularly, as opposed to conventional diets. Hide length was similar in groups C and E (111.82 and 111.43 cm respectively), as could be expected based on body weights and body conformation scores. Skrede and Ahlstrom [2004] obtained hides 109 cm in length from foxes weighing about 10 kg.

A morphological and biochemical analysis of blood (Tab. 4) indicated a significantly higher RBC in group E, which confirms that experimental diets had no negative impact on animals. There were no significant differences between the groups with regard to the other blood morphological parameters, except for WBC and HCT. WBC was lower in E foxes, which could be an indicator of their better overall health. HCT was higher in E group, being directly related to RBC. The activities of ALP and AST were at a comparable level in both groups. ALT level, however, was higher in C group.

Item	Group		
	С	Е	
12		XX	
RBC $(10^{12}/l)$	8.12 <sup>**</sup> ±0.58	8.78 <sup>**</sup> ±0.36	
$MCV (\mu m^3)$	51.50±1.50	51.30±1.55	
RDW (%)	9.70±0.53	$10.20 \pm 0.80$	
WBC (10 <sup>9</sup> /l)	$14.80^{x} \pm 2.32$	$12.10^{x} \pm 3.09$	
HGB (mmol/l)	9.00±0.70	9.50±0.42	
HCT (1/1)	$0.41^{x}\pm0.03$	$0.45^{x}\pm0.02$	
MCH (pg)	$1.00\pm0.09$	$1.00\pm0.06$	
MCHC (mmol/dl)	21.60±1.09	21.10±0.70	
ALT IU/l	$52.60^{xx} \pm 8.91$	$20.40^{xx} \pm 17.66$	
AST IU/l	$12.14 \pm 2.00$	11.83±1.22	
ALP IU/l	40.61±9.09	36.66±9.24	

 
 Table 4. Means and standard deviations (SD) for blood morphological and biochemical parameters

<sup>x,xx</sup>Within rows, means bearing the same superscripts are significantly different at  $P \le 0.05$  and 0.01, respectively.

Haematological parameters of blood of foxes fed dry diets with animal meals were determined also by Szymeczko *et al.* [1999]. They demonstrated that dry feed caused no changes in the morphological and biochemical indices of the peripheral blood of experimental animals compared to controls fed conventional diets. Lorek *et al.* [2002] reported that in foxes fed pellets RBC was 8.75 U/l. This is comparable to E group from the present study. It is generally known that poor diet can reduce the red blood cell production [Tauson and Neil 1992].

In a study by Szałkowska *et al.* [1999] the average level of ALP in blue foxes was 43.11 U/l being similar to that determined in the current experiment. Kopczewski *et al.* [2002] found that the average activity of AST was 38.20 U/l, while according to Lorek *et al.* [2002] it was considerably lower. In the present study the level of ALT was over twofold lower in E than in C group. Kopczewski *et al.* [2002] demonstrated that in foxes fed high-energy diets the average activity of ALT exceeded the value to be above the reference for this species given by Brandt *et al.* [1989]. The experimental data gathered by Nenonen *et al.* [2004] indicate higher levels of both ALT and AST of foxes.

Organ	Changa	Number of changes	
Organ	Organ Change		group E
Stomach	<ul> <li>acute catarrhal inflammation of the mucosa</li> </ul>	10	8
	- lymphoid cell infiltrates	4	4
	<ul> <li>excessive exfoliation of epithelial cells</li> </ul>	4	2
	- acute catarrhal inflammation of the mucosa	8	8
Duodenum	- necrosis of the apical parts of villi	7	4
	- stimulation of lymph follicles	10	10
Large intestine	- acute catarrhal inflammation of the mucosa	10	10
	- lymphoid cell infiltrates	10	10
	- stimulation of lymph follicles	10	10
	<ul> <li>excessive exfoliation of epithelial cells</li> </ul>	7	5
	- congestion, hemostasis	10	9
	- parenchymatous degeneration of hepatocytes	10	10
	<ul> <li>vacuolar degeneration of hepatocytes</li> </ul>	10	10
Liver	- adipose degeneration	10	10
	<ul> <li>focal necrosis of hepatocytes</li> </ul>	5	2
	- lymphoid cell infiltrates	10	10
	<ul> <li>eosinophilic cell infiltrates</li> </ul>	5	3
Pancreas	- congestion, hemostasis	10	10
	<ul> <li>focal necrosis of pancreatic cells</li> </ul>	4	1
	<ul> <li>increased acidophilia of pancreatic cells</li> </ul>	5	3
	- lymphoid cell infiltrates	1	1
Kidneys	- congestion of the cortical and medullary layers	10	10
	- parenchymatous degeneration of the epithelium of tubules	10	10
	- focal vitreous degeneration of convoluted tubules	6	6
	- vacuolar degeneration of tubular epithelium	9	8
	- focal necrosis of tubular epithelium	10	9
	- membranous glomerulonephritis	10	10

#### Table 5. Results of histopathological analysis

Table 5 presents the results of histopathological investigations. There were stated lesions of internal organs and digestive tracts in both groups of animals, the changes, however, being more often observed in group C.

The reason for histopatological changes were pathogenic factors present in feed, so they were more intensive in C than in E group, as the level of microbial contamination was lower in diets composed of animal meals than in those based on typical raw components, which was also observed by Weiss [1987]. Catarrhal inflammation, accompanied by stimulation of lymph follicles and lymphoid cell infiltrates, was found in the mucosa of the digestive tracts of both groups of foxes.

Inflammatory processes in the digestive tract contribute to the penetration of toxins and pathogens into the blood and damage the parenchymal cells. Such lesions are sometimes noted also in properly fed foxes [Lorek *et al.* 1999, Kopczewski *et al.* 2002]. The reasons for such changes may be different, but most often they are related to immunological disorders. Kopczewski *et al.* [2002] attributed renal lesions observed in foxes to nephrotoxic factors present in feed.

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The livers of foxes in both groups were afflicted to a similar extent, and the observed morphological changes could affect all functions of the body. Those changes included circulatory disturbances in the form of hemostasis as well as retrogressive changes, primarily parenchymatous and vacuolar degeneration (reversible lesions) and – to a lesser degree – adipose degeneration and necrosis of single hepatocytes (irreversible). Other authors also reported numerous histopathological lesions in the liver of foxes [Kopczewski *et al.* 2002]. The pancreases of foxes were congested, and increased acidophilia of the cytoplasm of exocrine cells as well as small necrotic foci in some animals were recorded, usually in group C. However, normal functions of pancreas were only slightly impaired by the above changes. Glomerulonephritis combined with retrogressive changes in the epithelium of renal tubules and circulatory disturbances in the form of congestion were noted in animals of both groups.

The investigation presented here has pointed out that growing blue foxes can be fed dry diets mixed with water, whose main components are animal meals. Experimental feeding had no influence on the final body weight of foxes, but positively affected fur quality. A morphological and biochemical analysis of blood and a histopathological analysis of some internal organs and sections of the digestive tract confirmed that experimental diets had no negative impact on the health status of animals.

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## Wpływ żywienia lisów polarnych pokarmem uzyskiwanym z mieszania składników suchych z wodą na cechy produkcyjne i zdrowie zwierząt

#### Streszczenie

Badania wykonano na 120 rosnących lisach polarnych (*Alopex lagopus*), podzielonych na zasadzie analogów na dwie grupy po 60 zwierząt Grupę kontrolną (C) żywiono mieszankami tradycyjnymi uzyskiwanymi z komponentów mrożonych. W grupie doświadczalnej (E) korzystano ze sproszkowanych składników suchych, głównie mączek zwierzęcych, śrut, tłuszczów i preparatu witaminowo-mineralnego, które przed podaniem nawilżano wodą, uzyskując pokarm o konsystencji pulpy. Kontrolowano wzrost zwierząt i oceniono uzyskane futra, zarówno przyżyciowo, jak i na podstawie cech skóry. Monitorowano obraz mikrobiologiczny mieszanek. Wpływ żywienia na zdrowie zwierząt oceniono na podstawie badań morfologicznych i biochemicznych krwi oraz zmian anatomopatologicznych przewodu pokarmowego i narządów wewnętrznych. Stwierdzono, że mieszanki podawane lisom w grupie E charakteryzował korzystniejszy obraz mikrobiologiczny niż mieszanki tradycyjne. Żywienie stosowane w grupie E nie spowodowało zróżnicowania końcowej masy ciała zwierząt, natomiast wpłynęło korzystnie na jakość ich okrywy włosowej (P<0.01). Nie udowodniono negatywnego wpływu żywienia na stan zdrowia lisów.