

## EFFECT OF DIETS WITH HIGH METHIONINE LEVELS ON GROWTH PERFORMANCE, HEALTH STATUS, NUTRIENT DIGESTIBILITY AND NITROGEN RETENTION IN ARCTIC FOXES

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### ABSTRACT

The aim of the present experiment was to evaluate the influence of diets with high methionine content on growth performance, health status, nutrient digestibility and nitrogen balance in Arctic foxes (*Vulpes lagopus*). The experimental materials consisted of 120 blue Arctic foxes divided into three equal groups. Control group (C) animals were fed diets with standard methionine content. Diets for experimental groups E1 and E2 were supplemented with liquid methionine at 3 and 6 g kg<sup>-1</sup> fresh matter. The following parameters were determined in the study: body weight, body conformation, feed intake, length of the rearing period. Blood was sampled for hematological and serum biochemical analyses. Pelt quality was evaluated. Two digestibility trials were performed. It was found that diets with increased methionine content improved the growth performance of Arctic foxes. Increased dietary methionine concentrations had no negative influence on the health status of foxes, and they supported protein digestibility and nitrogen retention. However, the highest dietary methionine level (6 g kg<sup>-1</sup> fresh matter) in group E2 did not lead to a further improvement in the analyzed parameters, compared with the methionine content of diets fed to group E1 animals.

**Keywords:** digestibility, health status, methionine, nitrogen retention, pelt quality, *Vulpes lagopus*.

### INTRODUCTION

The rations for carnivorous fur animals are often formulated to contain high amounts of offal-based feeds, which may lead to dietary amino acid imbalance. The amino acid content of the ration may vary depending on the amino acid content of feed ingredients. In the nutrition of farmed-raised Arctic foxes (*Vulpes lagopus*), an important role is played by sulphur-containing amino acids. Methionine and cystine are the first-limiting amino acids for fur animals, which have a significant effect on fur growth and quality (Dahlman *et al.*, 2004). Currently farmed Finnish blue foxes are characterized by increased pelt size and improved fur quality, compared with their ancestors (Peura *et al.*, 2005). Previous research has demonstrated that increased dietary inclusion levels of methionine contribute to improved growth performance and fur quality in Arctic foxes. In the experiments conducted to date, the methionine content of fox diets ranged from 4.0 to 10.0 g kg<sup>-1</sup> feed DM (Lorek *et al.*, 2002a; Dahlman *et al.*, 2002a,b, 2003, 2004; Matusevicius *et al.*, 2004, Gugolek *et al.*, 2012). Beneficial effects of supplementary methionine have also been observed in raccoon dogs (*Nyctereutes procyonoides*) (Liu *et al.*, 2012; Zhang *et al.*, 2012b). In mink (*Neovison vison*), the optimum methionine levels were found to be 13.87 - 16.36 g kg<sup>-1</sup>

<sup>1</sup>feed DM (Zhang *et al.*, 2012a). Higher inclusion levels of methionine in fox diets have not been tested.

The objective of this study was to evaluate the influence of diets with high methionine content on growth performance, health status, nutrient digestibility and nitrogen balance in Arctic foxes, and to identify the maximum recommended inclusion rates of this amino acid in diets fed to farmed foxes.

### MATERIALS AND METHODS

A total of 120 farmed blue foxes, the offspring of animals imported from Finland, were assigned to three equal groups (n = 40), with 20 males and 20 females per group. The groups were identical in terms of sex ratio and origin. The animals were placed in standard cages (1 m x 2 m x 0.8 m), with two animals (one male and one female) per cage, in the same pavilion, on a farm in the Province of Pomerania (northern Poland). The experiment began in June, when the animals were 10 weeks of age, and ended in December.

Feed and water were available *ad libitum*. The diets were composed of typical feed ingredients available on the Polish market (Table 1). The chemical composition, nutritional value and energy content of diets are shown in Table 2. Metabolisable energy concentration was determined based on digestibility coefficients,

using the following energy conversion factors: protein - 18.8 MJ kg<sup>-1</sup>, fat - 39.8 MJ kg<sup>-1</sup>, carbohydrates - 17.2 MJ kg<sup>-1</sup>. All diets were formulated to meet the Nutrient Requirements of Mink and Foxes (1982). The experimental factor was dietary methionine content. The diets administered to control group (C) animals (AC and BC) were supplemented with methionine at the recommended level (NRC 1982). The composition of diets was validated by chemical analysis. Diets A were administered from June to September, and diets B from October to December. Diets for groups E1 and E2 were supplemented with liquid methionine - ALIMET (88% pure) supplied by Adisseo, at 3 (AE1, BE1) and 6 g kg<sup>-1</sup> fresh matter (AE2, BE2), i.e. approximately 2 and 4 g methionine per 100 g total protein in the ration.

Foxes were weighed at the beginning and at the end of the experiment, before feeding, within an accuracy of 0.1 kg, with the use of an automatic-indicating scale. At the end of the furring period, the body size, body conformation, fur quality and coat characteristics of foxes were evaluated in accordance with the Polish Arctic Fox Standard (1999). Feed intake, expressed as the difference between the amount of feed supplied and consumed, was determined once a week. The length of the rearing period was expressed as the number of days from birth to slaughter.

At 24 weeks of age, blood for hematological and serum biochemical analyses was sampled (approx. 5 ml) from the small saphenous vein of 10 males and 10 females, randomly selected from each group. The analyses were performed by standard methods (Winnicka, 2004). White blood cell (WBC) counts, red blood cell (RBC) counts, hemoglobin (HGB), hematocrit (HCT), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentrations (MCHC), red blood cell distribution width (RDW), mean platelet volume (MPV), glucose (GLU) and urea (UREA) levels, and the activities of creatine kinase (CK), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined.

In August and October, two digestibility trials were carried out on 18 females selected from six litters, and divided into three equal groups (n=6). Their genetic origin was the same as that of the animals used in the production trial. Diet A was tested in August, and diet B was tested in October. The animals were placed in individual metabolism cages for the collection of urine and faeces. A five-day experimental period was preceded by a five-day adjustment period. Water was available *ad libitum*, and the animals received 600 g (3.9 MJ ME) of feed once a day, at the same time. Leftovers and faeces were collected every day, and were weighed within an accuracy of 1 g. Faeces samples were frozen. Next, samples of faeces and feed were partially dried and ground. 20% sulphuric acid was used to preserve urine

samples. The total volume of the collection was determined at the end of the experiment.

Foxes were slaughtered at the optimum date, when their coats were thickest, upon the approval of the Local Ethics Committee for Animal Experimentation (opinion No. 54/2007N), in accordance with the relevant EU regulations. Coat thickness was estimated with the use of organoleptic methods. Pelts were processed by standard methods, and were graded based on size, quality and external appearance according to the International Trading System. To facilitate comparison, the grades were assigned the following numbers: Saga - 1, B - 2, C - 3 (Hryckiewicz, 1994).

The following determinations were performed: nutrient content of feed, the proportion of nutrients excreted in faeces and urinary nitrogen content - by standard methods (AOAC, 2003); dry matter (DM) content - by drying at a temperature of 103°C; crude ash content - by mineralization in a muffle furnace (Czyłok, Jastrzębie-Zdrój, Poland) at a temperature of 600°C; total nitrogen - by the Kjeldahl method, in the FOSS TECATOR Kjeltec 2200 Auto Distillation Unit, ether extract content - by the Soxhlet method, in the FOSS SOXTEC SYSTEM 2043; crude fibre content - in the FOSS TECATOR Fibertec TM 2010 System. The coefficients of apparent nutrient digestibility were calculated using the formula:  $a-b/a$ , where:  $a$  - nutrient intake,  $b$  - faecal nutrient excretion.

The dietary levels of methionine and cystine were estimated with the use of the Biochrom 20 plus amino acid analyzer, Biochrom amino acid analysis reagents (Biochrom Ltd., Cambridge, England), and the following standards for amino acid analysis offered by Sigma-Aldrich: Amino Acid Standard Solution (AASS-18), L-methionine sulphone, L-cysteic acid.

Data were analysed statistically by one-way ANOVA and Tukey's HSD post hoc test, with the use of STATISTICA software (StatSoft Inc., 2007), at a significance level of  $p \leq 0.05$ . Nutrient digestibility and nitrogen balance parameters were expressed as arithmetic means  $\pm$  standard deviations ( $X \pm SD$ ). The results were compared within trials.

## RESULTS AND DISCUSSION

No significant differences were found in the average initial body weight of Arctic foxes between groups. At 26 weeks of age, the body weights of foxes were as follows: group C -  $11.5 \pm 1.0$  kg, group E1 -  $12.8 \pm 1.2$  kg, group E2 -  $12.4 \pm 1.0$  kg (Table 3). The final body weights of the animals examined in our study may be regarded as typical of Finnish giant foxes. In a study by Dahlman *et al.* (2003), the average body weight at slaughter of Arctic foxes of the Finnish type ranged from 10.68 kg to 11.47 kg, and in an experiment by Gugoleket *et al.* (2012) it reached 10.99-11.47 kg.

Dietary supplementation with methionine has been found to stimulate weight gain by enhancing protein synthesis in the body. Dahlman *et al.* (2002b) demonstrated that diets with a low content of methionine and lysine reduced the growth rate of Arctic foxes. In a study by Gugoleket *et al.* (2012), the final body weights of Arctic foxes fed diets supplemented with methionine increased by 0.26 and 0.48 kg. In mink, an increase in the methionine content of diets improved the weight gains of animals, but to a certain level only – a further increase in methionine concentrations had no influence on growth performance (Zhang *et al.*, 2012a).

Foxes of all groups scored the highest number of points for body size and conformation. Trunk length was higher in experimental than in control animals (group E1 – 78.3 cm, group E2 – 76.0 cm, group C – 71.5 cm). This parameter was comparable to that reported by other authors. In experiments performed on blue foxes, average trunk length was 69-72 cm and 66-69 cm, respectively (Dahlman *et al.*, 2002a, 2003).

In the present study, dietary methionine supplementation had no influence on colour type, the purity of coat colour, body size or constitution of Arctic foxes. However, differences in fur quality were noted. The absence of differences in colour type and the purity of coat colour could be attributed to adequate nutrition in all groups, and the fact that both traits are genotype-dependent. Quality is considered to be the most important attribute of fur. Foxes from group E1 had the highest-quality furs (difference between group E1 and group C - 0.9 points, difference between group E2 and group C - 0.8 points). A correlation between fur quality and increased dietary methionine content has also been reported by other authors (Dahlman, 2002b; Gugoleket *et al.*, 2012).

The overall score for body conformation was also higher in experimental groups E1 and E2 than in the control group. The results of experiments conducted by Zhang *et al.* (2012c) and Dahlman *et al.* (2002b) revealed that methionine supplementation improved the overall fur quality of blue foxes fed the lowest protein diet. In our study, higher levels of dietary methionine speeded up fur growth by 4-7 days. The rearing period was longer in group C than in groups E1 and E2 (203.4 days vs. 197.2 and 199.6 days, respectively; statistically significant differences). Average feed intake was comparable in all groups.

The values of pelt length were similar in all groups, but Arctic foxes from experimental groups tended to have longer pelts. Average pelt length was  $126.4 \pm 8.1$  cm in control group C,  $128.7 \pm 5.3$  cm in group E1 and  $128.4 \pm 5.0$  cm in E2. Handling and stretching affect pelt length. Pelt size is proportional to the final body weights and body conformation scores of foxes. Previous research has revealed a positive correlation between body weight and pelt length (Gugoleket *et al.* 2002). The pelt length

determined in our study was comparable with the values reported by Scandinavian researchers. In experiments conducted by Dahlman *et al.* (2002a,b, 2003), average pelt length ranged from 116 to 122 cm and from 114 to 117 cm, respectively. The pelts of foxes from experimental groups E1 and E2 were characterized by the best quality, comparable with the highest grade, Saga (1). As expected, the results of pelt quality assessment were consistent with body conformation scores because fur parameters are known to affect overall pelt appearance.

The hematological and serum biochemical parameters of Arctic foxes are shown in Table 4. There were significant differences between groups in MCV values, which reached  $59.6 \pm 2.6$  in group C,  $55.0 \pm 1.0$  in group E1, and  $56.8 \pm 1.1$  in group E2. Diets with increased methionine content had also a significant effect on RDW and MPV. Increased dietary methionine concentrations had no adverse influence on the health status of animals. The results of blood analyses were similar to those reported in earlier studies (Loreket *et al.*, 2002; Loreket *et al.*, 2005; Sabaet *et al.*, 2008). Blood urea levels were higher in group C than in experimental groups E1 and E2, which could be due to the more desirable amino acid profile of methionine-supplemented diets. The activities of liver-specific enzymes, ALT and AST, were lower in experimental groups (non-significant differences), which indicates that methionine contributed to regulating liver metabolism and promoted normal liver function in foxes. Similar results were reported for other carnivorous animals, cats (Fauet *et al.*, 1987) and rats (Rana and Chauhan, 2000). Methionine has many important physiological functions in the body, and it is required for the optimal growth and productivity of animals. Methionine participates in the methylation process and in the regulation of antioxidant status, and affects nutrient metabolism and cell function. Methionine and arginine are also known to play a vital role in epidermal keratinisation (Tesseraud *et al.*, 2009).

The results of digestibility trials are shown in Table 5. Protein digestibility was higher in groups E1 and E2 than in group C, and it reached the highest level in group E1. The digestibility coefficients of the remaining nutrients were similar in all groups. The digestibility coefficients determined in our study are specific to blue foxes. Protein digestibility did not increase in response to methionine levels exceeding 14.87 in diets A and 17.66 g kg<sup>-1</sup> DM in diets B. The coefficients of protein digestibility were high. Dahlman *et al.* (2003) reported protein digestibility of 78-83%. The cited authors observed no significant differences in protein digestibility between groups of blue foxes fed diets supplemented with methionine at 4 to 10 g kg<sup>-1</sup> DM, but they found that digestibility increased with an increase in methionine inclusion levels. In a study by Loreket *et al.* (2001), crude protein digestibility reached 86-87%, and it was

comparable with the values noted in groups fed diets without the addition of methionine (AC, BC).

Daily nitrogen balance is presented in Table 6. In both diets, nitrogen retention was significantly higher in groups E1 and E2 than in group C (for nitrogen retention calculated as % of N intake in diets A and B). The highest nitrogen retention calculated as % of digested N in diets A was noted in group E1 ( $45.8 \pm 4.8$ ), and in diets B in group E2 ( $51.6 \pm 3.1$ ). The effects of dietary methionine levels on nitrogen retention noted in our study are similar to those reported by Dahlman *et al.* (2002a, 2003) and Gugoleket *et al.* (2012) who also found that higher methionine concentrations in fox diets were

correlated with higher nitrogen retention. In mink, an increase in dietary methionine levels to 14 g kg<sup>-1</sup> feed DM supported nitrogen retention, but a further increase in methionine concentrations had no effect on retained nitrogen (Zhang *et al.*, 2012b). Such a trend was also noted in our study where no significant differences were found between groups E1 and E2. It appears that the optimal amino acid ratio improves the availability of nitrogen and protein, which was observed in a study by Zhang *et al.* (2012c) where the most satisfactory results were reported for diets supplemented with both methionine and lysine.

**Table1. Diet composition (gkg<sup>-1</sup> fresh matter)**

Ingredients	Diet					
	AC	AE1	AE2	BC	BE1	BE2
flatfish offal	30	30	30	0	0	0
hard filleted cod offal	30	30	30	42	42	42
hard poultry offal	241	241	241	168	168	168
soft poultry offal	402	402	402	362	362	362
cooked pork offal	42	42	42	84	84	84
preserved poultry blood	16	16	16	28	28	28
meat and bone meal	30	30	30	57	57	57
sour milk	5	5	5	5	5	5
extruded barley	15	15	15	18	18	18
cooked ground wheat	76	76	76	74	74	74
wheat bran	3	3	3	2	2	2
steamed potatoes	0	0	0	82	82	82
vegetable oil	2	2	2	3	3	3
fruit and vegetables	5	5	5	4	4	4
Arbocel*	2	2	2	2	2	2
vitamin and mineral supplement	1	1	1	1	1	1
liquid methionine	0	3	6	0	3	6
water**	100	97	94	68	65	62
total	1000	1000	1000	1000	1000	1000

\*Arbocel – crude fibre concentrate manufactured by Rettenmaier

\*\* water used for steam treatment and mixing with feed

**Table2. Chemical composition (gkg<sup>-1</sup>DM) and nutritional value (%) of basal diets**

Specification	Diet					
	AC	AE1	AE2	BC	BE1	BE2
DM (g/kg)	330.20	326.38	324.65	343.50	341.98	336.11
In DM:						
Ash	71.30	71.04	71.01	78.31	78.08	78.65
Crude protein	362.11	370.62	376.09	342.40	346.12	350.53
Crude fat	264.87	259.04	254.98	262.57	260.71	258.82
Crude fibre	22.65	22.05	21.89	26.78	25.69	25.10
N-free extractives	279.07	277.25	276.03	289.94	289.40	286.90
Methionine	7.09	14.87	22.07	10.13	17.66	24.44
Cystine	2.28	2.29	2.52	2.09	2.06	2.19
ME (MJ kg <sup>-1</sup> ) DM	19.61	19.84	20.02	19.41	19.33	19.33
% ME from:						
protein	30.45	32.16	32.17	28.44	29.62	30.15

fat	52.20	51.11	51.30	52.85	52.06	51.52
carbohydrate	17.35	16.73	16.53	18.71	18.32	18.33

C - control group, E1 - group supplemented with liquid methionine at 3 g kg<sup>-1</sup> fresh matter, E2 - group supplemented with liquid methionine at 6 g kg<sup>-1</sup> fresh matter.

**Table 3. Performance traits of foxes (mean±SD)**

Specification		C	Group E1	E2
Body weight (kg)	Initial (10 weeks)	3.1 ± 0.5	3.2 ± 0.5	3.1 ± 0.4
	Final (26 weeks)	11.5 ± 1.0 <sup>B</sup>	12.8 ± 1.2 <sup>A</sup>	12.4 ± 1.0 <sup>A</sup>
Body conformation (points)	Trunk length (cm)	71.5 ± 5.5 <sup>B</sup>	78.3 ± 5.7 <sup>A</sup>	76.0 ± 4.9 <sup>A</sup>
	Body size and constitution (0-6)	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0
	Colour type (0-3)	3.0 ± 0.0	2.9 ± 0.3	3.0 ± 0.0
	Colour purity (0-3)	2.9 ± 0.3	2.7 ± 0.4	2.9 ± 0.3
	Fur quality (0-8)	5.1 ± 1.3 <sup>b</sup>	6.0 ± 1.0 <sup>a</sup>	5.9 ± 1.4 <sup>a</sup>
	Total scores (0-20)	17.0 ± 1.4 <sup>b</sup>	17.7 ± 1.3 <sup>a</sup>	17.7 ± 1.5 <sup>a</sup>
Average feed intake (g/day)		910.0 ± 170.0	905.0 ± 160.0	903.0 ± 160.0
Duration of the rearing period (days)		203.4 ± 9.0 <sup>Aa</sup>	197.2 ± 7.5 <sup>B</sup>	199.6 ± 6.4 <sup>b</sup>
Pelt evaluation	Pelt length (cm)	126.4 ± 8.1	128.7 ± 5.3	128.4 ± 5.0
	Pelt quality (1-3)	1.5 ± 0.2	1.3 ± 0.6	1.4 ± 0.5

<sup>a,b</sup>Values with different superscripts are significantly different at P < 0.05.

<sup>A,B</sup> Values with different superscripts are significantly different at P < 0.01.

C - control group, E1 - group supplemented with liquid methionine at 3 g kg<sup>-1</sup> fresh matter, E2 - group supplemented with liquid methionine at 6 g kg<sup>-1</sup> fresh matter.

**Table 4. Hematological and serum biochemical parameters (mean±SD)**

Specification	C	Group E1	E2
WBC (10 <sup>9</sup> /l)	9.3 ± 0.9	14.2 ± 1.1	11.7 ± 0.5
RBC (10 <sup>12</sup> /l)	8.5 ± 0.5	9.2 ± 0.4	8.8 ± 1.0
HGB (mmol/l)	18.5 ± 1.3	18.9 ± 1.0	18.8 ± 2.3
HCT (l/l)	50.6 ± 3.5	50.9 ± 1.6	50.4 ± 6.1
PLT (10 <sup>9</sup> /l)	195.4 ± 70.9	154.8 ± 59.7	246.4 ± 116.7
MCV (fl)	59.6 ± 2.6 <sup>Aa</sup>	55.0 ± 1.0 <sup>B</sup>	56.8 ± 1.1 <sup>b</sup>
MCH (fmol)	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
MCHC (mmol/l)	22.6 ± 0.1	23.0 ± 0.6	23.1 ± 0.6
RDW (%)	15.4 ± 0.5 <sup>a</sup>	14.8 ± 0.4 <sup>b</sup>	15.1 ± 0.3
MPV (%)	10.5 ± 1.1 <sup>B</sup>	13.2 ± 1.1 <sup>A</sup>	11.9 ± 0.90
GLU (mmol/l)	18.8 ± 10.0	20.8 ± 9.0	19.7 ± 10.3
UREA (mmol/l)	2.6 ± 0.2	2.1 ± 0.5	2.2 ± 0.8
CK (U/l)	613.0 ± 223.1	558.8 ± 277.8	679.0 ± 414.7
AST (U/l)	193.8 ± 29.5	185.0 ± 20.0	184.0 ± 20.0
ALT (U/l)	632.8 ± 258.2	510.1 ± 196.4	415.2 ± 175.4

<sup>a,b</sup>Values with different superscripts are significantly different at P < 0.05.

<sup>A,B</sup> Values with different superscripts are significantly different at P < 0.01.

C - control group, E1 - group supplemented with liquid methionine at 3 g kg<sup>-1</sup> fresh matter, E2 - group supplemented with liquid methionine at 6 g kg<sup>-1</sup> fresh matter.

WBC-white blood cell, RBC-red blood cell, HGB-hemoglobin, HCT-hematocrit, PLT-platelets, MCV-mean corpuscular volume, MCH-mean corpuscular hemoglobin, MCHC-mean corpuscular hemoglobin concentrations, RDW-red blood cell distribution width, MPV-mean platelet volume, GLU-glucose, UREA-urea, CK-creatinase, AST-aspartate aminotransferase, ALT-alanine aminotransferase

**Table 5. Effect of diets with different methionine levels on nutrient digestibility (%) in foxes (mean±SD)**

Specification	Group		
	AC	AE1	AE2
DM	83.54 ± 1.02	87.56 ± 1.27	86.19 ± 1.12
Crude protein	87.72 ± 1.79 <sup>b</sup>	91.55 ± 1.09 <sup>a</sup>	91.07 ± 1.89 <sup>a</sup>
Crude fat	97.12 ± 0.23	98.34 ± 0.55	97.38 ± 0.65
N-free extractives	65.87 ± 2.74	64.98 ± 2.05	65.04 ± 1.16
Specification	BC	BE1	BE2
DM	86.71 ± 1.08	85.48 ± 1.15	84.92 ± 0.87
Crude protein	85.77 ± 1.79 <sup>b</sup>	89.05 ± 1.92 <sup>a</sup>	88.76 ± 1.34
Crude fat	98.17 ± 0.19	98.12 ± 0.45	97.99 ± 0.43
N-free extractives	67.12 ± 1.66	66.26 ± 0.91	65.02 ± 2.42

<sup>a,b</sup>Values with different superscripts are significantly different at  $P < 0.05$ .

C - control group, E1 - group supplemented with liquid methionine at 3 g kg<sup>-1</sup> fresh matter, E2 - group supplemented with liquid methionine at 6 g kg<sup>-1</sup> fresh matter.

**Table 6. Effect of diets with different methionine levels on N balance in foxes (mean±SD)**

Specification	Group		
	AC	AE1	AE2
N intake (g/day)	8.2 ± 0.1 <sup>B</sup>	9.0 ± 0.1 <sup>A,B</sup>	9.4 ± 0.1 <sup>A</sup>
Faecal N (g/day)	1.0 ± 0.1	1.1 ± 0.2	1.0 ± 0.1
Urinary N (g/day)	4.4 ± 0.2	4.3 ± 0.4	4.1 ± 0.1
Digested N (g/day)	7.2 ± 0.2 <sup>B</sup>	7.9 ± 0.2 <sup>A,b</sup>	8.3 ± 0.1 <sup>A,a</sup>
Retained N (retained) (g/day)	2.8 ± 0.4 <sup>B</sup>	3.6 ± 0.5 <sup>A</sup>	4.2 ± 0.3 <sup>A</sup>
N retention as % of N intake	34.0 ± 4.0 <sup>B</sup>	40.2 ± 4.7 <sup>A</sup>	45.2 ± 4.0 <sup>A</sup>
N retention as % of digested N	38.6 ± 4.8 <sup>B</sup>	45.8 ± 4.8 <sup>A</sup>	50.8 ± 3.0 <sup>A</sup>
Specification	BC	BE1	BE2
N intake (g/day)	8.6 ± 0.2 <sup>B</sup>	9.5 ± 0.1 <sup>A,B</sup>	10.1 ± 0.1 <sup>A</sup>
Faecal N (g/day)	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.1
Urinary N (g/day)	4.6 ± 0.3	4.5 ± 0.6	4.3 ± 0.2
Digested N (g/day)	7.6 ± 0.3 <sup>B</sup>	8.5 ± 0.2 <sup>A,b</sup>	9.0 ± 0.2 <sup>A,a</sup>
Retained N (g/day)	2.9 ± 0.5 <sup>B</sup>	4.0 ± 0.5 <sup>A</sup>	4.6 ± 0.4 <sup>A</sup>
N retention as % of N intake	34.2 ± 5.3 <sup>B,b</sup>	41.8 ± 5.4 <sup>a</sup>	45.8 ± 3.1 <sup>A</sup>
N retention as % of digested N	39.0 ± 4.9 <sup>B,b</sup>	46.9 ± 6.4 <sup>a</sup>	51.6 ± 3.1 <sup>A</sup>

<sup>a,b</sup>Values with different superscripts are significantly different at  $P < 0.05$ .

<sup>A,B</sup> Values with different superscripts are significantly different at  $P < 0.01$ .

C - control group, E1 - group supplemented with liquid methionine at 3 g kg<sup>-1</sup> fresh matter, E2 - group supplemented with liquid methionine at 6 g kg<sup>-1</sup> fresh matter.

**Conclusions:** Our findings indicate that dietary supplementation with methionine at 14.87 (AE1) and 22.07(AE2)g kg<sup>-1</sup> feed DM from June to September, and at 17.66 (BE1) and 24.44 (BE2) g kg<sup>-1</sup> feed DM from October to December, at relatively stable cystine levels, had a beneficial influence on growth performance, protein digestibility and nitrogen retention in blue Arctic foxes. Increased dietary methionine concentrations had no negative effect on the health status of foxes. The highest dietary methionine level in group E2 did not lead to a further improvement in the analyzed parameters, compared with the values noted in group E1.

## REFERENCES

- AOAC (2003). Official Methods of Analysis. 17th edn. Association of Official Analytical Chemists, Arlington.
- Arctic Fox Standard (1999). Central Animal Breeding Office, Warszawa.
- Dahlman, T., T. Kiiskinen, J. Mäkelä, P. Niemelä, L. Syrjala-Qvist, J. Valaja, and T. Jalava (2002a). Digestibility and nitrogen utilisation of diets containing protein AT different level and supplemented with DL-methionine, L-methionine and L-lysine in blue fox

- (*Alopexlagopus*). Anim. Feed Sci. Technol. 98: 219-235.
- Dahlman, T., J. Valaja, P. Niemelä, and T. Jalava (2002b). Influence of protein level and supplementary L-methionine and lysine on growth performance and fur quality of blue fox (*Alopexlagopus*). Acta Agr. Scand. A-AN. 52: 174-182.
- Dahlman, T., J. Valaja, T. Jalava, and A. Skrede (2003). Growth and fur characteristics of blue fox (*Alopexlagopus*) fed diets with different protein levels and with or without DL-methionine supplementation in the growing-furring period. Can. J. Anim. Sci. 83(2): 239-245.
- Dahlman, T., J. Valaja, E. Venäläinen, T. Jalava, and I. Palonen (2004). Optimum dietary amino acid pattern and limiting order of some essential amino acids for growing-furring blue foxes (*Alopexlagopus*). Anim. Sci. 78(1): 77-86.
- Fau, D., J.G. Morris, and Q.R. Rogers (1987). Effects of high dietary methionine on activities of selected enzymes in the liver of kittens (*Felis domesticus*). Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol. 88: 551-555.
- Gugolek, A., M.O. Lorek, and D. Załocka (2002). Studies on the relationship between the body weight, trunk length and pelt size in Arctic foxes. Czech J. Anim. Sci. 47(8): 328-332.
- Gugolek, A., T. Wyczling, P. Janiszewski, P. Sobiech, P. Wyczling, and M. Konstantynowicz (2012). The effect of dietary methionine levels on the performance parameters of Arctic Foxes (*Vulpeslagopus*). Ann. Anim. Sci. 12(3): 393-401.
- Liu, H., G. Li, W. Zhong, D. Li, F. Liu, and W. Sun (2012). Supplemental dietary methionine affects the pelt quality and nutrient metabolism of raccoon dogs (*Nyctereutesprocyonoides*). Asian J. Anim. Vet. Adv. 7: 61-67.
- Lorek, M.O., A. Gugolek, and A. Hartman (2001). Nutrient digestibility and nitrogen retention in arctic foxes fed a diet containing cultures of probiotic bacteria. Czech J. Anim. Sci. 46: 485-488.
- Lorek, M.O., A. Gugolek, and A. Hartman (2002). Effect of feeding pellets to arctic foxes on their performance and selected morphological-biochemical blood indices. Czech J. Anim. Sci. 47(8): 333-338.
- Lorek, M.O., A. Hartman, A. Gugolek, and P. Matusevicius (2005). Effects of synthetic amino acids on morphological and biochemical blood parameters and health status of arctic foxes. Vet. ir Zoot. 30(52): 54-59.
- Matusevicius, P., A. Januskievicius, A. Gugolek, and A. Zilinskiene (2004). Sintetiniometioninoefektyumaslapiu (*Alopexlagopus* L.). Vet. ir Zoot. 25(47): 71-75.
- NRC (1982). Nutrient Requirements of Mink and Foxes. National Research Council. Second revised edition by the National Research Council, Subcommittee on Furbearer Nutrition. National Academic Press, Washington.
- Peura, J., I. Strandén, and E.A. Mäntysaari (2005). Genetic parameters in Finish blue fox population: Pelt character and live animal grading traits. Acta Agr. Scand. A-AN. 55(4): 137-144.
- Rana, S.V.S. and A. Chauhan (2000). Influence of methionine and zinc on liver collagen in molybdenotic rats. Relationship with lipid peroxidation. Biol. Trace Elem. Res. 73: 85-91.
- Saba, L., B. Likos-Grzesiak, B. Nowakowicz-Dębek, H. Bis-Wencel, J. Martyna, and W. Wnuk (2008). Effect of synthetic antioxidant on biochemical indices in blood of arctic fox (*Alopexlagopus*). Annales UMCS, Lublin – Polonia Sectio EE, XXVI 3: 3-18.
- Statistica PL (data analysis software system), StatSoft, Inc. 2007. version 8.0. [www.statsoft.com](http://www.statsoft.com).
- Tesseraud, S., S. Métayer-Coustard, A. Collin, and I. Seilliez (2009). Role of sulfur amino acids in controlling nutrient metabolism and cell functions: Implications for nutrition. Brit. J. Nutr. 101(8): 1132-1139.
- Winnicka, A. (2004). Wartości referencyjne podstawowych badań laboratoryjnych w weterynarii. Reference values of basic research in veterinary medicine. (in Polish) Wydawnictwo SGGW Warszawa.
- Zhang, H.H., G.Y. Li, E.J. Ren, X.M. Xing, Q. Wu, and F.H. Yang (2012a). Effects of diets with different protein and DL-methionine levels on growth performance and N-balance of growing minks. J. Anim. Phys. Anim. Nutr. 96: 436-441.
- Zhang, H.H., A.G. Yue, F.H. Liu, X.Y. Cao, F.H. Yang, and G.Y. Li (2012b). Effect of growth performance and N-balance of growing raccoon dogs fed reduced crude protein, lysine and DL-methionine supplementation diets. J. Anim. Vet. Adv. 11: 2187-2190.
- Zhang, H.H., F.H. Yang, Q.K. Jiang, Z.G. Yue, X.M. Xing, W.L. Sung, and G.Y. Li (2012c). Effect of low-protein, DL-methionine and lysine – supplemented diets on growth performance of blue fox (*Alopexlagopus*) during the growing-furring period. Proc. X<sup>th</sup> Intern. Sci. Congress in Fur Anim. Prod. 25-31.