# EFFECTS OF SYNTHETIC AMINO ACIDS ON MORPHOLOGICAL AND BIOCHEMICAL BLOOD PARAMETERS, AND ON HEALTH STATUS OF ARCTIC FOXES

Manfred O. Lorek[1], Areta Hartman[1], Andrzej Gugołek[1], Paulius Matusevičius[2]

[1] *Department of fur – bearing animal breeding, University of Warmia and Mazury in Olsztyn, 10–718 Olsztyn, Oczapowskiego 5, Poland*

[2] *Department of Animal Husbandry, Lithuania Veterinary Academy, Tilžės str. 18, LT–47181 Kaunas*

**Summary.** The effect of synthetic amino acids supplementation on morphological and biochemical blood parameters, and the general health status of Arctic foxes was investigated. The experiment comprised 120 Artctic foxes from weaning to slaughter, which were divided into 2 groups – control (I) and experimental (II). The control foxes were fed normal diet given to all animals in the farm. The foxes in experimental group were fed diet supplemented with synthetic amino acids: methionine, lysine and threonine, in the amount of 2 g of each amino acid per 100 g of total protein content in diet. Before slaughter blood samples from five randomly selected females in each group were collected for morphological and biochemical analysis. After slaughter five carcasses of males of each group were selected randomly for postmortem examinations.

The results of this experiment demonstrated no significant effect of synthetic amino acid supplementation on the morphological and biochemical parameters in blood and had no effect on the internal organs and alimentary tract segments examined.

**Keywords:** Arctic fox, synthetic amino acids, diet, blood, internal organs.

# SKIRTINGŲ AMINORŪGŠČIŲ ĮTAKA POLIARINIŲ LAPIŲ SVEIKATINGUMUI, KRAUJO MORFOLOGINIAMS IR BIOCHEMINIAMS RODIKLIAMS

**Santrauka.** Moksliniai tyrimai atlikti norint nustatyti aminorūgščių naudojimo tikslingumą poliarinių lapių racionuose, jų įtaką morfologiniams ir biocheminiams kraujo rodikliams bei bendrai sveikatos būklei. Racionai buvo papildyti sintetinėmis aminorūgštimis. Analogų principu bandymui atrinkta 120 poliarinių lapių. Bandymas tęsėsi nuo nujunkimo iki skerdimo.

Bandomosios grupės (II) lapių racionai buvo papildyti sintetinėmis aminorūgštimis – metioninu, lizinu ir treoninu – po 2 g kiekvienos aminorūgšties 100-ui g pašaro baltymų. Kontrolinės grupės (I) lapės buvo šeriamos tuo pačiu racionu, ne papildytu aminorūgštimis. Bandymo pabaigoje prieš skerdžiant atrinkta po penkias iš kiekvienos grupės pateles kraujo morfologiniams ir biocheminiams rodikliams nustatyti. Iš vienos ir kitos grupės atsitiktinai skrodimui atrinkta po penkias patinų skerdenėles fiziologinei būklei nustatyti.

Tyrimų rezultatai parodė, kad, papildžius lapių racionus aminorūgštimis, morfologiniai ir biocheminiai kraujo rodikliai atitiko fiziologinę normą ir nedarė įtakos tirtiems vidaus organų ir virškinamojo trakto segmentams.

**Raktažodžiai:** lapės, šėrimas, augimas, aminorūgštys, kraujas, vidaus organai.

**Introduction.** Data on amino acid requirements of fur-bearing carnivorous animals are scant. Sulfuric amino acids – methionine and cystine - are thought to be especially important (Borsting and Clausen, 1996), as they are not only components of fur keratin, but also influence protein utilization. The feeding standards for carnivorous animals provide information on methionine, cystine and tryptophan requirements only (Barabasz et al., 1994). That is why studies concerning particular amino acids in diets for Arctic foxes are only attempts at determining their optimum levels.

The results obtained by Riis and Borsting (1998) indicate that the concentrations of exogenous amino acids in diets for minks can be increased by adding synthetic methionine and lysine. According to Skrivan (1980), diets for foxes and minks should be supplemented with synthetic methionine and lysine in the period of winter fur development. When diets whose protein content was at a level of 22.5% ME of the ration were supplemented with synthetic DL–methionine, fur quality of experimental foxes improved significantly, compared with control ones (Dahlma and Blomstedt, 2000). A positive effect of synthetic methionione and blood and feather meals (components rich in sulfuric amino acids) on fur thickness in Arctic foxes was confirmed by Zoń et al. (2000). This problem was also investigated by Lorek et al. (2001), who determined the effects of diet supplementation with synthetic methionine on nutrient digestibility and conformation of Arctic foxes. They found that adding synthetic DL–methionine to diets had a beneficial effect on crude protein digestibility and fur quality parameters.

Studies on nutrient digestibility should be complemented with histopathological examinations of selected internal organs and alimentary tract segments, because nutritional factors are often responsible for changes in these organs, as well as morphological and biochemical blood indices (Kopczewski et al., 2002; Lorek et al., 1997, 2000; Rotkiewicz et al., 1995).

**Material and Methods.** The present studies, dealing with the effects of the levels of selected amino acids added to diets for growing Arctic foxes on blood morphological and biochemical indices, and the general health state of the animals, constitute a part of investigations on diet supplementation with synthetic amino acids, carried out at the Department of Fur-bearing Animal Breeding, University of Warmia and Mazury in

Olsztyn, Poland. The experiment was performed under production conditions, on 120 growing Arctic foxes, in the period from weaning to slaughter. They were placed in a house, in standard cages for growing foxes, four animals of the same sex in each. During weaning they were allocated to two groups, with equal numbers of males and females coming from particular litters (30 ♂ and 30 ♀). Two feeding periods were distinguished: growth and development (July to Septamber) and furring (October to November).

Diet components were typical of north-eastern Poland (Table 1). The composition and nutrient value of diets fully satisfied the requirements of growing foxes (Barabasz et al., 1994). Over the experimental period the foxes were fed ad libitum and had free access to water. All animals were subjected to prophylactic procedures, such as preventive vaccination and deworming.

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

| Specification | Period | | | |
|---|---|---|---|---|
| | Growth and development | | Furring | |
| Feed components: | | | | |
| beef | 9,0 | | 4,0 | |
| horsemeat | 12,0 | | 2,0 | |
| game bones | 15,0 | | 3,0 | |
| raw hard poultry offal | 12,0 | | 40,0 | |
| cooked hard poultry offal | 14,0 | | - | |
| soft poultry offal | - | | 24,0 | |
| blood meal | 5,0 | | - | |
| milk powder | 1,0 | | - | |
| ground wheat | 12,0 | | 7,0 | |
| ground barley | 13,0 | | 8,0 | |
| wheat bran | 2,0 | | - | |
| steamed potatoes | - | | 8,0 | |
| green forage and vegetables | 5,0 | | 4,0 | |
| Guyofox Plus 0.1% Fe | 1 kg / t | | 1 kg / t | |
| TOTAL | 100 | | 100 | |
| Nutritive value: | | | | |
| Percentage of energy from: | | | | |
| protein | 35 | | 32 | |
| fat | 43 | | 43 | |
| carbohydrates | 22 | | 25 | |
| Digestible protein (g / MJ ME) | 20 | | 17 | |
| Metabolizable energy (MJ / 100 g diet) | 0,637 | | 0,531 | |
| Amino acids g/100 g crude protein | Group | | Group | |
| | I | II | I | II |
| threonine | 3,39 | 5,02 | 3,23 | 4,97 |
| methionine | 2,19 | 3,30 | 1,75 | 3,58 |
| lysine | 5,87 | 7,79 | 5,12 | 6,43 |

The foxes of the control group (I) were fed diets given to all animals on the farm. The diets for the foxes of the experimental group (II) were supplemented with synthetic amino acids: methionine, lysine and threonine, in the amount of 2 g of each amino acid per 100 g of total protein contained in feed. The amino acid content of control and experimental diets is given in part 2 of Table 1. The levels of amino acids in diets were determined each time with an automatic amino acid analyzer AAA – T 339 M MICROTECHNA.

Before slaughter blood was collected (approx. 5 ml from the foot vein) for morphological and biochemical analyses from five females of each group, selected randomly. The analyses included the following blood tests, determined by generally accepted methods: red blood cell count (RBC) count, mean corpuscular volume (MCV), white blood cell count (WBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), lactate dehydrogenase (LDH), urea, total protein, and total cholesterol (Winnicka 1997).

After slaughter five carcasses of males of each group were selected randomly for postmortem examinations. Specimens of the following organs were taken for histopathological examinations: liver, spleen, lymph nodes, kidneys, cardiac muscle, lungs, stomach, duodenum, jejunum, and large intestine. The specimens were fixed in neutralized 10% formalin at pH 7.4, and paraffin-embedded. The microtome sections obtained were stained with hematoxylin and eosine (HE) and PAS, according to the Mc Manus method. Analysis of quantitative changes in the sections was made by a comparative method.

The results of blood tests were elaborated statistically by an analysis of variance in an orthogonal design, using the computer program Statistica 6.0.

**Results and Discussion.** The results obtained (Table 2) show that statistically significant differences between the groups were found only in mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). MCV was higher in group I, and MCHC – in group II. The other morphological indices examined, i.e. RBC, HGB, HCT and MCH, were very similar in both groups, and their values remained within the physiological norms and were consistent with those reported by other authors (Brandt et al., 1989). The white blood cell count (WBC) was lower in the experimental group; the lack of statistical differences in this index resulted from its high variation, i.e. 50.43% in the control group, and 36.83% in the experimental one. Similarly high statistical variation was noted in total hemoglobin concentration (HGB).

Table 2. **Morphological blood indices**

| Specification | Statistical measures | Group | |
|---|---|---|---|
| | | I | II |
| RBC ($10^{12}$/l) | n | 5 | 5 |
| | x | 9,20 | 9,19 |
| | v | 5,15 | 6,54 |
| MCV ($\mu m^3$) | x | 58,2$^{x}$ | 55,74$^{x}$ |
| | v | 2,20 | 2,79 |
| WBC ($10^9$/l) | x | 14,26 | 11,74 |
| | v | 50,43 | 36,83 |
| HGB (mmol) | x | 8,46 | 8,44 |
| | v | 49,07 | 50,12 |
| HCT (l) | x | 0,53 | 0,51 |
| | v | 6,88 | 8,21 |
| MCH (pg) | x | 1,13 | 1,13 |
| | v | 2,87 | 2,69 |
| MCHC (g/dl) | x | 19,54$^{x}$ | 20,36$^{x}$ |
| | v | 2,25 | 2,13 |

$^{x}$ – $P \leq 0.05$,
$^{xx}$ – $P \leq 0.01$.

The results of the study suggest that the experimental factor (diet supplementation with selected amino acids) had no significant effect on the morphological blood indices analyzed. The values of these parameters were similar to those obtained by many other authors (Brandt et al. 1989, Szymeczko et al., 1999). In the case of foxes the interpretation of morphological blood indices is difficult because this problem had been discussed in detail by a few authors only. Hematological examinations provide the basis for laboratory diagnostics. However, they not always enable precise determination of changes taking place in the organism, so analyses of biochemical blood indices are often necessary.

Indicatory enzymes are characteristic of particular cell structures, despite they are not liver-specific, they are present in all types of cells. They may be of cytoplasmic origin, like aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH), or of mitochondrial origin, like aspartate aminotransferase (ASTm). Changes in the serum concentrations of these enzymes reflect the degree of cell damage (Winnicka, 1997). The serum levels of enzymes (Table 3) did not show statistical differences, which was probably caused by high variation within the groups. AST level was higher in group II by 18 IU/l, whereas ALT and ALP levels were higher in group I by 27.6 IU/l and 34 IU/l respectively. In the studies conducted by Kopczewski et al., (2002) average activity of ALT exceeded the value of 141.6 U/l, considered normal by Brandt et al. (1989). The level of this enzyme varied from 91.75 to 217.46 U/l. These authors determined also AST activity as mean of three blood collections, which exceeded the reference value of 38.2 U/l. Also in own investigations its level was higher in both groups. The level of lactate dehydrogenase (LDH) was almost twofold higher in the experimental group, but this difference was not confirmed statistically. This probably does not require any further comments, as the reference values for this enzyme in the case of dogs are 105 to 1683 U/l (Winnicka, 1997).

The level of urea – the main final product of protein metabolism – was also evaluated. Its serum concentration depends on protein supply with the diet, decomposition of endogenous proteins or the secretory function of the kidneys. It is assumed that blood urea concentration increases when glomerular filtration rate decreases by over 50% (Kopczewski et al., 2002, Winnicka, 1997). According to Brandt et al. (1989), urea level in foxes should be 1.0 to 1.5 mmol/l. In the experiment performed by Kopczewski et al. (2002) urea concentration exceeded considerably the reference value, reaching 16.24 to 17.41 mmol/l. The values recorded in the present study, given in Table 3, were close to the reference level in both groups, which indicates that diet supplementation with amino acids had no negative effect on the kidney profile.

Table 3. **Biochemical blood indices**

| Specification | Statistical measures | Group | |
|---|---|---|---|
| | | I | II |
| AST IU/l | n | 5 | 5 |
| | x | 43 | 61 |
| | v | 17,09 | 32,02 |
| ALT IU/l | x | 87,4 | 59,8 |
| | v | 47,74 | 87,97 |
| ALP IU/l | x | 68,4 | 34,4 |
| | v | 50,84 | 66,56 |
| LDH IU/l | x | 324,8 | 610,2 |
| | v | 67,33 | 67,16 |
| Urea mmol/l | x | 1,73 | 1,67 |
| | v | 13,36 | 23,00 |
| Total protein g/l | x | 60,4 | 65,6 |
| | v | 6,89 | 15,42 |
| Total cholesterol mmol/l | x | $6,08^{x}$ | $5,10^{x}$ |
| | v | 7,93 | 14,54 |

$^{x}$ – $P \leq 0.05$,
$^{xx}$ – $P \leq 0.01$.

Total protein level remained within physiological norms (60 – 80 g/l), and amounted to 60.4 g/l in group I and 65.6 g/l in group II. In the studies carried out by Kopczewski et al. (2002) this parameter ranged from 55.10 to 73.30 g/l. The level of total cholesterol was statistically significantly higher in the control group. According to Winnicka (1997), total cholesterol in the blood serum usually increases with disturbances in bile acid transportation, and decreases with liver function impairment. The results obtained by Kopczewski et al. (2002) do not indicate any effect of feeding on serum cholesterol level, which varied from 5.43 to 6.95 mmol/l, slightly exceeding the norm of 5.17 mmol/l (Brandt et al. 1989).

The results of numerous histopathological examinations of internal organs and selected segments of the alimentary tract in foxes and minks show that diet may influence their health condition. According to many authors, special role is played by energy level, most often connected with the fat content of rations (Kopczewski et al., 2002; Lorek et al. 1997, 2000; Rotkiewicz et al. 1995; Sławoń et al. 2000). Amino acids added to diets had a positive effect on the livers of the experimental foxes (Table 4), which were characterized by less advanced parenchymatous and vacuolar degeneration, concerning mainly hepatocytes surrounding central veins. In the control foxes hepatocyte damage was more serious and observed within whole lobules. Circulatory disturbances, in the form of congestion and hemostasis, could take place during slaughter. A similar phenomenon was described by Bieguszewski et al. (1990). Intense lymphocyte proliferation in splenic and lymph follicles, accompanied by congestion of lymphatic organs, were observed in the foxes of groups I and II. There were no differences between the groups as regards morphological changes in the kidneys. The number of histopathological changes was similar in both groups.

Histopahtological examinations revealed cardiac muscle damage in two experimental foxes. However, it was minor and observed in the animals with foci of pulmonary fibrosis and bullous emphysema, which means that respiratory disorders were responsible for insufficient oxygen supply to the cardiac muscle. These changes were not connected with feeding. The stomachs of both control and experimental animals were characterized by increased amounts of mucus on the mucosa, and similar intensity of congestion of the mucosa and submucosa. As regards the duodenal mucosa, it was normal and showed no changes in all experimental foxes fed diets with amino acids. Pathological changes were minor in these animals, also in the jejunum and large intestine.

Histopathological examinations indicated no significant differences in the alimentary tract morphology in the foxes. The mucosal barrier integrity was preserved in both groups, and the morphological changes recorded could be caused by various factors in particular animals, not necessarily by diet supplementation with synthetic amino acids. The factors affecting negatively alimentary tract functions may be for instance poor microbiological quality of feed components or fat excess in the ration. Rouvinen and Niemela (1992) noted advanced intestinal inflammation in foxes fed diets containing fish fatty acids.

**Conclusions.**

1. Higher levels of amino acids added to diets for growing foxes did not cause changes in the morphological and biochemical pictures of blood.

2. Diet supplementation with methionine, lysine and threonine had a positive effect on liver morphology and metabolism.

3. Diet supplementation with amino acids had no effect on the morphology of the other internal organs and alimentary tract segments in the experimental foxes.

Table 4. **Histopathological changes in internal organs (no. of animals)**

| Organ / changes | Group | |
|---|---|---|
| | I | II |
| Liver | | |
| - normal, no changes | 1 | 2 |
| - congestion, hemostasis | 5 | 4 |
| - parenchymatous degeneration of hepatocytes | 5 | 3 |
| - vacuolar degeneration of hepatocytes | 4 | 1 |
| - focal necrosis of hepatocytes | 1 | 0 |
| - focal lymphocytic infiltrations | 1 | 1 |
| Spleen | | |
| - congestion, hemostasis | 3 | 2 |
| - hemosiderosis | 2 | 4 |
| - excitation and enlargement of splenic follicles | 3 | 3 |
| Lymph nodes | | |
| - congestion, hemostasis | 3 | 5 |
| - edema of the medulla | 0 | 2 |
| - excitation and enlargement of lymphatic follicles | 3 | 2 |
| - presence of plasma cells | 1 | 0 |
| Kidneys | | |
| - congestion, hemostasis | 4 | 4 |
| - parenchymatous degeneration of convoluted tubule epithelium | 5 | 5 |
| - glassy degeneration | 1 | 1 |
| - proliferative-mesangial inflammation of renal glomerules | | |
| - extracapillary inflammation of renal glomerules | 0 | 1 |
| - congestion of renal glomerules | 0 | 1 |
| | 1 | 1 |
| Heart | | |
| - normal, no changes | 5 | 4 |
| - parenchymatous degeneration of the cardiac muscle | 0 | 2 |
| Lungs | | |
| - normal, no changes | 3 | 3 |
| - congestion, hemostasis | 3 | 3 |
| - acute bullous emphysema | 1 | 1 |
| - chronic bullous emphysema | 0 | 1 |
| - focal fibrosis | 0 | 1 |

Table 5. **Histopathological changes of selected segments of the alimentary tract (no. of animals)**

| Organ / changes | Group | |
|---|---|---|
| | I | II |
| Stomach | | |
| - increased amount of mucus on the mucosa | 2 | 4 |
| - congestion of the mucosa | 3 | 4 |
| - congestion of the submucosa | 3 | 3 |
| Duodenum | | |
| - normal picture of the mucosa | 3 | 5 |
| - increased amount of mucus on the mucosa | 1 | 2 |
| - congestion of the mucosa | 3 | 2 |
| - eosinophilic infiltrations | 1 | 0 |
| - lymphocytic infiltrations in the lamina propria | 0 | 3 |
| - lymphocyte atrophy in the lamina propria | 3 | 3 |
| - presence of numerous plasma cells | 1 | 1 |
| - necrosis of villus apexes | 1 | 1 |
| Jejunum | | |
| - normal picture of the mucosa | 4 | 4 |
| - deformed villi | 1 | 1 |
| - increased amount of mucus on the mucosa | 2 | 1 |
| - congestion of the mucosa | 5 | 3 |
| - congestion of the submucosa | 1 | 1 |
| - lymphocytic infiltrations | 3 | 1 |
| - plasmocyte infiltrations | 3 | 1 |
| - eosinophilic infiltrations | 1 | 0 |
| - necrosis of villi | 1 | 0 |
| Large intestine | | |
| - normal picture of the mucosa | 4 | 3 |
| - increased amount of mucus on the mucosa | 1 | 1 |
| - congestion of the mucosa | 2 | 3 |
| - edema of the submucosa | 0 | 1 |

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