

EVALUATION OF BLOOD BIOCHEMISTRY, MORPHOLOGY AND THE AMOUNT OF ISTHMUSES IN THE EOSINOPHILS NUCLEI FOR STANDARD DARK MINK (*MUSTELA VISION*)

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Summary. The aim of our study was to determine the difference of some blood morphology and serum biochemistry parameters in 7 month old (females and males) standard dark minks (*Mustela vision*). We estimated that the average number of red blood cells (RBC) in blood of standard dark mink females ($6.47 \pm 0.10 \times 10^{12}/l$) and white blood cells (WBC) ($6.70 \pm 0.19 \times 10^9/l$) were statistically significantly lower ($p < 0.001$) compared to males, respectively RBC ($9.41 \pm 0.24 \times 10^{12}/l$) and WBC ($9.32 \pm 0.14 \times 10^9/l$). The average of haemoglobin (Hb) (160.65 ± 0.89 g/l) in the blood of female minks was statistically significantly lower ($p < 0.001$) than in males (166.27 ± 0.94 g/l). The average of albumins (Alb) (33.84 ± 0.38 g/l), calcium (Ca) (2.45 ± 0.02 mmol/l), phosphorus (P) (1.85 ± 0.05 mmol/l), magnesium (Mg) (0.87 ± 0.02 mmol/l), glucose (Glu) (5.80 ± 0.18 mmol/l), aspartate aminotransferase (AST) (87.28 ± 4.42 TV/l) were statistically significantly lower ($p < 0.001$) than in males respectively Alb (42.36 ± 0.44 g/l), Ca (2.70 ± 0.02 mmol/l), P (2.78 ± 0.03 mmol/l), Mg (1.29 ± 0.02 mmol/l), AST (111.72 ± 3.38 mmol/l). The amount of eosinophils with two and three isthmuses in the nuclei for male minks was significantly lower ($p = 0.0000142$; $\chi^2 = 27.7$; $df = 4$) compared to females, but with one isthmus in nuclei was significantly lower for females ($p = 0.00047$; $\chi^2 = 22.2$; $df = 5$).

Keywords: *Mustela vision*, blood morphology, eosinophils, isthmus.

KRAUJO MORFOLOGINIŲ, BIOCHEMINIŲ RODIKLIŲ IR ŠAŠMAUKŲ SKAIČIAUS EOZINOFILŲ BRANDUOLIULOSE ĮVERTINIMAS STANDARTINIŲ TAMSIŲJŲ AUDINIŲ KRAUJYJE (*MUSTELA VISION*)

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Santrauka. Šio darbo tikslas – įvertinti 7 mėnesių tamsiųjų standartinių audinių (*Mustela vision*) patelių ir patinų kraujo morfologinių ir biocheminių rodiklių skirtumus. Nustatyta, kad standartinių audinių patelių kraujyje vidutinis eritrocitų skaičius ($6,47 \pm 0,10 \times 10^{12}/l$) ir leukocitų skaičius ($6,70 \pm 0,19 \times 10^9/l$) statistiškai patikimai ($p < 0,001$) mažesnis negu patinų, atitinkamai $9,41 \pm 0,24 \times 10^{12}/l$ ir $9,32 \pm 0,14 \times 10^9/l$. Hemoglobino (Hb) vidutinis kiekis patelių kraujyje ($160,65 \pm 0,89$ g/l) statistiškai patikimai ($p < 0,001$) mažesnis negu patinų ($166,27 \pm 0,94$ g/l). Patelių kraujo serume vidutiniai albuminų (Alb) ($33,84 \pm 0,38$ g/l), kalcio (Ca) ($2,45 \pm 0,02$ mmol/l), fosforo (P) ($1,85 \pm 0,05$ mmol/l), magnio (Mg) ($0,87 \pm 0,02$ mmol/l), gliukozės (Glu) ($5,80 \pm 0,19$ mmol/l), aspartataminotransferazės (AST) ($87,28 \pm 4,42$ TV/l) kiekis statistiškai patikimai mažesnis ($p < 0,001$) už patinų rodiklius, atitinkamai Alb – $42,36 \pm 0,44$ g/l, Ca – $2,70 \pm 0,02$ mmol/l, P – $2,78 \pm 0,03$ mmol/l, Mg – $1,29 \pm 0,02$ mmol/l, Glu – $9,14 \pm 0,17$ mmol/l, AST – $111,72 \pm 3,38$ mmol/l. Nustatyta, kad eozinofilų kiekis su dviem ir trimis šašmaukomis branduolyje patinų kraujyje statistiškai patikimai mažesnis ($p = 0,0000142$; $\chi^2 = 27,7$; $df = 4$) už patelių, tuo tarpu eozinofilų su viena šašmauka branduolyje mažesnis patelių kraujyje ($p = 0,00047$; $\chi^2 = 22,2$; $df = 5$).

Raktažodžiai: *Mustela vision*, kraujo morfologija, eozinofilas, šašmauka.

Introduction. The first reports about the values of minks haematology and serum chemistry were published in 1935 and 1967 (Kennedy, 1935; Kubin and Mason, 1967). In 1972 Fletch S. M. and Karstad L. H. examined 45 male and 73 female minks using anaesthesia with α -

agonist and xylasine. They studied some blood parameters of healthy minks (RBC, Hb, MCV (mean cell volume), MCH (mean cell hemoglobin), and WBC) and stated that there was no significant difference in some parameters of the colours types and between males and females minks

(Fletch and Karstad, 1972). Weis report was about blood morphology (hematocrit (HCT), Hb, RBC, RBC indices, WBC, leukocyte differential cell count, platelet count, reticulocyte count, and Heinz body count) and serum biochemistry (blood serum urea nitrogen (BUN), sodium (Na), potassium (K), chloride (Cl), total carbon dioxide (CO₂), Glu, Ca, creatinine (Crea) and P) value for adult standard brown minks. There were no statistically significant differences between male and female mink (Weiss et al, 1993).

The objective of performed study was to propose the value of blood morphology and serum biochemistry reference range also the eosinophils nuclei segmentation significance differences for 7 month old standard dark minks.

Materials and methods. *Animals:* Eighty five samples of standard adult dark mink blood (*Mustela vison*) (55 females and 30 males) were collected during the autumn, spring and summer in the mink farm. All minks were clinically healthy, timely vaccinated and dehelminthized, of same age (7 months) and genotype, and were fed with the same balanced food. The ration included meat (beef, chicken, pork), fish (most of the time – cod), ground grain, scolded flour (most of the time – yeasty), vegetable oil, vitamin complexes, acetic and lactic acids and blood flour. In order to create serum blood was drained before the planned slaughter from the nail capillary into a capillary tube with heparin (Reflotron, Germany) and collected by jugular venipuncture into vacuum-proof (BD Vacutiner, United Kingdom) with no preservatives.

Blood analysis: Investigation was realized at the laboratory of clinical tests in the Lithuanian Veterinary Academy. The blood film was stained by Papenheim method (Kraft and Dürr, 1997), with May-Grunvald and Giemsa

stain (Merck KGaA, Germany). The blood films and the amount of isthmuses of the eosinophils' nuclei were measured with the YS 100 microscope (Nikon, Japan) with the 100 x oil objective that magnifies 100 times. The RBC and WBC count was estimated using Neubauer chamber (Paul Marienfeld GmbH&Co.KG, Germany) (Kraft and Dürr, 1997). The total concentration of total proteins (TP), Alb, Ca, inorganic P, Mg, Urea, Glu, AST and alanine aminotransferase (ALT) in blood was determined using an automated blood analyser Hitachi 705 (Hitachi, Japan), using DiaSys (Diagnostic Systems GmbH, Germany) reagents.

Statistical analyses: Statistical analyses were performed using SPSS No13 for Windows. Means and standard errors of traits of blood parameters were calculated. Student's t-test ($\alpha=0.05$) was used to ascertain the existence of significant differences between the groups. The differences of isthmuses' frequencies of the eosinophils and neutrophils nuclei were analysed. Differences between distributions of groups were evaluated with chi-square (χ^2) test of homogeneity.

Results. The results of the blood morphology parameters are given in Table 1.

The comparison of the RBC, WBC, Hb was significantly different between the females and males ($p<0,001$). The results of different leukocyte numbers, means, median and range are given in Table 2.

The mean values of eosinophils' were significantly different between females and males (9.02 ± 0.396 and 7.70 ± 0.545 %). The amount of segmented neutrophils was significantly different by sex (42.69 ± 0.905 and 52.70 ± 1.210 % for females and males respectively).

Table 1. **Blood morphology for 7 month old captive standard dark mink**

Parameter	Females (n=55)			Males (n=30)		
	Mean±Sem	Median	Range	Mean±Sem	Median	Range
RBC ($10^{12}/l$)	6.47±0.104 ***	6.48	5.43–8.56	9.41±0.235	9.56	6.50–12.40
WBC ($10^9/l$)	6.70±0.190 ***	6.80	3.40–8.70	9.32±0.144	9.50	7.40–10.50

ˆ – $p<0.1$; * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$ (Student test)

Table 2. **Differential leukocyte counts for 7 month old captive standard dark minks**

Parameter	Females (n=55)			Males (n=30)		
	Mean±Sem	Median	Range	Mean±Sem	Median	Range
Basophils (%)	0.16±0.050	0	0–1	0.17±0.069	0	0–1
Eosinophils (%)	9.02±0.396 ˆ a	9	2–14	7.70±0.545	7	2–13
Young neutrophils (%)	0.09±0.054	0	0–2	0.17±0.069	0	0–1
Band neutrophils (%)	7.05±0.366	7	2–14	7.33±0.513	7	2–12
Segmented neutrophils(%)	42.69±0.905 ***	43	30–58	52.70±1.210	53	39–65
Lymphocytes (%)	40.13±1.004 ***	42	21–53	31.00±1.323	32	16–45
Monocytes (%)	0.53±0.113	0	0–3	0.63±0.140	0	0–2

ˆ – $p<0,1$; * - $p<0,05$; ** - $p<0,01$; *** - $p<0,001$ (Student test)

a – $p<0,05$; b – $p<0,01$; c – $P<0,001$ Wilkonson test

Table 3. **Blood serum biochemistry for 7 month old captive Standard Dark Mink**

Parameter	Females (n=55)			Males (n=30)		
	Mean \pm Sem	Median	Range	Mean \pm Sem	Median	Range
Albumin, g/l	33.84 \pm 0.384 ***	33.70	30.9 – 38.9	42.36 \pm 0.443	42.65	37.8 – 47.5
Total protein, g/l	72.42 \pm 0.728	72.50	66.9 – 80.6	74.20 \pm 0.695	75.60	66.0 – 80.0
Total calcium, mmol/l	2.45 \pm 0.016 ***	2.45	2.30 – 2.58	2.70 \pm 0.024	2.73	2.32 – 2.91
Phosphorus, mmol/l	1.85 \pm 0.045 ***	1.83	1.47 – 2.35	2.78 \pm 0.027	2.75	2.56 – 3.23
Magnesium, mmol/l	0.87 \pm 0.017 ***	0.85	0.74 – 1.07	1.29 \pm 0.019	1.27	1.14 – 1.57
Glucose, mmol/l	5.80 \pm 0.181 ***	5.54	4.35 – 7.55	9.14 \pm 0.171	9.05	7.0 – 11.40
Urea, mmol/l	7.55 \pm 0.427	7.30	4.70 – 13.70	8.27 \pm 0.533	8.75	3.90 – 15.20
Hemoglobin (g/l)	160.65 \pm 0.888 ***	160.00	149.0 – 175.0	166.27 \pm 0.942	168.00	156.0 – 175.0
AST, TV/l	87.28 \pm 4.421 ***	89.40	13.2 – 128.0	111.72 \pm 3.377	112.15	76.30– 166.00
ALT, TV/l	91.94 \pm 8.174 *	77.40	62.70 – 266.90	112.94 \pm 5.181	103.05	76.60– 212.60

‘ – $p < 0,1$; * - $p < 0,05$; ** - $p < 0,01$; *** - $p < 0,001$ (Student test)

The results of the blood serum biochemistry parameters are given in Table 3.

After counting the amount of isthmuses of the eosinophils nuclei it became evident that females have less eosinophils with one isthmus in the nuclei 20.4% (101/495) than males 37.7% (87/231). Males have less eosinophils with two 31.2% (72/231) and three 22.5% (52/231) isthmus in the nuclei Figure 1.

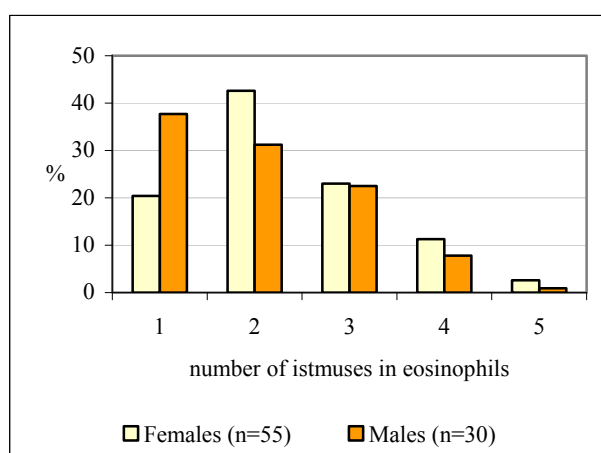


Figure 1 **The amount of the eosinophils repartition in male and female blood** ($p=0.0000142$; $\chi^2=27.7$; $df=4$)

Further isthmus research showed (Figure 2) that the higher percentage of females had statistically significantly reliable one 25.5% (14/55) or two 43.6% (24/55) eosinophils with one isthmus in the nucleus than males, respectively males 10.0% (3/30) and 30.0% (9/30). While higher percentage of males had three 30.0% (9/30), four 26.7% (8/30) or seven 3.3% (1/30) eosinophils with one isthmus in the nucleus, respectively females had three eosinophils 23.6% (13/55) and not four or seven. A higher percentage of females had statistically reliable four 23.6% (13/55), five 32.7% (18/55) or six 7.3% (4/55) eosinophils with two isthmuses in the nucleus were. While the higher percentage of males had one 13.3% (4/30), two 50.0% (15/30) or three 13.3% (4/30) eosinophils in the nucleus

with two isthmuses, respectively females had one 3.6% (2/55), two 20.0% (11/55) and three 12.7% (7/55) eosinophils. Also a higher percentage of females had no statistically reliable three 18.2% (10/55), four 7.3% (4/55), five 3.6% (2/55) or six 3.6% (2/55) eosinophils with three isthmuses in the nucleus were found, while the males had one 33.3% (10/30) or two 30.0% (9/30) eosinophils with three isthmuses in the nucleus. It was found out that the higher percentage of females had statistically reliable with one 45.5% (25/55), two 20.0% (11/55) or three 5.5% (3/55) eosinophils whose nuclei had four isthmuses, respectively males only had one 33.3% (10/30) and two 6.7% (2/30), none had three. Meanwhile, the higher percentage of males had statistically reliable four 3.3% (1/30) eosinophils with four isthmuses in the nucleus. The eosinophils with five isthmuses in nuclei were no statistically reliable between females and males.

Discussion. Fletch, evaluated some blood morphologies parameters and stated that there was no significant difference in the standard deviation of PCV, Hb or total RBC count of the colour types bled ($p > 0.1$) similarly between males and females ($p > 0.05$) (Fletch and Karstad, 1972). Our result of the blood morphology and serum biochemistry showed there were significant difference between the females and males for standard dark minks (Table 1 and 2). Hb concentration, RBC count, WBC count and the different types of leucocytes in the leucograms in our study were statistically significantly lower in females than males ($p < 0.001$). Also the eosinophils and lymphocytes count were statistically significantly lower in males than females ($p < 0.001$ and $p < 0.05$). Our results of the blood morphology and serum biochemistry were significantly different from the known Standard brown minks published values (Weiss et al., 1994). Weiss proposed that hematologic and serum chemistry reference values were no statistically significant differences between males and females minks. HCT, Hb concentration and RBC count in his study were 10 to 20% lower compared to Fletch and Karstad studies (Weiss et al., 1994).

Report of serum biochemistry data for standard dark mink is limited (Kubin and Mason, 1967).

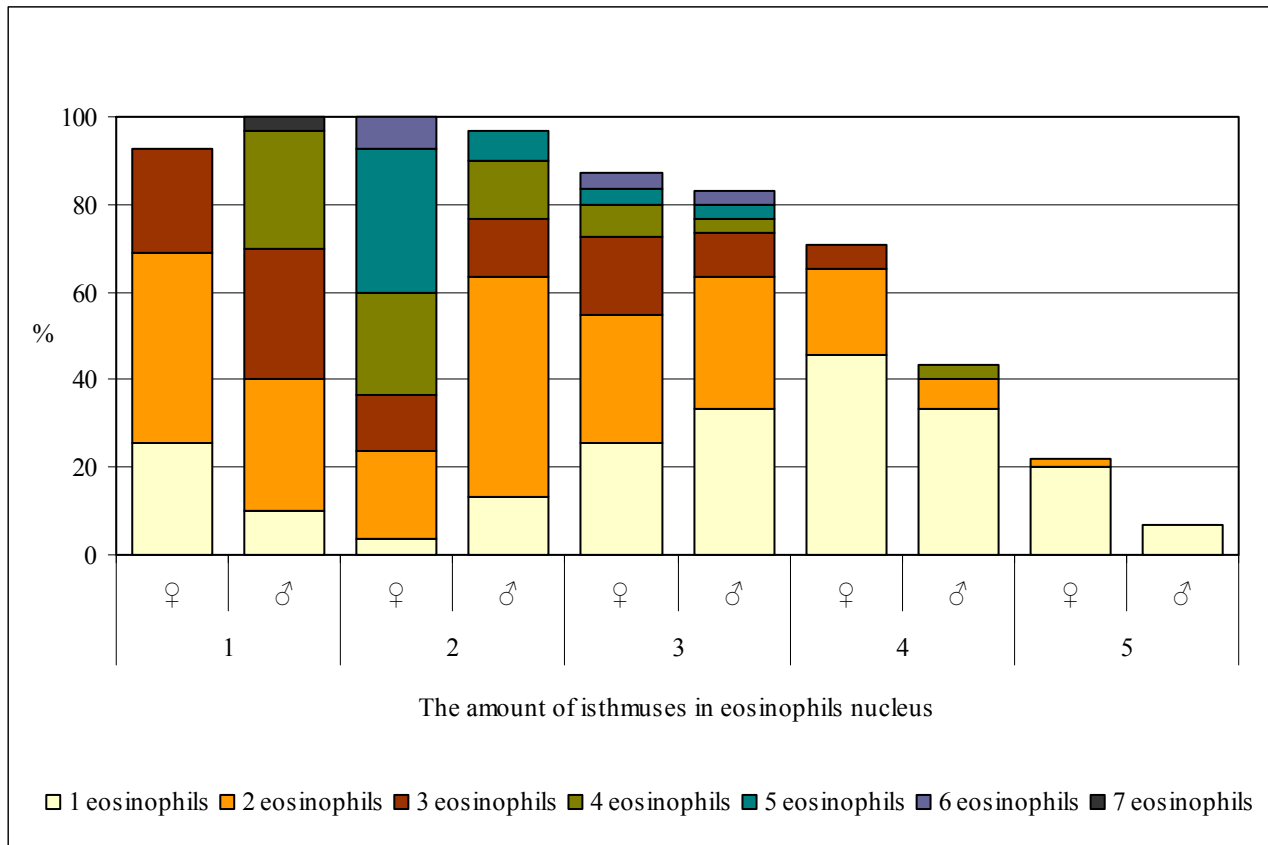
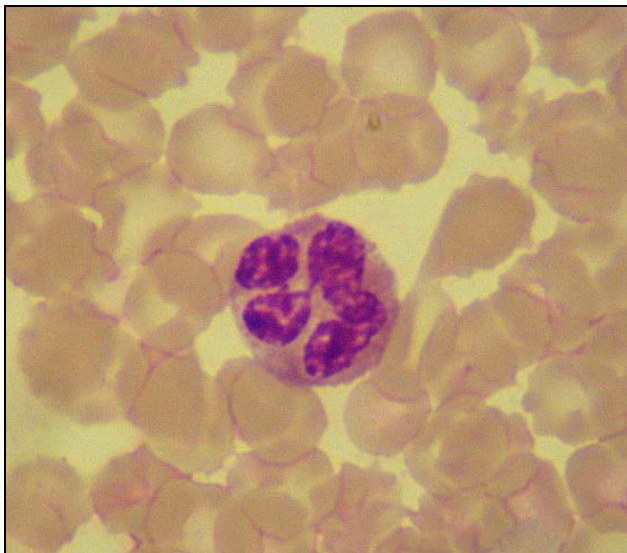
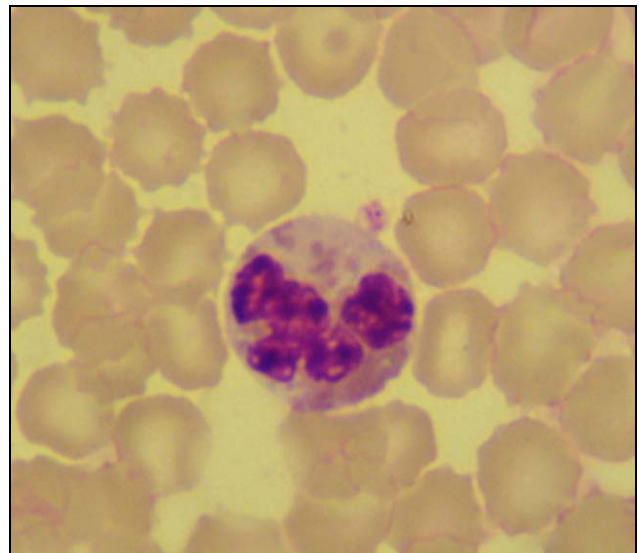


Figure 2. The amount of mink with isthmuses in the eosinophils nucleus. The values evaluated in percentage; ♀=female, ♂=male



Picture 1. Eosinophil with three isthmuses in the nuclei



Picture 2. Eosinophil with four isthmuses in the nuclei

During our study the average values of Alb, Ca, P, Mg and AST were statistically reliably lower ($p < 0.001$) than those of males. Our values of serum glucose of Standard dark mink females were statistically reliably lower ($p < 0.001$) than the serum values of Glu of mink males. The values for serum Glu in Weiss study were similar to

those of others in which blood samples were collected under anesthesia (Weiss et al., 1994).

The eosinophils nucleuses have a lighter blue colour. They also have few segments and filaments that are distinct. The eosinophils life period is 10–12 days (Schalm et al., 1975; Canfield, 1998; Willard et al., 1999). Leader in

his study determined that in normal eosinophils of mink the nuclei usually contained one, two or three lobes (Leader et al, 1963), sometimes nuclei may be unlobed (Canfield, 1998). Sparrevohn determined that the 70-80% of blood eosinophils has two isthmuses in the nuclei and other eosinophils one, three or neither isthmuses (Sparrevohn et al., 1967). In our study we found that eosinophils' nuclei contained one, two, three, four, five isthmuses (Picture 1 and 2).

Often the presence of hipersegmented eosinophils in blood may imply allergies or illnesses (Yamamoto et al., 2001). Also hipersegmented eosinophils used as atopic dermatitis marker.

Reagan (1998) stated that we can watch the hipersegmentation of nucleus, if the blood cells linger in the peripheral blood circulation more than their life period or if there is lack of vitamin B12 in organism. All minks in our investigation were clinically healthy wherefore we can suppose that male eosinophils regenerate faster than female. To confirm this proposition more thorough analyses are necessary.

In our study a different segmentation of the eosinophils of females and males was discovered. In the blood of females there were more eosinophils with segmented nuclei than in the blood of males.

Conclusions.

1. The values of standard dark minks (*Mustela vison*) red blood cells, white blood cells and haemoglobin are statistically significantly different between the females and males ($p < 0.001$).

2. It was determined, that the amount of eosinophils in the blood with two and three isthmuses in the nuclei in males is statistically significantly lower ($p = 0.0000142$; $\chi^2 = 27.7$; $df = 4$) than female eosinophils, whereas in females with one isthmus in the nucleus is statistically lower ($p = 0.00047$; $\chi^2 = 22.2$; $df = 5$) than males.

3. The average of albumin, total calcium, phosphorus, magnesium, glucose, aspartate aminotransferase is statistically significantly different between the females and males for Standard Dark minks ($p < 0.001$).

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