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TISSUE PATHOMORPHOLOGY AND IMMUNOHISTOCHEMISTRY IN MINK *(NEOVISON VISON)* FED BLOOD PLASMA SUPPLEMENTED DIET IN THE PERIOD OF PREPARATION FOR BREEDING

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Abstract

The research carried out at a mink farm aimed to determine the effect of blood plasma supplemented diet applied at the period preparing mink for reproduction on the animal organism. The studies included four groups of mink. The control group received a non-supplemented diet, while the experimental groups had feed with additive of 0.5%, 1.5%, and 2.5% of beef-pork plasma in the daily feed ration. The pathomorphological and immunohistochemical evaluation was performed on the liver, kidneys, lymph nodes, spleen, and bowel from all the groups. Pathomorphological and immunohistochemical changes of various intensity were observed in the examined organs from all experimental groups.

Key words: mink, blood plasma supplemented diet, internal organs, pathomorphology, immunohistochemistry.

New nutritional strategies for mink aim, among others, to increase an energy level in feed rations through an increased content of energy from fat. It is expected to improve reproduction performance and ensure high quality skins. However, such feeding may cause metabolic disorders at the cellular level, often imperceptible during a short production cycle of the species. The period of mink preparation for breeding is the time when animal is getting adapted to higher burden of the organism. At that time, mink needs a feed ration of low-caloricity and high-protein content, as well as appropriate hygiene and sanitary conditions. Some authors, however, recommend restrictive feeding routines at the preparatory period so as to ensure good body condition and welfare of animals (3, 4, 6, 13). These problems have been widely addressed but they still lack good solutions. Recently, blood plasma has been frequently incorporated into diet of many animal species, and its high nutritional value as a feed supplement was confirmed (7, 8, 9, 11, 14, 17). So far, blood plasma has not been used in carnivorous fur animal feeding, therefore, the present study was conducted to determine the effect of supplemental dietary plasma on mink organism during the preparatory period for the breeding.

Material and Methods

The study was performed on 120 pastel mink (*Neovison vison*) being at the preparatory period for the breeding. The animals were divided into four equal groups. The experimental group D1 received 0.5% of beef-pork plasma dietary additive to a daily feed ration, group D2 – 1.5% additive, group D3 – 2.5% additive, and the control group C was fed a non-supplemented feed. Nutritional value of a feed ration was tailored to the investigated period and satisfied the nutritional requirements (1). Throughout the study period, the animals were maintained under supervision of veterinary and animal husbandry staff and were provided with suitable prophylactic treatments. Animal feed ration included 60% of cod-fillet offals, 5% of mackerel heads, 20% of poultry bowels, 5% of poultry heads and paws, 1% of blood meal, 5% of extruded wheat, 1% of wheat bran, and 3% of water. The EM level (51.8% of protein, 37.1% of fat, 11.1% of carbohydrates) reached 1,110

kcal/feed kg. The basic components of blood plasma were protein (min. 70%), fat (max. 2.0%), fiber (max. 0.3%), and ash (max. 14.0%). The plasma supplementation started on 5th d before mating. The animals were slaughtered after the preparation period according to the consent provided by the Local Ethics Committee. The males after the post-mating period were slaughtered and sections of the liver, kidneys, lymph nodes, spleen, and bowel were collected from all animals of the studied groups for histopathological, histochemical, and immunohistochemical examinations. The tissue material was fixed in 10% neutral buffered formalin for paraffin block preparation, then sectioned and stained with haematoxylin and eosin. The histochemical examinations for neutral fats were performed in the liver and kidneys using Daddi`s Sudan method for lipids. The bowel and lymph node sections were additionally processed for immunohistochemical assays. The DAKO En vision and Dual Link Systems were applied. The reagent was visualised with a colour reaction by 3,3-diaminobenzidine (DAKO) as a chromogene. The following antibodies were applied: anti-rabbit polyclonal antibody CD3 (DAKO) diluted at 1:100 was used to identify T lymphocytes; anti-mouse monoclonal primary antibody CD79α (DAKO) at 1:100 dilution detected B lymphocytes; and finally, monoclonal primary antibody DEF diluted at 1:200 served to detect defensins. The quantitative study of B and T lymphocytes in the small intestine wall was performed using a computer assisted analysis of microscopy images (NIS Elements BR-2.20, Laboratory Imaging) coupled with a digital camera and light microscope. The percentage of positive cells in five fields of view including a hundred of cells at 20x magnification of the objective-lens were calculated.

Results

The internal organs collected post mortem from the control group of animals displayed the appropriate microscopic structure. The liver and kidneys showed fine vacuoles localised in the peripheral zone of the lobule and renal proximal tubular epithelial cells. The Sudan IV staining visualised the presence of orange-stained bodies and indicated the lipid nature of the vacuoles (Fig. 1). The liver obtained from the experimental group D1 showed a partially blurred lobular structure and the presence of swollen hepatocytes with fine vacuoles of lipid nature in the cytoplasm, localised in the peripheral zone of the lobules. In the central vein and sinusoidal spaces, a high number of red blood cells was noted. That evidenced the organ congestion. The presence of single inflammatory foci composed of mononuclear cells localising between the lobules and grouping around blood vessels was also detected. The kidneys collected from the same group, showed changes similar to the liver lesions, displaying small vacuoles of the lipid nature in the renal tubular epithelial cells as well as some parenchymatous degeneration. Tubular cells undergoing parenchymal degeneration changes had only faintly transparent cytoplasm and poorly visible cell nucleus. They often appeared to intend toward the centre making the tubular lumen decreasing and showing a star-like or slit-like shape. The boundaries between epithelial cells got partially blurred. Focal mononuclear cell infiltration was observed between the tubules. The renal cortex showed numerous haemorrhages. The spleen was found to be congested, which was manifested by a great amount of blood accumulated around the splenic follicles and splenic venous sinuses. The microscopic changes of the intestines were limited primarily to the mucous membrane. There were visible mixed inflammatory infiltrates. The immunohistochemical analysis revealed the dominance of T lymphocytes (51%) in the inflammatory infiltrations as compared to the control group (40%); while the reaction to the B lymphocytes involved 18% of the cells and was higher than in the control group (15%). The lymph nodes exhibited proliferative reaction properties. Partial blurring of the follicle structure was observed, as well as the lymphatic infiltrates in which the predominance of T lymphocytes responsible for development of cell-mediated immune response was demonstrated immunohistochemically (Fig. 2-4).

Group D2 displayed the most advanced histopathological changes in the examined organs. The most visible changes were observed in the structure of the kidneys. They were manifested by numerous inflammatory infiltrates between the tubules, increased number of vacuoles in the cytoplasm of renal tubules, and haemorrhages in the renal cortex (often associated with infection process).Vacuoles in the hepatocyte cytoplasm, stained orange with Sudan IV, often pushed the cell nucleus aside to the peripheral zone or even made it to disappear. They were localised not only in the peripheral zone of the lobule but in the central area as well. Many massive inflammatory infiltrates were also noted around the blood vessels and between the hepatic lobules. The intense inflammatory infiltrates extending into the mucosal, often surrounding the intestinal crypts, were also seen in the fragments of the intestines of mink from group D2. The immunohistochemical examination indicated substantial dominance of T lymphocytes (76%), whereas B lymphocytes were found primarily in the surface layer of the mucus and constituted 20% (Fig. 5).

The analyses of the histological sections from group D3 samples revealed the histopathological changes of intensity comparable to that observed in group D1. There were visible fat vacuoles in renal tubules and hepatocytes from the peripheral lobular zone as well as focal inflammatory infiltrates in the kidneys and liver. Intestinal mucus also displayed inflammatory infiltrates consisting predominantly of T lymphocytes (55%) and B lymphocytes (17%). Besides, abundant haemorrhages were observed in the renal cortex.

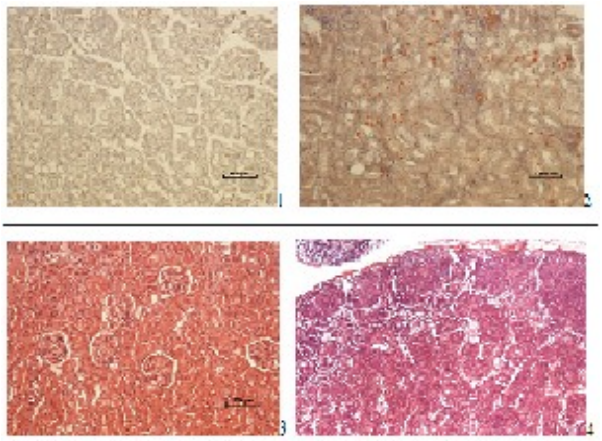

Fig. 1. Group C. 1 - expression of CD79α antigen labelling B lymphocytes in intestinal mucus, IHC stain, approx. 100x; 2 - reaction to the presence of Sudan-stained bodies in kidney epithelial cells, Sudan IV stain, approx. 100x; 3 - kidney, H.E., approx. 100x; 4 –lymph node, H.E., approx. 50x.

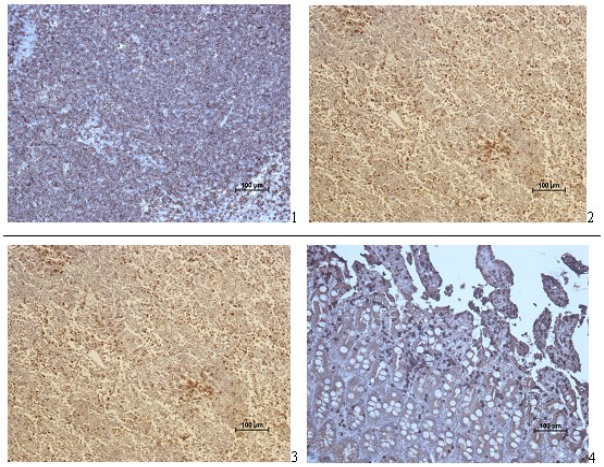

Fig. 2. Group D1. expression of antigen 1-CD3 identifying T lymphocytes in lymph nodes, IHC stain, approx. 100x; 2 – CD79α identifying B lymphocytes in lymph nodes, IHC stain, approx. 100x; 3 – CD79α identifying B lymphocytes in lymph nodes, IHC stain, approx. 100x; 4 – CD3 identifying T lymphocytes in intestinal mucus, IHC stain approx. 100x.

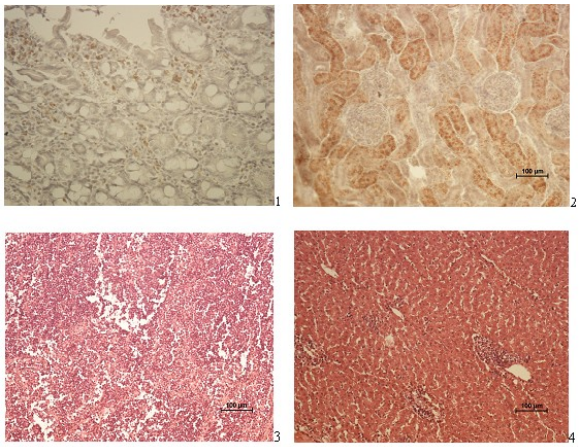

Fig. 3. Group D1. 1 – expression of CD79α antigen identifying B lymphocytes in intestinal mucus, IHC stain, approx. 200x; 2 – reaction to the presence of Sudan-stained bodies in kidney epithelial cells, Sudan IV stain, approx. 100x; 3 – reactive lymph node, H.E., approx. 100x; 4 – fine inflammatory infiltrates in the liver, H.E., approx. 100x.

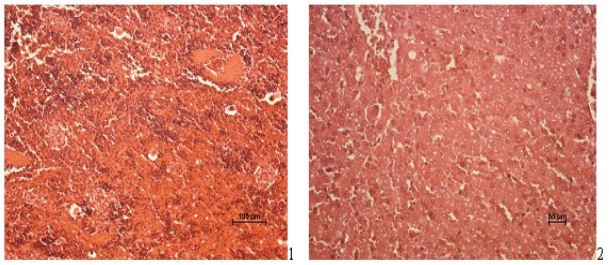

Fig. 4. Group D1. 1 – Congestion of the spleen, H.E., approx. 100x; 2 – congestion of the liver, H.E., approx. 200x.

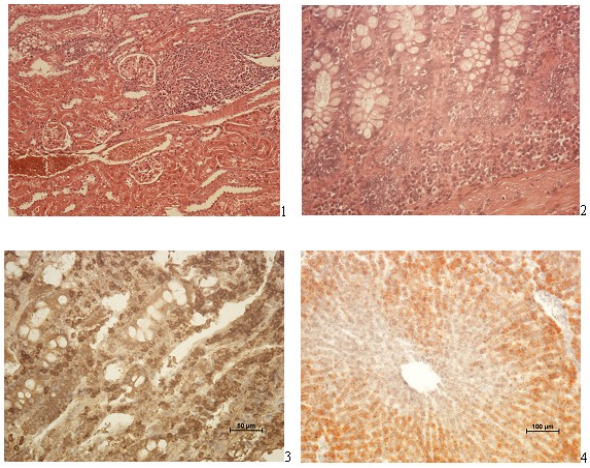

Fig. 5. Group D2. 1 – massive inflammatory infiltrates in the kidney, H.E., approx. 100x; 2 – inflammatory infiltrate surrounding intestinal crypts, H.E., approx. 200x; 3 – enhanced expression of CD4 antigen identifying T lymphocytes in intestinal mucus, IHC stain, approx. 200x; 4 – reaction to the presence of Sudan-stained bodies in hepatocytes, Sudan IV stain, approx. 100x.

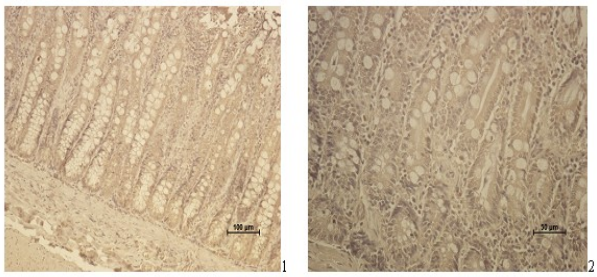

Fig. 6. Group D2. 1 – defensin expression in intestinal crypts and inflammatory cells, IHC stain, approx. 100x; 2 – defensin expression in intestinal crypts; IHC stain, 200x.

The immunohistochemical examination showed positive reaction to the presence of defensins in all groups. The reaction intensity differed between the groups. The most intensive reaction was observed in group D2 (Fig. 6). Pronounced expression of defensins was detected in the intestinal epithelium, intestinal crypts, and inflammatory cells. The reaction was granular and restricted mainly to the cell cytoplasm. In group D1 and D3, the expression was less intensive, yet noticeable in the same structures as in group D2.

Discussion

Blood plasma products are used in nutrition of a number of animal species because they are rich in specific proteins. The plasma proteins include ca 50% of albumin fraction, 25% of globulins, 5% of fibrinogen, and 20% of other proteins or peptides, including haptoglobin, transferrin, growth factors. The plasma products incorporated into pig diet was found to improve animal performance. In some cases in poultry, however, the preparations decreased bird body weight (7, 8, 11, 14). A histological analysis of the jejunal wall structure in poultry fed a diet supplemented with blood plasma, performed by Jamroz (7), demonstrated that all groups of birds had a normally functioning intestinal wall, which is contrary to the results obtained in the present study. Likewise, other authors (8, 9, 11, 17) did not note any differences in the mucus structure, height of intestinal villi, depth of crypts, or cell proliferation. The research conducted on mink showed inconsiderable histopathological changes in animals fed plasma additive as compared to the control mink with a regular diet characteristic for carnivorous fur animals (2, 12).

The immunological system of animals is able to inhibit excessive activation of immune response before tissues get injured. However, in numerous cases it shows tolerance to antigens. Immune reaction mounting is controlled by the highly completed system *via* both, direct cell to cell contact and cytokines regulating the response. The maintenance of immunological tolerance requires involvement of large number of immune cells (5). The peripheral lymphoid organs are sites where mature B and T lymphocytes are exposed to foreign antigens and initiate the adaptive immune reaction. The immunohistochemical analyses indicated the dominance of T lymphocytes, while B lymphocytes were commonly identified in the surface layer of the mucous membrane. The present infiltrates had no destructive potential but were organised similarly to the peripheral lymphoid tissue. Lymphocytes circulating in the blood stream and lymphatic system encounter the antigens they remember and distinguish subpopulations of these cells as well. The most important antigens include: CD3 (characteristic of T lymphocyte), CD79α (characteristic of B lymphocyte), CD4 and CD8 (subpopulations of T lymphocytes – helpers and suppressors, respectively), and CD34 (typical of blastic cells). Estimation of the percentage and absolute number of T lymphocytes (CD3) proves to be very important in quantitative evaluation of cell-mediated response, while application of antibody CD79α to label B cells enables to identify the mature and immature forms of B lymphocytes. Importantly, these antigens are of high value in histopathologic diagnosis of neoplasms (5, 15).

Defensins are recognised as potent peptides with antimicrobial properties as well as regulators of the immune system activity. They play a prominent role in innate and adaptive immunity being able to mediate acute inflammatory response. Intestinal defensins secreted by Paneth cells in the small intestines are extremely useful in preventing inflammatory conditions, which was confirmed in the experimental studies on mice (10, 18). This indicates their role in the activation of the immune system, as it was observed in mink fed a blood plasma supplemented diet. Valdovska and Pilmane (16) highlighted a strict correlation between a systemic cellular inflammation level and the intensity of defensin expression in mink liver that profoundly evidences defensin-stimulated cell proliferation as the compensation mechanism. The researches have confirmed the enhancement of the immune response. However, intensification or blockage of the functional capacity of lymphocytes and obtaining a balance between mediators of immune activation and immune suppression for optimal host defence are the essential elements of immunotherapy. Appropriate handling of these elements is critical because it can cause or trigger the irregularity of the immune system.

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