DE GRUYTER OPEN

DOI: 10.2478/fv-2018-0004

FOLIA VETERINARIA, 62, 1: 25—31, 2018

ADDITION OF DRIED BLOOD PLASMA TO FEED OF MINKS DURING LACTATION AND REARING OF KITS

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ABSTRACT

Studies on the effects of dried blood plasma on certain health parameters of mink (of the pastel variety) were conducted during lactation and the rearing of the kits. The study included two groups of mink (control group and experimental group). Animals in the experimental group received 0.5 % of dry blood plasma and the control group did not receive the plasma supplement. From the whole blood of both groups the direct and indirect haematological indices were recorded. Also, in both groups, the histopathological and immunohistochemical studies were performed on the: liver, kidneys, lymph nodes, spleen, and intestinal segments. From the experimental group some of the organs examined demonstrated slightly altered histopathology.

Key words: blood plasma; defensins; haematological markers; histopathological examination; immunohistochemistry; mink

INTRODUCTION

Birth and lactation are a particular burden for all mammalian females. Inappropriate nutrition during pregnancy and lactation can adversely affect the health of the mother, the offspring and the number of litters. Food and organizational errors can lead to: the loss of foetuses directly before labour; the newborns just after birth; or the death of the young. Breeders are striving for the best reproduction rates because only then can successful breeding be accomplished. Furthermore, the use of growth promoting and therapeutic antibiotics in a piglet's feed may generate antimicrobial resistance which can be transferred to humans. For this reason, the search for other products and alternatives to the use of antibiotics, began. The spray dried plasma seems to be the most promising [18].

In recent years, dried blood plasma has been used in many animal species and these formulas have confirmed their high value as a feed additive [9, 11, 12, 14, 15, 16]. They are rich in specific proteins and consists of about 50 % of

the albumin fraction, 25 % of globulins, 5 % of fibrinogen, and 20 percent of other proteins, including: haptoglobin, transferrin, growth factors and other proteins or peptides [11, 13]. However, they have not been used in the feeding of carnivores; that is why studies were conducted to determine the effects of plasma addition to feed on the histopathological and immunohistochemical image of mink's tissue *(Neovison vison)* during lactation and the rearing of the kits.

MATERIALS AND METHODS

This study included two groups of mink *(Neovison vison)* of the pastel variety. Each group consisted of 30 mink and all animals were 3 months old. The study was carried out during lactation and the rearing of the kits. During those time frames, dried blood plasma was added to the feed of the mink. The plasma used in the experiment (SDAP) is a ready-to-use product, manufactured using strictly defined procedures. The drying of plasma was carried out in the Anicet's BallTec dryer [13], at 55 °C, and then sterilized by heating to 200 °C. The resulting product in a dusty form was packed in paper bags, ventilated and stored in accordance with the manufacturer's instructions in cool and dark room. The basic composition of the plasma was: protein min. 70.0 %, fat max. 2.0 %, fibre max. 0.3 %, and ash max. 14.0 %. The experimental group D received 0.5 % of beef-pork plasma dietary additive to a daily feed ration and the control group C received no supplemental dietary plasma. The animals were provided with veterinary and zootechnical care and subjected to appropriate prophylactic treatment throughout the study period. The animals of both groups showed no signs of disease throughout the experiment. Food poisoning, diarrhea or other gastrointestinal symptoms were not observed.

Blood samples for haematological tests were collected from the females twice: during lactation and at the time of rearing. The blood samples were taken from vena saphena, and collected in Sarstedt S-Monovette tubes, in the morning, before the feeding of the animals. The following parameters were recorded: red blood count (RBC), haematocrit (Ht), haemoglobin (Hb), mean red cell volume (MCV) and mean corpuscular haemoglobin (MCH), and the mean corpuscular haemoglobin concentration (MCHC). In addition, the total number of white blood cells (WBC), red blood cell distribution width (RDW), thrombocyte (PLT), mean platelet volume (MPV), and the platelet anisocyte (PDW) were also recorded. The haematological parameters were determined using the MS-4vet.

After weaning, the females from both groups were slaughtered, according to the Local Ethical Commission (No. 48/2011). Samples of the following organs were collected: liver, kidney, lymph nodes, spleen, and the small intestine. They were subjected to the routine microscopic evaluation and immunohistochemical studies. The sections of the internal organs were collected from both the experimental and control animal groups. The tissue material was fixed in 10 % neutral buffered formalin for paraffin block preparation, then sectioned and stained by hematoxylin and eosin. The small intestine and lymph node sections obtained from all mink groups were additionally processed for immunohistochemical assays. The En vision and Dual Link Systems by DAKO were applied. The reagent was visualized with a color reaction by 3,3-diaminobenzidine /DAB/as /DAKO/ chromogene. The anti-rabbit polyclonal antibody CD3/DAKO/ diluted at 1 : 100 to identify T lymphocytes, anti-mouse monoclonal primary antibody CD 79alpha/DAKO/ at 1 : 100 dilution that detects B lymphocytes, and finally, monoclonal primary antibody DEF that serves to detect defensins diluted at 1 : 200 were also applied. The quantitative study of B and T lymphocytes in the small intestinal wall was performed using 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 was determined by counting 100 cells in 5 fields of view at 20x magnification of the objective-lens.

Results were statistically calculated using the STATISTICA software, based on the ANOVA test. For comparison of two groups, Wilcoxon signed-rank test was used.

RESULTS

Statistical analysis did not show any statistically significant differences in the blood haematological parameters of the control C and experimental D groups ($P > 0.05$).

The average number of red blood cells (RBC) was 9.7 x $10^{12}.l^{-1}$ in the control group and 10.5 x $10^{12}.l^{-1}$ in the experimental group, slightly exceeding the reference values reported by H u n t e r [10], who gave the range from 5.80 to 9.73 $10^{12}.l^{-1}$ and B e r e s t o v et al. [3].

The mean haematocrit (Ht) in the study ranged from 0.59 to 0.63 $l.l^{-1}$ and was slightly above the reference values of 0.35—0.56 $l.l^{-1}$ [3, 10].

The mean haemoglobin (Hb) levels ranged from 6.79 to 11.66 $mmol.l^{-1}$ and were within the reference values of 6.206—11.79 $mmol.l^{-1}$ given by H u n t e r [10], with the same upward trend for the females of the experimental group.

The mean red cell volume (MCV) values were 61.57—60.47 fl, and they were compatible with the limits of reference values of 35.1—75.0 fl, same as the mean values for the mean haemoglobin in red blood cells (MCH) of females in the control group (17.93 pg) and experimental group (17.37 pg). They were also compatible with the reference values 12.2—25.4 pg [3, 10].

The mean values of the mean haemoglobin concentration in the red blood cells (MCHC) were also consistent with the reference values of 28.6—38.1 $g.dl^{-1}$ [3, 10] and were 29.20 and 28.80 $g.dl^{-1}$, respectively.

The mean white blood cell count (WBC) in the study was 6.85 and 7.22 $10^9.l^{-1}$ and was also consistent with H u n t e r' s [10] reference values, which range from 3.80—12.20 $10^9.l^{-1}$ and with B i s - W e n c e l et al. [4].

Some of the parameters in the experimental group were slightly higher than in the control group, including erythrocytes, leukocytes, hematocrit, haemoglobin, PLT, MPV and PDW (Table 1).

The histopathological examinations of the internal organs taken from mink of the control group showed their correct microscopic structure. In the liver of mink from the experimental group, small inflammatory infiltrates were found around the vessels and between the lobules (Fig. 1). In the kidneys, deposits of crystalline salts in the kidney tubules and single inflammatory infiltrates were seen (Fig. 2). Slight focal destruction of the intestinal villi and the presence of inflammatory infiltrates located mainly in the mucous membrane were observed in the intestine of a few experimental animals (Fig. 3). No cases of diarrhoea or other gastrointestinal pathologies were reported in our study. The flock resistance was also satisfactory, requiring no additional pharmacological intervention. It seems that the observed changes might be connected with the animal intra-subject variability based on genetic predispositions to obesity. In agreement with the information presented in [7], the body condition score (BCS) correlated with the histopathological changes in the mink.

Table 1. Levels of selected haematological parameters in the blood of mink from both groups

Parameters	Control group			Experimental group			Statistical analysis	
	M	Me	SD	M	Me	SD	Z	P
RBC [$10^{12}.l^{-1}$]	9.70	10.25	0.97	10.50	10.25	0.65	-0.22	0.83
Hb [$mmol.l^{-1}$]	17.47	18.00	2.05	18.23	18.20	0.25	-0.22	0.83
Ht [$l.l^{-1}$]	59.77	62.90	7.04	63.23	62.90	1.23	0.22	0.83
MCV [fl]	61.57	61.40	1.36	60.47	61.40	4.67	0.22	0.83
MCH [pg]	17.93	17.70	0.59	17.37	17.50	1.11	0.65	0.51
MCHC [$g.dl^{-1}$)]	29.20	29.40	0.53	28.80	28.60	0.35	0.87	0.38
RDW [%]	10.40	10.20	1.21	9.47	9.30	0.67	0.87	0.38
WBC [$10^9.l^{-1}$]	6.85	3.36	7.40	7.22	7.93	1.62	-0.44	0.66
PLT [$G.l^{-1}$]	556.33	473.00	343.66	561.00	473.00	168.25	0.22	0.83
MPV [fl]	8.80	9.10	0.52	9.13	9.10	0.35	-0.44	0.66
PDW [fl]	8.70	8.90	1.51	9.57	9.80	0.68	-0.22	0.83

M — Mean; SD—standard deviation; Me—median; Z—Wilcoxon signed-rank test; P—test probability; RBC—red blood count; Hb—haemoglobin; Ht—haematocrit; MCV—mean red cell volume; MCH — mean corpuscular haemoglobin; MCHC—mean corpuscular haemoglobin concentration; RDV—red blood cell distribution width; WBC—the total number of white blood cells; PLT—thrombocyte; MPV—mean platelet volume; PDW—platelet anisocyte

The immunohistochemical studies confirmed the dominance of T lymphocytes in apparent infiltrates (Figs. 4 and 5). The lymph nodes showed T-cell hyperplasia (Fig. 6). The immunohistochemical studies conducted at this stage of research showed a positive reaction to the presence of defensins in the mink from the experimental group. The expression of defensins was slightly increased in comparison with previous studies in the intestinal epithelium, intestinal crypts and inflammatory cells [16], and the reaction was granular and mainly confined to the cytoplasm of the cell.

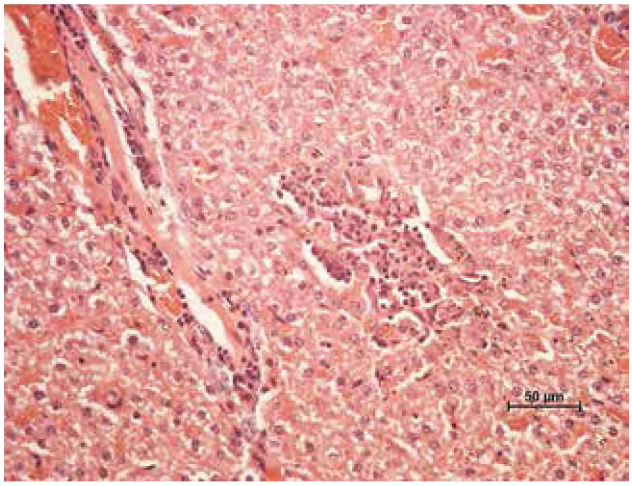

Fig. 1. Minor inflammatory infiltration in the liver of experimental group, HE stain. Magn. x200

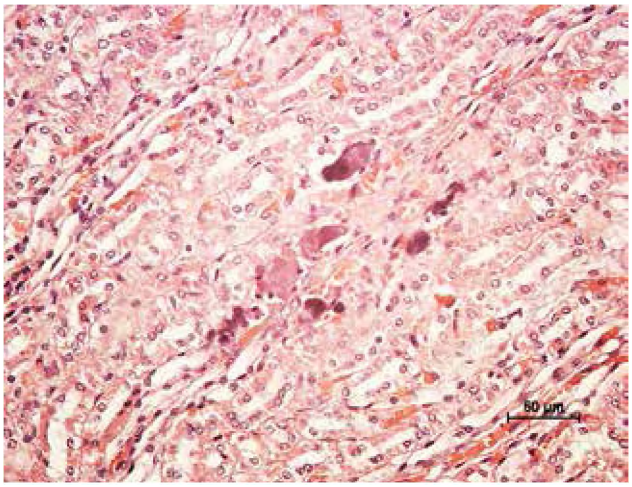

Fig. 2. Mineral salt deposits in the kidney of experimental group, HE stain. Magn. x200

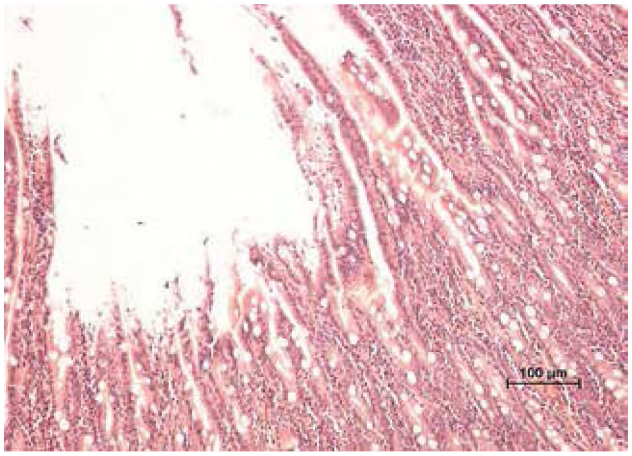

Fig. 3. Destruction of intestinal villi and presence of inflammatory infiltration in the mucous membrane of experimental group, HE stain. Magn. x200

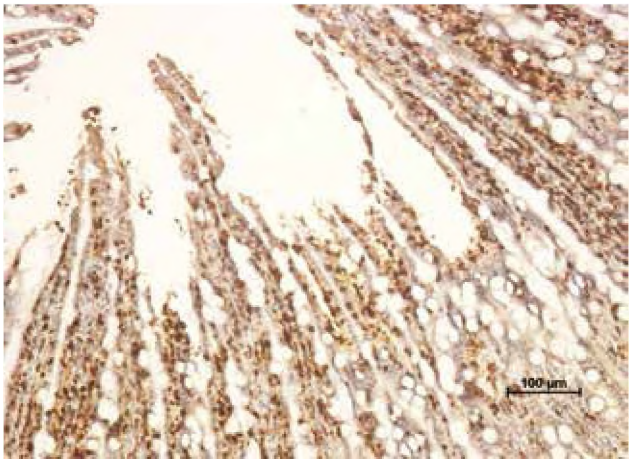

Fig. 4. Expression of CD3 antigen identifying T lymphocytes in intestinal mucus of experimental grup, IHC stain. Magn. x200

DISCUSSION

Commission Regulation (EC) No 1292/2005 amended the ban on the use of blood as a by-product in animal nutrition and therefore, the interest in searching for new sources of animal protein that could be used in animal feed increased. Dried animal blood plasma preparations have confirmed their unconventional value as a feed additive in growing animals. Dried blood plasma contains specific proteins, nucleotides, and immunoglobulins; therefore, the interest increased in using it as a feed additive in pigs and poultry [11, 12, 15]. Due to the immunoglobulin content, blood preparations can be used in the prevention and treatment of many diseases caused by intestinal pathogens. The

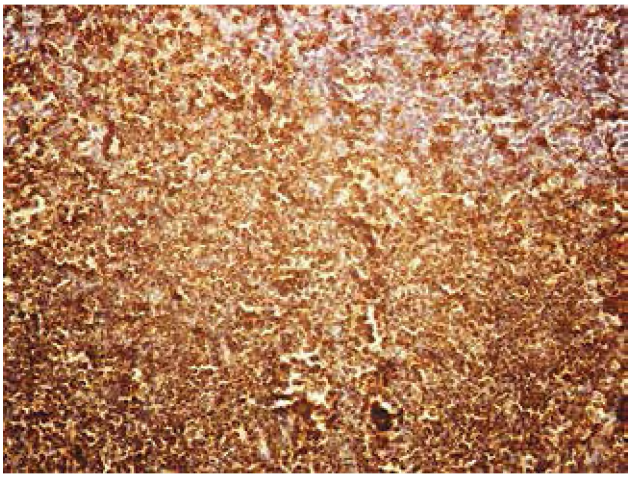

Fig. 5. Expression of CD3 antigen identifying T lymphocytes in the lymph node of experimental grup, IHC stain. Magn. x200

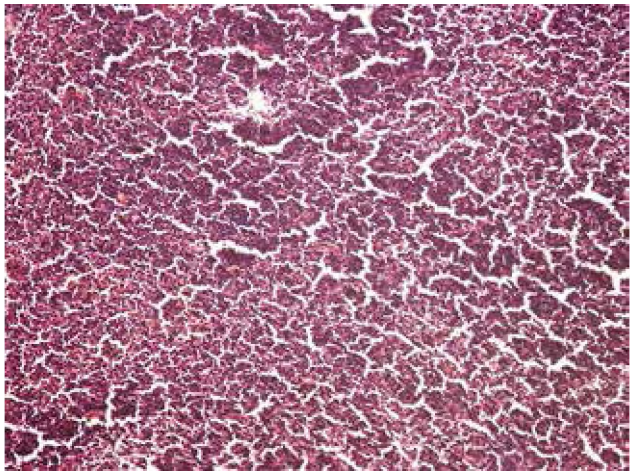

Fig. 6. Obliteration of lumps in the lymph node of experimental grup, IHC stain. Magn. x200

calves fed before weaning with dried plasma had better weight gain and were characterized by lower morbidity and mortality. Dried blood preparations have also been successfully used in feeding lambs, dogs, foxes, poultry and fish [1].

The basic system of feeding mink in Poland is based on high energy feed. The usage of energy-rich feeds with high levels of energy from fat is a prerequisite for obtaining high quality skins and better reproduction rates [4, 5, 6, 14, 16, 17]. This may adversely affect the health of the animals, especially by threatening the reproductive processes. Such nutrition leads to metabolic abnormalities which result in histopathological changes, especially in the liver and kidneys. Intensive nutrition of this kind can also cause a number of disease processes [6, 7, 8, 17].

The weaning period is a time when the functionality and integrity of the intestinal mucosa deteriorates. Young animals take more feeds to which the digestive tract is not enzymatically prepared [8, 21]. This can increase the amount of undigested feed in the intestines and increase the susceptibility to diseases. These problems are traditionally abolished by the sub-therapeutic use of antibiotics in doses as growth promoters. This can be successfully replaced by immunological modulation with Spray-Dried Animal Plasma (SDAP) bovine serum [23]. Results indicate that SDAP's components possess biological functions that have a positive impact on the digestive system of healthy adult cats. This influence persists even after the sterilization or canning [20]. Positive results of the dried blood plasma usage were obtained in the experiment on pigs and enough evidence supporting the safety of commercial spray dried porcine plasma in feeding pigs is available [18]. However, such results have not been confirmed in the poultry experiment [9]. The feed additive that can reduce the effects of mycotoxins is also spray dried plasma protein (SDPP). Inclusion of SDPP to pigs has been shown to improve the pig's performance. Weaning is a very stressful event and SDPP can benefit pig growth and decrease intestinal disorders. Evidence suggests that diets supplemented with SDPP have reduced diarrhoea and intestinal barrier dysfunction due to a reduction in inflammation caused by weaning stress [22].

It appears that the addition of plasma to young mink must still be tested with respect to the balanced daily ration of food intake. Protein-rich foods "enhanced" by plasma proteins in the early stages of life can modify the state of homeostasis and be considered a "ballast" for the condition of the internal organs. N o w a k o w i c z - D ę b e k et al. [18] showed that the addition of 0.5 % plasma to adult females during breeding preparation was acceptable to the organism and did not cause important changes in the internal organs. The results confirm the interaction of the defensins produced in Paneth's cells in maintaining intestinal homeostasis, which play an important role in innate and acquired immunity. Among the solutions positively influencing the difficult period of rearing young animals, dried blood plasma should be noted. Defensins, previously known as leukes and phagocytes, are cationic proteins belonging to the group of AMP proteins (antimicrobial peptides). The synthesis of these proteins occurs in many cells, mainly phagocytic in human and other animal intestinal cells. Defensins

as a proinflammatory mediator where dendritic cells participate in signalling from the surface of the mucosa to the basilar membrane in the gastric mucosa [23].

It has been shown that their antibacterial activity is revealed after about 3—4 hours, and that they possess a multistage mode of action. It consists in binding defensins to the membrane of the attacked cell, followed by its endocytosis and apoptosis. Antimicrobial, antiviral, antifungal and antiparasitic effects have been demonstrated and also, defensins induce the production of certain chemokines and activation of the complement, macrophages and mast cells. They bind and neutralize bacterial endotoxins and they participate in wound healing. Furthermore, defensins reduce stress by inhibiting glucocorticoid synthesis, and also act against cancer.

CONCLUSIONS

In conclusion, it should be stated that the proposed supplementation of blood plasma can contribute to improving the health of the animals and maintaining their good condition during the lactation and perinatal period and the kits' rearing. These studies however, need to be continued in order to determine the precise dosage of plasma supplements.

REFERENCES

1. **Banach, M., Makara, A., 2011:** Blood and its derivatives (In Polish). *Chemia czasopismo techniczne,* 108, 3—20.
2. **Barabasz B., Bielański P., Niedźwiadek S., Sławoń J., 1994:** Standards for feeding carnivorous and herbivorous fur Animals (In Polish). *IFiZZ PAN,* 28—29.
3. **Berestov, V. A., Blomstedt, L., Brandt, A., Juokslahti, T., Jorgensen, G., Kozhevnikova, L. K., et al., 1989:** Haematology and Clinical chemistry of Fur Animals. Ed. Asbjorn B. (Ed.), *Scientifur,* 10—18.
4. **Bis-Wencel, H., Saba, L., Kopczewski, A., Pyzik-Molęda, M., Likos-Grzesiak, B., Ondrasovic, O., 2004:** Shaping the reference values of selected white blood cells in the context of high energy diet (In Polish). *Zeszyty Naukowe Przeglądu Hodowlanego,* 72, 75—79.
5. **Bis-Wencel, H., 2006:** Effect of diet on selected mink reproduction indicators (In Polish). *Acta Scientiarum Polonorum, Medicina Veterinaria*, 5, 103—110.
6. **Bis-Wencel, H., Nozdryn-Płotnicki, Z., Saba, L., Zoń, A., Kopczewski, A., Sroka, A., 2006:** Effect of preservatives on histopathological image of internal organs of mink (In Polish). *Medycyna Weterynaryjna,* 62, 70—73.
7. **Bis-Wencel, H., Łopuszyński, W., Saba, L., Bryl, M., Rowicka, A., 2010:** Histopathological examination of selected internal organs of pastel minks in relation to oxidative state parameters. *Bulletin of the Veterinary Institute in Puławy,* 54, 651—654.
8. **Bis-Wencel, H., Bryl, M., Kowaleczko, M., Rowicka, A., Matyjaszczyk, M., 2011:** The bacteriological state of mink's kittening houses in perinatal period, *Annales UMCS*, Section EE, 24, 21—27.
9. **Donkoh, A., Atuahene, C C., Anang, D. M., Ofori, S. K., 1999:** Chemical composition of solar-dried blood meal and its effect on performance of broiler chickens. *Anim. Feed Sci. Technol.*, 81, 299—307.
10. **Hunter, D. B., 1996:** Mink hematology and clinical biochemistry. In **Hunter, D. B., Lemieux, N., (Eds.):** *Biology, Health and Disease,* 1st edn., Canada Mink Breeders Association. University of Guelph, Ontario, Canada, 7—1 to 7—5.
11. **Jamroz, D., Wiliczkiewicz, A., Orda, J., Kuryszko, J., Stefaniak, T., 2012:** Use of spray-dried porcine blood by-products in diets for young chickens. *J. Anim. Physiol. Anim. Nutr.,* 96, 319—33.
12. **King, M. R., Morel, P. C. H., Pluske, J. R., Hendriks, W. H., 2008:** A comparison of the effects of dietary spraydried bovine colostrum and animal plasma on growth on intestinal histology in weaned pigs. *Livest. Sci.,* 119, 167—173.
13. **Kowalski, Z., Makara, A., Banach, M., 2011:** Production technology for blood plasma and hemoglobin (In Polish). *Chemik*, 65, 5, 466—475.
14. **Langerkvist, G., Johansson, K., Lundeheim, N., 1994:** Selection for litter size, body weight, and pelt quality in mink *(Mustela vison):* correlated responses. *J. Anim. Sci.,* 72, 1126—1137.
15. **Marquez, E., Brancho, M., Archile, A., Rangel, L., Benitez, B., 2005:** Proteins, isoleucine, lysine and methionine content bovine, porcine and poultry blood and their fractions. *Food Chem.,* 93, 503—505.
16. **Nowakowicz-Dębek, B., Śmiech, A., Zoń, A., Bis-Wencel, H., Ondrasovicova, O., Wlazło, Ł., Wnuk, W., 2012:** Tissue pathomorphology and immunohistochemistry in mink *(Neovison vison)* fed blood plasma supplemented diet in the period of preparation for breeding. *Bulletin of the Veterinary Institute in Pulawy,* 56, 393—397.

17. **Nowakowicz-Dębek, B., Łopuszyński, W., Trawińska, B., Saba, L., 2009:** Effect of various management conditions on anatomo-histopathological changes in Arctic fox *(Alopex lagopus). Bulletin of the Veterinary Institute in Pulawy,* 53, 105—109.
18. **Nowakowicz-Dębek, B., Zoń, A., Jakubczak, A., Wnuk, W., 2013:** Haematological parameters of wild and farm mink, red fox and raccoon dog. *Medycyna Weterynaryjna,* 69, 40—42.
19. **Pérez-Bosque, A., Polo, J., Torrallardona, D., 2016:** Spray dried plasma as an alternative to antibiotics in piglet feeds, mode of action and biosafety. *Porcine Health Management,* 2, 16.
20. **Rodríguez, C., Saborido, N., Ródenas, J., Polo J., 2016:** Effects of spray-dried animal plasma on food intake and apparent nutrient digestibility by cats when added to a wet pet food recipe. *Anim. Feed Sci. Technol.,* 216, 243—250.
21. **Rouvinen-Watt, K., 2007:** Mink nursing sickness. *Fur Animal Research,* 15, 157.
22. **Weaver, A. C., Campbell, J. M., Crenshaw, J. D., Polo, J., Kim S. W., 2014:** Efficacy of dietary spray dried plasma protein to mitigate the negative effects on performance of pigs fed diets with corn naturally contaminated with multiple mycotoxins. *J. Anim. Sci.,* 92, 3878—3886.
23. **Żyłowska, M., Wyszyńska, A., Jagusztyn-Krynicka, E. K., 2011:** Defensins-peptides with antimicrobial activity (In Polish). *Post. Mikrobiol.,* 50, 223—234.

Received July 6, 2017
Accepted December 6, 2017