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BIOCHEMICAL PARAMETERS OF BLOOD SERUM OF DOGS WITH HEPATIC LIPIDOSIS

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Summary. Dog hepatic dystrophy (hepatic lipidosis) is a disease that mostly arises from inappropriate conditions of keeping and feeding. Triacylglycerols (TG) are accumulated in hepatocytes due to variety of toxic influences, lack of carbohydrates in nutrient budget. One of the main mechanisms of lipidosis that accompanied by hyperlipidemia is increased accumulation of triacylglycerols (TG) on the background of reducing the rate of their removal. Dog's fatty liver dystrophy is characterized by indistinct identify clinical features that requires using of laboratory and instrumental methods in diagnostics. Determination of lipid composition in dogs was not carried out in veterinary medicine practice in Ukraine although abnormalities of lipid metabolism play an important role in the development of liver lipidosis.

Keywords: dogs, liver, lipidosis, diagnostics, biochemical parameters, lipid diagram, treatment

Introduction. Liver dystrophy is a disease that in most cases arises from inappropriate conditions of keeping and feeding, especially of farm animals (Levchenko, and Halias, 2002; Chub, Levchenko and Sakhniuk, 2000; Vikulina, 2010; Nazaruk, Hutiy and Hufriy, 2012; Xenoulis and Steiner, 2010). Levchenko V. and Fasolja V. (2008), Soloviova L. M. (2004), Dykyi O. A. (2000), Fasolya V. P. (2008), Lokes P. I. (2013), Morozenko D. V. and Timoshenko O. P. (2012), etc. were studied the problem of dog's fat dystrophy.

While hepatic dystrophy (hepatic lipidosis), according to the medical literature, in hepatocytes due to variety of toxic influences, carbohydrate deficit in nutrient budget, which the body tends to eliminate, by gluconeogenesis, the triacylglycerol (TG) are accumulated (Center, 1996). It is known that the increasing of glucocorticoids secretion tends to fatting mobilization from depot to liver, where fast fat infiltration is happening, phospholipids synthesis is interrupted, the oxidation processes of long-chain fatty acids are suppressed; and degenerative changes in the parenchyma of organ are appeared. Enhanced synthesis TG on the background of reducing the rate of their elimination is one of the main mechanisms of lipidosis (Tennant and Center, 2008). Thus, it is inhibited the liver formation HDL, which is the main transport TG form of the liver cells. Besides the liver has ability to synthesize the lipid components with greater intensity than proteins, which is one of the main factors in the development of its fatty infiltration (Boomkens et al., 2004).

Therefore, disorders of lipid metabolism play the important role in disease of liver lipidosis. According to Xenoulis and Steiner (2010), in various dog's breeds while primary hyperlipidemia observed the different nature of lipid in various breeds dogs. These authors reported that in secondary hyperlipidemia, which occurs due to some diseases (pancreatitis, hypothyroidism, hyperadrenocorticism, diabetes, nephropathy with microalbuminuria, cholestasis, fatness), lipid diagram of dogs blood serum is changing in different ways, and in some cases the nature of these changes is not defined. The authors think that the study of this problem will clarify information on the diseases process that were mentioned above, to complete the diagnostic tests scheme, determine what orientation indicators of lipid metabolism and lipoproteins have in treatment of internal dog diseases, accompanied by hyperlipidemia.

Synthesis of albumin, factors of the blood coagulation system, vitamins (especially B, K), utilization of glucose and other biologically active substances are reduced along with hyperlipidemia. The processes of transamination and deamination, glucuronidation of many compounds, are disordered, that tends to change of detoxification function of the liver (Bass, Hoffmann and Dorner, 1976; Mahley et al., 1981).

Not clearly definite clinical features entail fat degeneration foci in dog liver. This circumstance requires the using for laboratory and instrumental diagnostic methods: ultrasound diagnostic (US) and biochemical researches, including indicators of lipid metabolism status. The last ones give opportunities more objectively qualitatively and quantitatively to assess the degree of metabolic disturbances.

Determination in dog lipid composition does not carry out in veterinary medicine practice in Ukraine

although abnormal features of lipid metabolism play an important role in the development of liver lipidosis.

The aim of work. Determination of diagnostic value of lipid metabolism indicators on the background of other biochemical tests in dog blood serum while liver fatty degeneration before and during treatment.

Materials and methods. The investigations were carried out of different dog breeds at the age of 3–7 years, about the similar weight, with a close nutrient budget. First 15 animals without clinical pathology features were selected (German breed and mongrel), males and females, 10 and 5 dogs respectively.

Blood samples were taken from the subcutaneous vein of the forearm or the lateral subcutaneous vein of the thigh. Indicators of this animal group have not been outside of the reference rules in the morphological composition of blood, urine and biochemical parameters — total protein, albumin, ALT, AST, alpha-amylase activity, glucose, urea, creatinine, total and direct bilirubin.

For the purpose of establishing the role of lipid metabolism disorders in lipidosis pathogenesis of dog liver were selected 24 sick lipidosis animals that have been examined with the use of clinical, laboratory and ultrasound methods at the time of admission to hospital and in 30 days after the treatment start.

Biochemical research of blood serum was carried out by standard approaches, described in handbooks by Levchenko (2010) and Kamyshnikov (2004). In addition of mentioned biochemical tests, in the blood serum was determined the content of triacylglycerols (TG), total cholesterol, cholesterol of lipoproteins of very low — HDL, low — LDL and high density — VLDL (Kamyshnikov, 2004).

Treatment of animals was carried out according to the following scheme: hepatoprotector 'Dyvopraid' tablets in dose 1 tablet per 5 kg of body weight 2 times a day before feeding — 30 days; 'Hepavikel' solution for injection — 1 ml per 10 kg of body weight subcutaneously 1 time per 7 days in dose 4 total injections (in 28 days period); dietary requirements: boiled rice, oatmeal or buckwheat; chicken and/or beef boiled meat, chopped equally mixed with porridge.

The calculation of food amount in nutrient budget was carried out in the following way: 15 g of meat per 1 kg of body weight per day, it should be 25% of the daily nutrient budget, 65% — porridge, the other 10% — vegetables (carrots or beets). The amount of food was 60 g/kg of body weight per day. Boiled water is not limited. The data, mentioned by Starchenkov (2001), were taken as a base diet.

All performed calculations were done by STATISTICA 7.0 (StatSoft, USA) program (Rebrova, 2002).

Results and discussion. According to anamnesis, the animals (100% of dogs) were suppressed for a long time, hyporexia was observed (70.8%), some individuals demonstrated anorexia (25%), vomiting (16.7%), fever (25%), enlarged spleen (8.3%), and anemia and icterus visible mucous membranes (of 20.8 and 79.2% respectively), low pain in liver palpation (54.2%), dyspepsia (45.8%), constipation (20.8 per cent).

Suppression, hyporexia and anorexia were indexes of intoxication syndrome as the result of damaged disinfect function of liver. Liver dysfunction, stomach, bowel violated gastrointestinal motility, that was accompanied by vomit and dyspeptic effect (Afanas'ev and Yurina, 1999), which were demonstrated by of liquid stool, often light-yellow color. While liver palpation animals demonstrated anxiety because of pain, the abdominal wall was tensed. Many of the dogs were suffered of anemia, that was proved by the pale color of the mucous membranes and their yellowness was defined in small number of sick animals that is generally inherent to dog liver disease, induced by non-invasive factors (Tennant and Center, 2008).

So suppression, inhibition, anemone visible mucous membranes and hyporexis were observed in animals with fatty hepatic dystrophy most often; quite often showed tends to pain during palpation; hepatomegaly, dyspepsia, and critically conjunctive, anorexia and constipation were observed much rarer, as well as small temperature increase in body. Vomiting and splenomegaly were detected in fewest quantities.

Thus, the clinical symptoms in the dog fatty hepatic dystrophy are not specific and it is needed further diagnostic procedure with use laboratory and instrumental investigations for diagnosis.

According to ultrasound diagnostic (US) in 91.7% of sick dogs were revealed equal diffuse increase of echogenicity of liver parenchyma with a homogeneous structure, which was medium — to coarse-grained, and capsule echo-structure of was — dense. Liver sides were rounded; the bile ducts are slightly dilated. Part of the animal had thickening of the gallbladder wall with moderate sediment or sediment inside. These dogs reveal fragmentation of the parenchyma structure on the echogram in the case of process aggravation (Lokes, Stovba and Karysheva, 2007).

As liver is functionally closely connected with the hematogenesis system, so in its diseases often occur significant changes in the morphological composition of blood because of imbalance of iron metabolism, vitamin B_{12}, folic acid, as well as erythropoietin, and others (Sysueva, 2009). Our results, therefore, point to the anemia syndrome in all sick dogs.

Analysis of leukocytosis indicators in sick dogs had been demonstrated that average total number of leukocytes due to stab forms of neutrophils was increased. Consequently, in part of dogs with hepatic dystrophy possible development of the inflammatory process, that is similar to ultrasound data (fragmentation of the liver parenchyma structure and the thickening of the gallbladder wall).

Diagram of lipid analysis in animals with symptoms of clinical pathology liver revealed dyslipoproteinemia, that was accompanied by increase TG level and total cholesterol due to increasing cholesterol content in LDL, on decline background of VLDL cholesterol, which is not typical for standard dog lipid indicators but similar to people lipid composition (Zagayko, 2009; Tennant and Center, 2008) (Table 1).

Table 1 – Lipid diagram of dog blood serum with liver lipidosis (mM/l), n = 24

Indexes	Total cholesterol	Triacylglicerols	Cholesterol VLDL	Cholesterol LDL	Cholesterol HDL
Clinically healthy animals					
M ± m	4.71 ± 0.26	0.74 ± 0.08	3.79 ± 0.25	0.58 ± 0.06	0.30 ± 0.04
Sick lipidos liver dogs before treatment					
M ± m	7.27 ± 0.36^{ooo}	2.51 ± 0.33^{ooo}	1.41 ± 0.14^{ooo}	4.71 ± 0.31^{ooo}	1.15 ± 0.15^{ooo}
Sick lipidos liver dogs after treatment					
M ± m	5.31 ± 0.08***	0.76 ± 0.08***	2.75 ± 0.08***	2.22 ± 0.10***	0.35 ± 0.03***

Note: * — difference of indicators before and after treatment, statistically proved at $p < 0.05$; ** — $p < 0.01$; *** — at $p < 0.001$; ^{o} — the difference indicators between the clinically healthy animals and animals before treatment, statistically proved at $p < 0.05$; ^{oo} — at $p < 0.01$; ^{ooo} — at $p < 0.001$

Table 2 – Biochemical parameters of dog blood serum with hepatic dystrophy

Index	ALT, u/l	AST, u/l	Creatinine, μM/l	Urea, mM/l	Total bilirubin, μM/l	Conjug. bilirubin, μM/l	Total protein, g/l	Alb., %
Clinically healthy animals (n = 15)								
M	32.6	29.4	91.4	6.2	5.7	2.0	65.2	44.7
m	6.05	4.92	11.56	0.75	1.16	0.15	2.64	1.17
Sick dogs before treatment (n = 24)								
M	79.2^{ooo}	70.0^{ooo}	84.2	4.6	14.6^{ooo}	8.2^{ooo}	68.2	38.6^{o}
m	4.28	4.07	4.28	0.28	0.94	0.72	1.65	1.12
Sick dogs after treatment (n=24)								
M	38.8***	42.70***	81.5	5.0	7.7***	3.7***	63.5	44.8*
m	2.63	2.19	1.79	0.10	0.43	0.33	3.26	1.45

Note: * — difference of indicators before and after treatment, statistically proved at $p < 0.05$; ** — $p < 0.01$; *** — at $p < 0.001$

Besides liver pathology, one of reasons of hyperlipidemia is cholestasis, which characterized by increasing of cholesterol and TG levels in blood serum. The liver is not able to dissimilate cholesterol and remove it out of the bile tracts, because it often contents the pathological form of lipoprotein X, which is very rich on cholesterol and comes into LDL composition (Xenoulis et al., 2008; Alegre et al., 1976).

Our results are confirmed by literature data about hepatic lipidosis characterized the growth of LDL cholesterol (as probable source of abnormal lipoprotein X) and total cholesterol proves the presence of cholestasis syndrome and correlates with bilirubin concentration increase (Alegre et al., 1976).

According to data of literature, in dog hepatic dystrophy occur isochronic moderate increase of AST and ALT activity that indicates the presence of cytology syndrome on the background of serum TG increase and cholesterol HDL (Kirk and Bonagura, 2005; Boomkens et al., 2004). Before treatment the activity of both transferases in the blood serum of sick dogs is also increased, by our results (Table 2).

Hepatocellular failure syndrome was not definitely revealed, except of reducing the part of albumins, as there was no specific decrease concentration of serum TG and cholesterol LDL, which is an indicator of significant damage of liver parenchyma.

Reduction or normalization of majority biochemical tests level was observed after the treatment, including positive changes in diagram of lipid composition. Basic etiological factors and pathogenetic links of the development of hyperlipidemia in dog hepatic lipidosis are shown in Fig. 1.

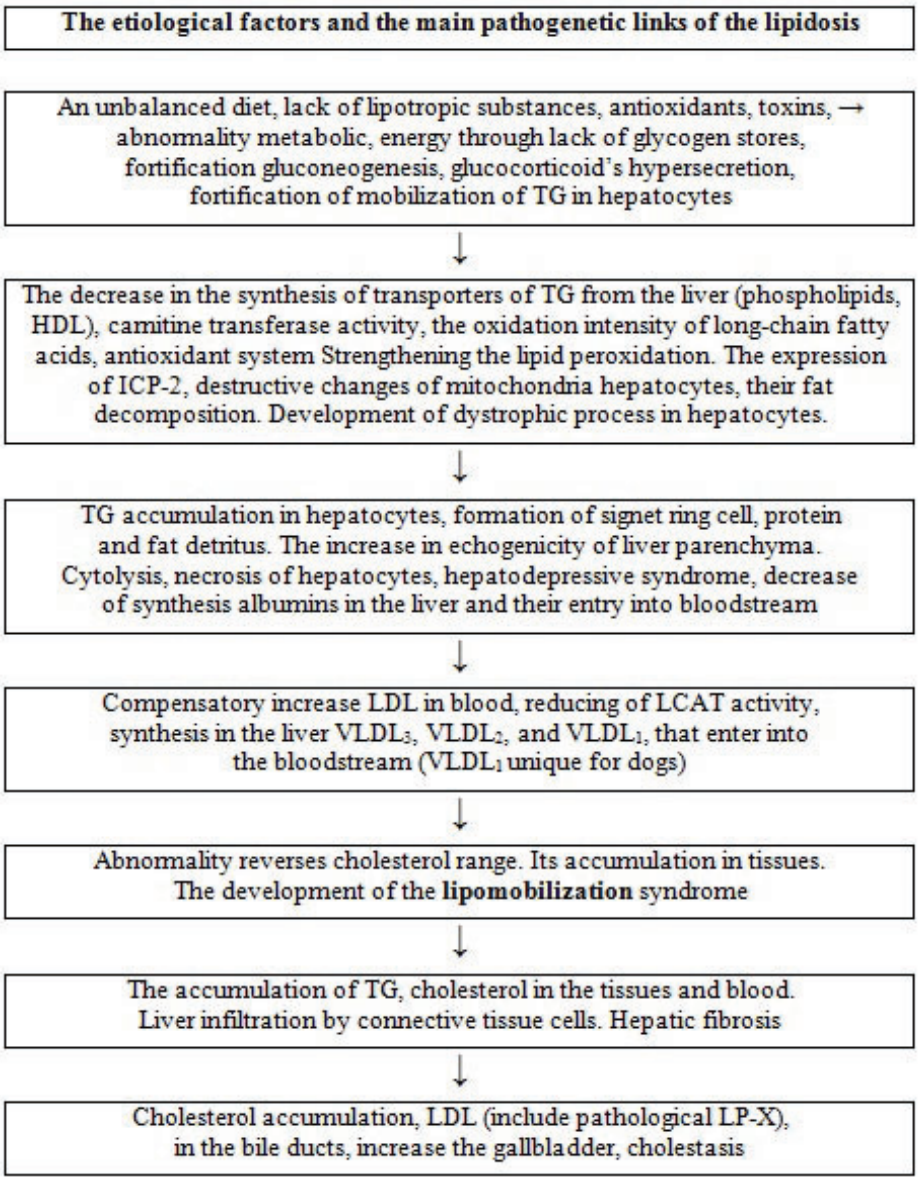

Figure 1. Etiopathogenesis hyperlipidemia hepatodystrophy for dogs

Conclusion. According to the clinical data, instrumental and laboratory parameters the hepatic dystrophy was detected in sick dogs. It was characterized by the most quantity of animals by the chronic course and significant changes in lipid indicators.

The most diagnostic informative value in dogs' hepatic dystrophy has cholesterol LDL 100%, triacylglycerols, cholesterol HPHD, VLDL — 87.5%, total cholesterol — 58.3% of the animals, in which the value of biochemical tests were out of the indexes in control group.

In other tests the most diagnostic informative value in dog hepatic dystrophy had conjugated bilirubin — in 100% of animals, ALT and total bilirubin — at 75 and AST — at 70.8%. The lowest was the diagnostic informative of albumin (at 48.5%), uninformative was urea and creatinine (at 20.8 and 0% of animals, respectively).

Evaluating of the treatment effectiveness for hepatic dystrophy by highly informative tests, demonstrated the limits of the indexes of the control group were total bilirubin and ALT (95.8% of sick animals), albumin (91.7%), AST (at 87.5%). The diagnostic significance was less total protein (75.0%) and conjugated bilirubin (29.2% of dogs) parameters.

These results are the basis for recommendations of all informative indicators as diagnostic criteria for the diagnosis and treatment of dog hepatic dystrophy.

References

Afanas'ev, Yu. I. and Yurina, N. A. (eds.) (1999) *Histology [Gistologiya]*. 5th ed. Moscow: Meditsina. ISBN 5225045235. [in Russian].

Alegre, B., Carrasco, D., Breto, M. D., Gras, M. and Berenguer, J. (1976) 'Semiquantitative determination of lipoprotein X (Lp-X): its usefulness in differential diagnosis of jaundice', *Revista Clinica Espanola*, 143(1), pp. 79–81.

Bass, V. D., Hoffmann, W. E. and Dorner, J. L. (1976) 'Normal canine lipid profiles and effects of experimentally induced pancreatitis and hepatic necrosis on lipids', *American Journal of Veterinary Research*, 37(11), pp. 1355–1357.

Boomkens, S. Y., Penning, L. C., Egberink, H. F., van den Ingh, T. S. G. A. M. and Rothuizen, J. (2004) 'Hepatitis with special reference to dogs. A review on the pathogenesis and infectious etiologies, including unpublished results of recent own studies', *Veterinary Quarterly*, 26(3), pp. 107–114. doi: 10.1080/01652176.2004.9695174.

Center, S. A. (1996) 'Hepatic lipidosis, glucocorticoid hepatopathy, vacuolar hepatopathy, storage disorders, amyloidosis, and iron toxicity', in Guilford, W.G., Center, S. A., Strombeck, D. R., Williams, D. A. and Meyer, D. J. (eds.) *Strombeck's small animal gastroenterology*. Philadelphia, PA: W. B. Saunders, pp. 766–801. ISBN 9780721637600.

Chub, O. V., Levchenko, V. I. and Sakhniuk, V. V. (2000) 'The results chromatography of volatile fatty acids of highly productive cows' [Rezultaty khromatohrafii letkykh zhyrnykh kyslot u vysokoproduktyvnykh koriv], *Bulletin of the Bila Tserkva State Agrarian University [Visnyk Bilotserkivskoho derzhavnoho ahrarnoho universytetu]*, 13(2), pp. 182–188. [in Ukrainian].

Dykyi, O. A. (2000) *Hepatic-dystrophy in dogs of service breeds (etiology, pathogenesis, diagnostics, treatment and prophylaxis) [Hepatodystrofiia u sobak sluzhbovykh porid (etiolohiia, patohenez, diahnostyka, likuvannia ta profilaktyka)]*. The dissertation thesis for the scientific degree of the candidate of veterinary sciences. Bila Tserkva: Bila Tserkva National Agrarian University. [in Ukrainian].

Fasolya, V. P. (2008) 'Diagnosis and treatment hepatopancreatic syndrome in dogs' [Diahnostyka i likuvannia hepatopankreatychnoho syndromu v sobak], *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyj [Naukovyi visnyk Lvivskoho natsionalnoho universytetu veterynarnoi medytsyny ta biotekhnolohii imeni S. Z. Gzhytskoho]*, 10(2(37), 1), pp. 366–372. Available at: http://elibrary.ru/download/elibrary_25981817_79066598.pdf. [in Ukrainian].

Kamyshnikov, V. S. (2004) *Handbook on clinical and biochemical research and laboratory diagnostics [Spravochnik po kliniko-biokhimicheskim issledovaniyam i laboratornoy diagnostike]*. 2nd ed. Moscow: MedPress-Inform. ISBN 5983220012. [in Russian].

Kirk, R. and Bonagura, D. (2005) *Kirk's Current Veterinary Therapy [Sovremennyy kurs veterinarnoy meditsiny Kirka]*. Moscow: Akvarium. ISBN 9785423802912. [in Russian].

Levchenko, V. and Fasolja, V. (2008) 'Spread of polimorbidinterual pathology in the dogs of service breeds' [Poshyrennia mnozhynnoi vnutrishnoi patolohii u sobak sluzhbovykh porid ta yii patohenez], *Scientific and Technical Bulletin of Institute of Animal Biology and State Scientific Research Control Institute of Veterinary Medical Products and Fodder Additives [Naukovo-tekhnichnyi biuleten Instytutu biolohii tvaryn i Derzhavnoho naukovo-doslidnoho kontrolnoho instytutu veterynarnykh preparativ ta kormovykh dobavok]*, 9(3), pp. 179–183. Available at: http://old.inenbiol.com/ntb/ntb2/pdf/3/2.pdf. [in Ukrainian].

Levchenko, V. I. (ed.) (2010) *Methods of laboratory clinical diagnostics of animal diseases [Metody laboratornoi klinichnoi diahnostyky khvorob tvaryn]*. Kyiv: Ahrarna osvita. ISBN 9789667906771. [in Ukrainian].

Levchenko, V. I. and Halias, V. L. (eds.) (2002) *Veterinary clinical biochemistry [Veterynarna klinichna biokhimiia]*. Bila Tserkva: Bila Tserkva State Agrarian University. ISBN 9667417409. [in Ukrainian].

Lokes, P. I. (2013) *The pathology of the liver and urinary system in domestic dogs and cats (clinical and biochemical status, pathogenesis, diagnosis, treatment) [Patolohiia pechinky ta orhaniv sechovoi systemy u sviiskykh sobak i kotiv (kliniko-biokhimichnyi status, patohenez, diahnostyka, likuvannia)]*. The dissertation thesis for the scientific degree

of the doctor of veterinary sciences. Kyiv: The National University of Life and Environmental Sciences of Ukraine. [in Ukrainian].

Lokes, P. I., Stovba, V. H. and Karysheva, L. P. (2007) *Ultrasound diagnosis of small animal diseases [Ultrazvukova diahnostyka khvorob dribnykh tvaryn]*. Poltava: Poltava State Agrarian Academy. ISBN 9789662088052. [in Ukrainian].

Mahley, R. W., Hui, D. Y., Innerarity, T. L. and Weisgraber, K. H. (1981) 'Two independent lipoprotein receptors on hepatic membranes of dog, swine, and man. Apo-B,E and apo-E receptors', *Journal of Clinical Investigation*, 68(5), pp. 1197–1206. doi: 10.1172/JCI110365.

Morozenko, D. V. and Timoshenko, O. P. (2012) 'Biochemical indices of connective tissue in pathogenesis, diagnosis and control of treatment efficiency hepatopathy of dogs' [Biokhimichni pokaznyky stanu spoluchnoi tkanyny u patohenezi, diahnostytsi ta kontroli efektyvnosti likuvannia hepatopatii sobak], *The Animal Biology [Biolohiia tvaryn]*, 14(1–2), pp. 411–419. Available at: http://nbuv.gov.ua/UJRN/bitv_2012_14_1-2_67. [in Ukrainian].

Nazaruk, N., Hutiy, B. and Hufriy, D. (2012) 'Features lipid peroxidation in the blood of the bullock affected by cadmium and nitrites' [Osoblyvosti perekysnoho okysnennia lipidiv u krovi bychkiv, urazhenykh kadmiiem ta nitrytamy], *Scientific and Technical Bulletin of Institute of Animal Biology and State Scientific Research Control Institute of Veterinary Medical Products and Fodder Additives [Naukovo-tekhnichnyi biuleten Instytutu biolohii tvaryn i Derzhavnoho naukovo-doslidnoho kontrolnoho instytutu veterynarnykh preparativ ta kormovykh dobavok]*, 13(3–4), pp. 250–254. Available at: http://old.inenbiol.com/ntb/ntb8/49.pdf. [in Ukrainian].

Rebrova, O. Yu. (2002) *Statistical analysis of medical data: using of STATISTICA applied package [Statisticheskiy analiz meditsinskikh dannykh: primenenie paketa prikladnykh programm STATISTICA]*. Moscow: MediaSfera. ISBN 5-90840134. [in Russian].

Soloviova, L. M. (2004) *A comparative estimation of methods of diagnostics and therapy of hepatodystrophy in dogs [Porivnialna otsinka metodiv diahnostyky i terapii hepatodystrofii u sobak]*. The dissertation thesis for the scientific degree of the candidate of veterinary sciences. Bila Tserkva: Bila Tserkva National Agrarian University. [in Ukrainian].

Starchenkov, S. V. (2001) *Diseases of dogs and cats [Bolezni sobak i koshek]*. Saint-Petersburg: Lan'. ISBN 5811404204. [in Russian].

Sysueva, A. V. (2009) *Morphological and functional changes of red blood cells in liver pathologies in small domestic animals [Morfofunktsional'nye izmeneniya eritrotsitov krovi pri patologiyakh pecheni u melkikh domashnikh zhivotnykh]*. The dissertation thesis for the scientific degree of the candidate of veterinary sciences. Moscow: Moscow State University of Applied Biotechnology. [in Russian].

Tennant, B. C. and Center, S. A. (2008) 'Hepatic Function', in *Clinical Biochemistry of Domestic Animals*. 6th ed. Elsevier, pp. 379–412. doi: 10.1016/B978-0-12-370491-7.00013-1.

Vikulina, G. V. (2010) *The state of connective tissue's biopolymers and lipid's exchange in clinically healthy and sick on bronchopneumonia young pigs [Stan biopolimeriv spoluchnoi tkanyny ta obminu lipidiv u klinichno zdorovykh i khvorykh na bronkhopnevmoniiu porosiat]*. The dissertation thesis for the scientific degree of the candidate of veterinary sciences. Bila Tserkva: Bila Tserkva National Agrarian University. [in Ukrainian].

Xenoulis, P. G. and Steiner, J. M. (2010) 'Lipid metabolism and hyperlipidemia in dogs', *The Veterinary Journal*, 183(1), pp. 12–21. doi: 10.1016/j.tvjl.2008.10.011.

Xenoulis, P. G., Suchodolski, J. S., Levinski, M. D. and Steiner, J. M. (2008) 'Serum liver enzyme activities in healthy Miniature Schnauzers with and without hypertriglyceridemia', *Journal of the American Veterinary Medical Association*, 232(1), pp. 63–67. doi: 10.2460/javma.232.1.63.

Zagayko, A. L. (2009) *Lipid metabolism and antioxidative-prooxidative state disorders under experimental metabolic syndrome [Porushennia obminu lipidiv ta prooksydantno-antyoksydantnykh protsesiv za eksperymentalnoho modeliuvannia metabolichnoho syndromu]*. The dissertation thesis for the scientific degree of the doctor of biological sciences. Kyiv: Bogomolets National Medical University. [in Ukrainian].