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THE ROLE OF ACUTE PHASE INFLAMMATORY PROTEINS IN THE PATHOGENESIS OF METABOLIC SYNDROME IN OBESE HORSES

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Summary. Metabolic syndrome and obesity in horses are closely related processes that are accompanied by chronic inflammation. Our research aimed to establish the normative indicators of acute phase inflammation proteins in obese horses. We conducted a study on twenty horses, ten of which were in the control group and ten were in the experimental group, showing signs of obesity. The Henneke scoring system was used to assess the body condition. This system ranks animals from one to nine, with one being exhausted and nine being very fat. Serum concentrations of circulating immune complexes, seromucoids, C-reactive protein, haptoglobin, total protein, and its fractions were determined. It was found that significant changes in protein metabolism occur in animals with obesity, namely: the content of circulating immune complexes, seromucoids, C-reactive protein, haptoglobin, and globulins significantly increase, indicating the development of inflammatory processes in horses due to obesity. The localization of these processes is associated both with obesity in animals and possibly with laminitis, the development of which is one of the pathogenetic links of metabolic syndrome

Keywords: laminitis, blood serum, protein metabolism

Introduction. Obesity in horses against the background of metabolic syndrome is characterized by metabolic disorders, which can lead to serious consequences for animal health, especially in case of a sedentary lifestyle or overeating (Carter et al., 2010).

According to studies, a certain proportion of horses are overweight, which increases the risk of developing obesity and metabolic syndrome. Some researchers believe that the etiology and pathogenesis of obesity and the development of metabolic syndrome in horses are similar to humans and include insulin resistance, hyperglycemia, hyperlipidemia, and hypertension (Morgan, Keen and McGowan, 2015).

The pathogenesis of the metabolic syndrome involves a complex interaction between hormonal, metabolic, and inflammatory processes (Durham et al., 2019).

This leads to impaired carbohydrate and fat metabolism, as well as changes in the function of the endocrine system, which can lead to the development of cardiovascular disease, obesity, laminitis, and other diseases (Karikoski et al., 2011).

The role of acute phase inflammatory proteins in the context of equine obesity has been little studied, although it is known that equine obesity is often associated with chronic inflammation, which plays a key role in its pathogenesis. Acute phase inflammatory proteins are important mediators of inflammation and may be important indicators of the degree of inflammatory response in obesity. Studying the role of acute-phase inflammatory proteins will allow us to understand the mechanisms by which obesity affects the metabolism and health of horses. They may be important biomarkers for diagnosing and assessing the severity of obesity in horses, as well as monitoring the effectiveness of therapy (Menzies-Gow, Harris and Elliott, 2017).

Acute phase inflammatory proteins are a group of proteins that are actively synthesized in response to various stressors such as trauma, infection, tumors, and other pathological conditions (Bilous and Kovalchuk, 2015).

They perform many essential functions, such as regulating the immune response, correcting metabolism, protecting against infection and shaping the response to tissue remodeling, antiviral protection, phagocytosis, regulation of apoptosis, and other processes related to the body's defense against harmful effects (Slivinska, Maksymovych and Shcherbatyy, 2017).

Acute phase proteins are actively involved in the body's immune and inflammatory responses and are synthesized by cells such as hepatocytes, monocytes, macrophages, and fibroblasts.

Importantly, inflammatory proteins can also participate in the formation of inflammasomes, protein complexes that play a key role in initiating the body's inflammatory cascade response.

The purpose of this study was to determine the levels of acute phase inflammatory proteins in normal and obese horses.

Materials and methods. The study was conducted on twenty horses of different breeds and sexes, from which age-matched groups were formed: clinically healthy animals (n = 10) and a control group with signs of obesity (n = 10). The horses were mostly of the Ukrainian Riding breed and partially crossbred on its basis. The body condition of each horse was scored on a 9-point Henneke scale after visual inspection by a veterinarian.

Feeding and housing conditions met the physiological needs of the animals. The diet contained the required amount of nutrients, and access to water and exercise was unrestricted. Regular clinical examination of the animals was performed, including the determination of basic physiological parameters and examination of organs and systems by general clinical methods.

The control group consisted of clinically healthy animals with normal physiological parameters.

The diagnosis of obesity was made according to the Henneke scoring system, where the first rank corresponds to emaciation and the ninth to a very fat animal, and the assessment was made by a veterinarian on the farm.

Blood was collected directly from the jugular vein into tubes for further biochemical studies. Blood was collected from the jugular vein on an empty stomach into 10 cm^3 Vacuette tubes for further serum collection.

The following biochemical parameters were determined in serum: concentration of total protein, albumin, total globulins, haptoglobin, and seroglycoids using reagent kits from PJSC 'Reagent' (Ukraine). The concentration of circulating immune complexes was determined as described Gołda et al. (2004) by precipitation of protein complexes antigen-antibody PEG-6000. Biochemical parameters were recorded using a Shimadzu UV-1800 spectrophotometer (Japan).

Experiments on animals were conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and in accordance with Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). The research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' under the current procedure.

Statistical analysis of the data was performed using Minitab v. 19 (Minitab Inc., USA). Based on the results of statistical processing, the table show non-parametric indicators, such as: median, quartiles Q1 and Q3. A significant difference between the study groups was established based on the calculation of the Mann–Whitney test ($p < 0.05$).

Results and discussion. The detailed results of serum concentrations of circulating immune complexes, seromucoids, C-reactive protein, haptoglobin, total protein, and protein fractions are presented in Table 1. At the same time, it should be noted that there was no significant difference between the ages of animals in different groups. Blood serum is a dynamic equilibrium system, which consists of 70.0% proteins, and the specificity of their metabolism reflects the state of almost all body tissues. In the animals of the experimental group, a significant increase in the score of body condition was recorded to a median score of 7.0 ($p < 0.05$), which corresponds to the score 'well-fed animal'. Thus, significant differences between the groups were found for almost each of the studied indicators.

Table 1 — Results of acute phase protein concentration in the control and obese groups

Indicators	Age, years	Body condition, points	Circulating immune complexes, mmol/l	Haptoglobin, g/l	C-reactive protein, mg/l	Seromucoids, g/l	Total protein, g/l	Albumin, g/l	Globulins, g/l
Clinically healthy horses, n = 10									
Median	11.5	6.50	116.00	0.462	3.19	2.19	65.50	34.0	32.0
Q_1	9.75	5.75	99.50	0.429	3.08	2.06	61.75	32.0	30.5
Q_3	12.25	7.00	129.00	0.477	3.63	2.26	72.00	36.0	36.0
Obese horses, n = 10									
Median	12.0	7.00*	160.00***	0.617***	5.62***	3.21***	75.00*	35.0	39.5***
Q_1	12.0	6.75	138.75	0.581	4.98	3.11	71.50	33.3	37.8
Q_3	14.0	8.00	170.00	0.678	5.92	3.61	76.50	36.3	42.0

Notes: * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.001$, compared to clinically healthy horses.

Circulating immune complexes (CICs) can be formed when antigens interact with antibodies in the blood. When these complexes are produced in excessive amounts or cannot be effectively cleared by the body, they can trigger an inflammatory response. Inflammation can result from the deposition of CICs in tissues, where they activate inflammatory processes, contributing to tissue damage and the further development of

inflammatory diseases (Tkaczenko et al., 2023). Thus, according to the results of our studies, the level of CICs in obese horses was increased by 37.9% and reached the level of 160.0 mmol/l ($p < 0.001$). It should also be noted that the concentration of CICs had a significant positive correlation (0.769) with the content of seromucoid, which may be associated with chronic inflammation, which is often observed in obesity. In addition, it is known that the processes caused by CICs deposition in tissues can affect metabolism, causing insulin resistance and other factors that contribute to the development of obesity (Stefaniuk-Szmukier, Piórkowska and Ropka-Molik, 2023).

Other important proteins in the acute phase of inflammation are haptoglobin and C-reactive protein (CRP). Haptoglobin is known to be elevated in horses with peritonitis or after surgery, can be produced by adipocytes, and is considered a marker of obesity (Johnson, 2002). In this study, we found a 33.5% increase in haptoglobin levels to 0.617 g/l ($p < 0.001$) compared to the control group. The haptoglobin level was also directly correlated with the age of the animals (0.771) and the level of CRP (0.781). It is known that CRP levels in horses increase several times during inflammation and obesity (Girardi et al., 2019).

CRP levels are increased in enteritis, pneumonia, and arthritis in adult horses and foals. In addition, CRP levels have been shown to correlate with inflammatory markers and to increase during experimentally induced laminitis; however, others have shown no difference in CRP levels in obese horses (Johnson et al., 2010). We found that CRP levels increased by 76.2% ($p < 0.001$) to a level of 5.62 mg/l, which is in full agreement with previous authors and may indicate both the development of animal obesity and the subclinical course of laminitis, which requires further research (Reynolds et al., 2019) (Zak et al., 2020). In addition, the development of the inflammatory process in horses of the experimental group is evidenced by an increase in the level of globulins by 25.8% ($p < 0.001$) against the background of an increase in the level of total protein by 14.3% ($p < 0.05$). It should be noted that the level of albumin did not undergo significant changes.

The role of seromucoids in distinguishing between acute and chronic inflammation is debated: they are indicated because they are a rather sensitive marker of inflammatory processes, during which their level increases several times. An increase in the level of seromucoids has been described in many inflammatory processes of bacterial and viral etiology, while other data suggest that seromucoids should be considered as a marker of chronic rather than acute inflammation (Galatyuk et al., 2018).

The results obtained in our study indicate that the level of seromucoids increased significantly by 47.2% ($p < 0.001$) to the level of 3.21 g/l in comparison with the control group. In addition, seromucoids are normal constituents of connective tissue, so when it is destroyed, they enter the bloodstream in significant amounts and are therefore considered markers of destructive and degenerative processes (Witkowska-Piłaszewicz et al., 2019). This is supported by correlation data on the direct dependence of seromucoid levels on body condition, circulating immune complexes, and haptoglobin and CRP. According to some reports, seromucoids also correlate with body mass index and adipose tissue content (Henneke et al., 1983) and adiponectin levels (Gołda et al., 2004). Therefore, in our opinion, seromucoid content can be used as an integral indicator of proteins in the acute phase of inflammation in obese horses.

Conclusions. Obesity in horses leads to a significant increase in acute phase inflammatory proteins. Determination of seromucoid concentration can be used as an integral indicator of acute phase inflammatory proteins in obese horses.

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