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DYNAMICS OF LIPID PEROXIDATION IN OBESE HORSES

Borovkov S. B., Boiko V. S.

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: serg_b78@ukr.net

Summary. Obesity is a pathological condition characterized by a specific pathogenetic process involving lipoperoxidation. Laboratory data on the levels of lipid peroxidation products in biological samples provide insights into the extent and severity of damage associated with this condition. This study aimed to investigate the impact of obesity on the intensity of lipid peroxidation processes as well as the compensatory activity of the antioxidant defense system in horses following influenza vaccination. In horses exhibiting signs of obesity, vaccination triggers oxidative stress, which is marked by excessive production of toxic lipoperoxidation products, specifically diene conjugates and malondialdehyde. On average, these levels were found to be 16.9% and 17.6% higher ($p < 0.01$) compared to those in horses with normal weight. The development of oxidative stress is regulated by antioxidant mechanisms, including catalase activity and total antioxidant activity, both of which were significantly reduced in horses with obesity. Specifically, these measures were lower by an average of 12.2% and 9.8% ($p < 0.01$) in the obese horses compared to their normal-weight counterparts. Markers of oxidative stress (content of diene conjugates and malondialdehyde), the activity of antioxidant defense enzymes, and total antioxidant activity in the blood of horses after vaccination are sensitive and informative indicators that can be used to assess the impact of vaccine prophylaxis, especially in animals with signs of obesity. Thus, obesity in horses significantly affects the levels of lipid peroxidation and oxidative stress, which can lead to serious health complications. Further research in this area may help develop effective strategies for preventing and treating obesity in horses, ultimately improving their overall health. Additionally, this research could serve as a foundation for future studies on the broader impact of oxidative stress on animal health

Keywords: metabolic syndrome, oxidative stress

Introduction. ***Relevance of the topic.*** Lipid peroxidation (LPO) is a critical biochemical process that can have serious consequences for the health of various animal species, including horses. This process involves the oxidative degradation of lipids, primarily polyunsaturated fatty acids, which leads to the formation of reactive by-products that can damage cellular structures and lead to many pathophysiological conditions. In recent years, the prevalence of obesity in horse populations has raised concerns about the dynamics of oxidative stress and its relationship to the LPO system. This article presents the results of a study of LPO mechanisms indicating the levels of LPO components in healthy and obese horses.

LPO is fundamentally characterized by the oxidative degradation of lipids, a process that begins when reactive oxygen species (ROS) interact with unsaturated fatty acids in cell membranes. This interaction leads to a chain reaction that ultimately results in the formation of lipid hydroperoxides and various aldehydes, including malondialdehyde. In addition, ROS, such as superoxide anions and hydrogen peroxide, significantly affect metabolic processes, as they are by-products of normal metabolic processes, and can accumulate to toxic levels under certain conditions, such as obesity. In the context of equine health, understanding the LPO system is very important as it is associated with various metabolic disorders, including insulin resistance and laminitis, which is particularly common in obese horses. In addition, oxidative stress resulting from an imbalance between ROS production and antioxidant defense can exacerbate the effects of LPO, leading to cellular damage, inflammation, and ultimately chronic health problems.

When assessing LPO levels in obese horses, several biomarkers serve as reliable indicators. One of the most studied markers is malondialdehyde (MDA), a byproduct of the oxidation of polyunsaturated fatty acids. MDA can be quantified in biological fluids such as plasma and urine. Elevated levels of MDA are often linked to increased oxidative stress and are correlated with various metabolic disorders in horses.

Research has shown that obese horses exhibit significantly higher levels of MDA compared to clinically healthy horses, indicating a heightened state of oxidative stress associated with their obesity. Therefore, monitoring these biomarkers can provide valuable information about the health status of obese horses and assist in guiding nutritional and treatment interventions to reduce oxidative damage.

The dynamics of LPO in obese horses are influenced by several factors, including diet, physical activity, and genetic predisposition. The composition of a horse's diet can significantly impact lipid metabolism and levels of oxidative stress. Diets high in omega-6 fatty acids, commonly found in grain-based feeds, can lead to increased production of ROS and, consequently, higher levels of LPO. In contrast, diets that are rich in antioxidants, such as vitamins E and C, can help reduce oxidative stress and lower LPO levels. Additionally, the role of physical inactivity cannot be overlooked, as obesity in horses is often linked to reduced physical activity. This lack of exercise can impair antioxidant defenses further and contribute to increased LPO. Regular physical activity has been shown to increase antioxidant enzyme activity, thereby reducing oxidative stress. In addition, genetic predisposition may affect the ability of an individual horse to respond to oxidative stress, leading to variability in LPO levels among obese horses. Genetic factors may influence metabolic pathways and the efficiency of antioxidant systems, emphasizing the need for a multifaceted approach to understanding and treating LPO in obese horse populations.

In conclusion, the dynamics of LPO in obese horses is a complex interplay of oxidative stress, dietary influences, physical activity, and genetic factors. The identification of reliable biomarkers, such as malondialdehyde and F2-isoprostanes, provides valuable information about the oxidative status of these animals and highlights the need for effective management strategies to mitigate the health risks associated with obesity. Understanding these dynamics is not only critical to improving equine health outcomes but also lays the foundation for future research into the broader impact of oxidative stress on animal health. As the prevalence of obesity in equine populations continues to rise, veterinarians and horse owners must recognize the importance of monitoring LPO and implementing appropriate dietary and lifestyle interventions to promote optimal equine health and well-being.

Analysis of recent research and publications. LPO is a group of biochemical processes characterized by the oxidation of unsaturated fatty acids that are part of cell membrane phospholipids due to the action of ROS (Titov et al., 2021) and can lead to damage to cell membranes, and is an important marker of oxidative stress, which plays a significant role in the development of various pathological conditions in animals. Studies show that LPO can be an indicator of both diseases and reactions to stressors (Angelidou, Ni and Fedorova, 2018). The main mechanism of LPO is initiation, which occurs under the influence of ROS, which can be formed as a result of metabolic processes or under the influence of exogenous factors such as radiation, toxins, or inflammation (Melnyk et al., 2024). ROS interact with unsaturated fatty acids, causing their oxidation and formation of peroxides, which are further decomposed into various toxic products, such as MDA and diene conjugates (DC) (Tiron and Vastyanov, 2023). These products can cause further cellular damage, activating inflammatory processes and contributing to the development of various diseases, including cardiovascular and metabolic disorders (Lyzogub et al., 2012).

However, the body has a variety of defense mechanisms that counteract these negative effects. The body's antioxidant system plays a key role in controlling LPO. It consists of enzymatic (e.g., superoxide dismutase, catalase) and non-enzymatic (vitamins C and E, glutathione) components that neutralize ROS and prevent their negative effects (Bobryk et al., 2021). However, when the level of ROS exceeds the capacity of the antioxidant system, ROS activation occurs, which can lead to oxidative stress and, as a result, cell death (Payenok, Kostiv and Hrytsyshyn, 2019). The main components of the antioxidant system are enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, which neutralize ROS and prevent cell damage (Said et al., 2020).

Studies show that the activation of LPO can be caused by healthy factors such as stress, toxic substances, and hypoxia. For example, in the case of chronic obstructive pulmonary disease, the level of LPO increases due to the influence of exogenous oxidants contained in polluted air (Ivchuk and Kovalchuk, 2019). Similarly, in the case of iron deficiency anemia in children, elevated iron can initiate an oxidase reaction, which leads to increased production of hydroxyl radicals and, consequently, cellular damage (Sherbatyuk, Gorishniy and Gorishniy, 2018). The body's antioxidant defense mechanisms also include non-enzymatic components such as vitamins (e.g., vitamin E) and flavonoids (e.g., quercetin), which can stabilize the prooxidant system and reduce LPO-induced damage (Mialiuk et al., 2022). Endogenous glutathione, which is a powerful antioxidant and participates in the detoxification of ROS, also plays an important role in protecting against oxidative stress (Mialiuk et al., 2022). In addition, research shows that various pathological conditions such as diabetes mellitus, neurodegenerative diseases, atherosclerosis, and obesity can lead to impaired antioxidant systems, which in turn contributes to the development of oxidative stress.

Obesity in horses is a complex phenomenon caused by the interaction of genetic, nutritional, and environmental factors. Understanding these factors is important for developing effective strategies to prevent and treat obesity in horses. Genetic factors play an important role in the development of obesity. Studies show that certain genetic markers may be associated with the risk of developing metabolic disorders that may lead to obesity (Borovkov, Timoshenko and Borovkova, 2023).

For example, horses with a hereditary tendency to accumulate fat are at a higher risk of developing obesity, particularly when they experience improper nutrition and a lack of physical activity (Sorokman, Popeliuk and Ushakova, 2021; Johnson, 2002). Genetic factors can also influence the metabolism of fats and carbohydrates, which play a crucial role in weight management (Musiienko and Marushchak, 2020). Nutritional factors are another significant contributor to obesity in horses. Improper feeding practices, such as excessive consumption of high-calorie feed, can lead to weight gain. High-calorie feeds that are rich in sugars and fats can contribute to obesity if not balanced with adequate physical activity (Terenda et al., 2023). Additionally, insufficient fiber intake can adversely affect digestion and metabolism, further promoting fat accumulation (Kotova et al., 2020). Studies show that excess body weight in horses can lead to increased levels of ROS, which in turn activates LPO processes. Increased levels of LPO result from the oxidation of unsaturated fatty acids, which are part of cell membranes, leading to their damage and impaired cell function. One study found that obese horses had elevated levels of LPO products such as malondialdehyde, a marker of oxidative stress. This suggests that obesity may lead to an imbalance between the body's prooxidant and antioxidant systems, which is characteristic of oxidative stress. Under conditions of oxidative stress, there is an increase in inflammatory processes, which can worsen the overall health of horses and, in particular, affect their productivity and viability (Borovkov, Timoshenko O. and Borovkova, 2023). Studies also show that obesity in horses can be associated with metabolic disorders, which are associated with elevated levels of insulin and leptin, which in turn can activate inflammatory mechanisms. This underscores the importance of controlling body weight in horses to prevent the development of oxidative stress and reduce the risk of comorbidities.

According to the literature, studies in various animal species, such as cattle, dogs, and pigs, indicate a complex interplay between genetic, nutritional, and environmental factors that contribute to obesity.

In cattle, obesity is often associated with metabolic disorders such as insulin resistance. Studies have shown that excessive energy intake, especially from carbohydrate-rich feeds, can lead to fat accumulation in the liver and other organs. An important role in the development of obesity is also played by inflammatory processes that are activated by the accumulation of adipose tissue. For example, macrophages in bovine adipose tissue have been found to promote inflammation, which in turn worsens metabolic parameters (Weisberg et al., 2003).

Obesity is also a serious problem in dogs, which is associated with low levels of physical activity and improper nutrition. Studies show that dogs that have access to high-calorie food and do not get enough physical activity are at an increased risk of developing obesity (Muñoz-Prieto et al., 2018). In addition, social factors, such as the owner's age and lifestyle, can also influence the risk of obesity in dogs (Suárez et al., 2022). Owners with a sedentary lifestyle are more likely to overfeed their dogs, which leads to an increase in body weight (Muñoz-Prieto et al., 2018).

Pigs, in particular mini pigs, are also used as a model for studying obesity. Studies have shown that a diet high in fat and sugar leads to increased fat mass and the development of insulin resistance (Niu et al., 2017). For example, a study in mini pigs found that a diet rich in saturated fat promotes fat accumulation in the liver and the development of metabolic disorders that resemble human obesity (Niu et al., 2017; Reyer et al., 2017). It has also been found that genetic factors, such as polymorphisms in genes related to fat metabolism, can influence the susceptibility to obesity in pigs (Reyer et al., 2017).

It should be noted that environmental factors, such as housing conditions and level of physical activity, also have a significant impact on the development of obesity, particularly in horses. Horses that are kept in confined spaces and do not have the opportunity for active movement are at an increased risk of obesity (Maksymovych, 2016). Studies show that horses that exercise regularly are less likely to develop obesity, as physical activity helps to burn calories and maintain a healthy body weight (Tkachova and Tkachenko, 2019). Thus, obesity in horses is the result of a complex interaction of genetic, nutritional and environmental factors. For effective prevention and treatment of this condition, it is necessary to take all these aspects into account when developing individualized feeding and exercise programs for each horse.

In such cases, the correction of body functions with the help of biologically active substances can be an effective strategy to reduce the damage caused by LPO (Myalyuk et al., 2022). Thus, the defense mechanisms against LPO are a complex system that includes both enzymatic and non-enzymatic components that interact to maintain homeostasis under conditions of oxidative stress. Further research in this area may help to develop new therapeutic strategies for the treatment of oxidative stress-related diseases and improve the overall health of animals.

The aim of the study. To investigate the peculiarities of LPO processes and compensatory activity of the antioxidant defense system in the body of horses after influenza vaccination.

Research tasks. To achieve the aim of the study, horses of mainly Ukrainian riding breed kept in state research farms of Poltava Region were used and the following tasks were set: to study the dynamics of LPO indicators (MDA, DC) and the level of antioxidant system activity (level of antioxidant activity (AOA) and catalase activity (CA)) in the blood serum of horses with obesity.

Materials and methods. Horses of mostly Ukrainian riding breeds, kept in state research farms in Poltava Region, were studied. The animals were divided into two groups: 9 clinically healthy horses and 9 horses with diagnosed obesity, the total number of studied animals was 18. The experimental studies were conducted at the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'.

The feeding and housing conditions met the physiological needs of the animals: the diet was balanced in terms of the main nutrients; the animals had constant access to water and the opportunity for active walking. All animals underwent a general clinical examination following standard methods. Physical condition and Body Condition Scoring (BCS) were assessed by two independent veterinary experts. Blood was taken from the jugular vein on an empty stomach into 10 ml

Vacuette tubes for further serum collection following biochemical methods. The study of blood serum was performed using a two-beam spectrophotometer of the research class Shimadzu UV 2600i/UV 2600 (Japan). The intensity of LPO processes was assessed by the formation level of its products — DC and MDA by extraction with a mixture of heptane-isopropanol as described by Stegniy et al. (2009). The state of the antioxidant system indicators was studied by catalase (EC 1.11.1.6) activity using H_2O_2 spectrophotometrically at a wavelength of 410 nm, as described by Korolyuk et al. (1988) and by total plasma lipid AOA, as described by Klebanov et al. (1988).

During the experimental studies outlined in this paper, all interactions with the horses involved in the research 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 under 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). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'.

Statistical data analysis was performed using the Minitab 19 software by Minitab Inc., utilizing a free trial version. The arithmetic mean (M) and the standard error of the arithmetic mean (m) were calculated. The probability of the difference between the arithmetic means of two variation series was assessed using the reliability criterion (td), Student's *t*-distribution tables, and the nonparametric Van der Waerden method.

Results and discussion. The major products of LPO are classified as primary and secondary. Primary LPO products, which include DC, are formed as a result of oxidation of polyunsaturated fatty acids at the stage of free radical formation. The appearance of DC indicates the formation of free radicals and thus the free radical mechanism of oxidation of polyunsaturated fatty acids, and at the same time serves as a signal for the formation of hydroperoxides (Melnyk et al., 2024). Secondary products of lipid-free radical oxidation are formed as a result of the destruction of polyunsaturated fatty acid hydroperoxides, producing a significant amount of MDA and ketones (Bobryk et al., 2021). MDA is a bifunctional aldehyde capable of forming Schiff bases with protein amino groups and acts as a cross-linking agent. Oxidation results in the formation of protein-insoluble lipid complexes called wear pigments or lipofuscin (Melnyk et al., 2024). Thus, MDA content is an indicator of the activity of oxidative processes caused by oxygen radicals (Segin, Hnatush and Gorishniy, 2016).

In this regard, the first stage of our research is to determine the content of DC and MDA. However, in the body, the antioxidant system, which consists of enzymatic and non-enzymatic components, plays a key role in controlling LPO. In order to reflect the state of the antioxidant system, we studied the CA and the level of AOA. The results are presented in Table 1.

Table 1 — Results of equine blood serum studies (M ± m; n = 10)

Observation period	Indicator			
	MDA, μmol/L	DC, ΔD	AOA, %	CA, μmol H_2O_2/L×min
Horses with normal body weight				
Before vaccination	3.67 ± 0.16	31.20 ± 1.06	75.70 ± 3.25	68.50 ± 1.42
7th day	4.08 ± 0.06$^{\bullet}$	34.19 ± 0.67	69.03 ± 6.06	65.07 ± 1.27
14th day	4.42 ± 0.06$^{\bullet\bullet\bullet}$	38.94 ± 0.61$^{\bullet\bullet}$	71.91 ± 5.22	64.39 ± 0.75
21st day	4.20 ± 0.08$^{\bullet\bullet}$	36.40 ± 0.69$^{\bullet}$	79.48 ± 3.21	72.26 ± 0.17$^{\bullet}$
28th day	3.83 ± 0.16	32.70 ± 0.97	81.22 ± 2.53	76.44 ± 1.66$^{\bullet\bullet}$
Horses with obesity				
Before vaccination	3.62 ± 0.11	30.5 ± 0.77	80.70 ± 4.66	70.40 ± 2.05
7th day	4.01 ± 0.03	34.03 ± 0.69$^{\bullet}$	70.69 ± 4.71	63.64 ± 0.9$^{\bullet\bullet}$
14th day	4.57 ± 0.03$^{*,\bullet\bullet}$	39.28 ± 0.48$^{\bullet\bullet\bullet}$	67.22 ± 8.39	61.10 ± 1.07$^{*,\bullet\bullet}$
21st day	4.78 ± 0.04$^{**,\bullet\bullet\bullet}$	40.93 ± 0.50$^{***,\bullet\bullet\bullet}$	65.93 ± 8.42	59.06 ± 0.92$^{***,\bullet\bullet\bullet}$
28th day	4.65 ± 0.12$^{\bullet\bullet\bullet}$	39.71 ± 0.76$^{***,\bullet\bullet\bullet}$	76.34 ± 2.58	66.17 ± 1.32$^{***,\bullet}$

Notes: * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.001$, compared to horses with normal body weight; $^{\bullet}$ — $p < 0.05$, $^{\bullet\bullet}$ — $p < 0.01$, $^{\bullet\bullet\bullet}$ — $p < 0.001$, compared to pre-vaccination.

According to the results presented in Table 1, horses with normal weight exhibited an increase in MDA levels by 11.2% ($p < 0.05$), 20.4% ($p < 0.001$), and 14.6% ($p < 0.01$) during the entire observation period compared to levels before vaccination. Similarly, the level of DC increased by 9.6%, 24.8% ($p < 0.01$), and 16.7% ($p < 0.05$) during the same period. These findings suggest that lipoperoxidation processes were activated, particularly on 7th and 14th days when antioxidant defense was suppressed. This is indicated by a trend toward decreased AOA and CA, along with an 8.8% reduction in total AOA on 7th day. Since the total AOA reflects the body's ability to inhibit the formation of peroxidation products, it suggests that after vaccination, there are insufficient antioxidants available in the horses' bodies to effectively manage the processes of peroxidation at a physiological level during the early stages of immunological reactions. Starting from the second half of the experiment (21st and 28th days), the effectiveness of antioxidant defense mechanisms begins to increase. This is evidenced by a significant increase in AOA and CA on 21st day, with an increase of 17.3% and 11.6% on 28th day ($p < 0.01$) for these indicators, respectively. This trend suggests a reduction in the intensity of oxidation chain reactions, likely due to the activation of natural antioxidant systems, which may result from changes in metabolic processes.

Thus, the study found that the use of the vaccine caused a temporary excessive formation of LPO products in the blood of animals, which was controlled by the activity of the antioxidant defense system and the balance of the enzymatic and non-enzymatic links of the antioxidant system. The results obtained are in line with the literature on the temporary effect of the vaccine on lipid metabolism and are also consistent with our previous studies (Elmallah et al., 2017, Borovkov and Boiko, 2024).

Obesity in horses is a serious and widespread problem that can lead to the development of metabolic disorders such as equine metabolic syndrome, which in turn leads to oxidative stress. Genetic predisposition, current management practices, pathological changes in adipose tissue, and changes in the microbiome are key factors contributing to the development of this condition (Reynolds et al., 2019).

In the study examining the intensity of LPO processes in obese horses following vaccination, the results showed significant changes (Table 1). There was an increase in both primary and secondary products of LPO — specifically, DC and MDA — throughout the entire observation period. The level of DC rose by 11.6% ($p < 0.05$), 28.8% ($p < 0.001$), 34.2% ($p < 0.001$), and 30.2% ($p < 0.001$) compared to the levels measured before vaccination. Similarly, the level of MDA increased by 10.8%, 26.3% ($p < 0.01$), 32.2% ($p < 0.001$), and 28.4% ($p < 0.001$) during the same timeframe.

Additionally, we found that these LPO products were elevated in comparison to horses with normal weight: DC levels increased by 12.4% ($p < 0.001$) and 21.4% ($p < 0.001$) on 21st and 28th days, respectively; MDA levels increased by 13.8% ($p < 0.001$) and 21.4% on the same days.

The excessive formation of membrane-altering toxic LPO products indicates a shift in the functional activity of the antioxidant system, which plays a crucial regulatory and prognostic role in protecting cell membranes. To investigate this, we studied the dynamics of AOA and CA in obese animals following vaccination.

Our findings revealed a decrease in AOA of 12.4%, 16.7%, and 18.3%, and a decrease in CA of 9.6% ($p < 0.01$), 13.2% ($p < 0.01$), and 16.1% ($p < 0.05$) on days 7, 14, and 21, respectively, compared to levels before vaccination.

Additionally, we observed a notable decline in total serum AOA in experimental horses with signs of obesity when compared to those with normal weight, with decreases of 6.5%, 17.0%, and 6.0% on days 14, 21, and 28, respectively. The reduction in CA in the obese group was also significant, exhibiting decreases of 5.0% ($p < 0.05$), 18.3% ($p < 0.001$), and 13.4% ($p < 0.001$) during the same period, correlating with the observed changes in AOA levels. In summary, the impact of vaccination on obese animals results in oxidative stress due to altered metabolic processes, which is reflected in the decreased CA and total AOA levels.

Conclusions. 1. When horses with signs of obesity are vaccinated, oxidative stress occurs in their bodies. This is accompanied by an excessive production of toxic lipoperoxidation products, specifically diene conjugates and malondialdehyde, which are, on average, increased by 16.9% and 17.6% ($p < 0.01$), respectively, compared to horses with normal weight. The development of oxidative stress is influenced by the body's antioxidant resources, specifically catalase activity and total antioxidant activity, both of which are reduced by an average of 12.2% and 9.8% ($p < 0.01$) in horses with obesity compared to those of normal weight.

2. Markers of oxidative stress, such as the levels of diene conjugates and malondialdehyde, along with the activity of antioxidant defense enzymes and total antioxidant activity in the blood of horses following vaccination, are valuable and sensitive indicators. These markers can be used to assess the effects of vaccine prophylaxis, particularly in animals showing signs of obesity.

3. Thus, obesity in horses has a significant impact on the level of lipid peroxidation and oxidative stress, which can lead to serious consequences for their health. Further research in this area may help to develop effective strategies for the prevention and treatment of obesity in horses, as well as to improve their overall health.

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