



## Marker changes in the blood lipidogram in the pathogenesis of some internal diseases of horses

**Mohammed Khalil Alhindy\***

PhD in Veterinary Sciences, Associate Professor

Al-Azhar University-Gaza

1277, amal Abdl Naser Str., Gaza, Palestine

<https://orcid.org/0000-0003-0678-8436>

**Sergii Borovkov**

PhD in Veterinary Sciences, Associate Professor

National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine"

61023, 83 Pushkinska Str., Kharkiv, Ukraine

<https://orcid.org/0000-0003-3021-2410>

**Olha Tymoshenko**

Doctor of Veterinary Sciences, Professor

State Biotechnological University, Kharkiv, Ukraine

61002, 44 Alchevskykh Str., Kharkiv, Ukraine

<https://orcid.org/0000-0001-9696-1698>

**Abstract.** Disorders in lipid metabolism in the bodies of horses (genus *Equus*) due to various factors often lead to the development of diseases, including those associated with the onset of metabolic syndrome. Therefore, determining marker changes in the serum lipid profile during metabolic disorders is a relevant issue in investigating the pathogenesis of the most common diseases in this species. The purpose of the study is to identify the features of lipid metabolism indicators in the serum of horses in a physiological state and in the case of the onset of colic, laminitis, and metabolic syndrome symptom complexes. The investigation of lipid metabolism indicators in the serum of these animals was conducted using an enzymatic colorimetric method with the use of a biochemical automatic analyzer COBAS C 311 ("Roche Diagnostics GmbH", Germany). It is established that in the lipid profile of the serum of clinically healthy Ukrainian Hutsul horses, the share of high-density lipoprotein cholesterol was 75.1%, low-density lipoproteins were 15.7%, and

### **Suggested Citation:**

Alhindy, M.K., Borovkov, S., & Tymoshenko, O. (2024). Marker changes in the blood lipidogram in the pathogenesis of some internal diseases of horses. *Ukrainian Journal of Veterinary Sciences*, 15(1), 9-23. doi: 10.31548/veterinary1.2024.09.

\*Corresponding author



Copyright © The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (<https://creativecommons.org/licenses/by/4.0/>)

very low-density lipoproteins were 9.2%. In cases of pathologies with colic symptom complex in the serum of horses, the share of high-density lipoprotein cholesterol significantly decreased, while low-density lipoproteins increased 3.9 times compared to healthy animals. In the lipid profile of the serum of clinically healthy ponies, the share of high-density lipoprotein cholesterol was 26.3%, low-density lipoproteins were 65.1%, and very low-density lipoproteins were 8.61%. In the case of laminitis in ponies, the dynamics of lipid profile indicators corresponded to those in horses. The established regularities regarding changes in the serum lipid profile in different representatives of the horse genus allowed specifying their role in the pathogenesis of certain prevalent pathologies. The results of the study are of practical value for application in clinical veterinary medicine and will contribute to conducting high-quality laboratory diagnostics of the most common horse pathologies (colic symptom complex, laminitis, and metabolic syndrome), characterised by lipid metabolism disorders

**Keywords:** pony; Ukrainian Hutsul breed; cholesterol; lipoproteins; colic symptom complex; laminitis; metabolic syndrome

---

## Introduction

Horse breeding is considered a substantial area within the agricultural sector and veterinary science. Modern horse breeding provides opportunities for the development of sports, tourism, and recreation and serves as a source of agricultural production. However, there is a negative trend in the development of the horse breeding industry. According to T.A. Yusiuk-Omelnytska (2023), in 2001, the total number of horses in Ukraine was 701.2 thousand, with 209.8 thousand on enterprises and 451.4 thousand in private households. By 2019, there was a significant decrease to 244.0 thousand, mainly due to a reduction in horses in private households. As of 2020, Ukraine has 79 institutions involved in breeding activities in horse breeding, including 20 horse farms, 46 breeding reproducers, 3 breeding centres, and 2 genetic control enterprises, as noted by O. Stetsiuk (2020). State horse farms concentrate 28.0% of the breeding population, while the rest is distributed among farms of various ownership forms.

Nevertheless, proper conditions for the maintenance and feeding of horses are often not observed, especially in recent years, which can have serious negative consequences for the

animals. Issues such as insufficient body weight or, conversely, obesity, musculoskeletal problems, digestive system issues, weakened immunity, and nervous disorders can be observed in horses. Therefore, it is crucial to create suitable conditions for the housing, proper nutrition, and regular veterinary care of horses to ensure their health and well-being.

Among the most common pathologies in horses related to metabolic syndrome is laminitis. Researchers like A. Reynolds *et al.* (2019) noted equine metabolic syndrome, classified in 2010 by the American College of Veterinary Internal Medicine as insulin resistance, general or regional obesity, and susceptibility to laminitis. Equine metabolic syndrome is similar to that in humans. It is classified by features such as visceral obesity, hypertriglyceridemia, glucose intolerance, low cholesterol levels, and high-density lipoproteins. A.C. Ericsson *et al.* (2021) compared metabolic syndrome in horses with that in humans, allowing the application of methodologies already developed in medicine for diagnosis and differential diagnosis of metabolic syndrome in horses. The state of hyperlipidemia in horses can develop

in various forms. Therefore, different terms are used to differentiate them based on the severity of these diseases. In this context, four terms are proposed, determined by the concentration of triglycerides in the horse's blood serum: hypertriglyceridemia, hyperlipidemia, severe hypertriglyceridemia, and hyperlipemia. Hyperlipidemia usually results from a negative energy balance, primarily caused by feed restriction, especially during periods of high energy demand, such as pregnancy, lactation, or anorexia induced by illness. However, other findings suggest that obese and stressed mares pose the highest risk of developing hyperlipidemia regardless of pregnancy status.

There are several factors that can cause disruptions in energy metabolism in horses, but the most common cause is insulin resistance, as described in most publications, including Z. Daradics *et al.* (2021) and J. Delarocque *et al.* (2021). Various studies have been conducted to demonstrate that horses with insulin resistance are prone to hyperlipidemia; however, the etiology of this condition remains unknown. Hyperlipidemia most often arises as a primary sign. However, through clinical trials, it has been proven that its occurrence is secondary, resulting from the development of pathologies in various systemic diseases leading to a negative energy balance. Among the most common pathologies in miniature horse breeds are enterocolitis, dental diseases, bacterial and parasitic infections, pneumonia, colic, and laminitis.

Therefore, the purpose of this study is to investigate lipid metabolism indicators in the blood serum of horses and ponies under normal conditions and in the case of colic, laminitis, and metabolic syndrome.

### Literature Review

In the last decade, lipid metabolism in horses and other animals has become the subject of in-depth research in global veterinary medicine. J.J. Kaneko *et al.* (2008) and C.M.M. Loos *et*

*al.* (2019) indicate that disorders in lipid metabolism are associated with diseases of the liver and biliary tract, kidneys, pancreas, and lungs. J. Delarocque *et al.* (2021) note that changes in lipid metabolism play a crucial role in the onset of dystrophic processes, endocrine pathology, and obesity, negatively affecting the animals' bodies and leading to the development of other pathologies. Equine metabolic syndrome has significant prevalence and is a current issue in horse breeding, as evidenced by a considerable number of publications. It is a complex disorder, with more questions than answers, as A.C. Ericsson *et al.* (2021) note that the main components of metabolic syndrome in horses are intense obesity, insulin resistance, and laminitis. This is complemented by the results of studies by N. Heliczner *et al.* (2017) and R. Lu *et al.* (2018), indicating that this syndrome encompasses a much broader range of disorders affecting energy metabolism, adipocyte function, promoting thrombosis, causing inflammation and oxidative stress, and altering the function of vascular endothelial cells in affected horses.

The role of adipose tissue in the development of metabolic syndrome is not limited to the excessive accumulation of nutrients. K. Marycz *et al.* (2018a; 2018b) show that adipokines released from adipocytes and other cells in adipose tissues, including leptin, resistin, adiponectin, visfatin, and apelin, as well as inflammatory cytokines, play a crucial role. A. Reynolds *et al.* (2019) state that disruptions in lipid metabolism, particularly enhanced lipid peroxidation, are one of the primary links in the stress response associated with metabolic syndrome. According to the research conducted in Ukraine, specifically by A. Andriichuk *et al.* (2014) and M.A. de Laat & D.M. Fitzgerald (2023), the causes of lipid metabolism disorders in farm animals were identified.

C.M.M. Loos *et al.* (2019) and Z. Daradics *et al.* (2021) developed and implemented sufficient diagnostic methods for this syndrome,

including biochemical ones. These methods include determining glucose, insulin, interleukins, and lipid exchange indicators in the blood, including lipoproteins. Researchers note that blood lipid profile indicators undergo specific changes and depend on the region, breed, and age characteristics of the animals.

Therewith, information regarding this problem in horses is lacking, although these animals often experience pathological conditions and diseases accompanied by disruptions in lipid metabolism, including lipoproteins – obesity, hepatic lipidosis, nephropathy, cachexia, tumors, lung diseases, and others. This is mentioned in individual studies by domestic researchers such as I.A. Zhukova (2014) and L.I. Posternak (2017).

R.B. Olley *et al.* (2019), N.P. Karikoski *et al.* (2022) and C. Cantarelli *et al.* (2018) emphasise that there is currently a lack of in-depth research on the role of lipid metabolism disorders in the development of pathologies in the internal organs of horses. The causative factors of these disorders may include the pathogenic influence of environmental factors, especially inappropriate conditions of maintenance and feeding, neurogenic stress, various physical, chemical, and other factors. Therefore, exploring the features of lipid metabolism disorders, determining the informativeness of their indicators for the diagnosis and assessment of the effectiveness of horse treatment for various internal diseases, is a relevant task for veterinary science and practice.

## **Materials and Methods**

During the study, Ukrainian riding breed horses and ponies housed at the State Biotechnological University Equestrian Sports Complex (Kharkiv) and at the Feldman Eco Park Public Organisation (Kharkiv region) were examined. Specifically, 10 Ukrainian riding breed horses, males and females, of sports direction, aged

6-10 years, in a state of relative rest (control group), 5 horses with colic syndrome, and 5 horses with symptoms of laminitis were examined. 5 clinically healthy ponies (control group) and 5 ponies with metabolic syndrome on the background of obesity were used for the study. Thus, a total of 30 animals were examined. Studies on these farms were conducted during 2021-2023. The research scheme included a minimal number of animals for statistical processing using non-parametric methods of statistical analysis, allowing the determination of the informativeness of indicators according to the stated research purpose.

Feeding and housing conditions corresponded to the physiological needs of the animals. The ration of animals was balanced with essential nutrients. All animals had free access to water and enjoyed walking. Animals underwent clinical examination according to generally accepted methods, including the determination of basic physiological indicators, the examination of major organs and systems using inspection, percussion, palpation, and auscultation methods. Only clinically healthy animals were selected for the control group. The diagnosis of metabolic syndrome, colic symptom complex, and laminitis was made comprehensively, considering the data of the medical history, clinical picture, and based on the conducted laboratory studies. In addition, for the diagnosis of metabolic syndrome, an oral glucose tolerance test with insulin was applied. Horses in the control group were examined during show jumping competitions, when their bodies were subjected to significant physical and emotional stress. Blood samples from the animals were obtained in a state of relative rest and 15 minutes after an entertainment show performance with a large audience and loud music accompaniment. Blood samples were taken from the jugular vein into Vacuette vacuum tubes with a volume of 10 cm<sup>3</sup> for further obtaining native blood, plasma,

and serum depending on the research methods for its biochemical analysis, which were conducted at the Research Institute of Experimental and Clinical Medicine, Kharkiv.

The research on biochemical indicators in the blood serum, including total cholesterol, triacylglycerols, high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL), was conducted using the photometric system COBAS C 311 (Germany) with the corresponding ion-selective electrodes. Total cholesterol was determined using an enzymatic colorimetric method, where cholesterol esters are hydrolysed by cholesterol esterase into cholesterol and fatty acids. Cholesterol oxidase catalyses the oxidation of cholesterol to form hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide acts on the oxidative coupling of phenol and 4-aminophenazone to form a red-coloured compound, the intensity of which determines the concentration of cholesterol. Cholesterol in lipoproteins was determined using a homogeneous colorimetric method based on the use of cholesterol esterase and cholesterol oxidase in the presence of surfactants that selectively absorb certain types of lipoproteins.

During the experimental research, all manipulations with the horses involved in the studies were conducted considering the basic principles of bioethics, in accordance with the Law of Ukraine No. 3447-IV (2006, February), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986), and the "General Ethical Principles of Experiments on Animals" adopted by the First National Congress on Bioethics (Procedure for conducting..., 2012).

Statistical analysis of the data was performed using the Minitab 19 program by Minitab Inc. The results of the statistical processing present non-parametric indicators in the

tables, such as Median, Quartiles  $Q_1$  and  $Q_3$ . The significant difference between the study groups was established based on the calculation of the Mann-Whitney criterion ( $P < 0.05$ ).

## Results and Discussion

The clinical condition of the horses before the start of the study corresponded to physiological parameters. The body temperature, pulse, and respiratory rate in horses were within the range of  $Me =$  quartile  $Q_1$  and  $Q_3$ , respectively. Cardiac auscultation revealed no changes in heart tones and murmurs, and lung auscultation detected vesicular breath sounds throughout the projection of the lungs on the chest wall. Therefore, these animals were selected into the control group for further investigation.

According to the results of H. Gehlen *et al.* (2020), the composition of the blood lipid profile in animals of different species shows significant differences. For instance, in pigs, the quantitative distribution of cholesterol fractions is similar to that in humans, namely, the share of low-density lipoprotein cholesterol accounts for about 50-60% of the total serum cholesterol. In rabbits and mice, on the contrary, almost all serum cholesterol is part of very low-density and high-density lipoproteins. According to P.G. Xenoulis *et al.* (2020) and F.H. Alonso *et al.* (2022), in the blood serum of dogs, cholesterol in high-density lipoproteins is 5-7 times higher than in low-density lipoproteins. In horses, as reported by E.M. Norton *et al.* (2019), similar to dogs and unlike humans, the highest proportion of cholesterol is noted in high-density lipoproteins, preventing the development of atherosclerosis in animals of these species. These data indicate significant variability in lipid metabolism indicators in the blood serum of different animal species, necessitating further examination. It can be assumed that such variability may result from the influence of different diets and genetically

determined differences in the mechanisms of lipoprotein and cholesterol metabolism in animals of different species.

Table 1 presents the results of the study of the lipid profile in the blood serum of clinically healthy resting horses.

**Table 1.** Lipid profile indicators in the blood serum of clinically healthy horses, n=10 (mmol/L)

| Indicator      | Total cholesterol | Triglycerides | HDL Cholesterol | LDL Cholesterol | VLDL Cholesterol |
|----------------|-------------------|---------------|-----------------|-----------------|------------------|
| Median         | 1.85              | 0.36          | 1.39            | 0.29            | 0.17             |
| Q <sub>1</sub> | 1.81              | 0.34          | 1.37            | 0.28            | 0.16             |
| Q <sub>3</sub> | 1.89              | 0.38          | 1.40            | 0.31            | 0.18             |

Source: developed by the author

According to the results shown in Table 1, it was found that in clinically healthy horses, the share of HDL cholesterol was 75.1%, while LDL cholesterol was 15.7%, and associated with VLDL cholesterol was 9.2%. This indicator is consistently unchanging in all examined animals, both in terms of the median value and when calculating individual quartiles. This aligns with the findings of most other researchers. For example, J.J. Kaneko *et al.* (2008) indicated that in the bodies of clinically healthy horses, high-density lipoproteins play a crucial role in lipid metabolism. This is explained by their ability to transport cholesterol alongside other representatives of this class through the circulatory system's organ network. According to their model, high-density lipoproteins exhibit an anti-atherogenic effect through their role in the reverse transport of cholesterol by removing it from macrophages and incorporating it into the early forms of these lipoproteins, followed by the transformation of free cholesterol into esterified form (cholesterol esters). Esterified cholesterol is then extracted from the plasma by the liver through high-density lipoprotein receptors and transported to the bile for subsequent intestinal excretion. Thus, high-density lipoproteins can perform functions related to transporting cholesterol

from tissues and the intima of blood vessels to the liver for the synthesis of bile acids and the elimination of excess lipids from the body. J. Delarocque *et al.* (2021) indicated that this mechanism provides regulation of cholesterol levels in the body. In addition, high-density lipoproteins exhibit antioxidant properties positively impacting the endothelium and the vascular system's condition. They also transport cholesterol for the synthesis of not only bile acids but also steroid hormones and vitamin D. This underscores the substantial functional aspects of lipid metabolism in horses, indicating the importance of high-density lipoproteins in maintaining effective cholesterol and other lipid exchange. Understanding these metabolic processes in clinically healthy animals can shed light on anomalies that occur in horses, especially those suffering from laminitis, where considerable changes in lipid metabolism and serum lipid profile are observed. Such studies open prospects for developing targeted therapeutic strategies to overcome characteristic lipid metabolism disorders affecting the development of internal diseases in horses.

It can be asserted that determining lipid metabolism indicators, along with other biochemical tests, should be applied not only in diagnosing internal diseases in horses but also

in assessing the condition of clinically healthy animals under physical or emotional stress, particularly during sports competitions or other stressful factors. In the current environment where demands on sports horses are steadily increasing, their bodies are not always able to withstand intense training and stress loads during competitions. Therefore, veterinary professionals need rapid and effective methods to assess the functional state and training capabilities of horses' bodies to maximise their adaptation to stress factors, adequately and timely correct possible disorders, and thus support their ability to endure intense loads and demonstrate high performance in competitions. This is precisely what prompted the determination of lipid metabolism indicators in sports horses during show jumping competitions.

The increase in the content of triglycerides in the blood serum is a specific indicator for exercising horses, as the primary source for working muscles during loading is the oxidation of free fatty acids released by their breakdown. Despite the consumption of free fatty acids by the muscles, the triglyceride content in the blood serum increases, and their synthesis in the liver also increases. Therefore, this increase is a specific sign for a horse undergoing emotional and physical stress during training. This elevation is a basis for a more in-depth analysis of lipid metabolism in sports horses and should be considered when assessing

lipid exchange indicators in horses. Therefore, an examination of lipid exchange indicators in major pathologies associated with its disturbances has been conducted.

Among the pathologies in horses that manifest substantial changes in lipid metabolism, researchers prefer a group of diseases accompanied by a colic symptom complex, caused by a sharp change in the diet when transitioning animals from stable to pasture. The diagnosis of the colic symptom complex was established comprehensively. According to the history, it is known that the animals were transferred to green fodder, which included mown meadow grass, replacing hay. They eagerly consumed the green mass of mown grass, and in the evening, clinical signs of digestive disorders in the form of colic were observed. These animals showed restlessness, constantly lying down and getting up, hitting their abdomen with their limbs, making pendulum-like movements with their bodies. At the same time, an increase in pulse and respiration rates was noted. Upon palpation of the abdominal wall, tension and tenderness were detected. Auscultation revealed the absence of bowel peristalsis sounds, and percussion indicated excessive gas accumulation. After blood sampling, the animals were given symptomatic treatment and transferred to a different diet. The lipid profile indicators in the blood serum of horses with the colic symptom complex are presented in Table 2.

**Table 2.** Lipid profile indicators in the blood serum of horses with the colic symptom complex, n=5 (mmol/L)

| Indicator      | Total cholesterol | Triacylglycerols | HDL Cholesterol | LDL Cholesterol | VLDL Cholesterol |
|----------------|-------------------|------------------|-----------------|-----------------|------------------|
| Median         | 2.55*             | 0.38             | 1.19            | 1.15*           | 0.21             |
| Q <sub>1</sub> | 2.32              | 0.36             | 1.09            | 1.05            | 0.18             |
| Q <sub>3</sub> | 2.93              | 0.46             | 1.32            | 1.29            | 0.32             |

**Note:** \* $P < 0.05$ , compared to clinically healthy horses (Table 1)

**Source:** developed by the author

From the indicators presented in Table 2, it can be inferred that with the clinical manifestation of colic in the blood serum of horses, there was an increase in the concentration of total cholesterol by 37.8% ( $P < 0.05$ ) compared to clinically healthy animals (Table 1), mainly due to a 3.9-fold increase ( $P < 0.05$ ) in the concentration of low-density lipoprotein cholesterol. Therefore, with a sharp change in the diet of these animals, the reverse cycle of cholesterol metabolism is likely to be disrupted. As a result, the intensity of cholesterol entering tissues and vessels increases, further deposition under the intima occurs, and the functioning of internal organs is disrupted. However, for colic symptoms caused by a sharp change in the diet, there were no noteworthy changes in the blood serum level of triglycerides and, accordingly, very low-density lipoprotein cholesterol. Thus, hypercholesterolemia developed in this pathological condition due to low-density lipoprotein cholesterol against the background of a relatively stable level of triglycerides.

Another fairly common pathology among all breeds of horses is laminitis. Its pathophysiology is not yet fully understood and is contradictory. Chronic laminitis is accompanied by persistent lameness, breakdown of anatomical structures of the hoof, including changes in the laminae, prolapse of the sole, and disruption of hoof horn growth. There is a separation of the basal membrane of the hoof bone and the epidermal lamellae lining the inner surface of the hoof capsule. Only a very limited number of horses restore their athletic condition after experiencing chronic laminitis and can return to a normal training regimen. Laminitis more often occurs as a complication of pathologies of internal organs that seem unrelated to the limbs, among which the most common are gastrointestinal pathologies, septic metritis, pneumonia, pleuritis, insulin resistance, and others. According to A.C. Ericsson *et al.* (2021), laminitis can also

occur against the background of hyperlipidemia. However, this mechanism in the pathogenesis of this pathology in horses is not sufficiently studied. Presumably, hyperlipidemia induces insulin resistance, suppressing the biological action of the hormone and reducing glucose intake into all cells of the body. This includes the lamellar keratinocytes of the horse's hoof, which have an extremely high glucose requirement.

Perhaps it is associated with hyperinsulinemia and "glucose starvation" of the hoof tissues, or perhaps with an increase in capillary pressure in the hoof vessels. Nevertheless, the final mechanism of laminitis development is not yet established. Therefore, five horses with clinical symptoms of laminitis were investigated, and a complex of biochemical indicators, including a lipid profile, was determined in their blood serum. The animals showed a change in gait and short steps, changes in body position, limb swapping to alleviate pain. Some animals had swelling in the hoof wall area, increased temperature in the hoof area. Unnatural positions were also noted; horses tried to reduce the load on the limbs, standing on the back of the hoof or even lying down. Difficulty in standing up was observed because horses with laminitis may have difficulty rising or even refuse to stand due to pain.

Considering this, the lipid exchange indicators in the blood serum of horses with laminitis were determined (Table 3). According to the research results, horses with laminitis showed a considerable increase in the serum concentration of total cholesterol by 34.1% ( $P < 0.05$ ) and low-density lipoprotein (LDL) cholesterol by 51.7% ( $P < 0.05$ ) compared to clinically healthy animals (Table 1). It is noteworthy that lipoproteins of this fraction transport cholesterol to tissues, and in normal circumstances, their level in the blood of animals is insignificant, unlike in humans, where this indicator depends particularly on dietary fat intake. The

elevation of cholesterol concentration in this fraction is a pathogenic factor in the develop-

ment of internal diseases in animals, particularly laminitis in horses.

**Table 3.** Indicators of the lipid profile in the blood serum of horses with laminitis, n=5 (mmol/L)

| Indicator      | Total cholesterol | Triacylglycerols | HDL Cholesterol | LDL Cholesterol | VLDL Cholesterol |
|----------------|-------------------|------------------|-----------------|-----------------|------------------|
| Median         | 2.48*             | 1.02*            | 1.56            | 0.44*           | 0.48*            |
| Q <sub>1</sub> | 2.27              | 0.85             | 1.49            | 0.39            | 0.39             |
| Q <sub>3</sub> | 2.77              | 1.07             | 1.68            | 0.55            | 0.54             |

**Notes:** \*  $P < 0.05$ , compared to clinically healthy horses (Table 1)

**Source:** developed by the author

However, an increase in the level of high-density lipoprotein (HDL) cholesterol, where cholesterol concentration is usually highest in clinically healthy animals compared to other lipoprotein fractions, is not observed in horses. High-density lipoproteins are known to facilitate the reverse transport of cholesterol from tissues to the liver, where it serves as a source for bile acid formation, vitamin D, and steroid hormones. Horses with laminitis experienced a substantial increase in the concentration of triglycerides and very low-density lipoproteins by 2.8 times ( $P < 0.05$ ), indicating the development of hyperlipidemia (Table 3). This condition arises due to the increase in cholesterol concentration, which is a cyclic alcohol by chemical structure and a neutral fat represented by triglycerides. This phenomenon is not observed in colic symptoms. However, this hyperlipidemia does not lead to the direct deposition of fats on the epidermal lamellae covering the inner surface of the hoof capsule in horses, as is characteristic in large ruminants.

Thus, in horses, hypercholesterolemia likely leads to microvessel damage in the distal part of the limb, accelerating dystrophic and destructive disturbances in hoof tissues and contributing negatively to the development of the pathological process. However, all the pathogenetic links of this process are currently

not definitively revealed. It is worth noting that direct deposition of triglycerides on the lamellae did not occur.

In horses, especially in ponies as their variety, metabolic disorders, primarily associated with obesity, are highlighted as a separate metabolic syndrome, often accompanied by laminitis, making it particularly important to inspect pathologies in horses. The existence of metabolic syndrome is considered established only in horses and ponies, and its presence in animals of other species remains an open question. For instance, N. Heliczek *et al.* (2022) noted that ponies with excess body weight exhibit large local fat deposits, especially in the withers and on the back, often accompanied by insulin resistance, a leading factor in the development of laminitis in horses, as described earlier. Moreover, the authors acknowledge that ponies, like horses, with metabolic syndrome are less prone to vascular complications, including the development of ischemic disease and atherosclerosis, compared to humans. This is primarily attributed to differences in lipoprotein metabolism, dietary habits, and different lifespans. The pathophysiological mechanisms linking metabolic syndrome to impaired insulin regulation and the onset of laminitis in these animals require further investigation. Various mechanisms are considered, including

the impact of reduced intensity of peripheral glucose metabolism on lamellar bone tissue, pro-inflammatory status associated with insulin resistance, accumulation of end products of glycation, effects mediated by insulin-like growth factor, vasoactive properties of insulin, and subsequent development of endothelial damage. Endothelial dysfunction likely plays a primary role in the development of laminitis. Nevertheless, whether laminitis in the presence of metabolic syndrome should be interpreted as a metabolic, cardiovascular, or inflammatory condition or as their combination remains a contentious issue.

Metabolic syndrome in ponies was diagnosed based on the analysis of anamnesis data, clinical presentation, morphometric indicators, and the results of biochemical blood serum studies. Insulin resistance in ponies was diagnosed using an oral sugar test, which is increasingly used by practicing veterinarians to assess insulin regulation disorders. Although this test quantitatively determines hyperinsulinemia and insulin regulation disorders in response to

oral glucose levels, its results were used to assess insulin sensitivity in horses and ponies. In these animals, pancreatic exhaustion is rarely observed. Conversely, it begins to produce significant amounts of insulin. The peculiarity lies in the fact that insulin toxicity may play a key role in triggering laminitis when exceeding its maximum norm values (100  $\mu$ IU/mL) by more than 3 times.

This aspect opens up new possibilities for further investigation and the development of treatment and prevention strategies for laminitis in ponies, considering the importance of metabolic syndrome in this process. Further exploration of the relationships between metabolic syndrome and the development of laminitis in these animals will contribute to the understanding of its mechanisms and allow the development of effective measures to preserve their health.

Table 4 provides the results of the examination of two groups of ponies – those without signs of obesity and those with massive fat deposits.

**Table 4.** Serum lipid profile indicators in ponies under normal conditions and with metabolic syndrome, n=5 (mmol/L)

| group No.      | Total cholesterol | Triacylglycerols | Cholesterol LDL | Cholesterol HDL | Cholesterol VLDL |
|----------------|-------------------|------------------|-----------------|-----------------|------------------|
| 1.Median       | 2.09              | 0.39             | 0.55            | 1.36            | 0.18             |
| Q <sub>1</sub> | 1.96              | 0.37             | 0.49            | 1.31            | 0.16             |
| Q <sub>3</sub> | 2.24              | 0.42             | 0.60            | 1.45            | 0.19             |
| 2.Median       | 4.57              | 0.62*            | 1.98*           | 2.30*           | 0.29*            |
| Q <sub>1</sub> | 4.31              | 0.58             | 1.85            | 2.21            | 0.25             |
| Q <sub>3</sub> | 4.81              | 0.69             | 2.09            | 2.39            | 0.33             |

**Notes:** 1. – clinically healthy animals; 2. – obesity, metabolic syndrome; \* $P < 0.05$  compared between groups

**Source:** developed by the author

According to the data in Table 4, in the blood serum of clinically healthy ponies, the content of total cholesterol and triglycerides did not significantly differ from the indicators in clinically healthy horses (Table 1). However,

unlike the latter, in animals without signs of obesity, a reverse correlation between the fractions of low-density lipoprotein cholesterol and high-density lipoproteins was observed – the amount of low-density lipoproteins exceeded

the content of high-density lipoproteins by 2.5 times. In healthy horses, on the contrary, the amount of high-density lipoproteins is 4.8 times higher than that of low-density lipoproteins. This indicates the existence of metabolic syndrome in animals, which may be analogous to that in humans. Defining metabolic syndrome in animals requires specifying their species affiliation, for example, metabolic syndrome in cats, dogs, horses, etc. In cases where there is insufficient objective evidence of the development of metabolic syndrome in animals, it is considered appropriate to use the term “metabolic dysfunction” to describe the condition of representatives of a certain species.

In the group of ponies with pronounced signs of obesity, significant hyperlipidemia was observed, confirming the presence of metabolic syndrome and being a risk factor for the development of laminitis. The increase in the levels of all lipid profile indicators in the blood serum of animals in this group confirms its importance in the formation of the specified pathological condition. The directionality of the ratio of low-density lipoprotein cholesterol to high-density lipoproteins was maintained, but an increase in the amount of low-density lipoprotein cholesterol by 1.2 times compared to high-density lipoproteins was noted (Table 4). This highlights the complex metabolic changes that occurred in the bodies of ponies with obesity and emphasises the need for further examination to uncover the molecular mechanisms of these changes and develop effective strategies for managing metabolic disorders in horses with obesity. Considering the complexity of the impact of these changes on the organism, further investigation will be a key stage in the development of therapeutic approaches and preventive measures for correcting metabolic disorders in the studied species. The obtained results on determining lipid metabolism indicators in horses and ponies with

colic symptoms, laminitis, and obesity suggest the feasibility of a more in-depth investigation of the role of lipids and lipoproteins in the development of internal diseases in horses as informative diagnostic tests.

## Conclusions

The conducted study disclosed that the serum lipid profile in clinically healthy Ukrainian Hutsul horses was characterised by the following composition: the share of high-density lipoprotein cholesterol was 75.1%, low-density lipoproteins were 15.7%, and very low-density lipoproteins were 9.2%. In cases of colic symptom complex induced by a sharp change in the diet, horses experienced an acute inflammatory process accompanied by hypercholesterolemia, due to an increase in the content of low-density lipoprotein cholesterol, compared to clinically healthy animals, with no changes in the content of triglycerides, very low-density, and high-density lipoproteins.

For laminitis as a chronic inflammatory process, horses exhibited hyperlipidemia due to an increase in the concentration of triglycerides, total cholesterol, very low-density lipoprotein cholesterol, and low-density lipoproteins, compared to clinically healthy animals, with no changes in the content of high-density lipoprotein cholesterol. In clinically healthy ponies, the content of total cholesterol and triglycerides in serum did not significantly differ from the indicators in clinically healthy horses. However, in horses, quantitative indicators of high-density lipoprotein cholesterol were 4.8 times higher than those for low-density lipoproteins; in ponies, on the contrary, the amount of low-density lipoprotein cholesterol was 2.5 times higher than high-density lipoproteins. This supports the idea of a tendency to develop metabolic syndrome in ponies, similar in course to that in humans. Ponies with metabolic syndrome showed hyperlipidemia due

to an increase in both serum triglyceride and cholesterol content in all types of lipoprotein complexes of the lipid profile. In diseased animals, the content of low-density lipoprotein cholesterol exceeded high-density lipoprotein cholesterol by 1.2 times.

The results of determining lipid metabolism indicators, as informative diagnostic tests, for colic symptoms, laminitis, and metabolic syndrome in horses and ponies suggest the

expediency of more in-depth further investigation of the role of lipids and lipoprotein complexes in the development of internal animal diseases.

### **Acknowledgements**

None.

### **Conflict of Interest**

None.

### **References**

- [1] Alonso, F.H., Behling-Kelly, E., & Borjesson, D.L. (2022). Lipoprotein profile of pleural and peritoneal transudates in dogs and cats. *Journal of Veterinary Internal Medicine*, 36(2), 464-472. [doi: 10.1111/jvim.16369](https://doi.org/10.1111/jvim.16369).
- [2] Andriichuk, A., Tkachenko, H., Kurhaluk, N., Tkachova, I., & Vartovnyk, M. (2014). [Blood biochemical parameters in horses involved in eventing under the influence of exercise](#). *The Animal Biology*, 16(1), 9-20.
- [3] Cantarelli, C., Dau, S.L., Stefanello, S., Azevedo, M.S., De Bastiani, G.R., Palma, H.E., & De La Côte, F.D. (2018). Evaluation of oral sugar test response for detection of equine metabolic syndrome in obese Crioulo horses. *Domestic Animal Endocrinology*, 63, 31-37. [doi: 10.1016/j.domaniend.2017.10.006](https://doi.org/10.1016/j.domaniend.2017.10.006).
- [4] Daradics, Z., Crecan, C.M., Rus, M.A., Morar, I.A., Mircean, M.V., Cătoi, A.F., Cecan, A.D., & Cătoi, C. (2021). Obesity-related metabolic dysfunction in dairy cows and horses: Comparison to human metabolic syndrome. *Life (Basel, Switzerland)*, 11(12), article number 1406. [doi: 10.3390/life11121406](https://doi.org/10.3390/life11121406).
- [5] de Laat, M.A., & Fitzgerald, D.M. (2023). Equine metabolic syndrome: Role of the enteroinsular axis in the insulin response to oral carbohydrate. *The Veterinary Journal*, 294, article number 105967. [doi: 10.1016/j.tvjl.2023.105967](https://doi.org/10.1016/j.tvjl.2023.105967).
- [6] Delarocque, J., Frers, F., Feige, K., Huber, K., Jung, K., & Warnken, T. (2021). Metabolic changes induced by oral glucose tests in horses and their diagnostic use. *Journal of Veterinary Internal Medicine*, 35(1), 597-605. [doi: 10.1111/jvim.15992](https://doi.org/10.1111/jvim.15992).
- [7] Ericsson, A.C., Johnson, P.J., Gieche, L.M., Zobrist, C., Bucy, K., Townsend, K.S., Martin, L.M., & LaCarrubba, A.M. (2021). The influence of diet change and oral metformin on blood glucose regulation and the fecal microbiota of healthy horses. *Animals*, 11(4), article number 976. [doi: 10.3390/ani11040976](https://doi.org/10.3390/ani11040976).
- [8] European convention for the protection of vertebrate animals used for research and other scientific purposes. (1986). Retrieved from [https://zakon.rada.gov.ua/laws/show/994\\_137#Text](https://zakon.rada.gov.ua/laws/show/994_137#Text).
- [9] Gehlen, H., Schwarz, B., Bartmann, C., Gernhardt, J., & Stöckle, S.D. (2020). Pituitary pars intermedia dysfunction and metabolic syndrome in donkeys. *Animals*, 10(12), article number 2335. [doi: 10.3390/ani10122335](https://doi.org/10.3390/ani10122335).

- [10] Heliczner, N., Gerber, V., Bruckmaier, R., van der Kolk, J.H., & de Solis, C.N. (2017). Cardiovascular findings in ponies with equine metabolic syndrome. *Journal of the American Veterinary Medical Association*, 250(9), 1027-1035. doi: 10.2460/javma.250.9.1027.
- [11] Kaneko, J.J., Harvey, J.W., & Bruss, M.L. (2008). *Clinical biochemistry of domestic animals*. California: Academic Press. doi: 10.1016/B978-0-12-370491-7.X0001-3.
- [12] Karikoski, N.P., Box, J.R., Mykkänen, A.K., Kotiranta, V.V., & Raekallio, M.R. (2022). Variation in insulin response to oral sugar test in a cohort of horses throughout the year and evaluation of risk factors for insulin dysregulation. *Equine Veterinary Journal*, 54(5), 905-913. doi: 10.1111/evj.13529.
- [13] Law of Ukraine No. 3447-IV "On Protection of Animals from Cruelty". (2006, February). Retrieved from <https://zakon.rada.gov.ua/laws/show/3447-15#Text>.
- [14] Loos, C.M.M., Dorsch, S.C., Elzinga, S.E., Brewster-Barnes, T., Vanzant, E.S., Adams, A.A., & Urschel, K.L. (2019). A high protein meal affects plasma insulin concentrations and amino acid metabolism in horses with equine metabolic syndrome. *The Veterinary Journal*, 251, article number 105341. doi: 10.1016/j.tvjl.2019.105341.
- [15] Lu, R., Yuan, T., Wang, Y., Zhang, T., Yuan, Y., Wu, D., Zhou, M., He, Zh., Lu, Y., Che, Y., Fan, J., Liang, J., & Cheng, Y. (2018). Spontaneous severe hypercholesterolemia and atherosclerosis lesions in rabbits with deficiency of low-density lipoprotein receptor (LDLR) on exon 7. *EBioMedicine*, 36, 29-38. doi: 10.1016/j.ebiom.2018.09.020.
- [16] Marycz, K., Michalak, I., & Kornicka, K. (2018a). Advanced nutritional and stem cells approaches to prevent equine metabolic syndrome. *Research in Veterinary Science*, 118, 115-125. doi: 10.1016/j.rvsc.2018.01.015.
- [17] Marycz, K., Weiss, C., Śmieszek, A., & Kornicka, K. (2018b). Evaluation of Oxidative Stress and mitophagy during adipogenic differentiation of adipose-derived stem cells isolated from equine metabolic syndrome (EMS) Horses. *Stem Cells International*, 2018, article number 5340756. doi: 10.1155/2018/5340756.
- [18] Norton, E.M., Schultz, N.E., Rendahl, A.K., McFarlane, D., Geor, R.J., Mickelson, J.R., & McCue, M.E. (2019). *Heritability of metabolic traits associated with equine metabolic syndrome in Welsh ponies and Morgan horses*. Retrieved from <https://beva.onlinelibrary.wiley.com/doi/10.1111/evj.13053>.
- [19] Olley, R.B., Carslake, H.B., Ireland, J.L., & McGowan, C.M. (2019). *Comparison of fasted basal insulin with the combined glucose-insulin test in horses and ponies with suspected insulin dysregulation*. Retrieved from <https://www.sciencedirect.com/science/article/abs/pii/S1090023319300905?via%3Dihub>.
- [20] Posternak, L.I. (2017). *Prospects and criteria of horse-breeding in Ukraine*. *Agricultural Science and Food Technology*, 2(96), 230-236.
- [21] Procedure for conducting research and experiments on animals by scientific institutions. (2012). Retrieved from <https://zakon.rada.gov.ua/laws/card/z0416-12>.
- [22] Reynolds, A., Keen, J.A., Fordham, T., & Morgan, R.A. (2019). Adipose tissue dysfunction in obese horses with equine metabolic syndrome. *Equine Veterinary Journal*, 51(6), 760-766. doi: 10.1111/evj.13097.
- [23] Stetsiuk, O. (2020). *Development of equestrian tourism infrastructure in Ukraine*. Retrieved from <https://sportsscience.ldufk.edu.ua/index.php/trends/article/view/989/959>.

- [24] Xenoulis, P.G., Cammarata, P.J., Walzem, R.L., Suchodolski, J.S., & Steiner, J.M. (2020). *Serum triglyceride and cholesterol concentrations and lipoprotein profiles in dogs with naturally occurring pancreatitis and healthy control dogs*. Retrieved from <http://surl.li/rktya>.
- [25] Yusiuk-Omelnytska, T.A. (2023). [Current state of the equine industry in state ownership. Development of the livestock industry: Innovations, issues, prospects](#). In *All-Ukrainian scientific-practical conference of scientists, teachers, and postgraduates* (pp. 54-56). Kharkiv: NBTU.
- [26] Zhukova, I.A. (2014). [A dynamics of the physiology state of sporting horse is at physical activities](#). *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies named after S.Z. Gzhytskyj*, 16(2), 120-124.

## **Маркерні зміни ліпідограми крові у патогенезі деяких внутрішніх хвороб коней**

**Мохамед Халіл Альхінді**

Кандидат ветеринарних наук, доцент  
Університет Аль-Азхар, Газа  
1277, вул. амал Абдель Насер, м. Газа, Палестина  
<https://orcid.org/0000-0003-0678-8436>

**Сергій Борисович Боровков**

Кандидат ветеринарних наук, доцент  
Національний науковий центр «Інститут експериментальної  
та клінічної ветеринарної медицини»  
61023, вул. Пушкінська, 83, м. Харків, Україна  
<https://orcid.org/0000-0003-3021-2410>

**Ольга Павлівна Тимошенко**

Доктор ветеринарних наук, професор  
Державний біотехнологічний університет  
61002, вул. Алчевських, 44, м. Харків, Україна  
<https://orcid.org/0000-0001-9696-1698>

**Анотація.** Порушення метаболізму ліпідів в організмі коней (рід кінь, *Equus*) за дії різноманітних чинників часто призводить до виникнення захворювань, у тому числі з розвитком метаболічного синдрому. Тому визначення маркерних змін ліпідограми сироватки крові за метаболічних розладів є актуальним питанням у дослідженні патогенезу найпоширеніших хвороб у цього виду тварин. Мета роботи полягала у визначенні особливостей показників обміну ліпідів в сироватці крові коней за фізіологічного стану та у разі виникнення симптомокомплексу кольок, ламініту і метаболічного синдрому. Дослідження показників обміну ліпідів у сироватці крові цих тварин здійснювали ензиматичним колориметричним методом із використанням біохімічного автоматичного аналізатора COBAS C 311 (“Roche Diagnostics GmbH”, Німеччина). Встановлено, що у

ліпідограми сироватки крові клінічно здорових коней української верхової породи частка холестеролу ліпопротеїнів високої щільності становила 75,1 %, ліпопротеїнів низької щільності – 15,7 %, ліпопротеїнів дуже низької щільності – 9,2 %. За патології з симптомокомплексом колек у сироватці крові коней частка холестеролу ліпопротеїнів високої щільності істотно зменшувалася, а ліпопротеїнів низької щільності підвищувалася у 3,9 раза порівняно із здоровими тваринами. При цьому, в ліпідограми сироватки крові клінічно здорових поні на частку холестеролу ліпопротеїнів високої щільності доводилось 26,3 %, ліпопротеїнів низької щільності – 65,1 %, ліпопротеїнів дуже низької щільності – 8,61 %. У разі виникнення в поні ламініту, динаміка показників ліпідного складу сироватки крові відповідала такій у коней. Встановлені закономірності щодо змін ліпідограми сироватки крові у різних представників роду коней дозволили уточнити їх роль у патогенезі окремих найпоширеніших патологій. Результати дослідження мають практичну цінність для застосування у клінічній ветеринарній медицині та сприятимуть проведенню якісної лабораторної діагностики найпоширеніших патологій в коней (за симптомокомплексу колек, ламініту та метаболічного синдрому), у патогенезі яких відмічаються порушення метаболізму ліпідів

**Ключові слова:** поні; Українська верхова порода; холестерол; ліпопротеїни; симптомокомплекс колек; ламініт; метаболічний синдром