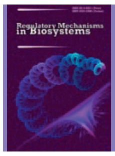

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Formation of cellular and humoral immunity in obese horses

S. Borovkov, V. Boiko*, V. Borovkova**, A. Paliy*, O. Pavlichenko**, Y. Shchepetilnikov**, D. Makhotina**

**Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine*
***State Biotechnological University, Kharkiv, Ukraine*

Article info

Received 15.02.2025
Received in revised form 17.03.2025
Accepted 26.04.2025

Institute of Experimental and Clinical Veterinary Medicine, Hryhoriia Skovorody st., 83, Kharkiv, 61023, Ukraine.
Tel.: +38-050-883-82-92.
E-mail: serg_b78@ukr.net

State Biotechnological University, Alchevskikh st., 44, Kharkiv, 61002, Ukraine. Tel.: +38-050-760-62-84. E-mail: vika_borovkova@ukr.net

Borovkov, S., Boiko, V., Borovkova, V., Paliy, A., Pavlichenko, O., Shchepetilnikov, Y., & Makhotina, D. (2025). Formation of cellular and humoral immunity in obese horses. Regulatory Mechanisms in Biosystems, 16(2), e25042. doi:10.15421/0225042

Obesity in horses is an increasingly important problem in modern veterinary medicine, which is often accompanied by metabolic syndrome and can affect the functional state of the immune system. The aim of the study was to investigate the effect of metabolic syndrome on the immune response of horses after influenza vaccination. For the study, 20 adult horses of different sexes and mixed breeds aged 8 to 16 years were selected, 10 of which were clinically healthy and 10 had signs of metabolic syndrome. All horses were routinely vaccinated with the commercially available equine influenza vaccine BioEquin FT, equine injectable suspension, Bioveta, Czech Republic. Serum samples were collected before vaccination and on the 1st, 2nd, 3rd, 4th weeks after vaccination. As a result of the study, haematological parameters and antibody levels were compared in horses with normal weight and obese horses before and after vaccination. The study showed significant changes in blood parameters depending on the metabolic status of the animals. Obese horses had slightly higher initial levels of leukocytes compared to animals with normal weight, but after vaccination this indicator decreased in both groups. The analysis of red blood cell content showed that in obese horses their level was significantly lower at all stages after vaccination compared to animals with normal body weight. A similar tendency was observed for haemoglobin and haematocrit, which remained 6–11% lower in obese animals after vaccination. It was also found that vaccination caused an immune response regardless of metabolic status with the formation of specific antibodies. The dynamics of the leukocyte blood count indicates an increase in the level of neutrophils in obese horses, especially on the 7th day after vaccination, and then a decrease on the 14–28th days. Horses with normal weight had a decrease in neutrophil count, indicating an activation of the immune response to the vaccine. An increase in eosinophils in the blood of obese horses was observed on days 21 and 28, as well as a decrease in lymphocytes and monocytes at all stages after vaccination, indicating a suppression of immune responses in obese animals. At the same time, these animals showed an increase in the level of globulins and circulating immune complexes, which may indicate the presence of chronic inflammatory processes. Thus, the study confirmed that obesity reduces the effectiveness of cellular immunity, although the humoral response remains stable. Prospects for further research include studying the mechanisms of obesity's effect on the immune system of horses to develop more effective vaccination regimens for obese animals.

Keywords: equine metabolic syndrome; vaccination; leukocytes; antibodies; blood proteins.

Introduction

Obesity is a widespread problem among horses worldwide. For example, in South East Australia, studies have shown that the overall prevalence of obesity among horses and ponies was 23.1%, with a significantly higher percentage in Shetland ponies at 71.5%, other pony breeds at 32.0%, and horses at 9.3% (Robin et al., 2015). In Ireland, a study of a local pony breed showed that 29.5% of animals had a body condition score (BCS) ≥ 7, and 68.5% had at least one of the following pathologies: increased neck fatness or regional excess body fat. In addition, 16.0% of ponies were diagnosed with hyperinsulinaemia, which negatively affects the significant activity of metabolic processes in this population (Giles et al., 2014). In the United Kingdom, a study showed that the prevalence of obesity among horses and ponies registered in veterinary clinics was 31.2%. Breeds such as draft, cob, local and Welsh breeds were more prone to obesity than thoroughbreds. In addition, animals used only for entertainment were more likely to be obese than those used in competition (Potter et al., 2016; Kosolofski et al., 2017). The study showed that animals with clinical signs of laminitis were more likely to be obese than those without such pathology (Kosolofski et al., 2017). In Canada, a retrospective study in the Saskatoon area showed that 20.3% of horses were classified as overweight and 8.3% as obese (Kosolofski et al., 2017).

The effect of obesity and metabolic disorders on the immune response in horses is an important topic for research, given the increasing number of obese animals. Studies show that obesity in horses is associated with various metabolic disorders, such as insulin dysregulation (ID) and hyperinsulinaemia, which can significantly affect the overall health and immunity of animals (Frasca & Blomberg, 2020; Shaikh et al., 2022; Hallman et al., 2023).

Obesity and related metabolic disorders lead to chronic inflammation, which can compromise the normal functioning of the immune system. Studies in humans and laboratory animals show that obesity causes increased levels of inflammatory cytokines such as TNFalpha, IL1, and IL6, leading to insulin resistance and other metabolic problems (Geerling et al., 2022; Al-Ansari et al., 2023). In a study on horses, high levels of TNFalpha and IL1 were found to be associated with reduced insulin sensitivity, confirming the presence of inflammatory processes in obese animals (Geerling et al., 2022).

Although most studies on the effects of obesity on the immune response to vaccination have been conducted on humans and laboratory animals, the results may be generalisable to horses. These studies show that obesity can reduce the effectiveness of vaccination due to impaired immune cell function, metabolic changes and chronic inflammation (Vick et al., 2007; Furtado et al., 2022; Al-Ansari et al., 2023). Obesity can also lead to alterations in the metabolism and function of immune cells, which reduces their ability to respond to vaccines. For example, obese people have a reduced immune response to vaccination against influenza, hepatitis B, tetanus, and rabies (Furtado et al., 2022). Similar mechanisms may be at work in horses. Obesity and metabolic disorders can reduce the effectiveness of the immune response to vaccination, making horses more susceptible to infection and reducing the effectiveness of preventive healthcare measures. This underscores the importance of controlling the weight and metabolic status of horses to ensure their health and welfare (Geerling et al., 2022; Shaikh et al., 2022). Therefore, obesity and metabolic disorders can affect the immune response to vaccination in horses, reducing their ability to develop effective immune defences and making them more susceptible to infectious diseases. Further research is required to better understand these mechanisms and develop strategies to improve equine health and immunity.

The determination of humoral and cell-mediated immune responses to influenza vaccination in horses is of high interest given the importance of effective vaccination to prevent an influenza outbreak. The humoral immune response involves the production of antibodies, whereas the cell-mediated response includes T-cell activity and the production of cytokines such as interferon gamma (IFN-γ).

Research shows that effective protection against influenza virus in horses is achieved through strong cellular and humoral immunity. However, despite updates to the influenza vaccine, the equine influenza virus continues to evolve and cause disease outbreaks even in populations with high vaccination rates (Oladunni et al., 2021). This is due to the fact that the influenza virus is able to cause mutations in the sites of antibody emulation, which avoids the protective effect of the host's immunity.

A study conducted in Australia evaluated the humoral and cell-mediated immune response to vaccination with a recombinant vaccine (ProteqFlu™). The results showed that antibodies produced after vaccination were cross-reactive to two H_3N_8 influenza virus subtypes, including the A/equine/Sydney/07 strain, confirming a humoral response. However, the cell-mediated response, as measured by IFN-γ levels in peripheral blood mononuclear cells, showed no significant differences between the groups of horses vaccinated with the accelerated and conventional regimens (Frasca & Blomberg, 2020).

Obesity can have a negative impact on the immune response to vaccination through metabolic and recovery mechanisms. Studies in humans and animals have shown that obesity impairs the immune response to influenza vaccination, reducing both humoral and cell-mediated immunity. This is due to systemic and cellular changes that disrupt the metabolism and function of immune cells, as well as increased levels of inflammatory cytokines such as TNFalpha and IL1, which leads to insulin resistance and other metabolic problems (Paillot, 2014; Gilkerson & Paillot, 2022).

Thus, for successful influenza vaccination in horses, it is important to consider both humoral and cell-mediated immune responses. Vaccines must stimulate the type of immunity to provide adequate protection. The effect of obesity on the immune response should also be considered as it may reduce vaccine efficacy and increase susceptibility to infection. Further research is needed to better understand these mechanisms and develop strategies to improve equine health and immunity.

Materials and methods

During the experimental studies presented in this paper, all manipulations with the horses involved in the research were carried out taking into account the basic principles of bioethics, in accordance with Article 26 of the Law of Ukraine 'On Protection of Animals from Cruelty' and the 'General Ethical Principles for Animal Experiments' adopted by the First National Congress on Bioethics (2012). Animals had free access to feed and water, and minimally invasive techniques were used for vaccination and blood sampling.

Horses kept in private stables in Kharkiv and Poltava regions were examined, and two groups were formed: 10 clinically healthy animals and 10 obese horses. Thus, a total of 20 animals were examined.

Feeding and housing conditions met the physiological needs of the animals. The animals' diet was balanced in terms of essential nutrients, and all animals had free access to water and were allowed to walk. All animals underwent a general clinical examination according to generally accepted methods. Body Condition Scoring (BCS) was performed by two independent veterinarians. All horses were routinely vaccinated with the commercially available equine influenza vaccine BioEquin FT, equine injectable suspension (influenzae equorum inactivatum virus, strain: A/Equi 2/Morava 95 (Eurasian type), H_3N_8 not less than 5 $\log_2$ HIT1 A/Equi 2/Brno 08 (American type, Florida 2 subline), H_3N_8 not less than 5 $\log_2$ HIT1 *Anatoxinum tetanicum purificatum* not less than 30 IU2), Bioveta, Czech Republic. Blood was taken before vaccination and on the 7th, 14th, 21st and 28th day after vaccination, from the jugular vein on an empty stomach into Vacuette vacuum tubes in the amount of 10 mL were used for further obtaining native blood and serum, depending on the research methods, for its morphological and biochemical study, which was carried out at the Diagnostic and Treatment Centre for Medical Experimental Research, Kharkiv, and at the accredited laboratory of the National Scientific Centre 'Institute of Experimental and Clinical Veterinary Medicine'. The laboratories have all the necessary licences and are certified in accordance with the certificate of conformity of measurement systems to DSTU ISO 10012:2005. The serum was analysed using a COBAS C 311 photometric system (manufactured by Roche Diagnostics GmbH, Germany) with ion-selective electrodes for the study of clinical and biochemical parameters of serum. The content of total protein and protein fractions was measured (albumin and globulin fractions: α_1, α_2, β, γ, circulating immune complexes, seromucoids and uric acid). Morphological studies of blood were performed using a Sysmex 330 haematological analyser (manufactured by Sysmex Corporation, Japan). Serological studies were performed in ELISA using test systems: IDEXX Influenza A Ab Test ELISA (USA), Ingezim Influenza A, manufactured by Ingenasa (Spain) and IDVet ID Screen Influenza A Antibody Competition Multi-species-FLUACA (France). The ELISA, reaction recording and interpretation of the results were performed according to the instructions of the test system manufacturers.

Statistical analysis of the data was performed using Minitab 19, Minitab Inc in a free trial version. Based on the results of statistical processing, the tables show the following indicators: mean value, error of the mean value for data analysis, mean ± standard deviation, collected before vaccination and on days 7, 14, 21 and 28; analysis of variance (ANOVA) was used to identify statistically significant differences between groups. In case of significant differences, the Tukey post-hoc test was performed.

Results

The study results included a comparative analysis of haematological parameters and antibody levels between normal-weight and obese horses before and after vaccination (Table 1).

The data analysis revealed that obese horses had slightly higher initial leukocytes values (8.10 ± 1.96) compared to horses with normal weight (7.54 ± 1.40), and on the 14th day after vaccination the difference was 8.8%. Also, after vaccination, this indicator slightly decreesed in both groups during the entire observation period: on average by 6.6% in animals with normal weight and by 7.7% in animals with signs of obesity compared to the level before vaccination.

In addition to transporting oxygen, red blood cells transport nutrients, maintain acid-base balance and participate in immune responses. As a result of our experiment, it was found that in obese horses, the initial level of red blood cells was significantly lower (7.02 ± 0.68, $P = 0.00057$) than in horses with normal weight (8.85 ± 0.86). This direction of changes remained during all stages after vaccination with a difference of 16.9% ($P = 0.0026$), 11.9%, 12.9% and 14.5%, respectively.

Red blood cells contain a protein with iron ions, haemoglobin, which ensures the main function of red blood cells – the transport of gases, primarily oxygen, which supports the processes of biological oxidation in the body. The level of haemoglobin directly depends on the number of red blood cells, which is consistent with the results of our experiment. Thus, the initial level of haemoglobin in obese horses was lower (124.65 ± 10.32, $P = 0.0039$) than in horses with normal weight (145.81 ± 16.34). This indicator differed by day 28 after vaccination by 11.1%, 6.3% and 8.4% ($P = 0.047$), respectively.

Based on the data in Table 1, we found a 20.2% ($P < 0.044$) decrease in platelet count on day 7 after vaccination in horses with signs of obesity compared to horses with normal weight. It should be noted that the number of platelets on the 21st day of the experiment in horses with normal weight was reduced by 14.2% compared to their number before vaccination. This direction of changes indicates that obesity affects the reduction of platelet activation, which in turn impairs communication with cellular components of the immune system and the complement system.

All horses had an immune response to vaccination regardless of their metabolic status, as evidenced by antibodies specific for influenza virus. The antibody content in obese horses slightly increased after vaccination, but remained lower than in horses with normal weight. Vaccinated horses with normal weight showed a more significant increase in antibody titres.

Inactivated or killed vaccines, such as those used in this study, tend to elicit a strong humoral immune response, but a weaker cellular immune response. This may have influenced the results obtained in this study, as the differences between obese and control horses were observed in cellular immune responses rather than humoral responses.

Table 1
Results of blood tests of horses (mean ± standard deviation, n = 10)

Index		Before vaccination	7th day	14th day	21st day	28th day
Leukocytes, $\times 10^{9}$/L	NWH	7.54 ± 1.40^{a}	7.04 ± 1.28^{a}	7.04 ± 1.18^{a}	7.04 ± 0.90^{a}	7.52 ± 1.64^{a}
	OH	8.10 ± 1.96^{a}	7.28 ± 1.01^{a}	7.66 ± 1.70^{a}	7.38 ± 1.37^{a}	7.36 ± 1.65^{a}
Erythrocytes, $\times 10^{12}$/L	NWH	8.85 ± 0.86^{a}	8.06 ± 0.99^{a}	8.06 ± 0.98^{a}	8.27 ± 1.09^{a}	8.81 ± 0.55^{a}
	OH	7.02 ± 0.68^{a}	6.70 ± 0.43^{a}	7.11 ± 0.71^{a}	7.20 ± 0.42^{a}	7.53 ± 0.51^{b}
Hemoglobin , g/L	NWH	145.81 ± 16.36^{a}	132.84 ± 18.11^{a}	133.01 ± 17.75^{a}	134.84 ± 19.22^{a}	145.04 ± 9.8^{a}
	OH	124.64 ± 10.34^{a}	118.06 ± 4.41^{a}	124.61 ± 11.64^{a}	127.33 ± 8.34^{a}	132.84 ± 6.3^{a}
Hematocrit , %	NWH	40.32 ± 4.21^{a}	37.16 ± 4.52^{a}	36.62 ± 4.22^{a}	37.42 ± 4.95^{a}	40.15 ± 2.35^{a}
	OH	34.52 ± 3.33^{a}	33.14 ± 1.87^{a}	36.53 ± 4.86^{a}	35.24 ± 2.43^{a}	36.90 ± 2.11^{b}
Platelets , $\times 10^{9}$/L	NWH	139.22 ± 19.85^{a}	132.21 ± 21.26^{a}	130.06 ± 23.13^{a}	119.46 ± 24.59^{a}	135.03 ± 35.6^{a}
	OH	126.81 ± 18.77^{a}	119.84 ± 18.72^{a}	128.82 ± 18.25^{a}	123.85 ± 27.33^{a}	134.81 ± 30.1^{a}
Antibodies to the influenza virus, unit	NWH	0.249 ± 0.066^{a}	0.241 ± 0.075^{a}	0.232 ± 0.071^{a}	0.226 ± 0.032^{a}	0.249 ± 0.088^{a}
	OH	0.201 ± 0.016^{a}	0.198 ± 0.011^{a}	0.299 ± 0.175^{a}	0.224 ± 0.016^{a}	0.219 ± 0.025^{a}

Notes: NWH – normal-weight horses, OH – obese horses; different letters indicate values which reliably differed one from another within one line of the table according to the results of comparison using the Tukey test with Bonferroni correction.

Thus, our data indicate that horses with signs of obesity develop leukocytosis, erythrocytopenia, decreased haemoglobin and haematocrit levels, and, in the early stages, thrombocytopenia after vaccination. This pattern of changes indicates the immunomodulatory effect of the vaccine, but a pathological condition such as obesity has a negative impact on the development of immunological reactions and homeostasis in general.

The first to respond to stress reactions caused by the administration of immunotropic drugs in the body of animals is the leukogram. When analysing the obtained data from the blood of horses, it was noted (Table 2) that in animals with normal weight, the level of band neutrophils changed with an increase of 3.53 times on the 7th day after vaccination, and 1.52 times on the 28th day compared to the number before vaccination. In animals with signs of obesity, the number of these neutrophils increased by 55.5% on day 7, and then (on days 14, 21 and 28) decreased by an average of 66.7%. The analysis of blood data from animals with signs of obesity against those of animals with normal weight demonstrates an increased number of band neutrophils both before vaccination by 4.53 times and after vaccination by 2.12, 3.14 and 1.53 times, respectively, on days 7, 14 and 21. Regarding the dynamics of segmented neutrophils: in animals with normal weight, a decrease of 18.7% was found on day 7 after vaccination, and in animals with signs of obesity, an increase of 43.2%, 25.2% and 27.2% was found compared to the number before vaccination. The analysis of segmented neutrophil data from animals with signs of obesity compared to animals with normal weight revealed a 12.3% decrease in the initial level, and from day 7 after vaccination, an increase of 7.3%, 28.8% ($P = 0.0091$) and 16.3% ($P = 0.0021$), respectively. Thus, the regenerative shift of neutrophils detected by us may indicate that the reactivity of the organism of horses with signs of obesity was maintained at a sufficient level.

Table 2
Dynamics of leukocyte blood formula in horses (mean ± standard deviation, n = 10)

Index		Before vaccination	7th day	14th day	21st day	28th day
Band neutrophils, %	NWH	0.4 ± 0.9^{a}	1.4 ± 1.1^{a}	0.2 ± 0.5^{a}	0.4 ± 0.6^{a}	0.6 ± 0.1^{a}
	OH	1.8 ± 1.3^{ab}	2.8 ± 1.3^{a}	0.6 ± 0.1^{c}	0.6 ± 0.1^{c}	0.6 ± 0.9^{bc}
Segmented neutrophils, %	NWH	57.6 ± 4.8^{ab}	46.8 ± 4.7^{a}	55.6 ± 9.9^{ab}	53.8 ± 5.8^{ab}	61.4 ± 4.4^{b}
	OH	50.0 ± 9.9^{a}	50.2 ± 13.8^{a}	71.6 ± 3.4^{b}	62.6 ± 3.7^{ab}	63.6 ± 7.8^{ab}
Eosinophils, %	NWH	0.4 ± 0.6^{a}	3.4 ± 0.9^{c}	2.4 ± 1.3^{ab}	0.8 ± 0.5^{bc}	1.2 ± 1.3^{bc}
	OH	2.0 ± 1.4^{a}	1.2 ± 0.8^{a}	1.8 ± 2.1^{a}	2.6 ± 1.3^{a}	1.6 ± 0.9^{a}
Lymphocytes, %	NWH	39.2 ± 4.2^{a}	44.6 ± 5.9^{a}	37.6 ± 9.6^{a}	42.0 ± 6.1^{a}	34.0 ± 2.4^{a}
	OH	43.8 ± 8.5^{a}	41.8 ± 14.1^{a}	23.0 ± 1.9^{b}	31.4 ± 5.6^{ab}	31.8 ± 8.0^{ab}
Monocytes, %	NWH	2.4 ± 2.1^{a}	3.8 ± 1.8^{a}	4.2 ± 2.2^{a}	3.0 ± 1.9^{a}	2.6 ± 1.7^{a}
	OH	2.4 ± 1.7^{a}	3.4 ± 1.8^{a}	3.2 ± 0.8^{a}	3.4 ± 1.7^{a}	2.4 ± 2.3^{a}

Note: see Table 1.

The dynamics of the studied haemogram indicates eosinopenia (2.83 ($P = 0.0039$) and 1.33 times) on the 7th and 14th day after vaccination, and then the development of eosinophilia (3.25 and 1.33 times) on the 21st and 28th day in the blood of animals with signs of obesity compared to the blood of animals with normal weight. However, in horses with normal weight, the level of eosinophils remained stably increased with a maximum difference of 8.52 times compared to the level before vaccination. In obese horses, the number of eosinophils decreased starting from the 7th day after vaccination and this pattern of changes persisted until the 28th day with an average difference of 25.0% compared to the pre-vaccination level. The main cells of the immune system, which are a type of white blood cell and are responsible for the acquired immunity, are lymphocytes and monocytes. The data in the haemogram table indicate a decrease in the number of lymphocytes and monocytes in horses with signs of obesity compared to horses with normal weight. As a result of the experiment, the following reductions were found: the number of lymphocytes by 6.3%, 38.8% ($P = 0.0011$), 25.2% ($P = 0.021$) and 6.4%; the number of monocytes by 47.8%, 28.8%, 25.3% and 15.3%, respectively. The data analysis of the number of lymphocytes and monocytes in horses with signs of obesity after vaccination compared to the pre-vaccination values also shows a decrease in the number of lymphocytes and monocytes, on average, by 34.4% and 10.8%, respectively. However, in horses with normal weight, after vaccination, an increase was noted in the number of lymphocytes and monocytes by an average of 11.3% and 41.6%, respectively, compared to the level before vaccination, indicating the active development of immunological reactions. Thus, significant changes in the quantitative indicators of the leukocyte blood count of obese horses compared with the dynamics of blood cells of horses with normal weight after vaccination demonstrate: neutrophilia; eosinopenia, which turns into eosinophilia; lymphocytopenia and monocytopenia, indicating suppression of the development of immunological reactions, especially on day 14 of the experiment. For a more complete and objective picture of the dynamics of the immune response to vaccination in obesity, it is important to provide data from experimental studies on the level of total protein and protein fractions, circulating immune complexes and seromucoids.

Table 3
Dynamics of biochemical parameters of blood serum (mean ± standard deviation, n = 10)

Index		Before vaccination	7th day	14th day	21st day	28th day
Total protein, g/L	NWH	68.07 ± 4.32^{ab}	64.32 ± 4.35^{a}	67.44 ± 5.34^{a}	71.44 ± 2.62^{ab}	74.85 ± 1.12^{b}
	OH	66.21 ± 2.74^{a}	65.32 ± 3.37^{a}	69.95 ± 2.48^{ab}	73.23 ± 2.52^{b}	74.34 ± 0.93^{b}
Albumin, g/L	NWH	43.91 ± 2.31^{a}	42.12 ± 2.51^{a}	41.83 ± 2.25^{a}	42.14 ± 1.75^{a}	43.12 ± 2.25^{a}
	OH	44.11 ± 1.53^{a}	43.15 ± 1.53^{a}	41.02 ± 2.44^{a}	41.81 ± 2.34^{a}	43.95 ± 1.71^{a}
Globulin, g/L	NWH	24.14 ± 5.74^{ab}	22.23 ± 4.93^{a}	25.65 ± 6.42^{ab}	29.36 ± 3.71^{ab}	31.81 ± 2.64^{ab}
	OH	22.12 ± 3.13^{a}	22.21 ± 3.26^{a}	28.92 ± 2.82^{b}	31.47 ± 3.51^{b}	30.14 ± 1.92^{b}
Circulating Immune Complexes, mg/mL	NWH	129.05 ± 28.41^{a}	141.42 ± 23.27^{a}	121.21 ± 31.44^{a}	208.63 ± 32.35^{b}	207.62 ± 22.65^{b}
	OH	126.86 ± 13.82^{a}	138.03 ± 12.42^{a}	123.23 ± 20.24^{a}	207.83 ± 28.05^{b}	192.04 ± 40.35^{b}
Seromucoids, mg/mL	NWH	1.68 ± 0.44^{a}	1.84 ± 0.21^{bc}	2.61 ± 0.46^{b}	2.54 ± 0.43^{b}	3.52 ± 0.54^{c}
	OH	1.80 ± 0.16^{a}	1.82 ± 0.13^{a}	2.63 ± 0.42^{b}	2.744 ± 0.22^{ab}	3.32 ± 0.49^{c}
Uric acid, μmol/L	NWH	39.42 ± 4.93^{a}	42.0 ± 11.9^{a}	35.13 ± 7.94^{a}	41.66 ± 8.13^{a}	59.14 ± 5.44^{b}
	OH	37.78 ± 4.92^{a}	43.35 ± 7.52^{a}	38.66 ± 4.61^{a}	39.94 ± 5.27^{a}	60.95 ± 4.94^{b}

Note: see Table 1.

In this study, it was found that in horses with normal weight, the level of total protein increased by 10.0% by day 28 compared to pre-vaccination levels. Obese horses showed an increase in total protein levels from 66.21 ± 2.74 to 73.23 ± 2.52 and 74.34 ± 0.93 (10.5% and 12.2%, respectively) on days 21 and 28. The increase in globulin levels in animals with normal weight was 21.6% and 31.8% on days 21 and 28, respectively. In obese animals, globulin levels increased by 30.7%, 42.1% and 36.2% on days 14, 21 and 28, respectively. At the same time, the level of albumin remained stable. The protein profile of obese horses showed a 12.9% increase in globulin levels on day 14 compared to animals of normal weight.

The study also showed that after vaccination in horses with normal weight, the level of circulating immune complexes increased by 9.6%, 61.7% and 60.9% on days 7, 21 and 28, respectively. In obese horses, these indicators increased by 8.8%, 63.9% and 51.4%. The level of circulating immune complexes correlated with the content of seromucoids, indicating a possible link with chronic inflammation, characteristic of obesity, and the development of suppressive reactions. In horses with normal weight, the concentration of seromucoids increased by 9.5%, 54.7% and 51.2% on days 7, 14 and 21, respectively, and on day 28, a maximum increase of 2.09 times was observed. In obese animals, seromucoid levels increased by 44.4%, 50.0% and 83.3% on days 14, 21 and 28. The initial level of seromucoids in obese horses was 7.1% higher, but their formation was less intense during the study, which may indicate a decrease in the functional activity of the liver.

In addition, it was found that in horses with normal weight, the concentration of uric acid increased by 50.1% by day 28 after vaccination, while in obese horses this indicator increased by 14.8% and 61.4% on days 7 and 28, respectively. An increase in uric acid levels in obese animals may indicate the development of metabolic syndrome, which is accompanied by oxidative stress and a decrease in immune response. This is consistent with the dynamics of the level of lymphocytes, eosinophils, monocytes, circulating immune complexes and seromucoids.

Discussion

The problems of veterinary science are multifaceted and include issues of both infectious and non-infectious pathology, which in most cases are common to animal husbandry (Paliy et al., 2021; Kolchyk et al., 2022; Rehman, 2023). Influenza vaccination is an important measure of protection against infection, especially for obese animals, which are at increased risk of severe disease. However, vaccination can cause various haematological changes, including changes in coagulation, risk of haematological abnormalities, and inflammatory reactions (Green et al., 2017; Shaikh et al., 2022). For example, our studies have shown leukocytopenia, which occurs at the expense of neutrophils and lymphocytes; it may be associated with apoptosis induced by interferon-α and indicates the immunomodulatory effect of the vaccine (Ato et al., 2013). It should also be noted that in horses with normal weight, the number of red blood cells after vaccination was reduced by an average of 8.1% compared to the pre-vaccination values, and in horses with signs of obesity, this trend was not observed. This is probably due to an excess of macrophages that destroy red blood cells because, with the help of the TLR9 receptor, they have the remnants of pathogenic structures from the vaccine on their surface and are perceived as a pathogen (Kawai & Akira, 2010; Huang et al., 2018; Lomikovska et al., 2020). The haemogram is completed with data on the level of haematocrit – the volume fraction of red blood cells in whole blood (the ratio of red blood cell volume to plasma), which depends on the number and volume of red blood cells. Usually, the values of haemoglobin, haematocrit and red blood cell count are interrelated and change in the same direction (Levchenko et al., 2017). The dynamics of the hematocrit level in our studies is consistent with the literature and had a decrease of 14.4% ($P < 0.05$) before vaccination, 10.8% on day 7 and 7.9% at the end of the experiment in obese horses compared to normal weight horses. However, horses with normal weight after vaccination had a decrease in haematocrit level during the experiment by an average of 8.1% compared to pre-vaccination, and this tendency was not observed in horses with signs of obesity.

Platelets are fragments of nucleated cells that play an important role in haemostasis. Activated platelets are the main source of sCD40L (CD154) in the blood. Platelet CD154 can interact with the CD40 receptor on endothelial cells, triggering an inflammatory response and enhancing immunoglobulin production by B lymphocytes. Platelet membrane and soluble CD154, in combination with other signals, can induce the maturation and activation of dendritic cells. They are also able to synthesise antigens in the context of MHC class I molecules and activate CD8+ T lymphocytes (Kerrigan et al., 2012; Kim et al., 2015).

Our data on the leukocyte profile indicate an increase in neutrophils, which, according to the literature, has a direct regulatory effect on the functional activity of T and B lymphocytes, which is realised through direct contact between these cells via PD-L1-PD1. Eosinophils provide a detoxifying effect by adsorbing immune complexes, fibrin, histamine, etc. The role of eosinophils is primarily to limit the damage caused by immune complexes. However, the eosinopenia described in our study is caused by an increase in adrenocorticoid activity, which leads to a delay in eosinophil retention in the bone marrow and characterises the development of stress, but eventually returns to normal levels or eosinophilia, which is associated with the influence of histamine-like substances and a special factor (eosinopoietin) secreted by lymphocytes during their antigenic stimulation, indicates the development of allergic reactions in animals with signs of obesity

(Kanda et al., 2015; De Castro et al., 2019). According to the literature, the introduction of vaccine antigens primarily activates cells of the innate immune system (dendritic cells, macrophages, neutrophils), which is consistent with our data. Further, activated dendritic cells and macrophages capture foreign agents and migrate to regional lymph nodes, where they stimulate antigen-specific responses of T- and B-lymphocytes (Leo et al., 2011). This fact explains the low level of lymphocytes against the background of low monocytes, and the decrease in monocytes reflects the presence of some dysfunction of the immune system, which may be associated with the pathogenetic features of the formation of obesity (Rekalova et al., 2016). It should also be noted that, according to the data in the literature, a decrease in lymphocyte proliferation indicates a low expression of the IL-2 gene in horses with signs of obesity (Lord et al., 2000) compared to metabolically normal controls.

The most important thing about the interaction between protein and the immune system is its important role in the formation of antibodies, which have a major function in protecting against viruses and infections (Hull-Nye et al., 2023; Korkh et al., 2024). In this study, we found a 10.0% increase in total protein levels in normal-weight horses on day 28 compared to pre-vaccination levels, and in obese horses, total protein changed from 66.2 ± 2.71 to 73.2 ± 2.49 and 74.3 ± 0.94 (10.5% and 12.2%, respectively) on days 21 and 28. This directionality of changes is due to the increased level of total globulins, which reflect the state of development of immune reactions in the body, or the development of the inflammatory process (Gardner et al., 2011). Thus, in animals with normal weight, an increase in globulin levels was found by 21.6% and 31.8% on the 21st and 28th day, respectively, compared to the level before vaccination. In animals with signs of obesity, after vaccination, the level of globulins increased by 30.7, 42.0 and 36.2% on the 14th, 21st and 28th day, respectively. At the same time, it should be noted that the level of albumin did not undergo significant changes. However, when analysing the protein profile data of horses with signs of obesity against the level of horses with normal weight, only the level of the globulin fraction increased by 12.9% on day 14 of the experiment.

One of the important indicators characterising the state of the humoral immune response is the level of circulating immune complexes, which are formed during the direct combination of antigens with antibodies. Due to the high antigen content, excessive formation of circulating immune complexes and their low elimination, the reticulo-endothelial system, which is responsible for their elimination, often develops a type 3 hypersensitivity reaction. At the same time, a high level of circulating immune complexes (especially those containing class G immunoglobulins) stimulates the suppressor activity of T-cells (Stepura et al., 2020). Analysing the data, it was found that after vaccination in horses with normal weight, the level of circulating immune complexes increased by 9.6%, 61.7% and 60.9%, respectively, on the 7th, 21st and 28th day, and in horses with signs of obesity - by 8.8%, 63.9% and 51.4%, respectively. It should also be noted that low-molecular-weight circulating immune complexes have the highest toxicity to body tissues, but the increased level of high-molecular-weight circulating immune complexes may be due to the activation of functional systems of polymorphonuclear leukocytes and mononuclear cells, as well as increased complement activity, which is a positive shift in the immune system (Krytsia, 2016; Kuznetsov, 2020).

Thus, the increased level of circulating immune complexes in our experiment may indicate both a disorder of immune homeostasis in the body of horses after vaccination and the development of immune reactions.

Analysing the data in the table, it should be noted that the concentration of circulating immune complexes was consistent with the content of seromucoid, which may be associated with chronic inflammation, which is often associated with obesity and reflects the expressed development of suppressive reactions that affect the humoral immune system, but at the same time its cellular factors are activated (Wylie et al., 2012; Menzies-Gow et al., 2016; Zhurenko & Zhurenko, 2018).

Thus, in horses with normal weight, the level of seromucoids after vaccination increases by 9.5%, 54.7% and 51.2%, respectively, on the 7th, 14th, 21st day, and on the 28th day, the maximum difference was determined to be 2.09 times. In horses with signs of obesity after vaccination, the increase was 44.4%, 50.0% and 83.3%, respectively, on the 14th, 21st and 28th day. Analysing the dynamics of seromucoid levels in horses with signs of obesity compared to those in animals with normal weight, it should be noted that the initial level of this indicator was 7.1% higher, and during the experiment, less active formation was recorded, which may indicate a reduced functional activity of the liver. Uric acid is the end product of purine metabolism, which is the main component of cellular energy reserves, such as adenosine triphosphate, as well as a component of deoxyribonucleic acid and ribonucleic acid (Li et al., 2023; Yu et al., 2023).

Today, uric acid is recognised as an alarmin, as well as an important and powerful mediator of type 2 immune responses involving epithelial cells, eosinophils, and monocytes. Uric acid also acts as an antioxidant in the body. It accounts for 50% of the total antioxidant capacity of the human body. Instead, in the cytoplasm or in an acidic/hydrophobic environment, uric acid is converted into a prooxidant agent and contributes to oxidative stress (Kim et al., 2010; Shao et al., 2019).

Hyperuricemia is often associated with clusters of metabolic syndrome, and therefore a number of authors define hyperuricemia as a component of metabolic syndrome. Increased uric acid concentration in metabolic syndrome, patients with gout or signs of abdominal obesity is associated with increased glucose levels after glucose loading and affects the reduction of high-density lipoprotein and increase in triglycerides (Heinig & Johnson, 2006; Krishnan, 2012).

As a result of the experiment, it was found that in horses with normal weight, the concentration of uric acid was increased by 50.1% on day 28 compared to the level before vaccination, and in horses with signs of obesity, the difference was 14.8% and 61.4% on days 7 and 28, respectively. Based on this analysis, it can be assumed that an increase in uric acid levels in horses with signs of obesity indicates the development of metabolic syndrome, which is consistent with the above indicators; oxidative stress and a decrease in the immune response, which is consistent with the data on the level of lymphocytes, eosinophils, monocytes, as well as circulating immune complexes and seromucoids.

Conclusion

In horses, regardless of their metabolic status, the reaction to vaccination is accompanied by an immune response at a sufficient level, as evidenced by antibodies specific for influenza virus. Vaccinated horses showed an increase in immunoglobulin titres. However, the metabolic status did not affect humoral immune parameters. In laboratory mice with diet-induced obesity, differences in humoral immune responses to influenza vaccination were observed (Chen et al., 2015). Similarly, an association between type 1 diabetes and the risk of influenza vaccination ineffectiveness has been observed in humans, but researchers have not found differences in humoral immune responses due to metabolic status. It is important to note that the pathophysiology of autoimmune type 1 diabetes differs significantly from type 2 diabetes and metabolic syndrome, the latter of which is most similar to metabolic syndrome.

It is considered possible that obesity plays a greater role in immune processes than metabolic status. Obesity in humans was a risk factor for lower circulating antibody levels three years after hepatitis vaccination and it was body mass index, not diabetes, that was positively correlated with a greater reduction in influenza antibody titres 12 months after vaccination (Sheridan et al., 2012). Obese people vaccinated against influenza, regardless of metabolic status, also have a higher incidence of influenza, even with similar serum antibody titres, compared to their non-obese patients of same age (Neidich et al., 2017). Furthermore, regardless of age and in response to influenza vaccination, obese people have lower antibody titres, a reduced percentage of memory B-lymphocytes, increased production of pro-inflammatory IL-6 by B-cells, and decreased production of anti-inflammatory IL-10 (Frasca et al., 2016).

Inactivated vaccines, such as those used in this study, tend to elicit a strong humoral immune response but a weaker cellular immune response. This may have contributed to the results observed in this study, as differences between horses with metabolic syndrome and control horses without metabolic syndrome were observed in cellular immune responses rather than humoral responses (He et al., 2006).

Low expression of IL-2 gene in influenza-stimulated peripheral blood mononuclear cells and peripheral blood cells indicates that horses with metabolic syndrome may have reduced lymphocyte proliferation (Lord et al., 2000) compared to metabolically normal groups of animals. The anti-inflammatory cytokines TNF-α and IFN-γ play an important role in antigen-specific cellular immune responses (Li & Verma, 2002). The absence of changes in TNF-α gene expression in influenza-stimulated peripheral blood mononuclear cells and over time in horses with metabolic syndrome, as well as low levels of IFN-γ gene expression in peripheral blood cells at weeks 1 and 3 compared to control horses without metabolic syndrome, further indicate a reduced cellular immune response to vaccination in these animals. Further evidence of a reduced cellular immune response in horses with metabolic syndrome is provided by differences in peripheral blood cells between vaccinated horses with metabolic syndrome and vaccinated control horses without metabolic syndrome for IFN-γ and IL-2. However, the data indicate that horses with metabolic syndrome are able to produce a marked humoral immune response to vaccination, but their ability to elicit a cellular immune response may be reduced.

References

Al-Ansari, A. S., Golding, E., Walshe, N., Mooney, C. T., & Duggan, V. (2023). Obesity and obesity-associated metabolic disease conditions in Connemara ponies in Ireland. Equine Veterinary Journal, 56(2), 273–280.

Ato, M., Takahashi, Y., Fujii, H., Hashimoto, S.-I., Kaji, T., Itamura, S., Horiuchi, Y., Arakawa, Y., Tashiro, M., & Takemori, T. (2013). Influenza A whole virion vaccine induces a rapid reduction of peripheral blood leukocytes via interferon-α-dependent apoptosis. Vaccine, 31(17), 2184–2190.

Chen, L. W., Zhang, W., Qiu, J., Zhang, X., Zhang, D., & Wei, H. (2015). The role of the microbiome in the therapeutic efficacy of probiotics: A review. Applied Microbiology and Biotechnology, 99(10), 3887–3896.

De Castro E Silva, F., De Oliveira, E., Ambrósio, M., Ayupe, M., De Souza, V., Gameiro, J., De Lima Reis, D., Machado, M., Macedo, G., Mattes, J., & Ferreira, A. (2019). High-fat diet-induced obesity worsens TH2 immune response and immunopathologic characteristics in murine model of eosinophilic oesophagitis. Clinical and Experimental Allergy, 50, 244–255.

Frasca, D., & Blomberg, B. B. (2020). The impact of obesity and metabolic syndrome on vaccination success. Interdisciplinary Topics in Gerontology and Geriatrics, 43, 86–97.

Frasca, D., Romero, M., Garcia, D., Diaz, A., & Blomberg, B. B. (2022). Obesity accelerates age-associated defects in human B cells through a metabolic reprogramming induced by the fatty acid palmitate. Frontiers in Aging, 2, 828697.

Furtado, T., Perkins, E., Pinchbeck, G., McGowan, C., Watkins, F., & Christley, R. (2022). Exploring human behavior change in equine welfare: Insights from a COM-B analysis of the UK's equine obesity epidemic. Frontiers in Veterinary Science, 9, 961537.

Gardner, E. M., Beli, E., Clinthorne, J. F., & Duriancik, D. M. (2011). Energy intake and response to infection with influenza. Annual Review of Nutrition, 31(1), 353–367.

Geerling, E., Hameed, M., Weger-Lucarelli, J., & Pinto, A. K. (2022). Metabolic syndrome and aberrant immune responses to viral infection and vaccination: Insights from small animal models. Frontiers in Immunology, 13, 1015563.

Giles, S. L., Rands, S., Nicol, C., & Harris, P. (2014). The prevalence of obesity and associated risk factors among domestic horses and ponies living at pasture. PeerJ, 2, e244.

Gilkerson, J., & Paillot, R. (2022). Assessment of humoral and long-term cell-mediated immune responses to recombinant canarypox-vectored equine influenza virus vaccination in horses using conventional and accelerated regimens respectively. Vaccines, 10(6), 855.

Green, W. D., Rebeles, J., Noah, T. J., Hudgens, M. G., Weir, S. S., MacIver, N. J., & Beck, M. A. (2017). Altered metabolism and function of T cells isolated from influenza vaccinated obese adults. The FASEB Journal, 31(S1), 434.3.

Hallman, I., Karikoski, N., & Kareskoski, M. (2023). The effects of obesity and insulin dysregulation on mare reproduction, pregnancy, and foal health: A review. Frontiers in Veterinary Science, 10, 1180622.

He, H., Orlando, R., Blanco, M. A., Pandey, R., Amzallag, E., Baraille, I., & Rérat, M. (2006). First-principles study of the structural, electronic, and optical properties of Ga_2O_3 in its monoclinic and hexagonal phases. Physical Review B, 74(19), 195123.

Heinig, M., & Johnson, R. J. (2006). Role of uric acid in hypertension, renal disease, and metabolic syndrome. Cleveland Clinic Journal of Medicine, 73(12), 1059–1064.

Huang, Z. Y., Perry, E., Huebner, J. L., Katz, B., Li, Y. J., & Kraus, V. B. (2018). Biomarkers of inflammation – LBP and TLR— predict progression of knee osteoarthritis in the DOXY clinical trial. Osteoarthritis and Cartilage, 26(12), 1658–1665.

Hull-Nye, D., Meadows, T., Smith, S. R., & Schwartz, E. J. (2023). Key factors and parameter ranges for immune control of equine infectious anemia virus infection. Viruses, 15(3), 691.

Kanda, A., Fleury, S., Kobayashi, Y., Tomoda, K., Julia, V., & Dombrowicz, D. (2015). Th2-activated eosinophils release Th1 cytokines that modulate allergic inflammation. Allergology International, 64(Suppl.), S71.

Kawai, T., & Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: Update on Toll-like receptors. Nature Immunology, 11(5), 373–384.

Kerrigan, A. M., Navarro-nuñez, L., Pyz, E., Finney, B. A., Willment, J. A., Watson, S. P., & Brown, G. D. (2012). Podoplanin-expressing inflammatory macrophages activate murine platelets via CLEC-2. Journal of Thrombosis and Haemostasis, 10(3), 484–486.

Kim, K., Li, J., Tseng, A., Andrews, R. K., & Cho, J. (2015). NOX2 is critical for heterotypic neutrophil-platelet interactions during vascular inflammation. Blood, 126(16), 1952–1964.

Kim, S. Y., Guevara, J. P., Kim, K. M., Choi, H. K., Heitjan, D. F., & Albert, D. A. (2010). Hyperuricemia and coronary heart disease: A systematic review and meta-analysis. Arthritis Care and Research, 62(2), 170–180.

Kolchyk, O., Illarionova, T., Buzun, A., Paliy, A., & Palii, A. (2022). Influence of probiotic microorganisms on microbial biofilms in feeds. Scientific Horizons, 25(1), 41–50.

Korkh, I., Boyko, N., Pomitun, I., Paliy, A., & Pavlichenko, O. (2024). Features of the formation of lambs' adaptive capacity in the first day of life. Veterinarska Stanica, 55(1), 63–77.

Kosolofski, H. R., Gow, S., & Robinson, K. A. (2017). The prevalence of obesity in a population of horses in Saskatoon and surrounding areas. Canadian Veterinary Journal, 58(9), 967–970.

Krishna, E. (2012). Gout and the risk for incident heart failure and systolic dysfunction. BMJ Open, 2(1), e000282.

Krytsia, I. P. (2016). The influence of immunomodulators on the performance of cellular immunity at the foals of saddle breeds. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, 18(3), 45–49.

Kuznetsov, A. (2020). The immune system and the nature of immune disorders in patients with seasonal allergic rhinoconjunctivitis (sark) with moderate and severe. The Journal of V. N. Karazin Kharkiv National University, Series Medicine, 39, 60–68.

Leo, O., Cunningham, A., & Stern, P. L. (2011). Vaccine immunology. Perspectives in Vaccinology, 1(1), 25–59.

Li, Q., & Verma, I. M. (2002). NF-κB regulation in the immune system. Nature Reviews Immunology, 2(10), 725–734.

Li, X., Sun, J., Bu, Q., Zhou, B., Li, L., Man, X., Zhao, L., Xu, Y., & Luan, H. (2023). Association between serum uric acid levels and clinical outcomes in patients with acute kidney injury. Renal Failure, 45(1), 2169617.

Lomikovska, M. P., Hayduchok, I. G., Potomkina, H. O., Zubchenko, S. O., Kril, I. Y., Ishcheykin, K. Y., & Chopyak, V. V. (2020). Peculiarities of tlr9 expression on immune competent cells in reactive arthritis patients with chronic epstein-barr virus infection. World of Medicine and Biology, 16(71), 83–88.

Lord, J. D., McIntosh, B. C., Greenberg, P. D., & Nelson, B. H. (2000). The IL-2 receptor promotes lymphocyte proliferation and induction of the c-myc, bcl-2, and bcl-x genes through the trans-activation domain of stat5. The Journal of Immunology, 164(5), 2533–2541.

Menzies-Gow, N. J., Harris, P. A., & Elliott, J. (2016). Prospective cohort study evaluating risk factors for the development of pasture-associated laminitis in the United Kingdom. Equine Veterinary Journal, 49(3), 300–306.

Neidich, S. D., Green, W. D., Rebeles, J., Karlsson, E. A., Schultz-Cherry, S., Noah, T. L., Chakladar, S., Hudgens, M. G., Weir, S. S., & Beck, M. A. (2017). Increased risk of influenza among vaccinated adults who are obese. International Journal of Obesity, 41(9), 1324–1330.

Oladunni, F. S., Oseni, S. O., Martinez-Sobrido, L., & Chambers, T. M. (2021). Equine influenza virus and vaccines. Viruses, 13(8), 1657.

Paillot, R. (2014). A systematic review of recent advances in equine influenza vaccination. Vaccines, 2(4), 797–831.

Paliy, A. P., Mashkey, A. N., Faly, L. I., Kysterna, O. S., Rebenko, H. I., & Palii, A. P. (2021). Ecology of zoophilic flies in livestock biocenoses of Ukraine. Biosystems Diversity, 29(3), 258–263.

Potter, S., Bamford, N., Harris, P., & Bailey, S. (2016). The prevalence of obesity and owner perceptions of body condition in leisure horses and ponies in Southeastern Australia. Australian Veterinary Journal, 94(11), 427–432.

Rehman, M. U. (2023). Editorial: Insights in veterinary infectious diseases: 2022. Frontiers in Veterinary Science, 10, 1179912.

Rekalova, E. M., Panasyukova, O. R., Matvienko, Y. A., Yasyr, S. G., & Singaievskyi, M. B. (2016). Imunolohichni pokaznyky yak indykatory zapalnykh protsesiv u khvorykh na khronichne obstruktyvne zakhvoriuvannia lehen ta khronichnyi bronkhit v fazi remisii [Immunological parameters as indicators of inflammatory processes in patients with chronic obstructive pulmonary disease and chronic bronchitis in remission]. Ukraynskyi Pulmonolohycheskyi Zhurnal, 3, 30–34 (in Ukrainian).

Robin, K., Ireland, J., Wylie, K., Collins, S., Verheyen, K., & Newton, J. R. (2015). Prevalence and risk factors for obesity in horses in the UK based on owner-reported body condition scores. Equine Veterinary Journal, 47(2), 196–201.

Shaikh, S. R., MacIver, N. J., & Beck, M. A. (2022). Obesity dysregulates the immune response to influenza infection and vaccination through metabolic and inflammatory mechanisms. Annual Review of Nutrition, 42(1), 67–89.

Shao, Y., Shao, H., Sawhney, M. S., & Shi, L. (2019). Serum uric acid as a risk factor of all-cause mortality and cardiovascular events among type 2 diabetes population: Meta-analysis of correlational evidence. Journal of Diabetes and its Complications, 33(10), 107409.

Sheridan, M. A., Sarsour, K., Jutte, D., D'Esposito, M., & Boyce, W. T. (2012). The impact of social disparity on prefrontal function in childhood. PLoS One, 7(4), e35744.

Stepura, N. N., Zamotayeva, G. A., Terekhova, G. N., & Volynets, I. P. (2020). Circulating immune complexes content in patients with diffuse toxic goiter complicated by autoimmune ophthalmopathy. Endokrynologia, 25(4), 305–309.

Vick, M. M., Adams, A. A., Murphy, B. A., Sessions, D. R., Horohov, D. W., Cook, R. F., Shelton, B. J., & Fitzgerald, B. P. (2007). Relationships among inflammatory cytokines, obesity, and insulin sensitivity in the horse. Journal of Animal Science, 85(5), 1144–1155.

Wylie, C. E., Collins, S. N., Verheyen, K. L., & Newton, J. R. (2012). Risk factors for equine laminitis: A systematic review with quality appraisal of published evidence. The Veterinary Journal, 193(1), 58–66.

Yu, L. L., Li, C. N., Fang, M. Y., Ma, Y., Wang, B., Lin, F. P., Liu, W. H., Tu, S. H., Chen, Z., Xie, W. X., Zhang, R. Y., Huang, Y., Zheng, C. H., & Wang, Y. (2023). Evaluating the effectiveness and safety of acupuncture on serum uric acid in asymptomatic hyperuricemia population: a randomized controlled clinical trial study protocol. Frontiers in Endocrinology, 14, 1218546.

Zhurenko, V. V., & Zhurenko, O. V. (2018). Vplyv zbudnyka kryptosporydiozu na biokhimichni ta imunolohichni pokaznyky krovi teliat [Influence of cryptosporidiosis agent on biochemical and immunological parameters of blood of calves]. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, 20(88), 136–140 (in Ukrainian).