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# Age and sex features of organism non-specific resistance of Ukrainian riding horse

# Вікові та статеві особливості неспецифічного організму опір українського верхового коня

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## ABSTRACT

It is established that the indices of non-specific resistance of Ukrainian Riding Horse depend on age and sex. The lowest indices of serum bactericidal activity, serum lysozyme activity, the content of γ-globulins were found in 6-month-old foals, for which, however, the phagocytosis completeness indices were concurrent with those of adult individuals of the corresponding sex. Hence, the foals are least protected in terms of humoral-type non-specific resistance. Indicators of bactericidal and lysozyme activity of serum, phagocytosis completeness index were higher for stallions. Indicators of geldings were higher than those for mares, but lower than those for entires. The higher total protein content is also characteristic for stallions; at the age of 6, 9, 12 - the content of albumins; for mares at the age of 1.5, 2, 3, 12 - the content of γglobulins was higher, the indicators of geldings in terms of albumin/globulin ratio were closer to those of mares of the corresponding age. Consequently, for Ukrainian Riding Horse, the indicators of organism non-specific resistance are higher for stallions and vary with age. These differences should be taken into account when working with this breed.

**Keywords:** phagocytosis completeness index, serum bactericidal activity, serum lysozyme activity, total protein and its fractions

## АНОТАЦІЯ

Встановлено, що показники неспецифічного опору українського верхового коня залежність від віку та статі. Найнижчі показники бактерицидної активності сироватки крові, сироватки крові лізоцимну активність, вміст γ-глобулінів було виявлено у жеребців 6-місячного віку, за які, однак, показники повноти фагоцитозу були одночасно з тими дорослих особин відповідної статі. Отже, жеребці найменш захищені умови неспецифічного опору гуморального типу. Показники бактерицидної та лізоцимної активності сироватки, повноти фагоцитозу індекс був вищим для жеребців. Показники поворотів були вищими, ніж для кобили, але нижчі, ніж у хоробрих. Більш високий вміст білка також характерний для жеребців; у віці 6, 9 років, 12 - вміст альбумінів; для кобил у віці 1,5, 2, 3, 12 років - вміст γглобулінів був вищим, показники залози за співвідношенням альбумін / глобулін були ближче до кобил відповідного віку. Отже, для українського верхового коня показники організму неспецифічні стійкість вища для жеребців і змінюється з віком. Ці відмінності повинні бути враховується при роботі з цією породою.

**Ключові слова:** індекс повноти фагоцитозу, бактерицидна активність у сироватці крові, активність лізоциму в сироватці крові, загальний білок та його фракції

## INTRODUCTION

Ukrainian Riding Horse (Ukrainian Saddle Horse) is a relatively young sports breed, with its raise in Ukraine started in 1945 to meet the needs of equestrian sport. In the world arena, it has become famous for the victories of its representatives in dressage, show jumping, triathlon (Hopka et al., 2004). The Ukrainian Riding Horses are well known to both domestic and foreign fans of equestrian sport, as they are worthy rivals of traditional breeds historically famous in Europe.

The breed is local and does not appear on the list of risk groups (Khadka, 2010). At the same time, the negative trends in horse breeding are noticeable in Ukraine, such as a livestock reduction, a decrease in activity in terms of breeding work, lack of adequate state funding for stud farms and reproducers. The realities of time make the owners of horses adapt to the current conditions, and therefore the prospect of horse breeding is under threat (Skabal, 2014).

Oleksandrivskyi Stud Farm is one of the few that has continued to work with Ukrainian Riding Horsefrom 1965 to date. Paying tribute to the traditions and established approaches regarding the peculiarities of breeding Ukrainian Riding Horse, the issue of maintaining their high performance remains vital. After all, the improvement of the breed by selecting individuals with highly reproduced and desirable qualities for cross breeding is not always comparable to the stable‘s high adaptive and protective properties of the horses' organism in the time frame (Sierszchulski et al., 2005).

The development of the breed, its competitiveness in the given initial parameters entirely depends on the skillful use and consideration of both physiological characteristics of the breed in general and individual representatives in particular.

The indicators of non-specific resistance of horses (Adams and Templeton, 1998; Adams and Schutta, 2010; Stefurak et al., 2016) are an important criterion for value, endurance and, at the same time, the potential of adaptive possibilities and uniqueness of the breed.

An organism‘s natural non-specific resistance is the ability of an organism to counteract exogenous negative factors of different etiologies by activating cellular and/or humoral immunity (Smorodintsev, 1961; Rumyantsev, 1998; Stalder et al., 1998; Tarocco, 2001; Atanasova et al., 2008; Malinova, 2016).

According to Hristev and Zapryanova (2017) the bactericidal activity of the blood serum is an indicator which identifies the good resistance of the organism to causes from various character.

A comprehensive study of natural resistance and understanding of the basic mechanisms of the functioning of the horses’ organism of all ages and sexes is a key to success in developing new and maintaining the existing livestock raising technologies. This is very important in the conditions of modern Ukraine, in the balance between the needs of horse breeding, limited resources and lack of proper conditions for the maintenance and development of the breed.

Therefore, the research objective was to study the age and sex characteristics of the natural resistance of the Ukrainian Riding Horse.

## MATERIALS AND METHODS

### *Animals*

The material for research was blood samples of clinically healthy horses (n=114) of Ukrainian Riding Horse taken in the summer of 2016 in horses grown at Oleksandrivskyi Stud Farm No. 174 (Likarivka village, Olexandria district, Kirovohrad region, Ukraine). The enterprise complied with all sanitation measures recommended by the Ministry of the Agro-Industrial Complex of the State Department of Veterinary Medicine of Ukraine. At Oleksandrivskyi Stud Farm, serological preventive studies of the horse population for various types of infections were carried out at least once a year. According to their results, the enterprise was considered to be safe and confirmed that it is located in a safe area with regards to infections. The vaccination schedule at the enterprise complied with the Ukrainian legislation and, at the same time, did not

contradict the experience of foreign colleagues (Simon et al., 2011).

The horse population was vaccinated against influenza (horses older than 1.5 years were vaccinated twice a year in March-April and September-October, horses 3-4 weeks old - before being weaned), they were revaccinated in 4-6 weeks, depending on recommendations of the vaccine manufacturer (Martinova, 2012). The vaccines were selected on the basis of the forecast of the expected strain of influenza.

Foals at the age of 6 months were vaccinated against tetanus using domestic and foreign vaccine, furthermore, an annual revaccination was carried out after 4 weeks and after 5 months. Broodmares were vaccinated before breeding.

The first vaccination against herpesvirus infections (foreign vaccines) was carried out at the age of 6 months, a second revaccination was carried out in 4 weeks, a third - three months after the first one, and subsequently every 6 months.

It should be noted that the foals (0.5 year) selected for the experiment were not vaccinated, they were scheduled to be vaccinated after the samples of blood were taken. Preventive deworming of animals was carried out twice a year in March and September, using preparations authorized by the Ministry of Agro-Industrial Complex. In view of the fact that the immunity status of each animal unit is specific and depends on many different factors, such as the vaccination status, previous diseases and current infections (including subclinical ones), in order to minimize the influence of such factors on the indicators under research and to standardize the experimental conditions, animals were selected only if their systematic inspection (once a week) during three months prior to the start of the research showed no symptoms of any clinical infections. The horses were kept on an adequate diet and in identical conditions. Animals under the age of three were divided into three groups. The first group is stallions, the second group is mares. Starting from 6 years old, the results for three groups - stallions, mares, geldings were analyzed. At the age of more than three years – geldings also participated in the experiment; they were kept at the Stud Farm due to different circumstances. Other age categories were formed as follows: foals of 6 months (just weaned), 1 year, 1.5 years; mares and stallions aged 2 and 3; mares (single), stallions (in the pre-mating period), geldings aged 6, 9, 12. The geldings selected for the experiment were gelded after puberty (at the age of 4), and therefore they retained the exterior characters of the stallions of Ukrainian Riding Horse.

The number of animals of the relevant age category and sex involved in the experiment are presented in Table 1.

***Serum proteinogramme for Ukrainian Riding Horse***

The samples of peripheral blood in horses were taken before early feeding by the method of jugular vein puncture according to the generally accepted method in testtubes with an anticoagulant (4% solution of sodium citrate) and without it. Blood samples were centrifuged to obtain serum. Peripheral blood samples were taken in each animal unit three times during the summer, the result of one animal was considered the average value of the indices obtained in these samplings.

***Biochemical parameters***

*Total protein*

Protein concentrations (g/l) in serum of blood were measured with the dye-binding Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA, USA) (Bradford, 1976) and a microtitration plate reader (Titertek Multiskan Photometer, Labsystems, Finland) using BSA as a standard (Reilas, 2001).

***Strains***

In order to determine the lysozyme, bactericidal activity of the serum, the phagocytosis completeness index, Micrococcus lisodecticus ATCC 10240 (acetone powder), Bacillus subtilis ATCC 6633, Escherichia coli XL-Blue, kindly provided by Yurii Fedkovych from the Chernivtsi National University (Ukraine), were used. In the work, diurnal testing cultures were used: the agar

**Table 1.** The number of animals of different age and sex involved in the experiment

| Mares | | Stallions | | Geldings | |
|---|---|---|---|---|---|
| Age of animals, years | n | Age of animals, years | n | Age of animals, years | n |
| 0.5 | 10 | 0.5 | 7 | 6 | 3 |
| 1 | 7 | 1 | 6 | 0.9 | 4 |
| 1.5 | 6 | 1.5 | 7 | 12 | 4 |
| 2 | 7 | 2 | 5 | | |
| 3 | 8 | 3 | 4 | | |
| 6 | 5 | 6 | 4 | | |
| 9 | 8 | 9 | 7 | | |
| 12 | 7 | 12 | 5 | | |

testing culture (from 5-6 test tubes) was washed with physiological solution until obtaining 2 billion suspension with a density of 0.1 at 625 nm. Then, 0.01 ml of 2 billion microbial suspension is added in 4.4 ml of meat-peptone broth and left in the thermostat for 24 hours (1 day). Then, a diurnal suspension standardized by McFarland (optical density of 0.1 at 625 nm) was used (Lalitha, 2004).

***Determination of Serum Bactericidal Activity against Bacillus subtilis ATCC 6633***

Serum bactericidal activity was determined according to Smirnova and Kuzmina (1966). The method is based on measuring the optical density of the beef-extract broth with the growth of B. subtilis ATCC 6633 with and without addition of the serum being studied. Procedure: 4.5 ml of sterile meat-peptone broth and 1 ml of blood serum are measured in the test tubes. 0.1 ml of diurnal 2 billion B. subtilis 6633 culture is added in each test tube. The control is a test tube with a broth and a culture without serum. The contents of the test tubes is mixed thoroughly and 2 ml is collected from each one using a sterile pipette to measure the optical density at 625 nm (the same optical density at which the standardization of culture is carried out). The mixture is placed in a thermostat at 39°C for 3 hours, after which it is measured again at 625 nm. As a result, two indicators are obtained: the first characterizes the initial optical density of the broth with culture and serum immediately after mixing; the second one - the optical density of the same mixture after 3 hours of incubation. With a weak serum bactericidal activity, the optical density increases dramatically due to generation of bacteria.

Bactericidal activity of serum was calculated using the formula:

$$BA = 100 - (De3 - De0) \times 100 / (Dk3 - Dk0),$$

where

BA - serum bactericidal activity, %

De3 - optical density of the experimental sample 3 hours after the start of the incubation;

De0 - optical density of the experimental sample at the beginning of the incubation;

Dk3 - optical density of the control (without serum) of the sample 3 hours after the beginning of the incubation;

Dk0 - optical density of the check sample at the beginning of the incubation

***Determination of Serum Lysozyme Activity against Micrococcus lisodecticus ATCC 10240***

The serum lysozyme activity was determined by the modified photonephelometric method (Sarukhanov et al., 2012). The method is based on measuring the difference in optical density of samples with serum and bacteria culture in 0.75 M sucrose without and with incubation (45 minutes).

Procedure for lysozyme activity: 0.03 ml of blood serum is introduced into test tubes with sucrose, then it is incubated at 56°C for 30 minutes to inactivate the complement. After cooling the samples, a 1.47 ml swab of standard testing cultures is introduced in each one and measured at the same wavelengths as the one for culture standardization (625 nm). The contents are shaken, incubated at 39°C for 45 minutes, and measured again at 625 nm.

The lysozyme activity was calculated based on the difference in the optical density of check and experimental samples using the formula:

$$LA = [(ODk - ODe)/ODk] \times 100,$$

where

LA - serum lysozyme activity, %

ODk - optical density of check samples (a mixture of serum and test culture without incubation);

ODe - optical density of experimental samples (a mixture of serum and test culture after incubation).

Determination of completeness of phagocytosis against Escherichia coli XL-Blue Completion of phagocytosis is determined on the basis of the absence of generation of bacteria inside of the leukocytes and the signs of their destruction. The experiment was conducted according to (Chumachenko et al., 1990). Procedure: An anticoagulant and 0.5-1 ml of blood are added into the centrifuge volumetric tube. Then, the same amount (by volume) of 2 billion suspension of bacteria (Escherichia coli XL-Blue) is added, mixed and placed in a thermostat at 37°C for 30 minutes. Then, a few drops of the mixture are added to a Petri dish with 2% meat-peptone agar and distributed along the surface of the medium with the help of a slide plate with sharpened edges (as in making an ordinary blood film). Plated dishes are placed in a thermostat at 37°C for the growth of bacteria and the onset of the third phase of phagocytic reaction, that is, the capture of bacteria by phagocytes. Exposure time is 3-6 hours. Then, the slide plate is heated over a spirit lamp, and, while placing the agar on the surface, lightly squeeze to a blood film located on the surface of the agar medium. The prepared films-prints are dried, fixed for 3 minutes with methanol and stained according to Romanowsky-Giemsa. When carrying out microscopic examination of preparations, a clear distinction between viable and non-viable microorganisms is seen. The first ones are bigger. In cases where the process of phagocytosis is complete, inside the leukocytes there are fragments of the destroyed cells, and the individual microbial bodies are much smaller than in leukocytes with incomplete phagocytosis.

The index of phagocytosis completeness was calculated as the percentage (%) of cells with completed phagocytosis out of 100 segmental neutrophils that were phagocytosed.

### *Determination of protein fractions of serum*

### *Turbidimetric Methods for Determining Total Protein*

Protein fractions in serum were determined by turbidimetric (nephelometric) method and expressed in %. The principle of the method is based on the fact that various protein fractions of serum are precipitated by phosphate solutions of different concentrations. The degree of turbidity of solutions determines the concentration of proteins in the experimental sample (Kondrahin, 2004). For horses, the values of protein content within the norm and their ratios within this method are presented in Table 2.

**Table 2.** Limits of physiological norm of protein content of various fractions in serum of horses according to turbidimetric method

| Albumins, % | α-Globulins, % | β-Globulins, % | β-Globulins, % | normal A/G ratio ranges |
|---|---|---|---|---|
| 35-45 | 14-18 | 20-26 | 18-24 | 0.5/1 – 0.8/1 |

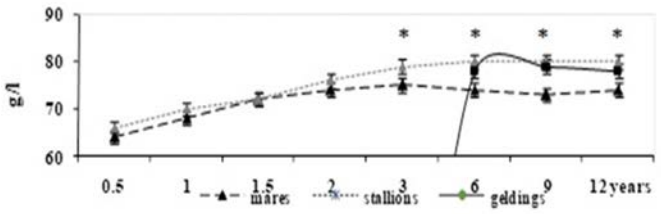
### *Statistical analysis*

Statistical processing of the results was carried out using Statistica 6.0 (StatSoft Inc, USA), ANOVA test. Values x±y represent standard deviation in all cases

## RESULTS

The morphological and biochemical blood indicators set for Ukrainian Riding Horse depends on sex and age. Up to three years, inclusive, there was an increase in the total protein content for the horses of both sexes, after

the age of 3 the variation of total protein content was negligible. The maximum values are set for stallions (Fig. 1).




* the difference between mares and stallions of the same age is significant, P<0.05

**Figure 1.** Total protein content in serum of Ukrainian Riding Horse

At the age of 6, 9, 12, albumin/globulin ratios for mares and geldings are lower than those at 1.5 years of age and compared with stallions of the corresponding age.

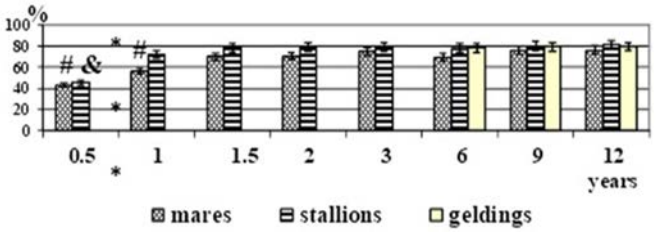
Although fluctuations of albumin/globulin fraction were found in the blood of puberty horses, these indices did not go beyond the limits of physiological values and were higher than the lower limit of norm (Table 1).

The content of γ-globulins, a fraction that includes the immunoglobulins G, M, D, A, E (Tothova et al., 2016), was the lowest for six-month-old mares and stallions - 18.23% and 18.52% respectively, sex differentiation was not found at this age for the γ-globulin content index (Table 3). At the same time, the values of γglobulins content for other age groups depended on sex - after puberty, the maximum values were found for mares (3, 6, 9 years), compared with stallions and geldings of the corresponding age.

Indices of the γ-globulin fraction content in horses of various sexes, found in the experiment, did not go beyond the limits of physiological values.

Indices of the α-globulin fraction content, with its many representatives being proteins of the acute phase, and therefore their content increasing in the first place with diseases, changed without expressed regularities. The maximum content of these proteins were recorded for mares and geldings at the age of six months. The Albumins, % α-Globulins, % β-Globulins, % γ- Globulins, % normal A/G ratio ranged 35-45 14-18 20-26 18-24 0.5/1 – 0.8/1 with also high levels of β-globulins content at this age; no logical changes in the studied periods of life of horses were recorded.

Study of indices of natural non-specific resistance of Ukrainian Riding Horse showed that the minimum values of serum bactericidal activity are recorded for 6-month-old foals (Fig. 2).




* the difference between mares and stallions of the same age is significant, P<0.05

# - the difference is significant compared with the indicators of blood serum bactericidal activity of mares of 1.5 – 12 years of age, P<0.05

& - the difference is significant compared with the indicators of blood serum bactericidal activity of stallions of 1-12 years of age, P<0.05

**Figure 2.** Serum bactericidal activity of Ukrainian Riding Horse of different ages

From the age of one year on, serum bactericidal activity differences in the horses of various sexes have been detected; for stallions' blood the ability to withstand in vitro culture B. subtilis is higher than that of mares and geldings. However, the statistically significant difference was found only in the first and the second year of life. Indicators of serum bactericidal activity of gelding's syndrome were at the level with the indicators of serum bactericidal activity of stallions of the corresponding age.

While studying the serum lysozyme activity, it was found that the age dynamics of this indicator are similar to the parameters of serum bactericidal activity - the lowest percentage of inhibition of M. lisodecticus is set for blood samples of six-month-old stallions and mares, whereas in one-and-a-half-year-old ones these figures were almost doubled and make up 22 - 38%, respectively (Fig. 3).

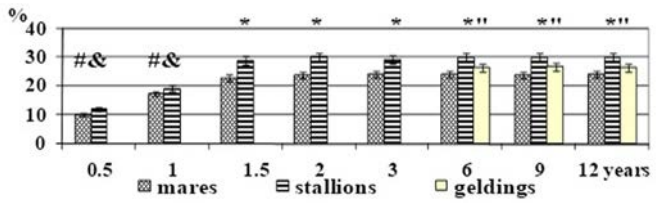
Cellular indices of organism non-specific resistance were high, starting with the first stage of research - already at the age of six months. No further recording of significant age-related fluctuations in indicators were conducted; however, it was observed that the phagocytosis

**Table 3.** Serum proteinogramme for Ukrainian Riding Horse

| Age of animals, years | n | Albumins, % | α-Globulins, % | β-Globulins, % | γ- Globulins, % | Alb./glob |
|---|---|---|---|---|---|---|
| | | | Mares | | | |
| 0.5 | 10 | 40.99±1.29 | 17.87±0.85 | 22.91±0.77 | 18.23±1.03 | 0.69/1 |
| 1 | 7 | 43.34±0.76 | 16.88±1.91 | 20.00±0.87 | 19.78±1.05 | 0.76/1 |
| 1.5 | 6 | 44.15±0.81 | 14.08±0.85 | 21.07±0.76 | 20.70±0.23 | 0.79/1 |
| 2 | 7 | 42.61±1.02 | 14.17±0.28 | 20.15±0.84 | 23.07±1.12 | 0.74/1 |
| 3 | 8 | 37.94±1.13 | 16.87±1.21 | 21.21±0.72 | 23.98±1.07 | 0.61/1 |
| 6 | 5 | 38.52±1.81 | 17.13±1.06 | 20.68±0.48 | 23.62±1.22 | 0.63/1 |
| 9 | 8 | 40.16±2.07 | 16.47±1.16 | 19.81±0.59 | 23.56±1.28 | 0.67/1 |
| 12 | 7 | 41.27±2.11 | 15.89±1.08 | 20.11±0.59 | 22.73±1.34 | 0.69/1 |
| | | | Stallions | | | |
| 0.5 | 7 | 40.85±1.21 | 17.81±0.61 | 22.82±0.38 | 18.52±0.29 | 0.69/1 |
| 1 | 6 | 41.44±0.79 | 16.98±0.62 | 22.67±0.31 | 18.91±0.38 | 0.71/1 |
| 1.5 | 7 | 44.22±0.65 | 15.58±0.57 | 20.41±0.18 | 19.79±0.48** | 0.79/1 |
| 2 | 5 | 43.18±1.12 | 16.04±0.54 | 19.69±0.29 | 21.09±0.49** | 0.76/1 |
| 3 | 4 | 39.87±1.09 | 15.37±0.49 | 23.18±0.74 | 21.58±0.81** | 0.66/1 |
| 6 | 4 | 41.33±1.11 | 15.37±0.68 | 21.30±0.23 | 22.00±0.89 | 0.70/1 |
| 9 | 7 | 42.97±1.34 | 14.59±0.69 | 20.63±0.90 | 21.81±1.56 | 0.75/1 |
| 12 | 5 | 43.18±1.33 | 15.13±0.74 | 21.77±0.65 | 19.92±1.21** | 0.76/1 |
| | | | Geldings | | | |
| 6 | 3 | 38.18±1.21* | 15.13±0.76 | 23.05±1.13 | 23.64±1.20 | 0.62/1 |
| 9 | 4 | 40.08±1.24* | 17.00±0.52 | 21.45±1.18 | 21.47±1.31 | 0.67/1 |
| 12 | 4 | 40.18±1.44* | 16.35±1.15 | 20.57±1.82 | 22.90±1.03 | 0.67/1 |

* the difference is significant compared to the albumin content in the serum of stallions of the corresponding age, P<0.05
** the difference is significant compared to the values of γ-globulins content in serum of mares of the corresponding age, P<0.05

completeness indices of mares were significantly lower than those of stallions. The phagocytosis completeness indices of geldings were lower than those of stallions, but higher than the phagocytosis completeness indices of mares.



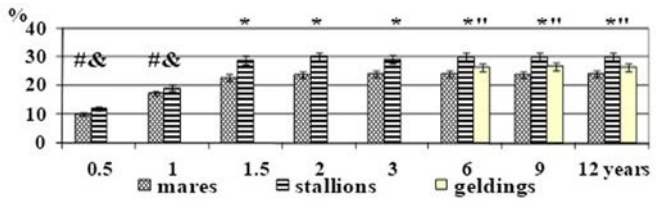

* the difference is significant between the indicators of blood serum lysozyme activity of mares and stallions of the same age, P<0.05

" the difference is significant compared with the indicators of blood serum lysozyme activity of mares of 1.5 - 12 of age

# - the difference is significant compared with the indicators of blood serum lysozyme activity of mares of 1.5 - 12 of age, P<0.05

& - the difference is significant compared with the indicators of blood serum lysozyme activity of stallions of 1-12 of age and geldings of 3-6 of age, P<0.05

**Figure 3.** Serum lysozyme activity of Ukrainian Riding Horse of different ages




* the difference is significant between the phagocytosis completeness indices of mares and stallions of the same age, P<0.05

**Figure 4.** Cellular indicators (phagocytosis completeness indices) of the natural blood resistance of the Ukrainian Riding Horse

## DISCUSSION

As a result of the conducted research, it was established that the indices of nonspecific blood resistance of Ukrainian Riding Horse are dependent on age and sex. The obtained results indicate that the period of formation of the maximum values of such indicators under research as serum bactericidal activity, serum lysozyme activity is 1.5 years (Fig. 2, 3). This is this age group that has the maximum albumin/globulin ratio (Table 3). As a result, the initial selection of horses - candidates for future reproduction, potential winners of sports competitions and geldings, can be started at this age, guided not only by such selection criteria as genealogy, constitution, endurance and other physical parameters, but also by taking into account the blood chemistry indices and thenatural non-specific resistance of the body of each animal unit.

It is known that for horses kept on stud farms, puberty comes in the period of 15-18 months (the general development of the organism occurs at the age of 4-5) (Hopka et al., 2004). Anabolic synthesis, the intensity of which is high for young animals, requires structural material that can be provided through albumins. Serum albumin is the major protein produced in the liver, which contains as much as 50% of the productive effort in any one moment. The concentration of this protein in the plasma has long been used as a bellwether of health and disease. It should be remembered that the serum albumin level is only the complex end result of synthesis, degradation, and distribution (Rothschild et al., 1977). Determination of albumin concentration and albumin/globulin ratio is a traditional method for diagnosing various types of diseases, with its informativity complementing information about the total protein and its fractions (Tothova et al., 2016). The high albumin content in the vascular system is associated with a normal fluid-and-electrolyte balance (Patil, 2006). Albumin represents a very abundant and important circulating antioxidant (Roche et al., 2008), provides transport of fatty acids (Van der Vusse, 2009), steroid hormones, including gonadal ones: 10-15% glucocorticoids are non-specifically linked to the albumin fraction (Ferreira de Medeiros, 2017). Probably, it is these properties of albumin that can be attributed to the high concentration of this simple protein in the blood of 1.5-year-old animal units that are reaching the stage of puberty in this age.

With age, there have been minor fluctuations of albumin content and, accordingly, albumin/globulin ratio, but also for 6-, 9-, 12-years-old horses they did not go beyond physiological values, and were higher than the minimum limit of the norm.

Thus, albumin synthesis is down-regulated and amino acids are shunted toward the synthesis of the positive acute phase proteins (Tothova et al., 2016). Since proteinograms did not record deviations in the distribution of proteins, this is another confirmation that the animals, in which samples of blood were taken, are clinically healthy.

Thus, differences were recorded between albumin content indices in mares, geldings and stallions - for geldings albumin content at the age of 6, 9, 12 was at the level of the same of mares and statistically different from that of stallions of the corresponding age for which albumin level, and respectively, albumin/globulin ratio was higher. On the one hand, it has long been known that steroid hormones play a role in the development and functionality of both. The removal of the testis through the castration has been used to significantly suppress the production of testosterone, which in turn can affect the synthesis and secretion of some of the plasma proteins (Fedorka, 2017).

Another reason for the similarity between the albumin content indexes of mares and geldings may be the comparable use of mares and geldings - they are both used in the enterprise for uniformly prolonged physical work, only some of the geldings for test. A more significant reserve of plasma proteins in the blood of stallions, primarily albumins, is justified because more energy-intensive processes are inherent in their use.

Total protein content indices in animals aged 0.5 were the minimum for the breed – 64.66 g/l protein for mares and stallions, respectively. Subsequently, the total protein content increased at the age of 1.5 and statistically did not differ from the level of adult animals. From the age of three, sex differences in the total protein content of stallions and mares were recorded - in stallions and geldings the studied values were statistically higher.

Sexual dimorphism at the level of total protein content is a phenomenon known to people (Markofskia and Volpi, 2011) and due to possible significant changes in the endocrine profile and excellent anabolic stimuli (feeding, exercise load) of animal units of different sexes. Almost all serum proteins are produced and secreted by hepatocytes (Tothova et al., 2016). The liver endocrine functions include the secretion of plasma proteins such as albumin and α- and β-globulins (Bergero and Nery, 2008). Therefore, differences in the content of total protein may be due to sexual differences in the functioning of the liver (Thapa and Walia, 2007; Tothova et al., 2016).

Serum bactericidal activity, serum lysozyme activity, and phagocytosis completeness indices were differentiated in terms of sex for all age categories, with their numerical values indicating the effectiveness of counteracting the pathogens. Throughout the entire period of research the indicators are higher for stallions of the Ukrainian Riding Horse, therefore, the mares are characterized by significantly lower potential of the Organism‘s natural non-specific resistance in terms of these criteria. At the same time, sex differences are recorded already at the first stage of research - starting from 6 months. These specifics should be taken into account when selecting the optimal regimes of keeping and breeding the Ukrainian Riding Horse.

Compared to stallions, reduced organism natural resistance at the level of serum lysozyme activity indices is also observed for geldings - 26.44% - 26.91% vs. 29.92% - 30.13% for stallions, at the age of 6, 9, 12, and is approaching the indicators of mares.

It is known that the lysozyme indicator is a value which depends on the breed of horses, and this indicator is dependent on the environment (Sotirov, 2004). Since lysozyme is a muramidase enzyme that hydrolyzes the cell walls of gram-positive bacteria (Sarukhanov et al., 2012), the lower titres of this bacteriolysin, correspondingly, indicate a lower anti-infection protection against microorganisms precisely with such a structure of the cell wall. It is interesting that during booster vaccination against influenza virus and Equine herpes virus 4/1 in horses and making different exercises (jumping hurdles on four consecutive days) by a group of experimental horses (assay group) it was found that regular exercises during the period of vaccinal antibody production did not significantly change lysozyme concentrations, but on the contrary improved the intensity of classical pathway of complement activation (Sotirov at al., 2004).

However, on the background of lower, compared with stallions, serum bactericidal activity, serum lysozyme activity, phagocytosis completeness indices established for mares and geldings, these horses had the higher content of γ-globulins, a fraction represented

by immunoglobulins. Consequently, the content of γ-globulins in the serum of horses is differentiated in terms of sex, and this difference is higher in favour of mares, statistically significant from the age of three (Table 3). The lowest content of γ-globulins was found in serum of 6-month-old foals. Probably this fact reflects the physiological norm of this age category.

Instead, the numerical ranges of the phagocytosis completeness index set for the youngest age group of foils were high and were also characteristic of adult horses. Their indices remained stably high during the research.

Thus, the cellular element of organism non-specific resistance, unlike humoral, is being formed at an early age. On the other hand, the division into humoral (Serum bactericidal activity, serum lysozyme activity in this study) and cellular (phagocytosis completeness index) factors of nonspecific resistance is rather conventional, as for example, agents that provide bactericidal characteristic of serum are produced by blood cellular elements or preformats that also provide natural resistance (Bitiukov and Minenkov, 2012). Therefore, it is obvious that the process of forming humoral protection must be preceded by the cellular one. Similar conclusions are found in the work of predecessors (Perryman and Jerzy, 1989). In some research (Maslianko, 1987; Motuzko and Nikitin, 1990), there is evidence that the formation of own factors of nonspecific resistance of farm animals starts from a three-month age. Thus, the data obtained by this study for the Ukrainian Riding Horse does not contradict the data set for horses of other breeds.

Since the humoral and cellular indices under research are not constant values, this should be taken into account when working with the breed. Moreover, it is concluded that the systematic study of the indicators of natural resistance of Ukrainian Riding Horse breed, both within the same population, and in comparison with other selective branches of the same breed (territorially isolated for a long time), will allow monitoring of the state of the breed at the level of counteraction to the exogenous factors of infectious etiology. This will assist in recording changes in the studied indicators and in the comparison of the influence of paratypic factors, training conditions, genetic effects, etc. All this will lay the foundation for the next steps on developing optimal conditions of keeping and training conditions for the immunization of animal units of all ages and sexes, increasing their endurance and improving the physical parameters, for the sake of sports and other achievemenets of the breed.

## CONCLUSIONS

Based on the obtained indicators, it is concluded that the age-related changes in the organism of Ukrainian Riding Horse are accompanied by an increase in the humoral factors of the organism natural non-specific resistance.

Serum bactericidal activity, serum lysozyme activity indices reach the level of adult animal units at the age of 1.5 and are differentiated in terms of sex - higher for stallions than for mares. The level of serum bactericidal activity, serum lysozyme activity indices of geldings is higher than that of mares, but lower than the level of stallions.

The least protected in terms of non-specific resistance of humoral type are the 6-month-old foals, but since the phagocytosis completeness index is high at this age, it is likely that the cellular element of organism non-specific resistance appears on the foreground of the young organism's resistance to pathogens.

Another cluster of indicators commonly used in biochemistry - the content of total protein in the serum, the distribution of its fractions, albumin/globulin ratio show both age-related and sex-related dependence. For stallions, the higher total protein content was characteristic, at the age of 6, 9, 12 - the content of albumins; for mares at the age of 1.5, 2.3 and 12 - the content of γ-globulins was higher, and the indexes of geldings in terms of albumin/globulin ratio were closer to that of mares of the corresponding age.

Consequently, it was discovered that sexual dimorphism and established age-related peculiarities at the level of non-specific resistance of the Ukrainian Riding Horse, determine its specifics and uniqueness. Since the

researched humoral and cellular resistance indices are not constant values, this should be taken into account when working with the breed.

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