Comparison of blood biochemical parameters of stallions of different age groups

M M Atroshchenko¹,², A M Shitikova², M G Engalycheva² and E Yu Borodkina¹

¹The All-Russian Research Institute for Horse Breeding, Divovo, Rybnovskii district, Ryazan region, 391105, Russia
²Ryazan State Medical University, 9 Vysokovol'tnaya St., Ryazan, 390026, Russia

E-mail: atromiks-77@mail.ru

Abstract. The analysis of biochemical parameters of blood serum of stallions of different age groups (n = 86) is carried out. It was found that with age there is an increase in the concentration of total protein, fibrinogen, direct and indirect bilirubin, while the group of young stallions aged 3-5 years is characterized by a higher value of alkaline phosphatase activity compared to the group of 6-10 years (p = 0.002) and a lower value calcium concentration compared with the group of stallions aged 16-22 years (p = 0.012).

1. Introduction
Environmental factors have a significant impact on the animal organism, directly or indirectly affecting its vital activity and quality of life. The body’s response to environmental factors can be different in degree and nature, mainly representing changes in the ecophysiological processes and ethology of the animal [1-3]. Such changes can be studied and controlled using various laboratory tests [4]. Hematological and biochemical parameters are widely used in the clinical diagnosis of organic, infectious and some parasitic diseases in horses. These indicators play a key role in monitoring the animal’s recovery during treatment and for assessing its metabolic status, as well as in programs for studying the health level of livestock of horse breeding farms. Timely and correct treatment can eliminate many problems and prevent further deterioration of the body’s condition [5]. The normal functioning of the body can be impaired by stress, trauma, pathological conditions caused by microorganisms, parasites, chemical or physical factors. Also, the sex, breed, seasonality and age affect the health of the animal [6]. The influence of the age of farm animals on various biochemical parameters of blood serum and sperm plasma is widely discussed, and much remains controversial [7-9].

The purpose of this study was to analyze the biochemical parameters of the blood serum of stallions of different age groups.

2. Material and methods
All procedures were carried out in accordance with the “European Convention for the protection of vertebrates used for experimental and other scientific purposes” ETS No. 123 (18 March 1986) and the Law of the Russia Federation on Veterinary Medicine No. 4979-1 (14 May 1993). The protocol of the present investigation was approved by the Local Ethics Committee of the All-Russian Research Institute for Horse Breeding (ARRIH), Ryazan Oblast, Russia.
The study was carried out on the basis of the Tersk stud No. 169 (Stavropol Territory), the Pochinkovsky and Perevozsky stud farms (Nizhny Novgorod region), the All-Russian Research Institute for Horse Breeding (Ryazan Region). Laboratory research was carried out in the cryobiology laboratory of the All-Russian Research Institute of Horse Breeding (Ryazan Region) and the Department of Biological Chemistry of the Ryazan State Medical University (Ryazan).

The experiments used 86 stallions of riding, trotting and heavy draft breeds, aged from 3 to 22 years. During the experimental studies, the conditions of feeding and feeding the stallions corresponded to the established standards.

A blood sample from each stallion from the jugular vein was taken once prior to morning feeding during the breeding season. Blood samples were centrifuged at 1500 rpm. for 20 min and the serum was stored at -18° C until analysis.

Determination of biochemical parameters in blood serum: total protein (TP), albumin, glucose, urea, cholesterol, triglycerides, fibrinogen, total bilirubin, direct and indirect bilirubin, creatinine, HDL and LDL cholesterol, potassium, sodium, chlorine, magnesium, calcium ions, the content of serum iron, uric acid, as well as the activity of enzymes: alkaline phosphatase (ALP), creatine phosphokinase (CPK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), α-amylase, lactate dehydrogenase (LDH), γ-glutamyltransferase (GHT) was carried out on an automatic biochemical analyzer Vegasys (Analyzer Medical System, Italy). To assess the statistical significance in the study groups, the nonparametric U Mann-Whitney test was used. Results are presented as median (Me), upper and lower quartiles [Q1; Q3]. Differences were considered statistically significant at \( p < 0.05 \).

### 3. Results and discussion

All stallions participating in the research were divided into four age groups (table 1). The first group included stallions aged 3-5 years, the second group— at the age of 6-10 years, the third - 11-15 years old, and the fourth group included older stallions, from 16 to 22 years old.

**Table 1.** Biochemical parameters of blood serum of stallions of different age groups, Me [Q1; Q3], \( (n = 86) \).

| Index                      | Group (age, years old) | 1 (3-5) \( n=15 \) | 2 (6-10) \( n=33 \) | 3 (11-15) \( n=20 \) | 4 (16-22) \( n=18 \) |
|----------------------------|------------------------|---------------------|---------------------|---------------------|---------------------|
| Total protein, g / l       |                        | 62.5 [60.0;65.7]    | 67.4 [62.5;73.1]    | 69.2 [65.3;71.7]    | 69.7 [66.8;72.0]    |
| Albumin, g / l             |                        | 37.0 [35.5;38.3]    | 39.3 [36.2;43.9]    | 40.9 [37.0;43.4]    | 40.7 [36.0;44.6]    |
| Globulins, g / l           |                        | 23.9 [21.8;28.3]    | 27.0 [23.3;33.8]    | 28.8 [22.7;33.2]    | 30.1 [24.7;33.5]    |
| “Albumin / globulins”, rel. |                        | 1.6 [1.2;1.8]       | 1.4 [1.2;1.8]       | 1.4 [1.2;1.7]       | 1.4 [1.1;1.7]       |
| Glucose, mmol / l          |                        | 4.1 [3.5;4.7]       | 4.5 [3.8;5.0]       | 3.6 [3.1;4.1]       | 3.6 [3.0;4.2]       |
| Fibrinogen, g / l          |                        | 2.2 [2.2;2.4]       | 2.6 [2.2;3.4]       | 3.1 [2.2;4.1]       | 3.1 [2.6;3.64]      |
| Urea, mmol / l             |                        | 4.5 [3.7;6.1]       | 6.0 [4.7;8.0]       | 6.7 [4.7;8.0]       | 6.4 [5.8;7.2]       |
| Cholesterol, mmol / l      |                        | 2.4 [2.1;2.9]       | 2.2 [1.9;2.5]       | 2.2 [1.9;2.5]       | 2.4 [2.1;2.6]       |
| ALT, U / l                 |                        | 10.0 [8.5;10.5]     | 10.0 [8.0;12.0]     | 8.5 [7.0;10.3]      | 10.0 [7.0;10.0]     |
| AST, U / l                 |                        | 206.0 [168.5;261.0] | 173.0 [150.0;205.0] | 188.5 [143.8;219.3] | 189.5 [160.0;196.8] |
| α-amylase, U / l           |                        | 36.0 [16.0;42.0]    | 18.0 [10.0;36.0]    | 17.5 [10.0;36.5]    | 27.1 [14.8;41.0]    |
| Creatinine, μmol / L       |                        | 130.0 [120.0;140.5] | 134.0 [107.0;160.0] | 144.0 [120.3;152.3] | 134.5 [117.0;152.3] |
| GHT, U / l                 |                        | 12.0 [10.0;15.5]    | 13.0 [12.0;15.0]    | 13.5 [10.8;16.8]    | 12.0 [10.3;15.5]    |
The concentration of total protein in the blood serum of the studied animals from different age groups was within the acceptable range for horses, amounting to 52-79 g/l [10]. It was also found that the concentration of total protein in the blood serum of group 1 stallions is statistically significantly lower compared to group 3 stallions (p = 0.010) and group 4 (p = 0.005). This indicator in stallions from group 2 was statistically significantly lower than in stallions of group 4 (p = 0.038). Thus, with age, there is a tendency for the concentration of total protein in the blood serum to increase. The data obtained are consistent with our recent studies [11], as well as with the study of Abeni F. et al. [12]. The increase in the concentration of total protein in the blood was clearly seen in the work of Ireland J. L. et al. In horses aged 30 years or more, these changes were accompanied by hyperglobulinemia and an albumin / globulin ratio below unity, which could be explained by the presence of inflammatory processes in the oldest horses [13]. Statistically significant differences in the concentration of globulins in animals of different age groups were not found. However, differences were obtained in relation to one of the proteins of the acute phase of inflammation - fibrinogen. The study of the concentration of fibrinogen in the blood of horses has been carried out over the past decades in order to diagnose various inflammatory diseases [14]. The concentration of this protein in the blood of young stallions (3-5 years old) differed by a lower level compared to animals aged 11-15 (p = 0.010) and 16-22 years (p = 0.001). In some of the studied stallions in groups 2-4, the concentration of fibrinogen was higher than the reference values according to Kaneko J.J. et al., constituting 2-4 g/l for horses [10]. However, an increase in the concentration of fibrinogen in the blood of animals with age may also be associated with age-related changes leading to a disruption in the breakdown of this protein and an increase in the time of its circulation in the blood, while the synthesis of fibrinogen by the liver itself may decrease [15].

Statistically significant differences in stallions of group 1 from older animals were also observed in the activity of enzymes. Thus, animals aged 3-5 years were characterized by the highest activity of alkaline phosphatase in comparison with other groups, however, statistical significance was obtained only in comparison with group 2 (p = 0.002). In a study by Mikniené Z. et al. similar data were obtained:

### Notes
- a Statistically significant differences between group 1 and group 2,
- b Statistically significant differences between group 1 and group 3,
- c Statistically significant differences between group 1 and group 4,
- d Statistically significant differences between group 2 and group 3.

### Table

| Parameter                  | Group 1 | Group 2 | Group 3 | Group 4 |
|----------------------------|---------|---------|---------|---------|
| CPK, U/l                   | 169.0 [152.0;194.0] | 180.0 [130.0;279.0] | 186.0 [146.3;244.3] | 192.0 [142.3;244.8] |
| LDH, U/l                   | 545.0 [473.0;617.0] | 417.0 [289.0;529.0] | 449.0 [368.5;581.0] | 481.0 [309.3;585.0] |
| ALP, U/l                   | 261.0a [246.0;304.5] | 190.0 [149.0;256.0] | 207.5 [174.0;338.8] | 228.0 [159.8;281.5] |
| Total bilirubin, μmol/l    | 24.5c [19.4;27.8] | 24.8 [20.5;33.0] | 30.0 [22.6;39.3] | 34.7 [25.1;42.0] |
| Direct bilirubin, μmol/l   | 7.1 [4.4;9.2] | 6.3 [5.0;9.3] | 5.3 [5.2;8.2] | 7.09 [6.2;9.3] |
| Indirect bilirubin, μmol/l | 15.4b [13.0;19.6] | 18.5 [14.2;26.7] | 23.9 [17.8;33.4] | 27.1 [18.7;34.4] |
| HDL cholesterol, mmol/l    | 1.6 [1.4;1.6] | 1.5 [1.4;1.7] | 1.5 [1.4;1.6] | 1.6 [1.4;1.8] |
| LDL cholesterol, mmol/l    | 0.7 [0.6;1.0] | 0.6 [0.4;0.9] | 0.6 [0.3;0.9] | 0.5 [0.4;0.9] |
| Triglycerides, mmol/l      | 0.2 [0.1;0.3] | 0.2 [0.1;0.3] | 0.2 [0.1;0.3] | 0.2 [0.2;0.4] |
| Uric acid, μmol/l          | 32.0 [25.5;35.0] | 26.0 [25.0;29.0] | 31.0 [28.0;34.8] | 28.0 [26.0;30.0] |
| Chlorides, mmol/l          | 94.0 [91.0;96.5] | 95.0 [90.0;100.8] | 94.4 [91.8;102.0] | 94.5 [91.2;98.0] |
| Magnesium, mmol/l          | 0.9 [0.8;0.9] | 0.9 [0.8;1.0] | 0.9 [0.8;1.1] | 0.9 [0.7;0.9] |
| Calcium, mmol/l            | 2.8 [2.7;2.9] | 3.0 [2.8;3.1] | 2.9 [2.7;3.1] | 3.0 [2.8;3.2] |
| Sodium, mmol/l             | 142.0 [139.5;144.0] | 140.0 [131.0;145.0] | 142.0 | 141.0 |
| [137.0;145.0] | [130.5;143.5] |
| Potassium, mmol/l          | 3.5 [3.1;3.9] | 3.4 [3.1;3.8] | 3.7 [3.4;4.0] | 3.3 [3.0;3.6] |
| Serum iron, μmol/l         | 38.5 [32.8;46.1] | 39.7 [37.2;41.0] | 45.3 [29.7;48.2] | 38.2 [35.0;43.4] |
the activity of alkaline phosphatase in foals was statistically significantly higher than in adults [16]. Various studies on the effect of age on bone metabolism have shown a negative correlation between horse age and alkaline phosphatase activity [16,17]. Apparently, the higher activity of alkaline phosphatase observed by us in the 1st group of stallions is associated with the involvement of the enzyme in the process of growth and synthesis of bone tissue, which occurs most actively at a young age.

In addition, the first group of stallions had lower blood calcium levels compared to older stallions from group 4 (p = 0.012). Calcium is a part of bone tissue, participates in muscle contraction, coagulation, cell adhesion, plays the role of a regulator in numerous enzymatic and hormonal processes [18]. Study by Berlin D. et al. showed that the concentration of ionized and total calcium in the blood of foals is statistically significantly lower than in adult animals [18]. Feldman F.B. et al. found that young animals assimilate dietary calcium more efficiently, in addition, they have a higher level of absorption of calcium and phosphorus compared to older animals [19]. Thus, our results are consistent with the data of other authors.

Besides, there was a tendency for the concentration of indirect and total bilirubin to increase with age. Thus, in group 1, compared with group 4, there was a lower concentration of total (p = 0.021) and indirect bilirubin (p = 0.005), and the concentration of indirect bilirubin in group 1 was statistically significantly lower than in group 3 (p = 0.038). In the scientific literature, there is a variety of data on the effect of age on the level of bilirubin in the blood. Thus, in the study by Tumbleson M.E. et al. there was a direct correlation between the age of cows and the concentration of total bilirubin in the blood serum [20]. Study by Guroze S.Y. et al. showed a decrease in the level of direct bilirubin with age, and an increase in total bilirubin in Arab purebred mares [8]. Mohri M. et al. reported that the concentration of bilirubin in the blood of calves was always within the reference range for adults [9]. Apparently, the effect of age on the concentration of bilirubin in the blood manifests itself in different ways in different species, and the sex of the animal also matters.

4. Conclusion
In the course of our study, the most statistically significant differences in biochemical parameters were observed between the group of young stallions (3-5 years old) and the group of the oldest stallions (16-22 years old). Thus, in group 1 (3-5 years), a lower level of concentration of total protein, fibrinogen, direct and indirect bilirubin was found in comparison with group 4 (16-22 years), while there was a tendency for these indicators to increase with age in investigated animals. In addition, group 1 (3-5 years old) was characterized by a higher value of alkaline phosphatase activity compared to group 2 (6-10 years old) (p = 0.002) and a lower value of calcium concentration compared to group 4 (16-22 years old) (p = 0.012), which may be associated with the involvement of alkaline phosphatase and calcium in bone metabolism, which is more intensive in young stallions.

Acknowledgements
The research was carried out with the financial support of Russian Science Foundation, Grant No: 20-16-00101. The samples of cryoconserved sperm were got from Bioresource collection “Cryobank of genetic resources” of the All-Russian Research Institute for Horse Breeding. The research was done using equipment of the Core Centrum of the All-Russian Research Institute for Horse Breeding.

References
[1] Alferov I V 2019 Biochemical peculiarities of blood serum of mares of the Yakut breed when using a grain from beer in the diet Krasgau Bulletin 7(148) 169-74
[2] Bagirov V A, Kalaschnikov V V, Zaitsev A M, Atroshchenko M M, Miroshnikov S A, Zavialov O A and Frolov A N 2017 Reproductive function in purebred arabian stallions as related to the levels of chemical elements in mane hair samples Sel'skokhozyaistvennaya Biologiya 52(6) 1184-93
[3] Zinovieva N A, Fisinin V I, Bagirov V A, Kostjunnina O V and Gladyr E A 2013 Bioresource centers as a form for conservation of animal genetic resources of agricultural purpose
Achievements of Science and Technology in Agriculture 11 40-41

[4] Bagirov V A, Iolchiev B S, Tadzieva A V and Klenovitsky P M 2015 Assessment of producers’ reproductive potential by means of laboratory research of the sperm Reports of the Russian Academy of Agricultural Sciences 1-2 51-54

[5] Miknienė Z, Maslauskas K, Kerzienė S, Kučinskienė J and Kučinskas A 2014 The effect of age and gender on blood haematological and serum biochemical parameters in Žemaitukai horses Vet. ir Zootech. 65 37-43

[6] Pošiváková T, Švajlenka J, Pošivák J, Pokorádi J, Hromada R, Korim P and Molnár L 2019 The influence of age on the activity of selected biochemical parameters of the mouflon (ovis musimon l.) Animals 15(9) 242

[7] Atroshchenko M M, Kudlaeva A M, Fomina M A, Kalashnikov V V, Zaitcev A M, Denisova O V, Navasardyants D G, Belonovskaya O S and Pasko A A 2019 Analysis of seminal plasma biochemical parameters and sperm cryostability in different age groups of stallions IOP Conf. Ser.: Earth and Environmental Science 341 012162

[8] Gurgöze S Y and İcen H 2010 The influence of age on clinical biochemical parameters in pure-bred arabian mares J. Equine Vet. Sci. 30(10) 569-74

[9] Mohri M, Sharifi K and Eidi S 2007 Hematology and serum biochemistry of Holstein dairy calves: age-related changes and comparison with blood composition in adults Res. Vet. Sci. 83 30-39

[10] MacNeill A 2008 Clinical biochemistry of domestic animals, 6th edition, ed J J Kaneko, J W Harvey and M L Bruss Vet. Clin. Pathol. 6 904

[11] Atroshchenko M M, Kudlaeva A M and Engalycheva M G 2020 Study of the influence of age on the biochemical parameters of blood serum of stallions Horse Breeding and Equestrian Sports 5 10-14

[12] Abeni F, Prà A D, Bertin G and Calamari L 2013 Serum protein fraction in mature horses and relationship with metabolic and hematological parameters J. Equine Vet. Sci 33(11) 905-11

[13] Ireland J L, McGowan C M, Clegg P D, Chandler K J and Pinchbeck G L 2012 A survey of health care and disease in geriatric horses aged 30 years or older Vet. J. 192(1) 57-64

[14] Crisman M, Scarratt W and Zimmerman K 2008 Blood proteins and inflammation in the horse Vet. Clin. North Am. Equine Pract. 24(2) 285-97

[15] Short K R and Nair S K 2000 The effect of age on protein metabolism Curr. Opin. Clin. Nutr. Metab. Care. 3(1) 39-44

[16] Gossett K A and French D D 1984 Effect of age on liver enzyme activities in serum of healthy quarter horses Am. J. Vet. Res. 45(2) 354-56

[17] Lepage O M, Marcoux M and Tremblay A 1990 Serum osteocalcin or bone Gla-protein, a biochemical marker for bone metabolism in horses: differences in serum levels with age Can. J. Vet. Res. 54(2) 223–26

[18] Berlin D and Aroch I 2009 Concentrations of ionized and total magnesium and calcium in healthy horses: effects of age, pregnancy, lactation, pH and sample type Vet. J. 181(3) 305-11

[19] Feldman F B, Zinkl G J and Jain N C 2006 Schalm's veterinary hematology Medicine – 2006 1232

[20] Tumbleson M E and Hutcheson D P 1971 Age related serum cholesterol, glucose, and total bilirubin concentrations of female dairy cattle Proceedings of The Society for Experimental Biology and Medicine 138(3) 1083-85