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Influence of Rank Stress on Behavior and Blood Indicators of A Young Horse

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ABSTRACT

The hierarchical structure in the community of young horses of the Hanoverian breed was studied, the social ranks of individuals were determined, the analysis of the behavioral repertoire of animals and the blood composition of horses occupying different hierarchical positions in the group was carried out. The influence of the hierarchical rank of animals on the ratio of different forms of behavior, as well as the effect of rank stress on the biochemical and hematological parameters of the blood of horses, was established. In the dominant individual, the proportion of such forms of behavior as exploratory activity, play activity and inactive forms of behavior is higher than in horses with a lower social status. The concentration of total protein, albumin and globulin fractions, as well as glucose, urea and creatinine, the activity of a number of enzymes (AST, ALT, and alkaline phosphatase) in the blood, as well as the red and white blood counts of low-ranking horses indicate an increased activity of the sympatho-adrenal system due to stress, experienced in competitive relationships in a group.

Key words: Horse behavior, Hierarchy, Sociogram of aggressive actions, Stress, Biochemical composition of shelter, Hematological parameters.

INTRODUCTION

In all branches of animal husbandry, including horse breeding, animals are exposed to various stress factors (Donnik et al. 2020) to the influence of which they are forced to adapt, since they are not able to use natural behavioral strategies while under human care in artificially created conditions. These stress factors include social interactions between individuals occupying different hierarchical positions in the community, which leads to the stress of all physiological and biochemical processes in the animal body.

A group lifestyle presupposes a different level of cooperation and competition of individuals within the animal community in a certain area, which results in the development of interpersonal relationships and their socialization. Each individual within the community must be considered as part of a network of social interactions that differ in strength, type and dynamics (Sueur and Mery 2017). Social behavior is fundamental to the survival of many animal species (Robinson et al. 2019), since such relationships involve the distribution of functional roles among members of society (Ivanov et al. 2013).

Such a strategy of behavior is based on various forms of interactions between individuals, each of which contributes to the viability of a given group, as a result of which the actions of its members become coordinated in space and time (Shimada 2020). As a result, the association of animals turns from a random accumulation into a structured community, which is based on hierarchical relationships, which are a complex of interconnected chains. Social rank affects almost every aspect of group life. The hierarchy serves as a universal mechanism for maintaining order in the coexistence of individuals, at the same time, in such a group, competition between animals for access to resources is constantly observed. Such a model of behavior sooner or later leads to conflicts of interests of the individual with other members of the community, which may have different manifestations (Ivanov et al. 2013).

Hierarchy between animals is formed in the course of conflicts, manifested in the form of direct clashes or demonstrations of threat, which can be no less effective than fights (Stanley 2018). With the establishment of hierarchical relations in the group, clearly expressed aggressive manifestations, as a rule, gradually give way to threats and demonstrations, which are a set of signal movements, postures and sounds used to exchange information between individuals (Ivanov et al. 2013; Wolter 2018). The higher the stage of evolutionary

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development a species occupies, the more complex and diverse the list of signs and signals that ensure the process of communication between individuals.

These species include the domestic horse, which, like its wild ancestors, belongs to social animals, in the community of which there are complex hierarchical relationships (Stachurska 2021) formed in the process of evolution (Waring 1983). This behavior strategy provides important benefits to horses.

Horses are capable of individually recognizing members of their community. In the community, horses constantly exchange communicative signals informing about intentions, current activities, social status, mood and emotions, as well as physical condition, and warn of dangers and other events of daily life (McGreevy and McLean 2010).

In the course of a horse's individual development, the prepubertal period, which precedes physiological maturity, is important. During this period, the animal's body undergoes a complex morphological and functional restructuring, accompanied by significant somatic and mental changes. At the same time, in the body of young animals, due to hormonal changes, there is an increased excitability, there is a high social activity of animals in the process of hierarchic relationships in society (Brubaker 2021). At this stage of ontogenesis, the social status of the horse is formed, in the course of the competitive struggle for which, young animals inevitably experience stress (Ivanov 2007). Rank stress creates stable emotions that induce arousal in the brain. Maintaining a dominant position in the hierarchy requires energetically demanding aggressive manifestations and physical exertion. Studies by a number of authors have shown that subordinates individuals have a higher level of stress hormone than dominant individuals (Schulte 2014; Sigurjónsdóttir 2019). There is an activation of the sympathetic nervous system, hypothalamic-pituitary-adrenal and renin-angiotensin systems (Lombard 2010). Due to the release of the hormone corticotropin from the hypothalamus, the release of adrenocorticotropic hormone from the pituitary gland is triggered, which, in turn, stimulates the release of corticosteroids from the adrenal cortex (Brown 1994). The glucocorticoid cortisol has a wide range of effects on all body systems, since most cells express cortisol receptors (Dhabhar 2014; Sauveroche 2020).

Due to the fact that the perception of a stress stimulus by an animal affects the intensity of physiological, biochemical, and behavioral processes, the response to stress can be assessed using some of these parameters as indicators (De Santis et al. 2017). Currently, there is no information in the literature on the biochemical and hematological blood profiles of animals occupying different hierarchical ranks. In this regard, the purpose of this work was a comprehensive physiological, biochemical and ethological assessment of the state of young horses under the influence of rank stress.

MATERIALS AND METHODS

The experimental protocol was approved by ethical committee of the Faculty of Animal Science and Biology Moscow Agricultural Academy named after K.A. Timiryazeva.

The research was carried out on a group of one-and-a-half-year-old filly of the Hanoverian breed on the basis of the Elitar horse breeding farm, located in the Podolsk district of the Moscow region. To analyze the ratio of different forms of behavior in the animals under study, one of the observation methods was used - the method of continuous logging (Lehner 1979). The fixation of behavioral patterns, such as exploratory, food, play behavior, sleep, nap, rest, was carried out during the period when the animals were in the levada. Hierarchical relationships in the group were studied using the method of constructing sociograms of aggressive actions, in which arrows indicated the direction and frequency of interactions between animals, followed by the calculation of the hierarchy coefficient for each individual (Ivanov et al. 2013). The thickness of the arrows is proportional to the number of aggressive actions and intentions of the animal towards members of the community (Fig. 1). Based on its value, each member of the community was determined by the social status (rank), and also the hierarchical structure in the community was determined. The biochemical parameters of the blood of the filly were determined using an automatic biochemical analyzer "LABIO-200", the hematological analysis of the blood of the animals was carried out using the hematological analyzer Abacus junior.

RESULTS

It has been established that the behavior of young horses as a whole corresponds to the structure of the behavioral repertoire of the species and this age group: exploratory behavior -54%, feeding behavior -35%, play behavior -8%. The ethological profiles of horses occupying different hierarchical positions in the community were determined. The dominant individual demonstrated exploratory behavior less frequently than low-ranking animals (by 7%), while play behavior and inactive forms of behavior (sleep, nap, rest) were recorded more often (by 1.6 and 4 times, respectively). Differences in the biochemical blood profiles of horses with different social status were revealed. In the dominant filly, the level of total protein (by 4.7%), albumin (by 6%), globulins (by 9%), urea (by 25.8%), activity of enzymes of alanine aminotransferase (by 12.4%) and lactate dehydrogenase (by 12.9%) were higher than those of other members of the group. In a low-ranking outcast horse, a number of blood biochemical parameters differed significantly from similar values of the dominant: the activity of aspartate aminotransferase was higher (by 15.5%), as well as the concentration of creatinine (by 8.5%), glucose (by 16.2%). Hematological parameters varied depending on the social status of the animals. The highest content of erythrocytes, hemoglobin and hematocrit in the blood was in a horse occupying the rank of an outcast, these indicators were higher by 6.8%, 8.3% and 10.8%, respectively, than in the dominant individual. At the same time, the osmotic stability of erythrocytes in the outcast was lower by 11%. The lymphocytic-granulocytic index in the horse, standing at the bottom step of the hierarchy in the studied community, was lower than in other members of the group by 22-65%, amounting to 0.68.

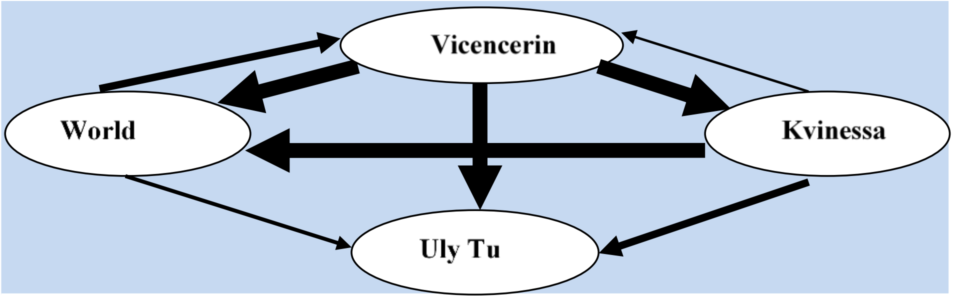

Fig. 1: Sociogram of aggressive interactions in the equine community.

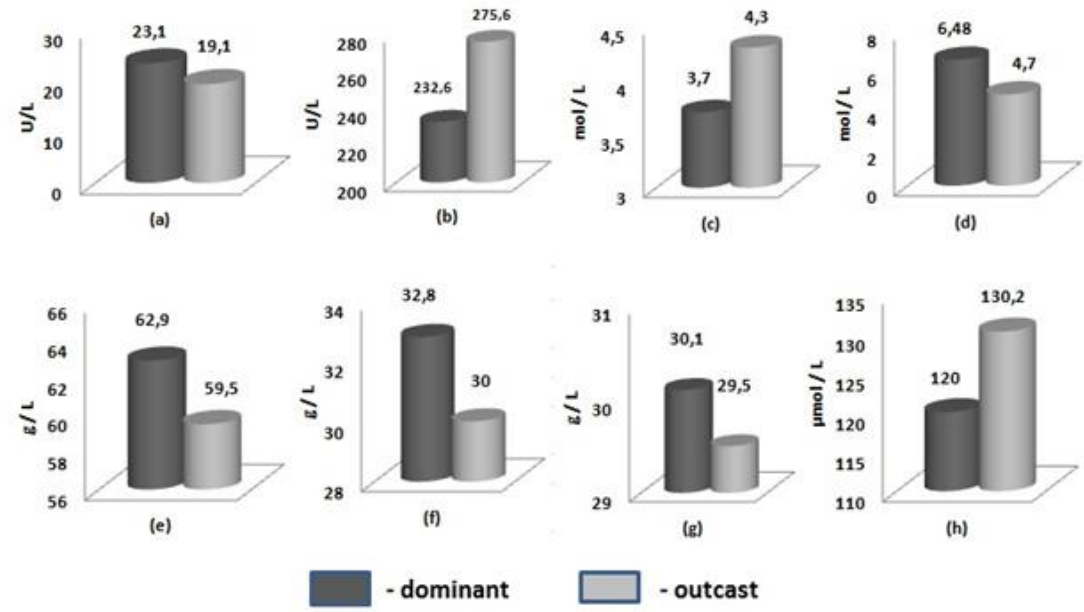

Fig. 2: Biochemical parameters of the blood of filly: (a) – alanine aminotransferase activity, (b) – aspartate aminotransferase activity, (c) – glucose concentration, (d) – urea concentration, (e) – total protein concentration, (f) – concentration of albumin fraction, (g) – concentration of globulin fraction, (h) - concentration of creatinine.

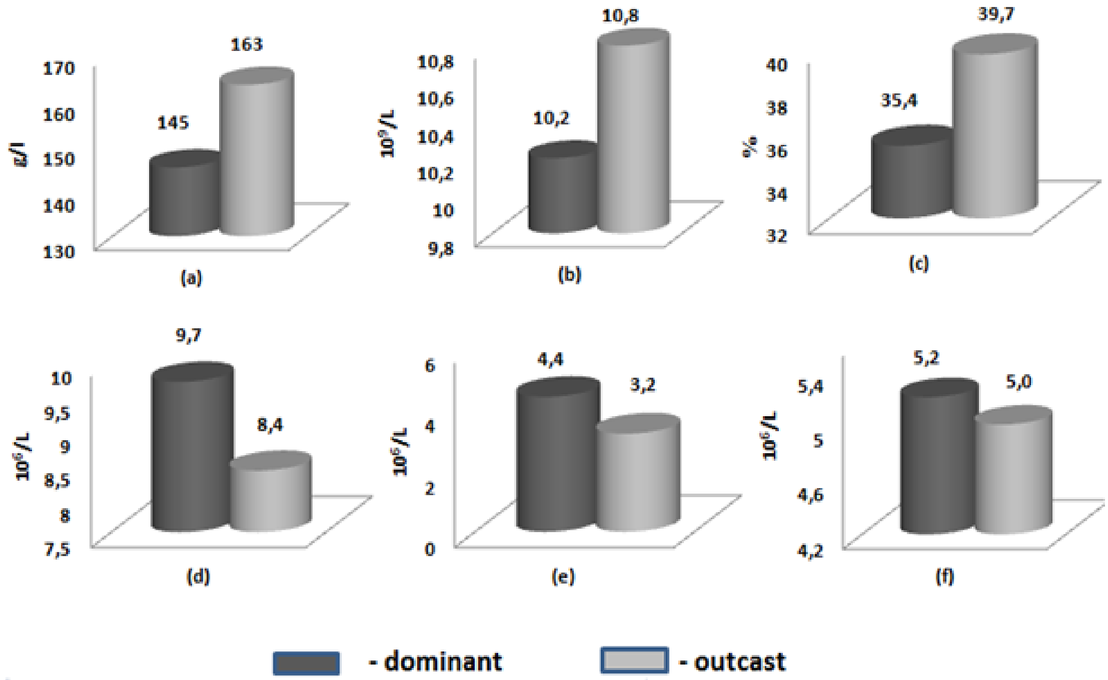

Fig. 3: Hematological parameters of filly blood: (a) - hemoglobin level, (b) - erythrocyte count, (c) - hematocrit, (d) - leukocyte count, (e) - lymphocyte count, (f) – granulocytes.

DISCUSSION

When studying the competing relationships between individual individuals in the studied community of filly, the number of agonistic actions of individual individuals relative to other members of the group, which were recorded using sociagrams of aggressive interactions, was of great importance. The data obtained using the sociogram formed the basis for determining the individual coefficients of the hierarchy, the calculation of which took into account the number of aggressive actions and intentions of an individual in relation to other members of the community, the number of recipients of these actions, as well as the number of aggressive manifestations of community members in relation to this individual and the number of animals initiating them (Fig. 1).

The calculated values of the coefficients of the hierarchy made it possible to rank the animals by social status. Among the members of such a group, one can distinguish a dominant (alpha individual), subdominants (beta individuals), which obey the dominant, but dominate over individuals of other ranks, subordinates (gamma of individuals), inferior to animals of higher ranks and dominating over lower ones, as well as those at a lower-level hierarchical ladder - outcasts (omega individuals).

In the group, the undisputed leader (dominant) was identified - the filly Vintserin, with the maximum value of this indicator 14. Individuals occupying an intermediate position in the hierarchical ladder were also identified: the subdominant filly Queen and the subordinate filly World Lady with hierarchy coefficients of 0.75 and 0, 5 respectively. The lowest social position in the community (outcast) was taken by the filly Will Two Win, whose hierarchy coefficient was 0, since this individual did not show aggression towards other members of the group and was exclusively the object of agonistic actions on the part of conspecifics (Fig. 1).

The research results showed that the behavioral repertoire of animals corresponded to normal values for this age group. It was found that the filly most often showed exploratory behavior, it accounted for 54% of the time during the observation period. This form of behavior is innate, manifests itself at different stages of ontogenesis and provides a biologically adequate orientation of the behavior of animals in a situation of novelty (Jastrzębska 2021). A high level of research activity allows young animals to develop and gain personal experience, receiving vital information, thereby ensuring the adaptation of the organism to a dynamic external environment, and ultimately being one of the survival mechanisms of the individual and the species as a whole (Ivanov et al. 2013). Horses use exploratory behavior to learn about hazards, food and water availability, social partners, resting places and convenient routes. Due to the specific anatomical and physiological characteristics of the digestive tract, feeding behavior occupies a significant share in the horse's behavioral repertoire. It is known that free-living horses spend 16-18 hours a day on grazing (Waring 1983). In the studied population, the share of feeding behavior was 35% of the observation time budget.

One of the significant forms of behavior in young animals is play, in the process of which animals study and improve many models of behavior, as well as their optimal physical development. Play behavior plays a leading role in shaping the individual and social behavior of horses. Ethological monitoring of fillies revealed that play behavior accounts for 8% of the time budget.

Inactive forms of behavior, which include sleep, nap and rest, are necessary for the restoration of many functions of the body, after its vigorous activity. The share of inactive forms of behavior in fillies for the entire observation period averaged 2.3%. This low value is due to the fact that horses are prey animals, their sleep is polyphasic, and the duration of one phase usually lasts more than 1 hour. This strategy allows them to effectively control their surroundings for self-preservation.

It should be noted that the ratio of different forms of behavior in individuals with different social status had some differences. Thus, the share of exploratory behavior in the dominant filly was 5-7% lower than in animals with a lower social status in the group. At the same time, this individual, on average, 1.6 times more likely to demonstrate play behavior and much more often in its behavioral repertoire were inactive forms of behavior, such as sleep, nap and rest. Such features in the behavior of a dominant individual are due to the fact that in a community the leader most often becomes the initiator of group games, and also has an advantage for rest (Ruckebusch 1972). Thus, the social status of horses in a group affects the ratio of different forms of behavior in an individual.

The body of animals responds to the impact of stress factors with compensatory-regulatory biochemical and physiological reactions, due to which the adaptation process takes place. Regardless of the nature of the stress-inducing effect, these adaptive mechanisms are stereotyped, and therefore, biochemical indicators can be used to diagnose stress and assess the adaptive capabilities of the organism (Sotnikova 2009). Animals with different social ranks experience different levels of stress, which is reflected in the biochemical parameters of the blood, which is a rather labile system that quickly responds to changes in the body due to the activation of the hypothalamic-pituitary and sympathoadrenal systems.

The blood biochemical composition of the studied animals turned out to be within the reference values, but at the same time, differences were noted in indicators in fillies with different hierarchical positions, which was most pronounced when comparing the data of the dominant individual and the outcast, i.e. animals with the highest and lowest social status.

An important component of blood plasma are proteins that perform various functions, participating in the construction of tissue proteins, transport of biologically active substances, and due to the globulin fraction, they form specific and nonspecific immunity, since its components are the body's defense factors (Sueur and Mery 2017).

In the dominant individual, in comparison with other animals of the group, due to the albumin fraction, a higher level of blood proteins was noted. The lowest content of total protein, albumins and globulins was found in the outcast, the difference in these indicators between this individual and the dominant was 6%, 9% and 2%, respectively, which indicates the suppression of anabolic processes in the animal with the lowest social status, due to inhibition of the protein-synthetic function of the liver under the influence of rank stress (Fig. 2).

Enzymes react very quickly to the action of any stressor, the activity of which reflects the rate of metabolism and energy during the formation of adaptive reactions (Eman et al. 2011). With sufficient reason, one can judge the processes of protein synthesis in the body by the activity of a number of enzymes. The liver is one of the most sensitive organs to the effects of stress, including rank stress (Solin and Lyashev 2016).

The enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), synthesized in it through α-ketoglutaric, oxaloacetic and pyruvic acids, provide a link between protein, carbohydrate and fat metabolism. Analysis of the parameters of the enzymatic activity of the blood of horses showed that the activity of alanine aminotransferase in the leading individual is higher than in the rest of the group and exceeds this indicator of the rogue by 17.3%, which indicates the intensity of protein metabolism in the dominant and is due to the priority access of the high-ranking individual to food resources. In the enzymatic apparatus of the liver, as a result of exposure to emotional stress, functional changes occurred, which caused an increase of 15.5% in the activity of aspartate aminotransferase in the outcast compared to the activity of this enzyme in the leader. Thus, by the activity of aminotransferases, one can judge the general direction of metabolic and energy processes in the body of animals and their adaptive reserves under stress (Fig. 2).

Exposure to stress factors also induces shifts in metabolic biomarkers (Fortunato et al. 2018). The end product of protein breakdown is urea, the concentration of which was the lowest in the blood of the outcast, and amounted to 4.7mol/L, which is 1.4 times lower than that of the dominant. This also indicates a lower intensity of synthetic processes, namely protein synthesis (Fig. 2).

Emotional stress due to the stress arising from rank conflicts between animals leads to a stable increase in the level of cortisol and adrenaline. Catecholamines play a fundamental role in the activation of metabolic pathways (Cuniberti 2012), which leads to an average increase of 14% in the blood glucose concentration of the subdominant, subordinate and outcast compared to the dominant (Fig. 2).

Another marker of the stress state of the body can be the concentration of creatinine in the blood of an animal. Stress contributed to the active production of creatinine in the body, its concentration in the outcast was 7.7% higher than in the leading individual (Fig. 2).

Maintaining the relative constancy of its composition, the blood stabilizes (homeostasis) the internal environment, which is necessary for the normal functioning of cells and tissues. The hematological parameters of the blood of animals reflect the state of the whole organism as a whole, as they are closely related to its various functions. The blood system belongs to the number of systems, the functional activity of which largely determines the adaptive capabilities of the organism under stress conditions (Austin et al. 2013). Psychological stress stimulates the inflammatory response, activation of macrophages, and the generation of reactive oxygen species (Gu et al. 2012).

The study found that the content of blood corpuscles in horses is influenced by their social status in the group. The highest content of erythrocytes and hemoglobin in the blood was found in a horse of the rank of an outcast, which is under the influence of psychological stress, accompanied by emotional stress, as a result of which, under the influence of sympathetic stimulation, the spleen releases the deposited portions of red blood cells into the bloodstream (Fig. 3).

The horse with the lowest social rank also has the highest hematocrit, which is the proportion of red blood cells in the total blood volume of the animal. Its value was higher than that of the dominant, subdominant and outcast by 6.8, 8.3 and 10.8%, respectively (Fig. 3).

The erythrocyte sedimentation rate in a low-ranking individual exceeds in value the level of this indicator in other animals in the group, which also reflects the state of tension in the body, leading to easier aggregation of erythrocytes and the acceleration of this process.

An indicator of the stress state of an animal is also the osmotic stability of erythrocytes, which characterizes the biological properties of cell membranes, which change their permeability and strength under significant mental and physical stress (Ivanov et al. 2013). The osmotic resistance of erythrocytes in the outcast was 11% lower than in the rest of the community. A decrease in the resistance of erythrocytes is accompanied by an increase in hemolysis and, as a result, leads to the stimulation of erythropoiesis, followed by the release of young, more resistant erythrocytes into the blood, as indicated earlier.

The quantitative composition of the peripheral blood corpuscles is maintained at a certain level, reflecting the physiological state of the organism, the degree of its reactivity and resistance to the action of external factors. The activation of the adrenal cortex, as the center of stress reactions, is accompanied by numerous changes in the composition of the blood. These changes (leukocytes, eosinophils, lymphocytes, blood sugar, blood viscosity, etc.) are indicators of the assessment of the stress state of animals, which make it possible to identify the negative impact of various stress factors on their body, to determine their intensity and duration of exposure.

An important role for assessing the state of the body, especially in chronic stress, is played by the analysis of the leukocyte blood count. The number of leukocytes in the blood of horses, regardless of their social status, did not go beyond the reference values, however, when examining the leukogram, that is, the percentage of individual forms of leukocytes, the lowest content of lymphocytes in the outcast was noted, while the number of granulocytes in him was sufficient high.

Granulocytes are considered as a component of a nonspecific defense reaction of an animal organism, phagocytic and cytotoxic activity. These white blood cells form a protective response of the body in different directions: they provide an inflammatory response, participate in the process of antigen presentation, provide the secretion of cytokines and the production of immuno-specific antigens. For the prompt diagnosis of stress, leukocyte indices are used, based on the determination of the ratio of white blood cells in the leukocyte formula. The lymphocyte-granulocyte index (IPH), which is the ratio of the level of lymphocytes to the total number of granulocytes (Tkachenko and Derkho 2014), is lower in a horse at the lowest rung of the hierarchical ladder than in individuals with a higher social status. For an outcast, this index has a value of 0.68, which is 22 - 65% lower than for other members of the studied community (Fig. 3).

Conclusion

An inevitable consequence of the social way of life, which serves as a fundamental attribute of the biology of many species, including horses, is the conflicts that occur within the group of animals (Blumstein et al. 2010). As a result of research, it was found that competitive relationships in a group of horses affect many life manifestations of animals with different social status, and especially in low-ranking animals. The level of social stress animals are exposed to is reflected at ethological, physiological and biochemical levels, causing shifts in metabolic homeostatic mechanisms. Differences in the enzymatic and metabolic activity of liver hepatocytes were revealed in high-ranking (dominant) and low-ranking (outcast) filly, due to stress caused by the social activity of animals. The ratio of the number of granulocytes and agranulocytes in an individual occupying the lowest rank indicates a decrease in the protective-adaptive reactions of the body of an animal in a state of chronic emotional stress when exposed to rank stress. Chronic social stress leads to a cascade of physiological, biochemical and behavioral changes in young horses that can threaten their wellbeing.

Author's Contribution

Ksenofontova A.A. the collection and analysis of ethological indicators, as well as the writing and submission of the manuscript. Voinova O.A. developed a research plan and experimental methodology, and also wrote a manuscript. Ivanov A.A. participated in the analysis of the results. Ksenofontov D.A. conducted laboratory tests and analyzed hematological parameters. Sakovtseva T.V. identified laboratory tests and analyzed biochemical parameters. All authors approved the final version of the manuscript.

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