The possibility of using amino acid supplements for stress prevention

E V Morgul\textsuperscript{1,3}, S N Belik\textsuperscript{1}, A R Morgul\textsuperscript{1}, I F Gorlov\textsuperscript{2}, M I Slozhenkina\textsuperscript{3} and A A Mosolov\textsuperscript{2}

\textsuperscript{1}Rostov state medical University, 29 Nakhichevansky lane, Rostov-on-don, 344022, Russia
\textsuperscript{2}Volga Region Research Institute of Manufacture and Processing of Meat-and-Milk Production, 400131, 6, Rokossovskogo street, Volgograd, Russian Federation
\textsuperscript{3}E-mail: ozoedu@mail.ru

Abstract. Every day, the modern human body is exposed to stress of various etiologies. One of the main effects of long-term stress is to accelerate the rate of premature aging. These processes affect the decrease in working capacity, reproductive capabilities, affect memory, emotions, behavior, vegetative reactions of a person, etc. The influence of unfavorable factors of professional activity (daily duty, psychoemotional overload, physical activity, violation of the diet) leads to early professional burnout. Therefore, it is important to search for and study the mechanisms of action of substances whose use for preventive purposes leads to a stable state of the body to stress. This article presents information on the study of the stress-detecting activity of a Proline-containing compound synthesized at the research Institute of pharmacology of the Russian Academy of medical Sciences. The experiment used male rats with a high level of anxiety of two ages - 3-month-old sexually Mature and 1.5-year-old. The animals were selected based on physiological tests: "Porsolt swimming test", "open field" test. Stress was simulated by forced 30-minute swimming at a water temperature of 28-30°C. Stress-detecting mechanisms of action of the amino acid compound were evaluated by indicators of the leukocyte formula and the content of biogenic amines. It was found that the development of the strategy of adaptation to the action of a stressful factor (30-minute swimming) depends on age. In young animals, when using an amino acid compound, a hormonal type of activation of the sympathoadrenal system was noted, which is the most effective. The body of 1.5-year-old rats reacted to external influence by activating the sympathoadrenal system along the mediator pathway and entering the depletion phase. The results of this study can be the basis for the use of amino acid compounds as a functional ingredient with a stress-inducing property in the production of food in the food industry.

1. Relevance of the study
Modern people are constantly in a state of chronic psychoemotional and environmental stress, which leads to a violation of homeostasis and the development of various diseases. Prolonged exposure to stress contributes to the activation of premature aging processes. Stress, as well as aging, is characterized by changes in behavior, decreased memory, performance, adaptive capabilities, and reproductive abilities [1]. Especially vulnerable are representatives of professions whose work is associated with unfavorable factors of professional activity (daily duty, psychoemotional overload, physical exertion,
dietary disorders), for example, medical workers, employees of locomotive crews, operators, firefighters, athletes, teachers, etc. [2]. One of the ways to prevent the state of stress and the development of pathological conditions caused by it is the systematic use of functional foods (AF) in diets that contain functional ingredients, the effectiveness of which is confirmed by published studies. Currently, FP enriched with probiotic and prebiotic components is widely used [3, 4], or containing natural components of animal and plant origin [5, 6]. At the same time, the question of using individual amino acids and their complexes as AF ingredients, which are powerful biological regulators that affect gene expression at various stages, including transcription, post-transcription processes, nuclear export and translation of Mature mRNA, is not considered [7]. in addition, many important metabolites (for example, purines/pyrimidines, neurotransmitters, etc.) are products of cellular metabolism of amino acids [8].

To prevent stress, we recommend using a peptide preparation as a functional ingredient. Regulatory peptides control physiological processes, starting with individual functions of specialized cells and ending with complex behavioral acts [9], and also affect the processes of accelerated aging [10]. These compounds include the Proline – containing peptide-ethyl ether N – phenylacetyl-L-prolylglycine synthesized in the research Institute of pharmacology of the Russian Academy of medical Sciences. This is a complex of amino acids: glycine, Proline and ether-phenylacetyl group, which work synergistically (1+1=3).

Glycine can be synthesized from several precursors, including serine, threonine, and choline [11]. Glycine is metabolically bound to the body's chemical components to a greater extent than any other amino acid. It is the main structural component of collagen-glycine residues unite the triple helix of collagen. Provides flexibility of active centers in enzymes. Glycine is one of the inhibitory neurotransmitters of the nervous system. As a rule, it balances the excitation and inhibition systems in the Central nervous system, stimulates mental activity, and helps to relieve the reaction to stressors [12].

Glycine regulates immune function, superoxide production, and cytokine synthesis, changing the intracellular level of Ca2+ [13]. Conjugation of bile acids in humans and pigs occurs with the direct participation of glycine, and therefore, glycine indirectly plays an important role in the absorption and digestion of fat-soluble vitamins and lipids. Glycine is used for the synthesis of creatine in the kidneys, takes part in the synthesis of proteins, purine nucleotides, heme, paired bile acids, keratin, and glutathione. Together, glycine plays a crucial role in the metabolism, growth, development, cytoprotection, immune response, and survival of humans and many other mammals [11]. The pronounced functional effects of glycine are due to its antihypoxic effect on models of histotoxic, hypercapnic and hypobaric hypoxia and the ability to regulate glutathione synthesis, and the introduction of a phosphorylated residue into the glycine molecule leads to an increase in the antihypoxic activity of this amino acid [14]. Glycine prevents stress-induced accumulation of lipid peroxidation products, reduces the concentration of Schiff bases of diene conjugates, activates endogenous antioxidant systems (superoxide dismutase, catalase, and ceruloplasmin), and has antiinflammatory effects on models of acute non-immune and chronic immune inflammation [15]. It should be noted that the maximum daily dose for the average adult should not exceed 15 g [16].

Numerous beneficial effects of glycine in combination with its insufficient de novo synthesis support the idea that glycine is a conditionally essential, as well as a functional amino acid for humans [11, 13].

Recently, there has been growing interest in research on Proline as a functional ingredient in food products, as well as its relationship with metabolic diseases, since unlike other amino acids, Proline has its own α-amino group in the pyrrolidine ring, and therefore it is the only proteinogenic secondary amino acid and has its own metabolic pathways [17, 18]. It has been established that Proline is a key regulator of numerous biochemical and physiological processes in cells. For example, Proline is a signal molecule, a sensor of cellular energy status, and a source of pyrroline-5-carboxylate (P5C) and a superoxide anion (free radical) involved in redox reactions in humans and animals [19]. In addition, Proline plays an important role in cell differentiation (embryonic stem cells), as well as in the growth and development of the fetus. It serves as the main substrate associated with the synthesis of polyamines (key regulators of DNA and protein synthesis, as well as cell proliferation and differentiation) in the small intestine [20,
Proline provides the synthesis of collagen, which is the main protein of connective tissue, and therefore is responsible for the normal functioning of the heart muscle, musculoskeletal system, skin elasticity and elasticity, provides rapid regeneration of ligaments, tendons and skin after damage, strengthens the arteries, eliminates blood clots, normalizes blood pressure, and prevents skin aging.

Thus, the development and production of PP containing regulatory peptides for the purpose of stress prevention may become a new priority for the food industry.

2. Methods and materials

The study was conducted on 80 mongrel white male rats. Prior to the experiment, the animals were kept in a vivarium at a temperature of 20-25°C with free access to water and food. While performing their work, they observed the ethical principles of animal experiments and the main provisions of the Helsinki Declaration. The experiment was performed in accordance with the European Convention for the protection of vertebrates used for experiments or other scientific purposes (Strasbourg, March 18, 1986), and the order of the Ministry of health of the Russian Federation No. 267 of 19.06.2003. The work was performed on the minimum allowed number of laboratory animals in accordance with the method of statistical processing of the results obtained.

The criteria for selecting animals were the level of anxiety and age. We selected rats with a high level of anxiety, as they are the most sensitive to external factors. To identify the level of anxiety, physiological tests were used: "Porsolt swimming test" [22], "open field" test [23]. For the "Porsolt swimming test", a container with a diameter of 50 cm and a depth of 40 cm filled with more than 70% water was used. Animals with a high level of anxiety are characterized by chaotic movements in the water, which lead to rapid fatigue, or passive swimming with a manifestation of numbness [22]. For the "open field" test, we used a rectangular chamber (40 x 50 cm) with plastic walls 50 cm high, the floor of which is divided into 10x10 cm squares. A 50 W lamp located at a height of 150 cm above the center of the cell was used as a stress factor. The rat was placed in a cell and its behavior was observed for 9 minutes and 5 days. Highly disturbed animals show a high level of defecation, low vertical and horizontal (or extremely high) activity, prolonged fading and prolonged grooming [23].

Groups were formed from highly anxious rats based on their age. The first group consisted of 3-month-old Mature males weighing 100-130 g; the second group consisted of 1.5-year-old rats weighing 350-480 g. Intact rats of both ages were compared with control groups. The experiment used an amino acid compound-ethyl ether N-phenylacetyl-L-prolylglycine, synthesized in the research Institute of pharmacology of the Russian Academy of medical Sciences. Moscow. The amino acid compound was given to rats per os with the usual Vivar feed ration, once at a dose of 5 mg/kg. The compound was used 1 and 24 hours before decapitation.

As a stress model, we used physical activity in the form of a forced 30-minute swim at a water temperature of 28-30°C, which is a strong stress factor for the selected experimental animals (rats) [22].

The stress-protective effect of the compound was determined by indicators of the leukocyte formula [24] and the content of biogenic amines [25]. Animals were decapitated, blood was collected with an anticoagulant, and the brain was extracted in the cold. To determine the leukocyte formula, blood smears were fixed with a solution of eosinmethylene blue according to May–Grunwald, and stained according to Romanovsky-Giemsa [26]. The number of shaped elements in the blood was calculated using a light microscope LABS CB2000CF 44231 (Celestron, China) and expressed as a percentage of the total number of white blood cells. The concentration of biogenic amines (norepinephrine, epinephrine, dopamine, serotonin) was evaluated using the reverse-phase method of high-performance liquid chromatography on a Liquochrom 312/1 chromatograph (Hungary) with A nucleosil C-18 column (SERVA, Germany). When studying the content of histamine in blood samples, it was separated from compounds that give color by the Pauli reaction on columns with CM-cellulose (Sigma, USA). The histamine content was measured by spectrofluorimetric method in reaction with orthophthalic aldehyde.

Statistical data processing was performed in the environment of integrated statistical software packages "Statistica 10". The arithmetic mean of the series M and the standard deviation m were calculated. The average values were compared using the student's t-test. The student's t-test was used.
for independent and dependent samples (p ≤ 0.05).

3. Results of the study
During the first day (1 hour, 24 hours) after application of the amino acid compound with food, a primary activation reaction was observed in 3-month-old rats (the first stage of the activation reaction). There was an increased content of lymphocytes to the upper half of the normal zone by 19.9% and a reduced number of segmented neutrophils (61.2%) to the lower limit of the norm (table 1). In 1.5-year-old rats, an orientation reaction was observed 1 hour after the use of the peptide. After 24 hours, the reaction of increased activation was recorded (table 2).

**Table 1. Effect of an amino acid compound on the leukocyte formula of 3-month-old rats (in % of the total number of white blood cells), M=m, n=10, p-significance of differences compared to the control.**

| Conditions          | Neutrophils   | Eosinophils | Monocytes | Lymphocytes |
|---------------------|---------------|-------------|-----------|-------------|
| Control             | 3.3±0.09      | 21.9±0.99   | 1.0±0.03  | 2.9±0.11    | 70.9±2.07  |
| Amino acid          | 2.7±0.73      | 9.2±0.15    | 1.1±0.31  | 3.4±1.25    | 85.8±1.15  |
| compound            | -18.0%        | -58.01%     | +11.3%    | +17.0%      | +21.2%     |
| 1 hour              | p>0.1         | p<0.05      | p>0.1     | p<0.05      |
| Amino acid          | 2.4±1.01      | 8.6±0.30    | 1.2±0.08  | 3.7±1.00    | 99.0±0.30  |
| compound            | -27.4%        | -61.2%      | +20.1%    | +28.0%      | +19.9%     |
| 24 hours            | p>0.1         | p<0.05      | p<0.05    | p>0.1       |
| Forced swimming     | 3.9±0.98      | 45.8±1.04   | 0.1±0.02  | 5.6±0.05    | 44.6±0.91  |
| (30 minutes)        | +18.2%        | +109.4%     | -90.0%    | +93.5%      | -37.0%     |
| Amino acid          | p>0.1         | p<0.05      | p<0.05    | p>0.05      |
| compound+            | 3.6±0.71      | 16.4±0.61   | 0.8±0.22  | 2.1±0.44    | 76.4±3.81  |
| forced swimming     | +9.5%         | -25.0%      | -20.1%    | -28.0%      | +8.3%      |
|                     | p>0.1         | 0.05<p<0.1  | p<0.1     | 0.05<p<0.1  |

**Table 2. Effect of an amino acid compound on the leukocyte formula of 1.5-year-old rats (in % of the total number of shaped elements of white blood), M=m, n=10, p-significance of differences compared to the control.**

| Conditions          | Neutrophils   | Eosinophils | Monocytes | Lymphocytes |
|---------------------|---------------|-------------|-----------|-------------|
| Control             | 5.9±0.30      | 13.3±0.37   | 5.8±0.34  | 4.5±0.32    | 70.5±2.11  |
| Amino acid          | 6.8±0.43      | 26.3±1.07   | 6.9±0.20  | 5.6±0.25    | 53.6±1.17  |
| compound            | +15.0%        | +98.2%      | +19.1%    | +25.3%      | -24.0%     |
| 1 hour              | p>0.1         | p<0.05      | 0.05<p<0.1| p<0.05      |
| Amino acid          | 4.8±1.10      | 7.5±0.90    | 4.2±1.41  | 3.4±1.32    | 81.2±0.33  |
| compound            | -19.2%        | -44.3%      | -28.0%    | -24.1%      | +15.2%     |
| 24 hours            | p>0.1         | p<0.05      | p>0.1     | p<0.05      |
| Forced swimming     | 7.3±0.32      | 16.8±0.83   | 6.7±0.14  | 5.5±0.31    | 63.7±3.59  |
| (30 minutes)        | +24.1%        | +26.3%      | +16.4%    | +22.2%      | -10.0%     |
| Amino acid          | p<0.05        | p<0.05      | 0.05<p<0.1| p<0.05      |
| compound+            | 4.8±0.35      | 6.6±0.31    | 4.4±0.27  | 3.5±0.18    | 80.7±5.86  |
| forced swimming     | 0.05<p<0.1    | p<0.05      | 0.05<p<0.1| p>0.1       |

After exposure to a stress factor (30-minute swim), the body of intact rats, regardless of age, developed a stress reaction. Immediately after swimming, 3-month-old males had a stage of acute stress
anxiety (table 1). In 1.5-year-old animals, after stress, an increase in the number of neutrophils by 26.5%, monocytes by 22.6%, and eosinophilia were recorded, which indicates depletion of the glucocorticoid function of the adrenal cortex and transition to chronic stress (table 2). When swimming, 1.5-year-old animals of the control group drowned many times. Bleeding from the eyes and nose was observed in 90.0% of the rats, and 10.0% of the animals died immediately after the end of the swim or 10 minutes after it. Prior use of an amino acid compound before a 30-minute swim prevented the development of a stress response in rats of both age groups. The leukocyte formula of 3-month-old rats reflected the development of the primary activation reaction. This was manifested by a decrease in the number of segmented neutrophils by 25.0 %, and the determination of the number of lymphocytes within the upper limit of normal (76.4%) (table 1). 1.5-year-old rats developed an increased activation reaction bordering on a stress reaction (table 2).

The monoaminergic mediator system is responsible for the development of adaptive processes and the formation of body responses to external factors, including stress [25]. The use of the peptide led to significant changes in the concentration of monoamines in the cerebral cortex and blood plasma. We found that the direction and severity of changes depend on the age of the animals. According to the concentration of monoamines 1 and 24 hours after the application of the amino acid compound, the development of the primary activation stage was noted in 3-month-old rats. In the cerebral cortex, after 1 hour, the serotonin content decreased by 27.8% (p<0.05) in the absence of changes in the level of other monoamines (table 3). Activation of the serotonergic system induces a complex adaptive response in water deprivation [25]. 24 hours after the application of the amino acid compound in the cerebral cortex, the content of norepinephrine and serotonin decreased by 42.9% and 57.7% (p<0.05), respectively (table 3). This fact indicates the activation of mechanisms for increasing the active resistance of the body, which is confirmed by the results of the leukocyte formula. A decrease in the NA/epinephrine ratio in blood plasma was recorded in blood plasma 1 and 24 hours after the drug administration, which indicates the predominance of the hormonal link in the regulation of the sympatho-adrenal system (table 3). The data obtained reflect an increase in the active resistance of the body by the type of activation reaction [24]. The use of the peptide in 1.5-year-old rats significantly changed the content of biogenic amines in the brain. After 1 hour, a significant increase in the level of serotonin and histamine was observed in the cerebral cortex of rats, and the ratio of NA/Ser decreased when compared with the results of the control group (table 4). Activation of the serotonergic system reflects the development of an adaptive training response. 24 hours after the use of the compound in 1.5-year-old rats, high levels of serotonin (70.5%, p<0.05), histamine (62.9%, p<0.05), and dopamine (48.2%, p<0.05) were recorded in the cerebral cortex (table 4). In the blood plasma of 1.5-year-old rats, 1 hour after the application of the amino acid compound, activation of the "stress-detecting" serotonergic system was noted (39.3%, p<0.05) with a decrease in the level of norepinephrine (25.1%, p<0.05) and dopamine (42.8%, p<0.05) without changes in the level of epinephrine and histamine. After 24 hours, an increase in the concentration of epinephrine (36.6%, p<0.05) was recorded in the blood plasma of 1.5-year-old animals with a high level of serotonin (29.1%, p<0.05) (table 4). The observed increase in the A/NA ratio indicates activation of the hormonal regulation of the sympatho-adrenal system, which reflects the development of the activation reaction and corresponds to the results of the leukocyte formula [24].
Table 3. The content of biogenic amines in the cerebral cortex (ng / g of tissue) and blood plasma (mcg / l) of 3-month-old rats (M±m, n=10, p-significance of differences compared to the control level, p<0.05, * - the level of adrenaline in the brain was not determined).

| Stru | Conditions | NA / Ser | Adrenaline | Noradrenaline | Dopamine | Serotonin | Histamine |
|------|------------|----------|------------|---------------|----------|-----------|-----------|
| control | 0.85 | * | 349.92 ± 9.05 | 238.32 ± 7.42 | 411.46 ± 14.13 | 50.99 ± 1.98 |
| Amino acid compound | 1.31 | * | 388.41 ± 10.03 | 209.72 ± 11.16 | 296.25 ± 8.35 | 52.01 ± 5.04 |
| 1 hour | | | +11.0% ± p>0.1 | -12.2% ± p<0.05 | -27.8% ± p<0.05 | +2.4% ± p>0.1 |
| Amino acid compound | 1.17 | * | 199.22 ± 7.02 | 207.34 ± 16.06 | 172.81 ± 9.09 | 56.12 ± 4.25 |
| 24 hours | | | -42.9% ± p<0.05 | -13.0% ± p>0.1 | -57.7% ± p>0.1 | +10.0% ± p<0.05 |
| Forced swimming | 0.48 | * | 167.96 ± 0.58 | 374.16 ± 0.97 | 349.74 ± 1.54 | 63.23 ± 7.09 |
| (30 minutes) | | | -52.0% ± p<0.01 | +57.3% ± p<0.01 | -15.1% ± p<0.01 | +24.2% ± p<0.01 |
| Amino acid compound+forced swimming | 1.81 | * | 171.46 ± 0.09 | 357.96 ± 0.74 | 91.30 ± 0.93 | 69.65 ± 3.75 |
| cortex | | | -50.8% ± p<0.01 | +50.2% ± p<0.01 | -77.8% ± p<0.01 | +36.6% ± p<0.01 |
| control | 1.99 | | 0.493±0.04 | 0.912±0.02 | 0.678±0.03 | 0.457±0.07 | 0.085±0.02 |
| Amino acid compound | 1.19 | | 0.601±0.02 | 0.704±0.03 | 0.470±0.24 | 0.592±0.04 | 0.091±0.04 |
| 1 hour | | | +22.1% ± p<0.05 | -23.3% ± p<0.01 | -31.2% ± p<0.01 | +30.0% ± p<0.05 | +7.4% ± p<0.01 |
| Amino acid compound | 0.99 | | 0.685 ± 0.03 | 0.672 ± 0.01 | 0.487 ± 0.06 | 0.678 ± 0.15 | 0.075 ± 0.02 |
| 24 hours | | | +40.3% ± p<0.01 | -25.9% ± p<0.01 | -28.0% ± p<0.01 | +48.1% ± p<0.01 | -13.9% ± p<0.01 |
| Forced swimming | 2.33 | | 0.700±0.09 | 1.310±0.12 | 0.861±0.21 | 0.562±0.05 | 0.119±0.09 |
| (30 minutes) | | | +43.0% ± p<0.05 | +43.9% ± p<0.05 | +27.4% ± p<0.05 | +23.3% ± p<0.05 | +40.4% ± p<0.05 |
| Amino acid compound+forced swimming | 2.47 | | 0.680±0.21 | 1.570±0.17 | 0.391±0.05 | 0.637±0.04 | 0.101±0.02 |
| blood | | | +39.7% ± p>0.1 | +73.1% ± p<0.05 | +42.7% ± p<0.05 | +40.4% ± p<0.05 | +19.0% ± p>0.1 |
Preliminary application of an amino acid compound before a 30-minute swim to 3-month-old rats in the cerebral cortex affected a decrease in serotonin levels by 77.8% (p<0.05), which is typical for increased motor activity. The concentration of norepinephrine decreased by 50.8% (p<0.05), dopamine increased by 50.2%, and histamine by 56.6%. In blood plasma, an increase in the level of epinephrine by 39.7%, norepinephrine – by 73.1% (p<0.05) was recorded, which is manifested in a high level of behavioral activity of the Central nervous system. The serotonergic system was activated by increasing the level of serotonin by 40.4% (p<0.05) and reducing the level of dopamine by 42.7% (p<0.05) (table 3). In 1.5-year-old rats, the level of norepinephrine increased by 51.7% (p<0.05) in the cerebral cortex under stress (30-minute swimming), and serotonin almost corresponded to the control data. The

### Table 4. The content of biogenic amines in the cerebral cortex (ng / g of tissue) and blood plasma (mcg/l) of 1.5 - year - old rats (M=m, n=10, p-significance of differences compared to the control level, p<0.05, * - the level of adrenaline in the brain was not determined).

| Structure | Conditions | NA / Ser | Adrenaline | Noradrenaline | Dopamine | Serotonin | Histamine |
|-----------|------------|----------|------------|---------------|----------|-----------|-----------|
| Cerebro | Amino acid 0.05 | * | 76.01 ± 10.03 | 230.31 ± 2.19 | 1560.09 ± 9.51 | 210.88 ± 17.34 |
| cortex | compound 1 hour | | 69.93 ± 4.31 | 204.98 ± 11.55 | 2137.32 ± 12.95 | 278.36 ± 5.49 |
| | Amino acid 0.03 | * | 85.97 ± -8.0% | 343.16 ± -11.3% | 2667.75 ± +36.9% | 343.52 ± +31.8% |
| compound 24 hours | p>0.1 | | p>0.1 | p<0.05 | p<0.05 |
| Forcing swimming (30 minutes) | 0.06 | * | 104.13 ± +36.8% | 92.12 ± 5.92 | 1622.49 ± +4.1% | 601.01 ± +185.0% |
| Amino acid 0.07 | * | 115.54 ± 0.35 | 115.16 ± 0.15 | 1716.09 ± 5.76 | 415.43 ± 8.01 |
| compound + forced swimming | p<0.05 | | p<0.05 | p<0.05 | p<0.05 |
| Blood | Control 1.93 | | 0.350 ± 0.02 | 0.905 ± 0.17 | 0.529 ± 0.08 | 0.468 ± 0.07 | 0.074 ± 0.01 |
| | Amino acid 1.06 | 0.420 ± 0.09 | 0.679 ± 0.04 | 0.303 ± 0.03 | 0.652 ± 0.03 | 0.085 ± 0.09 |
| compound 1 hour | +19.9% | p>0.1 | -25.1% | p<0.05 | -42.8% | p<0.05 | p>0.1 |
| | Amino acid 1.03 | 0.476 ± 0.03 | 0.624 ± 0.05 | 0.354 ± 0.06 | 0.604 ± 0.05 | 0.089 ± 0.18 |
| compound 24 hours | +36.6% | p<0.05 | -31.2% | p<0.05 | -33.0% | p<0.05 | p>0.1 |
| Forcing swimming (30 minutes) | 1.99 | 0.760±0.11 | 1.149±0.03 | 0.725±0.18 | 0.576±0.05 | 0.097±0.14 |
| Amino acid 2.37 | +117.1% | p<0.05 | +27.3% | p<0.05 | +37.0% | p<0.05 | p>0.1 |
| compound + forced swimming | 0.07 | +52.0% | 1.376±0.04 | +1.9% | 0.539±0.18 | +22.7% | 0.075±0.07 |
| Blood | | | | | | | |
concentration of histamine increased by 96.8% (p<0.05), and dopamine decreased by 50.6%. In blood plasma, an increase in the content of epinephrine by 54.7%, norepinephrine - by 52.0%, serotonin - by 22.7% (p<0.05) was observed (table 4).

4. Conclusion
When analyzing the ratio of components of the blood leukocyte formula and the balance of monoamines using an amino acid compound, the development of different strategies for adaptation to physical activity in 3-month-old and 1.5-year-old rats was revealed. The hormonal type of activation of the sympathoadrenal system in 3-month-old animals indicates the formation of a more effective adaptive response compared to 1.5-year-old rats. In 1.5-year-old rats, the sympathoadrenal system is activated along the mediator pathway, which is more typical for the transition of the stress reaction to the exhaustion phase.

The obtained data can become the basis for the use of amino acid compounds as functional ingredients with stress-inducing properties in the production of AF in the food industry.

References
[1] Fonken L K, Frank M G, Gaudet A D and Maier S F 2018 Stress and aging act through common mechanisms to elicit neuroinflammatory priming Brain Behav Immun 73 133-48
[2] Nazimko V A, Morgul E V, Petrova O A, Sheikhova R G, Kozina L S, Savenko M A and Lysenko d S 2012 Analysis of some indicators of biological age and adaptive capabilities of employees of locomotive crews Advances in gerontology 25(1) 57-62
[3] Kontareva V Yu, Belik S N, Morgul E V, Gorlov I F and Slozhenkina M I 2020 Yogurt enriched to correct intestinal microflora in dysbiosis IOP Conference Series: Earth and Environmental Science 548(8) 82051
[4] Kontareva V Yu, Belik S N, Morgul E V, Gorlov I F and Slozenkina M I 2020 The effect of prebiotic components on the quality of yogurt IOP Conference Series: Earth and Environmental Science 548(8) 82054
[5] Kryuchkova V V, Gorlov I F, Korneichuk K M, Belik S N and Mosolova N I 2020 Brine-ripened cheese enriched with vegetable ingredients: technology and quality IOP Conference Series: Earth and Environmental Science 548(8) 82063
[6] Kryuchkova V V, Gorlov I F, Belik S N and Kamlatsky 2020 As vegetable ingredients in functional fermented milk products IOP Conference Series: Earth and Environmental Science 548(8) 82092
[7] Beltrán Piña B G, González Castro M I and Rivas García F 2019 Influence of amino acids that come from the diet in the expression of genes Nutr Hosp 36(1) 173-82
[8] Rose A J 2019 Amino Acid Nutrition and Metabolism in Health and Disease Nutrients 11(11) 23-6
[9] Misiura M and Miltyk W 2019 Proline-containing peptides-New insight and implications: A Review Biofactors 45(6) 857-66
[10] Kozina L S, Lysenko A V, Finochenko T A, Morgul E V and Sheikhova R G 2007 The effect of regulatory peptides on aging intensity in rats with different anxiety level Advances in gerontology 20(3) 53
[11] Wang W, Wu Z, Dai Z, Yang Y, Wang J and Wu G 2013 Glycine metabolism in animals and humans: implications for nutrition and health. Amino Acids. 45(3) 463-77
[12] Oganesyan G A, Aristakesyan E A, Romanova I V, Vataev S I, Kozik V and Kambarova D K 2013 Questions of the evolution of the Wake-sleep cycle. Part 2: neurotransmitter mechanisms of regulation Biosphere 1 97-123
[13] Razak M A, Begum P S, Viswanath B and Rajagopal S 2017 Multifarious Beneficial Effect of Nonessential Amino Acid, Glycine: A Review Oxid Med Cell Longev 1716701
[14] Coleman D N, Lopreiato V, Alharthi A and Loor J J 2020 Amino acids and the regulation of oxidative stress and immune function in dairy cattle J Anim Sci 98(1) 175-93
[15] Chen Y H, Dong D C, Thompson H G, Shertzer D W and Vasiliou V 2013 Glutathione defense mechanism in liver injury: insights from animal models Food Chem Toxicol 60 38-44
[16] Diaz-Flores M, Cruz M, Duran-Reyes G, Munquía-Miranda C, Loza-Rodríguez H, Pulido-Casas E, Torres-Ramirez N, Gaja-Rodriguez O, Kumate J, Baiza-Gutman LA et al. 2013 Oral supplementation with glycine reduces oxidative stress in patients with metabolic syndrome, improving their systolic blood pressure Can J Physiol Pharmacol 91 855-60
[17] Wu G, Bazer FW, Burghardt R C, Johnson G A, Kim S W, Knabe D A, Li P, Li X, McKnight J R, Satterfield M C and Spencer T E 2011 Proline and hydroxyproline metabolism: implications for animal and human nutrition Amino Acids 40(4) 1053-63
[18] Carregaro F, Stefanini AC, Henrique T and Tajara EH 2013 Study of small proline-rich proteins (SPRRs) in health and disease: a review of the literature Arch Dermatol Res 305(10) 857-66
[19] Phang J M, Liu W, Hancock C and Harman M 2014 Proline metabolic signaling and parametabolic regulation. In: Nutrition and Epigenetics edited by Ho E and Domann F Boca Raton FL: CRC Press 299-322
[20] Holmgren A, Bouhy D and Timmerman V 2012 Neurofilament phosphorylation and their proline-directed kinases in health and disease J Peripher Nerv Syst 17(4) 365-76
[21] Ding J, Kuo M L, Su L, Xue L, Luh F, Zhang H, Wang J, Lin TG, Zhang K, Chu P, Zheng S, Liu X and Yen Y 2017 Human mitochondrial pyrroline-5-carboxylate reductase 1 promotes invasiveness and impacts survival in breast cancers Carcinogenesis 38 519-31
[22] Yankelevitch-Yahav R, Franko M, Huly A and Doron R 2015 The forced swim test as a model of depressive-like behavior J Vis Exp 2(97) 52-8
[23] Chen Z, Wei H, Pertovaara A, Wang J and Carlson S 2018 Anxiety- and activity-related effects of paracetamol on healthy and neuropathic rats Pharmacol Res Perspect 6(1) 00367
[24] Garkavi L X, Kvakina E B and Ukolova M A 1990 Adaptive responses and body resistance 224
[25] Bevilaqua F, Mocelin R, Grimm C Jr, da Silva Junior N S, Buzetto T L, Conterato G M, Roman W A Jr and Piao A L 2016 Involvement of the catecholaminergic system on the antidepressant-like effects of Alpinia zerumbet in mice Pharm Biol 54(1) 151-6
[26] Menshikov V, Delektorskaya L and Zolotnitskaya R 1987 Laboratory research methods in the clinic 368