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Effect of Nanobiocomposite Obtained by Means of Mechanochemical Synthesis from Non-Fruit Parts of Buckthorn on Seasonal Adaptive Rearrangements in Dwarf Hamsters

D. V. PETROVSKY[1], V. V. MAK[1], A. V. ROMASHCHENKO[1], G. V. KONTSEVAYA[1], I. E. KOLOSOVA[1], O. I. LOMOVSKY[2], P. ODONMAZHIG[4], ZH. AMGALAN[4] and M. P. MOSHKIN[1,3]

[1]*Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Pr. Akademika Lavrentyeva 10, Novosibirsk 630090 (Russia)*

E-mail: dm_petr@bionet.nsc.ru

[2]*Institute of Solid State Chemistry and Mechanochemistry, Siberian Branch of the Russian Academy of Sciences, Ul. Kutateladze 18, Novosibirsk 630128 (Russia)*

E-mail: lomov@solid.nsc.ru

[3]*Tomsk State University, Ul. Lenina 36, Tomsk 634050 (Russia)*

E-mail: mmp@bionet.nsc.ru

[4]*Institute of Chemistry and Chemical Technology, Academy of Sciences of Mongolia, Prospekt Mira 54-26, Ulaanbaatar (Mongolia)*

E-mail: j_amgalan@yahoo.com

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Abstract

The effect of nanocomposite obtained by means of solid-phase mechanochemical synthesis from non-fruit parts of buckthorn (buckthorn-based serotonin powder, BBSP) on the immunophysiological status of dwarf hamsters during autumn decrease of photoperiod was studied. It was shown that the addition of BBSP to food in curds has no effect on the amount of curds ate by the animals, does not eliminate the basic morphological changes developed in the animals under the conditions of decreasing daylight hours, does not affect the degree of ecdysis. At the same time, the addition of BBSP promotes reliable decrease in body mass, and increase in the spontaneous activity of the animals during the first four weeks of the experiment, conservation of the summer type of thermoregulation (high energy consumption for the compensation of the action of cold, and prevalence of chemical thermogenesis) and an increase in the humoral immune response to the introduction of sheep erythrocytes.

Key words: serotonin-containing powder, 5-hydroxytryptamine, buckthorn, dwarf hamsters, seasonal changes, humoral immunity, energy exchange, spontaneous locomotor activity

INTRODUCTION

For Russia, with its gross domestic product strongly dependent on the development of northern deposits, maintenance of health and working capacity of the inhabitants of northern and arctic regions is not only socially but also economically significant problem. Among the factors affecting human psychophysical welfare, the winter shortening of daylight hours occupies a significant place; artificial illumination cannot compensate for its physiological effects [1–3]. This is due to the extremely high threshold of the sensitivity of neurohormonal systems regulating diurnal rhythms of physiological functions to light action. The key role in the photoperiodic synchronization of the biorhythms of humans and other mammals is played by the nighttime secretion of melatonin. However, only 10–400 lux is sufficient to suppress this secretion for the majority of animal species, while the necessary intensity of illumination for humans exceeds 2000 lux and is comparable with illumination on a clear sunny day [5]. At the same time, according to the hygienic standards, illumination of the most important working zones is about 700 lux, and the intensity of domestic illumination does not exceed 300 lux [6].

As a consequence, seasonal disorders of the temporal coordination of physiological processes are observed in the inhabitants of Extreme North or Antarctica. These disorders are seasonal desynchronoses combined with changes in metabolism and a number of psychophysiological processes [3, 7]. Among the diversity of morphofunctional deviations coinciding in time with the onset of polar night, the role of photoperiod was unambiguously proved for seasonal affective disorders (SAD), which is a special form of depression characterized by decreased mental and physical working capacity, low spirits, drowse and increased appetite [8, 9]. According to the statistical data of American researchers [10], the frequency of SAD increases in proportion to the geographic latitude. SAD is based on the distortion of the balance of melatonin and its precursor serotonin due to the lack of illumination [22, 23]. The application of serotonin-containing preparations in combination with light-therapy removes the depression in many patients [13, 14] but these preparations are applied already at the stage of the clinical manifestation of SAD.

It is possible that more significant results might be achieved by affecting the processes of melatonin and serotonin exchange before the formation of depression symptoms. However, wide application of prophylactic means based on serotonin is limited by their high cost value when they are manufactured through traditional organic synthesis. At the same time, serotonin is present in substantial amounts in many plants, in particular in sea-buckthorn *Hippophae rhamnoides*; its content in non-fruit parts of the plant is much higher than in berries [15]. The shoots of *Hippophae rhamnoides* were used at the Institute of Solid State Chemistry and Mechanochemistry, SB RAS (Novosibirsk) to prepare nanobiocomposite by means of solid-phase mechanochemical synthesis. This composite is serotonin-containing powder based on sea-buckthorn (SCPS); it also contains water-soluble salts of 5-hydroxytriptamine (serotonin) and its derivatives [16].

It is impossible to carry out direct experimental test of SAD prophylactics using serotonin-containing preparations because of the absence of adequate genotypes of laboratory animals in which depression is formed under the influence of shortening photoperiod. The promising character of SCPS can be deduced from the results of the studies of seasonal changes at the background of this preparation in animal species with clearly pronounced photoperiodic regulation of annual biorhythms. Dwarf hamster *Phodopur sungorus* living in West Siberia belongs to the species of this kind. This animal is used as a natural resource for biological studies in research institutions of Moscow, Germany and the USA. It is sufficient to provide shortening of daylight hours for the formation of the complex of morphofunctional changes that are observed in hamsters in autumn [17]. Seasonal changes of fir, involution of gonads, changes in thermoregulation, suppression of immunity are recorded during this period [18–21].

The goal of the present study was to estimate the effect of SCPS on the processes of seasonal morphofunctional rearrangement in dwarf hamsters when passing from autumn to winter. The following indices subjected to annual variations were studied in mature males: changes of body mass and the mass of generative organs, colour of hair-coat covering, and diurnal rhythms of locomotor activity, thermoregulation and humoral immunity indices.

EXPERIMENTAL

Studies were carried out since October 18 to December 27, 2011. The age of male dwarf hamsters *Phodopus* was 4–6 months. Before experiment, the animals in the amount of 20 individuals were divided into two groups in the random manner (10 individuals in each group) and placed into individual cages where they were kept during the entire experiment under the standard vivarium conditions with natural photoperiod. The animals obtained food (palletized feed of Chara Co.) and water *ad libitum*. The bedding (wood chips) was changed once in two weeks.

During the first 7 days (week No. 0, preparatory stage) all the animals received fat-free curds in the amount of 3 g per animal, in addition to the basic food, at 16.00–17.00 h, during the transition from the light hours to dark. Later on (weeks Nos. 1–10), one group of animals (reference group, K) continued to receive curds, while the other group (experimental group, SCPS) received curds with SCPS added. The use of curds with the addition of ground fried sunflower seed kernels, as the products preferred by hamsters, allowed us not only to introduce SCPS in doses into the ration but also to control the amount of consumed additive. The additive was prepared in two stages. At first, 57 g of fat-free cruds was mixed with 3 g of seeds, and 30 g of this mixture was used to feed the animals of the reference group. We took 27 g of the remaining mixture and added 3 g of SCPS powder, mixed thoroughly and used to feed the animals of the experimental group. Serotonin-containing powder based on buckthorn contains 5-hydroxytriptamine about 1 % [15]. In our study, the diurnal portion of SCPS was 0.3 g per one hamster, which corresponds to 3 mg of 5-hydroxytriptamine. The consumption of the additive was monitored for all the animals by weighting non-eaten part left after 30 min from the start of feeding.

To monitor fur shed, the photographs of the dorsal part of animal body were taken every two weeks under standard illumination, standard exposure and diaphragm. With the help of built-in functions of the programme for processing digital images ImageJ, the index of the gray colour over the whole body was determined (grades 0 to 255): the larger is the index value, the lighter coloured is fur and hence the higher is shedding degree.

The activity of the animals was registered continuously during the entire experiment using the system of automatic monitoring developed by D. V. Petrovskiy, based on the infrared motion sensors; total activity per each 10 min of observation was determined.

At the 9th week of observation, the functional state of the cardiorespiratory system was examined in all the animals by evaluating the maximal aerobic productivity (MAP) on the basis of oxygen consumption in tests (cooling for 15 min in helium/oxygen (80 : 20) medium (heliox) at a temperature of 8–10 °C [22]. The animals were placed in the apparatus, which automatically recorded the amount of oxygen consumed by animals every minute. Rectal body temperature was measured with the help of special thermometer built in the apparatus. The thermometer was inserted into the rectum at a depth of 17 mm before and after the test. On the basis of test data, total energy loss ΔE in animals during the time of test was calculated using equation

$$\Delta E = \mathrm{OC}k_e + \Delta TCM/1000$$

where OC is total oxygen consumption during the test, mL O_2; k_e is scale coefficient equal to 20.36 J/mL O_2 [23]; ΔT is the change of rectal body temperature during test time; C is specific thermal capacity, accepted to be equal to the specific thermal capacity of water 4200 J/(K · kg); M is body mass, g. Specific total energy losses were determined as the ratio of total energy losses of an animal to its body mass $\Delta E/M$. Heat conductivity TC of the body was calculated using equation

$$TC = \Delta E/(t\Delta T_a M)$$

where t is test time, s; ΔT_a is the difference between rectal body temperature after the test and the temperature in test chamber, which was maintained constant during the test, °C.

The value of humoral immune response to a standard antigen dose was evaluated 24 h after the measurement of aerobic productivity. For this purpose, sheep erythrocytes (SE) were introduced intraperitoneally: 0.5 mL of 2 % SE suspension containing $4 \cdot 10^8$ cells/mL. At the peak of immune reaction (the 5th day after the introduction of SE) the animals were killed by cervical dislocation; the amount of antibody-forming cells (AFC) in spleen was determined by means of local hemolysis in liquid medium [24]. Blood parameters were measured in a Hema-Screen18 automatic hematological analyzer (Hospitex Diagnostics, Italy).

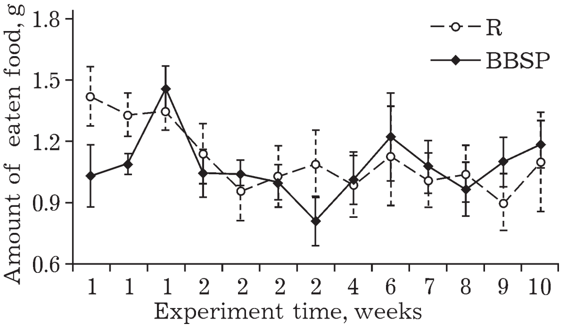

Fig. 1. Consumption of the food additive by the animals of the reference (K) and experimental (BBSP) groups during the experiment (food consumption was monitored during 3 days within the first week, 4 days within the second week, and later once a week).

Two-factor variance analysis was used for statistical data treatment. Average values are presented with the standard error. For normally distributed indices, the effect of controllable factors on the parameters under examination was evaluated with the help of multifactor variance analysis ANCOVA. To compare frequencies, Yates corrected χ^2 criterion was used. Two average values of normally distributed indices were compared relying on Student's t criterion, abnormally distributed values were compared using Mann–Whitney criterion.

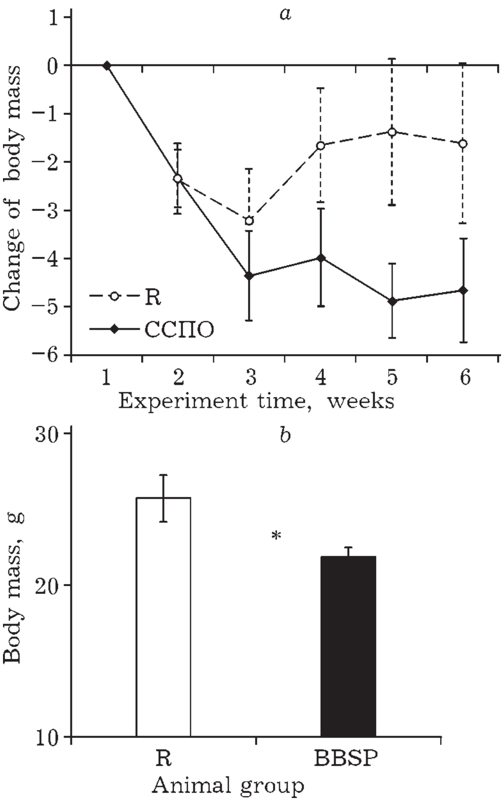

Fig. 2. Dynamics of body mass of the animals of reference (R) and experimental (BBSP) groups: a – mass change, b – body mass at the moment of killing. *$p = 0.04$ – reliable differences between the groups (Mann–Whitney criterion).

RESULTS AND DISCUSSION

The amount of curds eaten by the animals within 30 min after the start of feeding was found to be independent of the addition of BBSP (Fig. 1). According to the data of two-factor analysis, the amount of the food additive eaten is unaffected by the animal group, day of experiment and/or the interaction between these factors: $F_{1,219} = 0.37$; $p = 0.55$; $F_{12,219} = 0.31$; $p = 0.10$; $F_{12,219} = 0.15$; $p = 0.73$, respectively. As an average, the animals of the reference and experimental groups ate curds with the BBSP additive in the amount of (1.11±0.04) and (1.08±0.04) g/day, respectively. This level of BBSP consumption meant 0.11 mg of serotonin per animal daily. Survival of the animals of R group was 100 %, while the survival of the animals of BBSP group was 70 %. Three hamsters died at different moments from the start of experiment (7/10) but the inter-group differences in animal death were uncertain: Yates corrected $\chi^2 = 1.57$, d$f = 1$, $p = 0.21$.

At the background of the changing daylight duration, typical seasonal morphofunctional changes developed in the animals of both groups. In particular, the fur cover was detected to become more light-coloured (the influence of registration time $F_{5,99} = 11.25$; $p = 0.001$), expressed similarly in both groups ($F_{1,99} = 0.09$;

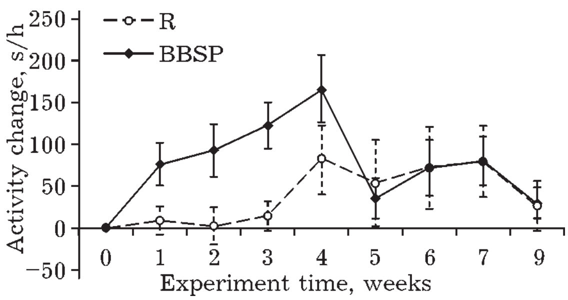

Fig. 3. Dynamics of the changes of spontaneous activity (nocturnal averaged over week) for the animals of the reference (R) and experimental (BBSP) groups.

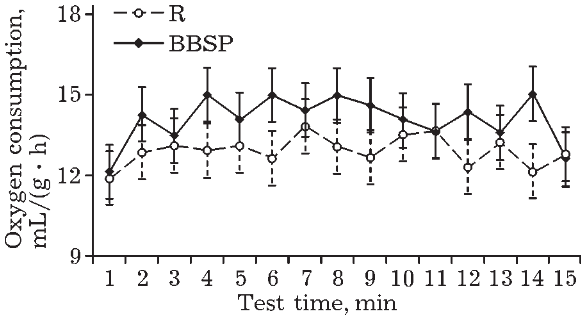

Fig. 4. Dynamics of oxygen consumption in the test of cooling in helium-oxygen environment for 15 min in the animals of reference (R) and experimental (BBSP) groups.

$p = 0.76$). At the same time, we observed differences in body mass changes (Fig. 2, *a*). Thus, according to the data of two-factor variance analysis, the addition of BBSP promotes more rapid decrease in mass, compared to the reference ($F_{1,85} = 8.04$; $p = 0.006$), which is expressed in reliable differences between the groups when weighing was carried out at the end of experiment (see Fig. 2, *b*).

The use of a set-up for automatic recording of locomotor activity allowed us to study spontaneous motion activity of the animals during the whole experiment. In connection with the fact that hamsters are characterized by nocturnalism, the changes of average nocturnal activity per week were monitored. Two-factor variance analysis revealed the effect of BBSP additive on the activity of animals ($F_{1,135} = 5.96$; $p = 0.02$); effects of time and factor interactions were unreliable ($F_{7,135} = 1.54$; $p = 0.16$ и $F_{7,135} = 1.08$; $p = 0.38$, respectively). The animals of the experimental group (BBSP) exhibited increased activity during the first weeks of the experiment, with peak values achieved within the 1-4th weeks from the start of experiment (Fig. 3), while the activity of the hamsters from the reference group remained almost unchanged.

The addition of BBSP into food has a substantial effect on the energy exchange in animals in the cooling test in helium-oxygen medium at a temperature 8–10 °C for 15 min. The variance analysis with repeated measurements (ANOVA repeated measure) showed statistically significant effect of BBSP additive on oxygen consumption during the test ($F_{1,240} = 14.56$; $p = 0.001$, Fig. 4). Along with the total higher energy exchange, the animals receiving BBSP exhibited reliably smaller number of short-time (1–2 min) episodes with a decrease in energy metabolism during the test. This was expressed as the minimal values of oxygen consumption since the 2nd minute till the 14th minute of the test equal to (10.9±0.8) and (8.5±0.7) mL/g of body mass for the animals of the experimental and reference groups, respectively ($p = 0.03$, Mann–Whitney criterion). Calculation of specific energy losses showed that they increase in the animals of the experimental group (Fig. 5, *a*), and this may be connected with an increase in body heat conduction (see Fig. 5, *b*). Body temperature of the hamsters of this group after the test was reliably lower in comparison with the animals of the reference group

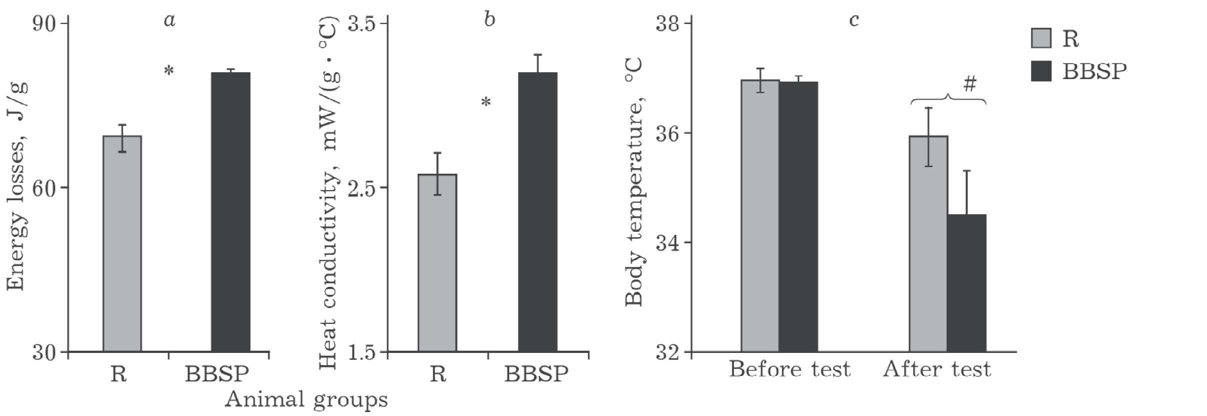

Fig. 5. Energy losses in the animals of the reference (K) and experimental (BBSP) groups in the test of cooling in helium-oxygen environment for 15 min: *a* – total energy losses during the test; *b* – heat conductivity of the body; *c* – body temperature before and after test. *$p = 0.005$; #$p = 0.02$ – reliable differences between the groups (Student's *t* criterion).

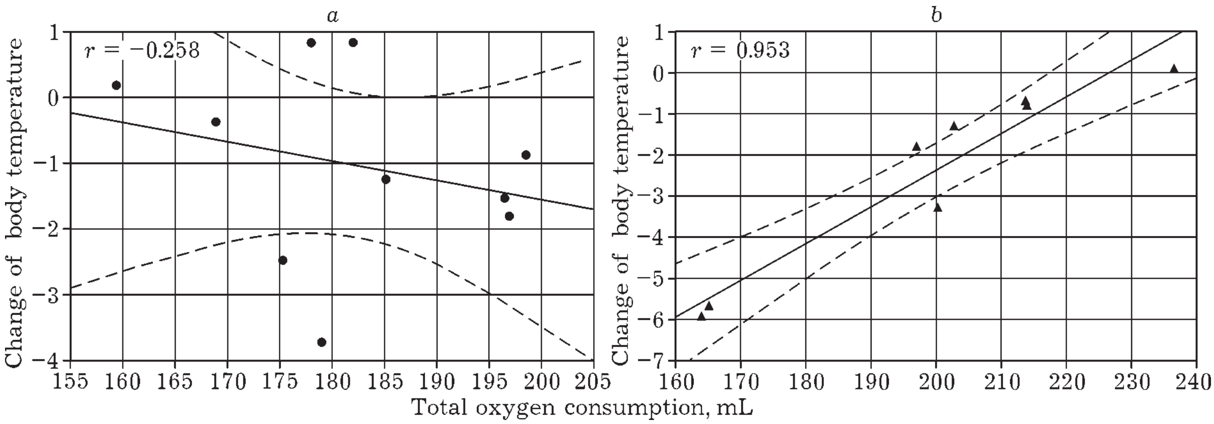

Fig. 6. Correlations between the change of body temperature and oxygen consumption during the test of cooling in helium-oxygen medium for 15 min for the animals of the reference (*a*) and experimental (*b*) groups.

(see Fig. 5, *c*). Correlations the changes of body temperature and oxygen consumption by the animals during the test were also to a high extent dependent on the addition of BBSP into food. No reliable interdependence between these parameters was revealed for the animals of the reference group (Fig. 6, *a*), while the change of body temperature for the animals of the experimental group correlated reliably with oxygen consumption (see Fig. 6, *b*). The highest oxygen consumption during the test was detected for the animals with minimal temperature decrease. The entire set of data provides evidence of different mechanisms of body temperature regulation in the animals of the groups under comparison. Heat formation in the hamsters receiving BBSP additive is enhanced through intensification of exchange processes, that is, "chemical" thermogenesis prevails, which is typical for summer. The animals of the reference group exhibit "physical" thermoregulation through the change of heat loss by the organism.

According to the results of investigations, the red blood values (the amount of erythrocytes, average volume of erythrocyte, hematocrit, hemoglobin level, average hemoglobin content in erythrocyte, average concentration of hemoglobin in erythrocyte) and white blood values (number of leucocytes, lymphocytes, granulocytes, thrombocytes, thrombocrit) did not differ in the animals of the studied groups.

To activate specific immune response, we used intraperitoneal introduction of non-replicating antigens (SE) to the animals of both groups. In both groups the introduction of antigen caused a decrease in the activity of the animals, which is one of the manifestations of painful behaviour (Fig. 7, *b*). At the same time,

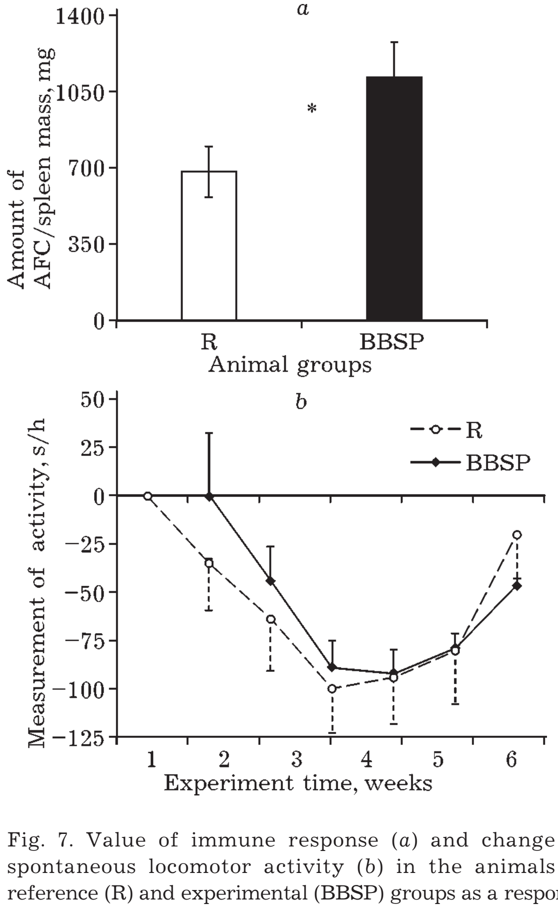

Fig. 7. Value of immune response (*a*) and change of spontaneous locomotor activity (*b*) in the animals of reference (R) and experimental (BBSP) groups as a response to the introduction of SE: $^{*}p = 0.05$ – reliable differences between the groups (Mann–Whitney criterion).

the value of immune response estimated from the number of AFC turned out to be 1.6 times larger for the animals of the experimental group than for the reference group (see Fig. 7, *a*).

CONCLUSION

Thus, the addition of serotonin-containing substance (nanobiocomposite obtained by means of solid-phase mechanochemical synthesis from non-fruit parts of buckthorn) with curds to feed dwarf hamsters does not abandon the major morphological changes developed in the animals with a decrease in daylight hours and does not affect the degree of shed. At the same time, the addition of this mechanochemical preparation into the food promotes a decrease in animal body mass, an increase in the spontaneous activity of the animals during the first four weeks of experiment, conservation of the summer type of thermoregulation (exhibited as high power consumption for the compensation of the action of cold and as predominance of chemical thermogenesis), an increase in the immune response to the introduction of SE. In other words, serotonin obtained with food slows down the development of functional changes that are characteristic of these animals under the conditions of shortened daylight hours. The effect of serotonin-containing substance on the programme of seasonal adaptations provides evidence of the reasonability of testing it as a prophylactic means to treat seasonal affective disorders.

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