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THE EFFECT OF FOOD ADDITIVE 'AQUACAROTIN' ON MAIN PHYSIOLOGICAL BODY PARAMETERS IN MODEL EXPERIMENTS ON ANIMALS

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Summary. The purpose of the work was to carry out experimental studies to determine the effectiveness of the domestic vitamin complex — dietary food additives with biologically active action (DFA BAA) 'Aquacarotin' (LLC 'Bionaftusia', Ukraine), which contains 2% of water-soluble β-carotene (provitamin A) in combination with water soluble vitamin E and vitamin C on physiological body parameters, such as body weight, body weight gain, and peripheral blood parameters. The research was conducted on 60 males of white non-breeding rats that had not reached the age of puberty, and achieved it in the process of research. The rats were grouped into control and main groups of 30 individuals in each group. The main group of animals received DFA BAA 'Aquacarotin' during 3 weeks in addition to main ration, and the control group of animals were kept on a standard rat diet. The influence of DFA BAA 'Aquacarotin' on organism systems was evaluated with body weight, body weight gain, and peripheral blood leukogram indices. The obtained data shows positive influence of DFA BAA 'Aquacarotin' on such physiological parameters as body weight, body weight gain, and hematopoiesis reflected by the peripheral blood indices. Biological activity in DFA BAA 'Aquacarotin' on body weight appears as its significant and accelerated gaining, and body weight increases directly in the process of growth. Active body weight gaining terminated when DFA BAA 'Aquacarotin' was stopped to use. It is valuable that after termination of food additives' introduction to animals' ration, the growth of their body weight slowed down and normalized, thus, it did not continue by itself, which further proves the controllability of the DFA effect, and makes it safe for consumption. DFA BAA 'Aquacarotin' impacts on myelocytic and monocytic leukopoiesis more significantly, which affects increasing of the neutrophil pool, and probably increases the pool of eosinophilic granulocytes and monocytes in peripheral blood stream while increasing of lymphopoiesis. Therefore, DFA BAA 'Aquacarotin' is controllable and therefore safe in regard of its effect on physiological parameters, animals' body weight gaining during growth, as well as peripheral blood parameters. The use of DFA BAA 'Aquacarotin' is beneficial for improving physiological body parameters (rapid increase in body weight), especially in the stage of growth. DFA BAA 'Aquacarotin' activates the myelocytic and monocytic leukopoiesis, which appears in increasing of macro- and microphage pool, as well as enhancement of their functional activity. The obtained data can be interesting and useful in medicine, agriculture, military affairs and nutrition technologies.

Keywords: vitamins A, E and C, dietary food additive 'Aquacarotin', body weight, body weight gain, white blood cells, peripheral blood leukogram

Introduction. The national economy of today is not without food additives all over the world (Raksha-Slusareva, 2014; Shubin, 2010). Dietary food additives (dietary food products) stand out among other dietary additives, which are introduced to food ratio for its enrichment with nutritious or useful (irreplaceable) ingredients (Raksha-Slusareva, 2014; Shubin, 2010). In such areas as agriculture, food medicine, industry, military affairs, and conventional life, dietary food additives with biologically active action (DFA BAA) or functional foods that contain DFA BAA are used. The need to use such DFA BAA like vitamins and vitamin complexes in all periods of organism's existence, especially during its period of growth, is undeniable (Maligina, Raksha-Slusareva and Popova, 2017; Shadrin and Gayduchik, 2016; Anjos et al., 2013).

The most essential vitamins in the period of growth and body formation are fat-soluble vitamins, such as A (β-carotene) and E (tocopherol) and water-soluble vitamin C (Shadrin and Gayduchik, 2016; Anjos et al., 2013; Aslam et al., 2017). Each of the abovementioned vitamins provides the functioning of many organs and systems in the body. Fat-soluble vitamin A, and especially vitamin E, are the most important bioantioxidants of blood lipoproteins and cell membranes and provide the stability of the latter. In this case, the antioxidant action of vitamins is known. Especially the antioxidant effect of vitamin C increases along with the use of tocopherol and retinol, and vitamin A protects vitamin C from oxidation. Vitamin A is a highly effective immune system stimulant, vitamin E is a natural immune regulator, and vitamin C helps to increase the body's resistance to adverse environmental effects. According to recent studies, not only vitamins A and E, but also vitamin C play a significant role, both in non-specific resistance of the organism, and in specific immune response (Alam and

Pawelec, 2012; Mora, Iwata and von Andrian, 2008; Bono et al., 2016; Ströhle and Hahn, 2009; Camarena and Wang, 2016).

Vitamin C refers to vitamins of seasonal, mostly summer, origin, although the development of modern scientific technology makes it possible to get its synthetic analogues into the body at any time of the year. The consumption of synthetic vitamins A and E eliminates almost constant shortage of food intake. Synthetic analogues of fat-soluble vitamins are difficult to metabolize by the body and often cause allergic reactions (Raksha-Slusareva, 2014; Shubin, 2010; Maligina, Raksha-Slusareva and Popova, 2017).

Native scientists of LLC 'Bionaftusia' created a new DFA BAA 'Aquacarotin' on the basis of nanotechnologies. It is a complex vitamin product, which contains 2% of β-carotene (provitamin A) combined with vitamins E and C in form water-soluble form. The micellar form of the product protects the body from side effects and overdose. The obtained water-solubility of vitamins A and E makes them easier to be digested and the body, especially in pathology, accompanied by metabolic disorders. Based on the above, the composition of the developed product should simultaneously enhance the potential of each other and increase positive effect on the body, immune system and cause antioxidant effect, as well as other action inherent to these vitamins. However, since the vitamin complex DFA BAA 'Aquacarotin' is water soluble, it is necessary to study all directions of its impact on the body and immune system components.

The aim of the research was to determine the influence of new DFA BAA 'Aquacarotin' on physiological indicators, such as body weight, body weight gain, and peripheral blood leukogram, which is a complex indicator of the body condition, in model experiments on animals.

Materials and methods. The research was conducted at the Department of Medical Biology, Microbiology, Virology and Immunology of the Donetsk National Medical University of the Ministry of Healthcare of Ukraine.

The research was conducted on 60 males of white non-breeding rats that had not reached puberty, and achieved it in the process of research. The rats were grouped into control and main groups of 30 individuals in each. Male rats with an initial weight of 100–140 g were used in this research. Animals were kept according to special requirements for rats' keeping (Zapadnyuk et al., 1983).

The main group of animals received DFA BAA 'Aquacarotin' counting 0.01 g of the product per day in addition to their diet. In this case, the daily dose of the DFA given to the animals was equal to the maximum daily dose of a human. On average, animals received 0.15 g of the DFA in 3 weeks of the DFA course. Control group of animals was kept on regular ration for rats.

The effect of DFA BAA 'Aquacarotin' on the physiological body systems was estimated with the indices of body weight, relative increase in body weight and peripheral blood leukogram.

Body weight and relative increase in body weight had been assessed before the start of the research in following dynamics: 2, 3 weeks and 3, 6, 13 weeks after the introduction of the food additive to animals' ratio, i.e. on 6, 9 and 16 weeks of the study.

Body weight and body weight gain were determined by weighing of animals on laboratory scales. Animal weight was determined by the formula (1):

$$A_G = W_T - W_O, \quad (1)$$

where A_G is absolute body weight gain of the animals; W_T — animal body weight at the end of reported period; W_O —animal body weight at the beginning of reported period (Stefanov, 2001).

Blood leukogram indices were evaluated with commonly used methods in examined animals of the main and control groups before start of the research, 3 weeks after use and 13 weeks after termination of taking DFA BAA 'Aquacarotin' (Bazarnova and Morozova, 1988).

Results were statistically analyzed using Statistica 6.0 software (StatSoft, USA) and Student's *t*-criterion. Significant differences were found between control and experiment at $p < 0.05$ (Lakin, 1990).

Results and discussion. The assessment of animals' status showed that the main group, which diet was amended with DFA BAA 'Aquacarotin' during 3 weeks, had differences by various physiological indicators, such as body weight and body weight gain, the content of leukocytes and elements of peripheral leukogroup, compared to the control group of animals which did not receive the DFA.

The data on changes in body weight in the control and main groups of animals in the dynamics of research are shown in Fig. 1.

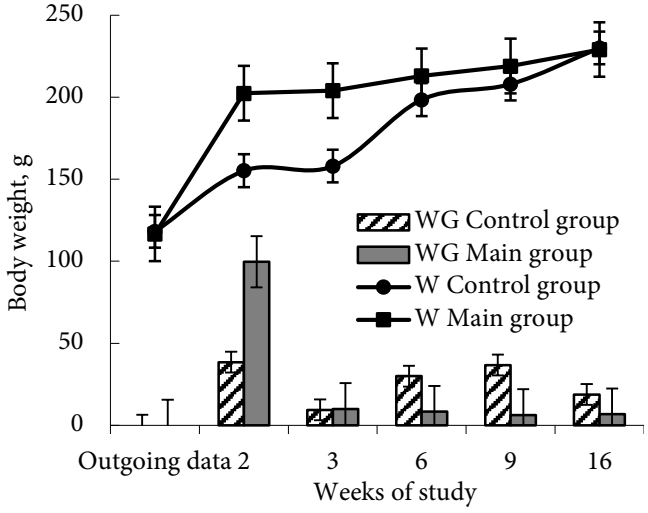

Figure 1. Changes in body weight (W) and body weight gain (WG) of in the main group of animals affected by DFA BAA 'Aquacarotin' and in the control group in the dynamics of research

As it can be seen from the given data, the initial animal body mass in the control and main groups almost did not differ before the experiment was started, and amounted 118.2 ± 2.5 g and 116.7 ± 5.3 g respectively, and the average amount was 117.5 ± 4.1 g. Fluctuations in the initial mass before the experiment in both studied groups were recorded within 100–140 g.

As for 2 weeks of study, the increase in the body weight in the main group was 99.7 ± 19.4 g. It was significantly and credibly higher than in control group 38.5 ± 5.4 g ($p < 0.05$). Fluctuations in body weight in the main group of animals after 2 weeks of the DFA inclusion to the diet was 150–260 g and was greater than that in the control group of animals (150–206 g). Animals' weight in the main group receiving DFA BAA 'Aquacarotin' increased from 116.7 ± 5.3 to 202 ± 19.4 g. Animals' weight in the control group increased from 118.2 ± 2.5 to 155.2 ± 5.7 g and was significantly and credibly less than in the main group ($p < 0.05$).

After 3 weeks, the body weight gain in animals in the main group was 10.0 ± 5.7 g, and probably decreased, compared to the data for 2 weeks of research ($p < 0.05$) and almost did not differ from that in the control group of animals (9.4 ± 3.4 g), which also was possibly reduced compared to those registered for 2 weeks. Fluctuations of body weight in the main and control groups were amounted 170–220 g and 150–205 g respectively. The weight of animals in the main group receiving DFA BAA 'Aquacarotin' for 3 weeks had no significant difference from indices of the previous research reference point amounted to 204.0 ± 9.6 g. Thereby, the animals' weight in the control group had only a slight increase trend and amounted to 158.0 ± 15.38 g, compared to the results obtained for 2 weeks of research. At the same time, animals' body weight in the main group during the third week of study remained significantly and credibly higher than in the control group ($p < 0.05$).

Since the possibility to control the effect is important for dietary food additives, and long influence of food additives on the body without control may be a negative factor that can, in particular, lead to obesity, we have continued the research on weight changes in animals after terminating the consumption of DFA BAA 'Aquacarotin' for next 13 weeks.

As can be seen from the data in Fig. 1, after 6 weeks of study (1.5 months) and 3 weeks after stopping adding DFA BAA 'Aquacarotin' to the diet, the animals' body weight gain considerably slowed down in the main group. The body weight gain in the main group was only 8.3 ± 4.8 g. Animals' body weight gain in the control group was conversely in trend of a significant increase at 30.0 ± 11.5 g ($p < 0.05$) and thereby, individual fluctuations in body weight in the main and control groups were very significant and amounted, 170–300 g and 150–270 g respectively. The body weight in the main group had slight increase trend and amounted to 213.0 ± 8.4 g. However, as at the previous reference research point, the body weight of the animals in the main group was bigger than that in the control group of animals 198.5 ± 9.47 g. At the same time, if in comparison to the results obtained during 3 weeks of research, the weight of animals in the main group during 6 weeks had only a tendency to increase, animals' weight in the control group during 6 weeks of research was significantly higher than at the previous reference point ($p < 0.05$).

After 9 weeks of animal monitoring and 6 weeks after termination of the DFA consumption, in comparison to previous research, the body weight gain in the main group decreased to 6.3 ± 2.5 g (2.81%), and the body weight gain of the control group of animals was 36.7 ± 12.3 g (5.03%), and conversely had significant increase trend. At the same time, on the 9th week of the research, the body weight gain in the control group significantly and probably outpaced such in the main group at $p < 0.05$. Individual fluctuations in body weight in the control group of rats, as during the previous research stage were 170–300 g, and were higher in animals of the control group (175–280 g). Animal body weight in the main group was 219.3 ± 89.4 g after 6 weeks upon the termination of the DFA course and hardly changed compared to previous figures.

Body weight in the control group significantly increased in comparison to previous research stage, and amounted to 208.5 ± 10.1 g. Possible differences between obtained and previous examinations' data for animals from the main and control groups for 9 and 6 weeks' observation period, as well as for 6 weeks after the termination of the DFA consumption have not been detected ($p > 0.05$).

After 16 weeks (4 months) from the beginning of experiment and 13 weeks after the DFA termination to animals of the main group, the body weight increase in it was almost at the level of the previous reference research point and amounted 6.8 ± 2.9 g (4.5%). Body weight gain in the control group at 18.69 ± 2.77 g (10%) was almost twice lower than previously, but significantly higher than in the main group during the last survey. Fluctuations in the body weight c in the main group of animals amounted to 200–310 g, and was 190–290 g for the control group. The body weight in the main and control groups, 13 weeks after the termination of the DFA for the animals in the main group and 16 weeks of research was 230.0 ± 9.1 g and 229.3 ± 8.4 g, respectively, almost had no difference between each other and reached the age norm for mature animals of this species.

Thus, the performed research shows that animals in the main group, which consumed DFA BAA 'Aquacarotin' with the diet only during the course of the additive consumption for up to 3 weeks, had probable and significant increase in body weight, both in relation to the initial data and control indices for the observation period, then, 3 weeks after its introduction, it slowed down, and was further regulated.

Data on changes of blood leukogram indices in the main group of animals under the influence of DFA BAA 'Aquacarotin' compared to the control animals that did not receive this food additive are given in Table 1.

Table 1 — Comparison of peripheral blood leukogram indices in the control group which were kept on the regular diet and the main group which received DFA BAA 'Aquacarotin'

Indices, g/l	Terms of study						Normal indices, g/l
	Before the DFA consumption		After 3 weeks upon adding the DFA to diet		After 13 weeks upon adding the DFA to diet		
	Groups of animals						
	Control (n = 30)	Main (n = 30)	Control (n = 30)	Main (n = 30)	Control (n = 30)	Main (n = 30)	
Leukocytes	9.87 ± 0.22	9.75 ± 0.90	11.24 ± 1.30	14.76 ± 1.90*			11.67 ± 0.37
Metaemyelocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.017 ± 0.005
Stab neutrophils	0.06 ± 0.02	0.04 ± 0.01	0.11 ± 0.01	0.22 ± 0.15	0.04 ± 0.02	0.09 ± 0.05	0.13 ± 0.01
Segmented neutrophils	1.23 ± 0.57	1.10 ± 0.8	2.40 ± 0.69	3.32 ± 0.90	1.96 ± 0.4	1.99 ± 0.90	2.79 ± 0.16
Eosinophils	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	1.02 ± 0.28*	0.12 ± 0.05	0.18 ± 0.08	0.25 ± 0.02
Monocytes	0.08 ± 0.02	0.06 ± 0.01	0.09 ± 0.03	0.48 ± 0.15*	0.12 ± 0.06	0.21 ± 0.09	0.14 ± 0.02
Monocytes	8.47 ± 0.28	8.53 ± 0.15	8.60 ± 2.75	9.42 ± 1.27	8.50 ± 1.27	9.78 ± 0.92	8.10 ± 0.25
Natural killers	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01

Note: * — $p < 0.05$ compared to the outgoing data.

As it can be seen from data in Table 1, the baseline indices for the content of leukocytes and peripheral blood elements leukogram did not differ among the animals in the main and control groups. After 3 weeks upon the consumption of DFA BAA 'Aquacarotin' in the food diet, the contents of leukocytes in the animals of the main group increased significantly, compared to the baseline ($p < 0.05$). Animals in the control group demonstrate only a tendency to increase the content of leukocytes. 25% of the animals out of the main group registered young forms of neutrophils in the peripheral blood.

The average number of young neutrophil cells does not exceed the upper limit standards. At the same time of studies on the animals in the main group, there was an increase trend in the number of stab neutrophils, and their content significantly increased in 50% of animals.

The average content of neutrophils reached the upper limit of the species norm, but increasing of stab neutrophil pool was not probable on benchmarks and indices for animals of the control group for a period of study ($p > 0.05$).

The pool of segmented neutrophils increased significantly, but not likely in relation to raw data and control group data.

In comparison to raw data of the main and control groups of animals, eosinophil and monocyte pool increased significantly and likely during examination period ($p > 0.05$). Increase trend for lymphocytic pool of the peripheral blood was also detected in the main group of animals.

The contents of leukocytes and peripheral blood leukogram are complex indices of the body status, and represent hematopoiesis. Therefore, this data demonstrates a positive impact of DFA BAA 'Aquacarotin' on a circulatory system and a whole organism. Herewith, it is important to underline the exact influence of the DFA on the activation of myelocytic and monocytic hematopoiesis, enhancement of non-specific resistance, as evidenced by increasing of monocytic pool, namely macrophages and monocytes, as well as natural killers.

13 weeks after the termination of the DFA addition to the diet, the content of peripheral blood leukocytes in animals of the main group had a downward trend, but remained higher than raw data and control group indices in terms of research. The content of stab neutrophils, segmented neutrophils, eosinophils, monocytes and lymphocytes in the main group of animals was higher than before adding the DFA to the ration and indices for a period of study, but decreased in comparison with these data, which were determined after completion of the DFA course. Trends related to change the leukogram, remained the same as to check them immediately after termination of the DFA. That is, for 13 weeks after stopping the use of DFA BAA 'Aquacarotin', its stimulating effect on peripheral blood remained, but decreased.

The obtained data suggest positive effect of DFA BAA 'Aquacarotin' to these physiological parameters in animals, like body weight gain and body weight, as well as the condition of blood formation, represented with peripheral blood. The biological activity of DFA BAA 'Aquacarotin' on body weight will give significant and express increase and relation to the volume of body weight of animals in the process of growth. Active gain of body weight stopped upon termination of DFA BAA

'Aquacarotin'. It is valuable that after the termination of additive's use, the animals' body weight gain slowed down and got normal, i.e. it did not continue after termination of the DFA, which indicates the control action on the DFA and safety of its consumption. DFA BAA 'Aquacarotin' more significantly influences myelocytic and monocytic hematopoiesis, and represents significant tendency to increase neutrophil pool, the pool of eosinophilic granulocytes, as well as monocytic pool of peripheral blood against the backdrop tendency to increase the pool of lymphocytes. Enhancing functional capacity of neutrophils influenced by DFA BAA 'Aquacarotin', namely the activation of biochemically caused bactericidal activity of neutrophils we detected earlier work (Raksha-Slusareva et al., 2017).

Conclusions. 1. DFA BAA 'Aquacarotin' is controllable, and therefore safe to influence the physiological parameters and weight, as well as body weight gain during growth, as well as peripheral blood indices.

2. The consumption of DFA BAA 'Aquacarotin' is useful to improve physiological characteristics of organism (rapid body weight gain), especially during growth.

3. DFA BAA 'Aquacarotin' activates myelocyte and monocyte hematopoiesis by increasing of macro- and macrophagic poos, as well as enhances their functional activity.

4. The data can be found interesting for being implemented in medicine, agriculture, military affairs, technology and food industry.

Prospects for further research. It will be used for deeper research of the dietary food additive effects with biologically active influence on cytomorphological changes in non-specific resistance and immunity system elements.

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