INVESTIGATION OF DETOXIFYING PROPERTIES OF BIOLOGICALLY ACTIVE ADDITIVE IN MEAT PRODUCTS

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***Abstract* – the results of comparative study of preparations of insoluble wheat food fibers Vitacel and the preparations of soluble food fibers: isolate of animal protein Tipro (Germany), inulin (Belgium), and biologically active additives based on them, in a chronic biological experiment have shown, that all of them have low level of alimentary sensibilization and don't cause inflammation processes.Inulin helps increase calcium level in bones and selenium in muscular tissue of laboratory animals and removes heavy metals more effectively, than the animal protein isolate and Vitacel.Vitacel is more effective with respect to cadmium than lead. Thus with combined effects of heavy metals, the use of combined preparations of food fibers is appropriate.The developed composition of biologically active additive in meat products ensures high level of heavy metals removal both from muscular tissue, and from heart, liver and bone tissue.**

***Index terms* - biologically active additives, biological evaluation, blood test, food fibers heavy metals, medical and biological evaluation**

I. INTRODUCTION

During last years a large part of Russian population had shown symptoms of immune deficiency and inadequate adaptation to unfavorable factors of environment, for example, to heavy metals contamination due to violation of nutrition structure and status. Therefore, a necessity of enrichment of daily diets with vitamins, mineral substances, microelements and food fibers arises, and this is one of the major causes of increasing requirements in biologically active additives (BAA) in foods, facilitating normalization of metabolic processes, detoxification of organism, supporting and strengthening of health, prophylaxis of diseases, acceleration of recovery processes.

Development and industrial production of functional and enriched meat products, helping detoxification of human's organism is one of the efficient measures of consequences reduction of heavy metals and other toxic substances environment contamination. The use of a ready multi-component BAA in the composition of meat products will allow intensify technological process.

II. MATERIALS AND METHODS

The purpose of investigations was validation in the experiments on animals of efficiency of biologically active additive introduced into meat product and helping to remove toxic substances from live systems under the effects of negative technogenic factors.

The preparation of insoluble wheat food fibers Vitacel and the preparations of soluble food fibers: animal protein isolate Tipro (Germany), inulin (Belgium), and ground meat were investigated.

Biological evaluation of the efficiency of food fibers and BAA based on them, for the case of lead and cadmium intoxication, was conducted by traditional experimental methods on growing white male rats during 30 days in vivarium of VNIIMP. The feed was mixed with the tested additive (at 2 g per one animal a day). Control groups were kept on common vivarium diets (15 g of porridge, 10 g of compound feed per day).

11 experimental groups of animals were formed, which were divided into 3 series. Each group consisted of 10 animals. The first series: group № 1 received the mixture of ground meat and food fibers (Vitacel and Tipro); group №2 – a mixture of ground meat and food fibers (Vitacel and inulin); the second series – group № 4 - a mixture of ground meat, food fibers (Vitacel and Tipro and a complex of vitamins, mineral substances (BAA1), containing mineral fortifier from egg shells, vitamins (B_1, B_2, PP, C), red palm oil Carotino as a natural source of carotenoids, tocopherols, selenopyran, ; group №5 a mixture of ground meat, food fibers (Vitacel) and BAA1; group №6 – a mixture of ground meat, food fibers (Vitacel, inulin) and BAA1; group № 7 – ground meat; the 3rd series: group №9 - BAA2, including BAA1, salt of prophylaxis grade, which in addition to sodium, contained potassium, magnesium, iodide casein, concentrate of phospholipids; group №10 – ground meat.

The control groups (3, 8, 11), respectively for the 1st, 2nd and 3rd series, received common vivarium diets. Solutions of heavy metals salts were added to the feeds of all groups at 1/100 LD50 for rats: cadmium sulfate – 0.6 mg/kg, lead citrate – 1.17 mg/kg. The level of heavy metals was determined by inversion voltammetry method. Mineral composition was determined on atom-absorption spectrophotometer "Perkin-Elmer". The level of selenium in organs and tissues of animals was determined by hydride atom-absorption method.

III. RESULTS AND DISCUSSION

The live weight gain of laboratory animals during the experiment decreased in the order as follows (Table 1):

The first series – vivarium diets > ground meat, Vitacel, inulin > ground meat, Vitacel, Tipro;

The second series – ground meat, Vitacel, BAA 1 > ground meat, Vitacel, Tipro, BAA 1 > ground meat, Vitacel, inulin, BAA 1 > ground meat > vivarium diets

The third series – vivarium diets > ground meat > BAA2.

Contrary to the first series of experiments, in the second one the weight gains in all the experimental groups were higher, than in the control, which indicates a positive effect of adding vitamins.

Clinical blood analysis of laboratory animals showed absence of positive effect or an insignificant negative effect of all the preparations on hemopoiesis function of rats (Table 1). Reduction of hemoglobin level to 17% can be explained by the capacity of food fibers to bind iron into insoluble complexes and remove them from organism.

Both in the 1st and the 2nd series of the experiment, hemoglobin level for the specimen with inulin was by 5.5% higher, than for the specimen with animal protein. This can be explained by the fact that the animal protein removes iron more effectively, or by a high sorption activity of inulin in relation to heavy metals which block iron assimilation.

As a result of analysis of WBC differential of animals in all the groups, juvenile neutrophils, monocytes and basophilic leukocytes were not found which suggested about the absence of inflammation process, immune conflict and food sensibilization.

Enrichment of specimens of the 2nd and 3rd series of experiment resulted in blood viscosity reduction as compared to the 1st series, which can be seen from ESR for 4, 5 and 6 specimens by 3, 5 and 8 times, respectively.

Table 1.Total blood analysis of rats

Index	Groups of animals									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	9	10
Erythrocytes, $\times 10^9$/μl	8.05	7.22	7.08	6.3	6.53	6.07	6.0	7.17	5.1	5.8
Hematocrit,%	72.0	75.0	79.0	73.0	87.0	60.0	69.7	70.0	46.7	51.8
Hemoglobin,g/dl	14.4	15.2	14.4	12.6	14.7	13.8	13.2	14.2	13.0	14.47
ERS,mm/h	0	0	0	0.3	0.5	0.8	0.1	0.1	0.13	0

Leukocytes,×10³/μl	30.8	32.7	24.0	14.1	22.7	12.1	12.1	20.7	4.2	6.53
Thrombocytes,×10³/μl	114.0	445.7	466.3	468.7	244.0	645.7	497.0	478.3	578.3	426.0
Neutrophils										
-juvenile,%	0	0	0	0	0	0	0	0	0	0
-stab,%	1.7	2.2	1.0	0	0	0	0	0.7	2.0	0.33
-segmented.,%	15.3	9.7	16.0	16.0	14.0	10.7	9.7	12.7	17.3	12.0
Eosinophils,%	1.0	0.7	1.7	0	0	1.3	0.3	0	0.66	0
Monocytes,%	0	0	0	0	0	0	0	0	0	0
Basophils,%	0	0	0	0	0	0	0	0	0	0
Lymphocytes,%	81.7	87.3	81.3	84.0	86.2	88.0	90.0	86.0	80.0	87.0

Studies on the accumulation of heavy metals in organs and tissues of laboratory animals have shown the highest levels of heavy metals for groups 7 and 10 (Table 2), which indicates a low sorption capacity of meat and the inevitability of accumulation of heavy metals both in human organism and in muscles and other vitally important organs when ordinary meat products are consumed under conditions of contamination with toxic substances.

In the first series specimens 1 and 2 were identical with regard to the level of reduction of lead in muscular tissue; for heart, liver and bone tissue the second sample was more effective, the level of reduction of cadmium of the second specimen was also higher for all organs and tissues.

The greatest detoxifying activity of the combination of inulin and Vitacel was confirmed in the second series of the experiment. The level of reduction of lead in liver, heart and bones for the 6th specimen with similar composition of food fibers and enriched with BAA1, was significantly higher, than for the fourth one, containing isolate of animal protein.

It should be noted a similar high level of lead reduction (up to 95%) for all the specimens of the 2nd series in muscular tissue.

Based on the investigations of the specimens of the 1st and 2nd series of experiments, the 6th specimen, additionally enriched with prophylaxis salt, iodine casein and phospholipids concentrate, was selected as the base in the development of detoxifying biological additive (BAA2).

For BAA2 the highest levels of removal of both - lead (81-95%) and cadmium (92-98%) - were found, for all organs and tissues, (Tables 2,3), which suggests about an efficacy of the developed biologically active additive with regards of heavy metals.

Table 2. Reduction of the level of lead in organs and tissues of laboratory animals, %

Organs and tissues of animals	Pb							
	Groups of animals							
	1	2	4	5	6	7	9	10
Muscular tissue	94	94	95	95	95	+16	95	30
Heart	49	64	33	55	83	22	81	33
Liver	26	54	62	29	92	+13	93	29
Bones	67	73	75	75	84	0	93	43

The level of lead in liver of the experimental animals of group №5, receiving the sample of ground meat with Vitacel, was by 87% higher than for group №6, receiving meat product with a set of soluble and insoluble food fibers (Vitacel, inulin). At the same time, Vitacel led to the highest reduction of cadmium level in muscle tissue (Table 3), which indicates the usefulness of combination of soluble and insoluble fibers when a complex of heavy metals is present.

Table 3. Reduction of cadmium level in organs and tissues of laboratory animals, %

Organs and tissues of animals	Cd							
	Groups of animals							
	1	2	4	5	6	7	9	10
Muscle tissue	58	80	85	99	65	+8,3	98	46
Heart	80	86	88	88	90	24	98	47
Liver	81	93	75	80	97	8,3	98	74
Bones	93	93	93	93	93	+50	92	8

The results of studies of calcium level in bones and selenium in muscular tissue of laboratory animals are presented in Table 4.

Table 4. Level of mineral substances in tissues of laboratory animals

Index	Groups of animals										
	1	2	3	4	5	6	7	8	9	10	11
Calcium, mg/kg	172.2	200.2	164.3	192.2	164.7	205.5	137.9	144.2	196.4	136.5	127.5
Selenium,μg/kg	9.7	11.4	8.3	13.6	11.7	19.7	6.2	6.8	19.0	10.1	8.2

The highest increase in calcium level in the bones (as compared to the control) was shown in the experimental samples 2, 6, 9, containing inulin: by 21.8%, 42.5 and 54.0%, respectively (Table 5). Samples 1 and 4, containing animal protein isolate promoted calcium assimilation to less degree: by 4.8% and 33.3%, respectively.

Table 5. Increase in the level of mineral substances in tissues of laboratory animals

Index	Groups of animals							
	1	2	4	5	6	7	9	10
Calcium, %	4.8	21.8	33.3	14.2	42.5	-4.4	54.0	7.1
Selenium, %	16.9	37.3	100	72.1	189.7	-8.8	131.7	23.2

The level of antioxidant – selenium - in muscular tissue of all groups is inversely proportional to the level of heavy metals in organs and tissues of rats, which can be explained by reduction of its consumption during complexes formation and removal of heavy metals from the organism.

The results of the investigations were used in the development of special products recommended for decreasing risks and prophylaxis of obesity, diseases of liver, cardiovascular diseases, and also for population living in megalopolises and unfavorable ecological situation.

IV. CONCLUSIONS

The results of the chronic biological tests of the preparations of food fibers on laboratory animals have led to the conclusion:

To prevent accumulation of heavy metals in live systems under the conditions of contamination with toxic substances, additional enrichment of meat products with detoxifying biologically active substances is necessary;

Absence of inflammation, immune conflict and food sensibilization of the animals consuming all the tested preparations of food fibers and biologically active additives based on them was found;

Inulin promotes increase of calcium level in bones by 21.8-54.0% and of selenium – by 37.3-189.7% in muscular tissue of laboratory animals;

Inulin removes heavy metals to a greater extent, than animal protein isolate and the preparation of insoluble wheat food fibers Vitacel.

- Vitacel is more effective with regards to cadmium, than lead
- It is more appropriate to use combined preparations of food fibers when the effects of heavy metals are present;
- The developed composition of biologically active additive in meat products formulation ensures high level of removal of heavy metals both from muscular tissue, and from heart, liver and bone tissue: lead by 95%, 81%, 93%, 93%; cadmium – by 98%, 98%, 98%, 92%, respectively.