Effect of antioxidants in a liposomal form containing organic iodine of the blood serum biochemical composition and the structure of muscle tissue formation of young rabbits

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Abstract. The study of the effect of antioxidants containing organic iodine on the formation of the enteral environment of California young rabbits is described in the article. A significant increase in the level of thyroxine in the experimental groups was in the 2nd by 6.7% and in the 3rd by 8.9% ($P<0.05$). In the third group, there was a significant tendency to increase the total protein in the blood serum: compared with the control, it increased by 3.39 units ($P<0.05$). This trend continues in the level of albumin at 3.58 units. ($P<0.05$). There was a significant increase in creatinine in young rabbits of both experimental groups by 20.51-28.89 units ($P<0.05$). There was a significant excess of iodine content in the meat of rabbits of group 2 by 81.0 mcg ($P<0.05$), and in animals of group 3-by 234.1 % ($P<0.05$). There was an increase in the number of muscle fibers in both experimental groups by 68.0 and 78.0% ($P<0.05-0.01$). The diameter of the muscle fiber of experimental animals was significantly lower by 13.1 and 17.3 % ($P<0.05$). The animals of the experimental groups formed meat with a more delicate structure and an increased content of valuable parts-muscle and fat tissue.

1 Introduction

Antioxidant vitality has been proven to have positive effects in rabbit breeding [1-2]. Antioxidant effects lead to improved animal health and increased growth of target tissues [3]. Toxins and fluctuations in ambient temperature lead to pathological changes: tissues are damaged; blood parameters change [2]. A positive effect on blood parameters in rabbits when using antioxidants has been proved [4]. Adverse factors contribute to an increase in free radicals, which can cause irreversible damage to cells [5].

The search, characterization, and application of natural antioxidants remain the focus of numerous research groups around the world. Natural antioxidants are now one of the most popular topics in the field of food and agriculture [6]. According to one theory, it is

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believed that the antioxidant properties of plants are developed in the course of evolution based on photosynthetic activity. Therefore, each species, subspecies, and variety of plants can be an object for evaluating the antioxidant potential [6]. An ideal antioxidant should be easily absorbed by the body and prevent the formation of free radicals at physiologically significant levels [7].

Useful properties of vegetable extracts in animal husbandry and poultry farming have recently been used more often [8, 9] due to low toxicity, pharmacological activity and economic viability [10-11]. Thus, feed additives of natural antioxidants can increase the efficiency of growing animals and poultry without compromising the quality of the products.

The use of the proper form of antioxidants remains an urgent problem. Antioxidants in their pure form, due to their low degree of solubility, are practically unable to overcome the barriers of cell membranes. The liposomal form will allow equally effective transportation of vital compounds in sufficient concentrations: water-soluble, fat-soluble, combinations of various antioxidants, etc. [12]. This form is widely used in medical practice [13].

Liposomal antioxidant action in poultry farming has been established to be effective [14-15]. Thus, the use of liposomal forms of antioxidants is an important part of further agroecological research in the field of agricultural production. These data will be aimed at ensuring environmental well-being in the course of further development of technologies in the agro-industrial complex [16].

The study of the effect of antioxidants in a liposomal form containing organic iodine on the formation of the biochemical composition of the blood serum of Californian young rabbits and the structure of muscle tissue has become the main aim of the research.

2 Material and methods of research

Research material: young rabbits of the California breed. Conditions for keeping animals: housing in cages. Feeding (main diet) - ready-made full-ration feed mixes to meet nutrient requirements. The young animals were weaned from the females rabbits at the age of 41st day. Young animals were housed in cages (4 young rabbits per cage). Young rabbits were distributed into three treatment groups: 1st (control group) - main diet (MD); 2nd (experimental group) - MD + "Polysol omega-3" (a liposomal form of antioxidants containing organic iodine of algae *Laminaria digitate* obtained from the White sea) at a rate of 250 g per ton of feed mixture in the form of granules; 3rd (experimental group) - MD + "Polysol omega-3" (a liposomal form of antioxidants containing organic iodine of algae *Cystoseira barbata* obtained from the Black sea) at a rate of 250 g per ton of feed mixture in the form of granules. Each group consisted of 20 young rabbits. Weighing of young rabbits was carried out individually in the morning before feeding: at birth, at weaning, on the 60th and 90th days. The experimental animals were offered feed according to physiological norms.

Before slaughter, blood samples were collected from the ear vein from the rabbits to determine total protein, albumin, glucose, alanine aminotransferase, asparaginaminotransferase, alkaline phosphatase, creatinine, urea, bilirubin, a-amylase, calcium, phosphorus. The studies were conducted on three animals from each group. The research was carried out on a biochemical analyzer *Vitalab Flexor E*. Thyroxine (T₄), triiodothyronine (T₃) and thyroid-stimulating hormone (TSH) was carried out on a biochemical analyzer *StatFax 3200*.

Determination of nitrogen by burning a suspension of concentrated sulfuric acid using a catalyst is a copper (II) sulfate. Organic nitrogen passes into ammonium ions and is leached. Distillation of the released ammonia into an excess boric acid solution occurs. Titration with hydrochloric acid to determine the amount of boric acidbound ammonia.

Calculation of the mass fraction of nitrogen in the product sample, based on the amount of formed ammonium. Determination of calcium by titrimetric method by forming a low-dissociated complex compound of calcium with a disodium salt of Ethylenediamine-N', N', N', N'-tetraacetic acid (Trilon B) in an alkaline medium and determining the equivalent point during titration using metal indicators. Determination of magnesium by flame atomic absorption spectrometry. Determination of iodine in meat was carried out by voltammetric method. Determination of selenium was carried out by wet burning of the sample with a mixture of nitric and perchloric acids and reduction of hexavalent selenium to Se^{+4} by the action of hydrochloric acid. The complex of selenic acid with 2,3-diaminophthalene-piazoselenol is determined by the amount of fluorescence. This value is proportional to the selenium content in the sample. Determination of cobalt by electrothermal atomic absorption spectrometry. Determination of copper was carried out by inversion-voltammetric measurement of this element concentration.

Histological studies of the muscle tissue of young rabbits were carried out in the histological laboratory of the Center for collective use "Molecular Biology" of the Medical Academy named after S.I. Georgievsky on samples taken from the thigh of the left hind paw of control and experimental animals. The material for histological studies was fixed in 10 % buffered formalin for 24 hours; then it was dehydrated and impregnated with paraffin in a LOGOS microwave *Mielstone* histoprocessor. From the manufactured paraffin blocks, serial sections with a thickness of 4 microns were made. These blocks were stained with hematoxylin and eosin. Using DM2000 microscope, sections were viewed and photographed with 10* and 40* lenses. Obtained photographs processed in the *ImageJ* was used to measure morphometric parameters. The number of muscle bundles in 100 mm², their diameter, the ratio of muscle and connective tissue in the field of view, and the thickness of the endomysium and perimysium were measured. Obtained values were statistically processed in Statistica 10. Portable using nonparametric methods and descriptive statistics methods. The differences significant if the probability of error was less than 0.05, 0.01 and 0.001 were considered.

3 Research results

Visual observations showed that the studied feed additive did not have any negative effect on the body of young rabbits. During the experiment, all animals were active and consumed feed well. Safety calculations revealed the positive effect of “Polysol omega-3” on the safety of rabbits (Table 1).

Table 1. Young rabbits safety in the experiment.

Indicator	1 st group	2 nd group	3 rd group
Number of heads	25	25	25
Cases of case in the experiment, heads	5	3	4
Safety, %	80.0	88.0	84.0

Cases of young animal deaths were recorded in all studied groups. Young rabbits from the experimental groups were less susceptible to various diseases. Safety of rabbits in the control group was 80 %; in the second experimental group - 88.0 %, which is 8.0 % more than in the control one. In the third experimental group, receiving feed additive “Polysol omega 3” based on the *Cystoseira barbata* obtained from the Black sea, rabbits' safety reached 84.0 %, which is 4 % higher than in the control group.

According to table 2, it can be concluded that the inclusion of the studied additives in the diet of the rabbits from the experimental groups had a positive effect on the dynamics of live weight growth.

Table 2. Dynamics of rabbits' live weight growth, $\bar{X} \pm Sx$.

Indicator	1 st group	2 nd group	3 rd group
Live weight at the beginning of the experiment (41 days), g	929.0±38.46	1035.38±51.24	910.67±25.28
Live weight at the age of 92 days (end of the experiment), g	2364.6±35.83	2396.0±47.29	2488.53±37.3
As a percentage of the control group	100.0	101.3	105.2
Absolute growth, g	1435.6±47.8	1361.6±56.91	1577.8±46.81
As a percentage of the control group	100.0	94.8	109.8
Average daily growth, g	28.15±0.94	26.7±1.21	30.9±0.89
As a percentage of the control group	100.0	94.8	109.7

The live weight of young rabbits from all groups was approximately at the same level - 910.0 - 1035.0 g at the beginning of the experiment. Young rabbits in the control group were inferior in terms of live weight to animals in the experimental groups by the end of the experiment. The largest increase in live weight was observed in young rabbits of the third experimental group ("Polysol omega 3" based on *Cystoseira brabata* from the Black Sea) - 1577.8 g, which is 9.8% more than in the control group. The average live weight of young rabbits in the third group was 2488.5 g, which is 5.2 % more than that of in the control one. At the same time, the absolute growth rate of the young animals of the second experimental group was 5.2% lower than in the control one and amounted to 1361.6 g compared to 1435.6 g in the control group. A similar situation was in terms of absolute average daily weight gain. In the second group, the absolute increase compared to the control one was by 5.2 % lower; in the 3rd group it was 9.7 % higher.

Thyroxine (T₄) and triiodothyronine (T₃) are thyroid hormones. They are produced and released by the thyroid gland and are catalysts for the formation and release of energy, controlling the synthesis of cellular proteins, in particular, enzymes [17]. Thyroid-stimulating hormone (TSH) is a glycoprotein synthesized by the pituitary gland, in the synthesis of which thyrotropin-releasing hormone is present as a catalyst [18].

The relationship between thyroid hormones and thyroid-stimulating hormone was noted: logarithmic dependence of the production of TSH and bound T₄ in humans is noted. In this case, it is established that a slight decrease in T₄ level increases the values of TSH [19]. However, TSH and T₃ levels are determined by 64 %. At the same time, there were no hereditary correlations between the content of TSH and human thyroid hormones [20-22].

Table 3 shows data on the content of thyroid hormones in the blood serum of rabbits of the studied groups.

Table 3. Thyroid hormones in young rabbits of the studied groups, X ±Sx (n=9).

	T4, nmol/ L		
X±Sx	13.94±0.47	14.87±0.28	15.18±0.39*
Cv, %	10.02	5.58	7.74
	T3, pmol/ L		
X±Sx	3.77±0.17	3.52±0.07	3.88±0.09
Cv, %	13.29	5.81	6.60
	TSH, mU/L		
X±Sx	0.30±0.01	0.31±0.01	0,32±0.01*
Cv, %	6.11	11.46	7.85
Here and further in all tables: differences are significant with the probability of statistical error: * - p<0.05; ** - p<0.01; *** - p<0.001			

The content of thyroxine in the experimental groups exceeds the same in the control one: 2nd - by 6.7 %; 3rd - by 8.9 % (p<0.05). According to the concentration of triiodothyronine, the difference between the groups varies slightly - from 3.52 pmol/l in the 2nd group to 3.88 pmol/l in the 3rd. The content of thyroid-stimulating hormone in the 2nd group prevails over the control group by 4.4 %, and the 3rd - by 8.3 % (p<0.05) (table 3).

Table 4 shows correlations within groups between indicators of thyroid hormone content. There was a significant positive relationship between T4 and T3 (0.60±0.30, p<0.05).

Biochemical parameters of blood serum of young rabbits on the background of feeding antioxidants in liposomal form with organic iodine content are set out in table 5.

Table 4. Correlation between indicators of thyroid hormone content within the study groups (n=9).

1 st group		
	T4	T3
T3	0,34±0,36	-
TSH	0,20±0,37	0,51±0,33
2 nd group		
T3	0,60±0,30*	-
TSH	0,48±0,33	-0,01±0,38
3 rd group		
T3	-0,07±0,96	-
TSH	0,47±0,54	-0,04±1,57

Table 5. Biochemical parameters of blood serum of young rabbits on the background of feeding antioxidants in liposomal form with organic iodine content, $\bar{X} \pm S_x$.

	Unit measurement	1 st group	2 nd group	3 rd group
Total protein	g/L	45.21± 0.72	46.23± 0.54	48.60± 1.16*
Albumin	g/L	42.80± 0.29	44.13± 0.44*	46.38± 0.99**
Glucose	mmol/L	5.13± 0.33	4.75± 0.19	4.50± 0.27
Alanine aminotransferase	U/L	56.48± 5.69	71.78± 4.77*	62.02± 7.60
Aspartate aminotransferase	U/L	80.84± 11.43*	78.17± 8.92	79.15± 9.34
Total bilirubin	gmol/L	0.26± 0.05	0.13± 0.03	0.24± 0.02
Direct bilirubin	gmol/L	0.15± 0.04	0.48± 0.04*	0.40± 0.05*
Urease	mmol/L	7.34± 0.69	8.17± 0.77*	9.12± 0.93
Alpha-amylase	U/L	236.88± 13.30	257.58± 19.39	288.67± 23.68
Alkaline phosphatase	U/L	153.13± 16.42	132.00± 7.60	127.50± 6.53
Phosphorus	mmol/L	2.30± 0.07	2.32± 0.04	2.91± 0.06*
Creatinine	gmol/L	59.96± 1.68	80.47± 2.07*	88.85± 2.54*
Potassium	mmol/L	5.09± 0.06	5.85± 0.10*	5.25± 0.05

The tendency to increase the total protein in blood serum in young rabbits of experimental groups increases. In the third group, this trend is reliable: in comparison with the control, it increases by 3.39 units. The increase in total protein was due to an increase in albumin levels by 3.58 units.

No fundamental regularities were found in the content of liver enzymes. Direct bilirubin is characterized by an increased content in the experimental groups by 0.25-0.33 units. There was an increase in creatinine in young rabbits of both experimental groups by 20.51-28.89 units.

Table 6 shows the content of certain micro- and macronutrients in rabbit meat in the experiment.

Nitrogen is contained in the protein compounds of the body. A significant advantage in nitrogen content by 0.15 mg/kg (4.2 %) ($p<0.05$) was noted in animals of the 2nd experimental group. There was a significant excess of iodine content in meat in the 2nd group of rabbits by 81.0 mcg (551.0 %) and in animals of 3rd group by 34.45 mcg (234.1 %) (table 6).

The young rabbits' muscles consist of striated muscle fibers united in bundles histologically level. In both groups, the transverse striation is well expressed, which indicates the functional activity of the tissue. The muscles are surrounded by a layer of connective tissue-endomysium, and the bundles - perimysium, between the collagen fibers of which adipocytes are located (fig. 1).

Table 6. Content of certain micro- and macronutrients in rabbit meat in the experiment (n=3).

	N, mg	Ca, mg	Mg, mg	I, mcg / kg	Se, mg / kg	Co, mg	Cu, mg
1 st group							
X ±S _x	3.53 ±0.0354	0.027 ±0.0014**	0.0175 ±0.0018	14.7 ±2.1920	0.0605 ±0.001***	0.0065 ±0.0004	0.5345 ±0.0209*
C _v , %	1.4	7.4	14.3	21.1	2.5	7.7	5.5
2 nd group							
X ±S _x	3.68 ±0.05*	0.0145 ±0.0004	0.015 ±0.001	95.7 ±7.071**	0.0645 ±0.0103	0.014 ±0.0042	0.4855 ±0.0534
C _v , %	1.9	3.4	13.3	10.4	22.5	42.9	15.6
3 rd group							
X ±S _x	3.63 ±0.05	0.0155 ±0.0004	0.0145 ±0.0004	49.15 ±5.20**	0.0435 ±0.0004	0.0185 ±0.0032*	0.4125 ±0.0343
C _v , %	2.1	3.2	3.4	15.0	1.1	24.3	11.8

Fig. 1. Histological section of the thigh muscle of rabbits in the experiment on the use of liposomal form of antioxidants with organic iodine content.

In the experimental groups, there was an increase in the number of muscle fibers by 68.0 - 78.0 % (p<0.05 - 0.01), which is reflected in an increase in the ratio of muscle and connective tissue. Diameter of the muscle fiber in the experimental groups, also, was a decrease by 13.1 - 17.3 % (p<0.05 - p<0.05). This fact indicates a finer structure of muscle fibers in young animals of experimental groups (table 7).

Table 7. Morphometric indicators of muscle tissue of control and experimental groups of rabbits.

Indicator	1 st group	2 nd group	3 rd group
Number of muscle fibers per 100 pm ²	32.56±1.38	55.00±2.98*	57.97±1.58**
The percentage of muscle	70.67±3.33	76.33±2.33	78.35±1.87
The percentage of stroma	29.67±3.67	23.67±2.33	21.96±2.04
Diameter of the muscle fiber, pm	33.55±1.05	29.16±1.05*	27.67±0.87*
Endomysium thickness, pm	6.30±0.23	6.17±0.33	6.04±0.25
Perimysium thickness, pm	19.19±1.11	24.03±1.97	25.62±1.45

Changes in the connective tissue component affect primarily the perimysium having little effect on the shell of muscle fibers. In perimysium, an increase in the accumulations of fat cells, particularly around the neurovascular bundles is also observed (fig. 1B).

4 Conclusions

The study of the effect of antioxidants in a liposomal form containing organic iodine allowed to get the following results. Young rabbits safety of in the experimental groups was higher by 8.0 and 4.0 percent than in the control one. Live weight at the age of 92 days of Young rabbits of experimental groups was higher by 1.3 and 5.2 percent in comparison with the control group of animals. Organic iodine affected the content of thyroid hormones. The significant increase of thyroxine in the experimental groups was in 2nd one by 6.7% and in 3rd one by 8.9% ($p < 0.05$). In the third group, the trend of increase the total protein in blood serum is reliable: in comparison with the control, it increases by 3.39 units ($p < 0.05$). This trend continues and in albumin levels by 3.58 units ($p < 0.05$). No fundamental regularities were found in the content of liver enzymes. There was an significant increase in creatinine in young rabbits of both experimental groups by 20.51-28.89 units ($p < 0.05$). There was a significant excess of iodine content in meat in the 2nd group of rabbits by 81.0 mcg and in animals of 3rd group by 234.1 %. the difference is significant in both cases ($p < 0.05$). An increase of number of muscle fibers in both experimental groups by 68.0 and 78.0 percent ($p < 0.05 - 0.01$) was noted. However, the tendency of percentage of muscle goes to increase by almost by 6 - 7 %. But the diameter of the muscle fiber of experimental animals reliably lower on 13.1 ($p < 0.05$) - 17.3 % ($p < 0.05$). Thus, the animals of the experimental groups formed the meat with a more delicate structure and an increased content of valuable parts are the muscle and fat tissue.

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