

Absorption of iodotyrosine from iodized milk protein in animals

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Received October 03, 2019; Accepted in revised form November 05, 2019; Published February 25, 2020

Abstract:

Introduction. One of the ways to solve the iodine deficiency problem is the addition of iodine to farm animal feed. It allows producing iodized livestock products. Promising sources of organic iodine are iodotyrosine-containing iodized milk proteins. Organic iodine accumulation in organs and tissues has not been sufficiently studied.

Study objects and methods. We determined iodotyrosine content in rat blood plasma and in pig muscle tissue. For this purpose, high performance liquid chromatography with mass spectrometric detection and cathodic stripping voltammetry were used.

Results and discussion. At the first stage of the study, we examined iodotyrosines in rat blood plasma after a single administration of iodized milk protein or potassium iodide (30 µg I/kg weight) at specific time intervals. A significant increase in the concentration of monoiodotyrosine and diiodotyrosine was recorded 4 and 24 h after the administration. At the second stage, we studied the accumulation of iodotyrosines in the muscle tissue of pigs during their fattening period (104 days). The diet of the control animal group included potassium iodide (0.6 mg I/kg of feed). The experimental groups A and B got iodized milk protein (0.3 and 0.6 mg I/kg of feed, respectively). Monoiodotyrosine content in the muscle tissue of pigs of the experimental groups was 3.0 and 5.2 times higher than that in the control group. Diiodotyrosine content was 4.9 and 8.2 times higher. In the experimental group A, iodine content in muscle tissues was 26% higher than that in the control group, in the experimental group B it was 72% higher. Calculations of iodine intake balance and its accumulation in muscle tissues showed that in animals whose diet included iodized milk protein, the iodine assimilation was much higher (0.70 and 0.53%) than in the control group (0.21%).

Conclusion. Iodotyrosines from iodized milk protein are absorbed by the gastrointestinal tract in an unchanged form and accumulate in muscle tissues. The findings give more clear understanding of physiological and biochemical mechanisms of organic iodine absorption in animals.

Keywords: Iodine, iodotyrosines, plasma, muscle tissue, iodized milk protein, absorption

Please cite this article in press as: Bolshakova LS, Lukin DE. Absorption of iodotyrosine from iodized milk protein in animals. *Foods and Raw Materials*. 2020;8(1):60–66. DOI: <http://doi.org/10.21603/2308-4057-2020-1-60-66>.

INTRODUCTION

Among non-metals, iodine is the most important biologically active microelement in human and animal nutrition. The main role of iodine in the body is synthesis of thyroid hormones, triiodothyronine and tetraiodothyronine, or thyroxine. The importance of these hormones is enormous, as they take part in various metabolic processes and influence the tissue growth and differentiation [1, 2]. Iodine deficiency leads to morphological and functional changes in the thyroid gland, to decreased thyroid hormones production and, as a result, to pathological conditions in humans and animals [3, 4]. Iodine deficiency has remained a problem for many countries, including Russia [5–7]. More than

half of the regions in the Russian Federation are iodine deficient, and 60% of the population suffers from iodine deficiency [8].

A decisive role in iodine deficiency prevention is given to the production of iodine-enriched foods of mass consumption (salt, milk, bread, meat products) [9–11]. One of the ways to produce iodized livestock products is addition of iodine to farm animal feed. The iodine content in milk can reach 500 µg/kg and more, in chicken eggs – up to 60 µg/egg [15–18]. Considering the accumulation of significant amounts of iodine in milk and eggs, the European Food Safety Agency (EFSA) has set a maximum level of iodine in feed: for dairy cows and small dairy ruminants – 2 mg/kg, for laying hens – 3 mg/kg of feed dry matter [18].

Table 1 Experiment scheme (stage I)

Group	Number of animals	Diet
Control	20	Balanced common diet (CD)
Experimental group A	16	CD + iodized milk protein (1500 µg/kg body weight)
Experimental group B	16	CD + potassium iodide (39 µg/kg body weight)

The iodine content in meat is directly related to its content in feed. However, even with significant concentrations of the trace element in the diet its content in muscle tissue of animals and poultry is much lower than in milk and eggs [19–21]. According to Flachowsky, the proportion of iodine absorbed from feed is 0.3% for pork and less than 1% for beef compared to 30–40% for milk and 10–20% for eggs [22]. Significant concentrations of iodine in milk and eggs are explained by the fact that not only the thyroid gland, but also exocrine glands, such as salivary, gastric, cervical uterine, and lacteous glands, can uptake iodine from the blood [23, 24].

The accumulation of iodine in the muscle tissue of animals and poultry when entering the body in an inorganic form is negligible. Iodine in the form of iodide ion is almost completely absorbed in the gastrointestinal tract, most part of it is used by the thyroid gland, some is captured by exocrine glands and leukocytes, and the rest is excreted from the body [25].

Currently, there is a growing interest in the study of organic forms of minerals. Iodine in organic molecules is more stable during feed storage. A number of studies have found a positive effect of organic iodine compounds on iodine accumulation in the body and the productivity of farm animals [26, 27]. He *et al.* showed that *Laminaria digitate* alga in the diet of pigs led to a 45% increase of iodine content in muscle tissue compared to the control group whose diet included iodine in the form of potassium iodide [28]. Promising sources of organic iodine are iodized milk proteins containing iodotyrosines. Iodotyrosines of milk proteins are analogues of natural compounds produced by the thyroid gland and involved in the metabolism of iodine. Iodine accumulation in organs and tissues entering as part of organic compounds has not been sufficiently studied.

The purpose of the work was to study the mechanism of absorption and accumulation of iodotyrosines, which are part of milk iodized protein, in the animal muscle tissue.

STUDY OBJECTS AND METHODS

The objects of the study were iodized milk protein (“Chemical Technologies”, Russia) and potassium iodide (“Iodobrom”, Russia). In iodized milk protein, the matrix for iodization is whey proteins, unlike in “Iodcasein”, where the matrix is milk casein.

Iodized milk protein is obtained by the Lublinskij *et al.* method [29], which involves mixing protein raw materials with an aqueous solution of inorganic iodine and enzyme treatment. A mixture of skimmed fresh milk and proteins of different natural origin is used as a protein raw material. In iodized milk protein, iodine is present in the form of mono- and diiodotyrosines, which are full analogues of natural organic iodine compounds. The content of total iodine in iodized milk protein is 2%, monoiodotyrosine – 1.32%, and diiodotyrosine – 0.64%.

The study included two stages. At the first stage, we studied the mechanism of iodotyrosine absorption. For that, mono- and diiodotyrosine content in rat blood plasma was studied after a single injection of iodized milk protein or iodide potassium. The study used Wistar rats aged 8–10 weeks obtained from a licensed source (Andreevka branch of Scientific Centre of Biomedical Technologies, Moscow, Russia). The experiments were performed in the vivarium of Gorbatov Federal Research Center of Food Systems of Russian Academy of Sciences, Moscow. All manipulations with the rodents were carried out in strict accordance with the protocol of research and the current regulatory documentation^{III} [30]. After five-day adaptation the animals were divided into groups (six animal units in each) and placed in plastic cages (“Tecniplast”, type IV S) on a fine wood chips litter. The rats had unlimited access to tap water and food. We used complete feed (by “Laboratorkorm”).

After the adaptation the animals randomly were divided into three groups. The scheme of the experiment is presented in Table 1.

The drug aqueous solutions were injected once with an intragastric probe. Dosages of drugs were 30 µg iodine/kg of body weight. After the injections, the animals were subjected to food deprivation for no more than 24 h.

Four animals from each group were subjected to euthanasia in a CO₂ chamber (VetTech, UK) 1 h, 4 h and 24 h after administration. Blood plasma was taken and stored at –30°C for future experiments.

Mono- and diiodotyrosine content in rats’ blood plasma was determined by liquid chromatography with mass spectrometry detection [31]. Samples were dried, degreased, and subjected to enzymatic hydrolysis using *Streptomyces griseus* proteases. The extraction and purification of iodotyrosines from the samples was performed by solid-phase extraction, followed by derivatization of the extract. Identification of the analytes was carried out according to the absolute retention time of chromatographic peaks of iodthyrosine recorded in the mode of monitoring of multiple reactions. The iodotyrosine concentration was determined based on the area of chromatographic peaks.

^I State Standard 31886-2012. Principles of Good Laboratory Practice (GLP). Application of the GLP principles to short term studies. Moscow: Standartinform; 2013. 10 p.

^{III} State Standard 33044-2014. Principles of good laboratory practice. Moscow: Standartinform; 2015. 12 p.

Table 2 Experiment scheme (stage II)

Groups	Diet	Monoiodo-tyrosine content, mg/kg feed	Diiodo-tyrosine content, mg/kg feed
Control	CD + potassium iodide (0.6 mg I/kg)	–	–
Experimental group A	CD + iodized milk protein (0.3 mg I/kg)	0.2	0.097
Experimental group B	CD + iodized milk protein (0.6 mg I/kg)	0.4	0.194

CD is balanced common diet

The results of the research were processed by parametric methods of variational statistics using the Student t-criterion for unrelated groups ($P < 0.05$) [32]. Arithmetic mean M , mean square deviation m , and the mean error of arithmetic mean σ were determined to calculate the reliability of the differences between the two samples.

At the second stage of the research we studied iodotyrosines accumulation in muscle tissue of large white pigs at the age of 4 months. Three groups of 20 animals each were formed. The selection of groups was carried out on the analogue principle. The duration of the experiment was 104 days. All the animals were kept on a balanced diet, fed twice a day. The diet included wheat, barley, corn, soybeans, peas, wheat meal, fish forage flour, yeast, as well as minerals and vitamins. The chemical composition of the feed was balanced by the main nutrients and depended on the fattening period. Access to water was free.

The control group received potassium iodide in the amount of 0.6 mg I/kg of feed, while the experimental groups A and B received iodized milk protein in the amounts of 0.3 and 0.6 mg I/kg of feed, respectively. The scheme of the experiment is presented in Table 2.

During the experiment we recorded average daily feed consumption, as well as initial and final weight of animals. At the end of the study, three pigs from each group were slaughtered and butchered. We calculated weight before the slaughter, kg; weight of hot carcass, i.e. weight after slaughter and visceration, kg; carcass yield, i.e. the ratio of the hot carcass weight to the weight before slaughter, %; chilled carcass weight (after 24 h storage at $4 \pm 2^\circ\text{C}$), kg; and mass of muscle tissue, kg. *M. longissimus dorsi* was sampled for chemical analysis. Iodotyrosine content in muscle tissue was determined by high performance liquid chromatography with mass spectrometric detection according to [31].

Iodine content was assessed on a TA-Lab voltammetric analyzer (“Tomanalit”, Russia). The method includes mineralization of samples and subsequent analysis of their aqueous solutions with the help of cathodic stripping voltammetry. During the mineralization process and subsequent ultraviolet

irradiation of the solution of the mineralized sample solution all forms of iodine are transformed into iodide ions. Iodide ions are concentrated on silver modified or mercury-film electrodes in the form of low-soluble sludge followed by cathodic reduction of the sludge with linear change of potential. The resulting cathodic peak at the potential minus (0.4 ± 0.05) B for the modified silver electrode and minus (0.3 ± 0.05) B for the mercury film electrode is an analytical signal. The content of iodide ions in the solution of the prepared sample is determined by the method of standard additives of the certified mixture of iodide ions.

The amount of iodine absorbed from feed (%) was calculated as a ratio of iodine accumulated in muscles during fattening to an amount of iodine consumed with feed. The amount of accumulated iodine in muscle tissue was determined by subtracting the amount of iodine in muscles at the beginning of the experiment from the amount of this trace element at the end. The initial amount of iodine was estimated based on the iodine content established at the consumption of inorganic iodine, taking into consideration the initial mass of muscle tissue. The latter was calculated based on the muscle tissue yield determined at the end of the experiment.

Statistical processing of results was carried out using the method of dispersion analysis ($P < 0.05$) [32]. The data are presented as arithmetic mean M and standard square deviation m .

RESULTS AND DISCUSSION

The mechanism of inorganic iodine compounds absorption is studied quite well. Iodine in the form of iodide ion is absorbed in the stomach and upper intestine for 30 min. The thyroid gland takes from 5 to 30% of iodine, some part is used by leukocytes and exocrine glands [25]. Organic iodide is believed to detach from the organic molecule in the liver and enters the blood in the form of iodide ion [33]. However, in the process of presystemic metabolism of iodized amino acids, in particular iodotyrosines, iodine detachment may not occur, and they enter the systemic blood flow unchanged. Absorption of organic selenium in the form of selenomethionine carries in a similar manner [34, 35].

To define the features of iodotyrosine metabolism in animals, we determined the concentration of monoiodotyrosine and diiodotyrosine in rats' blood plasma. We tested control sample (with no iodide), experimental sample A (with iodized milk protein), and experimental sample B (with potassium iodide). The results of the study are presented in Table 3.

Table 3 shows that the monoiodotyrosine concentration in the blood plasma of intact animals (control group) did not change throughout the experiment. After administration of iodized milk protein, the concentration of monoiodotyrosine did not differ from the control group in 1 h, but was

Table 3 Iodotyrosine concentration in rat blood plasma, ng/mL (n = 4)

Time, h	Groups								
	Control			Experimental group A (iodized milk protein)			Experimental group B (potassium iodide)		
	M	m	σ	M	m	σ	M	m	σ
Monoiodotyrosine concentration									
0	0.093	0.01	0.01	–	–	–	–	–	–
1	0.098	0.01	0.01	0.11	0.01	0	0.078	0.01	0.01
4	0.08	0.02	0.01	0.72*	0.07	0.04	0.1	0.02	0.01
24	0.085	0.01	0.01	0.31*	0.03	0.02	0.115*	0.01	0.01
Diiodotyrosine concentration									
0	0.05	0.01	0	–	–	–	–	–	–
1	0.048	0.01	0.01	0.043	0.01	0.01	0.035	0.01	0.01
4	0.04	0.02	0.01	0.27*	0.03	0.02	0.04	0.01	0.01
24	0.04	0.01	0	0.155*	0.01	0.01	0.05	0.01	0

* statistically significant differences ($P < 0.05$) from the indicator of the animals in the control group

significantly higher 4 and 24 h later ($P < 0.05$). In 4 h and 24 h monoiodotyrosine content in the sample A exceeded that in the control sample by 8 and 3.6 times, respectively.

In the sample B, a 35% increase in monoiodotyrosine concentration was recorded only after 24 h ($P < 0.05$). This can be explained by a more active synthesis of thyroid hormones after an increased iodine intake into the thyroid gland. Iodized tyrosines are formed in the gland during thyroglobulin proteolysis and can flow into the bloodstream along with hormones.

The content of diiodotyrosine and monoiodotyrosine in the control sample was at the same level during the experiment (Table 3). 4 and 24 h after administration of iodized milk protein, the concentration of diiodotyrosine in rats of the experimental group A exceeded that in rats of the control group by 6.8 and 3.9 times, respectively ($P < 0.05$). Introduction of potassium iodide did not cause an increase in diiodotyrosine content in rats of the experimental group B.

According to the finding, iodized milk protein increased significantly iodotyrosine content in rats' blood plasma. Concentrations of monoiodotyrosine and diiodotyrosine were maximal 4 h after administration of iodized milk protein. This is probably due to the fact that milk whey protein digestion takes 2–3 h. In the later period, the concentration of amino acids in the blood reaches maximum, and then it decreases, which is confirmed by our findings. It is also should be noticed that the content of monoiodotyrosine in blood was twice as much as that of diiodotyrosine. Such ratio of iodized amino acids consists with their content in iodized milk protein.

Thus, an increased concentration of iodized amino acids in rat blood plasma after taking iodized milk protein may indicate that monoiodotyrosine and

Table 4 Performance parameters of test pigs ($M \pm m$, n = 20)

Indicator	Groups		
	Control	Experimental group A	Experimental group B
Initial body weight, kg	47.55 ± 7.52	49.65 ± 7.77	48.95 ± 8.98
Final body weight, kg	113.25 ± 6.63	118.25 ± 7.41	115.59 ± 8.07
Average daily weight gain, g/day	631.69 ± 19.96	659.60 ± 18.27	640.81 ± 35.37
Average daily feed intake, kg/day	2.3 ± 0.19	2.3 ± 0.17	2.3 ± 0.12

diiodotyrosine are able to enter into systemic blood stream unchanged, without being deiodized in the liver during presystemic metabolism.

Getting into the systemic blood stream, amino acids begin to be distributed to various organs and tissues of the body. At the next stage, we studied the accumulation of iodotyrosines and the degree of iodine absorption in the muscular tissue of the pigs.

We did not find statistically significant differences between the control and experimental groups (Table 4).

The results of the research showed that iodine in organic form in the diet of pigs did not have a significant impact on the slaughter parameters of the animals (Table 5).

The yield of carcasses was the same in pigs of the control and experimental groups and amounted to 70%. The content of muscle tissue in pig carcages varied according to the iodine-containing supplement consumed. Muscle tissue yield of the animals in the experimental groups exceeded that in the control by 0.45% ($P < 0.05$), which indicates the positive effect of iodized milk protein on this parameter.

Table 5 Slaughter parameters and muscle yield of test pigs ($M \pm m$, n = 3)

Parameter	Groups		
	Control	Experimental group A	Experimental group B
Pre-slaughter weight, kg	110.09 ± 3.10	111.68 ± 2.79	110.21 ± 3.52
Hot carcass weight, kg	77.09 ± 2.07	78.18 ± 2.00	77.12 ± 2.50
Chilled carcass weight, kg	75.76 ± 2.07	76.87 ± 2.00	75.79 ± 2.49
Muscle tissue weight, kg	63.49 ± 1.82	64.76 ± 1.62	63.85 ± 1.95
Muscle tissue yield, %	83.80 ± 0.11	84.25 ± 0.12*	84.25 ± 0.21*

* statistically significant differences ($P < 0.05$) from the indicator of the animals in the control group

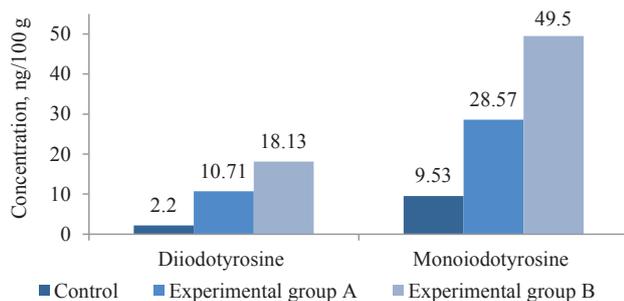


Figure 1 Iodotyrosine content in pig muscle tissue

When determining iodotyrosines in the muscle tissue of pigs (Fig. 1), it was found that contents of monoiodotyrosine and diiodotyrosine in the animals from the experimental groups were significantly higher than those from the control group. The content of monoiodotyrosine in the muscle tissue of pigs from the experimental groups A and B was by 3.0 and 5.2 times, and diiodotyrosine – by 4.9 and 8.2 times higher than in the control group, respectively. In the experimental groups, with the increase in the content of iodotyrosines in feed, their concentration in meat also increased, but not directly proportional to the increase in the amount of iodized amino acids consumed. The results recorded during this stage were obtained for the first time. For the last five years there have been no available data confirming or refuting our findings.

The content of total iodine in animals of the experimental groups was higher compared to that in pigs from the control group (Table 6).

At the same time, even in the experimental group A, where iodine content in the diet was twice less than in the control group, the concentration of iodine in muscles was 26% higher. In the experimental group B, with the equal content of iodine in the feed, the concentration of iodine in muscle tissue was 72% higher than in the control group.

Calculations of iodine intake balance and its accumulation in muscle tissue showed that in animals receiving iodine in the form of iodized milk protein, the degree of iodine absorption was much higher than that in the control group. According to Franke *et al.*, inorganic iodine absorption in the muscle/fat fraction is not more than 0.24%, which corresponds to our result recorded in the control group [36]. Besides, the researchers found a tendency of iodine absorption decreasing with an increase in its content in feed. This pattern was also found in our study. In the experimental group A, where iodine content in the diet was twice less, iodine absorption was higher than that in the experimental group B.

The data obtained confirm the assumptions of some authors about better absorption and more intensive accumulation of organic iodine compounds in animals. For example, Banoch *et al.* observed a similar effect

Table 6 Iodotyrosine content in pig muscle tissue

Parameter	Groups		
	Control	Experimental group A	Experimental group B
Total iodine, µg/100 g	8.83 ± 0.72	11.20 ± 0.98*	15.20 ± 0.62*
Amount of absorbed iodine in muscle tissue, % of injected amount	0.21 ± 0.03	0.70 ± 0.09*	0.53 ± 0.03*

* statistically significant differences ($P < 0.05$) from the indicator of the animals in the control group

when adding iodine-rich algae *Chlorella spp.* compared to potassium iodide. A noticeable effect of iodine introduced into the feed on the pork quality was not established [37].

CONCLUSION

The results of the study showed that iodotyrosines entering the body of animals in the form of iodized milk protein can be absorbed in the gastrointestinal tract in an unchanged form without iodine detachment and can accumulate in the muscle tissue. At the same time, there was a significant increase in the concentration of monoiodothyrosine and diiodotyrosine in the blood plasma of experimental animals. The content of iodized tyrosines in the muscle tissue of animals whose diet included iodine in the form of iodized milk protein significantly exceeded that in animals whose diet included inorganic iodine. In addition, it should be noted that the proportion of absorbed iodine from organic compounds is much higher than the absorption degree of inorganic iodine.

The findings can provide more clear understanding about physiological and biochemical mechanisms of organic iodine absorption in animals.

CONTRIBUTION

Concept and research design, statistical processing, and editing – L.S. Bolshakova. Collection and material processing, text writing – L.S. Bolshakova, D.E. Lukin.

CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

ACKNOWLEDGEMENTS

The authors express their gratitude to A.B. Lisitsyn, Academician of the Russian Academy of Sciences, Doctor of Technical Sciences, Professor, Laureate of the State Prize of the Russian Federation, and I.M. Chernukha, Doctor of Technical Sciences, Professor, for their methodical assistance with the research.

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