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ECOLOGICAL SAFETY OF MEAT PRODUCTS CONTAINING AGRICULTURAL HORMONES RESIDUES

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Meat and meat products belong to a group of foods consumed by population of the country systematically. Nowadays, for the intensification of agricultural production sometimes special chemical growth promoters are used leading to the accumulation of muscle mass in animals. Hormone regulators having pronounced adverse effect on human beings are among these substances (1).
Incorporation of small amounts of hormone identical preparations leads to intensive increase of muscle tissue in animals. It is said about so called "hormone technologies" for raising poultry, cattle and pigs in some countries abroad. Thus, injections of diethylstylbestrols at 10-100 mg/kg make it possible to have a quick weight gain at 5-25%, which seems attractive for producers.
Since 1989 The European Union has prohibited the use of any hormones in the production of meat; use of diethylstylbestrol is prohibited in Russia, however, a number of large countries of American continent use hormones for raising animals.
Up to 1996 the requirements contained in Russian regulations with regards to meat products allowed the residual level of three hormone preparations: estradiole, and testostrone not more than 0.0005 and 0.015% ppm, respectively, and no residual content of diethylstylbestrols was allowed at all (2).
The requirements of 1997 recommend to carry the control over the residues of zootechnical preparations according to recommendation of the Combined Committee of experts of FAO-WHO (3).
Two main groups of methods are used for the analytical determination of residues of hormone preparations: traditional chromatographic methods and modern immune methods. Among the requirements to the methods of express monitoring of foods the main are the sensitivity and selectivity of the method, and time and cost of analysis as well. From the indicated methods only ELISA meets all the requirements of the method of routine control (4).

Objective

The objective of the work was to study the possibility of carrying out a serial analysis of meat products for the content of hormone preparations by ELISA method with the use of standard immune-enzyme sets, for the determination of safety and quality of meat products (5).

Materials and methods

A set for immune -enzyme purification of extracts was used containing: immune-affinity column (cat. N SJ 2I94, "Randox Lab. Ltd.", England) for 10 determinations with the maximum absorption capacity over diethylstylbestrol 50 ng/column, a concentrated buffer solution for washing of the column, a concentrated solution for column storage, as well as a subsidiary set for the determination of stylbenes by immune-enzyme method (cat. N SJ 2152) for 96 determinations, including a microplate for 12x8 mountable cells with sensibilized to stylbenes antibodies, a buffer solution for washing the plate and dilution the samples, a protein conjugate, a substrate for staining the sample, chromogen for staining the sample, a set of standard solutions with the concentration of stylbene from 0 to 10 ng/ml and a photometer for the determination of optical density of the medium, in the cells of plates Strip Reader EL-301 produced by Biotek Instruments (USA).
The buffer solution for washing of the column was prepared by mixing of one volume of the concentrated solution of the washing buffer with 19 volumes of bidistilled water. The solution for the storage of the column was prepared by mixing one volume of concentrated solution of the storage buffer with 4 volumes of the bidistillate. .
The activated plate and the immune-enzyme column in the set are ready for use and stable during 2 years in case of storage at +2... +8 °C. In case of partial use of the plate the remaining part was evacuated together with the dessicant and was preserved at the same temperature in the refrigerator.
For the plates a concentrated buffer solution was used which was diluted by mixing the contents of the bottle with the concentrated buffer solution (amount 32 ml) with 970 ml of bidistilled water, the solution is stable during 30 days in case of storage at +2 ... +8 °C; the protein conjugate that had been prepared from the concentrated conjugate with the storage time 1 year, using the applied instruction on dilution by 1500 times; the diluted solution of the conjugate was used immediately after preparation, and the substrates for staining of the sample (substrates A and B) which before use were mixed in the ratio 1:1.
The sample was prepared as follows: 2.5g of the sample was homogenized with 15 ml of methyl-tretbutyl or sulfuric ether, the homogenate was agitated and separated by centrifugation during 10 min. with the velocity of rotation of the rotor 2000r/min, or settling of 12 ml of ether layer which was evaporated in the flow of compressed air. The dry residue was solved in 1 ml of chloroform added with 2 ml of 1 M solution of NaOH, the solution was agitated and 1 ml of water layer was separated with the further addition of 1 ml of 1 M solution of NaOH to the layer of chloroform and separation of 1 ml of water extract, which was neutralized by the addition 200 µl of 6 M solution of phosphoric acid to 2 ml of the extract. The whole amount of the neutralized solution was applied to immune-affinity column after its conditioning, which had been previously washed by 15 ml of the diluted solution of the washing buffer (the flow rate is less than 3 ml/min.). The extract was passed through the column by gravity, the column was washed by 5 ml of the diluted solution of the washing buffer and 5 ml of bidistilled water (the flow rate is less than 2 ml/min). The fraction of stylbene was eluated by washing the 3 ml column by 70% solution of ethyl alcohol in water. The final washing of the column was carried out by 10 ml of 70 % solution of ethyl alcohol in water and then it was poured with storage buffer.
100µl of diluted buffer solution and 25µl of that analyzed from the immune-enzyme column or standard sample was introduced by

means of the pipette in the appropriate recesses of the plate, which then was maintained in the dark at room temperature (+19 … +25 °C) during one hour, then by the pipette 75µl of the diluted solution of the conjugate was added in each recess of the plate, stored during 30 min, washed with 10-12 times diluted buffer solution and dried, then 125 µl of the mixture 1:1 substrates A and B was added.

The plate was maintained in darkness at the room temperature for 20 min., then 50 µl of 1 M solution hydrochloric acid was added to each recess, and the change of blue staining of the solution in the recess of the plate to yellow, was observed and then the optical density of the solution in the recess at 450 nm was measured.

Results and discussion

Usual analysis of toxicants by chromatographic method requires special physical and chemical purification of the extracts, obtained from the samples. As the chromatographs are the expensive instruments of high class, the requirements to the purity of the samples are rather high, and considerable time is needed for their obtaining.

In the described version of samples preparation with the use of immune-enzyme column, the operation of samples preparation is rather simple and quick.

As the method of sample preparation always includes the transfer of the specific toxicant into the solution, it seemed interesting to appreciate the real level of extraction of hormones by organic solvents. The investigations carried out have shown that the used ethers, being good solvents for diethylstylbestrols, make it possible as a result of a single extraction, to isolate 65-85 % of the substance bound in the sample; the second extraction adds more I5-25 %, and the third one allows to isolate the left 9-I5 % of the hormone. Thus, one can consider that in all the cases even a single extraction under appropriate conditions makes it possible to transfer to the solution immediately, at least, more than 2/3 of the analyzed substance. Subsequent analytical operations actually involved simple mixing of microamounts of several ingredients and allowed to determine the searched substances with the use of the spectrophotometer.

The current analytical procedures for quantitative assessment of the residual content of toxicants should give a reliable answer which substance is available and in what amount. When using the legal chromatographic analytical procedures, the identification of substances is done by the time of coming out of chromatographic peaks in the case of thin layer and HPLC, and in the case of use of GLC with mass spectrometry or spectroscopic ending, the confirming additional identification of the analyzed substance is carried out.

The problem sounds somewhat different if one wants to have answer to the question, what happens to hormonal or some other toxicant in meat and meat products during storage, i.e., what one should determine for reliable answer about that fate of the toxicant in the sample.

Analysis of structural peculiarities of complex organic toxicants shows that metabolic processes in live organisms as well as further processes of oxidative destruction lead to the decrease of the concentration, for example of diethylstylbestrol itself, and appearance of related chemical metabolites, differing from the initial substance by the presence of different organic substitutes. I.e. after some time depending upon the chemical stability of the toxicant it will either disappear or transform, and the toxicity of newly formed substances with the structure similar to the initial substance, will seem to be closer to the initial toxicant, which is also dangerous for a human being.

It is evident that in this case the used chromatographic analysis will give the answer about the substance itself, but will not identify the metabolites without special programs.

She selective analysis of meat products samples has shown that in 20 Russian samples, there were no diethylstylbestrol at all, in three Danish samples - 0, in three Canadian samples - 0, in two Argentina samples - 0; however, from 5 USA samples 2 contained diethylstylbestrol, from four Australian samples one contained diethylstylbestrol, and from 1 Austrian sample one contained diethylstylbestrol.

Taking into account that one can't fully trust to producers information, and the current practice of resales of meat to suppliers in different countries, the origin of products can't be determined exactly. The world experience shows that 1- 5% of all the meat products can be considered as containing hormones in amounts surpassing maximum allowable amounts.

Conclusions

Thus, in connection with the use of hormone growth promoters the control over its residual content is needed. The immune-enzyme analytical procedure can be regarded as the express method of quality evaluation of meat products in its certification for hormones level.

References

1. Ivankin A.N., Karpo B.S., Galkin A.V, Analysis of agricultural toxicants in meat industry by EL1SA method // Congress proceed. 44 ICoMST 1998, 30 Aug.- 4 Sept., 1998, V. 1. Barcelona, Spain, pp, 374-375.
2. Medical and biological requirements and Sanitary standards of quality of food materials and products, N, 5061-89. M.: MZ of USSR, 1990.
3. Sanitary rules and norms SanPin 2.3.27560-96. M: Goscomsanepidnadzor of Russia, 1997.
4. Eller K.I. Methods of quality control and safety of foods //Russian chemical magazine, 1994, No. 1.
5. Environmental. - Crumlin: Randox, 1993.