ANALYSIS OF AGRICULTURAL TOXICANTS IN MEAT PRODUCTS BY ELISA METHOD

Ivankin A.N., Karpo B.S.
All-Russian Meat Research Institute, Talalikhina 26, 109316, Moscow, Russia
Galkin A.V.
RANDOX company (Great Britain)

The development of modern industrial systems of animal and poultry production is directly connected with scientific research and implementation of intensive technologies of animals raising with the use of stimulating and other biogenic preparations. It leads to an increase of residues of veterinary preparations and pesticides in meat and meat products wich have unfavourable influence on human health.

"Hygienic requirements to food raw materials and food products. Sanitary norms and regulations (SanRen) 2.3.2 560-96" that are in force in Russian Federation since January 1, 1997 established of antibiotics (levomycetin and tetracycline) < 0,01 u/g, grisein < 0.5 u/g, bacitracin < 0.02 u/g.

With regards to poultry meat and milk the following limits were also established for the content of: streptomycyn <s 0,5 u/g and penicillin < 0,01 u/g. Depending on the product maximum content of antibiotics should not exceed (mg/kg) the following levels: benzylpenicillin - 0.004-0.05, spectionomycin - 0.,2-5, dihydrostreptomycin - 0.2-1, neomycin - 0.5-5, gentamycin - 0.1-1, chloro- and oxytetracyclin - 0.1-0.6, ceftiophur - 0.2-4.

Under the conditions of increasing competition among meat producers, the situation arises, when hormone preparations are used to increase the efficiency of production. Residues of hormonal preparations may be present in the raw meat after slaughter of the animals and get into human organism with meat products. Due to the risk of the excess quantity of hormones for human organism, the legislations establish the control procedures and limit levels of their application. It is not allowed to apply stilbenes in Russia. Meat products should not contain dietylstilbestrol accordingly. The content of estradiol and testosteron should not exceed 0.0005 and 0.015 mg/kg of product, respectively. Depending on the raw materials , maximum content of growth stimulants, steroids, and β-blocking agents should not exceed (mg/kg): 0.002-0.01 of zeranol, 0.002-0.01 of trenbolon, 0.03-0.005 of carbadox, 0.025-0.0005 of dexametason, 0.025-0.005 of corasolol.

Wided application of pest-killers in plants growing lead to siguficant contamination of agricultural lands and the environment with pesticides. Pesticides are poisonous substances, excess content of which are dangerous to the human health. In accordance with regulations SanRen 2.3.2. 560-96, safe level was set for chlororganic pesticides - DDT and its metabolites, as well as for hexachlorcyclohexan (lindan) - not higher 0.1 mg/kg of product, while the permissible concentration for the most of chlororganic, phosphororganic, and triazine pesticides is 0.01-1 mg/kg of product.

Analytical control of the level of dangerous biogenic substances was traditionally carried out by means of thin-layer chromatography, high performance liquing chromatography, liquid chromatography with mass-spectrometric detection, gas-chromatography with mass-spectrometric completion as well as immuno-enzyme method "ELISA", and radio-immune method (RIA) (I).

From all mentioned methods only ELISA is the most simple, reliable, can satisfy all requirements of the routine control, and makes it possible to reveal the content of harmful admixtures 0.1 mg/ml (mkg/kg).

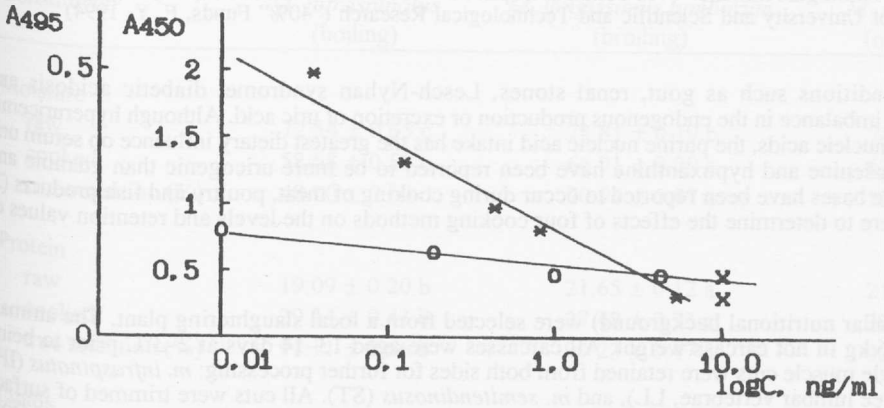
In these investigations samples of meat and meat products were used that had been presented for the certification tests under the system of certification "ИЦ ТЕСТ" - VNIIMP, and also specially contaminated meat samples comminuted with excess quantities of hormonal preparations under laboratory conditions. Diethylstilbestrol and estradyol 17 of β-production SIGMA (Germany) were used as standard samples of hormones. Besides some standard samples of hormones (OCO) were received for the test from the Institute of Nutrition (RAMN).

ELISA diagnostic sets (RANDOX Lab., Ltd., Great Britain) were used for the quantitative analysis. Results were compared with chromatographic data. Fig. 1 shows the dependence of the optical density of the dyed complex of diethylstilbestrol with conjugate obtained in the microtitre plates by RENDOX method. The character of the dependence of the change of optical density from the concentration, compared tj the standard, shows that the stilben hormone can be reliably determined in concentrations up to 10 mg/ml.

It is evident that up to 50 ng of stilbenes with 1-30 ng/ml concentration can be reliably determined by ELISA method.

Quantitative tests of meat products samples have shown that ELISA technology of RANDOX (2) with short duration of the analysis (2-3 hours for more than 100 samples simultaneously) and minimum sample preparation has a good sensitivity, allowing to determine concentrations of the controlled substances by 100 or even 1000 less than limiting permissible concentrations. According to the certificate, sensitivity of RANDOX set to some compounds being determined individually is (mkg/kg) : 0.5-5 for β-antagonists, 0,25-0,6 for corticosteroids, 0,25-1,25 for stilbenes (3).

Standard chromatographic analysis by thin-layer, gas-liquid, and liquid chromatography had inferiour indices with regards to minimum quantitative levels of substances analyzed by ELISA method.



Optical density A at 450 and 495 nm of stilben complex with conjugate RANDOX against stilben concentration in 70 % alcohol eluate.

* - a graduating curve for diethylstilbestrol standards according to RANDOX Specification at 450 nm on RANDOX reader;
o - a graduating curve for diethylstilbestrol standards (0, 0.05, 0.24, 0.56, 0.95, 4.6 ng/ml) according to RANDOX Specification at 495 nm on ECOM "Biotronic" photometer (Germany).
x - determinable concentration of diethylstilbestrol obtained in the sample treated in RANDOX immuneenzyme column (30 ng of toxicant in 3 ml of 70 % ethanol).
1 - concentration, ng/ml (log 10).

References

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