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EVALUATION OF TETRACYCLINE ANTIBIOTICS IN PORK MEAT

Abstract. In the present study, pork samples were examined for the detection of antibiotics from the tetracycline group by the immunoenzymatic method, using kits from the manufacturer R-biopharm and Biopanda Reagen. The antibiotics tetracycline, oxytetracycline and chlortetracycline were determined. The principle of the method involves the quantitative identification of the antibiotic present in the examined sample, where the fixed amount of antigen lined in the wells of the microtiter strips competes for anti-tetracycline, anti-oxytetracycline and anti-chlortetracycline antibodies which in turn are detected with the help of the enzyme conjugate. After incubation the wells are washed and the bound enzyme is visualized by adding the buffer solution. The measurement is performed photometrically at a wavelength of 450 nm after the addition of the stop solution. The absorbance value depending on the color developed is inversely proportional to the amount of antibiotic in the sample. The amount of antibiotics in the test sample can be interpolated from the standard curve constructed from the standards and corrected for sample dilution.

Introduction. Although introduced to veterinary medicine more than 60 years ago, tetracyclines are probably still the most commonly used antibiotics in animal husbandry. They have broad spectrum activity against Gram+ and Gram- bacteria. Since 1968, their use as growth promoters has also been discussed. But,

because scientists were alarmed by the state of high resistance of gram-bacteria, this led to the prohibition of various antibiotics, including tetracycline [3].

The desideratum comes from the fact that, in small quantities and through prolonged intake, antibiotics can have unwanted effects on humans and animals. Unwanted effects on the health of the consumer can be direct and manifest in the form of allergic or even toxic reactions [3].

The use of antibiotics or hormones in animals for therapeutic and growth-promoting purposes, if not applied according to regulations, can lead to the presence of traces of them as residues [4].

The monitoring of veterinary drugs in food products of animal origin, including pork, is an important element in the traceability chain as well as in ensuring their safety. Residues of commonly used tetracyclines, oxytetracycline, tetracycline and chlortetracycline can be detected in animal products such as meat, milk, eggs, honey. Quality control is considered the hallmark of the food industry, an essential condition for a product to be competitive [1].

For the detection of residues of various substances, screening tests are often used, capable of detecting the residue below the maximum allowed limits. Also, screening methods must avoid or minimize the number of false negative results as these will be considered compliant samples and not further analyzed [2].

Materials and Methods. As material, 8 pork samples were examined, purchased from the supermarket and the Chisinau municipal markets. The meat samples were packed separately in plastic bags and transported for research to the laboratory in a cooler bag with ice, where they were received and frozen at -20 °C until the research was carried out.

The preparation of research solutions was carried out according to the working protocol:

- REAGEN: for the preparation of 1xstandard diluent, 1 volume of 20x wash solution was homogenized with 19 ml of distilled water;

- BIOPANDA Reagents: 10 ml of concentrated wash buffer was homogenized with 190 ml of deionized water; 1 ml of concentrated assay diluents was homogenized with 9 ml of deionized water [8].

Chlortetracycline, oxytetracycline and tetracycline standard solutions are prepared according to the manufacturer's instructions [7].

Preparation of samples. At the first stage, the samples were homogenized. In the following steps, the working procedures of each kit's instructions were applied to each investigated antibiotic. 100µl of extract solution were used for the analysis. The standard solutions provided in the kit are concentrated and must be diluted 1:10 (1+9) with buffer solution. For the actual Elisa determination, follow the work protocol and measure the absorbance at 450 nm, compared to an blank. The average absorbance values of the standards and samples, respectively, are divided by the absorbance value of the first standard (zero standard) and multiplied by 100 [1].

Results. After the examination, the Ridawin program calculated the values of the concentration of the substance in the analyzed sample according to the absorbance, recording the semi-logarithmic plot and calculating the actual antibiotic concentration in the sample in µg/kg. The resulting concentrations of tetracycline, chlortetracycline and oxytetracycline each separately in µg/kg correspond to the absorbance of each sample and can be read from the calibration curve constructed by the software. To obtain the actual concentration of the substance in the sample, the obtained value must be multiplied by the dilution factor [1].

Conclusions

Following the research of the 8 samples, it can be observed that no residues of antibiotics from the tetracycline group were detected above the permissible limits. To eliminate the risk of contamination of products with antibiotic residues, it is necessary to monitor them.

References

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