RESEARCH ARTICLE **(Open Access)**

Assessment of stilbene residues in cattle through analytical control in Korca region

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Abstract

The use of substances having hormonal action is banned in Albania. However, sometimes forbidden drugs may be added to feeds for illegal administration or treatment to cattle for promoting increased muscle development or increased water retention and thus obtain an economical benefit. Residues of these substances may remain in meat and may pose a real threat to the consumer either through exposure to the residues. On this context use of stilbens as hormonal dugs in cattle is used in illegal way. Evaluation of stilbens residues in live cattle and beef meat samples remains a common objective of food control in Albania. Assessment of stilbene residues (diethylstilbestrol, hexerol and dienstrol) is carried out from 2012 to 2013 in 94 urine samples collected from cattle in region of Korca. Analytical control is performed by ELISA test as commercial product following the use instructions. Study results showed the positive results for stilbens group of substances in 8, 5% (8/ 94) of urine samples. Detection limit of ELISA test is respectively 0.15ng/ml for diethylstilbestrol, 0.25ng/ml for hexerol and 0.5ng/ml for dienstrol residues. 6 out 8 positive urine samples for stilbens residues contained diethylstilbestrol confirming as well use of hormones in cattle treatment.

Keyword: stilbens, residues, cattle, Korca, Albania

1. Introduction

Use of hormones as growth promoters in food producing animals is considered today as one of public health risk. There is illegal use of different substances having effect on growth rate on animal species as cattle, swine, poultry, sheep, goats ect. In cattle the use of hormones is limited to veal calves and beef cattle [2, 3, 6]. Some studies demonstrated that hormone treatment improves growth rate, nitrogen retention two months before slaughter. Beef cattle, including steers as well as heifers, were treated in large numbers, especially in the USA and the UK, with DES (diethylstilbestrol) or hexoestrol, administered orally, until the use of these compounds was restricted. Detection of stilbens residues as diethylstilbestrol were detected in beef and sheep livers in 1972 and 1973 [1, 2]. Use of diethylstilbestrol as a growth promoter for sheep and cattle, people exposed people to it at concentrations of up to 10 ppb in beef and mutton. According to Directive 96/22 EC, the EC prohibited the application, by any means to farm animals, substances having a thyrostatic, oestrogenic or gestagenic action for growth promotion purposes [3. 8] . The prohibition covers both the use of these hormones for domestic production and import of meat from animals treated with hormones for growth promotion purposes the third countries from. Some countries permit the use of the hormones in cattle as anabolic agents. In particular, the United States allows the use of 17 β-estradiol, testosterone, progesterone, and other substances for animal growth promotion individually or in combinations. The United States has opposed to the EU prohibition on the use of these hormones in animal production [2].

Diethylstilbestrol (DES), hexestrol and dienestrol (oestrogenic hormones) are collectively called stilbenes, which are banned worldwide for use in food producing animals. As promoting agents used in meat production, stilbenes are initiating the carcinogenic process at small concentrations in human. On this context use of effective screening methods play an important role on food safety. The groups of stilbenes, which are very efficient anabolic steroids, combined with a strong estrogenic activity, because of the potential carcinogenic effects of its residues on human health [8]. The administration of diethylstilbestrol to food-producing animals was prohibited. The European Community allows a limit of 2 ppb DES in urine or fish. Control of stilbens residues in live animals and

their products is remaining a permanent objective of food control authorities in Albania.

2. Material and methods

In region of Korca are collected samples from farms and slaughterhouses for control of residues of diethylstilbestrol, hexestrol and dienestrol. Urine samples are collected from live cattle in different locations. The object of study has been as well meat samples which are collected from slaughterhouses in Devoll, in Pogradec and in Kolonja districts. All samples are taken in aseptically way and are kept and transported in $4^{o}C$ to laboratory. The chosen procedure was ELISA essay as confirmatory test. Control laboratories applying this test can face a large number of samples in relatively short periods of time. Diethylstilbestrol-residues were determined by enzyme immunoassay (ELISA) as a screening system, which is simple, rapid, sensitive and cost-effective compared with traditional methods. MaxSignalTM Diethylstilbestrol (DES) ELISA Test Kit enables to detect diethylstilbestrol in animal matrices in response to customer concerns about food safety. The method is based on a competitive colorimetric ELISA assay. The drug of interest has been coated in the plate wells. During the analysis, sample is added along with the primary antibody specific for the target drug. If the target is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the drug attached to the well. The secondary antibody, tagged with a peroxidase enzyme, targets the primary antibody that is complexed to the drug coated on the plate wells. The resulting color intensity, after addition of substrate, has an inverse relationship with the target concentration in the sample. There are used three different antibodies to detect respectively diethylstilbestrol, hexerol and dienstrol residues in urine samples collected from farms and slaughterhouses in Korca region.

Samples were refrigerated at 2-4°C for no more than 1-2 days. For long period of store samples have been kept in freezing at -20°C. Frozen samples were thawed at room temps (20 – 25°C) or in a refrigerator before use. Detection limit of this kit for Diethylstilbestrol in urine is 0, 15pp/g. MaxSignalTM Diethylstilbestrol (DES) ELISA Test Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (assuming 12 wells for standards). The kit is storedat 2-8°C. The shelf life is 12 months when the kit is properly stored. The test was performed according to instruction for use.

3. Results and discussion

A survey carried in 2012-2013 in 94 urine samples collected from cattle in region of Korca evaluated the stilben's residues level. Analytical control is performed by ELISA test as commercial product. Study results showed the positive results for group of stilben substances in 8, 5% (8/ 94) of urine samples. 6 out 8 (75%) positive urine samples for stilbens residues contained diethylstilbestrol confirming as well use of hormones in cattle treatment**.** 2 other positive samples or 2% of total samples were contaminated with hexerol. The problem is then to discriminate between physiological concentrations and elevated hormone levels due to the administration of natural anabolics [1. 5, 6, 7]. This is not an easy task owing to the large variability described in the literature for the concentrations of the various steroids in different matrices. In the case of veal calves have established reference values for these substances in plasma and urine. The authors concluded that the best criterion to use to detect treated animals is the urinary or the plasma concentration of diethylstilbestrol, which increases after the treatment and remains for a long time [6]. They established levels for matrices, urine and plasma is 0.15 ppb/ml [4, 8]. In the instance of bulls, concentrations of diethylstilbestrol decreased in the plasma after treatment and detection of it sometimes is not easy. The National Residues Control Plan should guarantee the fulfillment of the requirements which are of importance to health of both humans and animals as well as to marketing of animals, foods and other products of animal origin.

Table 2. Assessment of stilbene residues (diethylstilbestrol and hexerol) in 94 urine samples collected from cattle in region of Korca in 2012-2013.

Urine samples	*No. samples*	*No. of positive samples (stilbens residues)*	*No. of positive samples (diethylstilbestrol)*	*No. of positive samples (hexerol)*
Farms	49	3	2	0
Slaughterhouses	45	5	4	2
Total	94	8/94 (8%)	6/94 (6.3%)	2/94 (2.1%)

4. Conclusion

The assessment of stilbens residues in live cattle and beef meat samples remains a common objective of food control in Albania. In 2012-2013, the control of 94 urine samples collected from beef cattle in Korca region is achieved by use of ELISA test. Study results showed the positive results for stilbens group of substances in 8, 5% (8/ 94) of urine samples. Diethylstilbestrol (DES) residues are found in75% of all positive samples demonstrating the use of this substance in treatment of the cattle as promoter growth.

5. References

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