Research Article

Healthcare

Keywords: stilbens, residues, calves, Korça, Albania.

Assessment of Stilbene Residues in Calves through Analytical Control in Korça Region in Albania

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Abstract

The hormonal substances are used for different proposes in animal treatment. Sometimes these substances are used for animal feeding or are used as growth promoters in cattle. Application of stilbenes as growth promoters in calves is done in illegal way. Residues of these substances in calve s' meat are exposing consumers with high level of concentration causing toxic syndromes associated with carcinogenesis in human. Control of stilbene residues in calves especially in in Albania is important because the calve s' meat is delicious food. This study evaluated situation of stilbene residues (diethylstilbestrol, hexerol) in 112 urine samples collected from calves in region of Korca. The analytical check is carried out in 2014 by using ELISA test as qualitative screening method for detection of stilbenes in urine samples. Diethylstilbestrol and hexerol residues are detected in 6, 2% (7/112) of urine samples collected from farms and slaughterhouses. The incidence of diethylstilbestrol residues in urine samples collected from farms was 4% (3/75) and 5.4% (2/37) in urine samples came from slaughterhouses. The incidence of hexerol in urine samples collected from different farms was 2.7% or 2 out 75. No positive cases of hexerol residues are confirmed in urine samples taken from slaughterhouses.

Introduction

New EU guidelines for identification and quantification of organic residues and contaminants are established to guarantee efficient control of prohibited growth promoting agents, as are steroids beta-agonists and other veterinary drugs (Heitzman, 2004). Exposure of human body with stilbens causes implications manifested with different toxic syndromes (Arts et al., 1991). The detection of a prohibited compound needs more information on residue structure and on biotransformation of registered veterinary drug regarding to the mass fraction and exceeds of the established Maximum Residue Limit.

The European Commission (EC) ban the production and importation of meat derived from animals treated with growth-promoting hormones in the early 1980s. These measures are related to natural hormones used for therapeutic purposes in treatment of animals (Scippo et al., 1993). In, 1989 the ban covered meat and meat products from animals treated with six growth promotants that are approved for use and administered in the United States, including estradiol, testosterone, progesterone, zeranol, trenbolone acetate and melengestrol acetate (FDA, 2009). Later, in 2003, the commission amended its policy approving permanently ban of 17β-estradiol.

The EU improved food safety policy, known as the precautionary principle, which supports taking protective action before there is complete scientific proof of a risk. The ban also effectively restricts trade of meat and meat products from countries that regularly treat farm animals with these growth promoters ((Heitzman, 2004).

The metabolism of diethylstilbestrol (DES) in calves has been reviewed recently. The substance is eliminated to a large extent in unaltered form. After oral administration of DES (diethylstilbestrol) in calves, 99.5% of this amount was excreted within 5 days after withdrawal. In liver extracts, this hormone is conjugated and it was found to be 75% and 25% respectively (Schilt et al., 1996). The activity of diethylstilbestrol was observed after withdrawal in kidney, liver, bile and urine for up to 5, 7, 9 and 11 to 12 days respectively (Scippo et al., 1993). The residues of DES in calves are detected until 98 days after the treatment. At the time of slaughter, levels were less than 0.1 ppb in muscle and fat, and 1 to 1.5 ppb in liver and kidney.

The residues of diethylstilbestrol are found in spleen, lung, adrenal glands and kidney, but less than level 0.5 ppb (Newbold et al., 2000). Other study on steers confirmed the diethylstilbestrol residues after 120 days of injection. Levels in liver, kidney, lungs and salivary glands were in the range of 0.07 to 0.13 ppb. In a recent study diethylstilbestrol metabolism in rhesus monkeys and chimpanzees, most of the substance was excreted with the urine (FDA, 2009).

Extracts in the organic and aqueous phase mostly contained unchanged diethylstilbestrol in the free and conjugated form respectively (Raun et al., 2000). Current evidence indicates that the oxidative metabolism of diethylstilbestrol leads to at least three compounds that may have cytotoxic or mutagenic activity, but these have not been identified as diethylstilbestrol metabolites in ruminants (Yobi et al, 2015). Derivates of hexerol are metabolic biotransformation of diethylstilbestrol in cattle organism. This study was focused on detection of DES and hexerol residues through control of urine samples collected from farms and slaughterhouses.

Material and methods

In region of Korca are collected samples from farms and slaughterhouses for control of residues of diethylstilbestrol, hexestrol. Urine samples are collected from live calves in different locations. As study material 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.

Results and discussion

Table 1. Control of stilbene residues (diethylstilbestrol and hexerol) in112 urine samples collected from calvesin region of Korca in 2014.

Urine samples	No. samples	No. of positive samples (stilbens residues)	No. of positive samples (diethylstilbestrol)	No. of positive samples (hexerol)
Farms	75	5/75	3/75	2/75
Slaughterhouses	37	2/37	2/37	0/37
Total	112	7/112 (6.2%)	5/112 (4.5%)	2/112 (1.8%)

This analytical check carried in 2014 evaluated the stilben residues level in 112 urine samples collected from calves in region of Korca. Analytical control is performed by ELISA test as commercial product. Study results showed the positive results for group of stilben substances in 6, 2% (7/ 112) of urine samples. 5 out 7 (71%) positive urine samples for stilbens residues contained diethylstilbestrol confirming as well use of hormones in calve treatment**.** 2 other positive samples or 1.8 % of total samples were contaminated with hexerol. Discrimination of physiological concentrations and elevated hormone levels due to the administration of natural anabolic is remaining difficult (Schilt et al., 1996). 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. In Netherland in 1984 samples of urine from slaughtered cattle were inspected for the presence of the stilbene derivatives diethylstilbestrol (DES), dienestrol (DE), and hexestrol (Jansen et al., 1985). From the fast screening stilbens residues were detected in 1.7% of urine samples. They established levels for matrices, urine and plasma is 0.15 ppb/ml. 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 (Scippo et al., 1994). In the instance of bulls, concentrations of diethylstilbestrol decreased in the plasma after treatment and detection of it sometimes is not easy. The excretion of DES in urine was faster for animals when are applied oil group of these substances. The DES content in urine

was decreased to the 1 µg/l level after 42 days for emulsion group of substances or 70 days for oil group of stlibens (Janesen et al., 1985). The excretion in feces was comparable to that in urine. After day 21 the excretion patterns of the two excreta were indistinguishable (Raun & Preston, 2002). The above discussion support monitoring initiatives of stilbens residues which are exposing the consumers periodically through consumption of meat.

Conclusions

This study carried out in 2014 concluded that stilbens residues are present in calves in region of Korca. Study results showed that there is illegal use of diethylstilbestrol in calves as growth promoter. Positive results for stilbens residues by analytical check of urine samples in calves are confirming the risk of stilbens residues as carcinogenic substances in humans. Calve meat should be under the monitoring and inspection by official veterinary service in Albania

References

1. Arts CJM, Van Baak MJ, Den Hartog JMP: Control system for detection of the illegal use of naturally occurring steroids in calves. *Journal of Chromatography 1991*, 564: 429-444.
2. FDA, U.S. Food and Drug Administration: The Electronic Orange http://www.fda.gov/cder/ob/default.htm and select Search by Active Ingredient and Discontinued Drug Products and search on diethylstilbestrol. *Book 2009*, Last accessed: 10/22/09.
3. Heitzman RJ: Commission of the Europ. Comm. Residues in food-producing animals and their products. Reference materials and methods. *Dziennik Ustaw* 2004, *91,* 456.
4. Schilt R, Stephany RW, Arts CJM, Frijns LMH: Estradiol levels in urine of veal calves as indicator of treatment: Possibility or fiction? *EuroResidue III. Conferece on Residues of Veterinary Drugs in Food.* Veldhoven, The Netherlands, 1996.
5. Scippo ML, Gaspar P, Degand G, Brose F, Maghuin-Rogister G: Control of the illegal administration of natural steroid hormones in urine and tissues of veal calves and in plasma of bulls. *Anal. Chim. Acta*, 1993, 275: 57-74.
6. Scippo ML, Degand G, Duyckaerts A, Maghuin-Register G: Control of the illegal administration of natural steroid hormones in plasma of bulls and heifers. *Analyst*, 1994, 119: 2639- 2644.
7. Newbold RR, Hanson RB, Jefferson WN, Bullock BC, Haseman J, McLachlan JA: Proliferative lesions and reproductive tract tumors in male descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis 2000*, 21(7): 1355-1363.
8. Raun AP, Preston RL: History of diethylstilbestrol use in cattle. *J Anim Sci* 2002. http://www.asas.org/Bios/Raunhist.pdf.
9. Jansen E. H. J. M. & Stephany R. W. Effective control for diethylstilbestrol in cattle in the Netherlands. *Journal Veterinary Quarterly*. 1985, 7: 35-38.
10. Yobi Kim, Hyo-Jin Kim, Soohee Kim, ByungJae So, Jae-Young Song, Sung-Won Park. Monitoring and surveillance testing for residual veterinary drugs and pesticides in domestic meat between 2012 and 2013. *J. Prev. Vet*. Med 2015; 39(3): 114-118.