HORMONE RESIDUES CONTROL
IN SLAUGHTERED ANIMALS IN POLAND IN 2000 - 2001

BARBARA WOŹNIAK

Department of Hygiene of Food of Animal Origin,
National Veterinary Research Institute, 24-100 Pulawy, Poland
e-mail: bwozniak@piwet.pulawy.pl

Examinations of anabolic hormone residues have been undertaken in Poland since 1990. In 2000 - 2001, 5393 animals (cattle, pigs, horses, rabbits, chickens, geese, turkeys, ducks and fishes) were tested for residues of diethylstilbestrol, hexestrol, dienestrol, zeranol, trenbolone, 19-nortestosterone and 632 cattle and swine for medroxyprogesterone acetate. Additionally, 1176 cattle were examined for natural 17β-estradiol and testosterone. Serum natural hormones were detected by the ELISA, and the remaining hormones by 2-D HPTLC. In 3 heifers and in 2 cows, the serum testosterone level exceeded the maximum level accepted by EC - 0.5 µg/l. Two bulls and 4 cows turned out to have increased content of 17β-estradiol in their blood. No remaining hormones were found. The National Residue Control Plan is useful in preventing the occurrence of undesirable residues in edible tissue of meat producing animals.

Key words: anabolic hormones, residues, meat producing animals.

Since the 1st January 1989, according to Directive 88/146/EEC replaced later by 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. 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. Unlike the EC, some countries permit the use of the hormones in cattle as anabolic agents. In particular, the United States allows the use of 17β-estradiol, testosterone, progesterone, zeranol, trenbolone acetate and melengestrol acetate for animal growth promotion individually or in combinations. The United States has opposed to the EU prohibition on the use of these hormones since its implementation.

In Poland, the use of hormones as growth promoters has been made illegal too (3). The examination of anabolic hormone residues started in Poland in 1990 in the frame of the National Residue Control Plan. The General Veterinary Inspectorate has been responsible for the establishment and execution of the Plan. The Plan must be conformed to the regulations laid down in Directive 96/23/EC and in Polish regulation of the Ministry of Agriculture and Food Economy of October 12, 1999. The Plan has to pay attention to two groups of substances, namely: the group of anabolic and prohibited
substances, and the group of veterinary drugs and contaminants. The number of samples collected is always based upon a percentage of the slaughtered animals in the previous year. According to the plan the residues of the hormones are determined in the urine, blood, fat and muscles of slaughtered animals and in the blood and urine of living animals and additionally at farms in drinking water. The examinations are carried out in the National Veterinary Research Institute in Pulawy and in five specialized veterinary diagnostic laboratories according to the requirements of the European Community.

Material and Methods

Sampling. In the years 2000 - 2001 the samples of urine, blood, muscles and fat were taken from animals in slaughterhouses situated in the whole country. The samples of drinking water, blood and urine of living animals were taken at farms. For cattle one half of the samples have been taken from live animals on the holding and one half at the slaughterhouse. In the case of swine, the minimum number of farms to be visited annually must represent at least one farm per 100 000 pigs slaughtered the previous year. For each category of poultry, equivalent of one-fifth of samples was taken at farm level. Samples were collected by official inspectors of the Veterinary Sanitary Inspection Service and sent to the relevant laboratories. Altogether 5393 samples from the animals were collected.

Samples preparation. Bovine serum samples were prepared and assayed for estradiol 17β and testosterone according to the ELISA protocol (R-Biopharm) in the National Veterinary Research Institute in Pulawy. The examinations of other hormone residues were carried out in the Institute and in five the regional veterinary laboratories, which used the same method and took part in the interlaboratory studies. The analysis of hormone residues in urine and tissues was based on the previously described method (7). The hormones were extracted from tissue samples with methanol, the metanol phase was degreased with n-hexane and extracted with diethyl ether. After evaporation of the ether layer, the residue was dissolved in acetate buffer and cleaned up by solid phase extraction on C18 column. From urine, the hormones were extracted with diethyl ether followed by the procedure corresponding to that for muscles. The samples were analysed by two-dimentional high performance thin layer chromatography and sprayed with sulphuric acid in ethanol. The detection limit for trenbolone was 0.5 µg/kg (l) and 2 µg/kg (l) for the remaining compounds.

Results

Altogether 3856 bovine and swine urine samples taken at farms and in slaughterhouses and 99 drinking water samples taken at the swine farms were examined for hormone residues. Neither the in farms nor in slaughterhouses the collected samples contained residues of diethylstilbestrol, hexestrol, dienestrol, zeranol, 19-nortestosterone and trenbolone. The results show (Table 1) that the blood of 11 cattle contained an excessive quantity of natural hormones. After farm sampling, 3 heifers turned out to have a higher quantity of testosterone in their blood and blood of one bull and 2 cows showed an excessive quantity of 17β-estradiol.
Table 1

Results of examination of hormone residues in slaughter animals in Poland in 2000 – 2001

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>cattle</th>
<th>swine</th>
<th>horses</th>
<th>poultry</th>
<th>rabbits</th>
<th>fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>urine</td>
<td>serum</td>
<td>fat</td>
<td>water</td>
<td>urine</td>
<td>fat</td>
</tr>
<tr>
<td></td>
<td>f+s</td>
<td>f+s</td>
<td>s</td>
<td>f</td>
<td>s</td>
<td>f+ s</td>
</tr>
<tr>
<td>Białażyok</td>
<td>219/0</td>
<td>57/0</td>
<td>18/0</td>
<td>171/0</td>
<td>7/0</td>
<td>24/0</td>
</tr>
<tr>
<td>Gdañask</td>
<td>264/0</td>
<td>67/0</td>
<td>40/0</td>
<td>183/0</td>
<td>22/0</td>
<td></td>
</tr>
<tr>
<td>Katowice</td>
<td>308/0</td>
<td>75/0</td>
<td>24/0</td>
<td>140/0</td>
<td>44/0</td>
<td></td>
</tr>
<tr>
<td>Poznań</td>
<td>312/0</td>
<td>73/0</td>
<td>54/0</td>
<td>281/0</td>
<td>20/0</td>
<td>27/0</td>
</tr>
<tr>
<td>Warszawa</td>
<td>427/0</td>
<td>84/0</td>
<td>44/0</td>
<td>240/0</td>
<td>24/0</td>
<td>16/0</td>
</tr>
<tr>
<td>Puławy</td>
<td>291/0</td>
<td>76/0</td>
<td>20/0</td>
<td>118/0</td>
<td>16/0</td>
<td>55/0</td>
</tr>
</tbody>
</table>

Total 1821/0 1176/11* 432/0 2035/0 200/0 56/0 1133/0 61/0 188/0

f - samples taken at farms; s - samples taken at slaughterhouse

* excessive quantity of 17β-estradiol - 3 samples, testosterone - 3 samples (f)

excessive quantity of 17β-estradiol - 3 samples, testosterone - 2 samples (s)
After the slaughterhouse sampling, an excessive concentration of 17β-estradiol was found in the blood of 2 cows and one bull, and testosterone in the blood taken from 2 cows. No gestagens such as medroxyprogesterone acetate were found in kidney fat. In poultry (chickens, geese, turkeys, ducks), rabbits and fish no residues of stilbenes, 19-nortestosterone, trenbolone and zeranol were found.

Discussion

A survey carried out in Poland in 2000 - 2001 demonstrated that the incidence of residues of anabolic hormones in tissues of slaughter animals is not a problem. In particular, urine samples of young cattle, cows, pigs, horses and muscles from poultry tested for the presence of residues of stilbenes, zeranol, trenbolone, 19-nortestosterone gave no evidence for the illegal use of these hormones in Poland. Only in 5 female bovine, the serum testosterone level exceeded the maximum level accepted by EC - 0.5 µg/l and the blood of 2 bulls and 4 cows contained an excessive quantity of estradiol-17β (above 0.04 µg/l). In previous years (1990 - 1998), 4857 urine samples were tested and only zeranol was found in one. In the mentioned period four cases of stilbenes and one of trenbolone were noted in poultry (1075 samples were analysed). In 1992-1998, 2051 cattle were examined for natural hormones: 17β-estradiol and testosterone. In 20 cases (females), the blood serum testosterone level exceeded the maximum accepted level and a higher concentration of 17β-estradiol was found in one case (8). Natural hormones are substances which are secreted in small doses by certain glands or during certain biological processes in the body. The hormones are transported to the target organs via the bloodstream, where they combine with specific receptors and generate physiological effects. The problem is then to discriminate between physiological concentrations and elevated hormone levels due to the administration of natural anabolics. 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 (4, 5). In the case of veal calves, Arts et al. (1) have established reference values for 17β-estradiol and testosterone in plasma and urine. They studied veal calves treated with a single injection of a testosterone-estradiol cocktail, and measured the concentrations of these hormones in the plasma and urine of treated animals. The authors concluded that the best criterion to use to detect treated animals is the urinary or the plasma concentration of 17β-estradiol, which increases after the treatment. They established decision levels of 1 µg/l and 0.02 µg/l in urine and plasma, respectively. For male veal calves levels of testosterone in blood plasma and urine varied widely. Scippo et al. (6) analysed steroid hormone concentrations in the plasma of adult cattle, before and after their treatment with natural steroid hormones, and established for them decision levels. For heifers, 17β-estradiol and testosterone concentrations in plasma are the best criteria to use to detect treated animals, with decision limit of 0.02 µg/l and 0.125 µg/l, respectively. In the instance of bulls, testosterone concentrations decreased in the plasma after treatment and the proposed decision limit is 1.5 µg/l. Action levels for 17β-estradiol and testosterone in plasma of cattle, which are dependent on age or sex are indicated by the EC (2). To exclude false-positive results, the decision level proposed by EC for 17β-estradiol in blood plasma was fixed at 0.04 µg/l in both male and female cattle. For testosterone, it was set up for female cattle at 0.50 µg/l and for male cattle at 10-30 µg/l.
depending on age. The reason of positive results for natural hormones obtained in Poland is unknown. It is possible that a mistake during sampling procedures occurred. Recently, the sampling system in Poland was modified and improved to eliminate any incorrectness. The National Residues Control Plan guarantees the fulfilment 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.

References