GRESS IN THE STRATEGY OF DISCRIMINATION OF MEAT-PRODUCING ANIMALS TREATED WITH WTH PROMOTERS*

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oduction

consumer has lost confidence in the food chain as a result of scandals reported in the media ecent years especially of the use of non registered products and even prohibited substances as growth promoters in meat production. The ban on the use of anabolic hormones in the EEC er states was a political decision devoid of scientific justification (MAGHUIN-ROGISTER,). The prohibition has not achieved his aim of protecting consumers since numerous tances are used illegally: artificial or natural anabolic hormones with androgenic, ogenic or gestagenic activities; ß-agonists; thyrostatic drugs; corticoids. macotoxicological effects can be expected from exogenous substances that depend on their re and dose. Recent massive intoxication in Spain caused by the presence of high centrations of clenbuterol residues in liver and in meat exemplifies that situation. The mentation of the EEC directive (88/146) "prohibiting the use in livestock farming of certain tances having a hormonal action", must be guarantied by an efficient system of control.

e somatotropine (BST, also called bovine growth hormone) is an efficient enhancer of milk or production. The safety of BST treatment is now well demonstrated both for animals and for human consumer of foodstuffs from animal origin. In the case of protein hormones like BST will be used as foodstuffs production enhancers, a strategy of control is required for the owing reasons: 1) the moratorium against BST applied in the EEC; 2) the risk for genetic ction of BST misuse in reproductive animals.

ible and efficient strategies of control are needed to reestablish the confidence and the tection of the consumers through official controls organised by the state department of iculture and of public health or through responsible label organisations involved in the lity assurance certification of foodstuffs. In this paper, criteria for the identification of le treated with artificial or natural anabolic hormones, with ß-agonists or with BST are ewed.

erial and methods

ificial anabolic hormones

rding to the commission decision (87/410/EEC) "laying down the methods to be used for ecting residues of substances having a hormonal action and of substances having a thyrostatic ion", the following methods can be used for the control of anabolics: immunoassay (IA), thin r chromatography(TLC), high performance liquid chromatography (HPLC), gas chromatography C), mass spectrometry (MS) and spectrometry (IR). Artificial hormones may thus be assayed in al tissues (muscle, liver, kidney, fat) or in excreta (urine, faeces) using immunochemical or nochemical methods. Immunochemical methods involve radio-immunoassay (RIA)(GASPAR and GUIN-ROGISTER, 1985; GASPAR et al, 1986), enzyme-immunoassay (EIA)(DEGAND et al., 1989) and miluminescent-immunoassay (CLIA) have been reported for the determination at the ppb (µg/kg) el of the main artificial estrogenic and androgenic compounds (stilbenes, methyltestosterone, testosterone, trenbolone, zeranol, ethinylestradiol). Generally, immunoassays are used as ening methods and the positive results are confirmed by a physicochemical method (MAGHUIN-LOYER, 1991b). High performance thin layer chromatography (HPTLC) (VERBEKE, 1979; DE BRABANDER VAN HOOF, 1990) has been applied to the identification of numerous anabolic hormones in meat, or urine samples. Mass spectrometry (MS) most often coupled to gas chromatography (GC-MS) its the reliable identification of residues by giving direct detailed informations about

their molecular structure (BERGNER-LANG and KACHELE, 1981, 1987; VAN PETEGHEM et al., 1987) is also very helpful in the identification of new compounds.

Natural sex steroid hormones, their metabolites and biosynthetic precursors.

In the aim to develop a system for the control of the illegal use of natural steroid hormones, have measured, by specific radioimmunoassays, 17 ß-estradiol, 17 alpha-estradiol, estro epitestosterone, and testosterone concentrations in urine of male and female veal calves and plasma of bulls. The samples originated from untreated and treated animals (veal calves treated by estradiol and/or testosterone-containing implants and the bulls by a single injec of 20 mg of estradiol benzoate and 200 mg of several testosterone esters).

For these endogenous compounds, data were statistically analysed using the Wilcoxon test evaluate the significance of the difference between treated and control (untreated) animals. decision limit was defined by the highest hormone concentration found in the control animals the treatment effect is an elevation of that concentration, the lowest hormone concentrat found in the control animals if the treatment effect is a diminution of that concentration.

ß-agonists

Among the most sensitive methods, we can enumerate: immunoassays (DELAHAUT et al., 1991; MEYER al., 1991; DEGAND et al., 1992a), HPLC (DEGROODT et al., 1989), GC-MS (VAN GINKEL et al., 19 Some are specific for the determination of clenbuterol, other can be used for the detection several ß-agonists: clenbuterol, salbutamol, cimaterol, mabuterol, terbutaline, mapenterol,... have developed an enzyme immunoassay with an anti-salbutamol antiserum that crossreacts w several ß-agonists (DEGAND et al., 1992b).

Bovine somatotropine

Immunoplates were successively coated with BST in 50 mM bicarbonate buffer, saturated with casein solution (25 mg/ml, 200 µl/well). After washing, they are incubated 2 h at 37°C in t presence of diluted plasma. Anti-BST antibodies bound to the plate are bring together with t second antibody (anti-bovine IgG coupled to horse radish peroxidase) and the enzymatic activi was measured after incubation with the substrate (H_2O_2, tetramethyl benzidine). IGF-BP profi was determined by western blotting according to Hardouin et al (1987).

Results and discussion

Using immunochemical (RIA, EIA) and physicochemical (HPTC, GC-MS) methods, non endogen synthetic steroids and related anabolic compounds like DES and zeranol can be detected in urin faeces, plasma or animal tissue samples with a limit of detection ≤ 2 ppb. The same holds t for ß-agonists, like clenbuterol, for which limits of detection as low as 0.2 ppb can be reach by immunoassay and HPLC.

An efficient strategy of control of the illegal use of these artificial growth promote generally involves the screening of large series of samples using immunoassay. Samples fou positive during the screening are then examined for confirmation by HPTLC, HPLC or GC-MS. Th procedure allows the examination of numerous samples at relatively low cost and the high expenses of time and money linked to more sophisticated methods like GC-MS is avoided f negative samples. Another advantage of such a control is to limit false positive results by usi two analytical methods based on totaly different principles: 1) recognition by an antibody; identification based on physicochemical (chromatography) or molecular (mass spectrometry properties. To keep the number of false negatives as low as possible, a general survey on limited number of samples must be organized in parallel with the main control in order t identify new compounds by using multiresidue methods preferably mass spectrometry.

Anabolic treatments with natural steroids is much more difficult to prove. The problem is then t discriminate between physiological concentrations and elevated hormone levels due t administration of natural hormones or their derivatives. Esters of estradiol or testosterone ca easily be identified in injection sites using HPTLC and, if they are discovered, the proof o illegal administration of hormone is established. In order to make possible the control in livin cattle of the illegal use of natural sex steroid hormones as anabolic, we have determined norma limits of hormone concentrations in urine of veal calves and in plasma of bulls. When the hormo

s found in treated animals were significantly different ($p<0.05$) from those found in eated animals, a decision level was established and the score of efficacy of that decision was calculated (i.e. the percentage of treated animals detected when that limit is ed). The results obtained are given in table 1. We agree with the conclusion of Arts et al.) for veal calves: the discrimination between untreated animals and veal calves injected estradiol containing solutions is possible with a decision limit of 1 ng/ml of 17ß-estradiol ine.

re involved in a research program on the development of immunochemical and physicochemical ods for the discrimination between cattle untreated or treated with bovine somatotropine T). Four companies are producing BST by genetic engineering. One preparation of BST has a cture identical to that of natural pituitary BST, the other three showing minor differences tructure that are localised at the amino terminal end of the sequence. These slight ctural differences can be helpful for the discrimination between untreated and treated le. Our strategy involves the examination of blood samples from suspected cattle for:
he detection of anti-BST antibodies using an ELISA. Our preliminary results indicate that -BST antibodies appear in the blood of treated (100µg BST/kg/day) veal calves after 28 or 36 s of treatment and after 12 weeks in cows treated 6 times at 15 days interval with 500 mg of

he determination of the insulin-like growth factors binding proteins (IGF-BPs). A limited rriment showed us, 4 days after treatment, a dramatic decrease in the concentration of IGF-BP2 a moderated increase in IGF-BP3 in the plasma of treated cows (treated with 500 mg of BST). preliminary observation was later confirmed in the literature (COHICK et al.,1992)
he quantitative (by immunoassay) and qualitative (by physicochemical methods) analyses in rt to establish decision levels or to demonstrate the presence of exogenous BST (DNA rec.BST).

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BLE 1. **LEVELS OF ENDOGENOUS STEROID HORMONES IN URINE OF VEAL CALVES AND IN PLASMA OF BULLS**

	Decision level	Score (%)			
ine of male and ale veal calves					
ss + conjugated hormone)					
17ß-estradiol	1 ng/ml	95			
17α-estradiol	23 ng/ml	58			
testosterone	0.5 ng/ml (female veal calves)	90			
	2 ng/ml* (male veal calves)	95			
sma of bulls		Days after injection			
ss hormone)		2	3	5	7
17ß-estradiol	40 pg/ml	100	100	64	45
17α-estradiol	70 pg/ml	36	9	-	27
estrone	7 pg/ml*	64	-	73	64
epitestosterone	1 ng/ml	27	27	-	9
testosterone	-	-	-	-	-

minimum level

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