

RESIDUAL STUDY IN LIVER AND BILE AFTER A GROWTH PROMOTING TREATMENT IN MALE BEEF CATTLE

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Overview

A residual study was carried out in male cattle implanted with REVALOR-XS[®] (trenbolone acetate and estradiol). At the end of treatment (70 days) liver and bile samples were analysed determining eleven steroids with liquid chromatography tandem mass spectrometry technique (LC-MS/MS). The results evidenced that in bile from treated animals the levels of the marker residue (α -trenbolone) are very high suggesting the possible usefulness of this biological fluid in the control of steroid abuse.

Animal experiment

Sixteen male beef cattle (10 - 14 months - Charolaise) were implanted for 70 days with REVALOR-XS[®] (Merck Animal Health) containing pellets of trenbolone acetate and estradiol. At the same time sixteen male beef cattle were kept as a control group.

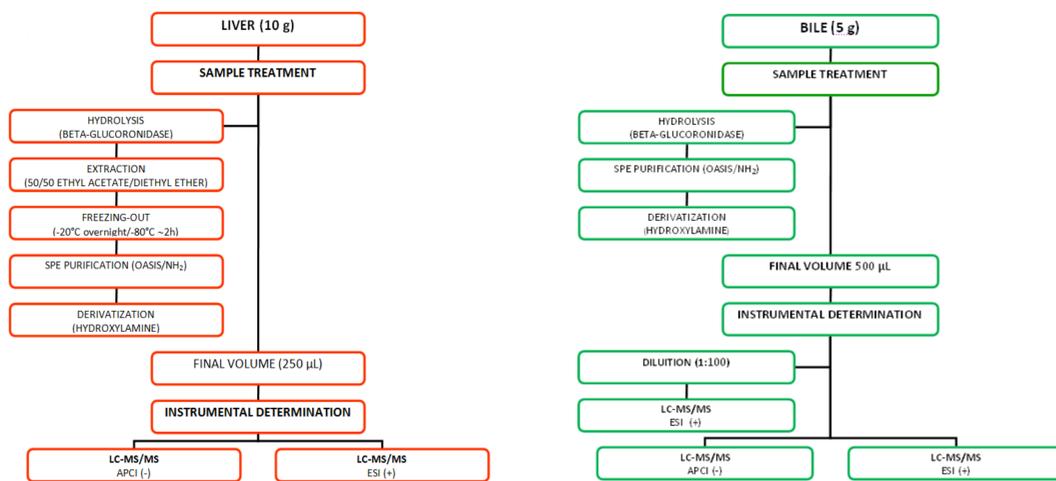
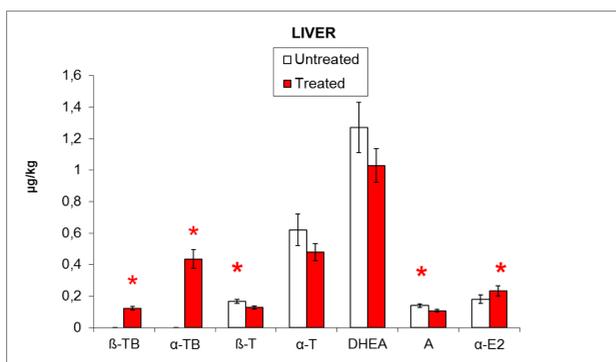


Figure 1. Flow diagrams of sample preparation

Figure 2. Steroid levels in liver



Introduction

The use of anabolic steroids is prohibited in food producing animals in the European Union. Because efficient control must consider both metabolic patterns and associated kinetics of elimination, in this work a residual study in bovine liver and bile after typical growth promoting treatment (trenbolone acetate and estradiol) is described. A selected group of eleven synthetic and natural steroids was determined: α - and β -estradiol (E2), α - and β -trenbolone (TB), androstenedione (A), dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT), estriol (E3), estrone (E1) and α - and β -testosterone (T).

LC-MS/MS Method

- Instrument: Thermo Electron Corporation HPLC Surveyor - TSQ Quantum ULTRA
- Column: Phenomenex Kinetex C18 (100 x 2.1 mm; 2.6 μ m)
- Flow-rate: 0.2 mL/min

Run A) Ionization mode HESI (+): Derivatised steroids (A, DHEA, DHT, T, TB, E1); Mobile phases: 0.1% Acetic Acid - Methanol

Run B) Ionization mode APCI (-): Underivatised steroids (E2 and E3); Mobile phases: Water - Methanol

Results

Liver - Both isomers of the administered synthetic steroid trenbolone were detected in liver of all the treated animals. The measured levels ranged between 0.06 and 1.1 μ g/kg for α -trenbolone and between 0.05 and 0.20 μ g/kg for β -trenbolone. Among the natural steroids α - and β -testosterone, DHEA, androstenedione and α -estradiol were found both in the treatment and in the control group (Figure 2).

Bile - The found natural steroids in bile were the same as in liver, but in addition estrone was detected (Figure 3). The concentrations of all analytes were generally higher than in liver. For an example, α - and β -trenbolone in implanted cattle ranged from 15 to 93 μ g/kg and from 0.08 to 0.77 μ g/kg, respectively.

Other matrices - At the end of the same animal experiment also plasma, urine, kidney and muscle were sampled. Relative concentrations of trenbolone isomers are shown in Figure 4.

Figure 3. Steroid levels in bile

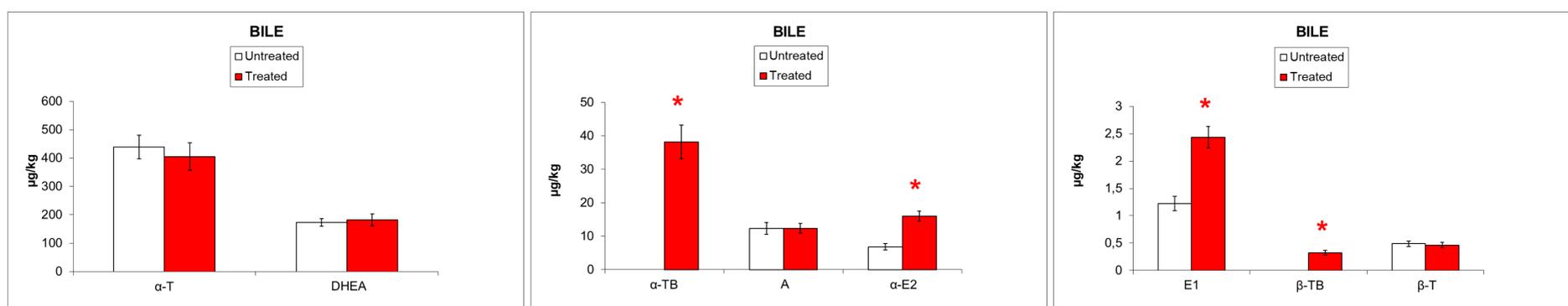
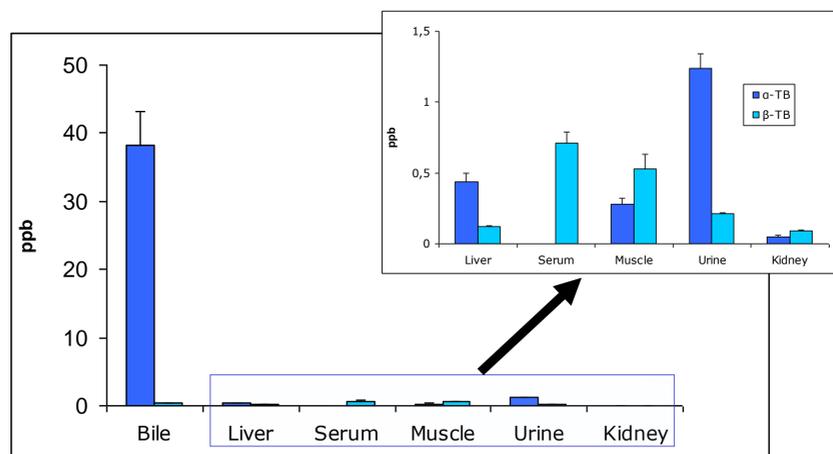
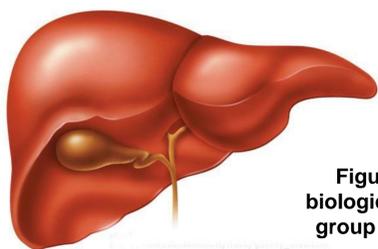


Figure 4. Trenbolone residues in biological fluids and tissues of treated group at the end of the experimental study with Revalor-XS[®]



Conclusions

Few data are available about the steroid levels in bovine bile following growth-promoting treatment. The high concentrations of the marker residue (α -trenbolone) found in bile of treated animals could suggest a new possible scenario in the control of steroid abuse complementing or replacing at the slaughterhouse conventional matrices such as urine or liver. Additional animal experiments changing substances, administration routes and withdrawal times are necessary to confirm the possible usefulness of this biological fluid in the control of steroid abuse.

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