ABUSE OF ANABOLIC AGENTS IN BEEF CATTLE: BILE AS A POSSIBLE ALTERNATIVE MATRIX FOR OFFICIAL CONTROL

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INTRODUCTION

The use of hormonal substances in food producing animals is banned in Europe since 1988. The contrast of hormone abuses can be improved by the implementation of new hyphenated techniques or by the application of well established analytical methods to new biological matrices where, hopefully, matrix interferences are reduced and/or residue concentrations are increased.

In this work the biliary excretion of a selected package of substances after two different in vivo growth-promoting treatments (zeranol and trenbolone/estradiol) was studied in cattle. In the first experiment (A), a first group was treated with zeranol and a second one received feed contaminated with zearalenone, the *Fusarium* mycotoxin that is metabolized to zeranol. In the second one (B), a control group and a group implanted with a combination of trenbolone acetate and estradiol were involved. Suitable LC-MS/MS methods applying either ESI or APCI sources were developed and validated to quantify the compounds of interest at trace levels. The final aim was to add new knowledge about the metabolic patterns and the associated kinetics of elimination in bile after growth-promoting practices.

MATERIALS AND METHODS

Experiment A

BILE

Enzymatic deconjugation *(Helix Pomatia)*

Solid Phase Extraction (SPE) (HLB) + clean-up (NH_2)

HPLC-MS/MS Analysis

	Zeranol treatment (group 1)	Zearalenone Treatment (group 2)
	Implant with 3x12 mg of zeranol pellets (36 mg/animal)	Each animal fed 3 mg/animal/die of zearalenone
	3 x	3 x
Day 1	Familiarization	
	Day 5	Day 5 - 21
Day 26	Abbatoir	

Experiment B

	Treatment Group	Control Group
	Implant with 200 mg of Trenbolone Acetate + 40 mg 17ß-Estradiol (pellets)	No treatment
	16 x	16 x
Day 1	Familiarization	
Day 15	+	\
Day 86	Abbatoir	

BILE

Enzymatic deconjugation *(Helix Pomatia)*

Solid Phase Extraction (SPE) (HLB) + clean-up (NH_2)

Derizatization step (NH_2OH 1,5M - 90°C - 60 min)

HPLC-MS/MS Analysis

RESULTS

Experiment A

Treatment	Animal	ZER (µg/kg)	TAL (µg/kg)	α-ZOL (µg/kg)	ß-ZOL (µg/kg)	ZON (µg/kg)	ZAN (µg/kg)
"Group 1"	1	12.7	20.7	6.3	54.1	9.8	10.0
Zeranol	2	14.1	23.5	5.3	54.9	7.1	6.6
(ZER)	3	14.2	21.4	3.2	42.4	5.2	8.6
	Mean	13.7	21.9	4.9	50.5	7.4	8.4
"Group 2"	1	<CCα	0.6	2.4	30.0	3.5	<CCα
Zearalenone	2	0.5	0.9	3.4	54.5	5.8	<CCα
(ZON)	3	0.8	1.7	5.9	56.4	7.4	<CCα
	Mean	0.4	1.1	3.9	47.0	5.6	<CCα

Animal feedstuff contaminated with *Fusarium spp.*

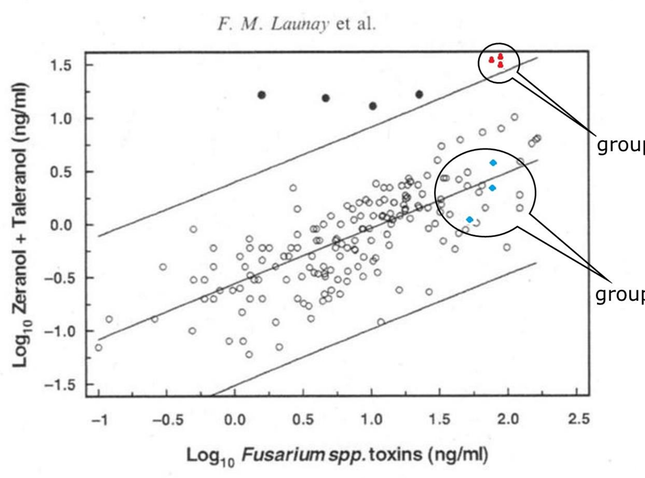
Metabolism ↓ in cattle

Zeranol naturally present in urine and bile

How to distinguish zeranol abuse from contamination with *Fusarium spp.* ?

Experimental evidences:

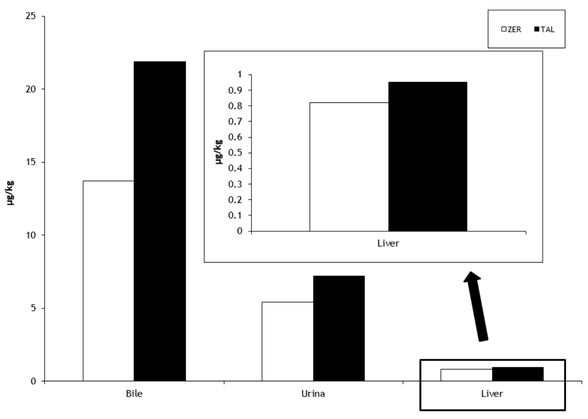
- ✓ ZER and TAL present in both groups
- ✓ ZER and TAL levels higher in implanted animals (group 1)
- ✓ Zearalanone (ZAN) increases in group 1



Criterion suggested **by Launay et al.** (2004) to discriminate abuse from contamination in urine here applied for the first time in bile with our experimental data:

✓**Group 1** samples data fell **out of** the confidence interval of the regression line
✓ **Group 2** samples data fell **inside** the confidence interval

ZER and TAL **concentration in bile higher** than in urine and liver collected at the same time.



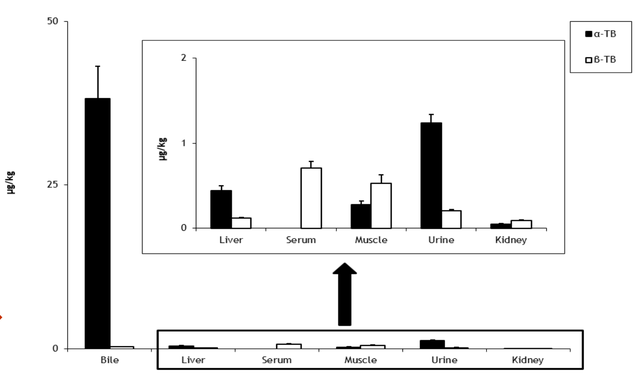
Experiment B

Group (animals)	Parameter	ß-TB (µg/kg)	α-TB (µg/kg)	α-E2 (µg/kg)
Control (16)	Mean	<CCα	<CCα	6.82
	Median	<CCα	<CCα	7.04
	Std dev	-	-	3.75
	Min	<CCα	<CCα	1.87
	Max	<CCα	<CCα	14.7
Treated (16)	Mean	0.33	38.2	16.0
	Median	0.29	30.4	15.1
	Std dev	0.16	20.0	6.18
	Min	0.08	14.7	4.73
	Max	0.77	93.1	30.7

Experimental evidences:

- ✓Trenbolone not found in the control group
- ✓α-estradiol (α-E2) found in the control group
- ✓ß-estradiol (ß-E2) never detected neither in control nor in treated group

α-trenbolone **concentrations in bile** two orders of magnitude **higher** than in liver, serum, muscle, urine and kidney.



CONCLUSIONS

The high concentrations of the marker residues observed in bile in both in vivo experiments compared with levels detected in urine and liver collected at the same time, corroborate the previous studies about the capability of this fluid to bioconcentrate certain anabolic substances. This suggests a new possible scenario in the control of hormone abuse at the slaughterhouse. The use of bile as target matrix could therefore permit to detect illegal hormone administration for a longer period and might represent a further tool to disclose the practice of using cocktails of anabolic compounds at very low doses.

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