

A statistical approach to detect abuse of natural hormones in cattle

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Introduction

There is lack of methods and operational criteria that can be used to detect the abuse of natural hormones. A testing strategy and criteria are needed to distinguish treated from non-treated animals. Traditionally, steroids are analysed in urine as free aglycons after deconjugation of the glucuronide and sulphate conjugates. Drawback of this deconjugation is that valuable information on the steroid profile in the sample is lost. From a theoretical point of view, administration of natural hormones will disrupt steroid profiles and will change the excretion patterns. The ultimate goal of this study is to “measure” these patterns including the conjugation state.

Analytical method

Recently we developed a method¹ to analyse the steroids free and conjugated in samples of urine. The analytical method is validated, natural hormones can be detected as low as at 7 ng L⁻¹. See figure 1 for the compounds measured.

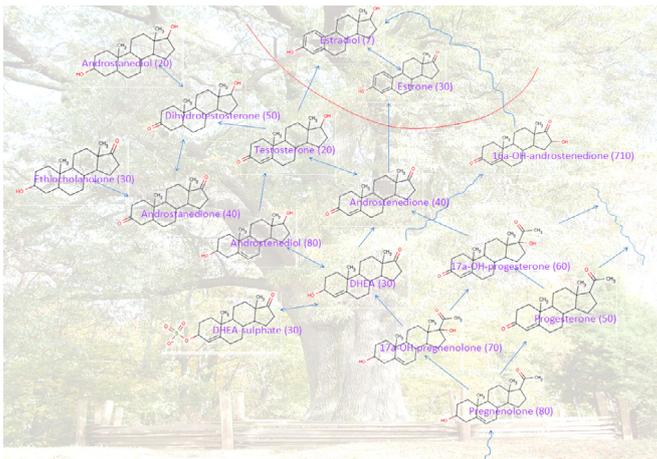


Figure 1. “Natural hormone tree” measured in urine. Sequential SPE was used for the isolation of free compounds and the glucuronide and sulphate conjugates. For detection GC-MS/MS is used. Between brackets C_α values in ng L⁻¹.

Samples used in this study

- In total 500 urine samples from bovine origin were obtained from Dutch routine control programs. Information on the animals such as age, gender, lactating status etc. was logged.
- Samples from animal experiments were used. Two animals were intramuscularly injected with estradiol-benzoate and testosterone-cypionate.
- Fourteen reference urine samples from guaranteed non-treated animals were collected.
- Six urine samples were obtained from cows treated with different steroid-esters. These six samples were obtained from a farm where steroid-esters were abused.

Statistics

Metabolic profiling is a promising approach² to identify the illegal use of natural hormones in cattle. A statistical model, discriminant analysis, has been used to model the data. A confusion matrix is used to evaluate the data. Confusion matrix displays the number of correct and incorrect predictions made (table 1). The fourteen and six “reference” samples were classified using the model (table 2).

Results and Evaluation

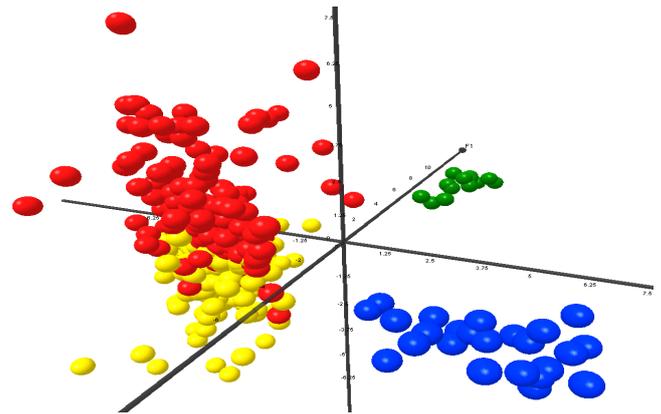


Figure 2. The data set was split into four groups for discriminant analysis, e.g. male, female (both routine), estradiol-benzoate and testosterone-cypionate samples.

Table 1. Evaluation of results by use of confusion matrix.

	Estradiol treated (%)	Testosterone treated (%)	Male (%)	Female (%)	Correct (%)
Estradiol treated	100	0	0	0	100
Testosterone treated	0	100	0	0	100
Male	0	0	91	9	91
Female	0	0	21	79	79

From figure 2 and table 1 it is concluded that the groups can be separated. There is some overlap between the male and female groups which can be expected since, especially the female group is not homogenous, e.g. lactating, non-lactating, pregnant etc.

Further evaluation was performed by classification of reference samples.

Table 2. Classification, steroid abuse (SA), female blank (FB) and male blank (MB).

	SA	FB	MB	MB	MB	MB																	
Estradiol treated									X														
Testosterone treated																							X
Male																						X	X
Female	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X					X

The steroid abuse animals are male animals. However, they appear in the female group, which indicates possible steroid abuse.

Conclusion and Discussion

- From the results obtained in this study it can be concluded that the used approach is a promising strategy to classify samples.
- Samples from “non treated” animals can be separated from animals treated with natural hormones.
- This strategy is not a confirmatory method. It should be treated as a screening method.
- More data from animals treated with natural hormones should be added to the data-set.
- Data-processing needs to be further refined.

References

- ¹ E.F. van Tricht, M.H. Blokland, S.S. Sterk and L.A. van Ginkel, Proceedings of the Euroresidues VI Conference. Federation of European Chemical Societies, Egmond aan Zee, Netherlands (2008), 611.
- ² C. Akre, L. van Ginkel, B. Le Bizec, H. De Brabander, W. Korth, J. Points, P. Teale, and J. Kay, Presence and metabolism of endogenous androgenic-anabolic steroid hormones in meat-producing animals: a review, Food Additives & Contaminants, (2009) Part A, 26:5, 640.