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The search for robust biomarkers of DHEA administration in bovine urine by metabolomic profiling: extending the control population

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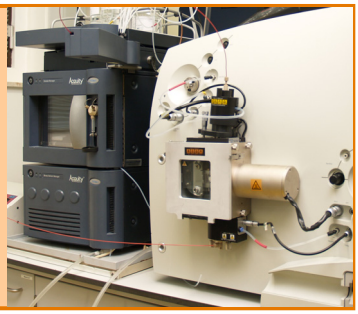
Introduction

In the European Union the use of all hormonal growth promoting substances in livestock production is prohibited [1]. The illegal use of natural steroids as growth promoters is very difficult to prove, due to the fact that the identity of the metabolites is often unknown and the endogenous levels of these metabolites are highly fluctuating. Recently, we have published a metabolomics approach for the identification of urinary biomarkers of DHEA administration to bovines [2]. In that study urines of treated and control animals were analysed by UPLC-ToF-MS and the full scan profiles compared. Using MetAlign pre-processing and alignment software [3] and multivariate statistics, the set of hundreds of differential mass signals between treated and control urines could be reduced to a set of 12 mass signals of potential biomarkers of DHEA treatment. In the present study the robustness of these markers is tested against a large (>140) control population of bovine urines, sampled from 50 farms throughout The Netherlands.

Materials and methods

- Male Frisian bovines (6) were treated with 1000 mg of DHEA daily for 7 days. Urine was collected at day -7 (control ◆) and at day 2, 5 and 7 (treatment ▲). Six untreated animals were included as controls (◆)
- Urines of 120 male bovines (age 10-45 weeks) were selected from a pool of urines available from routine monitoring and which had previously tested negative (controls ▲ ■ ●)
- All samples were purified by SPE (Strata X, 200 mg) in triplicate. Testosterone-d_3 and T-glucuronide-d_3 were included as internal calibrants
- Analysis was performed in a randomized order on a Waters LCT premier ToF-MS operating in ESI$^+$ and coupled to a Waters Acquity UPLC, equipped with an Acquity BEH 150 x 2 mm 1.7 µm analytical column
- Raw data were pre-processed and aligned with MetAlign software. Data sets were searched with Search-LCMS

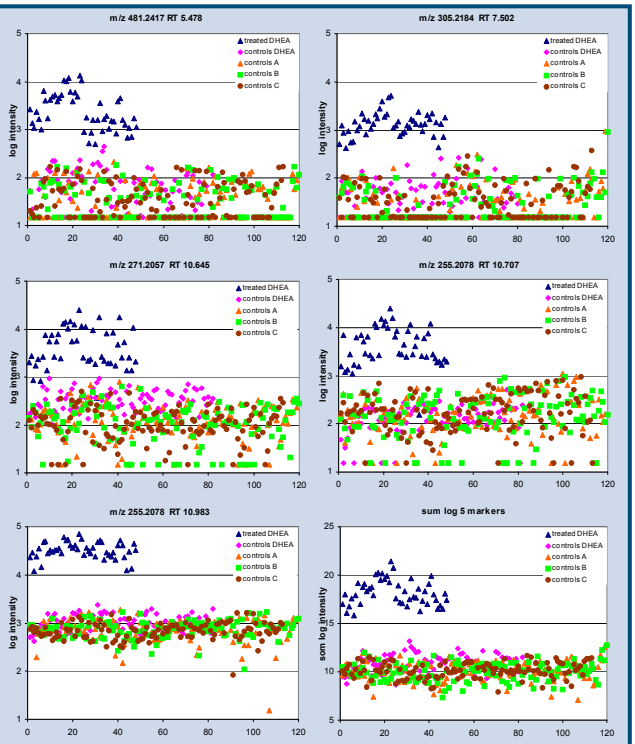


Results

- The analytical method proved very stable and robust during the complete period of analysis
- The complete set of data (48 injections from treated urines and 434 control injections) has been searched for 12 potential biomarkers of DHEA treatment. The intensities have been log transformed to visualise differences between the samples. For 5 of the potential biomarkers a good to excellent separation between treated animals and the extended control population is obtained
- For the strongest biomarker (*m/z* 255.2108, RT 10.98 min) a confidence interval (CI) of 4.2 σ is demonstrated between treated animals and controls
- Combination of biomarkers is possible by adding up the log intensities. The combination of the 5 selected biomarkers results in a separation between treated and controls with a CI of 3.47 σ, corresponding to a false positive/negative rate of 0.05%
- Tentative identification points to (partly) hydrogenated hydroxylated Phase II metabolites of DHEA (glucuronides)
- With the metabolomics approach it is possible to discriminate between urines of bovines treated with the prohormone DHEA and untreated bovines

Robust biomarkers for DHEA treatment of bovines

Biomarker RT (min)	Observed mass (Da)	Deviation (ppm)	Elemental composition biomarker	Fragment assigned	Elemental composition metabolite	Metabolite type
5.47	481.2423	-3.0	$C_{25}H_{37}O_9$	$[M+H]^+$	$C_{25}H_{36}O_9$	HO-androstene-ol-one-gluc
7.50	305.2138	-15.0	$C_{19}H_{29}O_3$	$[M\text{-gluc}+H]^+$	$C_{25}H_{36}O_9$	HO-androstene-ol-one-gluc
10.62	271.2056	-2.2	$C_{19}H_{15}O$	$[M\text{-gluc-}2H_2O+H]^+$	$C_{25}H_{38}O_9$	HO-androstene-diol-gluc or HO-androstane-ol-one-gluc
10.70	255.2095	6.5	$C_{19}H_{25}$	$[M\text{-gluc-}3H_2O+H]^+$	$C_{25}H_{40}O_9$	HO-androstane-diol-gluc
10.98	255.2108	11.8	$C_{19}H_{25}$	$[M\text{-gluc-}3H_2O+H]^+$	$C_{25}H_{40}O_9$	HO-androstane-diol-gluc



Log intensity of the 5 most robust biomarkers for DHEA treatment and the sum of the log intensity: treated animals vs extended controls

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