HIGH-RESOLUTION MASS SPECTROMETRY FOR METABOLOMIC PROFILING OF THE GLUCOCORTICOID STATUS OF HOLSTEIN-FRIESIAN COWS AFTER ADMINISTRATION OF PREDNISOLONE

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The anti-inflammatory properties of the natural glucocorticoid cortisol have led to the development of synthetic analogues, which exert even higher anti-inflammatory activities. Moreover, these drugs also induce body weight gain in production animals by improving feed intake and lowering feed conversion. Due to their growth-promoting effects, the use of synthetic glucocorticoids is strictly regulated in the European Union (CD 2003/74/EC). In the frame of the national control plans, which should ensure the absence of residues in food products of animal origin, in recent years, a higher frequency of prednisolone positive bovine urines has been observed. In an attempt to understand the origin of this prednisolone, an *in-vivo* study was conducted on adult Holstein-Friesian cows for further deepening the knowledge of the metabolism and distribution of prednisolone in cattle intended for meat production and to allow the characterisation of metabolites that may be used as a biomarker for exogenous administration.
Because of the complex nature of feces and urine as biological matrices, appropriate sample preparation procedures were required, but in terms of the metabolomic approach to be kept as generic as possible. To this extent, Plackett-Burman designs were successfully applied to develop two different sample preparations protocols. Metabolomic profiling was performed by using two different high resolution mass spectrometers: a stand-alone Orbitrap (Exactive^{TM}) and a ToF-MS/MS (TripleToF^{TM}). Targeted analysis of the known glucocorticoids was successfully validated according to CD 2002/657/EC. Decision limits and detection capabilities for prednisolone, prednisone and methylprednisolone in urine ranged from 0.1 to 0.5 µg L^{-1} and from 0.3-0.8 µg L^{-1}, respectively. For the natural glucocorticoids limits of detection and limits of quantification for dihydrocortisone, cortisol and cortisone ranged, respectively, from 0.1 to 0.2 µg L^{-1} and from 0.3 to 0.8 µg L^{-1}. In feces similar results were obtained.
The applicability of the analytical methods for untargeted metabolomic profiling was demonstrated by using ToxID^{TM}, Sieve^{TM} (Thermo Fisher Scientific), Simca^{TM} (Umetrics) and MetabolitePilot^{TM} (AB Sciex) software, enabling an efficient screening of the full scan data. A first screening was conducted on urine and feces samples collected from 2 cows and 2 calves after oral administration of prednisolone (1 mg kg^{-1} BW). Several prednisolone metabolites were identified, including 20β-dihydroprednisolone and 20α-dihydroprednisolone. The potential of these metabolites as a biomarker for illegal administration as opposed to endogenous formation will be further confirmed in a larger *in vivo* design.

Keywords: Biomarker, Metabolomics, High-Resolution Mass Spectrometry