

METABOLOMIC PROFILING OF THE GLUCOCORTICOID STATUS OF HOLSTEIN-FRIESIAN COWS BY U-HPLC-HR-ORBITRAP MS UPON ADMINISTRATION OF PREDNISOLONE

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The well-known anti-inflammatory properties of the natural glucocorticoid cortisol, has led to the development of synthetic glucocorticoid analogues, which exert even higher anti-inflammatory activities. Beside the anti-inflammatory properties, 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 (Council Directive 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 of the knowledge about 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, 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 each based on a two-step liquid-liquid extraction with *tert*-butyl methylether. For feces, this was followed by a solid phase extraction with C18 cartridges. Metabolomic profiling was performed using ultra-high-performance liquid chromatography coupled to full scan high resolution Orbitrap mass spectrometry by a combination of targeted and untargeted analysis. The targeted analyses were successfully validated according to CD 2002/657. Decision limits and detection capabilities for prednisolone, prednisone and methylprednisolone ranged in urine, respectively, from 0.1 to 0.5 $\mu\text{g L}^{-1}$ and from 0.3-0.8 $\mu\text{g L}^{-1}$. For the natural glucocorticoids limits of detection and limits of quantification for dihydrocortisone, cortisol and cortisone ranged, respectively, from 0.1 to 0.2 $\mu\text{g L}^{-1}$ and from 0.3 to 0.8 $\mu\text{g L}^{-1}$. In feces similar results were obtained.

The applicability of the analytical methods for untargeted metabolomic profiling was demonstrated by using ToXID, SieveTM (Thermo Fisher Scientific) and SimcaTM (Unimetrics) 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 with prednisolone (1 mg kg⁻¹ BW). Several prednisolone metabolites were identified, including 20 β -dihydroprednisolone and 20 α -dihydroprednisolone. The potential of these metabolites as a biomarker for illegal administration as opposed to endogenous formation will be further investigated in a larger *in vivo* design.