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Are saponins and sapogenins precursors of prednisolone? Preliminary results

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Article

Introduction

The Council Directive 96/23/CE allows the therapeutic use of the glucocorticoids prednisolone, methylprednisolone, betamethasone and dexamethasone, and the Commission Regulation No 37/2010/EU sets the maximum residue limits (MRLs) in bovine liver, muscle, fat and milk. However, their possible use as growth promoters is well known, due to e.g. their ability to increase weight gain or their synergistic activity with β-agonists. No MRLs are for urine so their presence at any concentration should not be allowed. In the last noughties, the frequency of detection of prednisolone in cow urine, especially in samples collected at the slaughterhouse, raised in Italy. The possibility of an endogenous production of prednisolone after stress events as the slaughtering was demonstrated (Pompa *et al.* 2011) on cows and its presence in bovine urine was shown to be related to a state of stress in the animals both at farm and slaughterhouse (Dusi *et al.* 2012; Bertocchi *et al.* 2013). The hypothesis that a contamination could lead to the presence of prednisolone in urine because of a likely microbiological dehydrogenase activity on cortisol was also shown in bovine (Arioli *et al.* 2010) and human (Bredehöft *et al.* 2012).

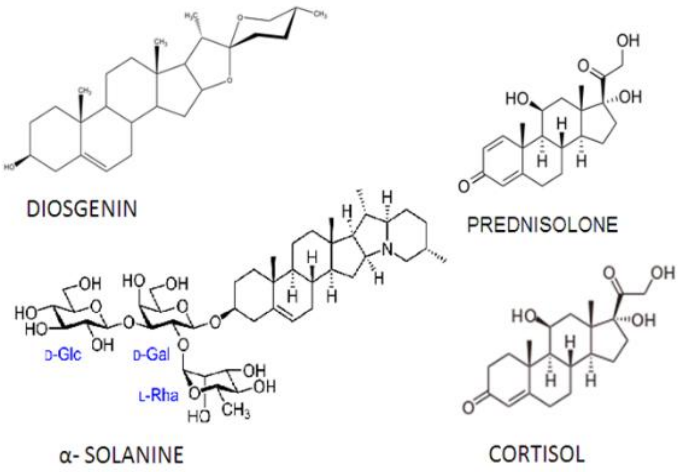

Figure 1 – Chemical structures of the considered molecules.

In this study, saponins and sapogenins with steroidal nucleus were analogously considered as possible cause of the neo-formation of prednisolone in the faeces-contaminated urine and in feed. Attention has been focused on α-solanine, a saponin contained in the Solanaceae, and diosgenin, an important starting material to obtain steroidal drugs such as cortisol, testosterone, estradiol, contained in many herbaceous plants as Dioscoraceae (Figure 1).

Material and Methods

Sample preparation - 500 mg of faeces or feed from the market were suspended in 500 mL of 0.9% NaCl, and gently mixed for a whole night. Three aliquots of 40 mL were collected from each suspension. An aliquot was spiked (100 ng/mL) with diosgenin, one with α-solanine and one was the control. At the times: t = 0; 1; 2; 4; 8; 24; 48 and 72h, 1 mL was collected, heated at 80 °C (15 min), fortified with the Internal Standard (Prednisolone d6, 10 ng/mL) and centrifuged at 3000xg (10 min). A 200 µL supernatant was collected and analysed.

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Sample analyses - HPLC-MSn was made with a LCQ DECA XP ion trap mass spectrometer equipped with a pump and an autosampler LC Surveyor (ThermoFinnigan, San Jose, CA, USA). The column was a 100mm×2.1mm id, 3µm Allure Biphenyl (Restek, Bellefonte, PA, USA). The mobile phase consisted of an aqueous solution of formic acid 0.1% (A) and methanol (B). A gradient was used: 40% eluent A and 60% eluent B from 0 to 15 min and 100% eluent B from 20 to 40 min. The acquisition was in the Electrospray Ionization (ESI) in the (+) mode for α-solanine and diosgenin, in the (-) mode for prednisolone. The data acquisition software was Xcalibur® by ThermoFinnigan. The Limit of Detection (LOD) were from 0.6 to 4 ng/mL, the Limit of Quantification (LOQ) were from 1 to 10 ng/mL.

Results

Diosgenin and α-solanine underwent a transformation in faeces. Their concentrations decreased over time. From t = 48h diosgenin was not detected while α-solanine was detected at a concentration lower than 30% of the initial one. After 72h also α-solanine was not detectable. The faecal suspension spiked with diosgenin, at t = 8h showed the presence of prednisolone at the concentration of 7.1 ng/mL (Figure 2). In the sample of faeces spiked with α-solanine, cortisol was detected at t = 24h with a concentration of 6.6 ng/mL (Figure 3). In the feed solanine did not transform while diosgenin steadily decreased to the 15% of the initial concentration. No corticosteroids were detected. In the control samples, no transformations were observed.

Figure 2 - Prednisolone neoformation in diosgenin-spiked faecal suspension.

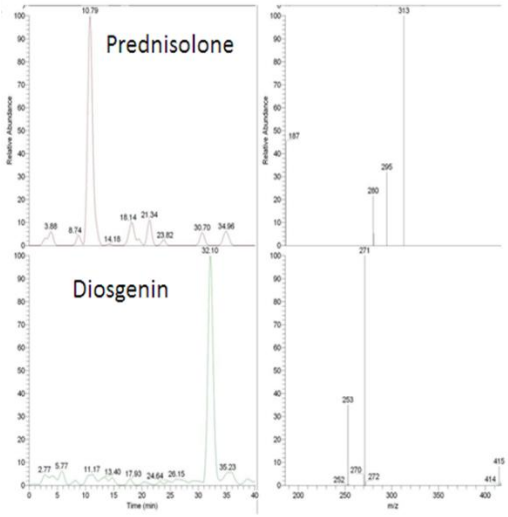
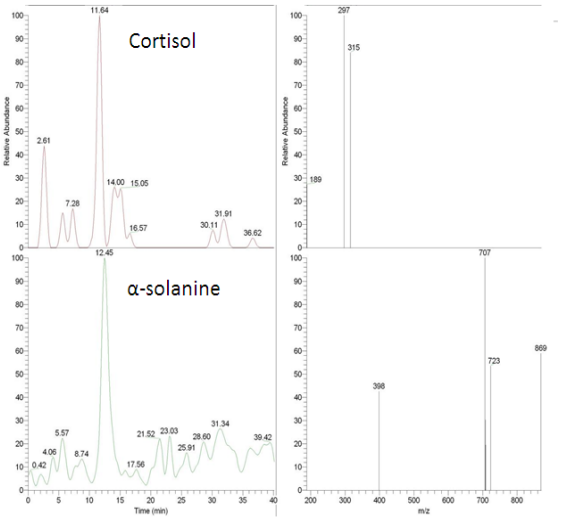


Figure 3 - Cortisol neoformation in α-solanine-spiked faecal suspension.



Discussion and Conclusions

The presence of corticosteroids in two faecal suspensions could show that the neo formation of prednisolone and cortisol from diosgenin and α-solanine, even if sporadic, may be possible.

It could be conceivable that the transformation event likely occurs in the presence of certain microorganisms, and is apparently random, as faeces are highly non homogeneous both in their rough composition and in the microflora present. Further studies are ongoing to investigate these preliminary conclusions.

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