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Optimization and validation of non-invasive HPLC-MS/MS method for stress quantification in free-living ruminants

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Wildlife management and conservation can benefit from a quantified understanding of physiological response of free-ranging animals to the various potential stressors. Non-invasive stress monitoring by fecal cortisol metabolites (FCM) determination has proven to be a powerful tool. That is, high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) has emerged as the most accurate method avoiding problems related to the non-specificity and matrix effects of the so-used immunoassays. In this work we have optimized, developed and validated a reliable method for 11-ketoetiocholanolone (11-k), a cortisol metabolite, quantification in ruminant's fecal samples by using and HPLC-MS/MS method. An appropriate extraction and purification procedure was developed to take into account the complex nature of feces. The method consisted in a primary fecal samples extraction with methanol and subsequent clean-up with hexane, followed by purification and pre-concentration of targeted metabolite with solid phase extraction (SPE). The final extract obtained was then analyzed by HPLC-MS/MS making used of a quadrupole-time-of-fly (Q-TOF) tandem mass spectrometer with an electrospray ionization interface operating in positive mode. An isotope internal standard was used in order to minimize matrix effect and to compensate the alterations of the analytical signal. After a rigorous optimization of both sample extraction and HPLC-QTOF parameters, the method was satisfactory validated and the best conditions were stablished. Matrix-matches standards were used for the calibration of the method. The limit of detection and quantification, referred to freeze-dried sample, were 13 and 40 µg kg-1, respectively. Recoveries in the range of 85-110% and RSDs not higher than 15%for the complete analytical procedure, including extraction and analysis, were achieved.

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