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CORTISOL DETERMINATION IN DAIRY COWS HAIRS BY NEAR-INFRARED (NIR), MID-INFRARED (MIR) AND RAMAN SPECTROSCOPY.

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Chronic stress in dairy cows can have negative impact on their emotional state, immunity, fertility and milk production. Measuring and assessing chronic stress in herds can help to objectify welfare status and to investigate strategies when problems are detected or suspected. However, robust, easy to use and cost effective methods to measure biomarkers are needed. Hair cortisol concentration has been highlighted as a good biomarker of chronic stress. Its analysis by enzyme-linked immunosorbent assay (ELISA) is robust but time-consuming and expensive.

Therefore, the development of spectroscopic technique based methods has become more and more interesting due to their rapidity, non-destructive aspect and cost-effectiveness. So, the first objective of this study was to assess the potential of three spectroscopic techniques: Near infrared (NIR), Mid-Infrared (MIR) and Raman to predict the cortisol content in hairs of dairy cows. After highlighting the best one, the second objective was to develop a more robust model with a higher number of samples analyzed using the selected technique.

As a preliminary step, a total of 134 hair powder samples from 30 cows were analyzed on bench-top spectrometers in order to acquire Near infrared (NIR), Mid-Infrared (MIR) and Raman spectra. The hair cortisol concentration of theses samples was determined by ELISA analysis. Hair cortisol ranged from 8.3 to 91.5 pg/mg of hair, with an average of 27.1 pg/mg of hair. The Partial Least Squares (PLS) models to predict hair cortisol provided R^2cv of 0.63, 0.62 and 0.52 and RMSEcv of 9.5, 9.2 and 11.1 pg/mg for NIR, MIR and Raman data respectively. The NIR and MIR had better performance than Raman spectroscopy. Further analysis will be carried out with MIR spectroscopy due to its performance and facility to implement.

In a second step, to improve the robustness of the model, 915 additional samples were collected from five different countries (Belgium, Luxembourg, France, Germany and Austria). A total of 1049 hair samples were analysed by ELISA and MIR spectroscopy. A variable selection was performed and different machine learning techniques were applied to build the best predictive model: Principal Component Regression (PCR), Partial Least Square (PLS), Elastic Net Regression (ENR) and Support Vector Machine Regression (SVM-R). A 10-fold cross-validation and a bootstrap validation were performed. The different machine learning approaches gave RMSEcv of 7.71, 7.39, 7.32, 7.41 pg/mg and RMSEv of 9.88, 8.14, 7.71, 7.99 pg/mg for PCR, PLS, ENR and SVM-R respectively. Elastic-net provided the best regression model for hair cortisol. The model is not efficient enough to strictly predict the hair cortisol concentration, but it could determine whether a cow is stressed or not.