**Application**

We aim to provide the foundation for developing a tool farmers can use to improve management strategies to prevent lameness, by developing an unbiased method for predicting lameness, complementing mobility scoring. Early detection of lameness allows for timely treatment improving animal welfare and sustainability of the dairy herd, consequently reducing economic losses of the dairy industry.

**Introduction**

Previous research has shown that metabolomics and lipidomics allow for the detection of potential metabolites and lipids that discriminate between lame and healthy cows using urine (Cardoso et al., 2024; Randall et al., 2023), milk (He et al., 2022), and blood (Dervishi et al., 2020). Randall et al. (2023) used untargeted liquid chromatography-mass spectrometry (LC-MS) and machine learning (ML) algorithms to predict lameness. In that study of one herd, the best-performing ML model predicted lameness with a mean accuracy of 82% at the time of lameness event. He et al. (2022), also utilised untargeted LC-MS and ML algorithms, achieving accuracies of 100% using a different cohort from the same herd. In this cross-sectional case-control study, we aim to validate the results obtained in previous research using a larger sample size of commercial dairy herds.

**Material and Methods**

A total of 849 (424 urine and 425 milk) cow samples (lame vs control; ≅ 1:1) were collected from 10 commercial dairy herds with cows scored using 0-3 mobility scoring (AHDB, 2024). Urine samples were collected via free-flow catch and both urine and milk were snap frozen into liquid nitrogen before storage at -80 °C. Cows receiving medication in the preceding 15 days and non-lame cows treated for lameness within the previous month were excluded. A suite of 5 ML algorithms, namely Random Forest (RF), Support Vector Machine (SVM), Partial Least Squares regression (PLS), multivariate adaptive regression spline (MARS) and Elastic Net (ENET), was applied for each herd dataset (1 urine and 1 milk dataset per herd) and to all herds (1 urine and 1 milk dataset across all farms, totalling 2 datasets). Mobility score outcomes were used to train the supervised models. The correlation coefficient between milk and urine prediction accuracies was calculated alongside the Student’s t-test to check for the significance between the two biofluids.

**Results**

Figure 1 shows the mean prediction accuracies by farm and for all farms for urine and milk cow samples. Overall mean prediction accuracies were higher for urine than milk, except for farm C, in which a higher accuracy was obtained for milk. When the data of all farms were included in the model, the predicted accuracy for urine was 66% and 61% for milk. At a farm level, the highest accuracy was 85% for urine and 77% for milk. Prediction accuracies using milk and urine showed a moderate positive correlation across farms (r = 0.43). A paired t-test revealed significantly higher prediction accuracies for urine compared to milk samples (t(10) = 3.68, p < 0.01).



Figure 1 - Mean prediction accuracies and corresponding standard deviations error bars by farm and for all farms for urine and milk samples

**Conclusions**

The combination of untargeted LC-MS with ML models shows promise to differentiate between lame and healthy cows based on a set of metabolites and lipids. However, a high inter-herd variability in classification accuracies was observed: given the results above, it wasn’t possible to establish a correlation with farm management or rule out confounding factors. Despite urine showing approximately 10% higher prediction accuracy, further work is required to weigh in practical considerations such as the ease of sample collection and respective stability/storage, overall costs, animal welfare implications and farm management compatibility.

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