DOES REARING SYSTEM IMPACT ON METABOLITE COMPOSITION OF DAIRY LAMB MEAT AS MEASURED USING RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY?

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I. INTRODUCTION

The dairy sheep industry is small but a growing part of the New Zealand agricultural sector [1]. Lambs from dairy sheep farming systems are either naturally reared (NR) through provisioning of maternal milk or artificially reared (AR) through feeding milk replacement formula, and weaned off milk from 4 to 6 weeks of age. Our previous study observed minor impacts of rearing systems on fatty acid profile and consumer liking of lamb meat [2], while it remains unknown whether two rearing methods would alter the metabolite profile of lamb meat. Rapid Evaporative Ionization Mass Spectrometry (REIMS) is an emerging tool for rapid authentication of muscles and meat quality assessment based on metabolite fingerprints [3]. This study aimed to determine the effects of rearing methods on metabolite changes, measured by REIMS, in two muscles from dairy lambs.

II. MATERIALS AND METHODS

A subset of twin-born East-Friesian-cross dairy lambs from a larger cohort study (n=96) were slaughtered at 18 weeks of age following natural rearing on the dam (NR; n=19) on pasture as twins or indoor artificial rearing (AR; n=19) on ad libitum milk replacer, as described in previous study by Pavan et al. [2]. All lambs were weaned off milk at 6 weeks of age and were managed on pasture thereafter. All lambs received a grain-based concentrate from 3 to 12 weeks of age and were finished on the same pasture. Lamb *m. longissimus thoracis* (LT) and *m. semitendinosus* (ST) muscles were collected from the same side of the animal at 24 h postmortem. Sub-samples were taken from the cranial end of both muscles and frozen at -80 °C for REIMS analysis. Lamb samples were thawed at 4 °C overnight and their metabolite features were determined in triplicate by the laser-assisted-REIMS (Waters, Wilmslow, UK) coupled to a quadrupole-time of flight (qToF) mass spectrometer (Waters Xevo® G2 qToF, Waters). The mass spectra were collected between *m/z* 500 and 1200 at 0.5 Hz in negative ionization mode with measurement times of <10 seconds/sample. Mass spectral features were mass corrected, aligned and library matched against the Human Metabolome Database and LipidMaps database. The mass features were analyzed by PCA (Principal Component Analysis), OPLS-DA (Orthogonal Projection to Latent Structures-Discriminant Analysis) and evaluated by ROC (Receiver Operating Characteristics) curves (SIMCA 16, Umetrics, Sweden). R^2 (cumulative) and Q^2 (cumulative) scores were generated to evaluate the robustness and accuracy of the OPLS-DA models. Individual features were considered different at $P_{adjr}<0.05$ based on t-tests adjusted for Benjamini-Hochberg false discovery rate.

III. RESULTS AND DISCUSSION

A total of 2,017 features were detected, of which 696 had tentative identifications based on library matching of high-resolution mass (mass error <5 ppm) after data cleanup (QC variation <30%). These features were used for further data analysis. The metabolite fingerprints differed between the two

rearing systems (OPLS-DA model: Q^2=0.56, R^2=0.63; AUC=0.998, Figure 1), suggesting REIMS can accurately discriminate lamb samples from AR and NR. However, absolute differences in metabolites were limited to 4 features (P_{adj}<0.05) tentatively annotated as 9-hexadecen-1-ol, octadec-11Z-enol, PA(P-16:0/0:0), and PE(P-16:0/20:5), which agreed with our previous observation that rearing systems played a minimal role in affecting the nutritional composition and quality of dairy lamb meat [1]. OPLS-DA (Q^2=0.77, R^2=0.80) and feature-reduced PCA models were more readily distinguished between muscle types, showing that the metabolite fingerprints of the two lamb muscles differed (Figure 2) regardless of the rearing system. LT and ST muscles can be accurately discriminated (AUC=0.94) by REIMS with 99 features (P_{adj}<0.05) driving the separation, of which the ions with the lowest P-values were mainly identified as phospholipids.

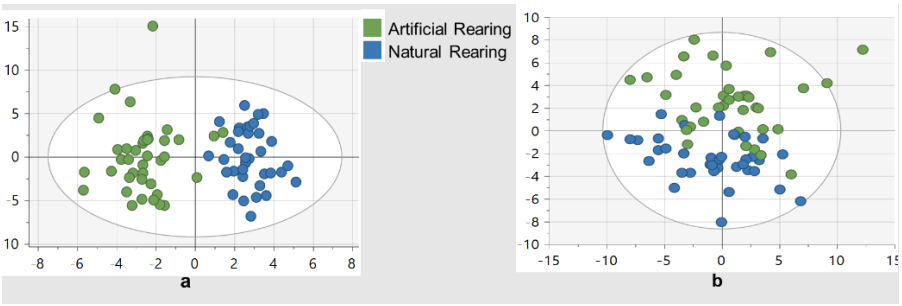

Figure 1. OPLS-DA (a) and feature-reduced PCA (b) score plots of lamb muscles from artificial rearing (AR) compared to natural rearing (NR) regardless of muscle type.

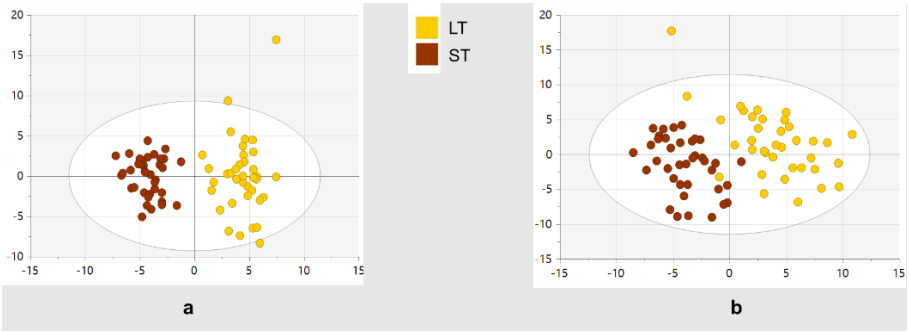

Figure 2. OPLS-DA (a) and feature-reduced PCA (b) score plots of lamb *m. longissimus thoracis* (LT) and *m. semitendinosus* (ST) regardless of the rearing regime.

IV. CONCLUSION

Results showed that rearing system had few major effects on the metabolite fingerprints of dairy lamb meat. Although AR and NR could be differentiated, overall they resulted in similar metabolite composition in meat. Further, REIMS fingerprinting can reliably and quickly discriminate muscle types, and to a lesser extent, different rearing regimes.

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