**The human and bovine milk metabolome at 2-weeks postnatal**

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**ABSTRACT**

**Objective**: Milk is an affordable, nutrient-rich food for infant growth and development, comprised of carbohydrates, fats, proteins, vitamins, minerals, and biologically active factors. During the first twelve months of life, human breast milk is the ideal nutrition source for healthy infants. After this development window, human breast milk is usually substituted with bovine whole milk for the sake of convenience. However, comparative investigation of the human and bovine milk metabolome from a similar lactation stage has not been extensively studied. The goal of this project is to interrogate the human and bovine milk metabolome at 2-weeks postnatal.

**Method**: Human milk (n=4) was collected at 2-weeks postpartum as part of the Breastfeeding and Early Child Health (BEACH) study. Mothers enrolled in the BEACH study were recruited between 36-38 weeks of pregnancy and planned to exclusively breastfeed their infant for at least 2-months. Similarly, bovine milk (n=10) was collected 2-weeks postpartum from primiparous Holstein dairy cows. Briefly, starting at 36 weeks of gestation, dairy cattle were housed in sand-bedded, shaded barns with access to fans and water soakers and fed a common transition cow total mixed ration. After calving, cows were treated similarly and milked twice daily. Human and bovine milk metabolomics was completed using high-resolution mass spectrometry and data analysis. The analysis was implemented using a Nextflow-based metabolomics pipeline on high performance university cluster. MZmine mass-spectrometry software, Python, R script and SECIMTools suite of tools were used for data processing and analysis.

**Results**: In the analysis 716 metabolites were identified in human milk and bovine milk samples that exceeded background signal produced by the blank control samples. Principle component analysis demonstrated that human milk metabolites were different from bovine milk. Specifically, we identified 9 features that were significantly enriched in lipid fractions that included "Creatine-D3" and "Glutathione". Cow milk metabolomic analysis is still ongoing, however we expect to find similar results in terms of metabolite volume between fat vs. skim and vs whole milk. We are planning to compare metabolite profiles between bovine and human milk samples to determine which metabolites are shared and which are unique in the different fractions.

**Conclusions**: These results highlight rigorous and reproducible data processing procedures are critical to elucidation of human and bovine milk metabolites. With a more comprehensive understanding of the metabolomic profile of human and bovine milk, we can develop better nutritional and health strategies that promote breastfeeding and dairy consumption.

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