

# Comparative analysis of bioactive oligosaccharide production in dairy cows using novel analytical techniques

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## Introduction

Milk oligosaccharides (OS) possess a multitude of bioactivities, including the abilities to act as prebiotics and prevent serious diseases in infants. Milk OS are highly concentrated in human milk but are much less abundant in bovine milk, and their presence in the dairy marketplace is scarce. With improved production and isolation strategies, these compounds could be recovered from dairy processing streams for use as ingredients in infant formula and adult therapeutics. Currently, little is known about variations in OS production among dairy cattle and the factors that impact milk OS abundance. Therefore, this study was developed with the objective of measuring milk OS abundance in a large sampling of dairy cattle by implementing novel techniques for high-throughput milk OS profiling. The OS data has been used for breed-level comparisons between Holstein and Jersey cows to identify differences in OS production. This phenotypic data will then be used in a genome-wide association study to identify genes that are potentially responsible for the measured OS variations. Examining the influence of genetics on bioactive compound formation will provide insights into ways that both milk OS abundance and the value of liquid milk production can be increased.

## Methods

A total of 634 milk samples from mid-lactation Danish Holstein and Jersey cows collected under the Danish-Swedish Milk Genomics Initiative were used for OS profiling and relative quantification. OS were extracted from the milk samples, isobarically labeled, and multiplexed prior to LC-MS/MS analysis to improve instrumental throughput. Tandem-mass spectrometry (MS) collision energies were optimized to generate reliable reporter ion signals from the isobaric labels that were used as a measurement of relative OS quantities. Relative abundances of individual OS and overall OS profiles were compared between the two breeds.

## Results

Analysis of test samples demonstrated that relative OS abundances could be accurately measured in real milk samples with a coefficient of variation below 10% for most OS. The application of isobaric labeling to milk OS analysis, which has been done for the first time in this study, has led to improved signal strength for large fucosylated OS during MS analysis and will allow this class of compounds to be

studied in greater detail. Unlike conventional methods that rely on analytical standards and measure relatively few OS, this technique reliably measures abundances for 16 OS in large sample sets without requiring standards. Significant differences in the abundance of 13 OS were identified between the breeds, with most OS having a greater abundance in the Jersey samples. Although Jersey milk had higher average OS abundances, the breed also demonstrated much higher variation between animals in OS production. This variation may represent a potential to harness genetic factors that impact OS abundances, and the connection between genetic polymorphisms and OS production is currently being elucidated in a genome-wide association study. In both breeds, correlations were observed between the abundance of specific OS pairs, which could provide clues into the underlying synthetic pathways.

Once complete, the results of this study will enable improved selection for desirable milk traits by identifying inter- and intrabreed variations in milk OS abundance and correlating these variations with genomic data. This dataset could lead to the development of personalized milk with enhanced bioactive content for specific human health needs.