

## Impact of dietary regimes across lactation on the metabolite composition of cow's buttermilk

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Buttermilk is a by-product in the manufacture of butter from cows' milk and is composed of a variety of micro and macronutrients<sup>(1)</sup>. Previous metabolomics research has detailed the impact of factors such as diet and lactation on cow's milk<sup>(2)(3)</sup>. Pasture-based farming practises are common in countries such as Ireland, due to environmental factors such as climate and soil type. Pasture feeding regimes are associated with not only improving overall nutrient profile, but also the fatty acid profile of cow's milk<sup>(4)</sup>. With a growing interest and demand in grass fed products, there is need to further explore the effect of grass feeding regime across lactation. Therefore, the objective of this study was to investigate the impact of dietary regimes across lactation on cows' buttermilk metabolome using liquid chromatography tandem mass spectrometry (LC-MS/MS). Buttermilk samples were obtained from 54 spring-calving cows fed different dietary regimes across lactation. Dietary regimes included a grass dietary regime where cows consumed perennial ryegrass at 95% of annual dry matter (DM) intake, a total mixed ration (TMR) diet where cows received maize silage, grass silage and concentrates on a DM basis or a partial mixed ration (PMR) diet, where cows consumed a diet of rotational grazing on pasture following morning milking with indoor housing of the cows at night with access to the TMR feed following evening milking. Metabolomic analysis of cow's buttermilk samples were performed using LC-MS/MS. Targeted LC-MS/MS analysis of the buttermilk samples was performed using the AbsoluteIDQ P180 assay. Multivariate statistical analysis of acquired dataset were performed using MetaboAnalyst. In total, 134 metabolites were identified in buttermilk samples including 16 amino acids, 12 biogenic amines, 13 acylcarnitine, 10 lysophosphatidylcholines, 68 phosphatidylcholines, 14 sphingomyelins and 1 hexose. Two-way ANOVA analysis determined that 92 metabolites were significantly different across lactation stage and dietary regime (FDR < 0.05), with 82 and 26 metabolites significantly associated with lactation stage and dietary regime respectively. The 26 metabolites associated with dietary regime included 19 phosphatidylcholine, 5 sphingomyelins and 2 acylcarnitines. Significant phosphatidylcholines included those with longer acyl chain lengths of which 81% had double bonds. Moreover, the 82 metabolites associated with lactation stage included 47 phosphatidylcholines, 8 lysophosphatidylcholine, 6 biogenic amines, 7 acylcarnitines, 10 sphingomyelins and 4 amino acids. Similar to dietary regime, significant phosphatidylcholines included those with longer acyl chain length of which 79% had double bonds. In conclusion, both dietary regime and lactation stage impact the metabolite composition in cows' buttermilk with a particular increased impact on lipid species. Future work will examine if these metabolites are also changed in other cow's milk products.

### References

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