In silico construction of a bovine milk proteome atlas to identify signatures of early lactation

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Take home message Among an atlas of 4510 proteins established by a computational aggregation, 65 proteins are specific of early lactation and potential biomarkers of negative energy balance.

Introduction Noninvasive methods based on easily available animal fluids (plasma, milk) and rapid analytical tools are needed to phenotype animal traits related to performance, welfare, health and product quality. Milk is an accessible biological fluid and its molecular characterization is a prerequisite to design tools for the phenotyping or the diagnosis of animal diseases (Roncada *et al.*, 2012). The sensitivity of proteomics technology made possible the identification of thousands of proteins in milk during the last 10 years. We hypothesized that computation of available proteomic data may enable the identification of milk protein biomarkers of traits such as energy balance and body reserve mobilization during early lactation, which are major drivers of dairy performance, robustness and functional longevity of dairy cows (Zachut and Moallem, 2017).

Material & methods Based on a computational approach, we aggregated datasets from 18 publications on milk proteomics from healthy cows whatever the husbandry factors studied. The lists of protein identifiers were extracted from articles and/or the supplementary data files, converted into gene names and aggregated in an atlas. This atlas was divided in 5 periods of the lactation cycle: colostrum (0-5 days in milk (DIM)), early lactation (5-21 DIM), lactation peak (21-60 DIM), mid-lactation (60-80 DIM) and late lactation (> 80 DIM). The early lactation period was narrow in order to ensure a negative energy balance of cows. Protein lists were mined using ProteINSIDE (http://www.proteinside.org/; Kaspric *et al*., 2015) allowing the identification of metabolic pathways in which proteins are implicated. ProteINSIDE webservice relies mainly on Gene Ontology (GO) enrichment tests (p value_Benjamini and Hochberg < 0.05) among the categories: Biological Process, Molecular Function and Cellular Component.

Results & discussion Among the 4510 different proteins identified in bovine milk, 65 gene names were identified exclusively in early lactation milk, 58 of which were annotated by 442 enriched GO terms. The most significantly ($p < 0.01$) enriched terms in the Biological Process category were “oxidation-reduction process”, 6 terms related to “transport” and 4 terms related to “immune system”. The most enriched GO terms in the Molecular Function category was “oxido-reductase activity”. The 58 proteins found in early lactation milk were mainly related to oxidative processes or oxidative metabolism such as mitochondrial cytochrome c oxidase subunit (COX5A, COX5B, COX7A2), aldehyde dehydrogenase family 3 member B1 (ALDH3B1), catalase (CAT), mitochondrial succinate dehydrogenase flavoprotein subunit (SDHA). Mitochondrial acetyl-CoA acetyltransferase (ACAT1) and mitochondrial phosphoenolpyruvate carboxykinase 2 (PCK2) were also found exclusively in early lactation milk. The biological significance of their presence in the milk remains to be defined. It may result from modifications of mammary epithelium permeability, may be linked to the secretion of metabolites related to oxidative pathways in milk during early lactation (communication Pires *et al.*, this symposium), and may constitute molecular signatures of physiological negative energy balance typical of this period. In agreement with these hypotheses, the abundance of PCK2 mRNA (a neoglugogenic gene expressed in liver) was increased in the mammary tissue of high genetic merit dairy cows (Weikard *et al.*, 2012), and may contribute to glyceroneogenesis during fasting or compromised feed intake, as already proposed for lipogenic tissues.

Conclusion The aggregation of milk proteomics from public data allowed the construction of a large milk protein atlas. This atlas can be mined to identify proteins that regulate the physiological adaptations and allow the phenotyping of dairy ruminants. Data suggest a proteomic signature specific of early lactation which is mainly related to “oxidation-reduction processes”. Two milk proteins associated to early lactation, ACAT1 and PCK2, may be biomarkers of negative energy balance typical of this period. Nonetheless, eventual associations remain to be confirmed by studies designed to relate their abundance, the energy balance and classic indicators of metabolic status in early lactating cows.

References

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