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Proteomics and metabolomics characterizing the pathophysiology of adaptive reactions to the metabolic challenges during the transition from late pregnancy to early lactation in dairy cows

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ABSTRACT

The transition from late pregnancy to early lactation is a critical period in a dairy cow's life due to the rapidly increasing drain of nutrients from the maternal organism towards the foetus and into colostrum and milk. In order to cope with the challenges of parturition and lactation, comprehensive adaptive reactions comprising the endocrine and the immune system need to be accomplished. There is high variation in this coping ability and both metabolic and infectious diseases, summarized as "production diseases", such as hypocalcaemia (milk fever), fatty liver syndrome, laminitis and ketosis, may occur and impact welfare, productive lifespan and economic outcomes. Proteomics and metabolomics have emerged as valuable techniques to characterize proteins and metabolite assets from tissue and biological fluids, such as milk, blood and urine. In this review we provide an overview on metabolic status and physiological changes during the transition period and the related production diseases in dairy cows, and summarize the state of art on proteomics and metabolomics of biological fluids and tissues involved in metabolic stress during the peripartum period. We also provide a current and prospective view of the application of the recent achievements generated by omics for biomarker discovery and their potential in diagnosis.

Biological significance: For high-yielding dairy cows there are several "occupational diseases" that occur mainly during the metabolic challenges related to the transition from pregnancy to lactation. Such diseases and their sequelae form a major concern for dairy production, and often lead to early culling of animals. Beside the economical perspective, metabolic stress may severely influence animal welfare. There is a multitude of studies about the metabolic backgrounds of such so called production diseases like ketosis, fatty liver, or hypocalcaemia, although the investigations aiming to assess the complexity of the pathophysiological reactions are largely focused on gene expression, i.e. transcriptomics. For extending the knowledge towards the proteome and the metabolome, the respective technologies are of increasing importance and can provide an overall view of how dairy cows react to metabolic stress, which is needed for an in-depth understanding of the molecular mechanisms of the related diseases. We herein review the current findings from studies applying proteomics and metabolomics to transition-related diseases, including fatty liver, ketosis, endometritis, hypocalcaemia and laminitis. For each disease, a brief overview of the up to date knowledge about its pathogenesis is provided, followed by an insight into the most recent achievements on the proteome and metabolome of tissues and biological fluids, such as blood serum and urine, highlighting potential biomarkers. We believe that this review would help readers to be become more familiar with the recent progresses of molecular background of transition-related diseases thus encouraging research in this field.

Abbreviations: AGP, α_1-acid glycoprotein; APP, acute phase protein; ATP, adenosine triphosphate; BHB, β-hydroxybutyrate; CoA, Coenzyme A; DIM, days in milk; GPC, glycerophosphocholine; NADP, nicotinamide adenine dinucleotide phosphate; NAFLD, non-alcoholic fatty liver disease; NEFA, non-esterified fatty acids; NNB, negative nutrient balance; PC, phosphocholine; PI, physiological imbalance; SAA, serum amyloid A; SARA, subacute ruminal acidosis; TCA cycle, tricarboxylic acid cycle; TG, triglyceride; VLDL, very low density lipoprotein

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1. Introduction

The transition period from late pregnancy to early lactation is a critical period in a dairy cow's life due to the rapidly increasing drain of nutrients from the maternal organism towards the foetus and into colostrum and milk. During this transition period, fetal growth reaches its exponential course during the last weeks of pregnancy and concomitantly the mammary gland parenchyma mass markedly grows [1]. After calving, the output of nutrients with milk exceeds the input by voluntary feed intake. The negative nutrient balance (NNB) resulting therefrom requires a massive mobilization of body reserves, mainly body fat but also protein. Albeit NNB is a common phenomenon in mammals, both the duration and the extent observed in modern high yielding dairy cows represent a biological extreme. To be able to cope with the challenges of parturition and lactation, comprehensive adaptive mechanisms comprising the endocrine and the immune system need to be accomplished. There is high variation in this coping ability and both metabolic and infectious diseases, summarized as "production diseases", may occur and have an impact on welfare, productive lifespan and economic outcomes. The incidence of such diseases is greatest during early lactation with hypocalcaemia (milk fever), fatty liver syndrome and ketosis (or acetonaemia) being the most common metabolic diseases. In case of infectious diseases, metritis and mastitis, attributable to the immune-compromised situation during the metabolic challenge, are most frequent. Fig. 1 presents the relationship between metabolic stress and disease development. Several studies have attempted to identify the causes and risk factors associated with the high incidence of health problems observed during the periparturient period [2–5], and systems biology approaches addressing the issue of the regulatory mechanisms of nutrient metabolism in lactation are published [6–8]. Beside environmental factors, production diseases also have a genetic component; e.g. for subclinical macromineral disorders and major clinical diseases the heritability reported were low to moderate [9].

To further investigate the complexity of these diseases, omics approaches which include multi-variate, and large-scale analyses, may be applied. Such studies gather information either at the level of the DNA, RNA, miRNA, protein or the metabolites, and provide a snapshot of the current condition in cells, tissues or body fluids (Fig. 2). However, variation in sampling times between different studies can yield different results and it is important to take this into account when interpreting omics results or planning further studies. The multivariate results from omics approaches require extensive bioinformatics resources that are mostly available online. Both proteomics and metabolomics have evolved as the functional continuation of transcriptomics in less than two decades, and have developed rapidly, due to improvements in technology and bioinformatics tools. General reviews about the application of proteomics and metabolomics to livestock science were published earlier [10–12]. Both proteomics and metabolomics involve the resolution of a complex mixture of compounds into components that can then be identified and characterized. For what concerns proteins, their identification always involves matching each the amino acid sequence to the respective encoding gene and thus depends of the sequence information available for the target species, but can also include the description of posttranslational protein modifications.

Two major mass spectrometry (MS) platforms are available for proteomics, following the mechanism through which ions are generated: these ion sources are termed matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). Before analysis, proteins are fractionated either by electrophoretic, for intact proteins, or chromatographic, for peptides generated after protein cleavage, techniques. Protein fractions are then digested, to generate peptides that can be further fractionate by chromatographic techniques and then characterized by MS, which can record the mass of analytes to generate information about their structure. The resulting data are further analysed with search engines, such as Mascot (Matrix Science Ltd), to generate in silico MS data for the specified genome sequence database.

Absolute protein quantification is difficult to achieve with proteomics techniques. On the contrary, relative quantitation can be achieved by gel-based methods, such as 2DE using semiquantitative protein stains, or protein labeling strategies, such as difference gel electrophoresis (DIGE) [13]. As an example, DIGE was applied to blood serum samples to identify potential biomarkers related to hypocalcaemia [14].

At the peptide level, relative quantification can be achieved by stable isotope-labeling approaches (iTRAQ) or by label-free comparison. Isolated proteins or tryptic peptides can be chemically labeled before separation (iTRAQ) [15]. Quantification of proteins by means of iTRAQ was used, among the others, to identify liver proteins related to physiological imbalance [16].

For metabolites, species-specificity is not an issue albeit relative quantities may differ. The major analytical approaches used in metabolomics rely on two techniques: MS and NMR techniques [17]. For MS-based techniques, samples are fractionated through

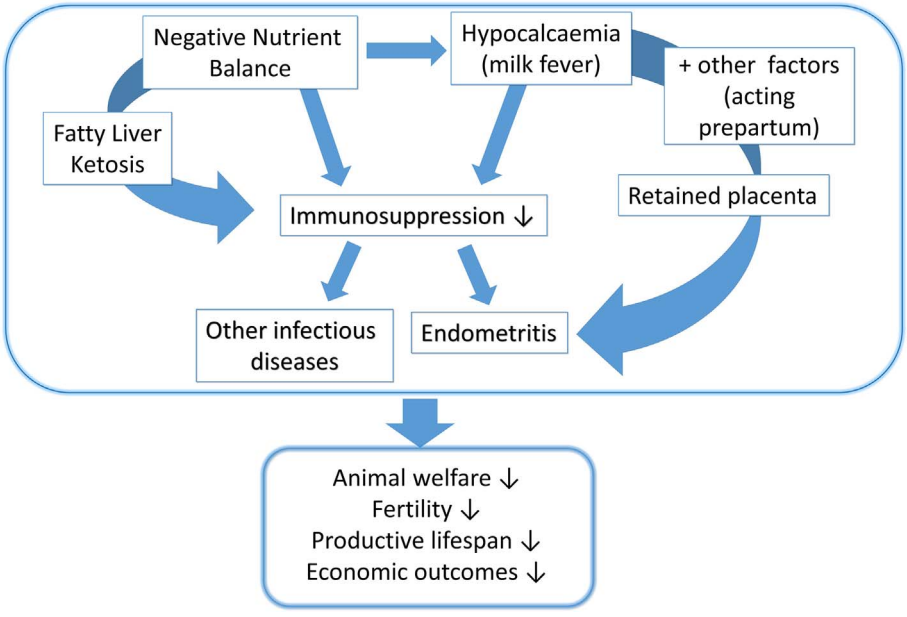

Fig. 1. The relationship between metabolic stress and disease development.
The figure presents a schematic flow of the events during the pathogenesis of peripartum related diseases.