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Review article

Application of nutrigenomics in small ruminants: Lactation, growth, and beyond

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ABSTRACT

Ruminants have a very special niche in the animal kingdom, and are the most important livestock species providing milk, meat, and wool for humans from consumption of highly-fibrous feedstuffs. Cattle, goat and sheep have been widely-used for years as models to study ruminal fermentation and the mechanisms whereby tissues utilize nutrients for milk synthesis, growth, wool accretion, and reproduction. The advent of high-throughput technologies to study an animal's genome, proteome, and metabolome (i.e., "omics" tools) offered ruminant scientists the opportunity to study multiple levels of biological information to better understand the whole animal response to nutrition, environment, physiological state, and their interactions. The omics revolution gave rise to the field of nutrigenomics, i.e. the study of the genome-wide influences of nutrition through alteration in mRNA, protein, and metabolite expression or abundance. This field of research is relatively new in ruminants, and particularly sheep and goats. Dietary compounds affect gene expression directly or indirectly via interactions with transcription factors including ligand-dependent nuclear receptors. New knowledge generated through the application of functional analyses of transcriptomic, proteomic, and metabolomic data sets in goat and sheep is discussed.

1. Introduction

Phenotypic variables have been the foundation of traditional nutritional science, where scientists have based their hypothesis and findings. However, as discussed in the "nutrigenomics technology" section, the recent advancements in molecular biology (and bioinformatics) have provided new tools to evaluate fundamental effects of nutrients on physiologic outcomes. The latter has allowed new fields of research such as nutrigenomics to bloom. Nutrigenomics has been described as "the study of genome-wide influences of nutrition" (Muller and Kersten, 2003), or how dietary nutrients can affect gene expression and consequently affect protein expression and metabolism of the entire organism.

2. Nutrigenomics technologies

2.1. Transcriptomics

Technological advances, such as microarray platforms and RNA-sequencing, allowed scientists to quantify the expression of almost the entire set of transcribed genes in a sample, hence, the term transcriptome. The transcriptome is the total transcribed RNA (i.e., mRNA, noncoding RNA, rRNA, and tRNA) in a cell or tissue, and reflects the cellular metabolic and non-metabolic response to a particular environment (e.g., diet, management, treatment) allowing a "still-frame" of cellular activity. Bovine microarrays were first used in small ruminants in 2007 during a nutrigenomic study of goat mammary transcriptome responses to feed deprivation (Ollier et al., 2007). This study highlighted genes responsible for the drop in milk protein, lactose, and fat secretion, and genes responsible for a slowdown in mammary cell proliferation and differentiation and/or an increase in programmed cell death leading to activation of early mammary involution.

Recently, RNA sequencing has replaced microarray platforms, and this technology has been used in omics studies since at least 2011. Despite the fact that none of those experiments had a nutrigenomics focus, this technology has been applied to study not only the transcriptome (Shi et al., 2015) but also the microRNAome (Li et al., 2012) in small ruminants such as goats and sheep (Jager et al., 2011; Cox et al., 2012).

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2.2. Gene reporter technology

Gene reporter technologies have been extensively used in the study of transcription factors and their involvement in cellular signaling cascades. The reporter used is often a gene that transcribes a protein with a readily-measurable phenotype that can be distinguished easily from a background of endogenous proteins (Alam and Cook, 1990). Normally, the reporter gene is linked to a promoter sequence through an expression vector (e.g. chimera plasmid) that is further transferred into cells, both *in vivo* or *in vitro*. When activated, the interaction between transcription factor and its *cis*-regulatory sequences (promoters, or response elements) included in the sequence of the reporter gene will elicit the expression of the reporter that can then be easily monitored.

In ruminant physiology these technologies have been used to address involvement of transcription factors in mammary metabolism and the response to nutrients and nutritional stages (discussed in Section 4). Goats have been recently used as models, for example, to characterize the activity and involvement of peroxisome proliferator-activated receptors (PPAR) and sterol regulatory element binding protein 1 (SREBP1) through the use of gene reporters (Xu et al., 2016a,b; Shi et al., 2017).

2.3. Proteomics

When considering the mRNA portion of the transcriptome, a direct hypothesis can be formulated on the protein expression of a cell, which due to the complexity of post-transcriptional regulations (e.g., alternate splicing, and phosphorylation or dephosphorylation), has a greater degree of complexity compared with the genome. Thus, proteomics has been developed to identify and differentially quantify protein species in complex biological samples.

The core technology is mass spectrometry (MS), which has been adopted by livestock scientists (Lippolis and Reinhardt, 2008; Sauerwein et al., 2014). In the past decade, various proteomics studies in sheep have addressed the proteome response during pathogen-related reproductive disease (Wu et al., 2014b; Du et al., 2016; Miao et al., 2016), digestive diseases (Athanasiadou et al., 2008; Nagaraj et al., 2012; Pisanu et al., 2017), or immunological disease (Marsh et al., 2006; Chiaradia et al., 2012) namely as a way to identify novel biomarkers. In addition, Al-Gubory et al. (2014) used this technique to understand basic physiological mechanism during the establishment of pregnancy in sheep. Restelli et al. (2014) used it to identify differences in the visceral and subcutaneous fat proteome in goats, also uncovering novel adipokynes in this species. Unlike large ruminants, proteomics use for nutrigenomics in small ruminants remains limited. Recently, Ren et al. (2016) investigated the effect of overgrazing on the sheep liver proteome, identifying a shift in energy resources from carbohydrates to proteins, causing an impairment of nutrient metabolism (protein and lipid) and immunity, which may be reasons for the reduced growth observed under this nutritional condition.

2.4. Metabolomics

Following the physiological flow of biological information processing and synthesis from gene expression to protein synthesis and metabolite changes, the analysis of the metabolome naturally completes and complements the transcriptome and proteome. The metabolome consists of the global profiling of metabolites in a sample, using high-resolution analysis together with statistical tools such as principal component analysis and partial least squares (Zhang et al., 2012). The small molecules detectable by this approach include peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins, polyphenols, alkaloids, and inorganic species.

Metabolomics studies may be conducted on a variety of biological fluids and tissue types with a number of different technology platforms, like nuclear magnetic resonance (NMR) and MS, with the latter becoming the technique of choice. Metabolomics applications, however, are almost inexistent in small ruminants, and only few recent examples exist in the published literature. Comparative studies of the metabolic profile of milk have been conducted in goat to address differences due to genotype or with other species (e.g. cow) (Scano et al., 2014; Caboni et al., 2016). Nutrigenomics applications have also been attempted in sheep, specifically in relation to maternal nutrient restriction during pregnancy, focusing on the global composition of umbilical venous blood (Sun et al., 2017) or on breed adaptability to harsh environmental conditions (Palma et al., 2016). The first study revealed a beneficial effect of dietary rumen-protected arginine or N-carbamylglutamate supplementation on mammalian reproduction to avoid detrimental effects of undernutrition during pregnancy. Their effect was associated with complex metabolic networks and signal transduction involving amino acids, protein, carbohydrate, energy, and lipid metabolism, as well as oxidative stress. In the latter case, both the liver and muscle metabolome were evaluated to investigate the response of different sheep breeds to seasonal weight loss due to pasture scarcity in the dry summer period. The data suggested that Dorper and Damara breeds are more tolerant to these conditions and, thus, more suitable than Merino for harsh environmental conditions.

3. Transcription factors in small ruminants

Dietary nutrients can alter gene expression in the short-to-medium term; however, these alterations or effects are carried out by specialized proteins within the cell, i.e. transcription factors (TF). TF are fundamental to the study of nutrigenomics; they can act as intermediaries between dietary nutrients and the ultimate alteration in gene expression. The TF can be activated directly or indirectly by dietary nutrients, and upon activation they translocate from the cytoplasm to the nucleus where they alter the transcription of specific target genes. The ability of TF to bind specific regulatory sites on DNA (i.e., response elements) to regulate gene expression confers these proteins a central stage in the field of nutrigenomics. It is because of this important role of TF in nutrigenomics that the accurate identification and characterization of TF that respond to specific dietary nutrients and to what extent these can be manipulated through dietary effects should be a focus of researchers in the near future.

3.1. General aspects

The normal cellular functions, as well as adaptations to external stimuli, are governed by a precise pattern of gene expression. In turn, the gene expression patterns are highly-regulated by the coordinated action of regulatory elements known as enhancers or *cis*-regulatory modules (Shlyueva et al., 2014). These contain short DNA motif sequences (i.e., 6–10 nucleotides) also known as response elements, that act as binding sites for TF. Once the TF bind a response element, they will recruit coactivators, chromatin remodeling proteins, and the RNA polymerase components.

Initial estimates indicated a range of 2000 to 3000 sequence-specific DNA-binding TF in the human genome (Vaquerizas et al., 2009). This discovery was followed by a comprehensive analysis of nearly 1500 manually curated TF, from which ~100 have been experimentally verified for their DNA-binding and regulatory functions (Vaquerizas et al., 2009). The combination of chromatin immunoprecipitation and DNA sequencing (ChIP-seq) has allowed the construction of databases such as ENCODE (Yip et al., 2012) and AnimalTFDB (Zhang et al., 2015) with current up-to-date information on verified TF. While ENCODE provides mainly information on human and rodent TF, AnimalTFDB offers an array of 50 animal species including *Bos taurus,* but no information specific to small ruminants is available. However, other web-based software such as LASAGNA (Length-Aware Site Alignment Guided by Nucleotide Association), which allows an automatic retrieval and analysis of TF binding sites (i.e., response elements) and related TF