NUTRIGENOMICS, A NEW DIRECTION FOR DAIRY COWS: A REVIEW

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Abstract

A fairly fresh area of studies is nutrigenomics in dairy cows. It is described as the research of nutritional genome-wide factors that alter gene expression. The capacity of nutrients to communicate with genes and modulate molecular processes that impact physiological functions is well recognized nowadays. This has resulted in increasing interest among researchers in exploring nutrition at a molecular level and developing two fields of study: nutrigenomics (evaluates the influence of nutrients on gene expression) and nutrigenetics (evaluates the heterogeneous individual nutrient response due to genetic variation). Due to their biologically significant positions during early postnatal life, fatty acids are one of the nutrients most studied. Fatty acids modulate transcription factors engaged in lipid metabolism regulation. The use of various sources of polyunsaturated fatty acids, starch concentrations, forage ratios and vitamins stands out among the options for dietary manipulation with the aim of modulating lipogenesis. Retinoic acid activates both receptors of retinoic acid (RAR) and receptors of retinoid X (RXR), causing epigenetic modifications in important adipogenesis regulatory genes. We are at the frontier of the nutrigenomics era in ruminants and original information firmly suggest that this science branch can play a critical part in future actions to feed better dairy cattle.

Key words: fatty acids, metabolism, milk, nutrients

INTRODUCTION

A new field of study that incorporates two distinct fields of studies called nutrigenomics and nutrigenetics has been created in latest years. Nutrigenomics is described as the research of "genome-wide nutritional factors" [18] and how this "influences the equilibrium between health and disease by changing the genetic makeup of an individual's expression and/or structure" [57] fat mammary synthesis remains an active study area with important progress in regulating lipid synthesis by bioactive fatty acids (FA). The theory of biohydrogenation created that diet-induced depression of milk fat (MFD) in the dairy cow is caused by inhibition of mammary synthesis of milk fat generated by particular FAs during ruminal biohydrogenation [27]. Trans-10, cis-12 conjugated linoleic acid was the first such FA to influence milk fat synthesis and its impacts were well described, including dose-response interactions. Coordinately down-regulated lipogenic ability and transcription of important mammalian lipogenic genes during MFD. For over a decade, researchers have been perplexed by the grounds of diet-induced MFD and highlights of important historical milestones unraveling the biology of low-fat milk syndrome have been evaluated elsewhere. Fat is the most variable element of milk in dairy cows, with many variables including genetics, physiological state, and climate affecting the quantity and structure [60]. Although the increased accessibility of DNA sequences in livestock has resulted in important progress in this area [59],most elements of molecular mechanisms engaged in dietary regulation of mammary lipogenesis in ruminants remain unsure. The FA secreted in cow's milk has distinct roots:

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FA up to C14:0 originates from de novo synthesis in the mammary gland, whereas FA with a length of chain higher than C14:0 originates from maternal diet or body reserves [17]. Linoleic acid (LA) and α-linolenic acid (ALA) are essential FA, meaning they cannot be synthesized in the organism and must therefore be acquired through the diet. Adipogenesis can be divided into several phases, including adipogenic engagement, adipogenic differentiation, and accumulation of lipids [16]. Vitamin A influences every adipogenesis stage. Retinoic acid, an active metabolite of vit. A, causes epigenetic modifications in adipogens, regulating their expression and the formation of adipocytes. Retinoic acid also decreases the accumulation of lipids [66]. Other nutrients, such as vitamin D, with retinoic acid modulates the signaling pathway to alter adipogenic differentiation and growth. Nutrients have profound effects on gene expression and differentiation of cells in general. Research is becoming more and more active in this field, which forms an exciting new research field called nutrigenomics [38].

METHODS OF THE NUTRIGENOMIC STUDY

Gene Expression. Transcription factors (TF) activation or inhibition results in more or less transcription of its target genes. Cis-regulatory elements which include the promoter of genes situated just upstream of the transcription starting point and cis-regulatory modules, including enhancers and silencers, which are visible from a few kilobases upstream of the transcription starting site, determine the short-to-medium-term regulation of gene expression [26, 63]. There are approximately 2,000 estimated different TFs in humans [35], which often work combinatorially, but for their DNA-binding and regulatory functions only around 100 have been experimentally verified [46]. Measuring the expression of recognized target genes can therefore be an indirect technique for testing whether a compound is an agonist or antagonist of a specific TF. When DNA is in the euchromatin framework, TF becomes available to the gene and its upstream areas, which are proteins that specifically bind brief DNA sequences (i.e., 6 to 12 nucleotides) called response elements situated in the gene enhancer areas [34]. Such an approach has been used in dairy cows to investigate PPARα and PPARγ [56] SREBP1 [70], and the LXRα [47]. Using gene expression has the benefit of not interfering with the cells ' standard biology and can be implemented in vitro and in vivo. However, there are some constraints to this strategy. Among these is the failure to differentiate whether the observed shift is a direct impact of the particular TF activation or inhibition or the impact is indirect through a secondary TF. However, the use of gene expression in nutrigenomic research is a lawful technique for indirectly studying TF activation [69].

Gene Reporter. The ability to monitor a TF activation is possible by producing a chimera plasmid by fusing the appropriate DNA coding for a promoter including the response element of the interest gene with the reporter gene sequence coding for the DNA sequence [37]. After the insertion (e.g. transfection) of the chimera into the cells, the response of the TF is evaluated by direct or indirect measurement of the expression of the gene reporter. Gene-reporter technology can be performed using temporary or permanent transfection methods. The initial use of gene reporter technology in bovine cells dates back more than 30 years [29] but has since been very limited. Due to the possibility of studying the activation of TF with great precision, the use of this technology is gaining momentum in nutritional studies.

Luciferase. Originally, luciferase was extracted from fireflies but is present in several other organisms. Generally speaking, the term luciferase and luciferin are used for the enzyme and substratum, respectively, generating bioluminescence upon reaction [40]. The most widely used: firefly luciferase and Renilla are bioluminescent proteins used for gene reporter assays. However, luciferase is arguably the most commonly used in mammalian cells for quantitative analysis of gene expression, and often the Renilla is used

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as an internal control for data normalization. This combination is due to the nearly ideal characteristics of these reporters: 1) mammalian cells do not contain luciferase or Renilla, 2) the two compounds remain inert within cells, and 3) the current generic assays for luciferase and Renilla are fast, easy to use and highly sensitive [42, 64]. Luciferase is by far the most widely used gene-reporter technology in dairy cow nutrigenomic studies [68], but has also been used to study bovine cell signaling [67],gene promoter region validation [59], gene expression of milk protein [20] and polymorphisms of single nucleotides [55].

Fluorescent Protein. The initial steps towards the use of fluorescent proteins in molecular biology were taken when Prasher (1992) sequenced and cloned the *Aequorea victoria* jellyfish green fluorescent protein (GFP). The big advantage of fluorescent proteins over luciferase is their ability to form internal chromophores without requiring other than molecular oxygen cofactors, enzymes, or substrates [43]. This advantage enables researchers to collect "true" data on a specific cellular activity in real time without harvesting the cells. No nutrigenomic studies have been published in dairy cows using fluorescent proteins to investigate TF activation to the authors ' knowledge [35,38].

KEY NUTRIENTS

Nuclear receptors are intracellular receptors that are activated by molecules of lipid signaling, including steroid hormones, thyroid hormones, retinoids, metabolites of vitamin D, and many others (tab. 1) [22, 36, 47]. They are also ligand-activated transcription factors that, by binding to their cognate DNA components, activate target gene expression. Dietary vitamin A is absorbed and transformed into retinal acid all-trans [50]. Retinoic acid acts as a ligand for receptors of retinoic acid (RARα, RARβ, and RARπ) (tab. 1) [56, 65]. They work with retinoid X receptors (RXRα, RXRβ, and RXRπ) to bind the target gene loci with retinoic acid reaction elements (RARE) [17]. Retinoic acid also activates the PPARβ/π orphan receptor to stimulate cell proliferation and lipid oxidation [51]. Thus, the biological impacts of retinoic acid are determined by the partitioning of retinoic acid between RAR and PPARβ/π. Two cellular retinoic acid binding proteins appear to control the partitioning of retinoic acid, with the protein II (CRABPII) binding cellular retinoic acid delivering retinoic acid to RAR and the protein type 5 (FABP5) binding fatty acid to PPARβ/δ [58, 62, 70]. Adipogenic progenitor cells express a elevated CRABP-II / FABP5 ratio, leading in RAR signaling dominance [62]. Because of the stage-specific expression of associated transcription variables, retinoic acid influences progenitor cells and mature adipocytes differently. Retinoic acid plays significant roles in both preadipocyte engagement and terminal adipocyte maturation as a metabolite of vitamin A (tab. 1) [5]. Decades ago, retinoic acid was discovered to encourage adipogenic engagement of embryonic stem cells in an in vitro adipogenesis model using embryonic stem cells. Consistently, the therapy of retinoic acid on embryoid-derived stem cells results in extended activation of the extracellular signal-regulated kinase-1 (ERK) pathway needed for adipogenic engagement [37]. Depending on the availability of RA, RAR / RXR heterodimers communicate with nuclear co-repressor proteins including retinoic acid and thyroid hormone receptor (SMRT) silencer and nuclear receptor corepressor (NCoR) silencer, or with coactivators such as (SRC)/p160 family and p300/CREB-binding protein (CBP) [11, 13]. Nuclear co-repressor proteins cause particular locus modifications in the chromatin structure that inhibit gene expression, while coactivators promote gene expression by recruiting ATP-dependent chromatin remodeling complex to loosen the structure, enabling gene expression to be initiated by RNA polymerase II. The PRC proteins dissociate quickly from RAR target genes in the presence of retinoic acid, forming permissive condition for gene expression, which in turn decreases the methylation of DNA in the respective promoters [30]. This may explain the promotional impact on preadipocyte gene expression of retinoic acid [34]. While

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retinoic acid encourages adipogenic engagement, vitamin A decreases the accumulation of lipids in mature adipocytes (tab. 1). Because vitamin A metabolite, retinoic acid, activates PPARα and PPARβ/δ in mature adipocytes, which induces oxidation of fatty acids and catabolism of lipids [52] it is not surprising that vitamin A decreases both lipid and adipocyte accumulation. Retinoic acid blocks late-stage adipogenesis by inhibiting C / EBPβ-mediated transcription and PPARγ activity, resulting in terminal differentiation of adipocytes. Bionaz et al. (2012) provided a comprehensive literature review of the effects of AA on milk protein synthesis. The conclusion of the review was that the activity of the main protein synthesis pathway, with mTOR as the central hub, is essentially inhibited in bovine mammary tissue and induced by cooperation between insulin, IGF-1, GH, AA (e.g. leucine) and glucose, leading to greater mammary protein translation [4]. Furthermore, the available information stated that the posttranscriptional modifications caused by AA, insulin, and glucose appear to fine-tune the protein synthesis, but a major impact on milk protein synthesis is matched by modifications in the mRNA expression of genes linked to the transport of glucose and AA absorption [22]. Appuhamy et al. (2014) assessed multiple essential AA alone or in conjunction with mTOR and AMPK phosphorylation on glucose and acetate in MacT cells in a subsequent research. The research verified the positive function in the milk protein synthesis and affirmed the beneficial impacts on activation of the mTOR pathway through phosphorylation of essential AA with a concomitant rise in casein synthesis [24]. Recent molecular studies have concentrated more on examining the nutrigenomic function of individual AA with main bovine mammary cells in milk protein synthesis in vitro [32]. Another study provided evidence that Arginine, a conditionally essential AA, is also capable of increasing the expression of casein genes and decreasing the expression of the translation inhibitor 4EBP1 when supplemented at a level equivalent to 2x the concentration found in casein [63].

Table 1 Nutrients that influence gene expression

Gene name	Gene symbol	Nutrient	Function
Retinoid X receptor, alpha	RARA	Retinoic acid	Development, differentiation, apoptosis, and CLOCK2 genes
Retinoid X receptor, beta	RARB	Retinoic acid	Morphogenesis embryonic, cell growth and differentiation
Retinoid X receptor, gamma	RARG	Retinoic acid	Growth and development of the skeleton
Peroxisome proliferator-activated receptor, alpha	PPARA	Fatty acids	Fatty acid metabolism, inflammation, and tissue regeneration
Peroxisome proliferator-activated receptor, delta	PPARD	Fatty acids	Fatty acid metabolism, epidermal proliferation, tissue regeneration
Peroxisome proliferator-activated receptor, gamma	PPARG	Fatty acids	Adipogenesis, lipogenesis, and insulin sensitivity
Nuclear receptor subfamily 1, group H, member 3	NR1H3	Oxysterols	Cholesterol homeostasis, inflammation
Vitamin D receptor	VDR	Vitamin D	Immune response
Nuclear receptor subfamily 1, group I, member 2	NR1I2	Vitamin E	Detoxification
Hepatocyte nuclear factor 4 alpha	HNF4A	Fatty acids	Development of the liver and kidney

NUTRIGENOMICS RELATION WITH MILK FATTY ACIDS

Recent studies in mammals such as rodents, cows, and humans have shown that lipids can control gene expression in the liver and mammary gland, helping to maintain adequate saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and

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polyunsaturated fatty acid (PUFA) levels in these tissues [16, 18, 32, 66]. Dietary lipids can function as lipogenesis regulators that interact with transcription factors including peroxisome proliferator-activated receptor (PPAR) and sterol-regulatory element binding protein (SREBP) transcription variables [13,37]. Both transcription factors are engaged in the regulation of the FADS1 and FADS2 genes (encoding for enzymes respectively 5 and 6 desaturases) and the ELOV-2 and ELOV-5 genes (encoding for elongase enzymes) [61, 68]. PPAR consists of a superfamily that includes PPARα, PPARπ and PPARβ/π [62,69]. SREBP is a family of transcription factors characterized as mediators of homoeostasis of cellular cholesterol and as regulators of biosynthesis and absorption of FA [10]. Three members of the SREBP family, SREBP-1a, SREBP-1c and SREBP-2, were identified [69]. Although SREBP-1c and SREBP-2 are structurally comparable, their regulation of hormones, nutrients and postnatal development in the liver is quite distinct. PUFA and their metabolites are the main FA that act at the level of the nucleus in conjunction with these transcription factors to regulate the lipogenic genes mentioned above [12, 20, 43]. Among the main short-chain fatty acids (SCFA) produced by rumen fermentation, butyrate is the most nutrigenomic data in dairy cows [28]. Butyrate impacts the expression of a big amount of genes in MDBK cells that are associated with cell cycle arrest, immune response, and signaling. Butyrate also impacted gene expression in ruminal papillae of dairy cows linked to glycolysis and lipogenesis [23, 67]. Surprisingly, information from a latest research suggested that SCFA, especially propionate, reduced anterior pituitary cell expression of GH (gonadotropic hormone) and prolactin (PRL) in milk cow [63]. Investigations on free fatty acid receptors (FFAR) in ruminants are relatively scarce. Zhao et al. (2014) have determined the expression of FFAR2 and FFAR3 in bovine mammary tissue during lactation and in mammary epithelial cells. The information from that research are indicative of those receptors that mediate increased intracellular Ca2 +, reduced cAMP, and increased mitogen-activated protein kinases (MAPK) phosphorylation. Hosseini et al. (2012) observed an increase in FFAR3 and FFAR2 during bovine adipogenesis in vitro but was not affected by insulin, propionate or β-hydroxybutyrate. However, there was an increase in FFAR3 expression due to propionate in cow white adipose tissue [49] despite the well-established decrease in adipogenesis frequency during early lactation [2].

THE BIOHYDROGENATION PROCESS

Milk cow lactating diets are small in fat content (about 4% –5%), with linoleic acid and linolenic acid predominantly PUFAs [32]. The ester connections are hydrolyzed when nutritional lipids reach the rumen (> 85 percent) followed by unsaturated FA biohydrogenation [19, 27]. Biohydrogenation is a conversion by rumen bacteria of unsaturated to saturated fatty acids. This intensive conversion also results in the creation of a number of conjugated linoleic acids and fatty acids trans 18:1, some of which are bioactive in the ruminant and other species when taken up by the mammary glands [41]. Biohydrogenation includes only a few species of rumen bacteria and performs these responses as a system for protecting against PUFA's poisonous impacts and/or matching the FA profile required for microbial development [32]. Rumen outflow of FA is primarily saturated free FA as a result of this comprehensive hydrolysis and biohydrogenation. However, some intermediate biohydrogenation compous, specifically CLA and trans-18:1 FA, also escape the rumen and are absorbed and used for the synthesis of milk fat. In modern exploatation for dairy, diet-induced MFD is often found and its occurrence involves two circumstances: an alteration in the rumen setting and a change in the population of bacteria that is often characterized by a reduction in rumen pH, and a nutritional source of PUFA [20]. As a result, there is a change in rumen biohydrogenation processes and completeness that improves the rumen outflow of intermediate biohydrogenation. The decrease in milk fat production during

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diet-induced MFD is therefore extremely associated with increased milk fat content of many trans-18:1 and CLA isomers [43, 59]. MFD's biohydrogenation theory suggested that MFD was triggered by inhibition of mammalian synthesis of milk fat by particular FAs generated in rumen biohydrogenation as intermediates [59]. Initial investigations used mixed CLA isomers and established proof of concept for the theory of biohydrogenation ; short-term infusion of CLA mixtures resulted in a dramatic reduction in the secretion of milk fat, which was reversed when supplementation ended [64]. Vyas et al. (2014) subsequently used comparatively pure isomers and proved that trans-10, cis-12 CLA abomasal infusion resulted in an instant reduction in the synthesis of milk fat, whereas cis-9, trans-11 CLA did not have any impact. However, comparisons between diet-induced MFD and trans-10, cis-12 CLA infusion suggested that extra intermediates for biohydrogenation needed to decrease the synthesis of milk fat [67]. Also of concern is the possible role of trans-18:1 isomers in regulating milk fat synthesis, partly because MFD is observed when abomasally infused big amounts of partially hydrogenated vegetable oils (PHVOs) [26]. Commercial use of trans-10, cis-12 CLA as a management instrument involves a CLA formulation that must have two features: it must provide protection for trans-10, cis-12 CLA from rumen bacteria changes, and it must eventually become accessible for absorption in the small intestine [25]. It will involve further inquiries to reconcile whether the various outcomes with trans-10 18:1 are linked to the use of physiological concentrations and/or the existence of other FAs in less pure preparations.

THE NUTRIGENOMICS ON FEED INTAKE LEVEL

Grala et al., 2013 noted that temporary feed restriction increases pyruvate carboxylase gene expression in milk cow liver. A powerful nutrigenomic impact was recorded in the liver of dairy cows due to prepartum feed intake (limited and high feed intake). In specific, cows experiencing prepartum feed limitation were noted to have a liver prepared to face the metabolic difficulties of the postpartum better [14]. Compared to properly fed livestock, extensive transcriptomic (is the set of all RNA molecules in one cell) impacts on the liver were also noted in early postpartum milk cows when exposed to limited grazing (60% equated to ideal forage level) [40]. The functional analysis revealed a general decline in the liver metabolism that could save energy for the other tissues. In addition, cholesterol synthesis was strongly inhibited, but PPAR signaling activation was noted [62]. In the liver of feed-restricted dairy cows, similar general nutrigenomic impacts were identified, but of a lower performances [3]. The nutrigenomic impact with the elevated intake prepartum (i.e., level amount of nutritional energy) was more acute in adipose tissue compared to the liver and significantly caused the gene networks engaged in triglyceride accumulation compared to a control group [11]. In 2 research in sheep and goats, feed limitation was noted to decrease the expression of multiple milk fat-related genes in mammary tissue [19]. At 2 wk postpartum, there was less phagocytosis and higher expression of several genes engaged in inflammatory response and metabolism from cows with elevated prepartum feed intake. Other nutritional elements are also increased or reduced in conjunction with energy content due to modifications in complete feed consumption [49]. Hence, the nutrigenomic impact of feed intake level is complicated, and a variety of TF is probably involved [1]. A system biology method should be used to account for this and the interaction between tissues.

CONCLUSIONS

One of the most comprehensive and successful examples of nutrigenomics in current animal science studies is the study of milk fat synthesis and its regulation by distinctive bioactive fatty acids. By depriving RXR required for adipogenesis, vitamin D metabolites decrease the formation of adipocytes during early growth of adipose. There are several transcription factors with

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high potential for nutrigenomic measures to fine-tune the dairy cows' metabolism to enhance efficiency, health, and quality of milk. The most powerful nutrigenomic compounds in the diet are the fatty acids. Other dietary elements have nutrigenomic roles, including the rate of nutrient consumption that can be used to prime the liver (and other tissues) to better meet metabolic difficulties, and AA, whose original studies disclosed an exciting nutrigenomic function in regulating the synthesis of milk protein. We anticipate that in the near future, practical nutrigenomic dietary interventions will probably not be accessible. More basic study requires to be carried out in order to reach practical applications. Data from nutrigenomics generated in dairy cows obviously underlines the fact that the present diet-building scheme for high-producing dairy cows is blind to the nutrigenomic impacts of nutritional compounds that are likely to alter their dietary requirements by influencing the animal's metabolism.

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