

Nutrigenomics – Impacts on Immunity, Health and Well Being

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Introduction

Over the last two decades, researchers have increasingly adopted molecular biology techniques and bioinformatics approaches to the study of ruminant nutrition and physiology. This has provided the field with novel and more robust information on how nutrients can affect animals at a cellular level, participating in the regulation of milk synthesis and meat quality and marbling (Osorio and Moisa, 2019). Specifically, dietary nutrients can alter gene expression directly or indirectly, affecting protein expression, metabolic and/or signaling status of cells, and, as a final consequence, can affect tissues, organs, and the entire organism (Bionaz et al., 2015).

The nutrient-genome interaction is at the basis of nutrigenomics as a science, expanding into how this relationship affects the balance between health and disease by altering the expression and/or structure of an animal's genetic makeup (Kaput and Rodriguez, 2004). The concept that food components can directly affect biological functions via genome-wide influences has led to a shift in our understanding of the field of nutrition, where dietary nutrients can no longer be considered just as basic building blocks for cells, tissues, and organs, or energy sources for metabolic processes. All nutrients, from simple fats, amino acids, and carbohydrates, to more complex phytochemicals, gained the status of "bioactives," capable of being detected by cellular sensors leading to a change of the cell biology (Muller and Kersten, 2003).

The Nutrient-Genome interaction

Dietary nutrients and bioactive compounds mainly interact indirectly with the animal's genome, as they don't directly bind with it or change its sequence. Rather, communication is mediated by specialized molecular proteins such as transcription factors for short- to medium-term regulation, and DNA or histone methyltransferases and histone acetyltransferases for medium- to long-term epigenetic effects (Osorio and Moisa, 2019).

Nutrigenomics is a relatively new branch of science in dairy cow research. Building upon data generated in other species, mainly humans and rodents, scientists have identified a short list of transcription factors with nutrigenomic potential in livestock species (Bionaz et al., 2015). This includes, but is not limited to, peroxisome proliferator-activated receptors (PPAR), sterol regulatory element-binding proteins (SREBP) and liver X receptors (LXR), which bind and are activated by fatty acids and metabolites of cholesterol. Similarly, proteins can be sensed by CCAAT/enhancer-binding protein (C/EBPs), and carbohydrates interact with SREBP and carbohydrate responsive element binding protein (ChREBP). Fat-soluble vitamins can also interact with the genome via retinoid X receptors (RXR) and retinoic acid receptors (RAR), vitamin D receptor (VDR), and pregnane X receptor.

The importance of these molecules and mechanisms, and their effect on dairy cow physiology is not always communicated to the dairy farmers, nutritionists, and industry consultants, even though some of the mentioned transcription factors have been linked to the production of milk components, and the well-known milk fat depression, a diet-induced production dysfunction. Most are aware that butterfat can be synthesized by the mammary gland using acetate, one of the three main volatile fatty acids produced by rumen fermentation, and by the uptake of circulating fatty acids which have been mobilized by adipose tissue. However, only few know of the role of PPAR γ , LXR α , and SREBP1 in regulating and stimulating fatty acid synthesis in response to specific nutrients (Bauman et al., 2011, Bionaz et al., 2015, Osorio and

Moisa, 2019). Furthermore, most are aware of the process of ruminal biohydrogenation and the effect that specific conjugated linoleic acids (CLA) have on milk fat synthesis. The effect of t10,c12 CLA on depressing milk fat synthesis via inhibition of SREBP1 was among the first and likely, the best-known, examples of nutrigenomics in dairy cows (Bauman et al., 2011).

Several fatty acids, both saturated (SFA) and polyunsaturated (PUFA), can regulate immune-related intracellular pathways and transcription factor activation, regulating inflammation onset, magnitude, and duration. (Sordillo, 2016). One of the central regulators of the inflammatory process is the transcription factor NF- κ B which regulates multiple innate and adaptive immune functions. NF- κ B induces the expression of various pro-inflammatory genes, including those encoding cytokines and chemokines, and participates in inflammasome regulation (Liu et al., 2017). In non-ruminant models, certain PUFA, such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), were found to have anti-inflammatory functions by inhibiting NF- κ B activation induced by gram-negative bacteria (Lee et al., 2010). Moreover, they can also indirectly affect this transcription factor through their interactions with other signaling pathways, including PPARs and SREBPs (Clarke, 2004). In contrast to PUFA, saturated fatty acids, such as lauric, myristic, and palmitic acids, were linked to NF- κ B activation, thus leading to a pro-inflammatory response (Erridge and Samani, 2009, Lee et al., 2010).

Liver X receptors, besides regulating fatty acid synthesis (Mu et al., 2021), have also been linked to the mammary gland inflammatory response (Hu et al., 2019). In vitro activators of LXR α inhibited the inflammatory response induced by gram-negative bacteria via inhibition of NF- κ B, leading to a lower expression and production of potent pro-inflammatory cytokines, such as TNF- α , IL-1 β , and IL-6 (Wang et al., 2018). Similar results were obtained in vivo, where synthetic activation of LXR attenuated mammary inflammation during induced mastitis in mice (Fu et al., 2014). Multiple natural molecules purified from plants or fungi, including phytosterols and terpenes, have been shown to activate LXRs (Komati et al., 2017), making their use a viable option to pursue when researching antibiotic-alternatives to mastitis in dairy cows.

Immunonutrition

Dairy cow rations are primarily formulated to meet production requirements, without truly dissecting what components of maintenance requirement might affect performance. There is substantial evidence indicating that the immune system is intimately involved with other mechanisms that allow cows to adjust quickly to the onset of lactation without suffering chronic disorders. In fact, cows that lag behind the rest of the herd in terms of production outcomes (including fertility) often display a greater inflammatory status and compromised liver function (Bionaz et al., 2007, Bertoni et al., 2008, Trevisi et al., 2012).

Dairy cows experience a period of immunosuppression around parturition, as several studies reported that both the innate and adaptive immune systems of peripartal cows are often compromised. For example, antimicrobial function and antigen response is reduced (Kehrli et al., 1989a, Kehrli et al., 1989b, Dosogne et al., 1999, Batistel et al., 2018), and consequently phagocytic activity by leukocytes is often (Ingvarsen et al., 2003, Batistel et al., 2018), but not always (Sander et al., 2011, Graugnard et al., 2012), reduced. Moreover, parturition influences the expression of multiple leukocyte genes required for normal function of these cells, including those involved in the classic immune response as well as normal cell growth, metabolism, and responsiveness to the blood environment (Burton et al., 2001).

However, this scenario is not so simple. Cows might be immunosuppressed when looking at some immune parameters, yet appear to mount an excessive immune response to pathogens that can cause more damage than benefit (Jahan et al., 2015, Vailati-Riboni et al., 2017c). For this reason, dairy scientists refer to the transition cows as “immunodysfunctional” rather than suppressed. Furthermore, transition cows were shown to display an overt inflammatory response related to pregnancy and lactation (Sordillo et al., 2009), even without signs of microbial infections and/or otherwise determined pathology (Bionaz et al., 2007, Bertoni et al., 2008). The conclusion for dairy producers is that, due to the combined results of immune and

metabolic dysfunctions, transition cows may be more susceptible to diseases like mastitis, but also to inflammation-associated tissue damage, than in other lactation periods.

Practical interventions

Since the immune system appears dysfunctional during early lactation, dairy nutrition researchers and the feed additive industry have placed some emphasis on developing dietary strategies and “immunostimulant” supplements to manage or reverse this scenario. In this section we are going to briefly discuss three nutritional strategies that were shown to alter dairy cow gene expression, positively impacting their immunity and health.

1. Energy density of dry cow

Traditional management provides “far-off” dry cows with a high-fiber/low-energy density diet (~1.30 Mcal/kg dry matter), while in the last month of gestation (“close-up” dry period) energy density is increased to approximately 1.60 Mcal/kg dry matter (NRC, 2001). The close-up value has been lowered to 1.50 Mcal/kg dry matter in the recent 2021 revision of dairy cows’ requirements (NASEM, 2021). However, studies from different research groups have demonstrated that prepartum overfeeding of energy often results in altered metabolic adaptation (Rukkwamsuk et al., 1999, Holtenius et al., 2003, Janovick et al., 2011, Ji et al., 2012, Ji et al., 2014, Khan et al., 2014), while exerting negative effects on postpartum health indices (Dann et al., 2006, Soliman et al., 2007, Graugnard et al., 2013, Shahzad et al., 2014).

Transcriptome profiling of neutrophils revealed that allowing cows free access to higher-energy diets during late pregnancy resulted in the alteration of pathways associated with the immune response and immune cell survival and proliferation (Moyes et al., 2014, Agrawal et al., 2015, Zhou et al., 2015). Furthermore, phagocytic activity of these cells was impaired, and early prepartal activation of inflammatory genes suggested a chronic state of compromised health (Graugnard et al., 2012, Moyes et al., 2014, Agrawal et al., 2015, Zhou et al., 2015). Transcriptomic studies further highlighted how overfeeding affects the whole system of the cow, as indicated by alterations in endoplasmic reticulum stress in hepatocytes, most likely as a consequence of greater activation of inflammatory pathways (Shahzad et al., 2014). Nutrigenomics mechanisms (i.e.: specific transcription factors) have not yet been clarified, but a recent study was able to provide evidence that a high-concentrate diet induced epigenetic (e.g., methylation) changes that contribute to the expression of immune-related genes in the livers of dairy cows (Chang et al., 2015).

Consensus among these research groups have prompted them to recommend limited use of a higher energy close-up diet, and keeping dry cows on a lower energy “far-off” type diet (around 1.30 Mcal/kg of dry matter) for the whole dry period. Of course, this recommendation has to take the herd body condition score (BCS) into account, as the effect of prepartum plane of nutrition is dependent on the BCS of the cow. Thus, these management tools need to be evaluated together to optimize a cow’s biological adaptations during the peripartum period. Omics analysis of the liver transcriptome highlighted how thin animals were more susceptible to prepartal nutritional management. Pathway expression patterns and metabolomics analysis suggested how overfeeding in late pregnancy should be limited to thin cows, while cows with optimal adiposity should be maintained on an energy-restricted diet (Vailati-Riboni et al., 2016a, Vailati-Riboni et al., 2016b, Vailati-Riboni et al., 2017a). The practical focus should be on reaching optimal BCS at the beginning of close-up, and maintaining cows on a slight feed restriction until calving.

2. Inclusion of yeast fermentation products

Fermentation products specifically derived from *Saccharomyces cerevisiae* (SCFP) have been reported to increase leukocyte function both in vitro and in vivo (Jensen et al., 2008, Magalhaes et al., 2008). Recently, new commercially available SCFPs for dairy cows and calves (NutriTek® and SmartCare®, Diamond V, Cedar Rapids, IA, USA) were the subjects of multiple publications illustrating a wide spectrum

of beneficial health and performance effects during immunological challenges, like dietary and respiratory infection, mycotoxins, heat stress, and the transition period (Alugongo et al., 2017, Harris et al., 2017, Knoblock et al., 2019, Olagaray et al., 2019, Velez et al., 2019, Mahmoud et al., 2020, McDonald et al., 2021, Klopp et al., 2022). The SCFP contain multiple vitamins and antioxidants, such as polyphenols, in addition to other bioactive compounds including fermentation end-products, β -glucans, and other components of the yeast cell wall, that have been shown to modulate the immune response in humans and animals by priming the innate and adaptive immune responses through activation of immune cells (Li et al., 2005, Volman et al., 2008).

When NutriTek efficacy against mastitis was tested in 25 large-scale commercial herds throughout the United States, its supplementation reduced incidence of mastitis in 64% of the dairies, with half of these also seeing a reduction in linear scores (Ferguson et al., 2018). To better understand the underlying mechanisms, researchers at the University of Illinois performed gene expression analysis of mammary gland samples collected from control or NutriTek fed dairy cows subjected to a mastitis challenge (Vailati-Riboni et al., 2021). Researchers induced subclinical mastitis by inoculating one quarter of each cow with 1,500 CFU of *Streptococcus uberis*, a gram-positive bacteria predominantly responsible for intramammary infection (Keane, 2019). NutriTek supplementation increased gene expression related to (i) a greater antimicrobial capacity of innate immune cells, (ii) the activation of cellular mechanisms to enhance mammary gland protection against side effects of inflammation, and (iii) the maintenance of tissue integrity and health (Vailati-Riboni et al., 2021). These activations resulted in a 4-fold lower somatic cell count (1,076,052 vs 179,415 cells/mL, control vs NutriTek) in the infected quarter of supplemented cows 36 h after infection. These boosted defenses allowed NutriTek fed cows to maintain higher feed intake and milk yield both during the challenge period and in the following month. Similar effects on somatic cell count were observed when NutriTek fed cows were subjected to heat stress. NutriTek fed cows had approximately 50% lower plasma cortisol, a stress-related and immunosuppressant hormone, and higher numbers of immune cells (9 to 26% more) in their blood, compared to the cows in control group. This led to a 36% lower somatic cell count during the intense heat stress challenge (Al-Qaisi et al., 2020).

Anecdotal reports have suggested that SCFP supplementation may also improve the outcome of bovine respiratory disease (BRD) in young calves, in dairy heifers when transitioning to group pens and freestalls, and in feedlot animals. Mahmoud et al. (2020) tested this hypothesis by supplementing neonatal Holstein x Angus calves daily with SmartCare and NutriTek, and challenging them at around 3 wks of life via aerosol inoculation with bovine respiratory syncytial virus (BRSV). Supplemented calves showed reduced clinical disease signs and had lower viral shedding, together with reduced lung pathology, and incidence of secondary bacterial infection. Immune cells gene expression was not investigated, but SmartCare and NutriTek enhanced innate cytokine production of circulating immune cells and had an immunoregulatory effect on innate immune function by cells in the airways. Supplementation also reduced virus-specific IL-17 secretion by cells isolated from the airways, leading to reduced neutrophil recruitment to the lungs. In the context of respiratory disease, limiting inflammation in the lungs is critical for minimizing lung tissue damage and promoting rapid recovery. Similar results were obtained from a follow-up experiment, testing the response to a coinfection of BRSV and *Pasteurella multocida* (McDonald et al., 2021). Again, SmartCare and NutriTek supplemented calves mounted a robust immune response and displayed lower severity of a viral-bacterial respiratory infection. As inflammation is an energy-demanding response, McDonald et al. (2021) also reported no detrimental metabolic consequences in supplemented calves, as these animals showed blood biomarkers changes favoring growth and development.

3. Rumen-protected methionine and choline as an immunostimulant

In addition to being considered one of the two most-limiting amino acids for milk production, methionine (Met), and several of its related metabolites (e.g. choline [Chol] and taurine), display an immunonutritional role, i.e. they help boost certain activities of the immune system in humans (Grimble and Grimble, 1998, Redmond et al., 1998, Grimble, 2006, Li et al., 2007). Since these properties have been tested on immunosuppressed human subjects with positive outcomes (Van Brummelen and du Toit, 2007), dairy scientists hypothesize that enhancing Met supply above what is recommended for optimal lactation performance (Lys:Met < 3:1) would have a positive effect on immune function in the transition period, where cows are in immunocompromised state.

Earlier results indicate the ability of Mepron[®], a commercially available rumen-protected form of methionine (Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany), to increase T lymphocyte proliferation *ex vivo* when supplemented at a rate of 30g/d (Soder and Holden, 1999). Since human lymphocytes seem to have an absolute requirement for Met to proliferate (Hall et al., 1986), these results were not unexpected. However, data on immune function were not generated. More than a decade later, researchers from the University of Illinois showed how the use of Smartamine (0.07-0.08% DM, Adisseo Inc., Antony, France), Metasmart (0.19% DM, Adisseo Inc.) or Mepron (0.09-0.10 % DM) during the transition period enhanced immune cell pathogen killing capacity (e.g., phagocytosis, and oxidative burst) (Osorio et al., 2013, Zhou et al., 2016, Batistel et al., 2018). Furthermore, Met supplementation optimized the immune response to bacterial components (Vailati-Riboni et al., 2017c) and helped mitigate the detrimental effects of close-up energy overfeeding (Vailati-Riboni et al., 2017b). Feeding rumen-protected Met, thus, helps dairy cows tackle the two aspects of periparturient immune dysfunction, hyporesponse (or suppression), and hyperresponse. Not many data have been generated on the effect of Chol supplementation on direct measures of immune functions, but some beneficial results have been observed (Vailati-Riboni et al., 2017c).

Changes in functionality of immune cells in response to supplementation with Met or Chol are often correlated to changes in their gene expression. Supplementation during the transition period can prime neutrophils, an important innate immune cell type, for mounting a better immune response upon pathogen challenge through fine-tuning of the cell transcriptome. The hyperactivation and response to stimuli observed in periparturient dairy cows (Jahan et al., 2015) has been linked to worsening of the animal oxidative status during this period (Vailati-Riboni et al., 2017b, Vailati-Riboni et al., 2017c). Zhou et al. (2018) observed how neutrophils had lower expression of oxidative stress-related genes in response to Met and Chol supplementation. This then led to a reduction in the expression of pro-inflammatory genes, indicating that a better redox status creates a more balanced immune response. Similar gene expression patterns were observed when supplementing methionine, choline, or taurine *in vitro* to neutrophils isolated from neonatal (3 wks old) Holstein calves (Abdelmegeid et al., 2017) or mid-lactation adult cows (Lopreato et al., 2019). Greater methylation of the promoter region of *PPAR α* occurred in the liver of cows supplemented with Met, leading to greater *PPARA* expression and upregulation of some of its downstream genes (Osorio et al., 2016). Activation of hepatic *PPAR α* has been associated with improved lipid metabolism and immune function, both of which were reported in companion publications from this study (Osorio et al., 2013, Osorio et al., 2014).

Through modulation of gene expression and consequent improvement of immune functions, Met and Chol supplementation to levels beyond milk production requirements demonstrate that maintenance and immune system requirements have to be integrated in our modern lactation diets. Supplementation can support practical aspects of herd health, such as reduction in the incidence of mastitis or adverse health events in general (Ardalan et al., 2010, Arshad et al., 2020).

Conclusions

The interface of immunity and nutrition is an emerging field of study. Relatively recently, they were considered separate entities, with the only focus on avoiding deficiencies in key nutrients. Now instead, the fields of nutrigenomics and immunometabolism show that targeted supplementation can boost immune function beyond what is considered normal or average, by modulating expression of cows' genetic potential. These concepts will have to be further applied in dairy science to better define maintenance requirements and develop more complete nutritional management of the dairy cow. As consumer scrutiny of the dairy industry's goods and production strategies increases, nutritional intervention (e.g., macro- and micronutrients, bioactive compounds, pre-, pro-, and postbiotics, phytonutrients) will lend themselves as preventative and prophylactic strategies to ensure food safety and security, while supporting animal productivity, health, and welfare.

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