Chapter 1 Gastrointestinal nematodes: Immune response, development of resistance, nutrition, and nutrigenomics in sheep

Capítulo 1 Nematodos gastrointestinales: Respuesta inmune, desarrollo de la resistencia, nutrición y nutrigenómica en ovinos

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Abstract

In tropical regions with warm and temperate climates, gastrointestinal nematode infections (GIN) are the main problems affecting the health and welfare of sheep. One of the strategies to control these infections is the selection of individuals genetically resistant to GIN. Such resistance implies the optimal development of the immune response, where animal nutrition plays an essential role. The objective is to describe the immune response, the development of resistance against GIN in sheep, and the use of nutrigenomics as a tool to evaluate the effect of dietary nutrients on the expression of genes involved in the immune response using a literature review. The conclusion is that for the proper functioning of the immune system, it is essential that animals have energy, protein, and micronutrients for cellular synthesis and other functions that demand a lot of energy during an infectious event.

Gastrointestinal nematodes, Immune response, Nutrigenomics, Sheep

Resumen

En regiones tropicales con climas cálidos y templados, las infecciones por nematodos gastrointestinales (NGI) son los principales problemas que afectan la salud y bienestar de los ovinos. Una de las estrategias de control de estas infecciones es la selección de individuos genéticamente resistentes a los NGI, dicha resistencia implica el óptimo desarrollo de la respuesta inmune, donde tiene un papel esencial la nutrición de los animales. El objetivo es describir la respuesta inmune, el desarrollo de la resistencia contra NGI en ovinos y el uso de la nutrigenómica como herramienta para evaluar el efecto de los nutrientes de la dieta en la expresión de genes implicados en la respuesta inmune, mediante una revisión bibliográfica. Se concluye que para el adecuado funcionamiento del sistema inmune es indispensable que los animales cuenten con nutrientes energéticos, proteicos y micronutrientes, para realizar la síntesis celular y otras funciones que demandan mucha energía durante un evento infeccioso.

Nematodos gastrointestinales, Respuesta inmune, Nutrigenómica, Ovinos

Introduction

Sheep production systems worldwide have something in common, their struggle against gastrointestinal parasites (especially nematodes), as they compromise animal health and productive performance due to stunting, weight loss, anemia, and even death of the most susceptible. Sheep can be simultaneously infected by different species of gastrointestinal nematodes (GIN). However, *Haemonchus contortus*, *Teladorsagia circumcincta*, and *Trichostrongylus colubriformis* are considered the most significant for their prevalence and pathogenicity (Ortolani et al., 2013). In the face of established anthelmintic resistance, identifying resistant animals by their immune response against GIN is one of the alternatives for infection control. The immune response is the result of a complex parasite-host interaction, where the host displaces all the defense mechanisms it possesses to eliminate the parasite. However, to survive, the parasite must not eliminate its host, which would be suicide, so the best option is to evade the immune response and establish a balance that allows it to subsist to develop chronic infections. In this balance, a factor that moves the faithful to one side or the other is the host's nutrition. Proper immune system function depends on energetic nutrients, proteins, and micronutrients, to perform cellular synthesis and other energy-intensive functions during an infectious event. The objective of this review is to describe the immune response, the development of resistance against GIN in sheep, and the use of nutrigenomics as a tool to evaluate the effect of dietary nutrients on the expression of genes involved in the immune response.

Gastrointestinal Nematodes

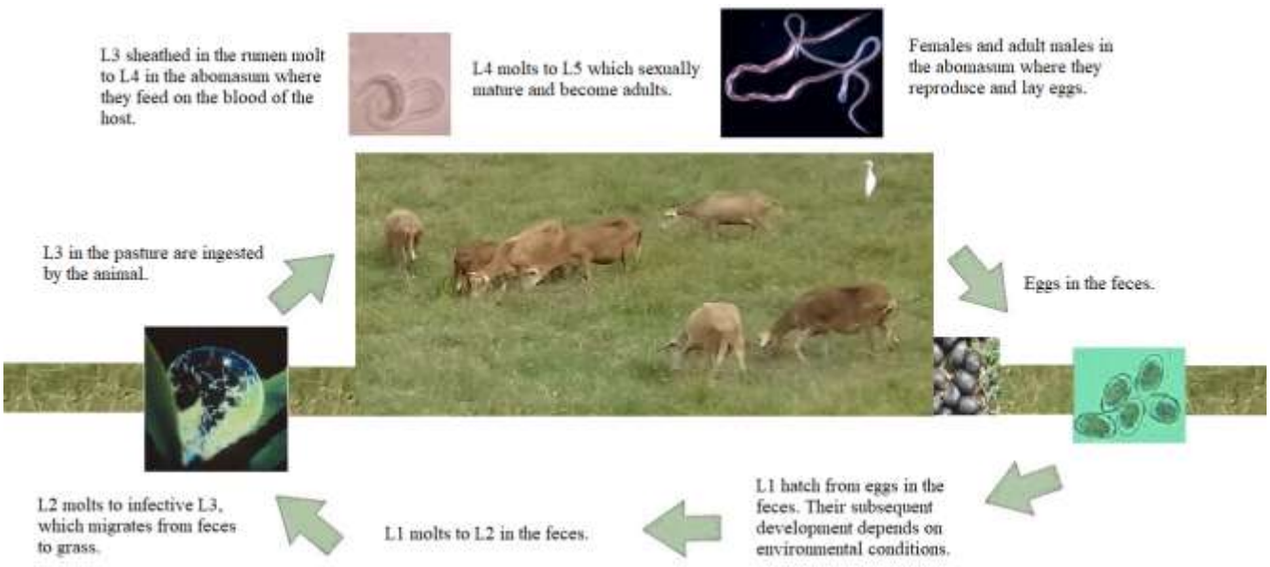
Life cycle

The life cycle of most GIN is monoxenous (it only requires one species to complete its life cycle) and comprises two phases. The first is a free-living phase (exogenous) directly influenced by the environment, and the second is a parasitic phase within the host (endogenous) influenced by the immune response generated by the infection (Urquhart et al., 1996).

The free phase begins with the elimination of eggs along with the feces. If environmental conditions are favorable (temperature between 10-35°C and more than 60% humidity), the embryonated eggs hatch, releasing the stage 1 larva (L_1), which molts to larva 2 (L_2) in 1-2 days. Both L_1 and L_2 remain in the feces feeding on organic matter and microorganisms present in the feces. The L_2 molts to L_3 (infective larva), retaining the cuticle (sheath) of the previous stage that protects it from the external environment. If the humidity conditions are suitable, the L_3 migrates towards the pasture waiting to be ingested by the host. The time elapsed between the hatching of L_1 to the appearance of L_3 is normally 5-6 days (there are variations due to the species). However, it can be delayed for several months if the temperature and humidity are not adequate (Urquhart et al., 1996).

The parasitic phase begins with the ingestion of grass with the L_3 by the host. The L_3 reaches the rumen, where they are released from the protective sheath and penetrate the mucosa of the abomasum to continue their development. After 4-5 days, the L_4 appear and after a new molt, they transform into preadults (L_5) that will sexually mature into adults that copulate and begin to produce eggs (in some species >5000 eggs per day) that are eliminated in the feces (Figure 1.1). The prepatent period is 2-3 weeks as long as the process of larval hypobiosis does not develop due to a host immune response or adverse environmental conditions (Urquhart et al., 1996; Besier et al., 2016).

Figure 1.1 Life cycle of the GIN Haemonchus contortus, showing the three main stages, host, feces, and pasture



Source: Image of the authors

Immune response

The immune response that sheep develop against GIN is the result of a complex interaction between defense mechanisms that have developed because of the evolution of the parasite-host interaction. These mechanisms (innate immune system and acquired immune system) are composed of organs, tissues, cells, and molecules that elaborate a coordinated response, which has allowed the host to identify the parasite, differentiate it from its components and eliminate it. Innate immunity is based on non-specific mechanisms of immediate action, lacking memory and which are responsible for fighting the infection at its onset with great efficacy. If these mechanisms fail to eliminate the parasite, they at least keep it under control while the mechanisms of acquired immunity develop, which require more time (Karrow et al., 2013).

Innate immunity

Innate immunity is characterized by non-specific mechanisms of immediate action, does not generate immunological memory, but is associated with the acquired or memory response. The cells involved in the innate response are responsible for stopping the infection during its onset.

These mechanisms fail to regulate the infection, but various specific signals are emitted by the innate response (e.g., polymorphonuclear cells, PMN) to attract specialized T and B lymphocytes to generate effective mechanisms to regulate the parasitic infection (Karrow et al., 2014).

Likewise, innate or non-specific immunity constitutes the first line of defense against pathogens, including parasites. Hosts have various cellular and molecular mechanisms that can recognize antigens to control infection and repair damaged tissue (Alba-Hurtado and Muñoz-Guzmán, 2013). The components of the innate response can be classified as external (physical and chemical barriers) or internal (soluble molecules and PMN cells, macrophages, among others). Physical barriers such as skin and mucous membranes prevent parasite entry through the integrity of their structures and reactions such as coughing, sneezing, intestinal peristalsis, and mucous secretion. Chemical barriers comprise pH, temperature, and enzymes that act by inhibiting the development or altering the structures of the parasite. Soluble factors or molecules include the complement system, immunoglobulins, and cytokines, which promote inflammation, cellular attraction, and/or activation corresponding to acquired immunity.

The cells involved in innate immunity are macrophages, neutrophils, and NK (Natural Killer) lymphocytes, which have the function of phagocytosis, antigen presentation, effector action, and cytokine release (Collado et al., 2008). The immune response has two distinct phases: the pathogen recognition phase and the effector or response phase, as described below.

Recognition phase: Cells of the innate immune system possess receptors called "pattern recognition receptors" (PRRs). These recognize specific molecular structures (not present in the host) on pathogens called "pathogen-associated molecular patterns" (PAMPs) that are microbial activators of the immune response. The main PRRs involved in the immune response against parasites are Toll receptors (TLRs) and C-type lectin receptors (CLRs), expressed by many cell types such as mucosal surface cells, antigen-presenting cells (APCs), T and B lymphocytes, macrophages, mast cells, neutrophils, and dendritic cells. Both PPR types identify PAMPs and DAMPs, which are molecules released by tissue damage or cellular stress (McRae et al., 2015). Likewise, they induce the inflammatory response, and PAMPs trigger the release of cytokines and other molecules that function as intracellular signals of the adaptive immune response (Balic et al., 2000; Balic et al., 2002).

Effector or response phase: After the parasite is recognized, to prevent the establishment and its pathogenic action, complement activation and fixation is initiated, leading to an inflammatory response, activation of phagocytosis by neutrophils/macrophages, and activation of B lymphocytes for the initiation of the humoral response (McRae et al., 2015). The complement system consists of about 30 proteins found in plasma and are activated by one of three pathways known as classical, alternative, and lectin. During activation, there are consecutive cascade reactions that generate active products, which also has biological actions such as assembly on the surface of the microorganism of the membrane attack complex (MAC) by forming ion and water permeable channels, stimulating lysis of the foreign agent, as well as the release of proinflammatory products that attract other effector cells of the immune system such as mast cells and basophils (Vijayasarathi et al., 2015).

However, the efficacy of these mechanisms against GIN may be weak due to the cellular components of the nematodes, such as lipids, chitin, surface and excretion, and secretion products that contribute to the evasion of the immune response.

Acquired immunity

Acquired immunity develops from cell signaling of the innate response and is characterized by the response specificity and generation of immunological memory (McRae et al., 2015). For the study, the three phases of the adaptive immune response are antigen recognition, lymphocyte activation, and effector response.

Antigen recognition: APCs are a key element in the immune response, by capturing pathogen-specific peptides/proteins which they present to Major Histocompatibility Complex class II (MHC II) molecules, specialized subpopulations of T lymphocytes (cells belonging to the "Cluster of Differentiation 4+", CD4+), to attract specialized cells such as T-helper (T_H) and cytotoxic (Tc) lymphocytes (Hein et al., 2010). APCs, macrophages, PMNs, contribute to the activation of cytokines that lead to the differentiation of T_H lymphocytes into T_H1 or T_H2.

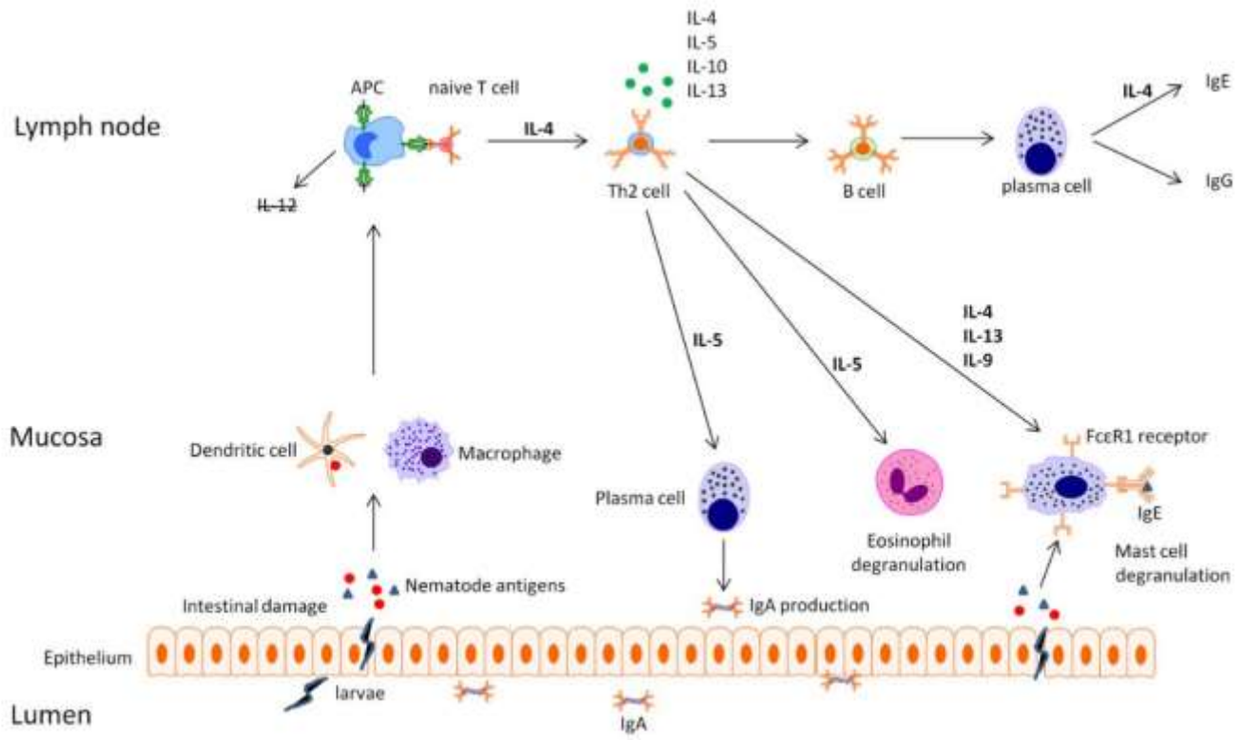
Lymphocyte activation: As specialized cells, lymphocytes are activated by specific antigens through B and T lymphocyte receptors (BCR, B cell, and TCR, T cell receptor). B lymphocytes are located in the blood and lymphoid organs and are responsible for the humoral immune response. In addition, they are characterized by synthesizing immunoglobulins (Ig) with action on antigenic receptors to send co-stimulatory signals from complement receptors or by T_H lymphocytes. The secreted Ig bind to the antigen and other cells of the immune system for pathogen neutralization (Balic et al., 2000; McRae et al., 2015).

Effector response: The T_H1 response is involved in infections caused by bacteria, protozoa, and viruses, but in recent years it is also associated with helminths, including gastrointestinal and pulmonary nematodes (Costa-Rodrigues et al., 2017). This pathway produces immune response genes such as interleukins 1, 2, and tumor necrosis factor-alpha (TFNα). Likewise, increased messenger RNA transcripts of immune system genes have been found in GIN susceptible/resistant sheep (Gill et al., 2000; Estrada-Reyes et al., 2017). The dominant immune response in GIN infections is mainly controlled by T_H2 class cytokines such as *IL-4, 5, 9, 10*, and *13* (Alba-Hurtado and Muñoz-Guzmán, 2013; McRae et al., 2015; Estrada-Reyes et al., 2017).

Development of resistance against *Haemonchus contortus* and other GIN

Resistance to nematodiasis is the ability of the individual to interact with and regulate parasite infection through the immune response (Stear et al., 1999). Numerous studies with GIN infected sheep have reported immunity against GIN associated with T_H2 cell response characterized by increased cytokines (*IL-4, 5, 13*), PMNs, mast cells, globular leukocytes, and IgA (Figure 1.2) (Shakya et al., 2009; Bowdridge et al., 2013). The small ruminants exhibit diverse mechanisms that control nematodiasis by reducing larval establishment through cell damage and adult fecundity (Rowe et al., 2008; Ortolani et al., 2013).

Figure 1.2 Immune response to GIN challenge in sheep. Image by McRae et al. (2015)



The immune response to gastrointestinal nematode challenge in sheep. Incoming larvae damage the intestinal mucosa, which leads to local inflammation and mast cell degranulation. Nematode antigens are taken up by APCs such as dendritic cells and macrophages. These cells subsequently migrate to the regional lymph nodes, where they present antigens to naïve T cells. T-cell differentiation results in the release of T_H2-associated cytokines and the recruitment of effector cells such as eosinophils and mast cells to the site of infection. It also initiates the adaptive immune response and the production of nematode-specific antibodies by plasma cells. Cytokines promoting a process are shown in bold.

Immune response against L_3

When *Haemonchus contortus* L_3 invades the abomasal mucosa, there is an increase in CD4+ T cells, TCR, and BCR during primary infection, which occurs approximately five days post-infection (Balic et al., 2000). In contrast, during repeated infections, significant recruitment can be observed in only three days (Balic et al., 2002), indicating a protective immune response. Consequently, parasitized animals show changes in the abomasal mucosa and local lymphoid tissue due to the effect of *IL-4, 5, 9, 10,* and *13* for their role in controlling nematodiasis.

IL-4 and *IL-13* are essential to maintain the T_H2 response and act as inducers of inflammatory processes. *IL-5* promotes the development of eosinophils and increases their toxicity, and indirectly is involved in the production of IgE together with *IL-9* to favor the growth of T_H lymphocytes and mast cells and potentiating the effects of *IL-4* in the production of IgE. *IL-10* has regulatory activity because it inhibits the synthesis of T_H1/T_H2 cytokines involved in inflammation. In addition, local and systemic production of IgA, IgE, and IgG is linked with the presence and control of nematodiasis (Gill et al., 1992). It is important to consider that depending on the parasitic invasion, it will be the described elements that are expressed in the animal's immune system which make it possible to describe two types of response: rapid expulsion (or immune exclusion) and delayed expulsion (Nisbet et al., 2016).

Rapid expulsion (or immune exclusion): This response occurs in animals hypersensitized by repeated larval infections over a prolonged period resulting in the expulsion of L_3 within 48 hours, e.g., before being implanted in the abomasal tissues (Balic et al., 2000). This response is characterized by a type 1 hypersensitivity reaction in the mucosa by degranulation by mast cells and globular leukocytes of proinflammatory mediators (proteases, prostaglandins, leukotrienes, histamine, and serotonin). These principally act by increasing mucosal permeability, increasing peristalsis, and facilitating the arrival of complement factors and antibodies to the intestinal lumen (Balic et al., 2002).

Eosinophils also play a relevant role in this type of response. An increase in the excretion of galectin-14 (a mediator released by eosinophils) in the mucus has been found, which promotes cell adhesion and increased viscosity, helping to prevent larval migration, and contributing to the rapid expulsion of infective larvae (Young et al., 2009).

Delayed expulsion: This response is characterized by the recruitment of eosinophils that destroy the L_3 through antibody-mediated cellular cytotoxicity, which occurs after about five days, e.g., after the establishment of larvae in the abomasal mucosa. The response is regulated by T_H2 cytokines, IgA, IgE, and IgG, which are directly involved in the expulsion of the larvae, thus decreasing the number of parasites in the abomasum (Balic et al., 2006).

Immune response against L_4

The development of L_4 in tissue is vital to continue the biological cycle of nematodes such as Haemonchus and Teladorsagia, because hypobiosis allows them to evade immune mechanisms by decreasing their metabolism (Gibbs, 1986).

Hypobiosis: Hypobiosis, also known as "tissue arrest," is a genetic faculty of the fourth stage of Haemonchus/Teladorsagia governed by chemotaxis of external and internal environmental factors of the host to modify the life cycle. During hypobiosis, there is a latency period with decreased metabolism observed in the L_4 in the abomasal mucosa where they accumulate. When there are adequate conditions for their development, they leave the mucosa in large numbers causing a very serious infection. The immunological response of the host and the presence of adult nematodes in large numbers creates overcrowding that inhibits L_4 by hypobiosis (Balic et al., 2000). An association has been observed between L_4 in hypobiosis and IgA as immunized animals developed hypobiosis, and non-immunized animals did not (Henderson and Stear, 2006).

Control of length and fecundity by IgA: Studies in *Telodorsagia circumcincta* have found that increased secretion of IgA specific for L_4 excretion/secretion products is associated with reduced L_4 length and thus decreased fecundity in the adult stage of the parasite (Ellis et al., 2014).

Galectin-11 in the control of L_4 growth: Galectins are lectin-like proteins with numerous biological functions such as regulation of immune response as lymphocyte adhesion and cytokine production. Preston et al., (2015) *in vitro* studies were the first to demonstrate that galectin-11 can bind to the pharyngeal region of L_4 of *Haemonchus contortus*, thereby inhibiting L_4 feeding, reducing its size and viability. They also found that galectin-11 affects adult nematodes, but not L_3. With these findings, new lines of research are being developed to study the mechanisms of action of galectin-11 in parasitic stages of GIN that will probably lead to vaccines (Laing et al., 2013).

Immune response against adults

The indications of resistance against adult *Haemonchus contortus* and other GIN are mainly expulsion and cell damage of adults and reduced fecundity of females.

Nematode expulsion: Expulsion of adult parasites usually happens after repeated larval infections and occurs through a non-specific mechanism of rapid rejection, or immune exclusion, or by the mechanisms of acquired immunity (Vijayasarathi et al., 2015).

Reduction in size and fecundity of females: The main manifestation of acquired immunity by the host against adult GIN is morphological change. As a result, they reduce their fecundity. The reduction in nematode size is associated with increased production of abomasal IgA specific for L_4, which correlates with a reduction in the length of adult females present and reduced fecundity, as demonstrated by a decrease in fecal egg count (Sinski et al., 1995; Stear et al., 1999). Female length is estimated to correlate between 60 and 70% with the number of eggs present in the uterus of female *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (Gruner et al., 2004). The mechanism involves eosinophils that have receptors for IgA and IgG and low-affinity receptors for IgE. Binding to IgA provides the most potent stimulus for degranulation of inflammatory mediators and cytokines such as *IL-5* that potentiate their cytotoxic effect (Balic et al., 2006).

Factors influencing resistance against GIN

Many factors influence the resistance of sheep to *Haemonchus contortus* and other GIN, some of which are host-specific, such as genetic constitution, breed, age, reproductive status, sex, and nutrition.

Genetic constitution: Genetic variation in resistance to *Haemonchus contortus* and other GIN has been confirmed in several studies, so genetic selection of naturally resistant individuals represents an option for the control of parasitic infections (Bishop, 2012; Emery et al., 2016).

Breed: Several breeds have developed natural resistance to GIN infections, such as Blackbelly, St. Croix, Florida Native, Gulf Coast Native, Thin Tail, Garole, Pelibuey, and Red Masaai (Amarante et al., 2009; Saddiqi et al., 2010; Jacobs et al., 2018; Estrada-Reyes et al., 2017). All have been studied to understand the immunological components involved and identify genes associated with resistance. Two quantitative trait loci (QTL) have been identified that have candidate genes, one on chromosome 3 near the interferon-gamma *(INFγ*) locus and another on chromosome 20 adjacent to the MHC encoding MHC II proteins involved in antigen presentation described above (Saddiqi et al., 2012; Karrow et al., 2014).

Age: Lambs aged three to six months are more susceptible to parasitic infections than adults. However, the high prevalence rate and the problem of anthelmintic resistance make it difficult to control nematodiasis in young and adults. The immunosuppression mechanisms observed in young ruminants are due to the poor response of CD4+ T lymphocytes, CD8+ T lymphocytes in lambs concerning adults (Miller and Horohov, 2006; Alba-Hurtado and Muñoz-Guzmán, 2013). In addition, it has been reported that IgA begins to be expressed from five months of age, confirming deficiencies in the T_H2 response in young animals (Shaw et al., 2013). However, in general, small ruminants are more susceptible than large ruminants to nematodiasis due to the delayed immune response observed. Therefore, it is important to select individuals with higher genetic potential against these pathogens.

Reproductive status: Temporary loss of immunity (decreased resistance) to GIN has been found in ewes close to lambing and during lactation manifested by elevated fecal egg counts that contaminate pastures, exposing newborn lambs to infection. This behavior is attributed to increases in progesterone and glucocorticoid concentrations during peripartum, nutritional factors, and lactation stress (Saddiqi et al., 2010; Emery et al., 2016).

Sex: Males are more susceptible to GIN infection than females. However, this difference does not occur before puberty. Immunity in males develops gradually from birth to adulthood, whereas resistance increases considerably after puberty in females. This susceptibility of males is attributed to androgens that modulate various aspects of host immunity (Toscano et al., 2019).

Nutrition, nutrigenomics, and epigenetics in the gene expression of the immune response against gin in sheep

Nutrition

Nutrition is vital for the development of immunity against GIN. We know that the quality of the feed influences the host's immune response and is under genetic control. Basically, feed with high protein content is essential to acquire maturity in the immune response. Under this priming, animals can meet their basic requirements for maintenance, growth, reproduction, and development of immunity. At the same time, those with poor nutrition will cause more individuals to be susceptible to GIN infection (Coop and Kyriazakis, 2001). Numerous studies have shown that protein-rich diets improve the host's ability to respond to the adverse effects of parasitic infection (Torres-Acosta et al., 2012).

GINs affect animal productivity through the reduction of voluntary intake, reduced diet digestibility, and inefficient absorption of metabolized nutrients. It has been proven that in small ruminants, as long as there is at least 80% of its metabolizable energy requirements, resistance to GIN is not compromised. On the other hand, alterations in protein metabolism are known to occur where there is a significant loss of endogenous protein in the digestive tract (Houdijk et al., 2012). There is evidence that immune system cells metabolize some non-essential amino acids (alanine and glutamine) (e.g., the host can synthesize them) to obtain energy to function. Apparently, it is an evolutionary advantage of the immune system not to depend on external sources of energy (Cruzat et al., 2018). Table 1.1 shows the nutrients and their relationship with the animal's immune function.

Table 1.1 Role of important nutrients in immune function. Adapted from Paul y Dey (2015)

Nutrient	Immunological function
Energy/lipids	Caloric malnutrition reduces cell-mediated immunity and antibody response. Changing the fatty acid composition of immune cells through diet affects phagocytosis, signaling in T lymphocytes, and antigen presentation capacity.
Protein/amino acids	Protein is necessary for the maturation of immune system organs. Specific amino acids are required for optimal immune function of gut-associated lymphoid tissue.
Zinc	It is crucial for the normal development and function of immune system cells (neutrophils, NK cells, phagocytosis, and cytokine production).
Copper	The deficiency affects the innate immune system.
Chromium	It reduces cortisol and increases immunoglobulins (especially IgM).
Iron	The deficiency involves the peripheral lymphoid system.
Selenium	They catalyze oxidation-reduction reactions and protect the host from oxidative stress.
Vitamin E	Influences the antibody-mediated neutrophil immune response. Stimulates lymphocytes.
Vitamin A	It influences the cellularity of lymphoid organs. Retinoic acid is essential for the migration of T and B lymphocytes to the intestine.
Vitamin D	It has inhibitory effects on the acquired immune response and a stimulatory effect on monocyte proliferation.
Vitamin C	Protects membranes against lipid peroxidation damage. Relieves the suppressive action of corticosteroids on neutrophils.
Vitamin B / Carotenoids	Riboflavin deficiency harms macrophage activity. It prevents oxidative damage in immune system cells. It is important in cell-mediated immunity and cytotoxicity.

In conclusion, adequate protein and energy intake, along with avoidance of micronutrient deficiencies (vitamins and minerals), are keys to strengthening animal immunity (Colditz, 2002: Paul and Dey, 2015).

Nutrigenomics

Nutrigenomics is an emerging science that studies the molecular relationships of dietary components that modify gene expression and/or structure. Thus, dietary nutrients can: a) act as ligands for the activation of transcription factors that favor receptor synthesis, b) be metabolized by primary or secondary metabolic pathways, altering the concentration of substrates or intermediates, and c) influence cell signaling. Nutrigenomics uses technologies from functional genomics (transcriptomics, proteomics, and metabolomics), bioinformatics, and molecular biology, with established nutritional and biochemical techniques (Benitez et al., 2017). We will look at some basic concepts for better understanding (Loor et al., 2015; Osorio et al., 2017).

– *Genome:* Complete set of genes of an organism or its organelles.

– *Transcriptomics:* Study of the transcriptome, e.g., the set of all RNA molecules, including mRNA, rRNA, tRNA, and non-coding RNA produced in cells, tissues, or organs.

– *Proteomics:* Study of the proteome, e.g., the whole set of proteins produced by a cell, tissue, or organ.

– *Metabolomics:* Study of chemical processes involving metabolites in a cell, tissue, or organ.

In recent years, studies have been conducted on the effect of nutrients on immune system gene expression. Ciliberti et al. (2015) investigated the effects of polyunsaturated fatty acid supplementation in the diet of dairy sheep. They evaluated gene expression in mononuclear cells, finding an overexpression of *IL-6*, an interleukin with proinflammatory and stimulatory functions for B lymphocytes in antibody production.

Elgendy et al. (2017) studied the effects of a high-zinc diet on the transcriptome of 15 sheep. The results indicate that 154 different genes were overexpressed in the transcriptome of sheep that received the treatment relative to the control. These genes were related to the immune system, various transductional signals, and processes related to membrane permeability. Elgendy et al. (2016) investigated the effect of dietary supplementation high in organic selenium on the transcriptome of 10 sheep. Blood samples were taken at the beginning and end of the experiment, from which RNA was obtained. Using qPCR, they found overexpression of T and B lymphocyte receptors, cytokine binding signals, and interleukins. The findings suggest that, in sheep, high dietary selenium supplementation leads to expression changes of several genes involved in immune system mechanisms (both acquired and innate) and provides further information on the transcriptional modulation capacity of selenium.

Epigenetics

Epigenetics studies heritable changes in DNA structure and organization that do not involve sequence changes and modulate gene expression in cells. Through epigenetic mechanisms, cells can mark which genes should be expressed, to what degree, and at what time. Moreover, epigenetic changes are not static and can be modified throughout the life of a cell. One of the main characteristics of epigenetic modifications is their reversibility (Murdoch et al., 2016).

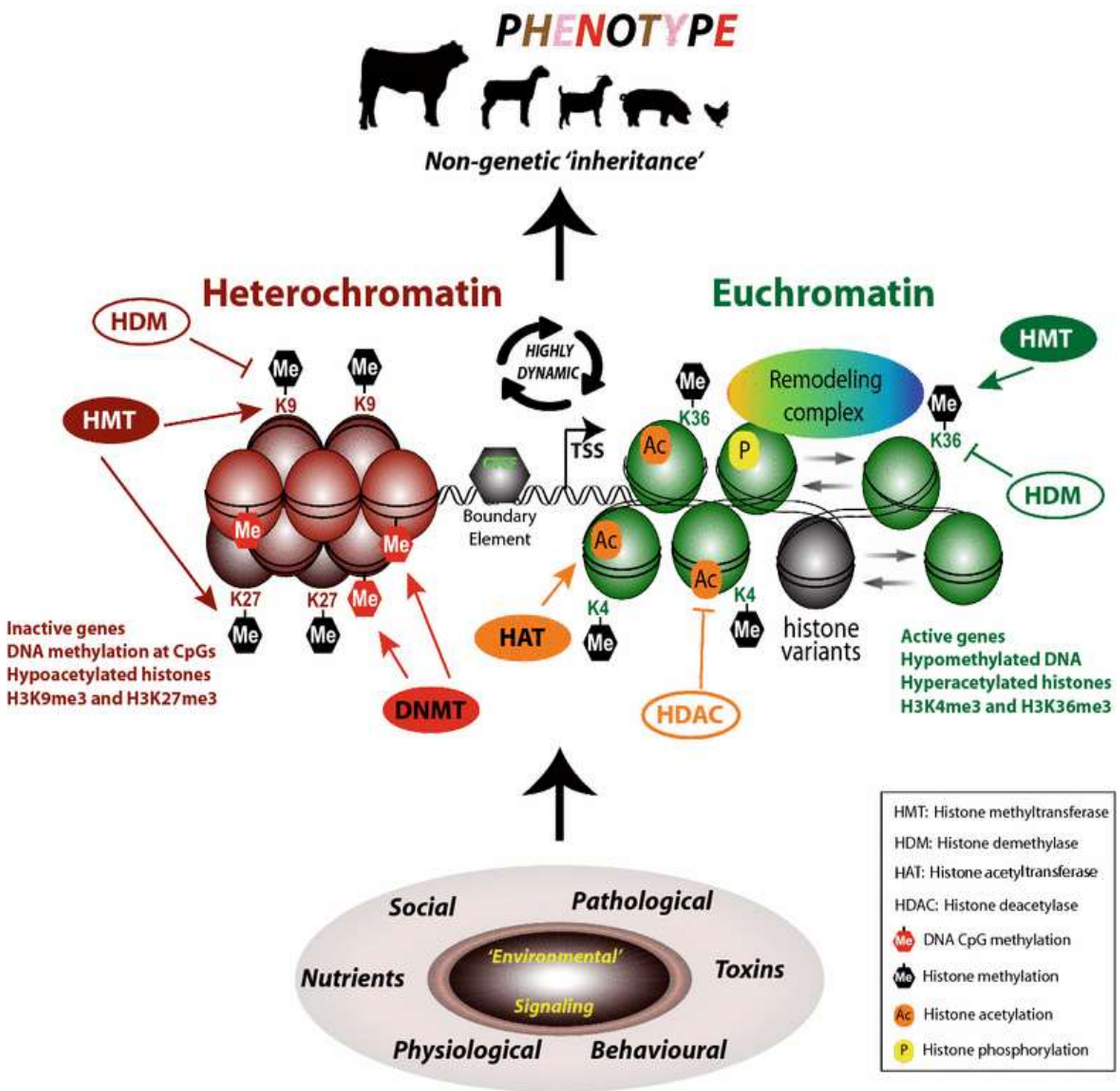
The epigenome (set of all epigenetic elements) can be influenced by environmental factors such as diet or stress and result in heritable changes in the phenotype (Figure 1.3). Epigenetic processes involve DNA methylation, chromatin remodeling, histone modifications, regulation of gene expression by non-coding microRNA, genome instability, and any other forces that modify the animal phenotype. These processes alter gene expression and can affect cell fate and phenotype plasticity as well as behavior. Several molecular mechanisms are involved, including paramutation, markers, imprinting, gene silencing, transposon silencing, X-chromosome inactivation, position effect, reprogramming, transvection, and maternal effects (Triantaphyllopoulos et al. 2016).

DNA methylation: Consists of adding a methyl group to the nucleotides (mainly cytosine) that make up the DNA sequence; these methyl groups act as recognition signals on the DNA, favoring the recruitment of proteins involved in the regulation of gene expression.

Histone modification: These proteins are involved in the compaction and organization of DNA within the cell nucleus. So specific amino acids of histones can be modified by adding acetyl, methyl, or phosphate groups, with combinations of histone modifications defining chromatin conformation and influencing gene expression.

microRNAs: These are small RNA that can silence genes by interfering directly in the transcriptional promoter regions of DNA or by binding to proteins to form transcriptional silencing complexes.

Figure 1.3 Chromatin modifications and remodeling events in livestock. Different environmental exposures trigger signaling pathways, which affect chromatin structure, thereby affecting gene expression leading to altered phenotype



Source: Image from Triantaphyllopoulos et al. (2016)

The effect of diet on epigenetic parameters in ruminants has not been explored much. Sinclair et al. (2007) investigated the effects of diet restricted in vitamin B6, vitamin B12, folic acid, and methionine in embryo donor ewes. The results indicate that the offspring of these ewes exhibited higher blood pressure, increased tendency to obesity, and insulin resistance compared to controls. They analyzed 1400 CpG sites (regions with a high concentration of cytosine and guanine pairs linked by a phosphate represented by p). They found that 4% (57 loci) were altered in more than two ewes on the restricted diet and 88% of these loci were unmethylated compared to controls. They concluded that reducing specific nutrients in the diet during the period close to fertilization could lead to alterations in DNA methylation in the offspring and modify their disease resistance.

Paibomesai et al. (2013) investigated the epigenetic mechanisms by which DNA methylation affects the adaptive immune system response (T_H1/T_H2) in peripartum female cattle. Concanavalin A was applied to CD4+ lymphocytes isolated from cows before and after parturition. Also, Concanavalin A plus dexamethasone was applied to CD4+ lymphocytes from mid-lactation cows. The response variable was interferon-gamma production (T_H1) and *IL-4* production (T_H2). The results indicate a decrease in DNA methylation in the gamma interferon promoter region and an increase in the *IL-4* promoter region. They conclude that the production of CD4+ lymphocytes is partially controlled through epigenetic modifications.

Conclusion

Nutrition modulates the expression of genes involved in the immune response of sheep infected with GIN, and the application of immunogenomic analysis to infection may lead to new treatment approaches. These new phenotypic markers can identify animals resistant to gastrointestinal parasites that affect sheep production worldwide.

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