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Chapter 1

Molecular Genetics and Genome Biology of Goats

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Abstract

Information on goat genome has led to a better understanding of the genetics of goats, its response to infection and the underlying immune response mechanism. Natural product-based therapeutic can therefore be utilized to target genes important for goat immunity. In this chapter, we have summarized the effect of diet and dietary supplements as immune modulators in goats. These modulators affect the expression of genes and secreted proteins associated with innate and adaptive immune response and homeostasis. Probiotics, mushroom extracts, plant polyphenol extracts, Sericea lespedeza (SL) and cowpea diet affect key molecular pathways including Toll-like receptor (TLR) pathway, Wnt signaling pathway and cytokine-mediated signaling pathway. Results from various studies reviewed in this chapter suggest that utilization of dietary immunomodulators has beneficial effects on goat health and production.

Keywords: blood, gene expression, transcription, translation, modulation, innate immunity, homeostasis

1. Introduction

The domestic goat (*Capra hircus*) is an important farm and companion animal species. They are descendants of the bezoar (*Capra aegagrus*) goat. The world goat population has been on the increase during the last three decades and is estimated to be 1 billion with the global genetic diversity characterized by more than 590 breeds [1]. Breeds have various advantageous characteristics such as adaptation to harsh environmental conditions, capacity to convert poor quality fibrous feedstuff into animal proteins and resistance to diseases. Known as 'the poor man's cow,' goats are primarily reared for meat production, but different breeds of goat are often used as a source of milk and wool. Goats are also used for carrying small loads and for land management and kept as pets.

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In the United States, production of small ruminants is a growing industry as a result of high demand for grass-fed or organically produced livestock [2]. Compared to all other livestock enterprise, goat production requires minimal capital input and low cost of breeding stock [3]. Globally, healthy goats are crucial for the long-term success of the goat industry. Production is negatively challenged by factors such as feed toxins, respiratory diseases (pneumonia) and other infectious diseases. Goats are also susceptible to viral diseases (foot and mouth diseases) and bacterial diseases (mastitis). Gastrointestinal (GI) nematode infection is considered the most important limiting factor in goat production systems around the world and results in huge economic losses to producers.

However, resistance to current drugs and lack of interest in developing new drugs by companies pose a challenge for sustainable ruminant production. The widely used approach for the treatment of infection by parasite is drug treatment. Measures used to reduce parasite infection include the reduction of stock density and the maximization of pasture to reduce parasite numbers. Plant-based anthelmintic is also being explored for use in the elimination of gastrointestinal parasites including extracts such as: garlic, neem, wormwood, tobacco, cowpea [4–6] and Sericea lespedeza [7]. Several other alternatives that have been proposed include the use of nonchemical additives such as probiotics [8] and prebiotics, the improved production practices and the use of genetics-based breeding schemes. Improvement in animal nutrition can also impact the immune response including gene response, protein synthesis, modification and degradation, metabolism, signal transduction and cellular proliferation [9]. The understanding of protective mechanisms regarding the initial steps of the host's response to pest or parasite-derived molecules that can correlate with resistance or susceptibility to pathogens needs to be explored. This understanding will aid the design of immunomodulatory strategies to induce a change in the magnitude of immune or nonimmune responses.

2. The goat genome

In the last decade, molecular genetics has led to the discovery of individual genes or candidate genes with substantial effects on our understanding of homeostasis and immunity. The goat genome has been sequenced, and raw sequences have been deposited in NCBI (GenBank, CapAeg_1.0 (GCA_000978405.1) under the accession no. SRA184825. Utilizing information on the goat genome enables a better understanding of the genetics of the goat and how it responses to infection and disease and fights it naturally. The sequencing of the goat genome has led to increased understanding of the genetics underlying immune response mechanism [10]. Genes significant for goat immunity can be targeted by using natural products and plant-based therapeutics for improving goat health. This eliminates the need for chemical treatment, buildup of antibiotic resistance and food insecurity concerns among goat consumers.

2.1. Innate immune system

The main function of the immune system is to distinguish between own cells and tissues from external cells and tissues in order to protect against infestation. The immune system has

various mechanisms to eliminate or withstand the impact of external agents. The animal's immune system is composed of two related functional elements: the innate immune system and the adaptive immune system [11]. Both function in coordination to protect against invading microorganisms [12]. Innate immunity is the first line of defense against organisms; it acts in a nonspecific way through anatomical barriers (skin, mucus membrane), secretions, cells and other elements. The adaptive immune system is the second line of defense which responds slower than the innate immunity system. Innate immune defense plays a key role in affording protection [13]. Unlike the innate immune system, the adaptive immune system has the ability to 'memorize' infectious agents allowing the adaptive immune system to serve as a rapid response system if pathological agents are encountered again [12]. The innate immune system consists of natural killer cells, T-cell and B-cell, basophils, eosinophils, monocytes, macrophages and polymorphonuclear neutrophils. These cells are called white blood cells or leukocytes and are also divided into two groups based on their morphology: granulocytes and agranulocytes. Granulocytes include eosinophils, neutrophils and basophils, and agranulocytes are lymphocytes (T and B cells) and macrophages [14]. A differential white blood cell count is an important tool used to provide clinical diagnosis and for monitoring of disease and blood disorders [15]. This system quantifies and differentiates white blood cells at one particular time giving an insight into infection and checking whether treatments are working [15].

2.2. Toll-like receptors

Animals live in a wide variety of microbe-rich environments, and hence, it is crucial to have a sensitive innate defense mechanism which relies in part by recognizing conserved molecules that are unique to some classes of potential pathogens [16]. It is very important to understand the innate immunity against microbial components and its critical role in host defense against infection. Toll-like receptors (TLRs) have been shown to participate in the recognition of pathogens by the innate immune system.

Toll-like receptors (TLRs) are a highly conserved group of proteins that have been identified in mammals [17]. The TLR family consists of 10 receptors: TLR-1-10, which are very important in the identification of microbes [18]. The coding regions within the goats, TLR-1-10 genes, have been sequenced and found to be conserved and highly similar in nucleotide composition [10]. With the discovery of Toll-like receptors (TLRs), studies have shown that pathogen recognition by the innate immune system is broadly specific, which relies on germline-encoded pattern-recognition receptors (PRRs) to detect relatively conserved components of pathogens referred to as pathogen-associated molecular patterns (PAMPs) [19]. The PAMPs recognized by TLRs include lipids, lipoproteins, proteins and nucleic acids derived from a wide range of microbes such as bacteria, viruses, parasites and fungi [20] which initiates a complex signaling cascade to activate a wide variety of transcription factors and inflammatory cytokines [18].

2.3. Cytokines

Cytokines are small proteins that transmit information from one cell to another. The analysis of cytokines secreted by immune cells in response to infectious agents is crucial to understand pathogenesis and immunity. Most cells in the body produce cytokines during inflammatory

processes which represent a large series of regulatory proteins of the immunologic system. Many cytokines are referred to as interleukins, a name indicating that they are secreted by some leukocytes and act upon other leukocytes. Two general patterns of cytokine secretion by such cells have been described. In the Th1 response, cytokines initiate cell-mediated reactions defined as the activation of macrophages to combat infectious pathogens by releasing IL-1, IL-2, IL-8, and IL-12 to activate inflammation [21]. In the Th2 response, T-helper cells activate B-cells; interleukins IL-4, IL-5, IL-6, IL-10 and IL-13 are released to counter infectious agents caused by extracellular organisms [21]. Studies have shown that the release of cytokines is essential for host survival from infection and is also required for tissue repair.

2.4. Wingless pathway

The Wingless (Wnt) signaling pathway is a conserved pathway in mammals. It involves Wnts, which are secreted glycoproteins that are associated with the Wnt-1 and Wingless gene products of Drosophila [22]. Activation of Wnt signaling happens when Wnt ligands binds to Frizzled receptors together with other receptors lipoprotein receptor-related protein (LRP) 5 and 6 [23–25]. About 19 Wnt ligands and 10 Frizzle receptors have been identified in metazoan mammals. The receptor-ligand interaction leads to downstream signal regulation which is categorized into two: canonical (Wnt/β-catenin) and noncanonical pathways. The former is dependent on β-catenin, but the latter is not. The noncanonical pathway is further subdivided into the planar cell polarity and the Wnt/Ca^{2+} pathways. The Wnt signaling pathway function in cellular processes includes cell proliferation, cell differentiation, cell migration, cell polarity and cell fate determination and has recently been implicated in stem cell renewal [26]. Wnt signaling has also been associated with various biological processes including adipogenesis, myogenesis, embryogenesis and meat quality. In addition, Wnt signaling has been associated with innate immune and inflammation responses via a cross talk with the TLR and NF-κB pathways [27, 28]. Therefore, a defective or deregulated Wnt signaling has detrimental effect on developing embryo (birth defects) and also affects a number of pathological disease conditions.

3. Methodologies for goat studies

3.1. Evaluation of phenotypic parameters

Various phenotypic characteristics are measured in goats following treatment with supplements or feeds in a study. Usually, body weight, body condition score and FAMACHA score are recorded periodically as a measure of effect on growth and health. Body weights are taken before morning feeding using a portable scale [8]. Body condition is scored on a scale of 1–5 by physical examination of the goat's body as described by Villaquiran et al. [29]. Blood samples collected aseptically are evaluated for packed cell volume (PCV) and white blood differential cell counts. PCV is widely used as an indicator trait for anemia. White blood differential counts are measured using the procedure described by Schalm et al. [30]. Fecal samples are collected directly from the rectum and evaluated for the number of parasite egg counts.

More specifically, the number of strongyle eggs and coccidia oocytes is measured using the modified McMaster method [31]. The fecal eggs counted are multiplied by 50, and resulting total is expressed as eggs per gram (epg) of fecal sample per animal [14].

3.2. Molecular techniques

The molecular effects of immunomodulators have been evaluated in goats at the gene transcription and protein levels using different techniques including real-time polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA).

3.3. Real-time PCR

Quantitative real-time PCR is used to measure messenger RNA (MRNA) levels [77]. For real-time PCR analysis, total RNA is isolated from whole blood cell pellets using Trizol method or QuickRNA MiniPrep Kit (Zymo Research) as per manufacturer's procedure. The concentration and purity of the RNA are checked on NanoDrop Spectrophotometer (ND-1000; Thermo Fisher). Mostly, a pure RNA typically yields a 260/280 ratio of ~2.0 and this is considered ideal. A 260/280 ratio below 2.0 suggests protein contamination [82]. In addition, the integrity of the RNA (RNA integrity number (RIN)) can be measured with a bioanalyzer, and a RIN <7.0 indicates a good RNA. Since RNA is not stable, it is converted into more stable complimentary DNA (cDNA) using cDNA conversion kits containing oligo (DT) and random primers, reverse transcriptase, and other needed reagents as specified in the manufacturer's manual. Real-time PCR is performed with reaction mixture comprising of cDNA template, primers and SYBR Green [78]. Housing-keeping genes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β-actin (*ACTB*), ribosomal protein L32 (RPL32), TATA sequence binding protein (TBP) and cyclophilin are used as internal controls for normalization of the RT-PCR data obtained [32]. The RT-PCR data are analyzed by calculating fold change in the expression of the specific genes tested using statistical approaches including the comparative C_T (also known as $2^{-\Delta\Delta C}{}_T$ or Livak's method).

3.3.1. Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) is a molecular assay used for analytical detection and quantification of specific antigens or antibodies in a given sample. It uses the concept of an antigen binding to its specific antibody which enables detection of antigens such as proteins, peptides and antibodies [33]. With ELISA, goat serum or plasma is evaluated for the levels of immune response and inflammation biomarkers such as cytokines, prostaglandin and immunoglobulins. Cytokines measured in goat serum following dietary supplementation include TNFα, IL-1β, IL-8, GCSF, GMCSF, Rantes and IFNγ [7, 8, 34]. The levels of secreted prostaglandin E, an eicosanoid and also an inflammation mediator have also been measured in goat serum and plasma with ELISA [34–36].

3.4. Effect of pathogen-associated molecular patterns

Goats rely on pasture as their main source of feed. Studies have been done to elucidate the effects of different PAMPs, microbe-associated molecular pattern (MAMP) and plant polyphenol

metabolite in animal feed on goat health. Pathogen-associated molecular pattern evaluated in goats includes the following: probiotics, mushroom, plant polyphenols, cowpea, lipopolysaccharide (LPS), peptidoglycan, nystatin and Sericea lespedeza (**Table 1**).

3.4.1. Probiotics

Probiotics has been studied and considered as health beneficial microorganism which plays a role in maintaining homeostasis. Previous studies have shown the use of probiotics to modulate gastrointestinal health. Liong [37] reported the resistance to infectious diseases in the gastrointestinal tract as a result of probiotics. Probiotics as a supplement in animal feed has shown to have a beneficial effect on milk yield, fat and protein content [38]. Ekwemalor et al. [39] reported the release of proinflammatory cytokines in goats orally drenched with probiotics (*Coriolus versicolor* [CV]). Previous study conducted by our research team looked at the molecular impact of probiotic administration on physical health parameters and activation of genes involved in homeostasis and immunity in goat blood. We reported that genes associated with innate and adaptive immunity were modulated as a result of probiotics treatment. Genes that were expressed are associated with the host response to bacteria, virus, T-cell activation, cytokines and inflammatory response. **Table 2** shows genes modulated as a result of probiotic modulation.

Modulator (s)	Sample type (s)	Cytokines	Innate immune response	Reference
Probiotics	Whole blood, serum	IL2, IL5, IL10, IL8, IL18	TLR4, TLR6, TLR7, TLR9	[80, 81]
Plant extract	Whole blood	—	TLR2	[54]
Cowpea	Whole blood, serum, plasma	TNFα, IL1α, ILβ, IL8 *IL10RA*, IL15, IP10, G-CSF, Rantes and IFNγ	TLR2	[34, 69]
Sericea lespedeza	Whole blood, serum	TNF-α, IFNr, GCSF, GMCSF, IL-1α, IP-10	TLR2 and TLR4	[7]
Mushroom	Neutrophils, whole blood, serum	IFNr, Rantes and granulocyte colony stimulating factor (GCSF). granulocyte macrophage colony-stimulating factor (GM-CSF)	TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10	[8]
Lipopolysaccharide	Mammary epithelial cells, whole blood, blood leukocytes	IL1B, CCL3 and IL8, *CCL2, CXCL6, IL6, CXCL8*	*PTGS2, IFIT3, MYD88, NFKB1, and TLR4*	[54, 83]
Peptidoglycan	Whole blood	—	TLR2	[54]
Lipoteichoic acid	Mammary epithelial cells	*CXCL6, CXCL8, CCL5*	*PTGS2, IFIT3, NFKB1, TLR4, and TOLLIP*	[84]

Table 1. List of immunomodulators tested on goats.

Category	Genes	Reference
Pattern recognition receptors (PPRs)	DDX58, NLRP3, NOD1, NOD2, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9	[8]
Cytokines	CCL2, CCL5, CSF2, CXCL10, IFNA1, IFNB1, IL18, 1L1A, IL1B, IL2, CXCL8, TNF	[8]
Innate immunity genes	APCS, C3, CASP1, CD14, CD4, CD40, CD40LG, CD8A, CRP, HLA-A, HLA-E, IL1R1, IRAK1, IRF3, IRF7, ITGAM, LY96, LTZ, MAPK1, MAPK8, MBL2, MPO, MX1, MYD88, NFKB1, NFKB1A, STAT1, TICAM1, TRAF6	[8]
Th1 markers & immune response	CCR5, CD80, CXCR3, IFNG, IL18, IL23A, SLC11A1, STAT4, TBX21, TLR4, TLR6	[8]
Th17 markers	CCR6, IL17A, RORC, STAT3	[8]
T-cell activation	CD80, CD86, ICAM1, IFNG, IL23A, 1 L6, SLC11A1	[8]
Treg markers	CCR4, CCR8, FOXP3, IL10	[8]
Adaptive immunity genes	CD40, CD40LG, CD8A, CRP, FASLG, HLA-A, IFNARI, IL1B, IL1R1, IRF3, IRF7, ITGAM, JAK2, MAPK8, MBL2, MX1, NFKB1, RAG1, STAT1	[8]
Inflammatory response	APCS, C3, CCL5, CRP, FOXP3, IL1A, IL1B, IL4, IL6, MBL2, STAT3, TNF	[8]
Defense response to bacteria	IFNB1, IFNG, IL23A, IL6, LYZ, MBL2, MYD88, NOD1, NOD2, SLC11A1, TLR1, TLR3, TLR4, TLR6, TLR9, TNF	[8]
Defense response to viruses	CD4, CD40, CD86, CD8A, CXCL10, DDX58, HLA-A, IFNARI, IFNBI, IL23A, IL6, NLRP3, TICAM1, TLR3, TLR7, TLR8, TYK2	[8]

Table 2. List of genes associated with innate and adaptive immunity.

Researchers have reported effects of probiotics in goats of which most effects have been attributed to an increase in the innate immune system and others in the acquired immune response. Leeber et al. [40] reported that probiotics have the properties to modulate host immune system through different signaling pathways of innate immune cells. The innate immune system functions by initiating a response to microorganisms or their components via pattern recognition receptors such as nucleotide-binding oligomerization domain-like receptors or TLR [41]. Previous studies conducted by Worku and Morris [42] and Worku et al. [7] have shown the expression of TLRs in whole blood.

When ligands bind to TLRs, they trigger at least two most important cell signaling pathways. One of the pathways involves MyD88, an adaptor protein which is shared by most TLRs. When this pathway is triggered, it leads to the activation of the transcription factor NF-κB which then results in the release of proinflammatory cytokines [10, 43, 44]. Ekwemalor et al. [8] reported that probiotics modulated the expression of genes in myeloid differentiation antigen 88 (MYD88)-dependent or MYD88-independent system, TLR-mediated signaling induction pathway, nuclear factor κB (NF-κB), cytokine-mediated signaling pathways and Wnt signaling pathway. **Table 3** shows the different genes that were expressed in the Wnt signaling pathway involved in canonical Wnt signaling, planar cell polarity, negative regulation, calcium signaling, cell growth and proliferation as a result of probiotics.

3.4.2. Mushrooms (Coriolus versicolor)

Mushrooms have been studied and are known for their nutritional and medicinal properties. They contain bioactive compounds which are of medicinal importance. There are several types of mushroom of which *Coriolus versicolor* (CV) is one of the studied types of mushroom because of its medicinal properties. They contain active ingredients such as polysaccharide krestin (PSK) and polysaccharide peptide (PSP) [45]. Eliza et al. [46] reported that extracts of CV have the potential of boosting suppressed immune function, extending the survival rate and improving quality of life. They exert their therapeutic effects by modulating the host's immune response. Zhou et al. [47] demonstrated their effect in stimulating the immune system and inhibition of cancer growth. Lull et al. [48] also reported their effect in activating T and B lymphocytes, macrophages, natural killer cells, and lymphocyte-activated killer cells, as well as promoting the production of antibodies and various cytokines. Results from research team showed that mushroom extracts of CV modulated the expression of 10 TLR in neutrophils and modulation of innate immunity through differential regulation of the secretion of serum proteins including cytokines and prostaglandin E2 to impact goat health [8].

3.4.3. Lipopolysaccharide, peptidoglycan and nystatin

Bacteria produce molecules such as lipopolysaccharide (LPS), lipoproteins, peptidoglycan and lipoteichoic acids (LTAs), and this serves as specific molecular signatures for different classes of bacteria [49]. Lipopolysaccharides (LPSs), also known as lipoglycans, are the main surface membrane of Gram-negative bacteria. LPSs comprise poly- or oligosaccharide region and lipid A, which is the main immunostimulatory part of LPS [50]. Lipopolysaccharide is recognized by TLR4 assisted by CD14 proteins [49].

Category	Modulator	Genes	Reference
Canonical WNT signaling	Probiotics	APC, AXIN2, CSNK1A1, DVL2, FZDI, FZD7, FZD8, GSK3A, GSK3B, LEF1, LRP5, NKD1, PORCN, RUVBL1, SFRP4, TCF7, TCF7L1, WIF1, WNT1, WNT2, WNT3A, WNT7B, WNT8A	[80]
Wnt signaling target genes	Probiotics	CCND2, WISP1	[80]
Planar cell polarity	Probiotics	DAAM1, MAPK8, VANGL2,	[80]
Proliferation	Probiotics	DAB2	
WNT signaling negative regulation	Probiotics	FBXW4, FBXW11, FRZB	[80]
Cell growth and proliferation	Probiotics	FOXN1, JUN, MMPZ, PPARD	[80],
WNT calcium signaling	Probiotics Nystatin	NFATC1, WNT5B, WNT5A	[35, 80]

Table 3. Differentially expressed genes on the Wnt signaling pathway in response to modulators.

Peptidoglycan and lipoteichoic acids are the major stimulatory components of Gram-negative bacteria and are recognized by TLR2 [51]. Pathogen recognition receptors, such as TLRs, have evolved to recognize these PAMPs and detect invading disease microbes [49].

The linkage between PAMPs, TLR, activation of the prostaglandin pathway and the promotion of Wnt signaling in inflammatory response has been studied [52, 53]. Previous work by Asiamah et al. [54] also indicates that TLR2 and Frizzled receptors are increased in response to bacterial cell wall components (lipopolysaccharide, peptidoglycan). Nystatin is a lipid raft inhibitor derived from the bacterium *Streptomyces noursei*, and in addition, a proinflammatory agent was also found to modulate TLR2 and Frizzled receptor in goat blood. These findings open a window into the innate immune mechanism and inflammatory response mediated through TLRs and Wnt in goats and may provide more understanding about disease resistance as well as aid in drug design and animal selection through breeding programs.

3.4.4. Plant polyphenols

Apart from plants being important feed resource for animal nutrition, they are also a rich source of polyphenol bioactive compounds that have beneficial health effects. Polyphenols (also known as phenolic compounds) are naturally occurring plant metabolites and are an integral part of both human and animal diet [55]. These compounds include flavonoids, tannins, phenolic acid and others [56]. Feed resources containing tannins have been reported to have both beneficial and detrimental effects on grazing animals. Tannin-rich plants have direct antiparasitic activity but might also act indirectly by increasing host resistance. These effects vary depending on the species of plant, parasite and host [57]. The antiparasitic potential of forage legumes (Fabaceae family), including sulla (*Hedysarum coronarium*) [58], sainfoin (*Onobrychis viciifolia*) [59], birdsfoot trefoil (*Lotus corniculatus*) [60], big trefoil (*Lotus pedunculatus)* [61] and Sericea lespedeza (*Lespedeza cuneata)* [62–64], herbs and fodder trees have been evaluated in goats.

The roles of polyphenol extracts from plants in the immune function have been reported on different cell types both *in vitro* and *in vivo* [65]. Polyphenol and other plant extracts demonstrated the ability to induce the release of both proinflammatory and anti-inflammatory cytokines, thus leading to the maintenance of the immune homeostasis in the host [66]. Epigallocatechin-3-gallate, a flavonoid found in green tea, has been shown to inhibit NF-κB activation induced by many proinflammatory stimuli [67]. Recently, there is a great interest in feed polyphenols due to their antioxidant capacity, inflammatory and immunomodulatory properties and their possible beneficial implications on animal health and production [7, 54, 68, 69, 79].

3.4.5. Sericea lespedeza

Sericea lespedeza (*Lespedeza cuneata*) is a leguminous plant with high tannin content. It has been studied extensively for its possible anthelmintic potential especially in small ruminants [60]. A study by Worku et al. [7] demonstrated the impact of Sericea lespedeza (SL) diet on innate immune response mediators in goats. More specifically, Sericea diet increased serum level of proinflammatory cytokines TNF-α, IFNr, GCSF, GMCSF, IL-1α and IP-10 ($P < 0.0002$)

and decreased ($P < 0.0001$) IL-8 and RANTES. In addition, results from gene expression analyses showed increased mRNA transcripts of cell surface receptors TLR2 and TLR4, and the cytokines IL-8, IL-10, IL-2 and INF-γ. Previous work by Asiamah et al. [54] also demonstrated that transcription of TLR2 and Frizzled receptor in goat blood is variably responsive to Sericea lespedeza. In summary, goats respond to plant extracts and may have an effect on the expression of innate immune markers. This may offer an avenue for the exploitation of plant-derived tannins to regulate inflammatory response and enhance goat innate response.

3.4.6. Cowpea

Cowpea (*Vigna unguiculata*, L. Walp) is a highly nutritious legume plant used as human food and feed for animals. It has been utilized as a supplement feed to enhance feed intake and improve productivity in ruminants fed low-quality roughage diets [70, 71]. Cowpea also contains polyphenol compounds including phenol acids, flavonoids and tannins [72]. Polyphenolic extract of cowpea has been shown to have potential impact on ruminant health via antioxidant capacity [68], anti-inflammatory properties [34, 73] and modulating expression of genes associated with immunity and homeostasis [6, 43, 69]. Treatment with cowpea extract downregulated the expression of proinflammatory cytokine TNFα (fold change (FC; treatment/control) = −43.39), IL1α (FC = −6.19), ILβ (FC = −3.62) and IL8 (FC = −1.25). Also, CPE modulated the expression of *IL10RA* (a receptor for IL10, an anti-inflammatory cytokine) and IL15 [73].

A study by Adjei-Fremah et al. [69] demonstrated the impact of cowpea forage grazing, particularly Mississippi Silver variety on growth, internal parasite burden, and markers of immunity in goats. Their study results showed a modulation in cytokines levels, TNF-α, IL-8, and IP10 decreased, whereas an increase in G-CSF, Rantes and IFNγ was observed. The total antioxidants in plasma also increased in the cowpea-grazed goats [34, 73]. Cowpea diet may therefore stimulate innate immune response in goats, and this will help the animals fight against infectious pathogens and diseases. The immunomodulatory potential of cowpea feed may be due to at least in part their polyphenols [68, 74, 75]. Phenolic compounds in animal feeds have antioxidant properties that prevent the damaging effect of free radicals and their metabolic by-products [76] and stimulate an immune response in animals [75].

4. Conclusion

These studies provide an insight into the utility of cross-reactive regents to understand the molecular genetics and genome biology of the goat and importance of dietary modulators as avenues for immune modulation and maintenance of homeostasis in the goat. Treatment with probiotics, mushroom extracts, PAMPS and plant-derived PAMPS resulted in differential expression of genes related to TLR signaling and WNT signaling pathway. Greater insight is provided into goat molecular genetics and genome biology, conserved and novel genes and signaling pathways. Gene expression and modulation has implications for the design and development of innovative therapeutics. Novel goat-specific and conserved gene expression patterns have been identified and provide insight into the utility of genome analysis for the better definition of the mechanism of action of modulators of gene expression in the goat for improved production and welfare.

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