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Evaluation of the Effect of Probiotic Administration on Gene Expression in Goat Blood

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Abstract

The objective of this study was to assess the molecular impact of probiotic administration on genes involved in homeostasis and immunity in goat blood. Following initial screening for infection, one-week post weaning, female SpanishXBoer goats were drenched daily with the recommended doses of FASTtrak microbial pack (Conklin Company Inc., Shakopee, MN) in 10 mL sterile water over an 8-week period. The control group were given sterile water. Blood samples were collected weekly. Total RNA was isolated from blood collected at the beginning of the study (week 0) and at the end of the study (week 8) using Tri-reagent and then reverse-transcribed to cDNA using the Ambion-Retroscript kit. Quantification of genes was performed in the CFX96™ Biorad Real-Time PCR detection system with the addition of the dye SYBR green. The cow Wingless (Wnt) signaling pathway, Human Innate & Adaptive Immune Responses and the Cow Inflammatory Cytokine & Receptors RT² Profiler™ PCR Arrays (QIAGEN, Valencia, CA) were used to profile the expression of 84 genes involved in each pathway. Probiotic treatment had no effect on body weight, body condition, fecal egg count and RNA concentration ($p>0.05$). Packed cell volume and FAMACHA scores were significantly improved by probiotic administration. Results from RT-PCR showed increased expression of genes in innate and adaptive immune response, cytokine and Wnt pathways in response to probiotics. Probiotics induced the expression of 32 genes involved in innate and adaptive immune response inflammatory cytokines, and 48 genes involved in the Wnt signaling pathway. This study provides evidence for a systematic effect of oral probiotic administration on expression of genes involved in immunity and homeostasis in goat blood.

Keywords: goat, probiotic, homeostasis, wingless

1. Introduction

Healthy goats are crucial for the long-term success of the goat industry. A major hurdle in the United States small ruminant industry is production losses due to gastrointestinal nematodes (Matthews et al., 2016; Kaplan et al., 2007). The common approach for control/treatment of infection is anthelmintic drug treatment. Increasing incidence of anthelmintic resistance has led to renewed interest in alternative parasite control strategies (Qadir et al., 2010). One of the alternatives that has been proposed is the use of probiotics (Broadway et al., 2014). Several types of probiotics have been discovered and it is very important to understand how they exert their beneficial effects on animals.

Probiotics are viable microorganisms that have positive effects on growth performance, nutrient synthesis, the microbial ecosystem, absorption, and the reduction in the incidence of intestinal infection and restoration of gut microflora after bouts of diarrhea (Khalid et al., 2011). Different probiotics such as bacteria, yeast, and fungi are used for manipulating rumen fermentation and the microbial ecosystem of the gastrointestinal tract of animals. The use of beneficial microorganisms to increase host defense is a new trend of improving health. Foods containing probiotics for human consumption have been marketed (Musa et al., 2009). Although the benefit of probiotics and its impact on health have been known for decades, research is still required to know the targets and mechanisms used by organisms to deliver their benefits in animal production.

The use of probiotics supplements to promote animal health is increasing. Various formulations of probiotic have been used as feed supplements for ruminants (Puniya et al., 2015). Administration of probiotics separately and in combination has significantly improved feed intake, feed conversion rate, daily weight gains and total body weight in goats, chicken, pig, sheep and equine (Chiofalo et al., 2004; Samli et al., 2007; Casey et al., 2007). Probiotics have been used to modulate gastrointestinal health such as improving lactose intolerance, increasing natural resistance to infectious diseases in the gastrointestinal tract, suppressing travelers' diarrhea and reducing bloating (Liong, 2007). Supplementing animal feed with probiotic has shown to have a beneficial effect on milk yield, fat and protein content (Kritas et al., 2006). In a previous work done by our research team, we reported the effect of oral administration of probiotics stimulated the release of pro-inflammatory cytokines (Ekwemalor et al., 2016a). Our recent results have also shown transcription activity in blood from probiotic treated cows (Adjei-Fremah et al., 2017; Adjei-Fremah et al., 2016b). The efficacy of probiotics has been found to be variable depending on survival rate and stabilities of strains, doses, frequency of administration, interactions with some medicines, health and nutritional status of the animal and the effect of age, stress and genetics of animals. Molecular investigations after treating the animal with probiotics may help understand the mechanism of action of probiotics.

The objective of this study was to assess the molecular impact of Probiotic administration on genes involved in homeostasis and immunity.

2. Materials and Methods

2.1 Animals and Housing

Ten female SpanishXBoer goats that were housed at the North Carolina Agricultural and Technical State University Farm were used in the study one-week post weaning. Animals were clinically healthy and not under any treatment. The experimental protocol used in this research was approved by the Institutional Animal Care and Use Committee.

2.2 Oral Administration of Probiotics

Following initial screening for infection, goats were drenched daily with 10 ml sterile water containing recommended doses of probiotics (treatment) or sterile water (control) over an 8-week period. FASTtrak microbial pack (Conklin Company Inc., Shakopee, MN) is a commercial probiotic supplement that was chosen because it had shown positive effects in goats. This product contained active dry yeast culture *Lactobacillus acidophilus, Saccharomyces cerevisae, Enterococcus faecium, Aspergillus oryza* and *fructooligosacharide*. Treatment was administered using a 50 mL conical tube.

2.3 Sample Collection

The body weight of each goat was measured on a portable weighing scale in kilograms before feeding in the morning. Body condition score was evaluated as described by Ekwemalor et al., 2016b. Blood and fecal samples were collected and evaluated once a week throughout the experiment. The Modified McMaster's technique (Whitlock, 1948) was used to measure fecal egg count. The numbers of strongyle eggs and coccidia oocyst were counted as described by Kaplan et al., (2004). The color of the conjunctival mucosa membranes of each animal was evaluated as classified into five categories according to the FAMACHA eye color chart (Kaplan et al., 2004).

Blood samples (10 mL) was collected from the jugular vein aseptically into tubes containing ethylenediaminetetraacetic acid for cell count analysis, Gel and Lithium Heparin (BD, Franklin Lakes, NJ) for serum collection and PAXgene tubes for RNA isolation from all 10 goats. Blood samples were processed within 2 hours of collection. Packed Cell Volume (PCV) was evaluated using an aliquot of blood in micro-capillary tubes and centrifuged for 5 min at 14,000 rpm in an IEC MB Micro Hematocrit centrifuge (Damon/IEC Division).

2.4 RNA Extraction

Total RNA was prepared as described from a previous study (Adjei-Fremah et al., 2016a; Asiamah et al., 2016). Briefly, 2.5ml of whole blood was transferred into PAXgene tubes and stored at -80^{0}C. Total RNA was isolated from samples collected at week 0 and week 8 of both control and treated groups according to the manufacturer's instructions (PreAnalytiX, BD, UK). The quantity and quality of RNA were measured with the ND-1000 UV/VIS Nanodrop spectrophotometer (260 nm and 260/280 nm respectively).

2.5 Real-time PCR

Reverse transcription was performed using Oligo (dT) primers with 2 ug of the total RNA from each treatment group using a Complementary DNA (cDNA) RETRO script Kit (Ambion Inc., Austin, TX) following the manufacturer's instructions as previously described by Adjei-Fremah et al., 2016c. The cDNA products were measured for purity and concentration using the Nanodrop spectrophotometer (NanoDrop Technologies).

Quantification was performed in the CFX96™ Biorad Real-Time PCR detection system with the addition of the dye SYBR green. The cow Wingless signaling pathway, Human Innate & Adaptive Immune Responses RT² Profiler™ PCR Arrays and the Cow Inflammatory Cytokine & Receptors Profile Array (QIAGEN, Valencia, CA) were used to profile the expression of 84 genes involved in each pathway. Gene expression was normalized using the housekeeping gene Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) and fold change in gene expression between test and control samples was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

2.6 Statistical Analysis

One-way Analysis of variance (ANOVA) was performed on all variables using the statistical analysis software package SAS version 9.3 (SAS Institute, Cary, NC) to evaluate differences between treatment and control groups. Variables included FAMACHA, PCV, body weight, body condition, FEC, and RNA concentration. A P-value of <0.05 was considered to be significant.

3. Results

3.1 Effect of Treatment on Physical Phenotypic Parameters

All animals used in this study were healthy and there was no sign of infection. Our study indicated that probiotics had no effect on body weight, body condition, FAMACHA, PCV and RNA concentration and fecal egg count ($p>0.05$). Goats in the treatment group had a FAMACHA score of 2 while goats in the control group had a FAMACHA score of 3. Percentage of PCV ranged from ranged from 20 to 32%.

3.2 Expression of Innate and Adaptive Immune Genes in the Control Group.

Innate and adaptive immune genes were analyzed using RT-PCR. At week 0, out of the 84 genes assayed, animals in the control group expressed 25 genes while 22 genes were expressed at week 8. There was a decrease in the number of genes expressed comparing week 0 to week 8. Genes that were expressed as a result of time were IL23A, MBL2, STAT3, TLR2 and TLR4. Other genes that increased were cytokine (IL1A [FC=2], CSF2 [FC=2]) Th17 markers (RORC, FC=14), Th1 marker (CCR5, FC=5) and innate immunity gene (MAPK1, FC=5). Other genes expressed remained the same at week 0 and week 8.

3.3 Expression of Innate and Adaptive Immune Genes in the Treatment Group.

At week 0, out of the 84 genes assayed in the treatment group, 41 genes were expressed while 67 genes were expressed at week 8. Thirty genes were induced as a result of probiotic treatment (Table 1). These are genes involved in the host response to bacteria, pattern recognition receptors, cytokines, inflammatory response and Th1 markers/Immune response. Some of the genes increased as a result of treatment (Table 2). The results point out an up-regulation of genes associated with innate and adaptive immune response.

Figure 1 illustrates the fold change in mRNA expression levels of selected genes comparison between control and probiotics-treated group. Toll-like receptors 4 and 6 had increased fold change in expression compared to the control group.

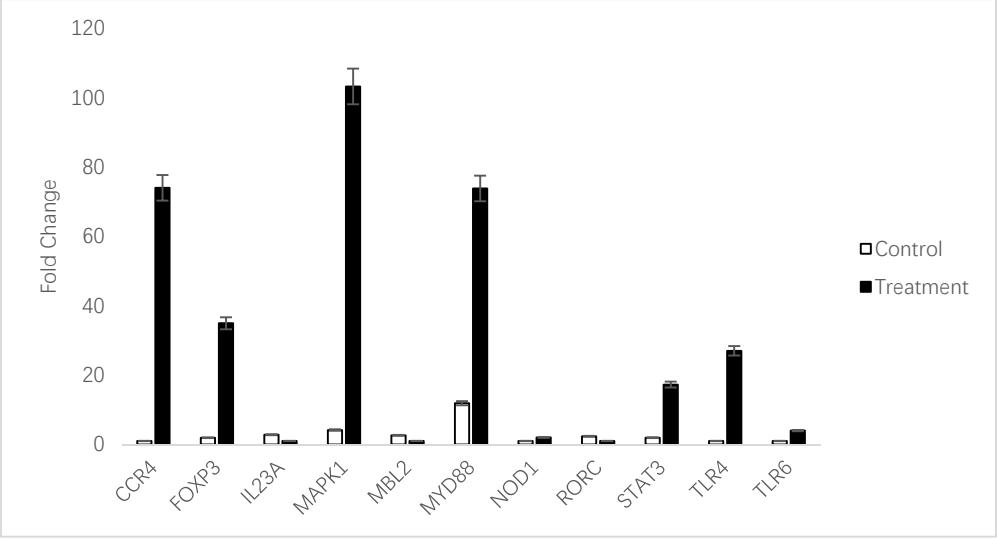

Figure 1. Fold change in gene expression of selected genes in the control and probiotics-treated groups

Table 1. Effect of treatment on the expression of genes associated with innate and adaptive immunity.

Genes	Function
Pattern recognition receptors	
NOD 2	Involved in gastrointestinal immunity and activating the NFKB protein
NLRP3	Plays a role in the regulation of inflammation, the immune response, and apoptosis
TLR2	Plays a role in pathogen recognition and activation of innate immunity.
TLR8	Plays a role in pathogen recognition and activation of innate immunity.
Cytokines	
CCL2	Involved in immunoregulatory and inflammatory processes
CCR6	Regulate the migration and recruitment of T cells during inflammatory and immunological responses
CXCL10	Stimulation of monocytes, natural killer and T-cell migration, and modulation of adhesion molecule expression
IFNB1	Involved in cell differentiation and defense against viral infection
IL1B	Mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis
IL4	A signal transducer and activator of transcription
IL6	Plays an essential role in the final differentiation of B-cells into Ig-secreting cells
Inflammatory response	
CCL5	Involved in immunoregulatory and inflammatory processes.
C3	Plays a central role in the activation of complement system
Defense response to virus	
CD4	Initiates or augment the early phase of T-cell activation
CD8A	Acts as a co-receptor with the T-cell receptor on the T lymphocyte to recognize antigens
DDX58	plays a major role in sensing viral infection and in the activation of a cascade of antiviral responses
HLA-A	Plays a central role in the immune system by presenting peptides derived from the endoplasmic reticulum lumen
Th1 marker & Immune Response	
CXCR3	Induces cellular responses that are involved in integrin activation, cytoskeletal changes, and chemotactic migration
STAT4	Essential for mediating responses to in lymphocytes, and regulating the differentiation of T helper cells
T-cell Activation	
ICAM1	Play roles in cell proliferation, differentiation, motility, trafficking, apoptosis and tissue architecture.
IFNG	Triggers a cellular response to viral and microbial infections
IL23A	Activate the transcription activator STAT4, and stimulate the production of interferon-gamma (IFNG)
Innate immunity genes	
IL1R1	Involved in many cytokine-induced immune and inflammatory responses
IRAK1	Plays a critical role in initiating innate immune response against foreign pathogens.
IRF5	Modulation of cell growth, differentiation, apoptosis, and immune system activity.
IRF7	Plays a critical role in the innate immune response against DNA and RNA viruses.
ITGAM	Plays a role in adherence of monocytes to stimulated endothelium
NFKB1	Stimulates the expression of genes involved in a wide variety of biological functions
STAT1	Mediates the expression of a variety of genes, which is thought to be important for cell viability in response to different cell stimuli and pathogens
Defense response to bacteria	
MYD88	Plays a central role in the innate and adaptive immune response.

Gene function information is from GeneCards (http://www.genecards.org/).

Table 2. Differentially expressed genes associated with treatment on Innate and Adaptive immune genes.

Gene	Function	Fold change
TLR4	This receptor has been implicated in signal transduction events induced by lipopolysaccharide (LPS) found in most gram-negative bacteria	27
TLR6	This receptor functionally interacts with toll-like receptor 2 to mediate cellular response to bacterial lipoproteins.	2
TLR7	Control host immune response against pathogens through recognition of molecular patterns specific to microorganisms	7
TLR9	They recognize PAMPs that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity	3
RORC	Key regulator of cellular differentiation, immunity, peripheral circadian rhythm as well as lipid	74
NOD1	This protein is an intracellular PRR that initiates inflammation in response to a subset of bacteria through the detection of bacterial diaminopimelic acid	2
MAPK1	This protein acts as a transcriptional repressor independent of its kinase activity	103
1L2	This protein is required for T-cell proliferation and other activities crucial to regulation of the immune response	3
IL5	Cytokine that stimulates the proliferation of T-lymphocytes	15
IL8	This chemokine is one of the major mediators of the inflammatory response	10
1L10	Inhibits the synthesis of a number of cytokines, including IFN-gamma, IL-2, IL-3, TNF and GM-CSF produced by activated macrophages and by helper T-cells	5
IL18	This gene is a pro-inflammatory cytokine that augments natural killer cell activity in spleen cells, and stimulates interferon gamma production in T-helper type I cells	118
CCR4	Play fundamental roles in the development, homeostasis, and function of the immune system	74

Gene function information is from GeneCards (http://www.genecards.org/).

3.4 Effects of Treatment on Cytokine Expression

At week 0, out of the 84 genes that were assayed, animals in the control group expressed 59 cytokines while animals in the treatment group expressed 60 cytokines (Figure 2). At week 8, animals in the control group expressed 26 cytokines while animals in the treatment groups expressed 51 cytokines. Forty-nine genes were both expressed in week 0 and week 8 (Figure 2). There was a decrease in the amount of cytokines expressed both in the control and treatment group. There was a time effect in the expression of cytokine CCL24, CCR4, CCR5, IL1B, LOC510185, MIF and OSM. Treatment had an effect in the expression of cytokine GR01, IL17F and LTB.

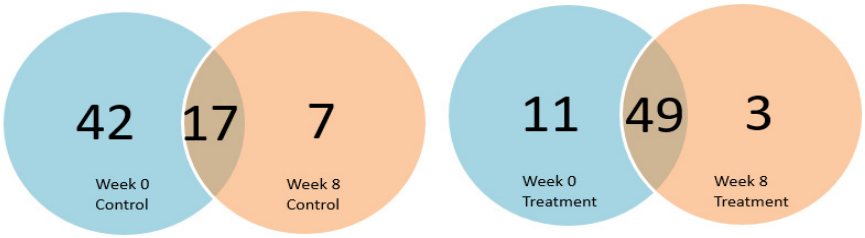

Figure 2. Venn diagram showing summary of response of treatment to cytokine genes after 8 weeks for control and treatment goats

3.5 Expression of WNT Genes

At week 0, animals in the control group expressed 54 genes while after 8 weeks 50 genes were expressed. There was a time effect in the expression of Wnt genes (DIXDC1, MYC and SOX17). Some of the Wnt genes increased as a result of treatment [AES (FC=3), CSNK2A1 (FC=2), CTBP1 (FC=3), KREMEN1 (FC=2), MMP7 (FC=2), SFRP1 (FC=2), VANGL2 (FC=2) and WIF1 (FC=3)]. There was a decrease in the amount of Wnt genes expressed in week 8 when compared to week 0 (Figure 3a).

At week 0, animals in the treatment group expressed 32 genes while 84 genes were expressed after 8 weeks. Treatment induced the expression of 41 genes involved in WNT-mediated signal transduction (Table 3). Some of the genes increased as a result of treatment (Table 4). There was an increase in the amount of wnt genes expressed in week 8 when compared to week 0 (Figure 3b).

Table 3. Effect of treatment in the expression of genes involved on WNT-mediated signaling pathway

Genes	Function
Canonical WNT signaling	
APC	Involved in cell migration and adhesion, transcriptional activation, and apoptosis
AXIN2	plays an important role in the regulation of the stability of beta-catenin in the Wnt signaling pathway
CSNK1A1	Involved in DNA repair, cell division, nuclear localization and membrane transport.
DVL2	play a role in the signal transduction pathway mediated by multiple Wnt proteins
FZD1	Receptor for Wnt signaling proteins
FZD7	Receptor for Wnt signaling proteins
FZD8	Receptor for Wnt signaling proteins
GSK3A	regulates proteins and transcription factors, such as JUN
GSK3B	Acts as a negative regulator in the hormonal control of glucose homeostasis
LEF1	Regulates T-cell receptor alpha enhancer function
LRP5	Acts as a co-receptor with Frizzled protein family members for transducing signals by Wnt proteins
LRP6	Acts as a co-receptor for Wnt and transmits the canonical Wnt/beta-catenin signaling cascade
NKD1	Functions as a negative regulator of the Wnt signaling pathway
PORCN	Involved in the processing of Wnt (wingless and homologue) proteins
RUVBL1	Involved in both ATP-dependent remodeling and histone modification.
SFRP4	Acts as soluble modulators of Wnt signaling
TCF7	plays a critical role in natural killer cell and innate lymphoid cell development.
TCF7L1	Participates in the Wnt signaling pathway
WIF1	play a role in embryonic development.
WNT1	Developmental processes, regulation of cell fate and patterning during embryogenesis
WNT2	Developmental processes, regulation of cell fate and patterning during embryogenesis
WNT3A	Developmental processes, regulation of cell fate and patterning during embryogenesis
WNT7B	Developmental processes, regulation of cell fate and patterning during embryogenesis
WNT8A	Developmental processes, regulation of cell fate and patterning during embryogenesis
WNT signaling Target genes	
CCND2	Regulator of CDK kinases
WISP1	Downstream regulator in the Wnt/Frizzled-signaling pathway.
Planar cell polarity	
DAAM1	Cell motility, adhesion, cytokinesis, and other functions of the cell cortex
MAPK8	Act as an integration point for multiple biochemical signals, and are involved in a wide variety of cellular processes such as proliferation, differentiation, transcription regulation and development.
VANGL2	Involved in the regulation of planar cell polarity
Proliferation	
DAB2	Adapter protein that functions as clathrin-associated sorting protein
WNT signaling negative regulation	
FBXW4	Recognizes and binds to some phosphorylated proteins and promotes their ubiquitination and degradation
FBXW11	Function in phosphorylation-dependent ubiquitination
FRZB	Modulator of Wnt signaling through direct interaction with Wnts.
Cell growth and proliferation	
FOXN1	Regulates the development, differentiation, and function of thymic epithelial cells
JUN	Transcription factor that recognizes and binds to the enhancer heptamer motif 5-TGA[CG]TCA-3
MMP7	Involved in the breakdown of extracellular matrix in normal physiological processes
PPARD	Ligand-activated transcription factor.
WNT calcium signaling	
NFATC1	Plays a role in the inducible expression of cytokine genes in T-cells
WNT5B	Ligand for members of the frizzled family of seven transmembrane receptors

Gene function information is from GeneCards (http://www.genecards.org/).

Table 4. Differentially expressed genes on the Wnt signaling pathway in response to probiotics

Gene symbol	Gene description	Fold change
AES	Amino-Terminal Enhancer Of Split	461
AXIN1	AXIN1	199
BCL9	B-Cell CLL/Lymphoma 9	267
BTRC	Beta-Transducin Repeat Containing E3 Ubiquitin Protein Ligase	208
CCND1	Cyclin D1	345
CSNK2A1	Casein Kinase 2 Alpha 1	343
CTBP1	C-Terminal Binding Protein	294
CTNNB1	Catenin Beta 1	220
DIXDC1	DIX Domain Containing 1	269
DKK1	Dickkopf WNT Signaling Pathway Inhibitor 1	217
DKK3	Dickkopf WNT Signaling Pathway Inhibitor 3	657
FRAT1	Frequently Rearranged In Advanced T-Cell Lymphomas 1	512
FZD3	Frizzled Class Receptor 3	1574
FZD4	Frizzled Class Receptor 4	6
FZD5	Frizzled Class Receptor 5	228
FZD6	Frizzled Class Receptor 6	90
FZD9	Frizzled Class Receptor 9	443
KREMEN1	Kringle Containing Transmembrane Protein 1	223
MYC	V-Myc Avian Myelocytomatosis Viral Oncogene Homolog	129
NLK	Nemo Like Kinase	491
PYGO1	Pygopus Family PHD Finger 1	1226
RHOA	Ras Homolog Family Member A	276
SOX17	SRY-Box 17	25
TLE1	Transducin Like Enhancer Of Split 1	375
WNT16	Wnt Family Member 16	317
WNT5A	Wnt Family Member 5A	367
WNT6	Wnt Family Member 6	302
WNT7A	Wnt Family Member 7A	125
WNT9A	Wnt Family Member 9A	4
CBY1	Chibby Family Member 1, Beta Catenin Antagonist	2
DKK2	Dickkopf WNT Signaling Pathway Inhibitor 2	199

Gene function information is from GeneCards (http://www.genecards.org/).

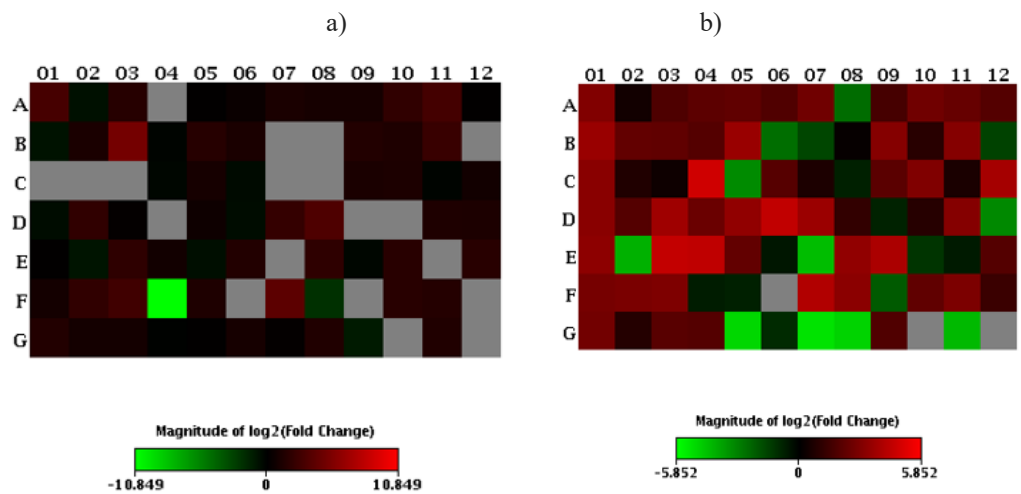

Figure 3. a) Heat map generated from RT-PCR array data reflecting gene expression values in control samples week 0 versus week 8. b) Heat map generated from RT-PCR array data reflecting gene expression values in treated samples week 0 versus week 8

4. Discussion

This study investigated the impact of oral administration of probiotics on gene expression in goat blood. The administration of probiotics has increased in animal agriculture. In this study, we evaluated the molecular impact of probiotic administration on physical health parameters and activation of genes involved in homeostasis and immunity in goat blood. Probiotics had no effect on the physiological parameters in goats. There was no effect of probiotics on body weight, body condition, FAMACHA and PCV, which remained within the normal range of healthy goats. Body weight and body condition are important in determining the health condition of an animal. The results from this study corroborated with the findings from earlier workers. A similar result was reported in which probiotics had no effect on body weight (Jinturkar et al., 2009, Gyenai et al., 2016, Whitely et al., 2009). In addition, previous studies conducted by Kumar et al. (2016) showed that supplementing probiotics to barbari goats had no effect on body weight. In contrast, studies conducted by Sharma (2008) reported that probiotics was effective in increasing body weight.

Treatment had no effect on fecal egg count. The lack of probiotic activity on *Haemochus contortus* and coccidia could be due to genera, species or strains (Gyenai et al., 2016). Detection of parasites eggs in fecal samples indicate that goats were infected. Fecal egg count is a parameter widely used to determine parasitic infection in animals (Kaplan et al., 2004). Previous study conducted by Gyenai et al., (2016) reported that probiotic mix had an effect on increase of egg per gram but in our studies treatment had no effect on fecal egg count. Treatment had no effect on FAMACHA scores and PCV. Goats in the treatment group had a FAMACHA score of 2 while those in the control group ranged from 2-3. This process clinically identifies anemic goats infected by parasitic pathogen, which reduces the number of treatments administered to goats (Kaplan et al., 2004). Treatment also had no effect on PCV which ranged from 20 to 32%. The normal range for PCV in goats is between 19-38 (Jain, 1986) which indicates that the goats were not anemic.

Probiotics modulated the expression of genes associated with innate and adaptive immunity. These are genes involved in the host response to bacteria, pattern recognition receptors, cytokines, inflammatory response and Th1 markers/Immune response. Studies have shown that probiotics have the properties to modulate host immune system through different signaling pathways of innate immune cells (Leeber et al., 2008). Most effects have been attributed to an increase in the innate immune response and to others an increase in the acquired immune response. The innate immune system initiates a response to microorganisms or their components via pattern recognition receptors such as Toll-like receptors (TLR) or nucleotide-binding oligomerization domain-like receptors (Kingma et al., 2011) and previous studies have reported TLRs expression in blood (Worku et al., 2016a, 2016b; Worku & Morris 2009). In our study, probiotics had an effect in the expression of TLR3 and TLR8 in goats, and increase in expression of TLR4, TLR6, TLR7 and TLR9. Several studies have reported the effect of probiotics on TLR (Adjei-Fremah et al., 2016b; Adjei-Fremah et al., 2017, Ekwemalor et al., 2016c, Tirumurugaan et al., 2010, Liu et al., 2016).

The activation of these receptors influences the nature of the subsequent adaptive immune response. Similar studies conducted by Adjei-Fremah et al., (2017) reported that probiotics modulated the expression of TLR 2, 6, 7 and 8 in cow but in our study probiotics modulated the expression of TLR 4, 6, 7 and 9 and induced the expression of TLR 2 and 8. Previous studies conducted by Bisanz et al., (2014) showed that probiotics had an immune-modulatory response on TLR2 which also corroborates with our study. TLR2 is specifically involved in the detection of gram-positive surface markers and also serves as an indicative of increased anti-bacterial innate immune system activity (Bisanz et al., 2014).

Binding of ligands to TLRs triggers at least two important cell signaling pathways. Several particular genes that were significantly modulated in our study are key components of the immune response pathway. One pathway involves MyD88, an adaptor protein shared by most of the TLRs that leads to the activation of the transcription factor NF-κB resulting in the release of pro-inflammatory cytokines (Raja et al., 2011, Adjei-Fremah et al., 2016, Liu et al. 2016). Probiotics induced the expression of genes involved in multiple signaling pathways such as TLR-mediated signaling induction pathway, nuclear factor κB (NF- κB), myeloid differentiation antigen 88 (MYD88) dependent or MYD88-independent system and cytokine mediated signaling pathways. Previous studies have suggested that probiotics exert their immune-modulatory effect through these pathways (Adjei-Fremah et al., 2017, Ekwemalor et al., 2016, Liu et al., 2016). Cytokines such as IL4, IL6, CCL2, CCR6 and IFNB1 were induced while IL2, IL5, IL8, IL10, IL18, CCR4 increased in expression as a result of probiotic treatment. Researchers have reported the expression of cytokine IL6 in chicken (Rajput et al., 2017) and IFNr, G-CSF in goats (Gyenai et al., 2016, Ekwemalor et al., 2016b) as a result of probiotic treatment.

The Wnt signaling pathway plays an important role in regulating a variety of biological processes. It is associated with cellular proliferation, differentiation, apoptosis, motility, and polarization of cells, in vertebrates and mammals (Villasenor et al., 2017). This pathway has been studied over the years in several species. In this study we report that probiotics had an effect in the expression of genes associated with the Wnt signaling pathway. All 84 genes were expressed of which probiotic treatment induced 48 genes after 8 weeks. These genes are involved in canonical Wnt signaling, planar cell polarity, negative regulation, calcium signaling, cell growth and proliferation. Ligands including WNT 1, 2, 3, 5, 7, and 8, and their receptors FZD 1, 7, and 8 were induced in response to probiotic treatment. Furthermore, probiotics treatment modulated the expression of Wnt target genes including TLE1, MYC, and CTNNB1. Previous study conducted by our research group reported the expression of wnt genes in cow as a result of probiotic treatment. In our study, MAP Kinase was activated in both Wingless and immune signaling pathways. This could be a point of cross-talk for both Wnt and TLR pathways. These kinases are a chain of proteins in the cell that communicates signals from a receptor on the surface of the cell to the DNA in the nucleus of the cell. Previous studies have reported that probiotics maintained homeostasis through enhanced MAPK activities (Segawa et al., 2011; Iyer et al., 2008). Increasing activity of MAPKs and their involvement in the regulation of inflammation make them potential targets for novel anti-inflammatory therapeutics. Results from the current study shows that probiotics treatments have molecular effect on homeostasis and help to improve the health of goats.

5. Conclusion

This work aimed to demonstrate the use of a system-wide approach to examine how oral administration of probiotics might modulate the expression of genes involved in homeostasis and immunity in goat. Our findings indicate probiotics had an effect on genes associated with innate and adaptive immunity and Wnt signaling pathway which could be helpful in maintaining and preventing diseases in goat production. Furthermore, to understand the detailed immune events occurring, and therapeutic potential, specific analysis of genes and pathways involved may are required.

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