Effect of probiotic supplementation on growth and global gene expression in dairy cows

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ABSTRACT

Use of probiotic supplements as a non-chemical approach to promote health has increased in animal production. The present study evaluated the effect of oral probiotic administration on growth and global gene expression profile in dairy cows. Lactating Holstein-Friesian cows received a daily dose (50 ml) of a commercial probiotic (containing Lactobacillus acidophilus, Saccharomyces cerevisiae, Enterococcus faecium, Aspergillus oryza and Bacillus subtilis) for 60 days. A microarray experiment was performed with blood collected at day-0 and day-60. Although probiotic supplementation had no effect on body weight, PCV and total protein concentration in plasma (P > 0.05), per cent lymphocyte count increased (P < 0.05), and per cent neutrophil count decreased (P < 0.05) in probiotic-treated animals. Gene expression analysis identified 10,859 differentially expressed genes, 1168 up-regulated and 9691 down-regulated genes, respectively, following probiotic treatment. Single experiment pathway analysis identified 87 bovine pathways impacted by probiotic treatment. These pathways included the Toll-like receptor (TLR), inflammation response and Wingless signalling pathways. Oral administration of probiotics to dairy cows had a systemic effect on global gene expression, such as on genes involved in immunity and homeostasis. The results of this study show that the utilization of probiotics in animal agriculture impacts genes important to dairy cow health and production.

1. Introduction

Utilization of probiotic supplements to promote health has increased in animal production (Song et al. 2012). Probiotics are considered as a safe alternative to antibiotics (Broadway et al., 2014). In livestock and poultry, probiotics enhance growth performance, feed conversion efficiency, microbial ecosystems and immunity (Lee et al. 2007; Dhama et al. 2008). In cattle, several positive influences of probiotics include improvement of production performance, stress reduction, stimulation of host innate immunity and maintenance of a constant lactic acid supply to rumen microbiota (Nocek et al. 2002). The positive impact of probiotics is due in part to production of antibacterial substances that are harmful to pathogenic microbes in the gut of animals (Dhama et al. 2008). Various multi or monostrains of probiotic microorganisms have been used as feed additives for ruminants (AlZahal et al. 2014; Punia et al. 2015) and poultry (Salaniheh et al. 2011).

Oral administration of probiotics influences health through immuno-modulatory activities in gut-associated lymphoid tissue, influence in innate and adaptive immunity and maintenance of immune homeostasis in man (Lebeer et al. 2008) and animal (Gyenai et al. 2016). Probiotics affect the immune response through multi-cellular signalling mechanisms and with possible cross-talk mechanism in modulating the host immune system (Dubey & Ghosh 2013). Studies have shown that consumption of probiotics induces local and systemic production of cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 12 (IL-12) and interleukin 18 (IL-18, Meyer et al. 2007; Ekwemalor et al. 2016a). The effect of a probiotic on transcriptional gene expression profile in poultry has been studied using microarray analysis and quantitative real-time polymerase chain reaction (PCR) techniques (Higgins et al. 2010). However, in dairy cows, the systemic effect of oral probiotic supplements on global gene expression is yet to be elucidated. The objective of the present study was to evaluate the effect of oral probiotic administration on growth and global gene expression profile in dairy cows using microarray analysis.

2. Materials and methods

2.1. Animal housing, feeding and sampling

All experimental procedures used were approved by the Institutional Animal Care and Use Committee (IACUC). Ten age-matched female Holstein-Friesian cows at mid-lactation were randomly selected from cattle housed at the North Carolina Agricultural and Technical State University dairy farm. Animals grazed on pasture and received a custom ration supplement...
with 14% protein (Union Grove milling Co. NC, USA) and water *ad libitum* throughout the study period.

### 2.2. Oral probiotic treatment

Five Holstein-Friesian lactating cows were given a daily dose of the commercial probiotic FASTtrak microbial pack (Conklin Company, Kansas City, MO, USA) mixed in 50 ml of sterile endotoxin-free water daily for 60 days. Endotoxin assay was performed as previously described by Adjei-Fremah et al. (2016a). The FASTtrak microbial pack (Conklin Company, Kansas City, MO) ingredients are: yeast culture (*Saccharomyces cerevisiae*), rice hulls, calcium carbonate, dried chicory root, dried *Enterococcus faecium* fermentation product, dried *Lactobacillus acidophilus* fermentation product, dried *Aspergillus oryza*, fermentation extract and dried *Bacillus subtilis* fermentation extract. Untreated animals (*n* = 5) receiving sterile endotoxin-free water (50 ml) only daily served as control. Body weight (BW) of animals was recorded weekly using a standard scale.

### 2.3. Blood sampling and analysis

Initial (Day 0) and end of study (Day 60) blood samples (50 ml) were drawn from the jugular vein aseptically from the probiotic-treated animals (*n* = 5) and control animals (*n* = 5) into Vacutainer tubes (BD Biosciences, San Jose, CA, USA) containing the anticoagulant Acid Citrate Dextrose. Blood samples were analyzed for Packed Cell Volume (PCV) and white blood cell differential counts (WBC) following the procedure described by Asiamah et al. (2016). PCV was measured using a microhematocrit centrifuge (Damon/IEC division). To conduct WBC, a thin blood smear was prepared on sterile glass slides in triplicate for each animal. Wright staining was used for histological cell staining. Duplicate counts of 100 cells were evaluated for different white blood cell types microscopically. Total protein concentration in blood plasma was measured using the Pierce bicinchoninic acid assay (BCA) kit (Thermo-Scientific, Waltham, MA). The cRNA yield were checked with the NanoDrop ND-1000 Spectrophotometer (Thermo-Scientific, Waltham, MA). The Cy3-labelled cRNA, 1.63 μg (specific activity > 10.0 pmol Cy3/μg cRNA) was fragmented and hybridized to Agilent Bovine (v2) 4 × 44k oligo-microarrays (G2519F) for 17 h at 65°C rotating in an Agilent hybridization oven following the manufacturer’s instructions. The microarray used had 44,000 (44k) cow transcripts. The array slides were scanned on the Agilent DNA Microarray Scanner (G2505B) using the one-color scan default settings for 4 × 44k array slides.

### 2.6. Microarray data processing and analysis

Scanned images were analyzed with Feature Extraction Software 10.10.1.1 (Agilent Technologies, Santa Clara, CA) using default parameters (GE1-v5_95_Feb07) to obtain processed signal intensities. Data normalization and statistical analysis were performed using GeneSpring software 13.0 (Agilent Technologies, Santa Clara, CA). Fold changes in gene expression, Student’s *t*-test, gene ontology and hierarchical cluster analysis were generated using the GeneSpring GX 13.0 software. Hierarchical cluster analysis involved differentially expressed genes with a cutoff of twofold change and a statistically significant difference in expression at *P* < 0.05. GeneSpring pathway analysis was used to conduct single experiment pathway analysis of the study results with a fold change cutoff of ≥ 2, *P* < 0.05.

### 2.7. Microarray GEO accession number

The data from this experiment have been deposited at the NCBI Gene Expression Omnibus (GEO) database under accession no. GSE75240 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc = GSE75240).

### 2.8. Real-time PCR

Total RNA from the individual animal samples (0.5 µg each, *RIN* > 7) was reverse transcribed into cDNA using the RT² first strand kit (QIAGEN, Valencia, CA). The cow Wnt signalling RT² Profiler PCR Array (QIAGEN, Valencia, CA) and the human innate and adaptive immunity array with 84 test genes related to Wingless (Wnt)-mediated signals transduction and immunity, respectively, were used. The genes profiled on the innate and adaptive immune array included genes of the Toll-like receptor (TLR) pathway, cytokines and chemokine receptors, inflammation response, NF-κB signalling, apoptosis, innate immune response and defense response to bacteria. Real-time PCR was performed using RT² SYBR Green Mastermix (QIAGEN, Valencia, CA) on the CFX Connect real-time system (Bio-Rad, Hercules, CA). Real-time PCR workflow was per manufacturer’s manual (Qiagen, Valencia, CA). All reactions were performed in triplicate. PCR was performed as follows: denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s.
Real-time data analysis normalization was performed with the housekeeping gene Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) and fold change in gene expression was calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). The Student’s t-test was used to determine the statistical significance of fold change in genes expressed.

2.9. Statistical analysis
Data were analyzed using SAS version 9.3 (SAS Institute, Cary, NC). One-way Analysis of variance (ANOVA) was performed on BW, PCV, WBCs and total plasma protein concentration, a P-value of < 0.05 was considered significant. The results are presented as mean ± SEM.

3. Results

3.1. Health parameters and blood cell count
All the experimental animals were healthy at the start of the study, and no incidence of disease occurred. The average BW and PCV measured at day 0 was 1616 kg and 28%, respectively. Oral supplementation of probiotic had no effect on BW, PCV and total protein concentration in plasma at the end of the study (P > 0.05). However, the PCV, an indicator of health status, increased at the end of the study period in both probiotic-treated (32%) and control animals (33%). Oral administration of probiotics slightly impacted per cent leucocytes counts. Per cent lymphocyte count increased (P < 0.05), and % neutrophil count decreased (P < 0.05; Figure 1(a)). In the untreated control group, per cent leucocytes count (Figure 1(b)) remained unchanged at the end of the study (P > 0.05).

3.2. Gene expression in control group
In the untreated animals (comparing Day 0 and Day 60), out of the 44,000 gene transcripts on the bovine (v2) array, 31,653 genes were expressed, 6026 up-regulated and 25,527 down-regulated gene transcripts, respectively. The hierarchical cluster analysis in Figure 2 depicts the differentially expressed genes (with a cutoff at twofold change, P < 0.05) in the no-probiotic animals. Global gene expression analysis in the untreated group revealed over expression of proinflammatory cytokine IL-1B (interleukin-1 beta: FC = 4). On the other hand, downregulation of gene transcripts of anti-inflammatory cytokines, namely IL-4 (interleukin-4: FC = −4), IL-5 (interleukin-5: FC = −32), IL-6 (interleukin-6: FC = −14) and IL-10 (interleukin-10: FC = −3), was observed. All known 10 bovine TLRs (TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9 and TLR10) were expressed but downregulated in the no-probiotic control group. Decreased expression of Wingless (Wnt) signalling genes, including Wnt1, Wnt2B, Wnt3, Wnt4, Wnt5A, Wnt5B, Wnt7A, Wnt8B, Wnt9A and Wnt10A, was observed in untreated animals. However, the expression of Wnt6 increased threefold (Table 2).

3.3. Effect of probiotic treatment on global expression
Global gene expression analysis identified 10,859 expressed genes, 1168 up-regulated genes and 9691 down-regulated genes in peripheral blood collected from animals after 60 days of probiotic administration compared to day 0. The hierarchical cluster analysis in Figure 3 shows the differentially expressed genes (with a cutoff at twofold change, P < 0.05) in the probiotic-treated group. Supplementation with probiotic increased the expression of genes associated with innate and adaptive immunity such as cytokines and chemokines, TLRs and other stress-related signalling molecules that are relevant in inflammation response and maintaining homeostasis (Table 3).

Pathway analysis results generated from the GeneSpring Pathway Analysis software identified 87 bovine pathways significantly (P < 0.05) associated with probiotic exposure for 60 days (Table 1). These included Wnt signalling; TLR signalling; prostaglandin synthesis and regulation; inflammation response; and TNF-α, nuclear factor kappa B (NF-Kb) and mitogen-
activated protein kinase (MAPK) pathways. TLRs expressed included TLR2, TLR6, TLR7 and TLR8; however, their expression levels decreased. Probiotics treatment inhibited the expression of NOD-like receptors (NLR) associated genes such as nucleotide-binding oligomerization domain-like 1 (NOD1) and NOD2.

Microarray analysis identified increased expression of chemokine C-X-C receptor type 2 (CXCR2) and a decreased expression of CXCR1 and CXCR7 in the probiotics-treated group. As expected, chemokine C-X-C motif ligand 8 (CCL8) was found to be upregulated. A number of systemic immune markers such as cluster of differentiation (CD) 3D, CD3E, CD19, CD40LG, CD44, CD46, CD53, CD55, CD72, CD74, CD80, CD163, CD5 and CD151 expression were regulated by probiotics treatment. With the exception of CD46 and CD74, all others mentioned were down-regulated in mRNA expression level. Wnt signalling pathways genes Dishevelled Segment

**Table 1.** Bovine pathways associated with probiotics treatment in dairy cow.

| Pathways | P-value a |
|----------|-----------|
| Bt_Wnt_Signalling_Pathway_WP1016_71410 | 2.86E-06 |
| Bt_Inflammatory_Response_Pathway_WP1040_78599 | 0.037348315 |
| Bt_Toll-like_receptor_signalling_pathway_WP1067_63387 | 7.17E-05 |
| Bt_Prostaglandin_Synthesis_and_Regulation_WP995_71430 | 0.0016666 |
| Bt_TNF-alpha_NF-kB_Signalling_Pathway_WP1047_63380 | 2.42E-05 |
| Bt_TNF-alpha_NF-kB_Signalling_Pathway_WP1047_63380 | 2.42E-05 |
| Bt_Apoptosis_WP1018_67048 | 4.56E-05 |
| Bt_MAPK_Cascade_WP1009_71415 | 0.002298732 |
| Bt_B_Cell_Receptor_Signalling_Pathway_WP1025_67068 | 1.35E-08 |
| Bt_MAPK_signalling_pathway_WP998_72096 | 1.53E-08 |
| Bt_Wnt_Signalling_Pathway_and_Pleripotency_WP1010_72100 | 2.53E-07 |
| Bt_Nuclear_Receptors_WP1068_69983 | 4.65E-05 |
| Bt_Growth_Hormone_Receptor_ (GHR)_Signalling_WP2891_79676 | 0.003089916 |
| Bt_EGFRI_Signalling_Pathway_WP978_67181 | 1.47E-12 |
| Bt_Regulation_of_Actin_Cytoskeleton_WP1062_72126 | 2.96E-08 |
| Bt_DNA_Replcation_WP983_71396 | 0.03307927 |
| Bt_Complement_Activation_Classical_Pathway_WP977_63463 | 0.001562093 |
| Bt_IL-4_signalling_Pathway_WP1055_63474 | 7.18E-04 |
| Bt_Type_IL_interferon_signalling_(IFNG)_WP1017_63372 | 0.001210297 |
| Bt_Translation_Factors_WP965_63460 | 1.34E-04 |
| Bt_Pentose_Phosphate_Pathway_WP1028_63381 | 0.012532177 |
| Bt_Iron_metabolism_in_placenta_WP2908_79168 | 0.019142887 |
| Bt_G13_Signalling_Pathway_WP1063_72019 | 0.005602178 |
| Bt_Hedgehog_Signalling_Pathway_WP1022_79162 | 0.037964767 |
| Bt_Growth_Hormone_(GH)_Signalling_WP2890_78846 | 0.036808405 |
| Bt_T_Cell_Receptor_Signalling_Pathway_WP1011_63335 | 2.45E-08 |
| Bt_Apoptogensis_WP987_79147 | 2.56E-13 |
| Bt_IL_3_Signalling_Pathway_WP1031_63382 | 3.34E-06 |
| Bt_Nucleotide_GPCRs_WP1042_63394 | 0.033363406 |

aP-value < 0.05 was considered significant

3.4. Real-time PCR

Quantitative real-time PCR was performed to validate involvement of selected pathways (Wnt signalling pathway and innate and adaptive pathway) genes based on their association with the immune response. Genes regulated by probiotics treatment included members of the Wnt signalling pathway; out of 84 genes tested, 58 genes were differentially expressed. The expression of Wnt5A and Wnt8A from the microarray analysis results was validated by RT-PCR results.

Evaluation of 84 genes associated with innate and adaptive immunity identified differentially expressed genes due to probiotics supplementation. Out of the 84 immune-associated genes tested, 52 genes were expressed in Day 0 samples. Sixty-four genes were detected on the probiotic group (Day 60), compared to the control group (Day 0). Probiotics treatment activated the transcription of 12 new genes, which included CD55, Conserved Helix-Loop-Helix Ubiquitous Kinase (CHUK), Heme Oxygenase 1 (HMOX1), IFNGR1, IFNGR2, IL-37, Neutrophil Cytosolic Factor 4 (NCF4), Lymphocyte Antigen 96 (LY96), NF-kB1, Peptidoglycan Recognition Protein 2 (PGLYRP2), Platelet-Activating Factor Receptor (PTAFR), TLR4 and TLR8. The expression of TLR2 and TLR8 on the probiotic microarray was confirmed in the RT-PCR results. The genes expressed on the microarray and confirmed by RT-PCR for the untreated and probiotic-treated groups have been highlighted on Tables 2 and 3, respectively.

4. Discussion

The use of probiotics in animal production for the promotion of health has increased. In the present study, the systemic effect of a commercial multistrain probiotic supplement was evaluated in bovine peripheral blood. Probiotics supplementation changed the cellular profile and global gene expression in blood. Under pasture condition and overtime, untreated animals (Day 60) expressed 31k genes. Probiotics treatment modulated the transcription of only 10k genes in the blood. Previous in vitro and in vivo studies have also reported that probiotic supplement affects gene expression profile. Administration of Lactobacillus casei, Bifidobacterium breve or Escherichia coli (Shima et al. 2008) or L. plantarum (Panigrahi et al. 2007) induced differential gene expression. Furthermore, the differential gene expression was dependent on the type of bacterial strain used (Higgins et al. 2010). Using whole blood enables understanding of the systemic impact of probiotic use. Previous studies have used whole blood samples to evaluate transcriptional profiling in response to treatment with probiotics (Jacquier et al. 2015; Lau et al. 2015).

The observed differences in gene expression particularly in the probiotics-treated group may be due to the response to...
Table 2. Differentially expressed genes, function and fold change in expression in untreated animals (no probiotics) identified by microarray analysis.

| Gene    | Gene functiona | Fold changeb |
|---------|----------------|--------------|
| Cytokines |                |              |
| IL-1R1  | Receptor for interleukin-1 alpha, interleukin-1 beta and interleukin-1 receptor antagonist. Involved in cytokine-induced immune and inflammatory responses | 3c |
| IL6ST   | Signal transducer shared by many cytokines | 3 |
| IL12B   | Acts on T and natural killer cells | (−17)c |
| IL6     | Involved in inflammation and the maturation of B cells | (−14)c |
| IL12A   | Cytokine required for T-cell-independent induction of interferon (IFN)-gamma | (−37)c |
| IL13    | An immunoregulatory cytokine produced primarily by activated Th2 cells | (−32) |
| IL10    | Cytokine with pleiotropic effects in immunoregulation and inflammation | (−3)c |
| Chemokines |                |              |
| CXCR1   | A receptor for interleukin 8 (IL8) | 3 |
| CXCL8   | Mediator of the inflammatory response and chemoattractant | 4 |
| CXCL2   | Protein involved in immunoregulatory and inflammatory processes | (−24) |
| Toll-like receptors (TLRs) |                |              |
| TLR1    | Pathogen recognition and activation of innate immunity. | (−12)c |
| TLR2    | A member of TLR relevant for pathogen recognition and activation of innate immunity. Mediates host response to Gram-positive bacteria and yeast via stimulation of NF-kappaB | (3)c |
| TLR3    | Pathogen recognition and activation of innate immunity | (−10)c |
| TLR9    | Preferentially expressed in peripheral blood leucocytes and has a fundamental role in pathogen recognition and activation of innate immunity | (−8)c |
| Wnt signalling |                |              |
| WNT6    | A member of the WNT gene family regulates cell fate and patterning during embryogenesis | 3 |
| WNT2B   | Regulates cell growth and differentiation | (−36)c |
| WNT5B   | Regulate cell fate | (−14)c |
| WNT3    | WNT gene family member, regulate cell fate and patterning during embryogenesis | (−12)c |
| WNT9A   | Regulate cell fate and patterning during embryogenesis | (−7)c |
| WNT1    | Regulate cell fate and patterning during embryogenesis | (−25)c |
| Transcription regulators |                |              |
| NFkBIL1 | A divergent member of the I-kappa-B family of proteins | (−100)c |

aGene function information is from GeneCards (http://www.genecards.org/).
bFold change in gene expression of ≥2.0, P < 0.05) was considered significant.
cGenes confirmed by RT-PCR analysis.
(−) indicate downregulation; all others were upregulated.

Table 3. Differentially expressed genes and fold change in expression in probiotics supplement animals identified by microarray analysis.

| Gene    | Gene functiona | Fold changeb |
|---------|----------------|--------------|
| Cytokines |                |              |
| IL16    | Chemoattractant, a modulator of T-cell activation | 45 |
| IL6     | Induces a transcriptional inflammatory response through interleukin 6 receptor, alpha | (−5)c |
| IL10RA  | Inhibits the synthesis of proinflammatory cytokines | (−4)c |
| CCL3    | Small inducible cytokine that functions in inflammatory responses | 13 |
| CCL19   | Antimicrobial gene involved in immunoregulatory and inflammatory processes | 156 |
| Chemokines |                |              |
| CXCR2   | Receptor for interleukin 8 (IL8) and mediates neutrophil migration to sites of inflammation | 139 |
| CXCR4   | A CXC chemokine receptor | (−5)c |
| CXCR1   | A receptor for interleukin 8 (IL8) | (−17) |
| CCL2    | Chemotactic activity for monocytes and basophils | (−14)c |
| CXCL8   | Mediator of the inflammatory response and chemoattractant | 8 |
| Toll-like receptors (TLRs) |                |              |
| TLR6    | Pathogen recognition and activation of innate immunity | (−3) |
| TLR2    | Pathogen recognition and activation of innate immunity | (−14)c |
| TLR7    | Pathogen recognition and activation of innate immunity | (−22) |
| TLR8    | Pathogen recognition and activation of innate immunity | (−8)c |
| Wnt signalling |                |              |
| WNT8A   | A member of WNT gene family regulates cell fate and patterning during embryogenesis | (−9)c |
| WNT5A   | Regulate cell fate and patterning during embryogenesis | (−9)c |
| WNT10B  | Regulate cell fate and patterning during embryogenesis | (−8) |
| KREMENS | Component of a membrane complex that modulates canonical WNT signalling | 23 |
| DVL1    | Regulates cell proliferation | 34 |
| PRICKLE3| Protein coding gene | 4 |
| Transcription regulators |                |              |
| MAP4K3  | Protein that activates key effectors in cell signalling | 144 |
| MAP3K8  | Activate both the MAP kinase and JNK kinase pathways | 41 |

aGene function information is from GeneCards (http://www.genecards.org/).
bFold change in gene expression of ≥2.0, P < 0.05) was considered significant.
cGenes confirmed by RT-PCR analysis.
(−) indicate downregulation; all others were upregulated.
microbe-associated molecular patterns (MAMPs). The MAMPs are recognized by pathogen recognition receptors (PRRs) such as TLRs, Nucleotide oligomerization domain (NOD)-like receptors (NLRs), Mannose receptor, Cytosolic DNA sensors and RIG1-like receptors (RLRs) (Janeway & Medzhitov 2002; Franchi et al. 2008). This establishes an interaction between the probiotic microorganism and the animal host, thus activating the innate immune response (Bridger et al. 2010). Previous studies have defined TLR expression in blood (Worku et al. 2016a, 2016b; Worku & Morris 2009). In the probiotic-treated group, TLR2, TLR6, TLR7 and TLR8 genes were detected but were down-regulated in expression. The involvement of TLRs with different probiotic strains has been reported. The probiotic bacterium Lactobacillus casei influenced innate immunity through an increased expression of the TLR2 receptor (Galdeano & Perdigon 2006). Similarly, the immunoregulatory activity of Bifidobacteria in bovine intestinal epithelial cells was TLR2 dependent (Murata et al. 2013). As expected, similar results were obtained in probiotic-treated animals presented in this study, four different TLRs (TLR2, TLR6, TLR7 and TLR8) were expressed and this may possibly be due to the multistrain composition of the commercial probiotic used.

Stimulation of TLR results in activation of multiple signalling pathways such as nuclear factor-κB (NF-κB), mitogen-activated protein kinases (MAPKs) and type-I interferon (IFN) response (Akira et al. 2006). More specifically, previous studies have suggested that multistrain combination probiotic may possibly elicit immuno-modulatory effect through NF-κB and MAPKs pathways (MacPherson et al. 2014; Ekwemalor et al. 2016b). The commercial multistrain probiotic used in the present study activated these pathways and, treatment modulated the expression of genes involved in these pathways. Activation or inhibition of NF-κB has been suggested as a requirement for pathogenicity (Higgins et al. 2010). Probiotic administration consisting of Lactobacillus and Streptococcus activated NF-κB and induced IL-6 and TNFα (Miettinen et al. 2000). Previous study in probiotic-treated chicks showed decreased expression of IFNγ (Haghhighi et al. 2008) and a similar effect was observed in cow blood in our study.

Upon activation of these pathways, particularly NF-κB, the expression of several cytokines was observed in the present study. Generally, stimuli provided by certain probiotic strains induce cytokine mRNA expression in leukocytes (Shida et al. 2009). In a similar study, treatment with probiotic Lactobacillus salivarius ssp. salivarius CECT5713 modulated the expression of cytokines and an anti-inflammatory effect was reported (Arribas et al. 2012). The interaction between probiotics and TLRs triggers expression of cytokines and may activate the innate immune system through myeloid-differentiation-primary-response-gene (MyD88)-dependent or MyD88-independent system. Results from this study showed inhibition of the MyD88 gene in the probiotic-treated animals. This suggests probiotic activation of innate immunity, possibly through a MyD88-independent mechanism. This deduction corroborates previous findings reported by Cekic et al. (2011) and Jacquier et al. (2015).

Wnt signalling pathway has been associated with cellular and biological processes and maintenance of homeostasis. Probiotics are known to contribute to the homeostasis of the gastrointestinal tract bacterial flora (Signorini et al. 2012); however, under inadequate initial bacterial colonization situations, probiotic supplements can also ensure a balanced immune response (Kalliomäki & Walker 2005). The Wnt pathway was significantly associated with our study results based on pathway analysis. Probiotic supplements modulate the expression of Wnt signalling genes as presented in the RT-PCR results; the underlying effect may result in homeostatic conditions in dairy cows and thus contribute to growth and production.

5. Conclusion

This study provides evidence for the impact of oral probiotic administration on gene expression in cow blood with possible systemic effects through pathways involved in immunity and homeostasis. Cross-regulation and activation of TLR and WNT subsequent to the recognition of probiotic-derived MAMPs through the TLR pathways may be important in cows as in other organisms. The modulation of the WNT pluripotency pathway may have implications for cell-mediated immunity and for adipogenesis in milk and meat from cows. A thorough evaluation of the impact of probiotics in animal production and health should include an assessment of the impact on gene activation in blood. Further definition of the interaction between the genes and pathways involved may aid in the design of the most effective probiotics for optimum dairy production and health.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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