Microarray analysis of the effect of Cowpea (*Vigna unguiculata*) phenolic extract in bovine peripheral blood

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**ABSTRACT**

In this study, the effect of polyphenolic extracts from cowpea (*Vigna unguiculata*) on global gene expression in bovine peripheral blood was investigated. Blood collected from Holstein-Friesian cows (*n* = 10) was treated with 10 µg/mL of cowpea phenolic extract (CPE) and subsequently used for transcriptional profiling using the Agilent bovine (v2) 4 × 44 K array. Calculation of fold change in gene expression and pathway analysis was conducted using the GeneSpring GX software 13.0. Real-time quantitative PCR was performed to validate the microarray data. Phenolic extracts of cowpea impacted global gene expression and resulted in 3170 differentially expressed genes (*p* < .05); 1716 genes were upregulated and 1454 genes were downregulated. Exposure to CPE impacted 66 pathways (*p* < .05) including the Wnt signalling pathway, Toll-like receptor pathway, inflammation response pathway, MAPK cascade pathway, prostaglandin synthesis and regulation pathway, cell cycle pathway, insulin signalling pathway, and the adipogenesis pathway. Expression of immune markers such as CD40, CD68, Toll-like receptors, and Wnt signalling changed. Exposure to CPE modulated expression of genes associated with immunity and homeostasis. Transcriptional profiles of the response to polyphenols may aid in the design of targeted diets to influence animal production and health and thus requires further study.

**1. Introduction**

Dietary nutrient and non-nutrient constituents in animal feed are essential for enhancing ruminant productivity and health (Dawson 2006). Non-nutrient components in animal feed include flavonoids and non-flavonoids such as phenolic acids and Proanthocyanidins (Tsao 2010). These anti-nutritional phe- nolic constituents in animal diets affect biological processes via activation and regulation of multilcalar pathways and have antioxidant properties (Tarapore et al. 2012). Consumption of antioxidant-rich feed prevents the damaging effect of free radicals and their metabolic by-products (Surai 2014) and stimulates an immune response in animals (Karasawa et al. 2011). The impact of diet and secondary dietary bioactive substances on gene expression termed nutrigenomics examines nutrient-gene interactions (Müller & Kersten 2003). Nutrients and non-nutrient components such as flavonoids impact the transcriptome, and subsequently affect the proteome, metabolome, and epigenome (Afman & Muller 2006).

Cowpea (*Vigna unguiculata* [L.] Walp), a legume predominately cultivated and consumed as food for humans and feed for animals, is rich in proteins and phenolic compounds (Zia-Ul-Haq et al. 2010, 2013). The nutritional and phenolic composition of cowpea seeds has been characterized (Cai et al. 2003; Gupta et al. 2010). Phenolic constituents in cowpea include phenolic acids such as protocatechuic acid, p-hydroxybenzoic acid, caffeic acid, p-coumaric acid, ferulic acid, 2,4-dimethoxybenzoic acid and cinnamic acid (Cai et al. 2003); flavonoids including quer cetin, myricetin, and kaempferol glycosides (Ojwang et al. 2012); anthocyanins and proanthocya- nidin predominantly catechin-O-glucoside (Ojwang et al. 2013). Polyphenols within cowpea have antioxidant (Siddhuraju & Becker 2007), anti-inflammatory (Ojwang et al. 2015), and antican cer (Gutiérrez-Uribe et al. 2011) properties. Cowpea research has focused on varietal selection for traits of interest (Hall 2004), and studies focused on the benefits of cowpea diet and pheno- lic constituents on health are increasing. With advances in bio- technology and the advent of various ‘omics’ technologies, new opportunities in cowpea research are possible to aid our understanding of the role and impact of cowpea on nutrition and health in both man and animals. Studies pertaining to the effect of a cowpea-based diet and its polyphenols on livestock health are inadequate. It is important to elucidate the molecular effect of polyphenols from cowpea on animals to enhance our understanding and utilization of cowpea in livestock production. The present study evaluated the effect of cowpea phe- nolic extracts (CPEs) on the bovine transcriptome in peripheral blood using microarray analysis. The *in vitro* effect of cowpea polyphenols extract on global gene expression was examined using the Agilent bovine v2.4 × 44 k array in a one-colour microarray experiment. The microarray experiment compared two...
groups; cowpea polyphenol extract treated blood to untreated control samples.

2. Materials and methods

2.1. Preparation of crude CPE
The procedure used for the preparation of crude CPE was as previously described by Adjei-Fremah et al. (2015). Briefly, methanolic extracts were prepared from leaves of Mississippi silver, a dual-purpose cowpea variety commonly used in the southern USA. The extracted crude CPE was concentrated and dried with a vacufuge (Eppendorf) to evaporate the methanol. The actual dry weight of the crude extract was determined (6.7 mg). The crude CPE extract was dissolved in phosphate buffered saline (PBS, pH 7.4).

2.2. Blood sampling

Age-matched, female, Holstein-Friesian cows at mid-lactation (n = 10; Body weight = 1653 ± kg) were selected from cattle housed at the North Carolina Agricultural and Technical State University dairy farm. Whole blood was collected aseptically from the jugular vein into vacutainer tubes (BD Biosciences, San Jose, CA, USA) containing the anticoagulant Acid Citrate Dextrose. All experimental procedures used were approved by the Institutional Animal Care and Use Committee of North Carolina Agricultural and Technical State University. All solvents and diluents used in this study were tested for endotoxin with the ToxinSensorTM Chromogenic LAL Endotoxin Assay Kit following the manufacturer’s protocol (GenScript, Piscataway, NJ, USA) as previously described by Adjei-Fremah et al. (2016).

2.3. Treatment of bovine blood with CPE

The procedure used for stimulation of whole blood with CPE was as previously described by Adjei-Fremah et al. (2015). Bovine whole blood (10⁷ viable cells/mL) was treated with 10 μg/mL CPE, and untreated control samples were maintained in PBS. Treatment conditions used were as described by Worku and Morris (2009). Treated and control samples were incubated for 30 min at 37°C, 95% humidity and 5% CO₂. After incubation, the samples were centrifuged at 700 g for 5 min at 4°C to obtain the cell pellet. Tri-reagent (Sigma-Aldrich, St Louis, MO, USA) was added to the cell pellet for total RNA isolation.

2.4. Extraction of total RNA

Total RNA was extracted using the ZR whole blood RNA mini-prep kit (ZYMO RESEARCH, Irvine, CA, USA) following the manufacturer’s recommendations. The concentration and purity of the isolated RNA were quantified using a NanoDrop-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA integrity number (RIN) was determined with the Agilent 2100 Bioanalyzer using the RNA 6000 Nano chip. All samples were stored at −80°C until used for microarray analysis and real-time PCR analysis.

2.5. Microarray analysis

All procedures and reagents used for the microarray experiment were as recommended by the manufacturer (Agilent Technologies, Santa Clara, CA, USA). Pooled RNA 0.5 μg (RIN > 7) was used to prepare Cyanine-3 (Cy3)-labelled cRNA, which were purified using RNAeasy columns (QIAGEN, Valencia, CA, USA). A NanoDrop ND-1000 Spectrophotometer (Thermo-Scientific, Waltham, MA, USA) was used to check for dye incorporation and cRNA yield. The Cy3-labelled cRNA was fragmented and hybridized to bovine (v2) 4 × 44 k Oligo arrays (G2519F) for 17 h at 65°C in a rotating hybridization oven. The microarray used had 44,000 (44 k) cow genes represented on the array. After hybridization, the arrays were washed at room temperature and then dried immediately. The slides were scanned on the DNA Microarray Scanner (G2505B) using the one-colour scan default settings for 4 × 44 k array slides.

2.6. Normalization and analysis of microarray data

Feature extraction software version 10.10.1.1 (Agilent Technologies, Santa Clara, CA, USA) was used to obtain process signal intensity values using default parameters (GE1-v5.95_Feb07). Data normalization and statistical analysis were performed using GeneSpring GX software version 13.0 (Agilent Technologies, Santa Clara, CA, USA) to generate fold changes (FC) in gene expression and hierarchical cluster analysis. Fold changes in gene expression calculated were filtered at a cut-off of ≥2 (p < .05) to obtain a list of significantly upregulated and downregulated genes. Hierarchical cluster analysis was generated using differentially expressed genes data with a cut-off of twofold change and statistically significant difference in expression (p < .05). GeneSpring Pathway Analysis software version 13.0 was used to conduct single experiment pathway analysis of the results with a fold change cut-off of ≥2, p < .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. GSE75239. http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE75239.

2.8. Real-time PCR

The microarray data were validated using real-time quantitative PCR which was performed with a CFX Connect real-time system (Bio-Rad, Hercules, CA, USA). Primers for 10 differentially expressed genes observed from the microarray results, TLR2, TLR4, IL6ST, SLC11AI, C3, CSF1, TNFSF4, NFKB1A, STAT1, and CCL3, as well as the reference gene GAPDH, as listed in Table 1 were designed using Primer3 software version 0.4.0 (Untergasser et al. 2012) and were commercially sequenced by Eurofins MWG Operon (Louisville, KY, USA). Table 1 shows information on primers and primer sequences used. Total RNA of the individual samples (0.5 μg each, RIN > 7) was reverse transcribed to cDNA using oligoDT primers (Ambion, Austin, TX, USA). Samples were prepared for real-time PCR amplification reaction in a final volume of 20 μl including
200 ng template, 250 nM primer, and 10 ul SsoAdvance SYBR green Mastermix (Bio-Rad, Hercules, CA, USA). The polymerase chain reaction 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. All reactions were performed in triplicate. For data analysis, normalization was performed with the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase.

3. Results

3.1. Change in transcriptional profile in blood in response to CPE

Global gene expression analysis identified 3170 genes that were differentially expressed out of 44 k transcripts. Of these, 1716 genes were upregulated and 1454 genes were downregulated. Differentially expressed genes were considered with a fold change cut-off of ≥2, p < .05. Figure 1 depicts a hierarchical cluster analysis of the differentially expressed genes (with a cut-off of twofold change, FC, p < .05). Treatment with CPE increased the expression of genes associated with innate and adaptive immunity such as cytokines and chemokines, Toll-like receptors (TLRs), and stress-related signalling molecules that are relevant for inflammation response and maintaining homeostasis. Tables 2 and 3 show a list of selected genes upregulated and downregulated in expression, respectively. TLRs regulated and downregulated in expression, respectively. TLRs like receptors (TLRs), and stress-related signalling molecules associated with adaptive immunity such as cytokines and chemokines, Toll-like receptors (TLRs), and stress-related signalling molecules that are relevant for inflammation response and maintaining homeostasis. Tables 2 and 3 show a list of selected genes upregulated and downregulated in expression, respectively. TLRs expressed included TLR2 (FC = 18.0), TLR10 (FC = 4.5), TLR4 (FC = −3.9), and TLR3 (FC = −5.45). Cowpea polyphenol treatment inhibited the expression of NOD (Nucleotide oligomerization domain)-like receptor-associated genes such as NOD1 and NOD2 and increased expression of immune markers such as CD40 (FC = 302.16), CD68 (FC = 19.33), CD151 (FC = 86.95), and CD19 (FC = 110.19).

Furthermore, expression of cytokines and cytokine receptor genes such as IL4R (FC = 5.87), IL10RB (FC = 79.05), IL2RA (FC = 11.23), and IL6ST (FC = 6.57) increased. However, the expression of genes such as IL1B (FC = −9.73), IL21 (FC = −8.20), IL32 (FC = −3.53), and IL1B (FC = −3.0) decreased. Chemokine coding genes including CXCL2 (FC = −4.03) and CCL3 (FC = −3.20) had decreased expression, whereas increased expression of CCL4 (FC = 5.05) gene was recorded. Modulation of expression of the genes NFKB1A (FC = 45.32), JAK1 (FC = 151.77), MAPK14 (FC = 59.90), MAPKAP1(FC = 13.79), MAPKAPK3(FC = 2.16), MAP2K1 (FC = 33.72), CSF1 (FC = −13.62), WNT1 (FC = −9.88), LRPS (FC = −4.84), LEF1 (FC = 17.88), TCF7 (FC = 142.54), PRIL2E2 (FC = −18.58), NCF1 (FC = 69.87), VANG1L1 (FC = 3.49), and SELB (FC = 16.08) was also observed. Some proinflammatory genes including SELB (FC = 16.08), LY2Z (FC = 78.42), and IL1B were upregulated in expression.

3.2. Pathway analysis

Pathway analysis results generated using the GeneSpring Pathway Analysis software identified 66 bovine pathways significantly (p < .05) associated with CPE exposure (Table 4). These pathways included the Wnt signalling pathway, TLR pathway, inflammation response pathway, prostaglandin synthesis and regulation pathway, and mRNA transcription initiation pathway. Three receptor pathways including TLR, T-cell receptor signalling, and TGF (Transforming growth factor) beta receptor signalling pathways were significantly associated with CPE pretreatment of cow blood. Pathways such as cell cycle, G1-to-S cell cycle control, apoptosis, and Wnt signalling pathways were identified in cow blood following treatment with CPE. Pathways associated with biological processes such as adipogenesis, glycogen metabolism, fatty acid beta-oxidation, and calcium regulation in the cardiac cell were also identified.

3.3. Validation of microarray data

The results of microarray analysis were validated using real-time PCR on 10 selected genes TLR2, TLR4, ILST, SLC11A1, C3, CSF1,
Table 2. List of selected upregulated (25 out of 1716) genes in bovine peripheral blood in response to treatment with phenolic extract from cowpea (Vigna unguiculata) leaves. All GeneBank ID are specific for Bos taurus.

| Gene ID       | Genes          | Description                                             | Fold change | p-value |
|---------------|----------------|---------------------------------------------------------|-------------|---------|
| NM_001077900  | STAT1          | Signal transducer and activator of transcription 1      | 5.62        | .0021   |
| NM_001205743  | VANGL1         | VANGL Planar Cell Polarity Protein 1                    | 3.5         | .0036   |
| NM_001045868  | NFKBIA         | Nuclear factor of kappa inhibitor, alpha (NFKBIA)       | 45.0        | .000023 |
| NM_174358     | IL2RA          | Interleukin 2 receptor, alpha                           | 11.1        | .0024   |
| NM_001099186  | TCF7            | Transcription Factor 7 (T-Cell Specific, HMG-Box)       | 142.5       | .000017 |
| NM_001076918  | TLR10          | Toll-like receptor 10                                   | 4.5         | .0018   |
| NM_174197     | TLR2           | Toll-like receptor 2                                     | 18.0        | .001    |
| NM_001075142  | IL4R           | Interleukin 4 receptor                                    | 6.0         | .0006   |
| NM_001076975  | IL10R          | Interleukin 10 receptor, beta                            | 79.0        | .000027 |
| ENSBTAT00000002395 | PRICKLE2 | Prickle Homolog 2                                      | 60.0        | .000021 |
| NM_001066295  | IL6ST          | Interleukin 6 signal transducer                         | 6.5         | .00075  |
| NM_001206495  | ILF3           | Interleukin enhancer binding factor 3                   | 10.0        | .00042  |
| NM_0010105611 | CD40           | CD40 molecule                                            | 302.2       | .0000028|
| NM_001045902  | CD68           | CD68 molecule                                            | 19.3        | .00048  |
| NM_001245998  | CD19           | CD19 molecule                                            | 110.2       | .000021 |
| NM_001075147  | CCL4           | Chemokine (C-C motif) ligand 4                          | 5.1         | .002    |
| NM_001062634  | JAK1           | Janus kinase 1                                           | 151.7       | .0000028|
| NM_001130752  | MAP2K1         | Mitogen-activated protein kinase kinase 1               | 33.7        | .0000059|
| NM_00102174   | MAPK14         | Mitogen-activated protein kinase kinase 14              | 59.9        | .000033 |
| NM_174119     | NCF1           | Neutrophyl cytotoxic factor                              | 60.7        | .00092  |
| NM_174182     | SELL           | Selectin                                                | 16.1        | .000093 |
| NM_174652     | SLC11A1        | Solute carrier family 11 (Proton coupled metal ion transporter) | 19.3       | .00013  |
| NM_001081603  | MAPKAP1        | Mitogen-activated protein kinase associated protein 1    | 13.7        | .002195 |
| BC11137       | LRF1           | Lymphoid enhancer binding factor 1                      | 17.88       | .0184   |
| NB_180999     | LYZ2           | Lysozyme c-2                                            | 78.4        | .003203 |
| NM_001035347  | CD151          | CD151 molecule                                           | 86.9        | .03168  |

*Fold change presented are significant, p < .05.

Table 3. List of selected downregulated (15 out of 1454) genes in bovine peripheral blood in response to treatment with a phenolic extract from cowpea (Vigna unguiculata) leaves. All GeneBank ID are specific for Bos taurus.

| Gene ID       | Genes          | Description                                             | Fold change | p-value |
|---------------|----------------|---------------------------------------------------------|-------------|---------|
| NM_001008664  | TLR3           | Toll-like receptor 3                                     | −5.5        | .0022   |
| NM_174198     | TLR4           | Toll-like receptor 4                                     | −3.9        | .0016   |
| NM_001205757  | IL10RA         | Interleukin 10 receptor alpha                            | −4          | .00017  |
| NM_198832     | IL21           | Interleukin 21                                           | −8.2        | .00043  |
| XM_002703050  | IL32           | Interleukin 32                                           | −3.5        | .0034   |
| NM_174093     | IL1B           | Interleukin 1, beta                                     | −3.0        | .001    |
| NM_001034735  | CD74           | CD74 molecule, major histocompatibility complex, class II invariant chain (CD74) | −3.13       | .00015  |
| NM_174511     | CCL3           | Chemokine (C-C motif) ligand 3                          | −3.2        | .0022   |
| NM_174299     | CCL2           | Chemokine(C-X-C motif) ligand 2                         | −4.0        | .00052  |
| NM_001040469  | C3             | Complement component 3                                  | −4.1        | .00081  |
| AY181987      | CSF1           | macrophage colony-stimulating factor                    | −3.0        | .001    |
| NM_001114191  | WNT1           | wingless-type MMTV integration site family, member 1    | −10.0       | .00026  |
| XM_6144220    | LRPS           | low density lipoprotein receptor-related protein 5      | −4.8        | .001    |
| ENSBTAT000000029105 | PRICKLE2 | Prickle Homolog 2                                      | −18.6       | .0014   |
| NM_00103477   | MAPKAPK3       | Mitogen-activated protein kinase-activated protein kinase 3 | −2.16       | .03275  |

*Fold change presented are significant, p < .05.

TNFSF4, NFKB1A, CCL3, and STAT1. The expression of TLR2, IL6ST, SLC11A1, NFKB1A, and STAT1 genes was upregulated, whereas TLR4, C3, CCL3, CSF1, and TNFSF4 were downregulated. The gene expression patterns observed in the RT-PCR analysis were consistent with the microarray data as shown in Figure 2. The expression of Wnt signalling pathway genes such as LRPS (FC = 4.00), LEF1 (FC = 1.00), TCF7 (FC = 1.00), and VANGL2 (FC = 16.00) has also been confirmed in a previous study by Adjei-Fremah et al. (2016) and their results were consistent with microarray analysis in this study.

4. Discussion

Polyphenols found in animal feed are beneficial for health (Landete 2012). Forages rich in phenolic constituents have immunoregulatory effects in ruminants and thus are considered useful for enhanced animal health and wellbeing. In the present study, the effect of crude CPEs on the global transcriptional profile in bovine peripheral blood was evaluated using microarray analysis. In vitro studies provide a window to study the possible effect of supplementation with cowpea feed on livestock. Our results demonstrated activation and influence of CPE on transcription in cow blood. Treatment with CPE impacted global gene expression and modulated the expression of genes associated with cellular processes, biological activities, and the immune response. Other studies using bovine blood neutrophils have shown the effect of plant natural product such as citrus-derived oil (Garcia et al. 2015) and tomato polyphenols (Gyenai et al. 2012) on gene expression. The impact of forages rich in polyphenol such as Sericea lespedeza on gene expression has been studied in goats (Worku et al. 2016; Asiamah et al. 2016).

Pathway analysis was performed to provide mechanistic insights into the underlying biology of the effect of CPE on
the bovine transcriptome. Exposure to CPE was associated with activation of multiple cellular pathways in cow blood. These included the ruminant immune response, cellular processes, and biological processes such as adipogenesis, insulin signalling, glycogen metabolism, acid beta-oxidation, and calcium regulation in the cardiac cell pathways.

Increased understanding of the bovine innate immune response is needed to aid in control of inflammatory diseases. In the present study, cowpea polyphenols modulated the expression of innate immune response genes. Plant-derived polyphenols regulate innate immune response gene and inflammation biomarkers (Gyenai et al. 2012). Pattern recognition receptors recognize microbial components referred to as pathogen-associated molecular patterns (PAMPs) to initiate host defense against infection. TLRs link pathogen recognition with induction of innate and adaptive immunity. In cattle, 10 TLRs are expressed which have also been detected in bovine peripheral blood (Menzies & Ingham 2006). The current study identified TLRs are expressed which have also been detected in bovine peripheral blood cells’ exposure to treatment with phenolic extract from cowpea (Vigna unguiculata) leaves.

Table 4. Summary of selected bovine pathways (35 out of 66) associated with cowpea peripheral blood cells’ exposure to treatment with phenolic extract from cowpea (Vigna unguiculata) leaves.

| Bovine pathway                       | p-Value    |
|--------------------------------------|------------|
| Bt_Cytosplasmic_Ribosomal_Proteins   | 0.00000002 |
| Bt_mRNA_processing                   | 0.00000008 |
| Bt_IL-6_Signalling_Pathway           | 0.00000017 |
| Bt_T_Cell_Receptor_Signalling_Pathway| 0.000069   |
| Bt_TGF-beta_Receptor_Signalling_Pathway| 0.00014   |
| Bt_Translation_Factors               | 0.000034   |
| Bt_Insulin_Signalling                | 0.000087   |
| Bt_TNF-alpha_NF-kB_Signalling_Pathway| 0.000071   |
| Bt_Glycolysis_and_Glucogenogenesis    | 0.00039    |
| Bt_Cell_cycle                        | 0.00075    |
| Bt_Delta-Notch_Signalling_Pathway    | 0.00065    |
| Bt_Toll-like_receptor_singalling_pathway| 0.00048   |
| Bt_Calcium_Regulation_in_the_Cardiac_Cell| 0.00001  |
| Bt_IL-2_Signalling_Pathway           | 0.00048    |
| Bt_MAPK CASCADE_WP1009_71415         | 0.00079    |
| Bt_Type_Il_interferon_singalling_IFNG| 0.00043    |
| Bt_IL-4_Signalling_Pathway           | 0.00054    |
| Bt_G1_to_S_cell_cycle_control_WP1078_7625 | 0.0001  |
| Bt_Adipogenesis_WP87_79147           | 0.0056     |
| Bt_IL-3_Signalling_Pathway           | 0.0020     |
| Bt_Oxidative_phosphorylation_WP994_63467 | 0.0082   |
| Bt_TGF_Beta_Signalling_Pathway       | 0.0010     |
| Bt_Eicosanoid_Synthesis_WP792_78598  | 0.017      |
| Bt_Notch_Signalling_Pathway          | 0.0013     |
| Bt_Apoptosis_WP1018_67048             | 0.061      |
| Bt_Glycogen_Metabolism_WP1073_71850  | 0.049      |
| Bt_IL-9_Signalling_Pathway           | 0.039      |
| Bt_IL-5_Signalling_Pathway           | 0.026      |
| Bt_IL-7_Signalling_Pathway           | 0.008      |
| Bt_Inflammatory_Response_Pathway     | 0.016      |
| Bt_Fatty_Acid_Beta_Oxidation_WP1061_79782 | 0.1       |
| Bt_Eukaryotic_Transcription_Initiation_WP1066_79155 | 0.23    |
| Bt_Wnt_Signalling_Pathway_NetPath    | 0.23       |
| Bt_Prostaglandin_Synthesis_and_Regulation_WP995_71430 | 0.05 |
| Bt_p38_MAPK_Signalling_Pathway       | 0.05       |

**Figure 2.** Validation of expression of selected genes from the microarray data using real-time PCR. Ten differentially expressed genes in bovine peripheral blood in response to treatment with 10 µg/mL of phenolic extracts from cowpea (Vigna unguiculata). Five genes upregulated in expression included TLR2, SLC11A1, NFKB1, STAT1, IL6ST, and genes downregulated in expression were TLR4, C3, CSF1, TNFSF4, and CCL3.

rich in flavonoids have an effect on TLR gene expression and protein expression (Pérez-Cano et al. 2014). Oral treatment of Wistar rats with a hydroalcoholic extract of Achyrocline satureoides, a rich source of quercetin and luteolin, decreased TLR4 expression on neutrophils (Barioni et al. 2013). In a similar study, a cocoa diet, a rich source of procyanidins among other flavonoids, induced an upregulation of TLR2 and TLR7 and downregulation of TLR4 and TLR9 in small intestine tissues (Pérez-Berezo et al. 2012). Exposure to CPE resulted in increased expression of a nuclear factor of kappa inhibitor, alpha (NFKB1A), and MAPK14 and MAP2K1 genes. Thus, recognition of CPE by a TLR-dependent mechanism may be associated with the observed effect.

Cell cycle, cell proliferation, apoptosis pathways, and the Wnt signalling pathway were also responsive to CPE treatment. In cow blood, pretreatment with CPE activated these pathways and modulated the expression of associated genes. These pathways are present and function in normal cow blood as expected. The Wnt signalling pathway functions in cellular processes including cell fate determination, motility, polarity, primary axis formation and organogenesis, and stem cell renewal (Yamaguchi 2001). In cancer studies, the anti-proliferative effect, induction of cell cycle arrests, or apoptosis potential of polyphenols has been identified as chemopreventive mechanisms (Garcia-Lafuente et al. 2009). The observed results indicate that CPE can be used to modulate cell proliferation to control disease associated with inflammation and cancer, warranting further study using cancer cell lines.

In the human diet, cowpea food is considered as low glycemic index (GI) food (Foster-Powell & Miller 1995). Low GI foods reduce post-prandial blood glucose and insulin responses in normal and diabetic patients (Collier et al. 1998). Studies have shown that cowpea food and its polyphenols may have an anti-diabetic effect. The anti-diabetic potential of plant polyphenols (Rizvi et al. 2005) has been suggested to be via inhibition of glucose absorption in the gut or its uptake by peripheral tissues (Matsui et al. 2002). Results from the present study identified insulin signalling and glycogen metabolism pathways to be significantly associated with CPE.
pretreatment of cow blood. Studies have shown that cowpea peptides have the ability to activate insulin signalling in skeletal muscle cells (Barnes et al. 2015). Venâncio et al. (2003) suggested that a protein with similar molecular mass and amino acid sequence homologous to bovine insulin is present in the cowpea plant.

Polyphenols in animal diets may also be capable of modulating genes associated with biological processes that impact animal products such as meat and milk (Waghorn & McNabb 2003; Vasta & Luciano 2011). Phenolic compounds have been shown to influence the expression of genes associated with adipogenesis (Hsu & Yen 2007). In this study, although the effect of CPE was examined in blood, pathway analysis revealed a possible effect of treatment on the adipogenesis pathway. Our study may provide a molecular basis for the effect of cowpea-based diets on marbling in cows. The Wnt antagonist SFRP4 gene expression was observed in response to CPE. Studies by Jeong et al. (2013), associated with SFRP4 gene expression to increased intramuscular fat deposition in the longissimus dorsi muscle. On the other hand, cowpeas use as feed sources have also been reported to promote marbling and is associated with desirable meat quality in taste panels (Schmidt et al. 2013). The wingless pathway has also been shown to affect bovine marbling and adipogenesis (Du et al. 2010). Thus, by looking at the effect of CPE in blood this study provides an approach to help elucidate CPE effect on early gene expression with possible implications for downstream effects on marbling. Much study is therefore required to ascertain the possible connection between cowpea feed, meat quality, and Wnt signalling via in vivo and in vitro studies using mesenchymal cells.

5. Conclusion

Microarray analysis of the effect of CPE on bovine blood shows that exposure to CPE in blood, as may result during supplementation with cowpea in animal feed, has potential benefits for modulation of pathways associated with activation of innate immunity, adipogenesis, and homeostasis. These results contribute to the mechanistic understanding of the molecular impact of polyphenols such as CPE in animal feed and offer avenues for the development of functional feed supplements to promote animal health and product quality.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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