Differential gene expression in heterophils isolated from commercial hybrid and Thai indigenous broiler chickens under quercetin supplementation

Tanakrit Khampeerathuch², Acharaporn Mudsak³, Suphakit Srikok³, Soulsack Vannamahaxay⁵, Suwit Chotinun⁴,⁵ and Phongsakorn Chuammitri⁴,⁶

¹Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand;²Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand;³Department of Livestock and Fisheries, Faculty of Agriculture, National University of Laos, Vientiane, Laos;⁴Department of Food Animal Clinics, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand;⁵Excellent Center in Veterinary Biosciences (ECVB), Chiang Mai University, Chiang Mai, Thailand;⁶Integrative Research Center for Veterinary Preventive Medicine, Chiang Mai University, Chiang Mai, Thailand

ABSTRACT
Chicken heterophils serve as the first-line defence in chicken innate immunity. The present study aimed to explore the effects of quercetin, a herbal phenolic substance known for its inflammatory modulation, on mRNA expression patterns in chicken heterophils. Isolated heterophils from two genetically distinct chicken lines, hybrid commercial and Thai indigenous chickens, Pradu Hang Dam, were in vitro co-cultured with live intestinal pathogenic Salmonella enteritidis under Phosphate-buffered saline (PBS) or quercetin supplementation. Quantitative real-time RT-PCR was performed to investigate the effects of quercetin on mRNA transcripts of 12 genes. The results showed that heterophils from hybrid chickens treated with quercetin had higher relative expression levels of IL6, ILBL1, ILBL2, IL18, CCL4, LITAF, and MIF. Unlike hybrid chickens, Pradu Hang Dam chickens had down-regulation of cytokine genes, IL1B, IL6, ILBL1, ILBL2, IL12B, and IL18, and chemokines, CCL4 and MIF. In addition, NOS2, IL17A, and TGFβ1 were up-regulated in this lineage. In conclusion, our findings emphasize the modulatory effects of quercetin to down-regulate most pro-inflammatory genes, whereas it up-regulates anti-inflammatory gene (TGFβ1) in Pradu Hang Dam chickens.

1. Introduction
Heterophils serve as the first line of innate immunity in chicken and avian species. In fact, chicken heterophils are among the terminally differentiated cells that can swiftly secrete many cytokines and chemokines during the activation phase when encountering external pathogens such as Salmonella spp., Escherichia coli, and Eimeria spp. (Mellata et al. 2003; Hong et al. 2006; Redmond et al. 2009; Chranova et al. 2011). In response to bacterial infection, heterophils are being expressed and secreted as a major source of pro-inflammatory cytokines such as IL-1β, IL-6 and IL-8, as well as chemokines in order to communicate between avian innate, adaptive immune cells and other affected tissues (Sick et al. 2000; Kaiser et al. 2005; Redmond et al. 2009). Chicken heterophils may be primed and later activated at the site of infection. The elevation or reduction in gene expression of heterophils after being stimulated with bacterial microbial-associated molecular patterns and other pathogens are nicely described with in-depth explorations (Kaiser et al. 2005; Chiang et al. 2008). The collection of knowledge regarding what type of cytokines, chemokines, antimicrobial peptides, and macromolecules are produced in response to Salmonella spp. are clearly reported in the literature (Withanage et al. 2004; Kaiser et al. 2005).

To date, many cytokines and chemokines initially identified in mammals have also been identified, characterized, and re-evaluated in chickens for their presence and functional conservation to the mammalian system (Kaiser et al. 2005). The constitutive expressions of pro-inflammatory cytokines and other genes in chicken appeared to be relevant in promoting, mounting, or putting host innate cells on alert, which leads to tissue inflammation and inevitable damages later (Swaggerty et al. 2004).

To manipulate and to resolve the inflammatory process occurring during an initial phase of bacterial infection or bacterial-causing inflammation, many studies have applied the natural bioflavonoid from plants, such as quercetin, which are ubiquitously present in many plant products (Formica and Regelson 1995; Wadsworth and Koop 1999). From various in vitro and in vivo studies, it has been revealed that quercetin possesses the effects of anti-inflammation, anti-oxidation, antiviral as well as other activities (Alzoreky and Nakahara 2003; Cho et al. 2003; Chuammitri et al. 2015; Nair et al. 2006). Many studies in human and mammalian neutrophils from the aspects of...
anti-inflammation at a molecular level provide an insight into the
effects of this compound that quercetin greatly reduced gene
expression, especially targeted genes that were involved in
early immune responses or pro-inflammatory cytokines, including
IL-1β, IL-6, TNF-α, MIP-1α/β, MIP-2, IL-8, IL-6, and iNOS,
which is vital to the host defence mechanism in mammals
(Cho et al. 2003; Liu et al. 2005; Geraets et al. 2009; Boesch-Saadatmandi
et al. 2011; Askari et al. 2012; Chan et al. 2012; Das et al. 2012; Freitas et al. 2014; Chuammitri et al. 2015; Huang et al. 2015). In this regards, the knowledge of quercetin pertaining
to chicken heterophils is inadvertedly established. This
prompted us to question how this substance would exert its
immunomodulatory effects in chicken heterophils from two
genetically different chickens. To improve our understanding of
chicken heterophils in response to bacterial infection at molecu-
lar and gene expression levels, we have utilized isolated hetero-
phils from commercial hybrid chickens and the Thai indigenous
chicken (TIC) breed, Pradu Hang Dam, as innate cell models.
Chinese heterophils from commercial hybrid chickens and native chickens were performed according to a previously
described technique with a slight modification (Chuammitri et al. 2015). In this regards, the knowledge of quercetin pertaining
to chicken heterophils is inadvertedly established. This
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lar and gene expression levels, we have utilized isolated hetero-
phils from commercial hybrid chickens and the Thai indigenous
chicken (TIC) breed, Pradu Hang Dam, as innate cell models.

2. Materials and methods

The reagents ethylenediaminetetraacetic acid (EDTA) powder,
Hank’s balanced salt solution without calcium and magnesium
and phenol red (HBSS-CMF), Histopaque, methylcellulose
trypan blue, and quercetin hydrate (QH) were ordered from
Sigma-Aldrich (St. Louis, MO, USA). Foetal bovine serum (FBS), Gibco RPMI 1640 medium with phenol red, and RNAlater
were obtained from Life Technologies (Carlsbad, CA, USA).

2.1. Birds

Commercial hybrid broiler chickens (Hybrid) aged range from 6
to 7 weeks (n = 36) were reared on a local farm in Chiang Mai,
Thailand. Native chickens (Gallus domesticus), Pradu Hang
Dam (Pradu) (Dorji et al. 2011; Akaboot et al. 2012), were
reared on a Pradu Hang Dam Farm in Chiang Mai under pro-
motions from the Research and Development Network Center
for Animal Breeding (native chicken), Department of Livestock
Development, Ministry of Agriculture and Cooperatives, Thai-
land. Eight-week-old chickens (n = 50) were used in this exper-
iment. Pradu chickens had no vaccination history against
Salmonella. Whole blood from hybrid or Pradu chickens were collected from wing veins (3 ml). Samples were mixed with EDTA
solution (1% final concentration; Blood:EDTA v/v ratio was
10:1). Samples from groups of five to six individuals were pooled.
The animal study protocol has been reviewed and approved by
The Faculty of Veterinary Medicine, Chiang Mai University,
Animal Care and Use Committee (FVM-ACUC) Ref. No. S11/2558.

2.2. Chicken heterophil isolation

Isolations of chicken heterophils from healthy hybrid or
Pradu chickens were performed according to a previously
described technique with a slight modification (Chuammitri
et al. 2011). The purity of cells was more than 90% as
determined under a microscope. The trypan blue was used to
determine heterophil viability and cell density was adjusted to
1 × 10^6 cells/ml.

2.3. Quercetin

QH with purity of ≥95% by HPLC and containing ≥2.9% water
as determined by Karl Fischer was used. Details regarding the
preparation of stock quercetin solution (5 mM) and working solu-
tion (50 μM) were previously described (Chuammitri et al. 2015).
This concentration was previously determined to be safe to use
for stimulation of chicken heterophils (Nojit et al. 2014).

2.4. Salmonella enteritidis propagation

Salmonella enteritidis was kindly provided by Dr Prapas Patcha-
nee, Faculty of Veterinary Medicine, Chiang Mai University. Sal-
monella enteritidis was freshly prepared by the colony picking
method and propagation in Luria-Bertani Broth (LB broth,
Caisson Laboratories, North Logan, UT, USA) at 37°C, 120 rpm
in shaking incubator until the log phase was reached. The
growth curve regression equation: y = (OD_{595}) × (5.09 × 10^{9})
+ (−8.3 × 10^{8}) was used to calculate bacterial concentration
(CFU/ml). The bacterial number was adjusted to approximately
10^8 CFU/ml prior to use in the experiment (Chuammitri et al.
2011). The Salmonella isolates were identified by WHO National
Salmonella and Shigella Center (NSSC) at the Department of
Medical Sciences, Ministry of Public Health, Thailand (Boonkhot
et al. 2015).

2.5. Heterophil stimulation

Heterophils (1 × 10^6 cells) were aliquoted into a 24-well cell culture plate (duplicate wells). Subsequently, heterophils were
co-cultured with 10 multiplicity of infection of S. enteritidis
(1 × 10^7 bacteria) in serum-free RPMI-1640 medium. A total of
50 μM quercetin or Phosphate-buffered saline (PBS) were sup-
pplemented and incubated at 41°C with 5% CO_2 for 60 min.
After stimulation, cells from two wells were combined,
washed, and spun. Heterophil cell pellets were resuspended in
RNAlater as per the manufacturer’s instructions in order to
preserve RNA for further analysis.

2.6. RNA extraction and complementary DNA synthesis

Heterophils in RNAlater were centrifuged at 11,000 rpm for
1 min and the liquid portion was discarded. Total RNA was
extracted and purified by NucleoSpin RNA (Macherey-Nagel,
Bethlehem, PA, USA) according to the manufacturer’s rec-
ommendations. RNA concentration was quantified using a DU
730 nanoVette UV/VIS spectrophotometer (Beckman Coulter).
Complementary DNA (cDNA) was reverse-transcribed from
2 μg of total RNA by Tetro cDNA Synthesis Kit (Bioline,
Taunton, MA, USA). Briefly, 20 μl reaction mixture contained
4 μl 5× RT Buffer, 1 μl 10 mM dNTP mix (2.5 mM each), 1 μl
Oligo(dT)18 (10 μM/μl), 1 μl Random Hexamer (10 μM/μl), 1 μl
RNase Inhibitor (10 U/μl), 1 μl Tetro Reverse Transcriptase
(200 U/μl), 2 μg Total RNA, and DEPC-treated H_2O. The cDNA
synthesis was carried out by LongGene A200 (LongGene...
Table 1. Sequence of the primers used in real-time quantitative RT-PCR.

| No. | Gene name | Accession no. | Primer Sequence | Annealing temp (°C) | Product size (bp) |
|-----|-----------|---------------|-----------------|---------------------|------------------|
| 1   | IL1B      | NM_204524     | Forward: GCC TCA CAG TCC TTC GAC ATC | 60 | 230 |
|     |           |               | Reverse: CGG CTC ATC ACA CAC GAC ATG T | | |
| 2   | IL6       | NM_204628     | Forward: TGG TCG CCT TTC AGA AGT ACC | 60 | 123 |
|     |           |               | Reverse: TTA TCA CCA TCT GCC GGA TCG | | |
| 3   | IL8L1     | NM_205018     | Forward: CCT CAT TGC AAG AAT GTG GAA G | 60 | 124 |
|     |           |               | Reverse: TAT TGG TGT CTG CCT TGT CCA G | | |
| 4   | IL8L2     | NM_205498     | Forward: TGG TTT CAG CAG CTC TGT CG | 60 | 193 |
|     |           |               | Reverse: AAG CAC ACC TCT CTG CCA TCC | | |
| 5   | IL12B     | NM_213571     | Forward: GTG GCT CTC ACT GAT AAA TCT G | 60 | 218 |
|     |           |               | Reverse: CAG GTC ACA GTT CCA TTT GTG C | | |
| 6   | IL17A     | NM_204660     | Forward: TTT CTG CAT ATG GAA AGG TG | 60 | 144 |
|     |           |               | Reverse: CCT GTG TCA TGG TGA TGC | | |
| 7   | IL18      | NM_204608     | Forward: AGG AGG TTA AAT CTG GCA GTG | 60 | 103 |
|     |           |               | Reverse: TCT TCT ACC TGG AGG CTG AAT G | | |
| 8   | CCL4      | NM_204720     | Forward: TCT GCC TCT TCT CAT GAC TTC | 60 | 166 |
|     |           |               | Reverse: GAA GAT GAT GGC AGG CTT TGA | | |
| 9   | LITAF     | NM_204267     | Forward: AGG AGG TGA AAT CTG GCA GTG | 60 | 193 |
|     |           |               | Reverse: TGG TTT CAG CTG CTC TGT CG | | |
| 10  | MIF       | NM_001305091  | Forward: TAC ATA GCC GTG CAT ATC GTA C | 60 | 238 |
|     |           |               | Reverse: ATG CAA AGG TGG AAG CTG TC | | |
| 11  | NOS2      | NM_204961     | Forward: AGT TGT GCA TGG ATG TGC GG | 60 | 211 |
|     |           |               | Reverse: TCA AGG AGC ATG TGG GCA AC | | |
| 12  | TGFβ1     | JQ423909      | Forward: TGG GTA TGG GCC CAA AGA GCT G | 60 | 101 |
|     |           |               | Reverse: ACA CAG AAG AGA TGG TGC GG | | |
| 13  | GAPDH     | NM_204305     | Forward: AAG GGC ATC CTA GGA TAC ACA G | 60 | 107 |
|     |           |               | Reverse: TGG TCA TTC AGT GCA ATG CC | | |

Scientific Instruments, Hangzhou, China) by incubating the samples at 25°C for 10 min, 45°C for 30 min, followed by heating at 85°C for 5 min, chilled on ice, and stored reaction at −20°C until further use.

2.7. Primers

Primers for genes, IL6, IL8L1(K60; IL8), IL8L2 (CXCL8; EMF-1; CXCL12), IL12B (IL-12p40), IL17A (pChl-17), IL18, CCL4 (K203, MIP-1β), NOS2 (iNOS), LITAF (TNF-α), MIF, TGFβ1 (formerly known as TGFβ4; Halper et al. 2004) and reference gene (GAPDH) (Hong et al. 2006) were designed using Primer3 Plus (Untergasser et al. 2007). The primer sequence of chicken IL1B (IL-1β) was described previously (Higgs et al. 2006). The real-time PCR primers were synthesized by Macrogen (Seoul, Korea). The sequences of oligonucleotide primers used in this study were indicated in Table 1.

2.8. Quantitative real-time RT-PCR

Quantitative real-time RT-PCR (qRT-PCR) was performed to investigate the effect of quercetin on pro-inflammatory cytokines, chemokines and other related gene expressions in chicken heterophs. The quantification of cytokine or chemokine mRNAs was performed in triplicate on ABI 7300 Real-Time PCR System equipped with SDS Software v1.4 (Life Technologies). qRT-PCR was performed using SensiFAST SYBR Hi-ROX Kit (Bioline) as per the manufacturer’s instruction. The reactions were run for 40 cycles in every tested gene. Next, dissociation curve analysis (Tm) (Figure S2) and 2% agarose gel electrophoresis were used to check real-time PCR product specificity (Chuammitri et al. 2017). Analysis of relative gene expression (RQ) was calculated from the C of the target gene and GAPDH. The expression levels (fold difference) were reported using 2 method (Livak and Schmittgen 2001).

To generate standard curves for cytokine, chemokine, and GAPDH-specific reactions, qRT-PCR products were purified by NucleoSpin Gel and PCR clean-up kit (Machery-Nagel). One hundred nanograms of purified qRT-PCR DNA were serially diluted (10 −1 to 10 −6 ) in sterile RNase-free water. Regression analysis of the mean values of qRT-PCRs for the log10 dilution was used to create standard curves (Liu et al. 2010) (Figure S1).

2.9. Data analysis

The collected data were subjected to screening for potential outliers by robust regression and outlier removal (ROUT) methods (Motulsky and Brown 2006). The normality test, D’Agostino–Pearson omnibus test, was applied to raw data preceding various statistical methods: unpaired two-tailed Student t test or Mann–Whitney test. Statistical significance was achieved when p < .05. Data presentations are displayed as mean with standard error (mean ± SE). Graphical representations from analysing data are built using GraphPad Prism (GraphPad software, San Diego, CA, USA). Heat maps of the average gene expression levels were generated by Genesis 1.7.6 using an average linkage method with Euclidean distance to present gene expression patterns (Stum et al. 2002). Selected genes from differential expression data were further created for a network of genes involved in inflammatory processes by CytoScape 3.0 with GeneMANIA plugin (Montjo et al. 2010).

3. Results

3.1. Standard curves of pro-inflammatory cytokines, chemokines, and other mRNAs in real-time PCR reactions

In order to access the efficiency of current PCR experiments, we have generated a standard curve of log10 serially diluted DNA for the different reactions. The linear regression between
the $C_r$ values and the amount of input DNA fitted with a straight line (Figure S1). The $R^2$ values of IL1B, IL6, IL8L1, IL8L2, IL12B, IL17A, IL18, CCL4, LITAF, MIF, NOS2, TGFB1, and GAPDH were determined. All $R^2$ values exceeded 0.99, and the equations of respective regression line of each gene were also given.

3.2. Effects of quercetin on the expressions of pro-inflammatory cytokines, chemokines, and other mRNAs in hybrid broiler chickens

Heterophils from hybrid chickens treated with quercetin had higher relative expression levels of IL6, IL8L1, IL8L2, IL18, CCL4, LITAF, and MIF (Figure 1), but only two tested genes, IL8L1 and CCL4, had significantly higher relative expression levels when compared to non-stimulated heterophils. Of the 12 chosen genes for assessment in quercetin treatment cells, IL1B, NOS2, IL12B, and TGFB1 had lower relative expression levels compared to non-stimulated cells. IL1B, IL12B, and TGFB1 genes had statistical differences of expressions (Figure 1).

3.3. Gene expression patterns in indigenous Thai breed (Pradu Hang Dam) chickens under the effects of quercetin

In heterophils from Pradu chickens, 7 of 12 pro-inflammatory genes were down-regulated when supplemented with

![Figure 1](image-url)
quercetin (Figure 2). On the other hand, only 3 of 12 tested genes were up-regulated and 2 genes were slightly changed. The expressions of cytokine genes, IL1B, IL6, IL8L1, IL8L2, IL12B, IL18, and chemokines, CCL4 and MIF, were down-regulated. Specifically, only IL1B, IL8L1, IL8L2, IL12B, CCL4, and MIF genes in quercetin supplementation were significantly different from non-stimulated control. NOS2, IL17A, and TGFB1 were up-regulated. No change in expression level has been observed in IL6 and LITAF genes from heterophils of Pradu chickens supplemented with quercetin or cells without stimulation.

3.4. Comparison of gene expression patterns in commercial breeds and indigenous Thai breed chickens under the effects of quercetin

From gene expression data explored from different chicken origins, we have noticed that some genes shared the authenticated features in induction of pro-inflammation or involved in some parts of inflammatory processes conducted by heterophils under quercetin supplementation. Venn diagrams extracted from the results in gene expression patterns depicted the overlapping and underlying genes that could be affected by quercetin. In Figure 3, some genes, IL6, IL17A, and LITAF,
participated in the pro-inflammatory process induced by the presence of Lipopolysaccharides (LPS). On the other hand, pro-inflammatory cytokines, $\text{IL1B}$ and $\text{IL12B}$, were profoundly decreased in both chicken origins. This finding may be due to the action of quercetin. The rest of studied genes appeared to be changed in the opposite directions (Figure 3).

Gene expression patterns of quercetin-stimulated heterophils from two distinct broiler chicken origins are shown in Figure 4. The heat map generated from individual data points of heterophils in hybrid chickens and Pradu chickens was connected by the similar patterns of up- or down-regulated genes. In this study, quercetin could facilitate in up-regulation of putative anti-inflammatory gene, $\text{TGFB1}$, in Pradu chicken; however, this phenomenon was conversely found in commercial hybrid chickens (Figure 4).

We further analysed the gene network that may be engaged in controlling pro-inflammatory cytokines and chemokines using CytoScape. Regardless of up- or down-regulations of the studied genes, we have input the gene annotation terms using publicly available human genes to find co-expression, co-localization, pathway, or physical interactions with the genes of interest in our studies. The gene network generated from CytoScape also discovered the relationships of the studied genes with other genes that can be correlated, for example response to molecule of bacterial origin, response to LPS, response to bacterium, positive regulation of cytokine production, leukocyte-mediated immunity, and inflammatory response (Figure 5).

4. Discussion

To date, there are no intensive studies of the effects of quercetin on immune cells in avian species. The searchable literature emphasized the efficacy of these plant compounds only in promoting meat production and growth performance of chickens.
The present study provides novel information regarding the anti-inflammatory effects of quercetin in chicken heterophil gene expressions from two genetically different chickens: commercial broiler and TIC. We previously reported the effects of quercetin hydrate on heterophil functions from broilers on ROS production, phagocytosis, and heterophil extracellular traps of the gram-negative bacteria *S. enteritidis* (Nojit et al. 2014). To the best of our knowledge, this is the first report to evaluate the anti-inflammatory effects of quercetin on chicken innate immune cells with respect to its influence on gene expression. According to Dorji et al. (2011), the similarity in genetics and genetic distance of two meat-type chickens (broiler and Pradu) has been addressed. Pradu chickens were already genetically determined in their meat production traits, and the authors suggested that these two chicken lineages were closely related than other Thai native chickens.

Bacterial invasion often triggers the innate immunity by releasing pro-inflammatory cytokines, antimicrobial peptides, or enzymes from innate immune cells. Pro-inflammatory cytokines and chemokines have a crucial role in initiating an innate immune response and assist in generating a local inflammatory response (Hughes et al. 2007). Pro-inflammatory cytokines, such as IL-1β, IL-6, and IL-8, were involved in *S. enteritidis* infection (Cheeseman et al. 2007; Abasht et al. 2008). The effects of quercetin on *S. enteritidis*-stimulated heterophils in this study revealed clear instances that many inflammatory cytokine/chemokine genes could be successfully suppressed only in native chickens. In comparison, the commercial chicken breeds showed no decline in inflammation, as depicted by the up-regulation of genes after quercetin supplementation.

As reported by Malek et al. (2004) regarding Fayoumi, native chicken of Egypt, many candidate genes were possibly controlling response to *S. enteritidis*, e.g. TLR4, CD28, and MD-2. Moreover, heterophils from this native chicken respond more robustly to *in vitro* *S. enteritidis* infection by expressing IL-6, GM-CSF, and anti-inflammatory cytokines (IL-10 and TGF-β4) (Cheeseman et al. 2008). In this study, quercetin also exerted anti-inflammatory action by up-regulation of TGF-β1 in Pradu, which was consistent with the results from the aforementioned work. By analogy with the broilers with a long history of genetic selection to increase growth or reproduction, native chickens may have been favourable for the reduction or suppression of inflammatory responses. The importance of genetic background in chicken aids in a strong anti-inflammatory nature, but the supplementation of known immunomodulatory substances such as quercetin or other derivatives could be of great interest in mounting an immune response in poultry.

The increase in pro-inflammatory gene expression (i.e. IL-6 and IL-8) has also been suggested to be associated with the resistance to extraintestinal *S. enteritidis* (Kogut et al. 2003). From our observations, the expressions of IL6, IL17A, and LITAF in Pradus’s heterophils stayed above the basal levels.
even after exposing to quercetin. This finding may imply the process in which cells readily prepare for \textit{S. enteritidis} response by generating a handful of inflammatory mediators. Indeed, quercetin was actively involved in LPS-stimulated inflammation at the early phase of infection. Even though the suppressive properties of quercetin on inflammation are actually exerted, higher level of presenting inflammatory mRNAs could eventually overcome the \textit{in vitro} actions of quercetin. Another study on two Malaysian indigenous chicken breeds extrapolated that iNOS genes may contribute to the resistance to \textit{S. enteritidis} (Tohidi et al. 2012). That finding is also in accordance with our data in term of the increase in NOS2 gene expression in Pradu chickens receiving quercetin, but not in hybrid commercial chickens. Quercetin up-regulated NOS2 gene in Pradu heterophils because the product of this gene, nitric oxide, helped generate an environment that is extremely hostile for the bacterium (\textit{S. enteritidis}).

Many reports clearly demonstrated and have agreed that quercetin interferes with signalling proteins, such as p38, Akt, IκBα, IκBβ, NF-κB, and AP-1, which are mainly involved in the process of signal transduction and gene transcriptions after LPS stimulation in mammals (Chang et al. 2013; Endale et al. 2013; Yang et al. 2014). The bacterial component, LPS, in the current study was a major player in initiating inflammatory signals in this particular assay setting. Nevertheless, quercetin could possibly lessen pro-inflammatory gene expressions via inhibition of key transcription factors (Boesch-Saadatmandi et al. 2011), but the actual signalling pathway in chicken heterophils remained unanswered.

As we were anticipating, quercetin attenuates more than half of studying pro-inflammatory, inflammatory cytokines, and chemokine genes in our assay settings. We asked whether those down-regulated or up-regulated genes from our results are connected to genes within the pathways of inflammation. One way for an in-depth exploration of information is to \textit{in silico} predict a group of genes already intensively studied by others (Kaiser et al. 2005; Szmolka et al. 2015). The results of gene network analysis reveal many annotated genes that envisage the immune response manipulated by quercetin. In conclusion, our data emphasize the modulatory effects of quercetin in controlling pro-inflammatory cytokines, anti-inflammatory cytokine, chemokines, and other genes involved in inflammatory processes. The importance of these findings needs further investigation in terms of exploiting in \textit{in vivo} study.

5. Conclusions

The results of this study emphasized that quercetin could potentially down-regulate pro-inflammatory genes such as \textit{IL1B} along with anti-inflammatory \textit{TGFBI}. Quercetin had also exerted its effects on promoting the expression of pro-inflammatory cytokines such as \textit{IL6} and other chemokines such as \textit{IL8L1, IL8L2, CCL4, IL18, MIF}, but only exerted a slight increase in \textit{IL17A} and \textit{LITAF} in chicken heterophils.

Disclosures statement

No potential conflict of interest was reported by the authors.

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