Flavonoids and other plant substances enhance the immunity of the animal

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Past concept of food:
- survival, hunger satisfaction, and absence of the classical nutrient deficiency diseases

New concept—Functional foods:
- better health and well-being
- reduce the risk of chronic illness and conditions such as cardiovascular diseases, cancers and obesity
Flavonoids as Functional Foods

**Primary Actions:**
- Antioxidation
- Immunomodulation
- Anti-inflammatory
- Antimicrobial

**Secondary Actions:**
- Anticancer effects
- Cardioprotective effects
- Gastrointestinal protective effects
- Production performance
two large groups of flavonoids

Isoflavone (Soy flavonoid):
Occurrence: Soybeans and soy products
Example: Genistein

Flavanones (Citrus flavonoid):
Occurrence: Citrus fruits and tomatoes
Example: Hesperidin
Overview of flavonoids research

- Most studies have been performed in vitro using high concentrations compared with the amounts possibly present in animal tissues or the diet.
- Some animal studies have also been performed, mostly on growth performance, the results varied between experiments and between animals.
Objective

To elucidate the role and mechanisms of action of hesperidin (a member of citrus flavonoids) and genistein (a member of phytoestrogens) in immunity in vivo (broilers)

Exp. 1, Hesperidin, genistein, separately

Exp. 2, Hesperidin, genistein, individual and combined, +/- LPS challenge
Exp. 1, Treatments

- Control 0mg flavonoid/kg feed
- Hesperidin, 2.5, 5, 10, 20 mg/kg feed
- Genistein, 2.5, 5, 10 mg/kg feed

Measurements

- Growth performance, not affected
- Immunity parameters
Anti-inflammatory Parameters

- Prostaglandin E2 (PGE2)
- Leukotriene B4 (LTB4)

Hepatic gene expression
- Cyclooxygenase-2 (COX-2)
- Inducible Nitric Oxide Synthase (iNOS)
Network of inflammation

ARACHIDONIC ACID

- Lipoxygenase
  - Hydroperoxyeicosa tetraenoic acids (HPETEs)
    - O₂
    - H₂O
    - Leukotriene A₄ (LTA₄)

- Cyclo-oxygenase
  - Prostaglandin G
    - Peroxidase
    - Prostaglandin endoperoxide (PGH₂)

- Prostaglandin endoperoxide synthase (PGES)

Leukotrienes (LTB₄, LTC₄)

Prostaglandins (PGD₂, PGE₂, PGF₂α, PGL₂)

Inflammation
Day 21: Elevated PGE2 in GS3 group

Day 42: Hesperidin and Genistein decreased PGE2 except elevation in (GS1) group
**Day 21**: Elevated LTB4 in GS3 group

**Day 42**: Hesperidin and Genistein decreased LTB4 except no change in (GS3) group
Day 21: Hesperidin decreased COX-2 expression
Genistein ↑ expression in GS1 while down regulated in GS3

Day 42: No effect of Hesperidin
Genistein response was reversed from day 21
**Day 21:** low level Hesperitin decrease, high level increase; Elevated iNOS expression in all genistein

**Day 42:** dose-dependent decrease
Immune-modulating effects

- Secretory IgA (sIgA)
- Intraepithelial lymphocytes (IEL)

Gene expression

- Interferon-gamma (IFN-γ)
- Interleukin-10 (IL-10)
- Interleukin-4 (IL-4)
**Day 21:** high level hesperidin ↑ slgA; low level genistein ↑ slgA in GS1, high level ↓

**Day 42:** low level hesperidin ↑ slgA production; all genistein ↓ slgA
Hesperidin: increased IELs on both 21 and 42 days

Genistein: high level GS3 increased IELs
Th1 and Th2 cytokines and Immunity
**Day 21:** low level hesperitin reduced IFN-γ expression

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### Hepatic IFN-γ

#### Day 21

<table>
<thead>
<tr>
<th>HD1</th>
<th>HD2</th>
<th>HD3</th>
<th>HD4</th>
<th>GS1</th>
<th>GS2</th>
<th>GS3</th>
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<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>100</td>
<td>150</td>
<td>a</td>
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Hesperidin supplementation

<table>
<thead>
<tr>
<th>IFN-γ expression (% of control)</th>
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Genistein supplementation

#### Day 42

<table>
<thead>
<tr>
<th>HD1</th>
<th>HD2</th>
<th>HD3</th>
<th>HD4</th>
<th>GS1</th>
<th>GS2</th>
<th>GS3</th>
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<tr>
<td>0</td>
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Hesperidin supplementation

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<th>IFN-γ expression (% of control)</th>
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Genistein supplementation

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**Day 42:** high level hesperidin ↑ expression; high level genistein ↓ in expression
Day 21: Hesperidin ↑ Expression in HD3 group, low level genistein ↑ expression

Day 21

IL-10

Day 42: all hesperidin ↑ expression; high level genistein ↑ expression
All treatments had no effect, except high level hesperidin suppressed IL-4 gene expression at 21 days.
Summary

- Overall, growth was not affected
- Generally, the anti-inflammatory and immunity responses were shifted towards positive direction; however, an increasing trend for iNOS, sIgA and IFN-γ on 21 day and decreasing trend for LTB4, iNOS, COX-2, sIgA and IL-10 on 42 days
Hesperidin suppressed eicosanoid synthesis and modulate immune related gene expression.

Genistein was not efficient immunity enhancer; however, an ↑ sIgA production and ↑ IL-10 mRNA gene expression with low dose of genistein (2.5 mg/kg) was observed on 21 days.
A strong relationship between dietary genistein concentration and COX-2, and IL-10 was observed with decreasing trend on day 21 and increasing trend on day 42 of broiler life.

Overall:
Flavonoids effects were animal age specific, and dose-dependent.
Exp. 2, Treatments

720 birds, 6 groups

<table>
<thead>
<tr>
<th>CON</th>
<th>6 x20=120</th>
<th>Basal diet</th>
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</thead>
<tbody>
<tr>
<td>G5</td>
<td>6 x20=120</td>
<td>Basal diet + 5mg/kg genistein</td>
</tr>
<tr>
<td>H20</td>
<td>6 x20=120</td>
<td>Basal diet + 20mg/kg hesperidin</td>
</tr>
<tr>
<td>*GH5</td>
<td>6 x20=120</td>
<td>Basal diet + 5mg/kg genistein / hesperidin</td>
</tr>
<tr>
<td>*GH10</td>
<td>6 x20=120</td>
<td>Basal diet + 10mg/kg genistein / hesperidin</td>
</tr>
<tr>
<td>*GH20</td>
<td>6 x20=120</td>
<td>Basal diet + 20mg/kg genistein / hesperidin</td>
</tr>
</tbody>
</table>

GH: Genistein/Hesperidin (1:4)
Half numbers of birds LPS challenge on day 16, 18 & 20

Measurements

Growth performance
Antioxidant activity
Immunity parameters
Lipid metabolism
Effect of genistein and hesperidin on growth performance +/- LPS

Body weight gain, g

Diet P = 0.064
LPS P = 0.056
D x LPS P = 0.033

Diet P = 0.074
LPS P = 0.957
D x LPS P = 0.765

- LPS  + LPS

21 day

42 day
Effect of genistein and hesperidin on plasma antioxidant status +/- LPS

**T-AOC, U/ml**

Diet P < 0.001  
LPS  P < 0.001  
D x LPS P = 0.383

Diet P = 0.001  
LPS  P = 0.221  
D x LPS P = 0.553

![Data representation graph](image-url)
Effect of genistein and hesperidin on plasma antioxidant status +/- LPS

MDA, n mol/ml

21 day, Diet P < 0.01  
42 day, Diet P < 0.01

- LPS  + LPS  - LPS  + LPS
Effect of genistein and hesperidin on plasma antioxidant status +/- LPS

SOD, U/ml

21 day, Diet P < 0.01
LPS P < 0.05

42 day, Diet P < 0.01
LPS P < 0.01
Effect of genistein and hesperidin on immune organ indices (relative organ weight) +/- LPS

Combined effect

21 day, No effect

42 day, Diet P < 0.01

Thymus, g/g

- LPS  + LPS  - LPS  + LPS
Effect of genistein and hesperidin on immune organ indices (relative organ weight) +/- LPS

Bursa, g/g

Diet P < 0.01
LPS  P < 0.01
D x LPS P < 0.01

LPS  P < 0.05
Effect of genistein and hesperidin on immune organ indices (relative organ weight) +/- LPS

Spleen, g/g

Diet P < 0.01
LPS P < 0.05
Diet P < 0.05

- LPS
+ LPS

21 day
42 day
Effect of genistein and hesperidin on T lymphocyte transformation rate (TLTR)

Combined effect

Diet $P < 0.001$
LPS $P < 0.001$
Diet $P < 0.001$
Effect of genistein and hesperidin on phagocytic index +/- LPS)

Combined effect

Diet P <0.001
LPS  P <0.001
D x LPS  P <0.001

Dose-dependent
Effect of genistein and hesperidin on intestinal intraepithelial lymphocytes (iIELs)

Duodenum

Diet P <0.001

Diet P <0.001
Effect of genistein and hesperidin on intestinal intraepithelial lymphocytes (iIELs)

jejunum

Number of jejunal iIELs

<table>
<thead>
<tr>
<th>Diet</th>
<th>LPS (-) 21 d</th>
<th>LPS (+) 21 d</th>
<th>LPS (-) 42 d</th>
<th>LPS (+) 42 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H20</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>GH5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Diet P <0.001
LPS  P <0.001

Diet P <0.001
Effect of genistein and hesperidin on neutrophil adhesion rate +/- LPS

Combined effect

Diet P <0.001
LPS P <0.001
Effect of genistein and hesperidin on serum anti-NDV (Newcastle disease virus) antibody titers

21 day, Diet P <0.001
LPS  P <0.001

42 day, Diet P <0.001

- LPS    + LPS
- LPS    + LPS
Effect of genistein and hesperidin on serum anti-AIV (avian influenza virus) antibody titers

21 day, Diet P <0.001

Con - LPS  G5 - LPS  H20 - LPS  GH5 - LPS  GH10 - LPS  GH20 - LPS

42 day, no effect

- LPS  + LPS  - LPS  + LPS  - LPS  + LPS  - LPS  + LPS
Effect of flavonoids on serum lipid profile

- **total cholesterol (CHO)**
- **triglyceride (TG)**
- **high density lipoprotein cholesterol (HDLC)**
- **low density lipoprotein cholesterol (LDLC)**
Effect of flavonoids on tissues total cholesterol (CHO) and triglyceride (TG)
Effect of flavonoids on fatty acid composition of breast muscle in broilers (% total fatty acids)

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>G5</th>
<th>H20</th>
<th>GH5</th>
<th>GH10</th>
<th>GH20</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>ΣSFA</td>
<td>37.06 ± 0.34 a</td>
<td>34.89 ± 0.2 7bc</td>
<td>36.02 ± 0.23 ab</td>
<td>34.89 ± 0.15 bc</td>
<td>35.14 ± 0.39 bc</td>
<td>34.15 ± 0.22 c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ΣMUFA</td>
<td>45.58 ± 1.43 a</td>
<td>43.58 ± 0.7 4ab</td>
<td>43.66 ± 0.81 ab</td>
<td>41.73 ± 0.85 ab</td>
<td>40.65 ± 0.32 b</td>
<td>40.97 ± 0.75 b</td>
<td>0.013</td>
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<tr>
<td>ΣPUFA</td>
<td>22.40 ± 0.23 b</td>
<td>23.99 ± 0.3 ab</td>
<td>23.76 ± 0.76 ab</td>
<td>27.12 ± 0.53 ab</td>
<td>27.88 ± 2.95 ab</td>
<td>30.31 ± 1.72 a</td>
<td>0.018</td>
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<tr>
<td>Σn-6</td>
<td>19.13 ± 0.21 b</td>
<td>20.94 ± 0.3 2ab</td>
<td>21.20 ± 0.68 ab</td>
<td>24.74 ± 0.68 ab</td>
<td>25.35 ± 2.61 ab</td>
<td>27.37 ± 1.60 a</td>
<td>0.006</td>
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<tr>
<td>Σn-3</td>
<td>3.26 ± 0.02a</td>
<td>3.04 ± 0.02 ab</td>
<td>2.56 ± 0.07bc</td>
<td>2.38 ± 0.19c</td>
<td>2.53 ± 0.14bc</td>
<td>2.94 ± 0.17a bc</td>
<td>0.002</td>
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<tr>
<td>n-6/n-3</td>
<td>5.85 ± 0.04c</td>
<td>6.87 ± 0.11bc</td>
<td>8.27 ± 0.05c ab</td>
<td>10.59 ± 1.19a</td>
<td>10.36 ± 1.16a</td>
<td>9.31 ± 0.43a b</td>
<td>0.002</td>
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<tr>
<td>PUFA/SFA</td>
<td>0.604 ± 0.01 b</td>
<td>0.687 ± 0.0 1ab</td>
<td>0.660 ± 0.03 ab</td>
<td>0.780 ± 0.04 ab</td>
<td>0.805 ± 0.10 ab</td>
<td>0.887 ± 0.05 a</td>
<td>0.017</td>
</tr>
</tbody>
</table>
Summary

Genistein and hesperidin could notably improve the antioxidative status.

Both flavonoids seemed to improve the cellular, humoral and mucosal immunity of the animal.

Both compounds seemed to affect lipid metabolism with more health-promoting metabolites.
The effects of genistein and hesperidin were dose-dependent and animal age specific.

Generally, combined effect of genistein and hesperidin was more profound than individuals, especially with LPS challenge, indicating mutual interaction of both compounds.
Thanks for attention!