Immune response to phytonutrients in pigs – antioxidant response

Yanhong Liu
University of California, Davis
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Outline

• Phytonutrients – plant extracts
  • Anti-inflammatory effects
• Weaning stress – oxidative stress
• Antioxidants – plant extracts?
• Overall summary
• Future research
Phytonutrients- plant extracts

• Extracted from parts of plants or synthesized
• Concentrated, hydrophobic, volatile aroma
• Mixtures of secondary plant metabolites
• Liquid or powder
• Phenolic compounds
Anti-inflammatory effects

In vitro

LPS-stimulated porcine alveolar macrophages

Liu et al., 2012
Frequency of diarrhea

Sham

Control vs. plant extracts
$P < 0.05$

E. coli

Control vs. plant extracts
$P < 0.05$

Pig days with diarrhea score $\geq 3$
1, normal; 5, watery diarrhea

Liu et al., 2013
Possible mechanism for reduced diarrhea

Ileal villi height (d 5 PI)

MUC2 in ileal mucosa (d 5 PI)

- Possibly improved gut barrier function!

Liu et al., 2013, 2014
Plant extracts reduced systemic inflammation caused by *E. coli* infection

White blood cell counts

Serum TNF-α

Liu et al., 2013
Plant extracts reduced gut inflammation caused by *E. coli* infection

Liu et al., 2013
Plant extracts reduced gut inflammation caused by \textit{E. coli} infection

\textbf{The Prostaglandin Pathway}

\begin{itemize}
  \item Arachidonic acid
  \item TNF-\(\alpha\)
\end{itemize}

\begin{itemize}
  \item Cyclooxygenase-2 (COX-2)
  \item PGG2 → PGH2
  \item PGG2 → PGG2
  \item TXA2
  \item PGF2\(\alpha\)
  \item PGE2
  \item PGD2
  \item PGI2
\end{itemize}

\textbf{Inflammation!}

\textit{Liu et al., 2014}
Summary

Anti-inflammatory effects

• Suppressed the production of inflammatory mediators in vitro

• Reduced diarrhea and enhanced disease resistance of weaning pigs

• Possible mechanisms
  • Gut barrier function
  • Gut mucosa immunity
  • Systemic immunity
  • Reduced oxidative stress?
Weaning stress

• Maternal separation
• Environmental change
• Increased exposure to pathogens
• Social hierarchy stress
• Move to solid feed
• Transportation stress
Oxidative stress in weaning pigs

- Poor immunity
- Weak intestinal structure
- Other stresses
- Metabolic stress
- Weaning stress
Oxidative stress

• An excessive production of reactive oxygenated species that cannot be counteracted by the action of antioxidants (Pisoschi and Pop, 2015)

• A disturbance in the prooxidant to antioxidant balance in favor of the oxidant species, leading to potential damage (Sies et al., 1991)
Reactive oxygen species (ROS)

- Free radical and non-free radical oxygen molecules
  - Hydrogen peroxide ($\text{H}_2\text{O}_2$)
  - Superoxide ($\text{O}_2^-$)
  - Singlet oxygen ($\text{1/2 O}_2$)
  - Hydroxyl radical ($\cdot\text{OH}$)

McDaniel, 2013
Reactive oxygen species (ROS)

- Internally generated sources
  - Mitochondria
  - Xanthine oxidase
  - Peroxisomes
  - Inflammation
  - Phagocytosis
  - Arachidonate pathways
  - Injury

Valko et al., 2006
Reactive oxygen species (ROS)

• Dual roles in biological system
  • Low concentration - defend against infectious agents
  • High concentration – important mediators of damage to cell structures, including lipids, proteins, and nucleic acids
• Balance is very important!
# Biomarkers for oxidative stress

<table>
<thead>
<tr>
<th>Free radicals acceleration</th>
<th>• $H_2O_2$, NO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antioxidant status</strong></td>
<td>• Tocopherols, ascorbic acid, uric acid</td>
</tr>
<tr>
<td></td>
<td>• Glutathione (GSH and GSSG), etc.</td>
</tr>
<tr>
<td><strong>Antioxidant enzyme activities</strong></td>
<td>• Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), 8-hydroxyl-2-deoxyguanosine (8-OHdG), catalase (CAT), Inhibitory hydroxyl ability (IHA)</td>
</tr>
<tr>
<td><strong>Lipid peroxidation</strong></td>
<td>• Malondialdehyde (MDA)</td>
</tr>
</tbody>
</table>

_Kadiiska et al., 2015_
Systemic oxidative stress caused by weaning

- Increased free radicals in serum
  - $\text{H}_2\text{O}_2$, NO
- Reduced antioxidant enzyme activities in serum
  - GSH-Px, SOD
- Increased lipid peroxidation in serum
  - MDA

Zhu et al., 2013
Oxidative stress in GI tract caused by weaning

- Reduced digestive enzyme activities in jejunum
  - Sucrase, Maltase, Amylase, Lipase
- Increased caspase concentrations in jejunum
  - Caspase-3, caspase-8, caspase-9
- Increased lipid peroxidation and decreased antioxidant enzymes activities

Zhu et al., 2012, 2013
Oxidative stress in liver caused by weaning

- Increased free radicals
  - $\text{H}_2\text{O}_2$, NO
- Increased oxidative injury
  - MDA, 8-OHdG
- Reduced antioxidant enzyme activities
  - GSH-Px, SOD, IHA
- Enhanced hepatic enzyme activities
  - Aspartate aminotransferase (AST), alanine aminotransferase (ALT)

Luo et al., 2016
Oxidative stress in brain

- Increased lipid peroxidation
- Decreased GSH level and GSH/GSSG ratio
- Reduced antioxidant enzyme activities
  - IHA, SOD, GSH-Px, CAT
- Rat data, need verify in pigs

Hong et al., 2016
Antioxidants

- Stable molecules, donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage (Lobo et al., 2016)

http://normsfarms.com/what-are-antioxidants/
Antioxidants – Level 1

Initiator → R· → ROO· → Non-radical products

Initiator → R· → ROO· → Non-radical products

ROOH → RH → ROOH + A·

• Preventive antioxidants
  • Suppress the formation of free radicals; SODs, CAT, GSH-Px

Amorati et al., 2013; Lobo et al., 2016
Antioxidants – Level 2

• Radical-scavenging antioxidants
  • Suppress chain initiation and/or break the chain propagation reactions, such as vitamin C and E

Amorati et al., 2013; Lobo et al., 2016
Antioxidants – Level 3

- Repair antioxidants
  - Remove oxidatively modified proteins, such as proteolytic enzymes

Amorati et al., 2013; Lobo et al., 2016
Type of antioxidants

• **Endogenous antioxidants**
  - Enzymatic antioxidants (*SODs, CAT, GSH-Px*)
  - Non-enzymatic antioxidants (*ascorbic acid, Glutathione, melatonin, vitamin E, uric acid*)

• **Exogenous antioxidants**
  - butylated hydroxytoluene (*BHT*), butylated hydroxyanisole (*BHA*), Se and vitamin E
  - Plant extracts
Plant extracts & antioxidant effects

• Phenolic compounds (carvacrol, thymol, eugenol, etc.)

• Other volatile constituents (e.g., sulfur-containing components of garlic or onions)
# Total phenols content

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Total phenols Gallic acid equivalent (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clove</td>
<td>899</td>
</tr>
<tr>
<td>Thyme</td>
<td>784</td>
</tr>
<tr>
<td>Oregano</td>
<td>764</td>
</tr>
<tr>
<td>Rosemary</td>
<td>225</td>
</tr>
<tr>
<td>Sage</td>
<td>123</td>
</tr>
</tbody>
</table>

Viuda-Martos et al., 2009
Antioxidant activities
DPPH method

IC$_{50}$, mg/mL

Thyme, Oregano, Clove, Sage, Rosemary, Ascorbic acid, BHT

IC$_{50}$: concentration (mg/mL) for a 50% inhibition

Viuda-Martos et al., 2009
Antioxidant activities
TBARS assay

IC\textsubscript{50}, μg/mL

Thyme | Oregano | Clove | Sage | Rosemary | Ascorbic acid | BHT
---|---|---|---|---|---|---
80 | 20 | 10 | 40 | 60 | 1 | 0

IC\textsubscript{50}: concentration (μg/mL) for a 50% inhibition

Viuda-Martos et al., 2009
Antioxidant activities
Ferrous ion-chelating (FIC) assay

EC₅₀, mg/mL

Thyme  Oregano  Clove  Sage  Rosemary  Ascorbic acid  BHT

EC₅₀: concentration (μg/mL) for a 50% chelating effect

Viuda-Martos et al., 2009
Antioxidant activities
FRAP (Ferric reducing antioxidant power) assay

TEAC, μM Trolox/mL

- Clove
- Ascorbic acid
- BHT
- Oregano
- Thyme
- Rosemary
- Sage

TEAC: Trolox equivalent antioxidant capacity

Viuda-Martos et al., 2009
In vitro antioxidant effects summary

- **DPPH**: Clove > Thyme > Oregano > Sage > Rosemary
- **TBARS**: Oregano > Clove > Thyme > Sage > Rosemary
- **FRAP**: Clove > Oregano > Thyme > Rosemary > Sage
- **FIC**: Rosemary > Sage > Thyme > Clove > Oregano

• Results obtained from different in vitro methods are variable

*Viuda-Martos et al., 2009*
In vitro methods for antioxidant activities summary

- Chemical-based antioxidant activity

**Pros**: simple and fast

**Cons**: not consider certain parameters in complex cell environments; mechanisms of antioxidants are not only by scavenging free radicals
Lipid peroxidation assay

- **Lipid peroxidation**: the oxidative degradation of lipids. In this process, free radicals take electrons from the lipids, resulting in cell damage
- Sensitively detect the concentration of MDA present in a variety of samples (liver and brain)
- One of most widely accepted assays for oxidative damage
Cellular antioxidant activity

- Very attractive testing method to support antioxidant research prior to animal studies
- Shows high physiological quality in antioxidant measurements
- Applied to product extracts, foods, dietary supplements
- Cheaper compared with animal studies
In vivo animal trials

• Highly recommended!
  • Dose effects
  • Mechanisms of action
  • Different stress conditions
Overall summary

• Reducing oxidative stress should be taken into account to promote pig health and production, especially in weaning stage

• Anti-inflammatory effects of plant extracts have been confirmed both in vitro and in vivo

• Plant extracts are potential antioxidants that can be added to animal feed
Future research

• Correlations between chemical-based methods, lipid peroxidation assay, and cellular antioxidant assay should be conducted to provide theoretical guidance in rationally screening anti-oxidant components

• More research are needed to verify the antioxidant activities of plant extracts supplemented to animal feed
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http://animalnutr-ansci.faculty.ucdavis.edu/