Soy Bioactives Fed to Disease-Challenged Pigs

Ryan N. Dilger, PhD
University of Illinois at Urbana-Champaign
Department of Animal Sciences
Division of Nutritional Sciences

World Pork Expo, June 7, 2017
• 30 MMT domestic use of SBM in livestock feed annually
• 85% of global soybean crop processed into oil and meal
Nutritional Value of SBM

- High-quality protein (47% CP) with amino acid patterns that complement cereal grains
- Lower fiber and higher energy value compared with other oilseeds

Source: United Soybean Board
Soy Isoflavones (ISF)

- Phenylpropanoid-derived compounds that have estrogen-like activity

Genistein
~50% of total soy ISF

Daidzein
~40% of total soy ISF

Glycitein
~10% of total soy ISF

Equol
microbially-derived from daidzein
Metabolism of Soy Isoflavones

• Bioactivity: equol > genistein > daidzein
• Only 30% of adults can produce equol
• Circulating ISF profiles differ between species

* J. Nutr. 136:1215
ISF and Immune Benefits

• ISF known to modulate cells associated with intestinal permeability and immune function

• ISF have 1,000-fold lower potency compared with estrogen, but evidence suggests ISF can exert anti-inflammatory and anti-viral effects

What does all this mean for animal ag?
What is Immunity?

• Integrated set of mechanisms designed to counteract homeostatic disturbances and preserve integrity of the host

• Immune system judges danger by:
  – Location
  – Looks
  – Damage
Components of the Immune System

**Innate Immunity**
- Epithelial barriers
- Phagocytes
- Dendritic cells
- Plasma proteins
- NK cells

**Adaptive Immunity**
- Naive B cell
- Antibodies
- Naive T cell
- Effector T cells

**Timeline**
- Hours: 0, 6, 12
- Days: 1, 3, 5
- Time after infection: 0 – 5 days
Cost of Immunity

Goal is to minimize the frequency, duration, and magnitude of immune response.
Systemic Immune Signaling

• **Proinflammatory cytokines**, hormones, neural inputs

• Repartitioning of nutrients *away* from productive efforts and *toward* immune function using key intracellular signaling pathways

• Most profound effects of:
  – Interleukin-1β (*IL-1β*)
  – Tumor necrosis factor-α (*TNF-α*)
  – Interleukin-6 (*IL-6*)
PRRSV and Swine Production

- Porcine reproductive and respiratory syndrome virus (PRRSV) is highly prevalent in the swine industry
- Affinity for alveolar macrophages
- Disease manifests as reduced growth performance, with annual production losses in growing pigs estimated at $664 million
- Nutritional intervention may be helpful in the management of the PRRSV

1. Benefits of feeding purified isoflavones (ISF) to PRRSV-infected pigs (Greiner et al., 2001)

2. Benefits of higher SBM concentrations recognized in field studies (Johnston et al., 2010)

3. Benefits of higher SBM confirmed under disease containment settings using PRRSV-infected pigs (Rochell et al., 2015)
Previous Study Design

Uninfected

LSBM  →  HSBM

Infected

LSBM  →  HSBM

1Pigs intranasally-inoculated with $1 \times 10^5$ 50% tissue culture infective dose of PRRSV

1Pigs weaned @ 21 d

Start dietary treatments

Inoculation

End @ 49 d
Previous Diet Formulation

<table>
<thead>
<tr>
<th>Ingredients, %</th>
<th>LSBM</th>
<th>HSBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>46.13</td>
<td>35.57</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>17.50</td>
<td>29.00</td>
</tr>
<tr>
<td>Dried whey</td>
<td>14.95</td>
<td>14.95</td>
</tr>
<tr>
<td>DDGS</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Poultry by-product meal</td>
<td>7.00</td>
<td>7.00</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.60</td>
<td>0.24</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.27</td>
<td>0.16</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>0.08</td>
<td>0.03</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.15</td>
<td>-</td>
</tr>
<tr>
<td>L-Valine</td>
<td>0.10</td>
<td>-</td>
</tr>
<tr>
<td>Other(^1)</td>
<td>3.22</td>
<td>3.07</td>
</tr>
</tbody>
</table>

\^1Other includes choice white grease, vitamins, minerals, and choline chloride

- Diets were isocaloric and contained equal SID Lys, Met, Trp, Thr, and Val

- Analyzed CP content:
  - LSBM: 22.8%
  - HSBM: 26.7%

- Total ISF content:
  - LSBM: 700 mg/kg
  - HSBM: 1,246 mg/kg
Summary of Previous Research

• Overall ADG of pigs fed HSBM tended to be greater during the 2-week post-inoculation period.

• Pigs fed HSBM exhibited decreased haptoglobin at 3 DPI, decreased serum PRRSV load at 14 DPI, and decreased serum TNF-α concentrations.

• Remained unclear whether effects of added SBM were due to ISF, amino acids, or other individual or combined factors.
Recent Study

• **Hypothesis**: Consumption of dietary soy ISF will elicit immune benefits in PRRSV-infected weanling pigs

• **Objective**: To quantify the effects of soy ISF on growth, clinical, and immune outcomes in pigs challenged with an acute PRRSV infection
Soy-derived Ingredients

• Two sources of refined soy protein
  1. **Soy protein concentrate, control** (Arcon® AF; ADM)
  2. **Value-added soy product** (HP 300; Hamlet Protein)

• **Soy ISF concentrate** (NovaSoy® 400; ADM)
  (ratio of ISF mixture equivalent to that in whole soybeans)

### Analyzed ISF Content of Ingredients (mg/kg)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Arcon® AF</th>
<th>HP 300</th>
<th>NovaSoy® 400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genistein</td>
<td>0</td>
<td>908</td>
<td>177,317</td>
</tr>
<tr>
<td>Daidzein</td>
<td>0</td>
<td>1,314</td>
<td>201,902</td>
</tr>
<tr>
<td>Glycitein</td>
<td>0</td>
<td>196</td>
<td>22,983</td>
</tr>
<tr>
<td><strong>Total ISF</strong></td>
<td>0</td>
<td>2,417</td>
<td>402,203</td>
</tr>
</tbody>
</table>
Study Design

Uninfected
- Control
  - No ISF

PRRSV-infected
- Control
  - No ISF
  - ISF
- HP 300
  - No ISF
  - ISF

-7 DPI
Pigs weaned @ 21 d of age

0 DPI
Inoculation*

14 DPI
End @ 42 d of age

DPI = days post-inoculation

*Pigs intranasally-inoculated with $9.3 \times 10^3$ 50% tissue culture infective dose of PRRSV
### Diet Formulation

<table>
<thead>
<tr>
<th>Ingredient, %</th>
<th>Control</th>
<th>HP 300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>62.32</td>
<td>59.08</td>
</tr>
<tr>
<td><strong>Soy source</strong></td>
<td><strong>17.50</strong></td>
<td><strong>20.80</strong></td>
</tr>
<tr>
<td>Dried whey</td>
<td>12.00</td>
<td>12.00</td>
</tr>
<tr>
<td>PBM(^1)</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Other(^2)</td>
<td>4.18</td>
<td>4.12</td>
</tr>
</tbody>
</table>

**Calculated composition**

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Control kcal/kg</th>
<th>HP 300 kcal/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME, kcal/kg</td>
<td>3,472</td>
<td>3,465</td>
</tr>
<tr>
<td>CP, %</td>
<td>21.3</td>
<td>21.3</td>
</tr>
<tr>
<td>SID Lys</td>
<td>1.34</td>
<td>1.34</td>
</tr>
<tr>
<td>SID Met + Cys</td>
<td>0.73</td>
<td>0.73</td>
</tr>
<tr>
<td>SID Trp</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td>SID Thr</td>
<td>0.86</td>
<td>0.86</td>
</tr>
<tr>
<td>SID Val</td>
<td>0.88</td>
<td>0.88</td>
</tr>
</tbody>
</table>

- Diets formulated on an equal SID basis using values provided by ingredient manufacturers
- Diets were pelleted (1/8” diameter) and fed in a single phase during the 21-d study
- NovaSoy\(^\circ\) addition: 0.34-0.41%

\(^1\)Poultry by-product meal
\(^2\)Other includes choice white grease, crystalline AA, vitamins, minerals, and choline chloride
<table>
<thead>
<tr>
<th>Item</th>
<th>ISF</th>
<th>Control</th>
<th></th>
<th>HP 300</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>CP, %</td>
<td></td>
<td>21.3</td>
<td>21.3</td>
<td>21.3</td>
<td>21.3</td>
</tr>
<tr>
<td>Lactose, %</td>
<td></td>
<td>8.8</td>
<td>8.8</td>
<td>8.8</td>
<td>8.8</td>
</tr>
<tr>
<td>TDF, %</td>
<td></td>
<td>11.9</td>
<td>11.8</td>
<td>11.8</td>
<td>11.8</td>
</tr>
<tr>
<td>ISF, mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genistein</td>
<td></td>
<td>52</td>
<td>757</td>
<td>291</td>
<td>947</td>
</tr>
<tr>
<td>Daidzein</td>
<td></td>
<td>0</td>
<td>625</td>
<td>358</td>
<td>969</td>
</tr>
<tr>
<td>Glycitein</td>
<td></td>
<td>34</td>
<td>69</td>
<td>48</td>
<td>125</td>
</tr>
</tbody>
</table>

**Total ISF**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>HP 300</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>87</td>
<td>1,451</td>
<td>697</td>
<td>2,041</td>
</tr>
</tbody>
</table>
Experimental Outcomes

1. Growth performance (-7, 0, 7, 14 DPI)
2. Rectal temps (0, 3, 6, 8, 12, and 14 DPI)
3. Blood/serum (0, 3, 6, 8, 12, and 14 DPI)
   - RBC measurements and WBC counts w/ differential, and PRRSV load (0, 3, 6, and 14 DPI)
   - PBMC isolation for immunophenotyping (12 DPI)
Successful infection (0.43°C ↑ in rectal temps), but no growth performance differences due to diet

*Difference ($P < 0.05$) between uninfected and infected groups fed the control diet
Serum PRRSV Load

Lighter bars = no suppl. ISF; darker bars = suppl. ISF

Differential effects of ISF at early time-points, and no treatment differences at 14 DPI

*ab* Bars without a common superscript letter differ (*P* < 0.05)

Higher Ct value = *lower* concentration of virus

All uninfected pigs tested negative for PRRSV (Ct > 35) at all time-points
Clinical Outcomes

**Hematocrit**

- **P-values**
  - DPI: < 0.001
  - Soy source: 0.87
  - ISF: 0.008
  - Interaction: 0.75

- Days Post-Inoculation

**Neutrophils**

- **P-values**
  - DPI: < 0.001
  - Soy source: 1.00
  - ISF: 0.05
  - Interaction: 0.22

Lighter bars = no suppl. ISF; darker bars = suppl. ISF

**ISF elicit benefits in RBC and WBC proportions**

Uninfected pigs exhibited no change in hematocrit and reduced neutrophil fractions throughout the study, indicating stable health status post-weaning.

*Difference (P < 0.05) between uninfected and infected groups fed the control diet*
Peripheral blood mononuclear cells (PBMC) fraction contains immune cells that are involved in both humoral and cell-mediated immunity. These cells exhibit both effector and regulatory roles.

<table>
<thead>
<tr>
<th>Type</th>
<th>Surface Marker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (total)</td>
<td>CD3</td>
</tr>
<tr>
<td>Helper TC</td>
<td>CD3/CD4</td>
</tr>
<tr>
<td>Cytotoxic TC</td>
<td>CD3/CD8</td>
</tr>
<tr>
<td>Memory TC</td>
<td>CD3/CD4/CD8</td>
</tr>
</tbody>
</table>
**Immunophenotyping**

Flow cytometer used to count cells expressing cell surface markers

<table>
<thead>
<tr>
<th>Type</th>
<th># Cells</th>
<th>% of TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total TC</td>
<td>31,743</td>
<td>100.0</td>
</tr>
<tr>
<td>Helper TC</td>
<td>10,679</td>
<td>33.6</td>
</tr>
<tr>
<td>Cytotoxic TC</td>
<td>5,578</td>
<td>17.6</td>
</tr>
<tr>
<td>Memory TC</td>
<td>1,844</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Technique permits quantification of differentiated and activated immune cell fractions
Treatment Effects on T Cells at 12 DPI

No change in total T cell numbers or proportion of memory T cells due to experimental treatment.

Cytotoxic T Cells  
**CD3⁺/CD8⁺**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control HP 300</th>
<th>PRRSV-infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Infected</td>
<td>20</td>
<td>30</td>
</tr>
</tbody>
</table>

- **Soy source:** 0.53
- **ISF:** 0.46
- **Interaction:** 0.51

*Difference (P < 0.05) between uninfected and infected groups fed the control diet.

T Helper Cells  
**CD3⁺/CD4⁺**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control HP 300</th>
<th>PRRSV-infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Infected</td>
<td>15</td>
<td>30</td>
</tr>
</tbody>
</table>

- **Soy source:** 0.78
- **ISF:** 0.01
- **Interaction:** 0.20

30% increase in proportion of T helper cells in pigs receiving supplemental ISF suggests benefits to adaptive immunity.

Lighter bars = no suppl. ISF; darker bars = suppl. ISF.
Conclusions

• Differential response of serum PRRSV load to ISF and soy source may be partly explained by an increase in helper T cells, thereby suggesting involvement of the adaptive immune response in disease progression

• $T_h^1$ vs. $T_h^2$ skew

Innate immunity cytokine profile shapes differentiation pattern of helper T cells
Upcoming Study

- Control vs. ISF-supplemented diets
- Group-housed pigs raised to market weight
- Quantify growth performance, duration of PRRSV shedding, development/efficiency of adaptive immunity, and carcass characteristics
Questions?

Acknowledgements

- Mark Berhow, USDA ARS
- Brooke Smith (PhD student)
- Many grad students

Research Support

- United Soybean Board
- Hamlet Protein
- ADM

Ryan N. Dilger, PhD
rdilger2@illinois.edu
Ph: (217) 333-2006
go.illinois.edu/RNDilgerLab
Estrogen Exposure

Estrogen equivalents per 3 oz. serving

<table>
<thead>
<tr>
<th>Category</th>
<th>Estrogen produced per day, ng</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepubertal girl</td>
<td>54,000</td>
</tr>
<tr>
<td>Adult man</td>
<td>136,000</td>
</tr>
<tr>
<td>Adult woman</td>
<td>630,000</td>
</tr>
<tr>
<td>Pregnant woman</td>
<td>19,600,000</td>
</tr>
</tbody>
</table>