Nutritional strategies to improve gut health status in farm animals

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Agenda

• Immune activation and alteration of digestive physiology
• Plasma amino acids during challenge
• Fate of undigested CP/AA
• Amino acid digestibility during enteric disease
• Dietary interventions
• Conclusions
Factors affecting AA digestibility

**Endogenous Factors**
- Species
- Breed/strain
- Gender
- Physiological state

**Exogenous Factors**
- Pathogens and disease?
- Stress?
- Anti-nutritional factors
- Dietary AA balance
Dynamic changes in AA digestibility in *Salmonella* challenged pigs

Lee, 2012
Anti-nutritional effect of fermentable protein in the hindgut

Indigested protein flow in hindgut

Protein fermentation

Low capacity to digest protein

Undesirable bacteria

Intestinal lesions, inflammation

Pathogenic bacteria

Lactic and bifido bacteria

Growth depression

Fermentation metabolites, bacteriotoxins

BC-VFA, Amines, Ammonia, p-cresol, indole, skatole, High pH

Scouring, mortality & morbidity

E. coli, Chlostridia, Salmonella, Campylobacter, etc.
Fermentation of AA in the hindgut

Phe $\rightarrow$ Tyr

Trp $\rightarrow$ Indol acetate

Bacterial fermentation $p$-cresol

Associated with chronic diseases in humans

Meijers y Evenepoel 2011
Bacterial protein fermentation metabolites reduce pig growth

Piglets of 4 weeks of age, consuming the same diet, all in good health.

Piglets with the LOWEST protein fermentation have the BEST growth

Piglets with the HIGHEST protein fermentation have the LOWEST growth

Piglets of 4 weeks of age, consuming the same diet, all in good health.

Yokoyama et al., 1982
Cycle of poor gut health

Poor Gut Health

- Digestive Failure
- Excess Nutrients to Hindgut
- Barrier Failure & Acute Phase Response
- Inflammation & Oxidative Stress
Immune activation

• Inflammatory cytokines
  – TNF-α, IL-1β, IL-6
• Induce fever
• Reduce feed intake
• Stimulate
  – Skeletal muscle proteolysis
  – Liver protein synthesis
  • Produce acute phase proteins

Kawai and Akira, 2010
Protein synthesis and accretion in immune challenged pigs

Orellana et al., 2002; Escobar et al., 2004

* $P < 0.05$

Days after PRRSV inoculation

Protein accretion, g/d DM basis

Control

PRRSV

* $P < 0.002$

Protein Synthesis (%/day)

Control

LPS

* $P < 0.05$

LD

Gastrocnemius

Orellana et al., 2002; Escobar et al., 2004
Immune activation and metabolism

Pathogens → Immune cells → Cytokines → Chronic inflammation

Muscle → Liver → CNS → Lymphatic → Other tissues

ROS → IkB → NF-κB → cytokine mRNA

AA
Changes in plasma amino acids with LPS in fed pigs

Price, 2011
Negative AA balance: SAL > LPS

AA balance, µmole/kg BW/ h

Negative for the entire trial

* P < 0.04

Price, 2011
Positive AA balance: LPS > SAL

* $P < 0.04$

Positive for the entire trial

Price, 2011
Changes in plasma Phe with LPS in fed pigs

Less AA absorption means more AA flow to the hindgut

Price, 2011
Cellular events in enteritis

- Poor digestive and absorptive capacity
- Local Inflammation:
  - Influx of phagocytes, hyperemia
  - Oxidative burst: iNOS $\rightarrow$ oxidative stress
  - Loss of osmotic balance, secretion of fluid
- Oxidative and osmotic stress $\rightarrow$ gut barrier failure
- Dissemination of endotoxin, antigenic macromolecules and opportunistic pathogens
- Systemic innate immune response
  - Acute phase proteins
  - Muscle catabolism
Digestibility vs. bioavailability

Intake → Absorption → Digestibility → Metabolism → Use for growth, etc. → Bioavailability

To feces → Bioavailability

van de Waterbeem and Gifford, 2003
Dysbacteriosis: failure to maintain a stable microbiota

• A shift in gut micro flora in favor of organisms that do not normally predominate (Schoorel et al, 1980).
  – First associated in with AGP withdrawal in EU
  – Also referred to as small intestinal bacterial overgrowth (SIBO)

• Symptoms:
  – Decreased feed intake, diarrhea, undigested feed, wet litter
  – Reduced performance but little mortality
  – Thinning and ballooning of the SI & watery, viscous digesta, sticky droppings
  – Altered mucous production, epithelial cell (and some liver), necrosis
  – Bacteria exist naturally in colon or cecum, something triggers overgrowth

• Clostridium overgrowth is a form of dysbacteriosis
Overgrowth of *Clostridium perfringens*

- **Subclinical Necrotic Enteritis**
- **Gangrenous Dermatitis**
- **Hemorrhagic Bowel Syndrome- HBS**
Dysbacteriosis
Other Signs of Poor Gut Health

- Wet-Caked Litter
- Foot Pad Lesions
- Loss of Pigmentation
- Synovitis
- Poor Uniformity
- Dirty Eggs
Enteric diseases: colitis

- Inflammation of large intestine
- Grower diarrhea (12-40 kg)
- Infection:
  - Bacterial, viral, parasite
  - Non-specific (Thomson, 2009)
  - NSP, undigested protein
- Rapid transit, overeating
  - Reduced absorption due to inflammation.
  - AGP removal, pellet vs. mash

Mild, no weight loss

Severe, with weight loss
Effect of Age on AA Digestibility

Lower AA digestibility in younger broilers compared to roosters

García et al., 2007
Swine: Age and AA digestibility
Soybean meal, 48% CP

Young pigs have lower AA SID
- AA SID < 20 kg BW
- AA SID > 20 kg BW

Pedersen et al., 2015
Protease deficiency in weaned pigs

↑ Flow of indigestible protein to the hindgut

↓ Digestible amino acids supply

Lindemann et al., 1986
Enteric diseases: piglet diarrhea

- Also known as diarrhea or enteritis
- Occurs 0-4 wks, most common 3-5 days post weaning (PWD)
- Bacterial, viral, parasites, etc.
  - Growth of opportunistic bacteria
  - Intestinal damage due to infection
- Predisposing nutritional conditions
  - Lack of feed intake at weaning, inflammation
  - Underdigestion and increased nutrients in the undeveloped hindgut

- Watery feces
- Gray-brown color
- Undigested feed
The balancing act of dietary crude protein

Crude protein, % diet

ADG (g/d)

E. coli (CFU/g digesta)

ADG 0-21d

Ileal E. coli

Nyachoti et al, 2006
The role of a protease

- Exogenous proteases
- Complements endogenous proteases
- Hydrolyzes
  - Proteins of vegetable
  - Proteins of animal origin
  - Allergenic proteins
  - Anti-nutritional factors
- Improves amino acid digestibility
Protease increased growth rate and feed efficiency

Piglets supplemented with protease grew faster and more efficiently compared to no enzyme supplementation

Wang et al., 2011
Protease increases nutrient digestibility

Main effects
Protein level: $P=0.45-0.56$
Protease: $P<0.01$

Wang et al., 2011
Protein digestibility at various points along the small intestine with and without a protease

Liu et al., 2013
Fermentable protein as an antinutritional factor

Dietary proteins: The sooner they are digested, the least chance to become an ANF.
Protease improves the intestinal environment

Ammonia nitrogen in cecum (mg/L)
- Main effects
  - Protein level: $P<0.01$
  - Protease: $P<0.01$

Protease reduces protein fermentation and hindgut pH.

Wang et al., 2011

Cecum pH
- Main effect
  - Protease: $P<0.14$
Protease improves the intestinal environment

Protease limits growth of undesirable bacteria and enhances growth of beneficial bacterial species.

Wang et al., 2011
# Broiler: Protease Modifies Microbiota

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Species Richness</th>
<th>Species Evenness</th>
<th>Shannon-Wiener Index (H’)</th>
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<tbody>
<tr>
<td>Protease</td>
<td>59.500 A</td>
<td>0.639 A</td>
<td>2.607 A</td>
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<tr>
<td>Neg Ctrl</td>
<td>48.750 AB</td>
<td>0.385 B</td>
<td>1.500 B</td>
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<tr>
<td>Pos Ctrl</td>
<td>36.750 B</td>
<td>0.327 B</td>
<td>1.193 B</td>
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<tr>
<td>P-value</td>
<td>0.0557</td>
<td>0.0255</td>
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<td>SEM</td>
<td>5.658</td>
<td>0.07</td>
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**Bacterial 16S rRNA sequencing analysis**

*Species richness:* total number of species within a habitat or community.

*Species evenness:* relative contribution of each species to total number of individuals in a community.

*Species diversity:* a measure of both richness and evenness

Escobar, et al., 2016
Protease improves fecal score

Main effects
CP: $P=0.01$ from 8 to 21 d
Protease: $P<0.01$ at all ages

The use of protease in a high protein diet allows to obtain the same fecal score than in the low protein diet

Wang et al., 2011
Protease reduced 14-day broiler mortality irrespective of diet

P value:
Nutrients: 0.17; Protease: 0.029; Nutrients x Protease: 0.93
PBM, *C. perfringens*, and barrier function in broilers

Protease reduced *C. perfringens* and improved gut barrier function.
Trypsin Inhibitor and urease in SBM samples from Brazil - 2015

Novus Lab, 2015.
TI in SBM collected in various countries around the world

Novus, 2016
Trypsin Inhibitor relationship between (TI) AA digestibility in vivo

### Soybean Meal 46% CP

<table>
<thead>
<tr>
<th></th>
<th>mg/g</th>
<th>0.5</th>
<th>1.5</th>
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<tr>
<td>T. l.</td>
<td>kg/ton.</td>
<td>total</td>
<td>dig.94%</td>
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<td>Lis.</td>
<td>28,67</td>
<td>26,95</td>
<td>24,08</td>
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<tr>
<td>Met.</td>
<td>6,72</td>
<td>6,32</td>
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<tr>
<td>TSAA</td>
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<td>12,97</td>
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<tr>
<td>Tre.</td>
<td>18,04</td>
<td>16,96</td>
<td>15,15</td>
</tr>
<tr>
<td>Trip.</td>
<td>6,29</td>
<td>5,91</td>
<td>5,28</td>
</tr>
</tbody>
</table>

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Summary

• Enteric disease
  – Reduce nutrient digestion and absorption
  – Increase nutrient flow to the hindgut
  – Increases bacterial fermentation
  – Alters in gut microbiota

• Undigested nutrients have similar effects
Nutritional strategies to improve gut health status in farm animals

• Feed the host and the microbiota
• Minimize protein fermentation in hindgut
  – Formulate for lower dietary indigestible protein
  – Use crystalline amino acid supplementation to balance dietary AA profile
  – Use exogenous protease
• Monitor ingredients for anti-nutrients content
  – TI, phytate, NSP, toxins, allergens, etc.
• Include fermentable carbohydrate for hindgut
  – Prebiotics: chicory/inulin, cassava, MOS, FOS, etc.
• Consider probiotic or symbiotic supplementation
• Reduce oxidative stress – dietary AOX
Thank You
Obrigado
Merci
감사합니다
Danke Schoen!
Đảm ơn lắm
Gracias
Danke
Thank You
Asante sana
Cảm ơn lắm