Dietary phytonutrients as alternatives-to-antibiotics in agricultural animals: Mode of action in modulating cross-talks among immunity, disease resistance and gut microbiota

LILLEHOJ, HYUN
ANIMAL BIOSCIENCES AND BIOTECHNOLOGY LABORATORY
BELTSVILLE AGRICULTURAL RESEARCH CENTER,
U.S. DEPARTMENT OF AGRICULTURE, AGRICULTURAL RESEARCH SERVICE
BELTSVILLE, MD, USA

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Speaker(s)</th>
<th>Institution(s)</th>
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<tbody>
<tr>
<td>09:00-09:30</td>
<td>Dietary phytonutrients as alternatives-to-antibiotics in agricultural animals: Mode of action in modulating cross-talks among immunity, disease resistance and gut microbiota</td>
<td>Hyun Lillehoj</td>
<td>Animal Biosciences and Biotechnology Laboratory, Agricultural Research Service, USDA, Beltsville, Maryland, USA</td>
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<tr>
<td>09:30-10:00</td>
<td>Dietary phytonutrients enhance disease resistance in swine</td>
<td>Yanhong Liu</td>
<td>University of California, Davis, USA</td>
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<td>10:00-10:30</td>
<td>Phytoneutrients as alternative feeding strategy to improve performance of cattle without using an antibiotics</td>
<td>Sergio Calsamiglia</td>
<td>Animal Nutrition and Welfare Service, Universitat Autónoma de Barcelona, Spain</td>
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<tr>
<td>10:30-11:00</td>
<td><strong>COFFEE BREAK: POSTER SESSION</strong></td>
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<td>11:00-11:30</td>
<td>Impact of dietary tannins on rumen microbiota in bovine</td>
<td>Mariano E. Fernández Miyakawa</td>
<td>Instituto de Patobiología, Centro Nacional de Investigaciones Agropecuarias, Instituto Nacional de Tecnología Agropecuaria, Buenos Aires, Argentina</td>
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<td>11:30-12:00</td>
<td>Meta-analysis of broiler research shows that VariumTM results in feed efficiency equal to antibiotics</td>
<td>Fang Chi</td>
<td>AMLAN International, Chicago, USA</td>
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<td>12:00-12:45</td>
<td><strong>Session 3 Expert Panel</strong></td>
<td>Hyun Lillehoj</td>
<td>Agricultural Research Service, USDA, USA</td>
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<td>Yanhong Liu</td>
<td>University of California, USA</td>
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<td>Sergio Calsamiglia</td>
<td>Universitat Autònoma de Barcelona, Spain</td>
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<td>Ron Cravens</td>
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<td>Mariano E. Fernández Miyakawa</td>
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<td>Jamie G. Nickerson</td>
<td>Aviagen inc., USA</td>
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<td>12:45-14:00</td>
<td><strong>LUNCH: POSTER SESSION</strong></td>
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</table>
Consumers want a food system that assures food is safe, affordable, healthful and sustainable

NO ANTIBIOTICS ever

1. zero added antibiotics
   no human use, no animal use, no ionophores
2. no exceptions
   create a sustainable supply chain for every customer, every where, every day
3. verified and certified partner with the USDA for accountability

NO ANIMAL ANTIBIOTICS

Antibiotics are not always the answer
Antibiotics as growth promoters: Many different types of pathogens infect chickens during their lifetime. Use of antibiotics is increasing.

Record-High Antibiotic Sales for Meat and Poultry Production

Antibiotic overuse is breeding new resistant strains of bacteria that infect people. But industrial farms haven’t gotten the message.

In 2011, 29.9 million pounds of antibiotics were sold in the United States for meat and poultry production.

3.9 times greater

Yet, in the same period, only 7.7 million pounds of antibiotics were sold to treat sick people in the United States.

Enzymes
Organic acid
Probiotics, Prebiotics
Phytogenics
Antimicrobial growth-promoters
Antioxidants
Coccidiostats
Mycotoxin binders
Minerals/vitamins
Signs of poor gut health

Dibner, J. 2010, Int. Coccidiosis Conf. 10, Guangdong, China
Sensing and sensory-linked effectors in the gut
Neuronal, endocrine and immune

Furness, JB, 2013: Nature Reviews Gastroenterology/Hepatology
Why do we want healthy gut?

- **Structural Intestinal Integrity**
  - Effective Digestion and Absorption of Food

- **Balanced and Stable Intestinal Microbiota**

- **Balanced Immune Response**
  - Absence of GI Illness
Dysbiosis and subclinical enteritis

- Inflammation and acute phase immune response
- Oxidative and osmotic dysfunction
- Gut barrier dysfunction
- Impaired nutrient synthesis (vitamins, minerals, SCFA)/impaired energy metabolism
Mechanisms of Antibiotic Growth Promoters (AGP)

1. Anti-microbial
2. Reduce subclinical infections
3. Reduce microbial use of nutrients
4. Reduce growth-depressing microbial metabolites
5. Enhance uptake of nutrients due to histologically thinner gut in antibiotic-treated animals
6. Reduce pro-inflammatory cytokines/chemokines
7. In germ-free mice, antibiotics increase in adiposity
8. In human newborns, antibiotics is associated with obesity

Low-level effect of antibiotic growth promoters on body weight gains in broilers

(Oh, ST, and Lillehoj, HS. 2017)
Beneficial effects of antibiotic alternatives

- Improve microbial flora
- Affect GI mucosa
- Stimulates enzyme secretion
- Growth/Reproduction
- Competitive exclusion/Enteric pathogen
- Mucosal protection: Innate and adaptive immunity
- Nutrient digestion
- Energy efficiency
- Maintenance
**Table 1: Infections where new or improved vaccines would significantly reduce the need for antibiotic use in chickens**

<table>
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<tr>
<th>Key syndrome</th>
<th>Primary pathogen(s) (disease)</th>
<th>Antibiotic use</th>
<th>Commercial vaccine exists</th>
<th>Major constraints to use of vaccine / vaccine development</th>
<th>Vaccine research priority</th>
</tr>
</thead>
</table>
| Systemic (Broilers)   | *Escherichia coli* (Yolk sac infection, airsacculitis, cellulitis)                               | High           | Yes                      | • Omphalitis: secondary bacterial infection – not a disease one can immunize against  
  • Strain coverage limited  
  • Airsacculitis, cellulitis: vaccines available, e.g. live aerosol vaccine. However, Serotype coverage limited and field efficacy variable | High                     |
| Systemic (Broilers, Breeder, Layers) | *Escherichia coli* (airsacculitis, cellulitis, salpingitis and portonitis) | High           | Yes                      | • Strain coverage limited                                                                                               | High                     |
| Enteric (Broilers, Breeder, Layers) | *Clostridium perfringens*, type A (necrotic enteritis)                                         | High           | Yes                      | • Toxoid vaccine for layers providing only short-lasting passive immunity  
  • Research needed to achieve active immunity  
  • Improved and/or more convenient (mass vaccination) vaccine needed for broilers                                           | High                     |
| Enteric (Broilers, Breeder, Layers) | *Coccidiosis* (secondary bacterial infections)                                                 | High           | Yes                      | • Lack of cross-protection  
  • Strains must be matched to infectious agent  
  • Current vaccines are not attenuated and can produce low dose infection  
  • Sub-unit vaccines have not been successful                                                                                     | High                     |
| Enteric (Broilers, Breeder, Layers) | *Infectious Bronchitis virus* (secondary bacterial infections)                                | Medium         | Yes                      | • Issues with strain matching and strain coverage  
  • High mutation rate of virus                                                                                                 | Medium                   |

* does not cover autogenous vaccines

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Report of the meeting of the OIE AD HOC Group on prioritization of diseases for which vaccines could reduce antimicrobial use in animals, Paris, April 21-23, 2015

<table>
<thead>
<tr>
<th>Bacitracin</th>
<th>GP, NE, CO</th>
</tr>
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<tbody>
<tr>
<td>Virginiamycin</td>
<td>GP, NE</td>
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</table>
Coccidiosis and Necrotic Enteritis


Antibiotic alternatives-Mechanisms?

Essential oils key in fight against antibiotic use
Feb 4, 2016 1227

Essential oils are a key solution to an alternative for antibiotic growth promoters (AGPs), this was shown by research carried out by US food giant, Cargill. But what do essential oils achieve that other non-medicated feed additives don’t?

Carbohydrate = 250 grams
Protein =100 grams
Fat = 60 gram
Phytonutrients =1.5 grams

Walsh et al., 2007
ncbi.nlm.nih.gov/Pubmed
Phytonutrients/phytochemicals: Broad name for a wide variety of compounds produced by plants, found in fruits, vegetables, beans, grains, and other plants.

Function of Phytochemicals

1. Anti-oxidants; carotenoids, polyphenols
2. Stimulates innate immunity
3. Enhance adaptive (memory) immunity; turmeric
4. Stimulate enzymes; Indole from cabbage
5. Anti-bacterial; allicin from garlic
6. Promote beneficial bacterial growth
7. Hormonal activity; isoflavones from soy
8. Physically block pathogen binding; Proanthocyanidins
9. Serve as signal transduction agents
10. Epigenetic regulation (Isoflavone, turmeric)
Phytochemicals act on receptors on entero-endocrine cells to release hormonal messengers

<table>
<thead>
<tr>
<th>Phytonutrient</th>
<th>Effects</th>
<th>Receptors</th>
</tr>
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<tr>
<td>Cinnamaldehyde</td>
<td>Improves nutrient efficiency, flavour, immune enhancement</td>
<td>TRPA1, TRPV3</td>
</tr>
<tr>
<td>Allicin and other garlic products</td>
<td>Increased nutrient assimilation</td>
<td>TRPA1, bitter taste</td>
</tr>
<tr>
<td>Eugenol</td>
<td>Anti-inflammatory and <strong>anti-oxidant properties</strong>, flavour</td>
<td>TRPV1, TRPV3 + ?</td>
</tr>
<tr>
<td>Capsicum</td>
<td>Immune stimulant, <strong>mucosal repair</strong>, improved mucosal blood flow</td>
<td>TRPV1</td>
</tr>
<tr>
<td>Curcumin (tumeric)</td>
<td>Immune stimulant, anti-inflammatory, <strong>anti-oxidant</strong>, flavour</td>
<td>ROS, COX-2</td>
</tr>
</tbody>
</table>
Hormesis is a biological phenomenon whereby a beneficial effect (improved health, stress tolerance, growth or longevity) results from exposure to low doses of an agent that is otherwise toxic or lethal when given at higher doses.

Outbreaks of Clostridial infections have been associated with certain drug use, disruption in gut microbiota, gut barrier failure, bacterial translocation, inflammation and acute death.

Li, Lillehoj et al., 2010, Avian Pathology 39:255; Li, Lillehoj et al., 2010, Avian Pathology 39:247
Oh, ST and Lillehoj, 2016. The role of host genetic factors and host immunity in necrotic enteritis. Avian Pathology. In press
Host intestinal genes differentially affected by Necrotic enteritis in broiler chickens

Avian Coccidiosis:

Coccidiosis are ubiquitous, they are present wherever chickens are reared (traditional, industrial, label or organic/bio farms). Nine species of Eimeria have been described in chicken.
Balance of Th17 and Treg cells in Coccidiosis

**In gut immune system...**

- **Dendritic cells (APC)** present the antigen to the T cells.
- **Naïve CD4+ T cells** differentiate into either Th17 or Treg once in contact with dendritic cells.

**Th17 and Treg cells have opposite actions on intestinal immunity.**

- **Th17 cells** play a key role in intestinal inflammation through induction of pro-inflammatory cytokines.
- **Treg cells** suppress a variety of physiological and pathological immune responses in the intestine.
IL-17, as a potential therapeutic target of coccidiosis

IL-17

• is pro-inflammatory cytokine produced by Th17 cells.
• induces various pro-inflammatory cytokines and mediates inflammatory response.

Administration of IL-17A neutralizing antibody induce
- Enhanced weight gain
- Reduced oocyst shedding
- Increased heterophil infiltration
- Reduction of cecal lesion
- Inhibition of *E. tenella* schizont and merozoite development

Del Cacho et al., 2014; Zhang et al., 2013

Flow diagram for screening immunoactive phytochemicals

- In vitro screening
  - Macrophage activation
  - Lymphocyte stimulation
  - Cytokine
  - Tumor cytotoxicity
  - Direct killing of pathogens

- In vivo feeding trial
  - Nutrigenomics

- Select synergistic plants

- Randomized Performance analysis in commercial broilers

- Disease challenge
In vitro and in vivo effects of purified phytochemicals on *Eimeria* and *C. perfringens*
NUTRIGENOMICS to identify 3 phytonutrient combination that reduced negative effects of avian coccidiosis

Phytochemical doses that we used were not cytotoxic to pathogens.
Dietary effects of phytonutrients on coccidiosis

Dietary Capsicum and turmeric oleoresins decreased inflammatory cytokine transcripts and mitigated gut damage due to necrotic enteritis.

Improved resistance to *Eimeria acervulina* infection in chickens due to dietary supplementation with garlic metabolites

Duk Kyung Kim¹, Hyun S. Lillehoj¹*, Sung Hyen Lee¹, Erik P. Lillehoj² and David Bravo³

**White phytochemicals**

<table>
<thead>
<tr>
<th>Phytonutrient</th>
<th>Benefits</th>
<th>Found in</th>
</tr>
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<tr>
<td><em>Allicin</em></td>
<td>Boosts immunity; helps lower high cholesterol; helps control high blood pressure; reduces the risk of heart attacks; reduces the risk for spread of cancer (particularly stomach and colon cancer)</td>
<td>Garlic, onions, leeks, scallions, chives</td>
</tr>
</tbody>
</table>

*Provided by the National Cancer Institute, www.cancer.gov*
Garlic effects: *in vitro*

**In vitro EA sporozoites killing**

Allium hookeri, a member of the family Alliaceae is found in Ceylon, Greece, Southern China, and India and has been used by locals to treat cough and cold and to heal burns and wounds (Sharma et al., 2011).

A. hookeri contains an abundance of organo-sulphur compounds, volatile sulphur compounds, proteins, prostaglandins, fructans, vitamins, and polyphenols as well as Allicin.

Allicin and organic sulphur compounds are known to reduce cholesterol levels, decrease the risk of heart attack, and exert anti-inflammatory effects (Bae and Bae, 2012; Kim et al., 2012).
Allium hookeri on LPS-induced intestinal inflammation

(A) JAM
- Mean normalized mRNA
- Control, Control, 1%, 5%, 1%, 5%
- Basal diet, Root, Fermented root

(B) Occludin
- Mean normalized mRNA
- Control, Control, 1%, 5%, 1%, 5%
- Basal diet, Root, Fermented root

(C) ZO1
- Mean normalized mRNA
- Control, Control, 1%, 5%, 1%, 5%
- Basal diet, Root, Fermented root

(D) MUC2
- Mean normalized mRNA
- Control, Control, 1%, 5%, 1%, 5%
- Basal diet, Root, Fermented root

Leaky Gut Progression
- Stress, Inflammation, Food Intolerances, Immune System Issues

Intestinal Barrier
- Tight Junction Assembly
- Membrane, Epithelial Cell

Intracellular Junctions
- Tight Junction, Adherens Junction, Gap Junction
- Caspase-1, MyD88, TRAF6, NLRP3, IL-1β
Interplay of nutrition, gut microbiota and immunity

Dietary fermentable fiber and SCFAs can shape the immunological environment in the lung and influence the severity of allergic inflammation.
Effect of phytochemicals on gut microbiota diversity in broilers

From plants to novel anti-parasite therapies

The 2015 Nobel Prize winner in Medicine to Dr. Youyou Tu concerning novel therapies against infections caused by Malaria protozoa.

Malaria-mosquito-borne disease caused by single-cell parasites, which invade red blood cells, causing fever, and in severe cases brain damage and death. It claims more than 450,000 lives

Strong evidence to suggest that the primary activator is an iron source. Multi-faceted nature of cellular response to artemisinin in Plasmodia and tumor cells includes DNA damage which is dose dependent.

-Resistance
-Bioavailability
-Cost and limitation
-Mechanism of action