Intestinal Disease Challenges and Immunological Evaluations in Poultry: Assumptions, Limitations, and Applications

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Food Security: A Global Challenge

- The world’s population is expected to reach 9 billion people (a 3 billion person increase) by 2050.

- An estimated 70% increase in worldwide food production will be required.

- Existing land and resources cannot account for the increase—technological advancement is required.
Food Security: Role of the Commercial Poultry Industry

1. Increased meat production to feed growing world population

2. Changes in production practices — reduced use of antimicrobial growth promoters (AGPs) and drug-related forms of chemotherapy
Changes in Production Practices

- **Technological Improvements:**
  - Improving existing practices
  - Developing new technologies
  - Genetic improvements to plants and animals
Changes in Production Practices

- **Chemotherapy / conventional drug administration:**
  - Widespread throughout animal agriculture
  - Influence on broiler industry success for 50+ years
  - Antimicrobial growth promoters - AGPs
  - Therapeutic antibiotics
  - Anticoccidial drugs

- Chemotherapy in commercial operations is changing
Historical Perspective: AGPs in Animal Agriculture

- Originated mid-1900s: dietary antibiotic administration at sub-therapeutic levels

- Common in world-wide in animal agriculture

- Very effective growth promotion
AGP Benefits

- Weight gain
- Feed efficiency
- Enteric disease suppression
- Meat quality improvements
- Improved overall production efficiency
Current Perspectives: Antibiotic usage in Animal Agriculture

- Advocacy for discontinued use:
  - World Health Organization
  - European Union
  - United States-Food and Drug Administration
  - Consumer / non-profit groups

- EU Ban on AGPs January 1, 2006

- Objective: reduce antibiotic usage in animal agriculture to reduce resistance to antibiotics used in human medicine
Impact of AGP Removal in the EU

- Impact to date of EU removal of AGPs--current data suggests:
  - Reports of reduced performance (weight gain and feed efficiency)—price increases
  - Increased incidence of enteric disease (Necrotic Enteritis in broiler chickens)—health suffers
  - Therapeutic drug use often increases
  - EU ban on AGP usage drives search for alternatives
Current AGP Status in the U.S.

- U.S.-Food and Drug Administration published a Draft Guidance (June 28, 2010) advocating the removal of AGPs from animal production within the US.

- FDA Guidance 209, 213, and VFD
  - antimicrobial usage for animal health
  - not growth promotion
  - therapeutic usage only
  - Phase in December 2016

- This legislation represents significant change to chemotherapy in commercial poultry production in the U.S.
Response of US Industry to Current Antibiotic Legislation
Conclusions on Antibiotic Usage

- AGP and antibiotic usage will decline in many areas of animal agriculture around the globe.

- To sustain present levels of production, effective alternatives must exist.

- If not, therapeutic drug use will increase and potentially surpass AGP usage levels.
Approved Anticoccidials (US)


<table>
<thead>
<tr>
<th>Trade or Empirical Name, Approval Label (Manufacturer)</th>
<th>Trade Name</th>
<th>First Approval by FDA</th>
<th>Drug Withdrawal (Days before Slaughter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfamethazine, 0.015–0.025% (Merck)</td>
<td>SQ, Sulquin</td>
<td>1948</td>
<td>10</td>
</tr>
<tr>
<td>Nitrofurazone, 0.0055% (Hess &amp; Clark; Smith-Kline)</td>
<td>nfz, Amifur</td>
<td>1948</td>
<td>5</td>
</tr>
<tr>
<td>Arsanilic acid or sodium arsenilate, 0.04% for 8 days (Abbott)</td>
<td>Pro-Gen</td>
<td>1949</td>
<td>5</td>
</tr>
<tr>
<td>Butynorate, 0.0375% for turkeys (Solvay)</td>
<td>Tinostat</td>
<td>1954</td>
<td>28</td>
</tr>
<tr>
<td>Nicarbazine, 0.0125% (Merck)</td>
<td>Nicarb</td>
<td>1955</td>
<td>4</td>
</tr>
<tr>
<td>Furazolidone, 0.0055–0.011% (Hess &amp; Clark)</td>
<td>nf-180</td>
<td>1957</td>
<td>5</td>
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<tr>
<td>Nitromide, 0.025% + sulfanilamid, 0.03% + roxarsone, 0.005% (Solvay)</td>
<td>Unistat-3</td>
<td>1958</td>
<td>5</td>
</tr>
<tr>
<td>Oxytetracycline, 0.022% (Pfizer)</td>
<td>Terramycin</td>
<td>1959</td>
<td>3</td>
</tr>
<tr>
<td>Amprolium, 0.0125–0.025% (MSD-AGVET)</td>
<td>Amprolium</td>
<td>1960</td>
<td>0</td>
</tr>
<tr>
<td>Chlortetracycline, 0.022% (American Aureomycin Cyanamid)</td>
<td>Zoamix</td>
<td>1960</td>
<td>(See feeding restrictions)</td>
</tr>
<tr>
<td>Zoalene, 0.004–0.0125% (Solvay)</td>
<td>Zoamix</td>
<td>1960</td>
<td>(higher levels, 5 days)</td>
</tr>
<tr>
<td>Amprolium, 0.0125% + ethopabate, 0.0004/0.004% (Merck)</td>
<td>Amprolium Plus, Amproli-Hi-E</td>
<td>1963</td>
<td>0</td>
</tr>
<tr>
<td>Buquinol, 0.00825% (Norwich-Eaton)</td>
<td>Bonaid</td>
<td>1967</td>
<td>0</td>
</tr>
<tr>
<td>Clopidol or meticlorpindol, 0.0125–0.025% (A. L. Laboratories)</td>
<td>Coyden</td>
<td>1968</td>
<td>0 days at 0.0125%; 5 days at 0.025%</td>
</tr>
<tr>
<td>Decoquinate 0.003% (Rhone-Poulenc)</td>
<td>Decoquinate</td>
<td>1970</td>
<td>0</td>
</tr>
<tr>
<td>Sulfalexamine, 0.0125% + ormetoprim, 0.0075% (Hoffmann-La Roche)</td>
<td>Rofenaide</td>
<td>1970</td>
<td>5</td>
</tr>
<tr>
<td>Monensin, 0.01–0.121 % (Elanco)</td>
<td>Coban</td>
<td>1971</td>
<td>0</td>
</tr>
<tr>
<td>Robenidine, 0.0033% (American Cyanamid)</td>
<td>Roben, Cycost</td>
<td>1972</td>
<td>5</td>
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<tr>
<td>Lasalocid, 0.0075–0.0125% (Hoffmann-La Roche)</td>
<td>Avatec</td>
<td>1976</td>
<td>3</td>
</tr>
<tr>
<td>Salinomycin, 0.004–0.0066% (Agre-Bio)</td>
<td>Bio-Cox</td>
<td>1983</td>
<td>0</td>
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<tr>
<td>Halofuginone, 3 ppm (Hoechst-Roussel Agri-Vet)</td>
<td>Stenorol</td>
<td>1987</td>
<td>5</td>
</tr>
<tr>
<td>Narasin, 54–72 g/T (Elanco)</td>
<td>Monteban</td>
<td>1988</td>
<td>0</td>
</tr>
<tr>
<td>Maduramicin, 5–6 ppm (American Cyanamid)</td>
<td>Cygro</td>
<td>1989</td>
<td>5</td>
</tr>
<tr>
<td>Narasin + nicarbazine, 54–90 g/T (Elanco)</td>
<td>Maxiban</td>
<td>1989</td>
<td>5</td>
</tr>
<tr>
<td>Semduramycin, 25 ppm (Pfizer)</td>
<td>Aviax</td>
<td>1995</td>
<td>0</td>
</tr>
<tr>
<td>Diclazuril, 1 ppm (Schering-Plough)</td>
<td>Clinicox</td>
<td>1999</td>
<td>0</td>
</tr>
</tbody>
</table>
Anticoccidial Drugs: Chemotherapy for Coccidiosis Control

- Avian coccidiosis: enteric disease affecting all commercially reared poultry worldwide
- Significant economic impact
- Traditional control measure: chemotherapy-anticoccidial drugs
- Rapidly emerging control measure: vaccination
Chicken *Eimeria* are ubiquitous in nature. All species except *Eimeria tenella* are still found in wild jungle fowl in Southeast Asia.
Emergence of Coccidiosis Vaccination in U.S. Broiler Production

% Vaccinated

Data courtesy of Dr. Blayne Mozisek, Merck Animal Health
Challenges to Coccidiosis Vaccination

- **Reluctant acceptance** by commercial industry
  - Immunity is developed by controlled vaccine infection (vaccine vs field strain immunity)
  - **Negative impact** on weight gain and FCR early in production (small bird operations)

- **Research to improve performance:**
  - Understanding immune response to *Eimeria*
  - Dietary modifications
  - Probiotic administration
Transitioning from Chemotherapy

- **Primary Objective:**
  - Maintenance of a healthy intestinal microenvironment
  - Efficient nutrient absorption for production efficiency

- **Obstacles:**
  - Avian Coccidiosis
  - Necrotic Enteritis
Healthy Gut: Protective, Structural, and Metabolic Function

- Intestinal Architecture
- Enterocyte Maturity
- Mucin Barrier
- Normal Microflora
- GALT
Compromised Gut

- Intestinal Architecture
- Enterocyte Maturity
- Mucin Barrier
- Normal Microflora
- GALT
Opportunities for Maintaining Intestinal Integrity

Vaccination

Diet Additives
- Prebiotics
- Probiotics
- Plant Extracts

Nutritional Strategies with Enteric Disease
- Enzymes
- Nutrient Levels
Sustainable Poultry Production with Reduced Chemotherapy: Options

- Effective alternatives to AGPs are emerging:
  - Probiotics or direct fed microbials (DFMs)
  - Prebiotics
  - Phytogenics
  - Essential oils
  - MOS, FOS, and related oligosaccharides / yeast cell wall products

- Effective vaccines exist currently and new vaccines are being developed
Additional Research is needed to better understand mode of action of alternative products (probiotics, prebiotics, phytogenics, MOS, yeast cell wall products) and vaccines within intestinal microenvironment:

- Impact on normal microflora
- Impact on immunity development
- Impact on production efficiency
- Implementation in commercial production systems
Probiotics and Gut Health

Probiotics

- Competitive exclusion
- Gut development
- Growth parameters
- Nutrient Utilization

(Fedorka-Cray et al., 1999: Williams, 2005: GuerinoDanan et al., 2001; Awad et. al., 2008)
Probiotics and Immunity

- Lymphocyte Proliferation
- Cytokine secretion
- Phagocyte Activation
- Eimeria resistance

(Lillehoj and Trout, 1996; Farnell et al., 2006; Dalloul et al., 2003; Dalloul et al., 2002)
Probiotic Influence on Broiler Performance – Coccidiosis Vaccination

Comparison of body weights on Day 44:
- **Control**: 2.65 Kg (b)
- **Ionophore**: 2.74 Kg (a)
- **Vaccine**: 2.62 Kg (b)
- **Vaccine + Probiotic**: 2.75 Kg (a)
Probiotic Influence on Broiler Performance – Coccidiosis Vaccination

Feed Conversion Ratio (FCR)

Day 44

- Control
- Ionophore
- Vaccine
- Vaccine + Probiotic

Values:
- Control: 1.89
- Ionophore: 1.85
- Vaccine: 1.88
- Vaccine + Probiotic: 1.83

Significance:
- a
- b
Effects of Probiotics on Mortality

**Cumulative Mortality**

- Probiotic reduced mortality ($P < .05$)
- Vaccine increased mortality ($P < .05$)

% Mortality

Experimental Groups:
- Control: 4.1
- Probiotic: 2.3
- Vaccine: 8.0
- Vaccine + Probiotic: 4.1
Effects of Probiotics on Intestinal Lesions

![Bar chart showing lesion scores for different experimental groups.]

- **Control**: 0.93
- **Probiotic**: 0.34
- **Vaccine**: 0.43
- **Vaccine + Probiotic**: 0.53

Legend:
- Blue: Control
- Red: Probiotic
- Yellow: Vaccine
- Green: Vaccine + Probiotic
Capsaicinoids and Enteric Disease Prevention

- Capsaicinoids are the pungent, vasoactive compounds present in chili peppers.
- Many lines of evidence suggest capsaicin and related compounds can influence enteric pathogen challenge by affecting pro-inflammatory responses.
Capsaicin fed PRIOR to Salmonella Challenge

(McElroy et al., 1994)

* P<0.05
Capsaicin fed FOLLOWING *Salmonella* Challenge (Woolsey et al., 1994)

* P<0.05
Investigation into **Phytase and Calcium Involvement in Necrotic Enteritis**

**Phytase**
- 0 FTU
- 1000 FTU

**Calcium**
- 0.6% (low)
- 0.9% (high)

**Phosphorus (NPP)**
- 0.30% (low)
- 0.45% (high)
Necrotic Enteritis Mortality (D 0-35)

% Mortality

- Calcium (P=0.0147)
- Phosphorus (P=0.3805)
- Phytase (P=0.0006)

Paiva et al. 2013
Daily Flock Mortality

![Graph showing daily mortality over time for different calcium levels (Low Ca, Standard Ca, Total Mortality).](image)
Calcium Source and NE Mortality (Days 0-21)

Paiva et al. 2013

0.60% Ca
0.90% Ca

P = 0.0383
Ca appears to be an factor in the pathogenesis of necrotic enteritis:
- Likely related to toxin activity
  - Alpha toxin
  - Net B

Alpha toxin
- Ca dependent C-terminal region which is necessary for binding

Net B Toxin
- Pore forming toxin

Kennedy et al., 2009
Vaccines and Intestinal Immune Responses

- New vaccines will continue to be developed as we transition away from chemoprophylaxis.
- Improved vaccine development will require a complete understanding of immune responses in GALT:
  1. Adaptive immunity (antibody and lymphocyte mediated responses)
  2. Innate immunity (innate cells and tissues)
  3. Non-traditional mechanisms (barrier nature of mucosal membranes)
Mucosal Immunity
(Macpherson and Harris, 2004)
Research Interests:
Enteric Immunity in Poultry

Study “non-traditional” immune elements:

- Barrier nature of the mucosal epithelium
- Innate cell involvement (mast cells, eosinophils)
- Epithelial antigen recognition / responsiveness

Adaptation of these elements to a model of enteric infection in the chicken
IEC Pathogen Signaling
Flagellin from *Salmonella* Typhimurium + Neutrophil Recruitment

10.1038/mi.2009.9
Gut Hypersensitivity Responses in the Chicken

Lamina Propria

Antigen

Mast Cell

Neuronal Synapse

De novo Synthesis

Degranulation

5-HT / HIS

Chloride Secretion

PGE / LTE

Na\(^+\), Cl\(^-\)

IEC or Enterocyte

Mucosal Epithelium

PSA
Chicken Intestinal Epithelial Cells (IEC)

- Obligate involvement of the IECs in invasion or colonization by pathogens in the chicken gut.

- To date, little consideration of chicken IEC as effector cells in host-responses resulting in immunity.

- In mammals, IEC have been demonstrated to
  - Act as APCs
  - Chemokine & cytokine production
Chicken Intestinal Epithelial Responses to Antigen Challenge

*Represents a statistically different $\Delta I_{sc}$ ($P<0.05$)
Success in live production of commercial poultry over the past several decades can be attributable to:

- Production Best Management Practices
  - Preventative disease programs (biosecurity and vaccination)
  - Good management, husbandry, housing
  - Adequate diet and nutrition
  - Genetic improvements
- Emerging alternatives to traditional drug use: probiotics, prebiotics, phytogenic products
- Judicious use of antibiotics and other traditional forms of chemotherapy
Conclusions—Vaccines and Dietary Alternatives to Chemotherapy

- At least in the U.S., we are beginning to transition away from chemotherapy in the traditional sense.
- Vaccines and alternatives to chemotherapy will become more common in commercial production systems.
- Research on application and best management practices will be key to successful implementation of new approaches.