Potential of plant-derived antimicrobials for controlling zoonotic and food-borne diseases

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Zoonotic Diseases

- About 75% of recently emerging infectious diseases affecting humans are diseases of animal origin, and approximately 60% of all human pathogens are zoonotic.
- Food-borne diseases
- Wide range of animal reservoirs
- Emergence of antibiotic resistance
Plant-Derived Antimicrobials (PDAs)
An alternative approach

- Phytophenolics
- Plant defense mechanism
- Wide spectrum of biological effects
- Bacterial resistance low

Burt et al., 2004; Ohno et al., 2007; Wollenweber, 1988
Plant-derived antimicrobials (PDAs)

- Trans-cinnamaldehyde (TC)
- Carvacrol (CR)
- Thymol (TH)
- Eugenol – (EU)
- Caprylyc acid – (CA)

Cinnamon oil
Oregano oil
Clove oil
Coconut oil
Efficacy of PDAs for reducing egg-borne transmission of *Salmonella* Enteritidis
Salmonella

- Major food-borne pathogen worldwide
- Highest incidence rate for *Salmonella* infections\(^1\)
- Total estimated annual cost – 4 billion USD\(^2\)

\(^1\)CDC 2010, \(^2\)Scharff, 2012
Salmonella outbreaks in the US

- **Salmonella**
- **Listeria monocytogenes**
- Pathogenic **E. coli**
- No reported outbreaks
Foods associated with *Salmonella* outbreaks

- **Poultry**: 29%
- **Eggs**: 18%
- **Pork**: 12%
- **Beef**: 8%
- **Vine vegetables, fruits, and nuts**: 13%
- **Other †**: 20%

Salmonella Epidemiology

- Chicken – reservoir host
- *Salmonella* Enteritidis (SE) – most common *Salmonella* from poultry\(^1\)
- Consumption of raw or undercooked eggs
- 100 billion table eggs are produced annually

\(^1\) Marcus et al., 2007; \(^2\) AMI, 2009; \(^3\) CDC, 2010
**Salmonella** chicken link

Primary colonization site – cecum

- Other sites – crop, intestine, cloaca
- Internal organs – liver, spleen, oviduct

Transmission routes:

- **Horizontal** – bird to bird
- **Vertical** – Transovarian - bird to yolk

(macrophages involved in systemic spread)\(^1,2\)

\(^1\) Gast et al., 2007; \(^2\) Gantois et al., 2009
Controlling *Salmonella*

Ideal Intervention Strategy

- Economically viable
- Practical for farmers to adopt
- No toxicity
- Organic farming
- Environmentally friendly
- No bacterial resistance development
Rationale

Supplementation of PDAs

Reduce cecal colonization

Reduce oviduct colonization

Reduce fecal contamination of shelled eggs

Reduce yolk and membrane contamination

Control salmonellosis
120 White Leghorn layer chickens
25 & 40 weeks of age
Treatments

1% TC control (No SE, 1% TC)
1.5% TC control (No SE, 1.5% TC)
Positive control (SE, No TC)
1% TC (SE, 1% TC)
1.5% TC (SE, 1.5% TC)

SE-28, SE-21, SE-12, SE-31, SE-90
Protocol

TRANS-CINNAMALDEHYDE FEEDING PHASE

Weeks

1  2  10

Day 1
Grouping
of birds

Day 10
Challenge
with
SE

Day 14
Egg
Collection
starts

Day 12
Challenge
check

Daily egg collection

Weekly fecal swab

Day 70
End of trial
Collection
of tissues

End of trial
Collection
of tissues

Collection
of tissues
RESULTS
Effect of TC on SE contamination of eggs *, week 1

*Treatments with different superscripts significantly differed from each other at $P < 0.05$
Effect of TC on SE contamination of eggs *, week 2

*Treatments with different superscripts significantly differed from each other at $P < 0.05$
Effect of TC on SE contamination of eggs *, week 3

*Treatments with different superscripts significantly differed from each other at \( P < 0.05 \)
Effect of TC on SE contamination of eggs *, week 4

*Treatments with different superscripts significantly differed from each other at $P < 0.05$
Effect of TC on SE contamination of eggs *, week 5

*Treatments with different superscripts significantly differed from each other at $P < 0.05$
Effect of TC on SE contamination of eggs *, week 6

*Treatments with different superscripts significantly differed from each other at $P < 0.05$
Effect of TC on SE contamination of eggs *, week 7

*Treatments with different superscripts significantly differed from each other at $P < 0.05$
Effect of TC on SE contamination of eggs *, week 8

25 WEEKS

40 WEEKS

*Treatments with different superscripts significantly differed from each other at $P < 0.05$
Effect of TC on SE contamination of eggs (N=2195) cumulative data

No change in egg production due to TC supplementation

*Treatments with different superscripts significantly differed from each other at $P < 0.05$
Effect of TC on SE in Chicken Organs*

*\(p < 0.05\)
Impact of research

In-feed supplementation of TC could be used to reduce egg-borne transmission of SE and improve microbiological safety of eggs.
Attenuation of *Vibrio cholerae* infection using plant molecules
Vibrio cholerae (VC)

- Causative agent of human cholera
- Toxin-mediated watery diarrhea
- Life threatening dehydration and electrolyte imbalance
- Extreme cases lead to kidney failure and death
- Serogroups 01 and 0139
- Globally - excess of 300,000 cases; 7500 deaths
Cholera: Route of Transmission

- Feces
- Contaminated Soil
- Unsanitized Water
- Food
- Human
Cholera toxin

CTB → Cholera toxin B subunit

CTA1 and CTA2 → Cholera toxin A subunit
Treatment/Control

- Oral Rehydration Therapy
  - Fluids supplemented with electrolytes and salts

- Antibiotics
  - First antibiotic resistant strain – 1970 (Kitaoka et al., 2011)
  - Multiple drug resistant *Vibrio cholerae* (Das et al., 2008; Akoachere., 2013)

- Vaccine

- Oral vaccine – Not fully effective (WHO Guidelines, 2012)

Safe and Effective alternative strategy needed
Effect of CR, TH and EG on cholera toxin production

Strains of *Vibrio cholerae* used in this study – VC 11623; BAA-25870 and BAA 2163
Protocol for quantification of cholera toxin

1. **V. cholerae with or without PDAS**
2. Culture supernatant collected after 24 hours
3. Dilution
4. Serial dilution
5. Cholera toxin custom kit (KPL protein detector kit)
6. Std curve, & Quantification by colorimetry

**Steps:**
- Collect culture supernatant after 24 hours.
- Dilute the supernatant.
- Perform serial dilution.
- Use cholera toxin custom kit for quantification.
- Generate standard curve and quantify by colorimetry.
Effect of CR, TH and EG on cholera toxin production (VC 11623)
Effect of CR, TH and EG on cholera toxin production (VC 569b)
Effect of CR, TH and EG on cholera toxin production (VC 2163)
Summary and Future Studies

- PDAs could be potentially added in oral rehydrating solution to control *V. cholera* infection in humans
- Validate the *in vitro* results in a mammalian *in vivo* model
- Characterize and delineate the mechanism of action CR, TH, and EG
Controlling aflatoxins in chicken feed using carvacrol and trans-cinnamaldehyde as feed additives
Aflatoxins (AF) in feed

- Fungal toxins in feed ingredients
- *Aspergillus* spp. - *A. flavus, A. parasiticus*
- Routes of contamination of grains:
  - Pre-harvest, post-harvest, and transportation
  - Processing of feed ingredients
  - Formulated feed after processing
Why study AF?

Aflatoxin-contaminated feed

Consumption by Chickens

Aflatoxin residues in chicken products

Carcinogenic and hepatotoxic effect

Economic losses to producers

Impact on Human health

Impact on Chicken performance

Bbosa et al., 2013; Herzallah et al., 2013
Aflatoxicosis in chickens

- **Acute aflatoxicosis:**
  - 50% mortality within 48 hours

- **Chronic aflatoxicosis:**
  - Decrease egg production
  - Decrease hatchability
  - Liver necrosis

Thrasher, 2012; Hamilton and Garlich, 1971
Materials and Methods

A. flavus NRRL 3357 or A. parasiticus NRRL 4123 or NRRL 2999 (5 log\(_{10}\) CFU/g)

200 g chicken feed

CR 0%, 0.4%, 0.8%, 1.0%

TC 0%, 0.4%, 0.8%, 1.0%

Incubated at 25°C for 3 months

0, 1, 2, 3, 4, 8, 12 weeks

Aflatoxin concentration

Farag et al., 1989; Razzaghi-Abyaneh et al., 2008
Effect of CR on *A. flavus* aflatoxin production in chicken feed

*Treatments differed significantly from the control (n = 6) (p<0.05)*
Effect of CR on *A. parasiticus* aflatoxin production in chicken feed

* All treatments differed significantly from the control (n = 6) (p < 0.05)
Effect of TC on *A. flavus* aflatoxin production in chicken feed

*Treatments differed significantly from the control (n = 6) (p<0.05)*
Effect of TC on *A. parasiticus* aflatoxin production in chicken feed

*Treatments differed significantly from the control (n = 6) (p<0.05)*
Protective effect of CR and TC on decreasing aflatoxin-induced cytotoxicity in hepatocytes
Hepatotoxic effect of aflatoxins

- Aflatoxin consumption by chicken
  - Acute Aflatoxicosis
    - Liver hemorrhage
  - Chronic Aflatoxicosis
    - Fatty liver
    - Liver necrosis

Doerr et al., 1983
Materials and Methods

- Human Hepatocytes (ATCC HepG2)
- 24 hours incubation to form monolayer in 96-wells plate
- 3 ppm or 10 ppm aflatoxin
- Control 0%
- CR 0.05% & 0.1%
- TC 0.05% & 0.1%
- Incubate for 18 hours at 37°C
- Cytotoxicity % using LDH assay

Reddy et al., 2006; Zhou et al., 2006
Efficacy of CR in reducing aflatoxin-induced cytotoxicity in hepatocytes

* All treatments differed significantly from the control ($p < 0.05$)
Efficacy of TC in reducing aflatoxin-induced cytotoxicity in hepatocytes

* All treatments differed significantly from the control ($p < 0.05$)
Summary

- CR and TC reduced aflatoxins in chicken feed
- CR and TC did not change feed composition
- CR and TC significantly decreased aflatoxin-induced cytotoxicity in hepatocytes ($p < 0.05$)
Concluding remarks

• Determine the stability of CR and TC in chicken feed during manufacturing process

• Field trials in chicken farms
THANK YOU!

QUESTIONS?