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)

Cinnamon oil

- Carvacrol (CR)
- Thymol (TH)

Oregano oil

- Eugenol –(EU)

Clove oil

- Caprylic acid –(CA)

Coconut oil

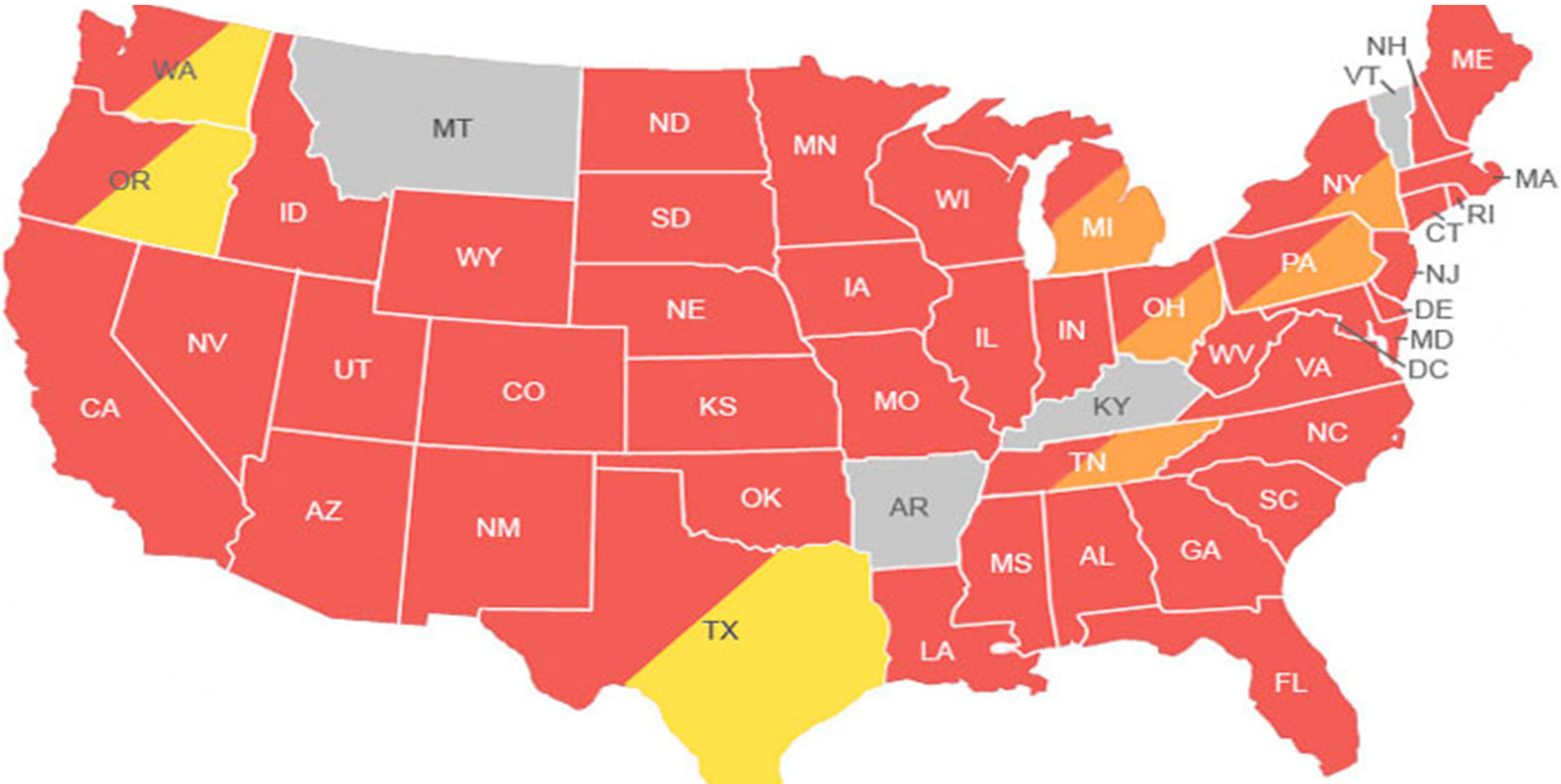
Efficacy of PDAs for reducing egg-borne transmission of *Salmonella* Enteritidis

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



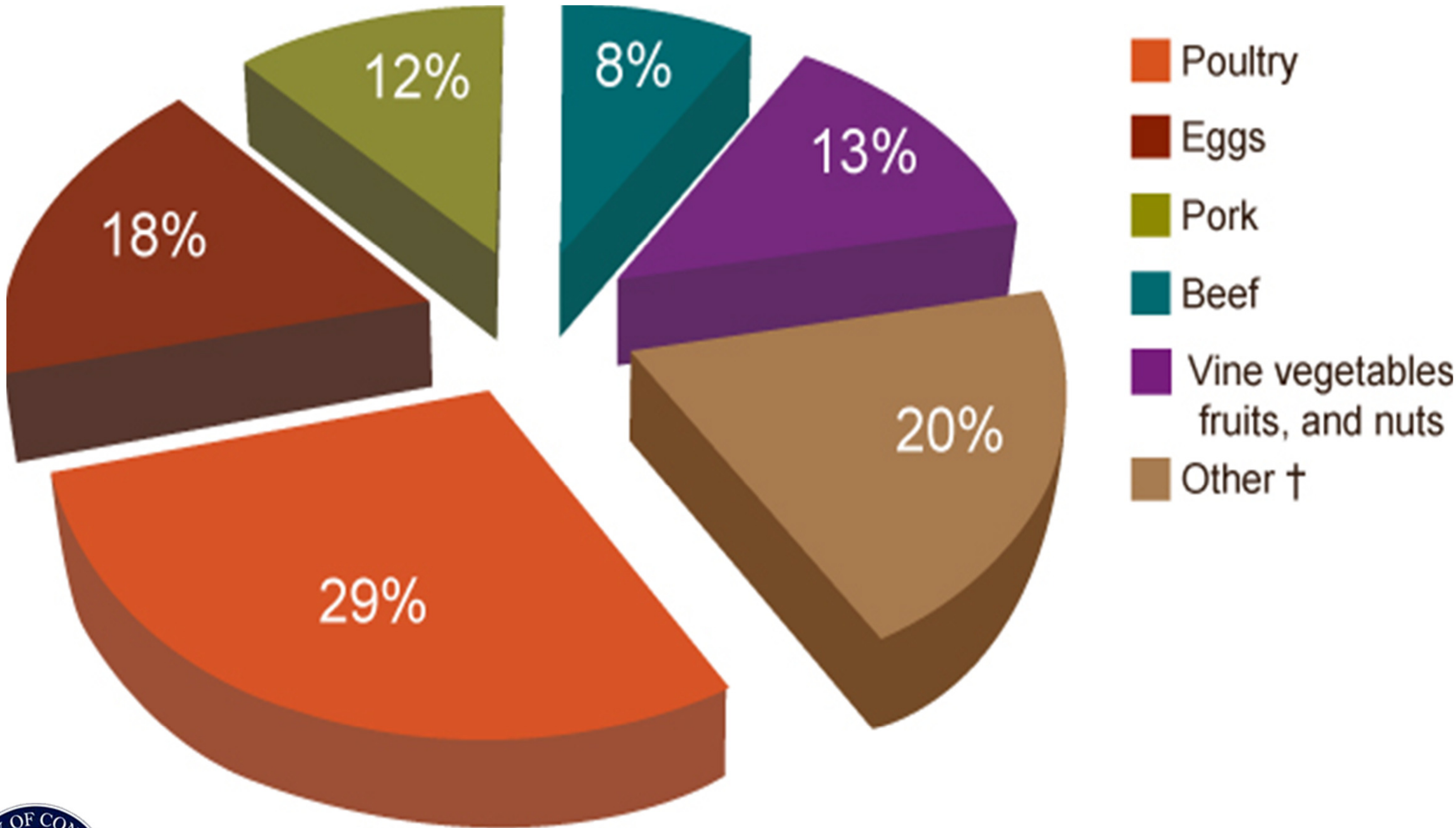
Salmonella

Pathogenic *E. coli*

Listeria monocytogenes

No reported outbreaks

Foods associated with *Salmonella* outbreaks



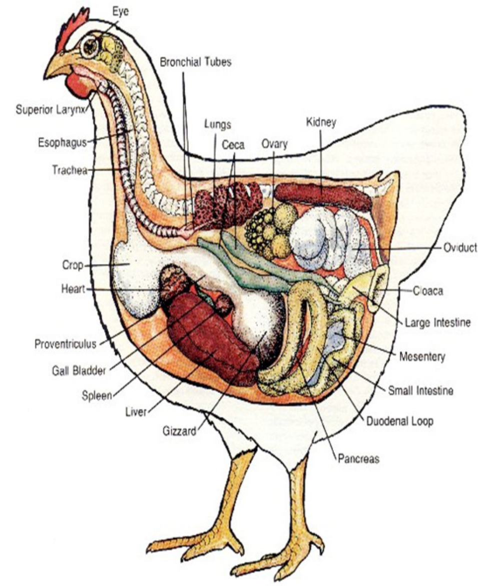
Source: CDC National Outbreak Reporting System, 2004–2008

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 fecal contamination of shelled eggs

Reduce oviduct colonization → Reduce yolk and membrane contamination

↓

Control salmonellosis

Challenge experiment in chickens

- 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)

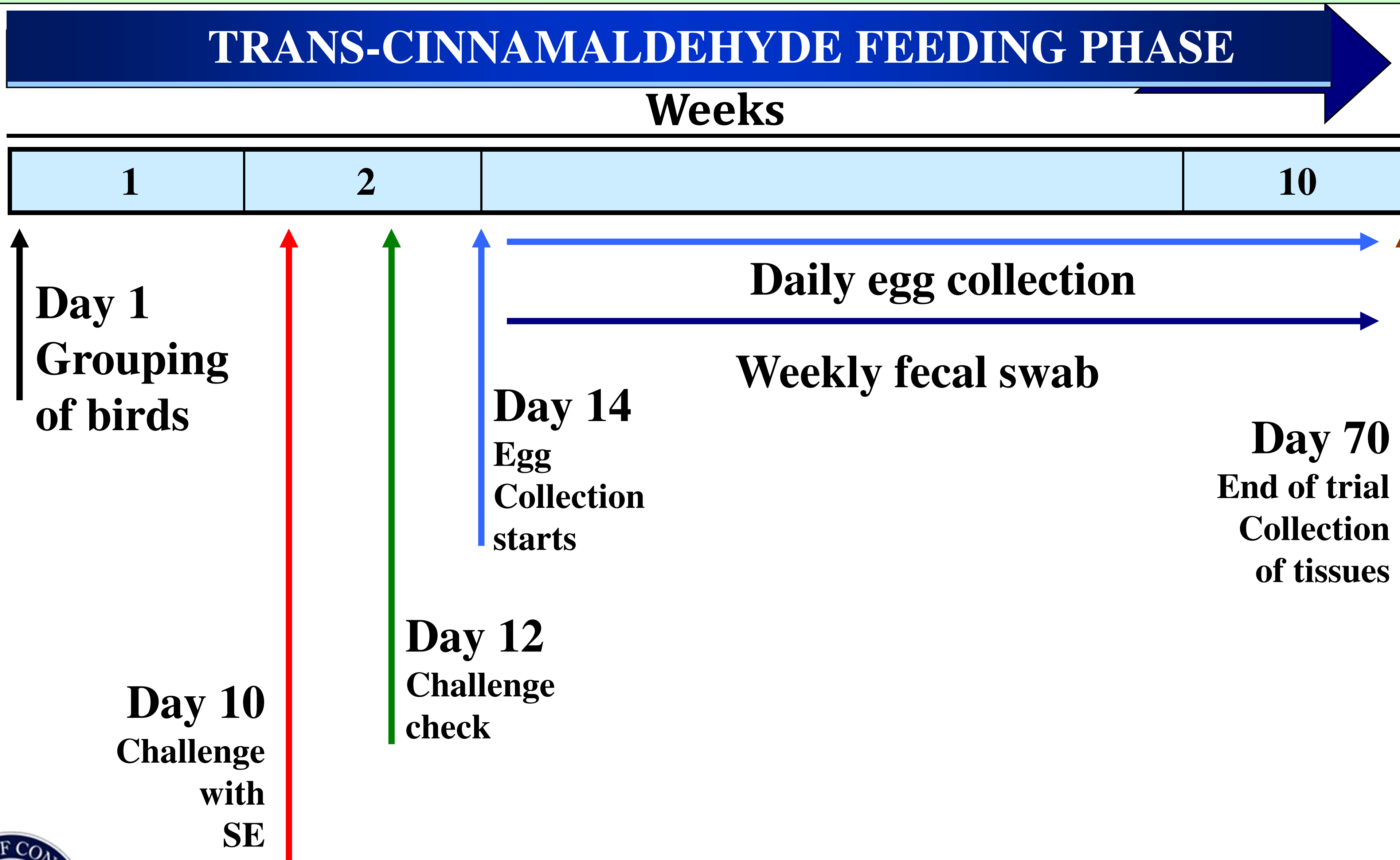
Positive control (SE, No TC)

1% TC (SE, 1% TC)

1.5% TC (SE, 1.5% TC)

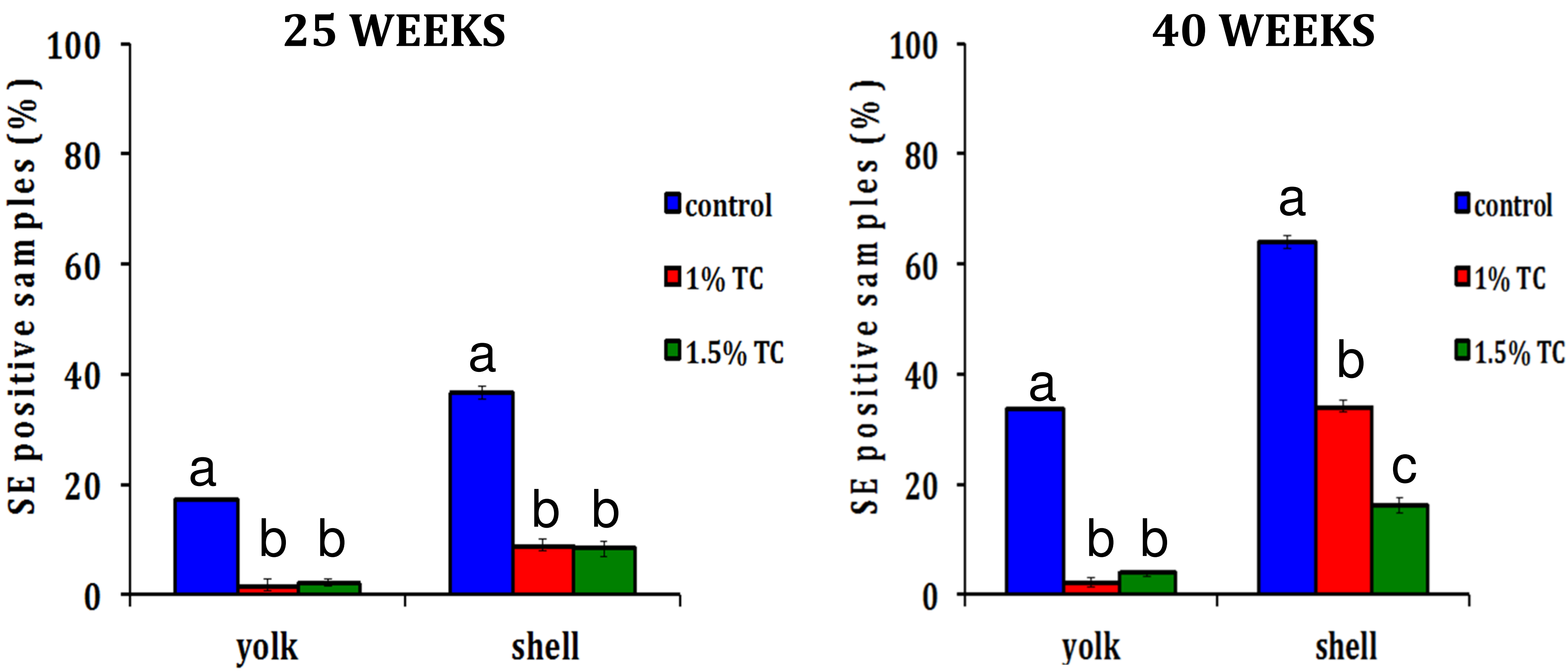
SE-28, SE-21, SE-12, SE-31, SE-90

Protocol


TRANS-CINNAMALDEHYDE FEEDING PHASE
Weeks
1
2
10
Day 1
Grouping of birds
Day 10
Challenge with SE
Day 12
Challenge check
Day 14
Egg Collection starts
Daily egg collection
Weekly fecal swab
Day 70
End of trial
Collection of tissues

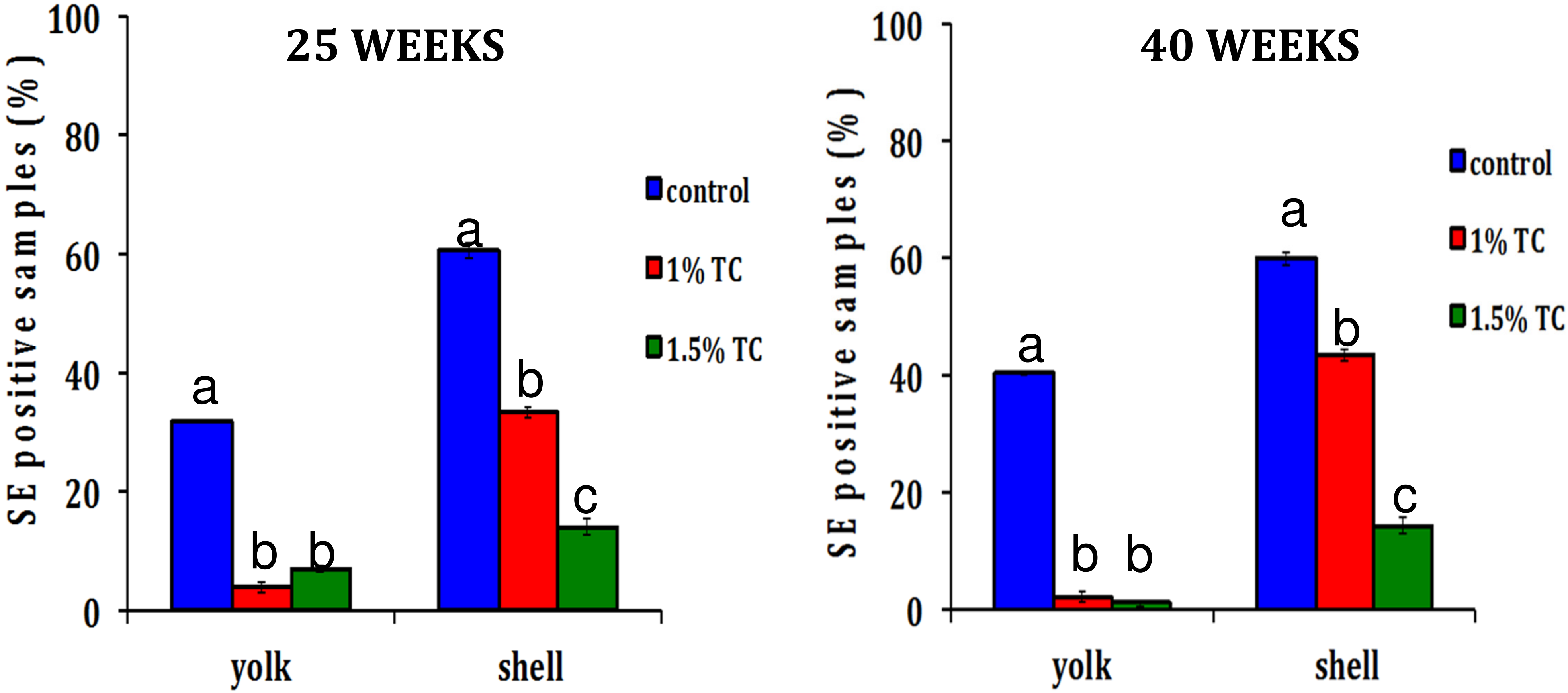
RESULTS

Effect of TC on SE contamination of eggs *, week 1



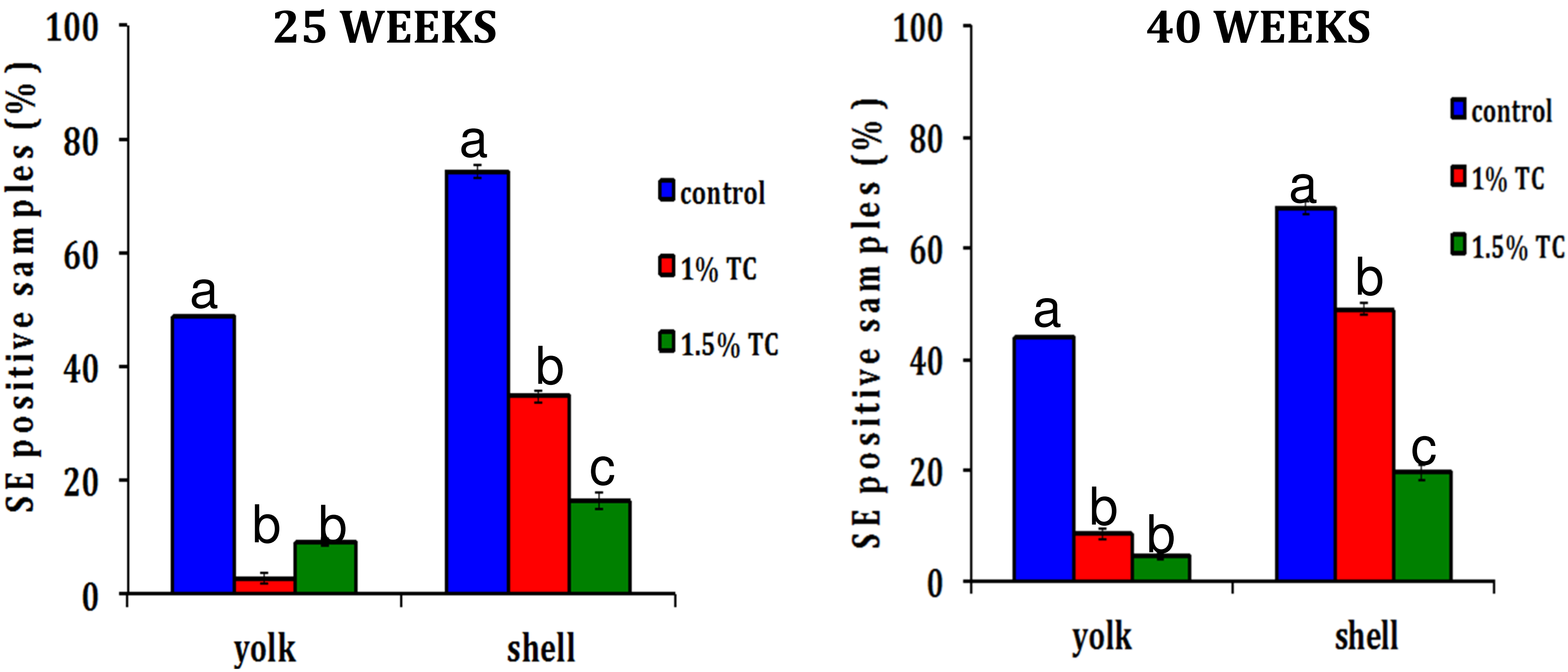
*Treatments with different superscripts significantly differed from each other at $P < 0.05$

Effect of TC on SE contamination of eggs *, week 2



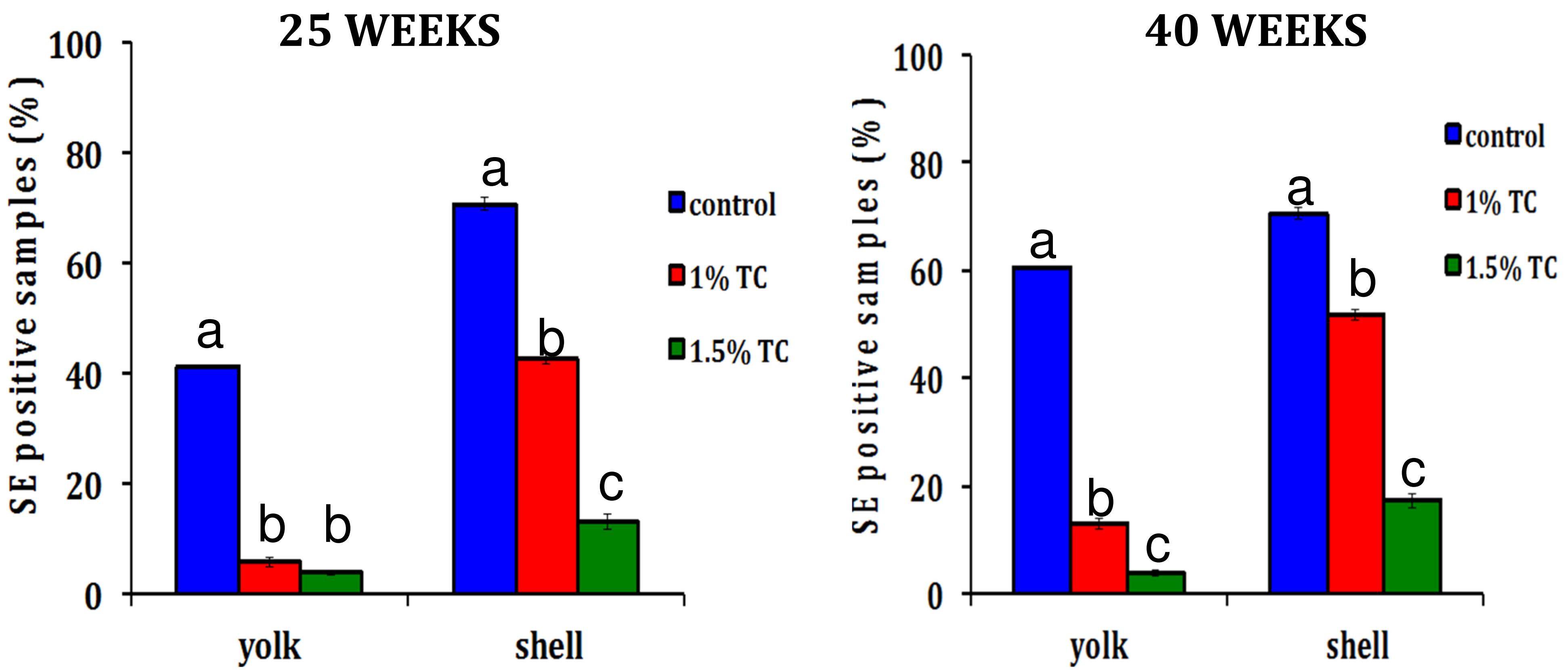
*Treatments with different superscripts significantly differed from each other at $P < 0.05$

Effect of TC on SE contamination of eggs *, week 3



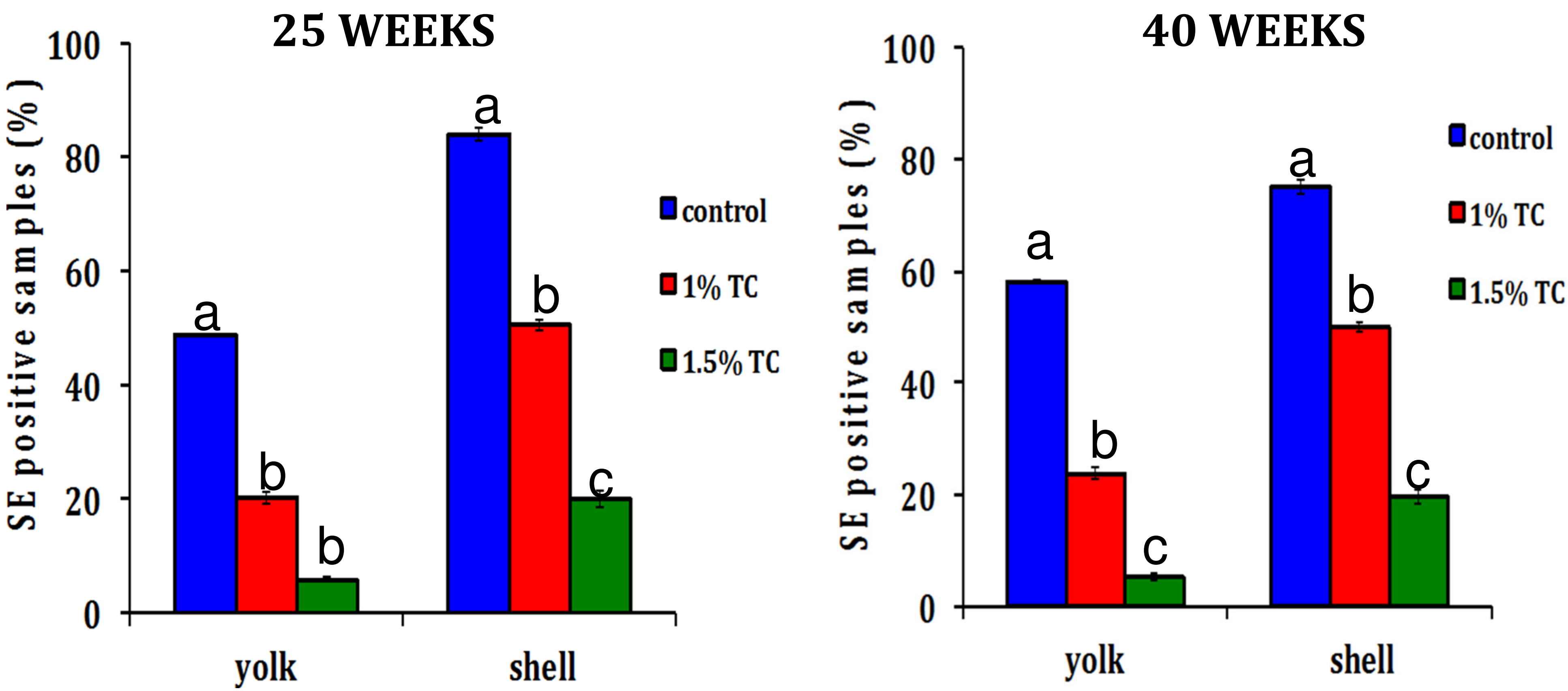
*Treatments with different superscripts significantly differed from each other at $P < 0.05$

Effect of TC on SE contamination of eggs *, week 4



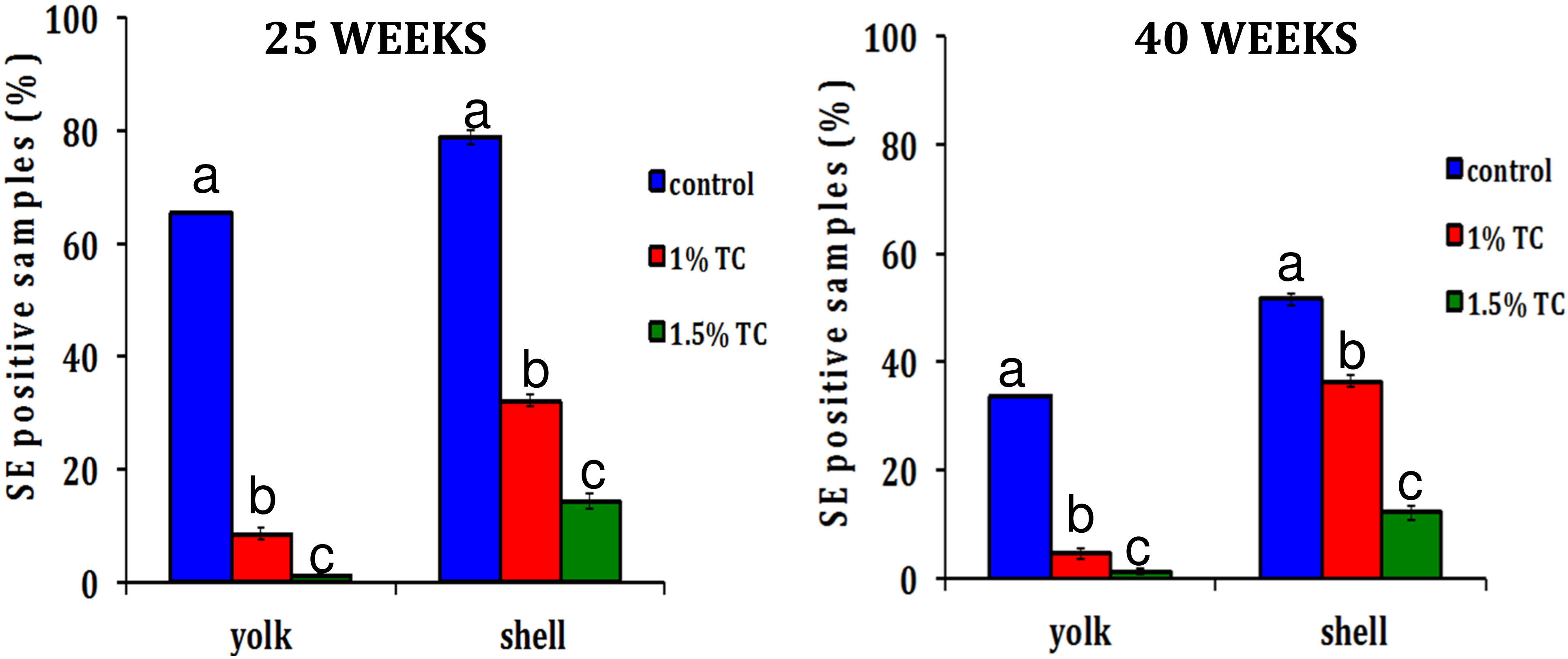
*Treatments with different superscripts significantly differed from each other at $P < 0.05$

Effect of TC on SE contamination of eggs *, week 5



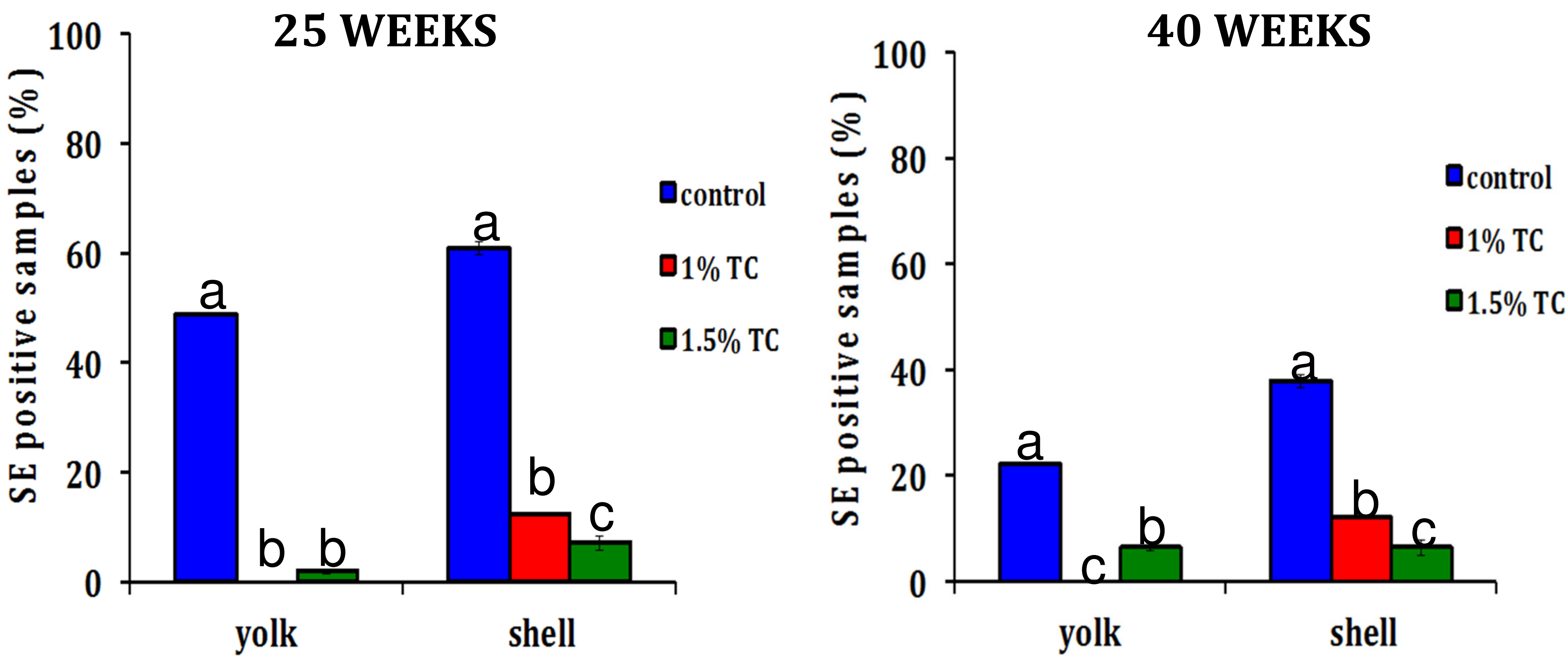
*Treatments with different superscripts significantly differed from each other at $P < 0.05$

Effect of TC on SE contamination of eggs *, week 6



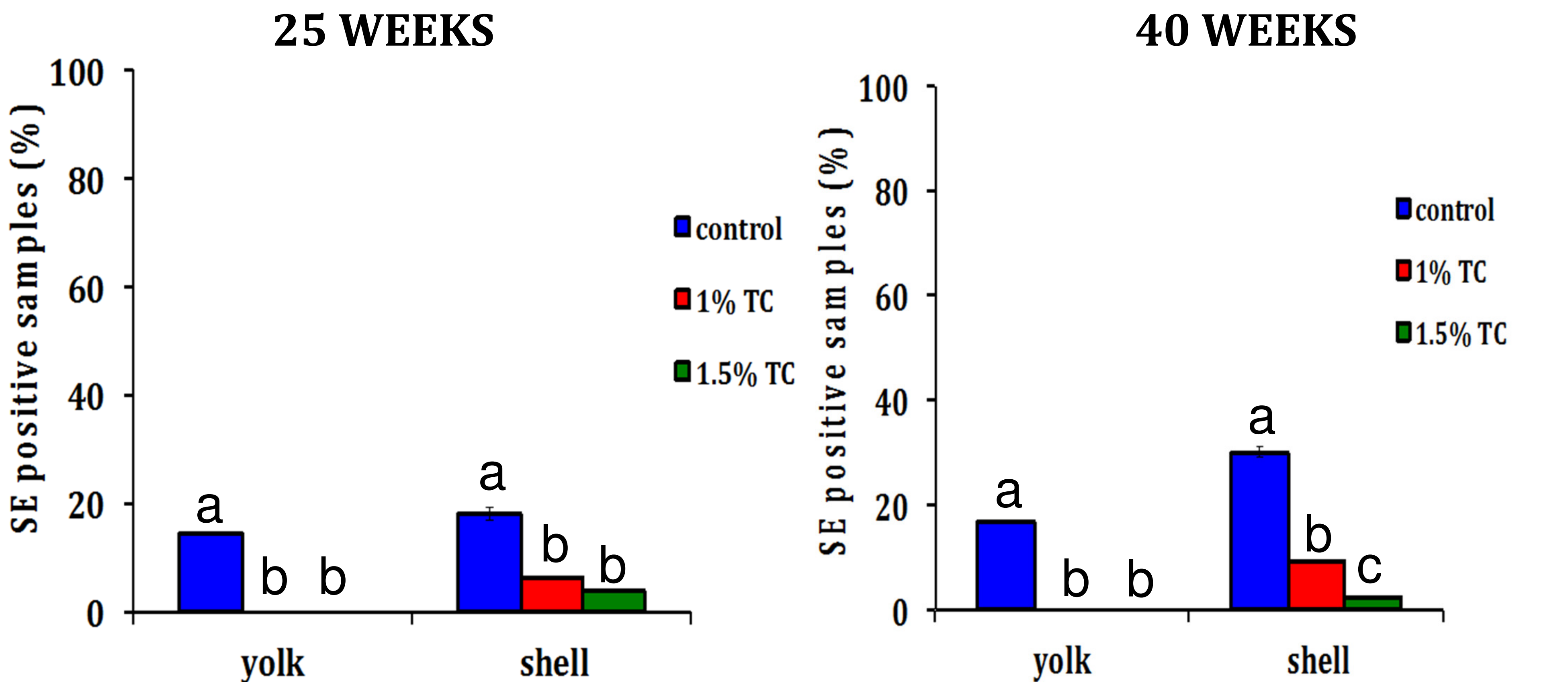
*Treatments with different superscripts significantly differed from each other at $P < 0.05$

Effect of TC on SE contamination of eggs *, week 7



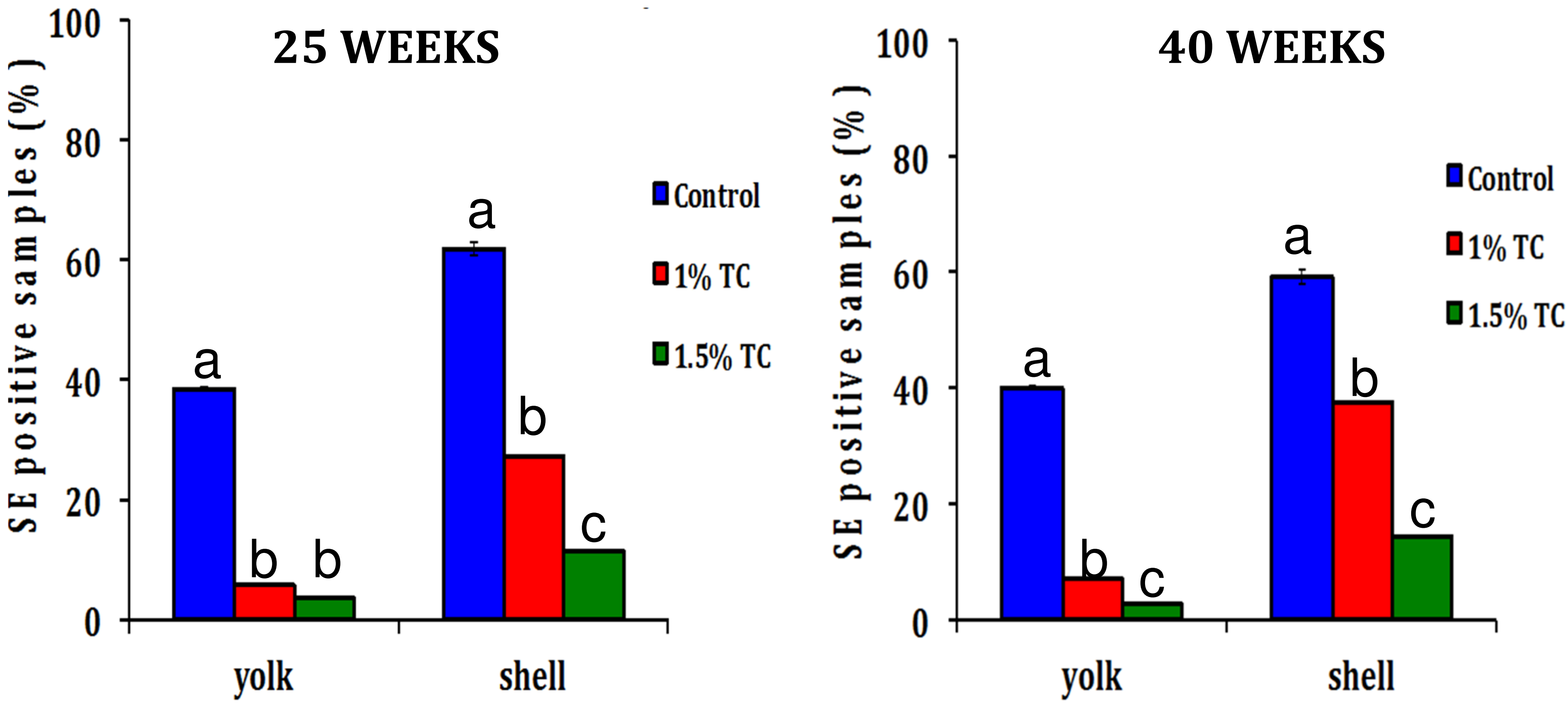
*Treatments with different superscripts significantly differed from each other at $P < 0.05$

Effect of TC on SE contamination of eggs *, week 8



*Treatments with different superscripts significantly differed from each other at $P < 0.05$

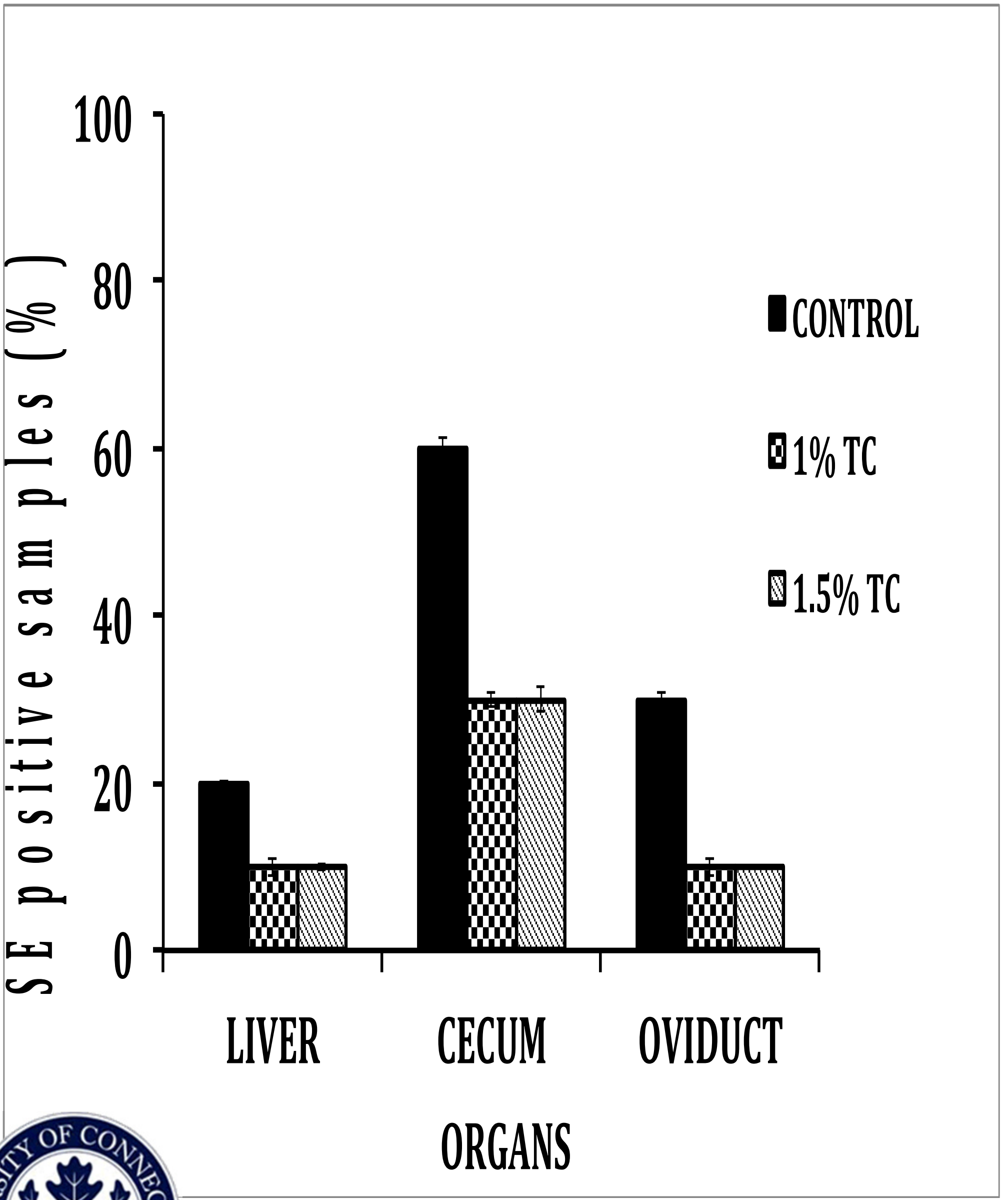
Effect of TC on SE contamination of eggs (N=2195) cumulative data

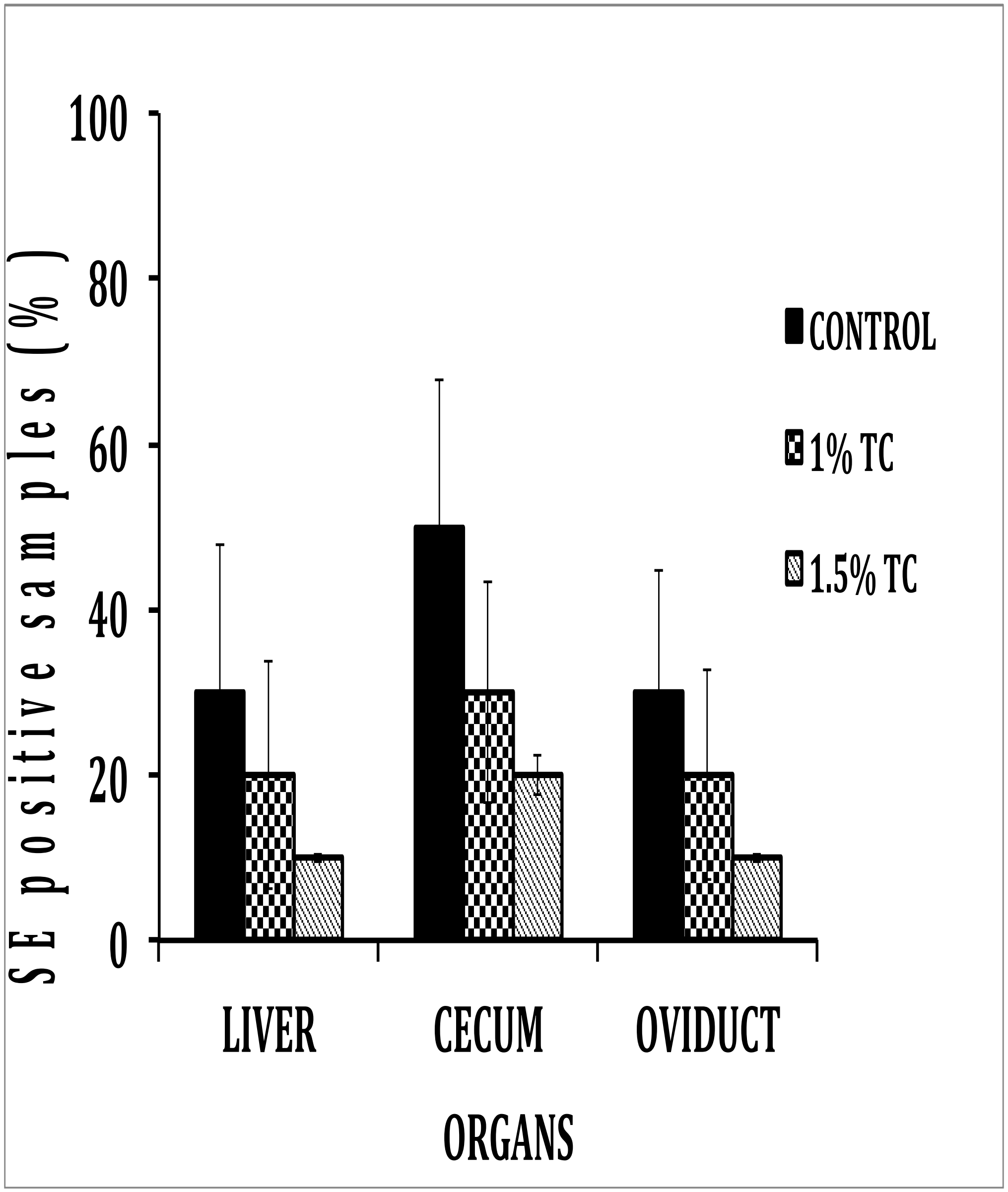


No change in egg production due to TC supplementation

*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

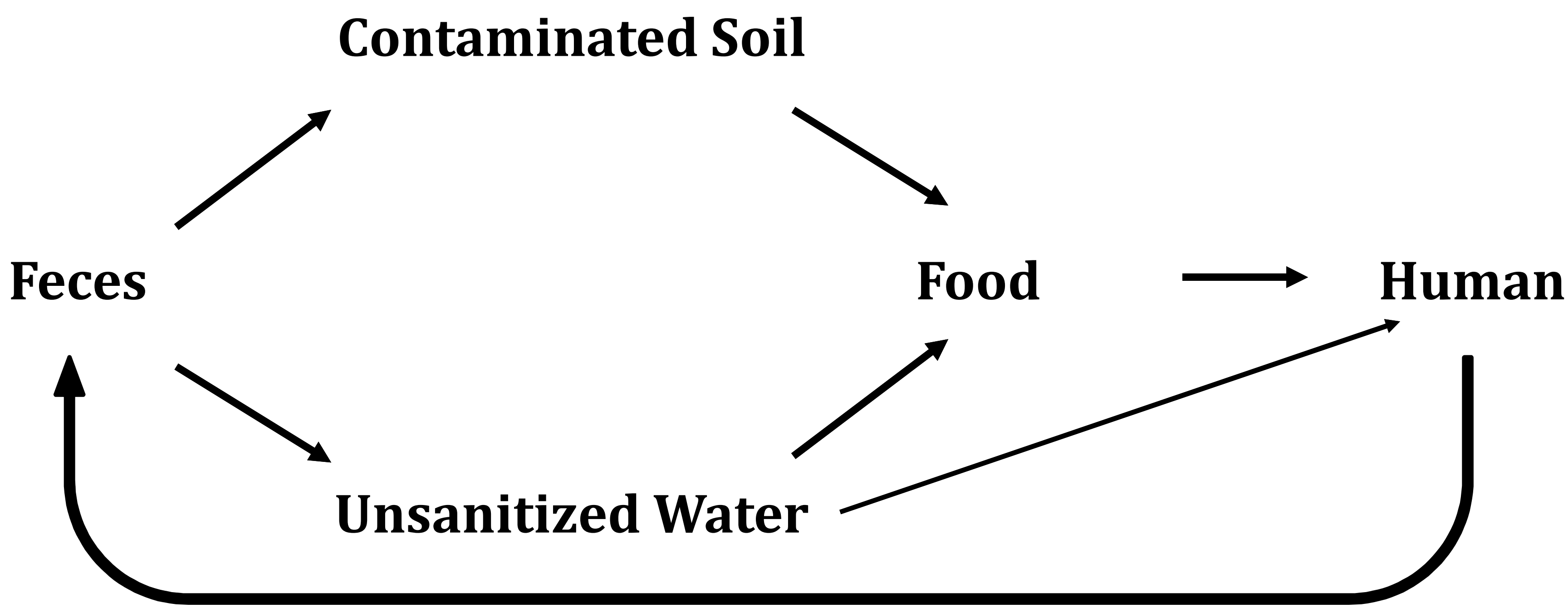
In-feed supplementation of TC could be used to reduce egg-borne transmission of SE and improve microbiological safety of eggs.

Attenuation of *Vibrio cholerae* infection using plant molecules

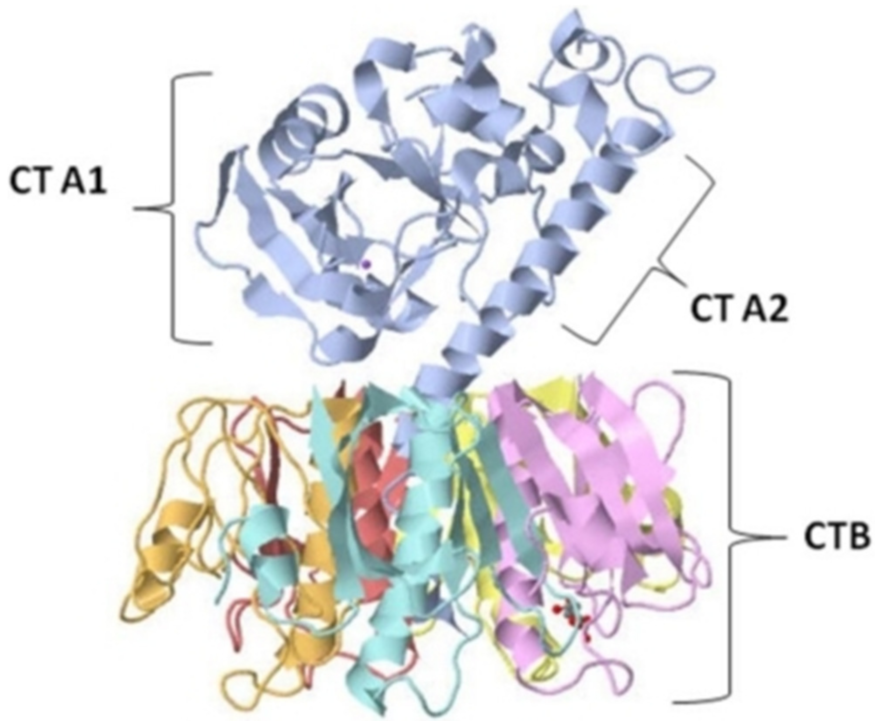
Vibrio cholerae (VC)

- Causative agent of human cholera
- Toxin-mediated watery diarrhea
- Life threatening dehydration and electrolyte imbalance
- Extreme cases lead to kidney failure and death
- Serogroups O1 and O139
- Globally - excess of 300,000 cases; 7500 deaths

Cholera: Route of Transmission



Cholera toxin



CTB → Cholera toxin B subunit

CTA1 and CTA2 → Cholera toxin A subunit

Treatment/Control

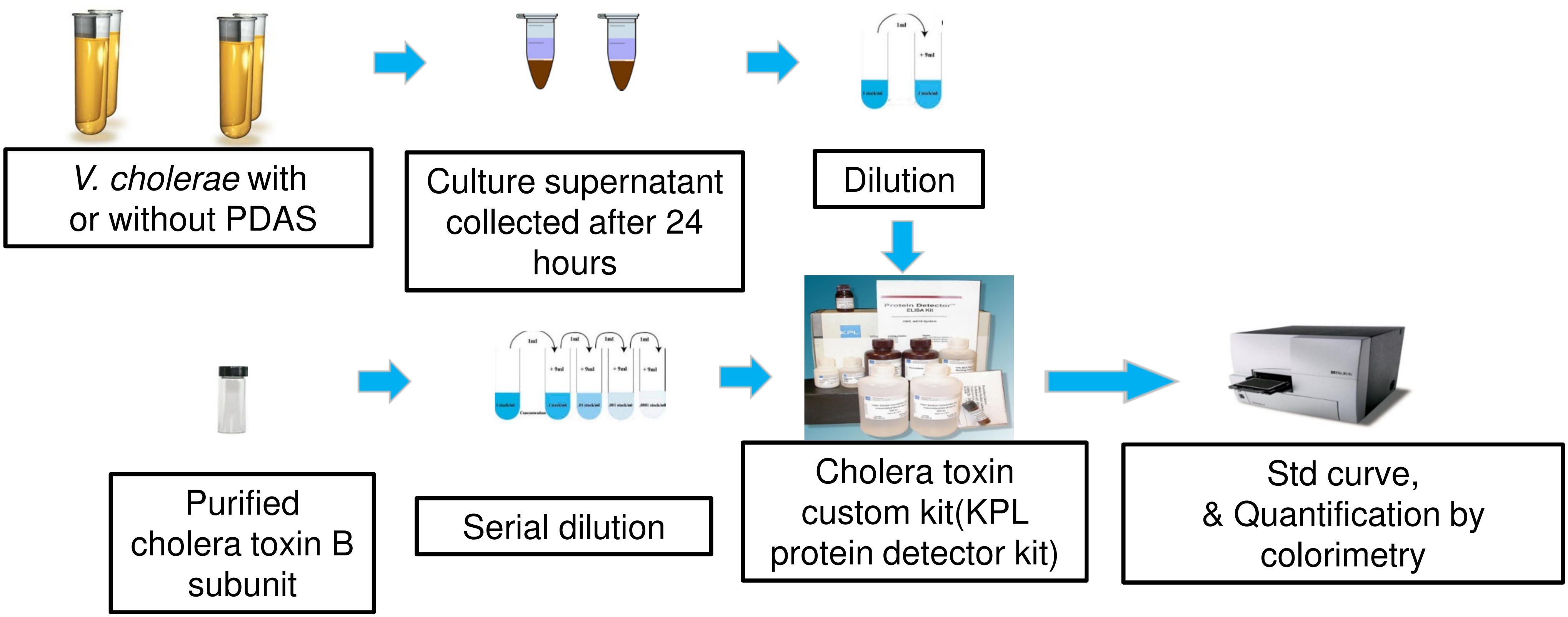
- **Oral Rehydration Therapy**
    - **Fluids supplemented with electrolytes and salts**
- **Antibiotics**
    - **First antibiotic resistant strain – 1970 (Kitaoka et al., 2011)**
    - **Multiple drug resistant *Vibrio cholerae* (Das et al., 2008; Akoachere., 2013)**
- **Vaccine**
- **Oral vaccine – Not fully effective (WHO Guidelines, 2012)**

Safe and Effective alternative strategy needed

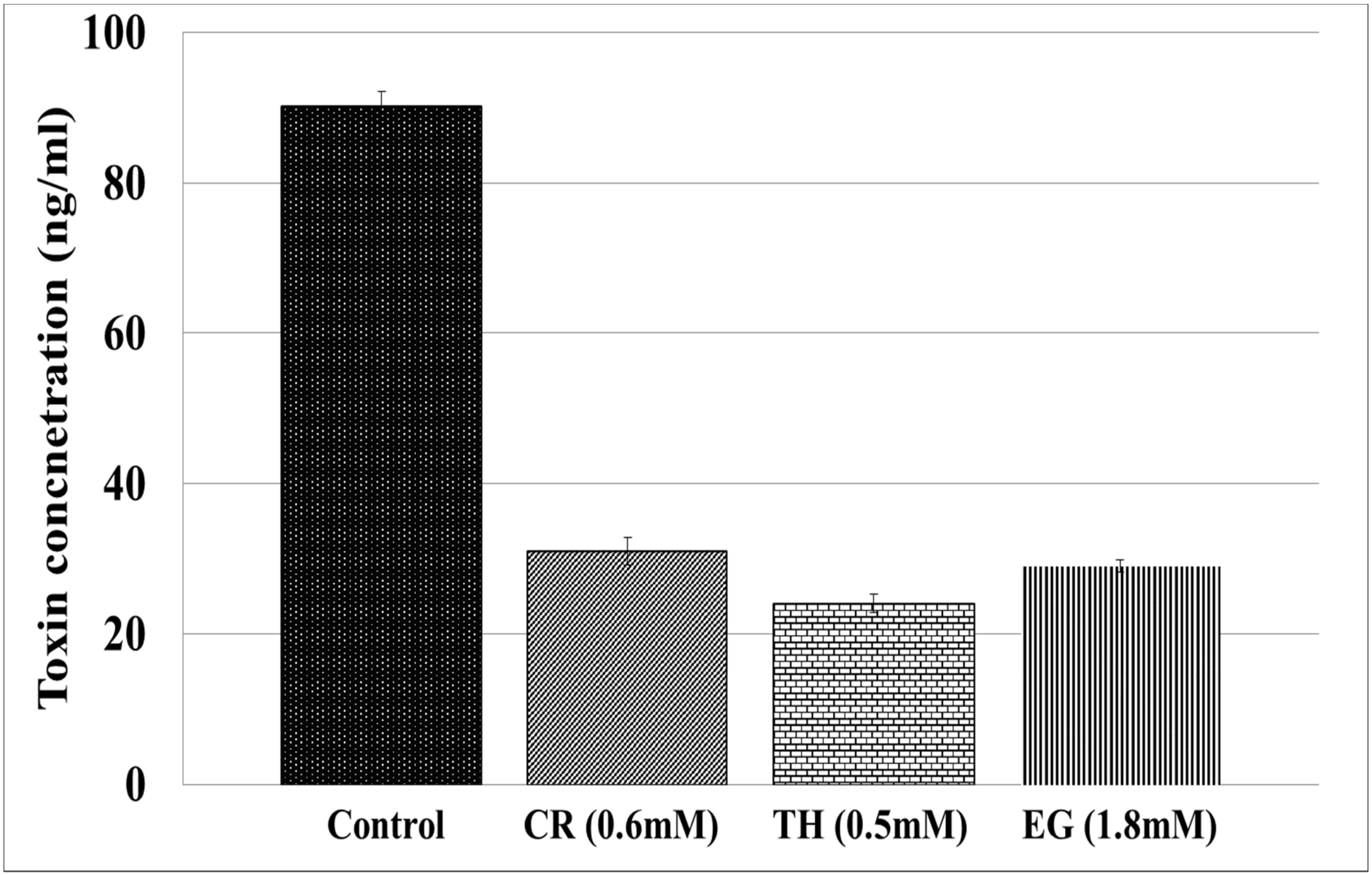
Effect of CR, TH and EG on cholera toxin production

Strains of *Vibrio cholerae* used in this study – VC 11623; BAA-25870 and BAA 2163

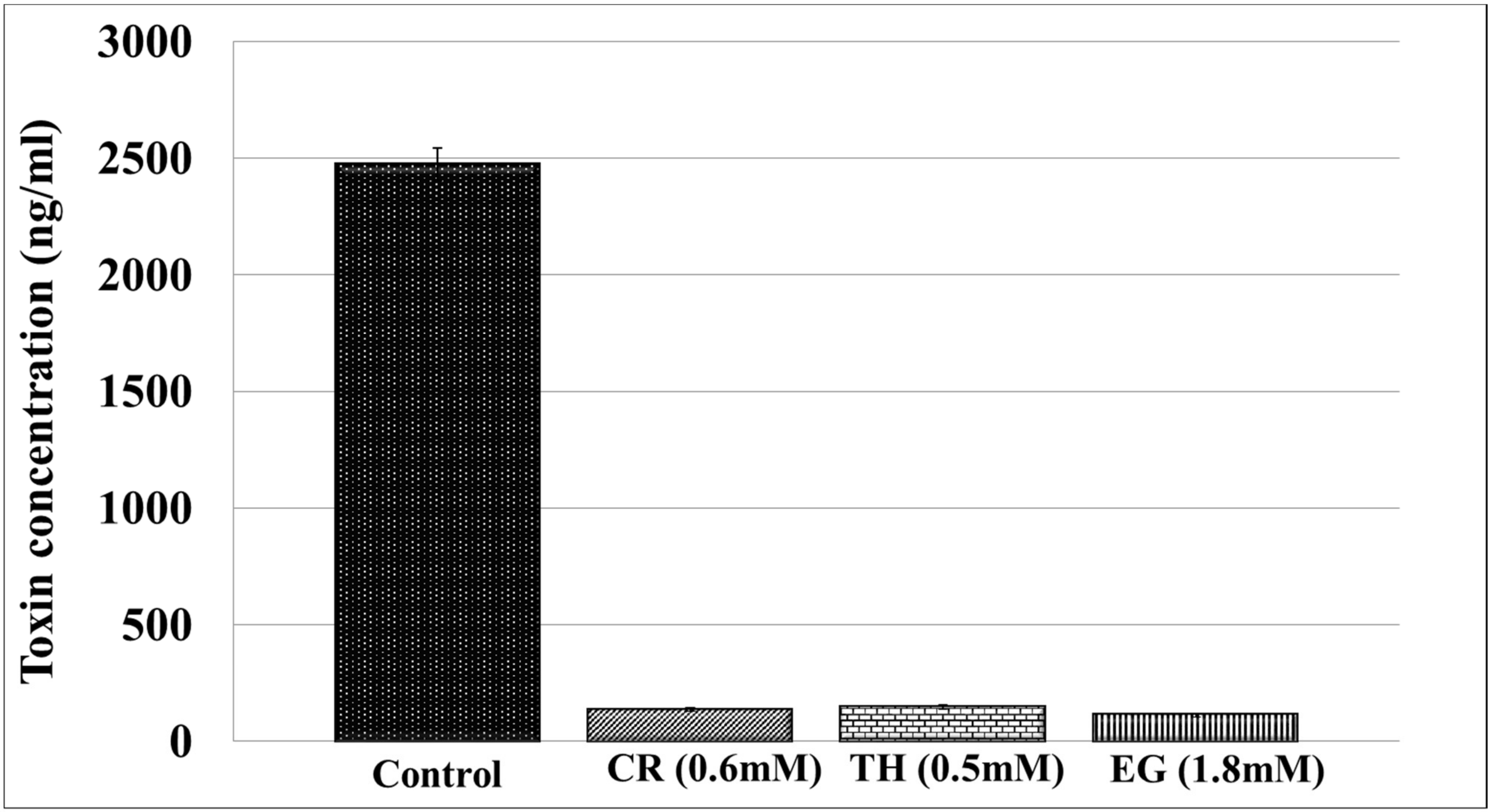
Protocol for quantification of cholera toxin



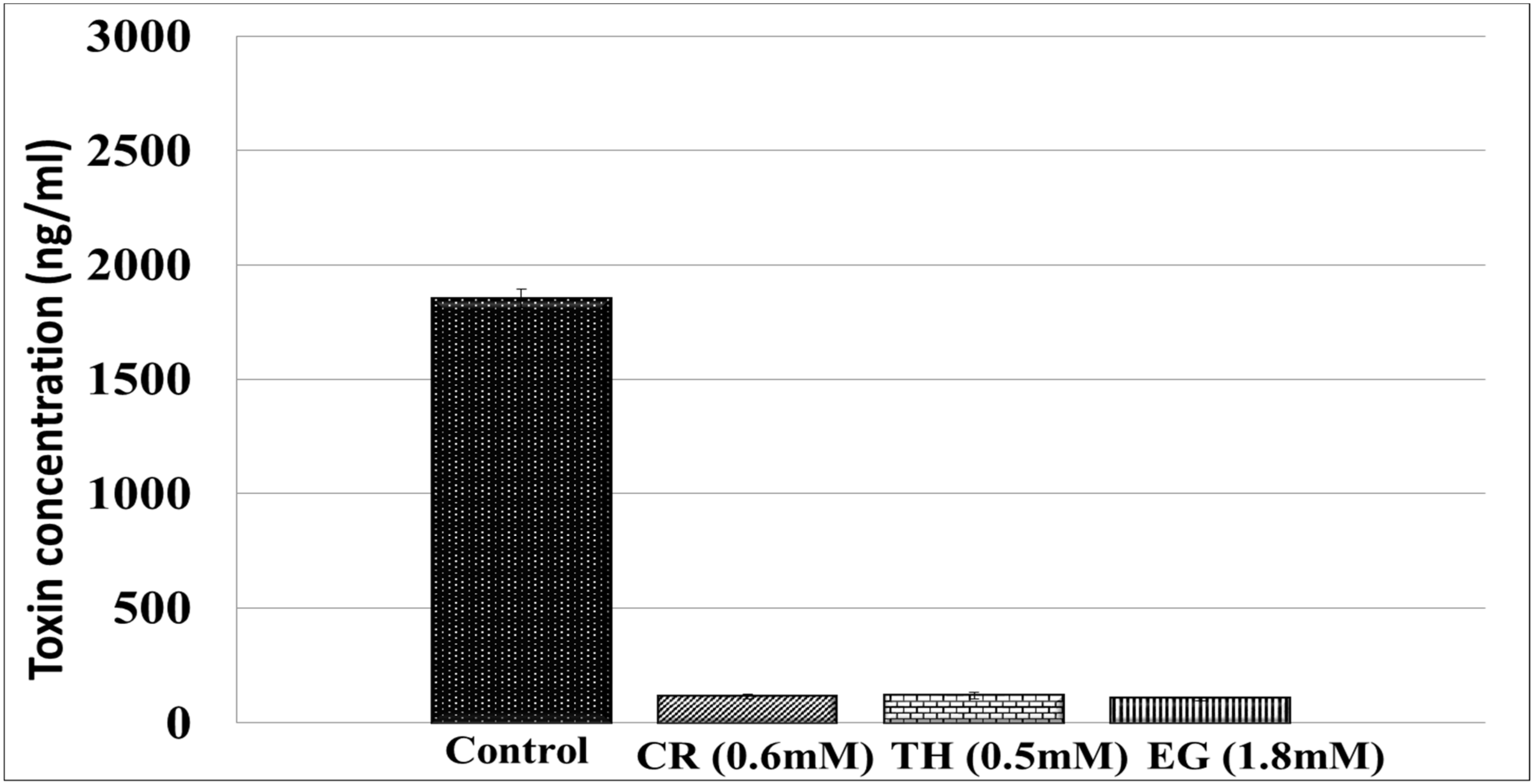
Effect of CR, TH and EG on cholera toxin production (VC 11623)



Effect of CR, TH and EG on cholera toxin production (VC 569b)



Effect of CR, TH and EG on cholera toxin production (VC 2163)



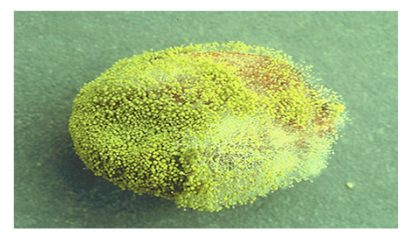
Summary and Future Studies

- 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

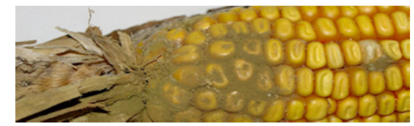
Controlling aflatoxins in chicken feed using carvacrol and trans-cinnamaldehyde as feed additives

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

↓

Impact on Human health

Economic losses to producers

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

Poor body weight gain

Fatty liver syndrome

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

↓

Incubated at 25°C for 3 months

↓

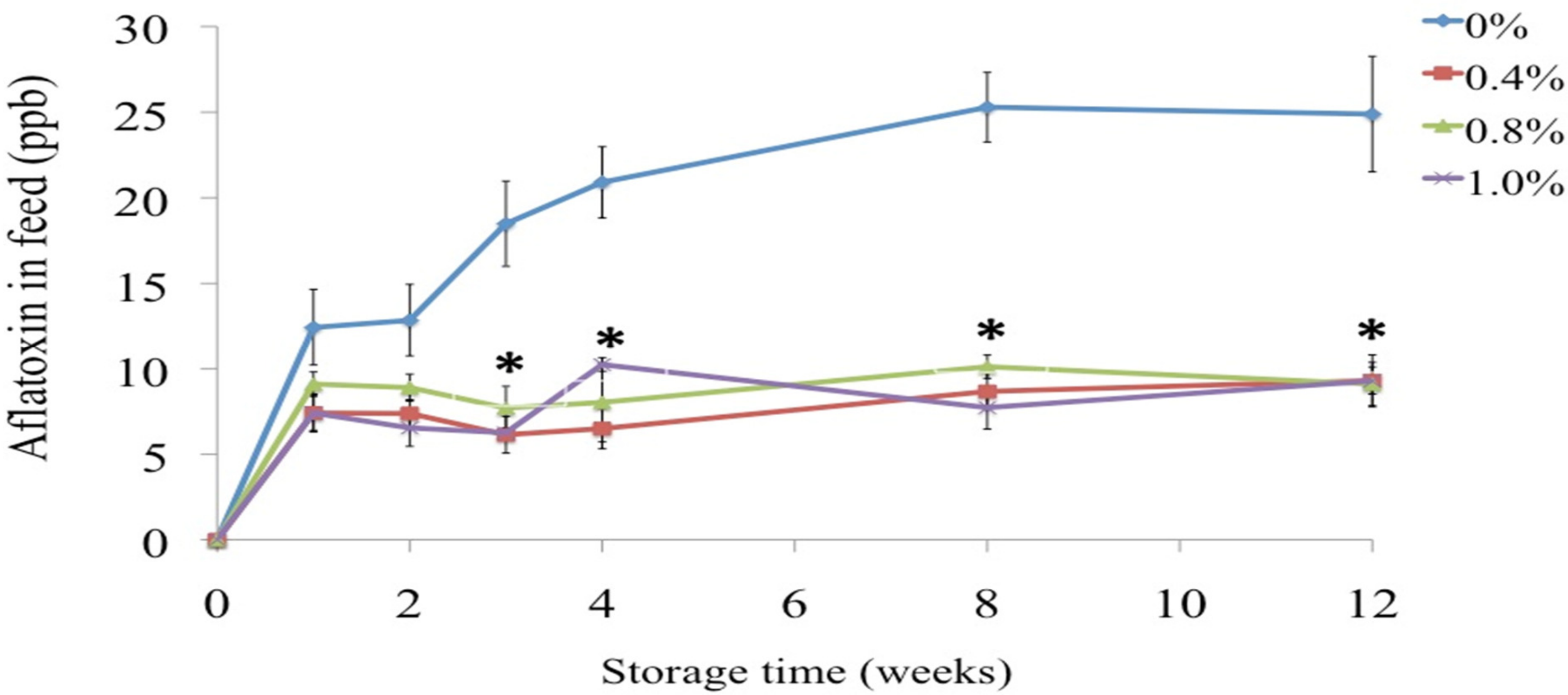
0, 1, 2, 3, 4, 8, 12 weeks

↓

Aflatoxin concentration

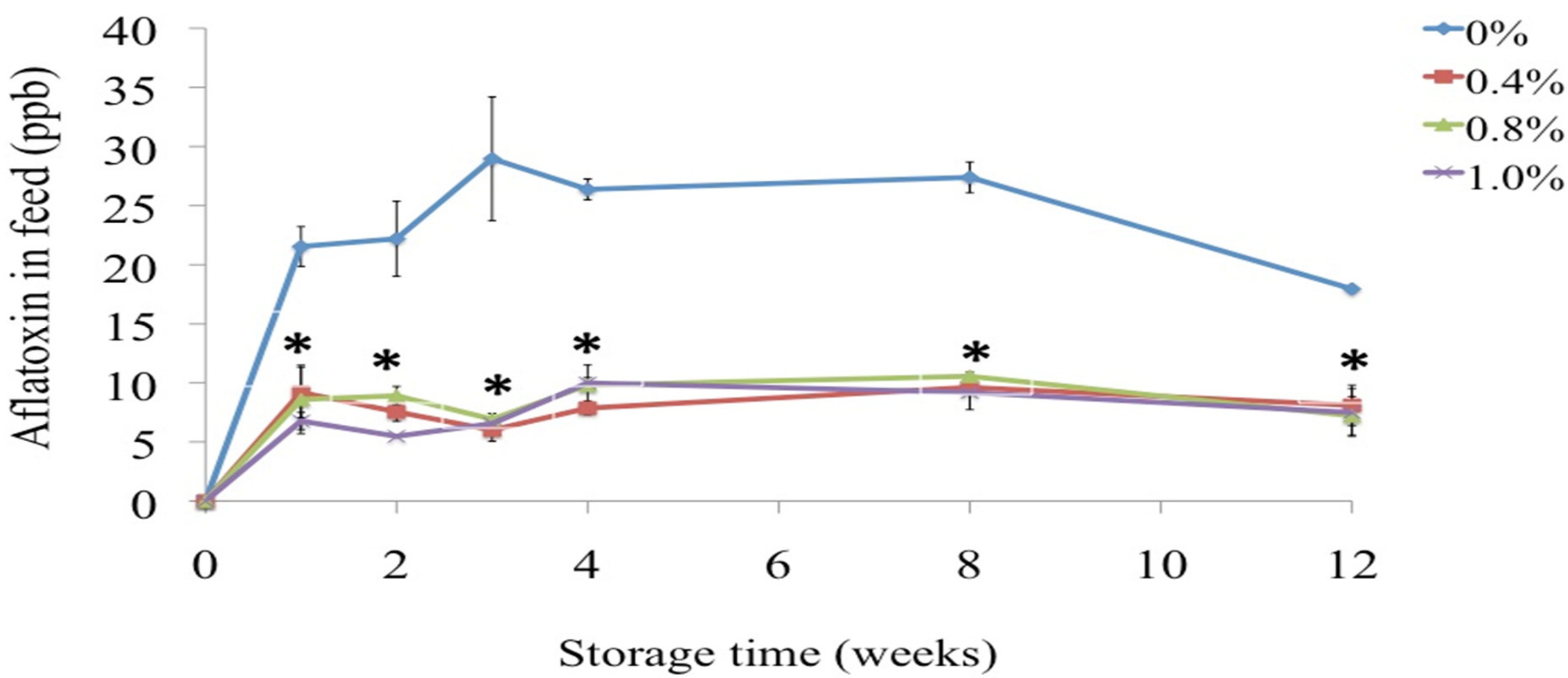
Farag et al., 1989; Razzaghi-Abyaneh et al., 2008

Effect of CR on *A. flavus* aflatoxin production in chicken feed



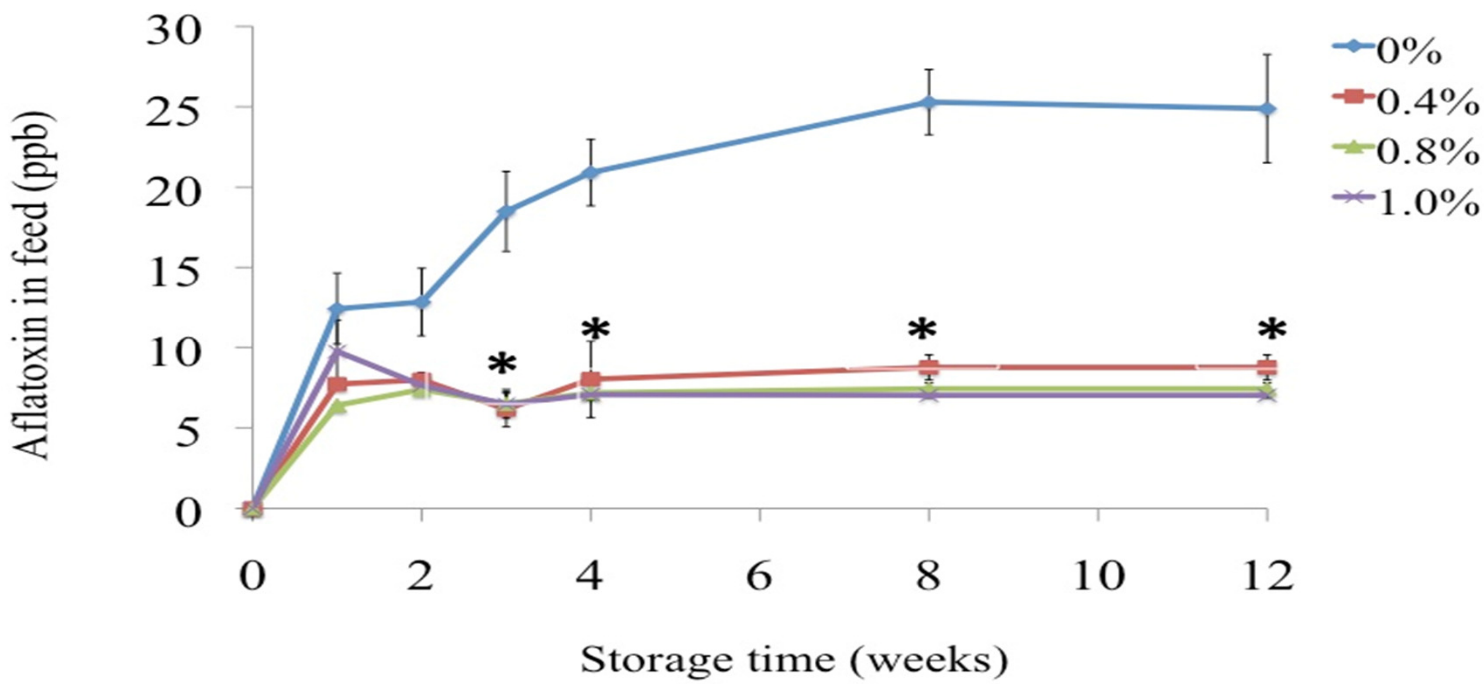
*Treatments differed significantly from the control (n = 6) ($p<0.05$)

Effect of CR on *A. parasiticus* aflatoxin production in chicken feed



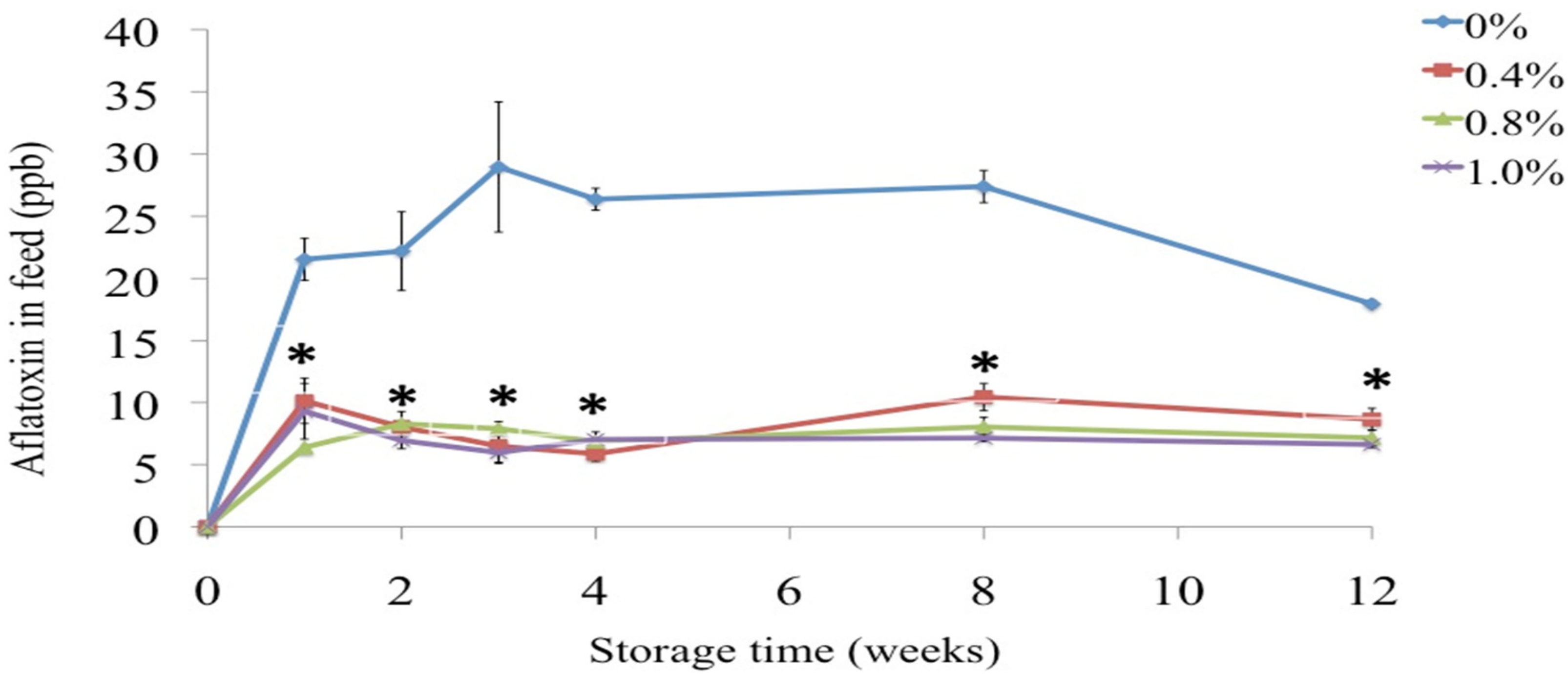
* All treatments differed significantly from the control (n = 6) (p<0.05)

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

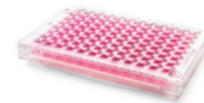
↓ Chronic Aflatoxicosis → Fatty liver

↓ Liver necrosis

Doerr et al., 1983

Materials and Methods

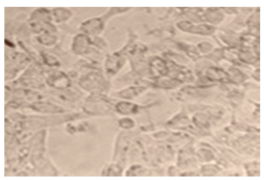
Human Hepatocytes (ATCC HepG2)

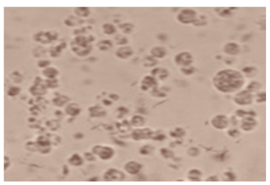


↓

24 hours incubation to form monolayer in 96-wells plate

↓





3 ppm or 10 ppm aflatoxin

↓

Control	CR	TC
0 %	0.05% & 0.1%	0.05% & 0.1%

↓

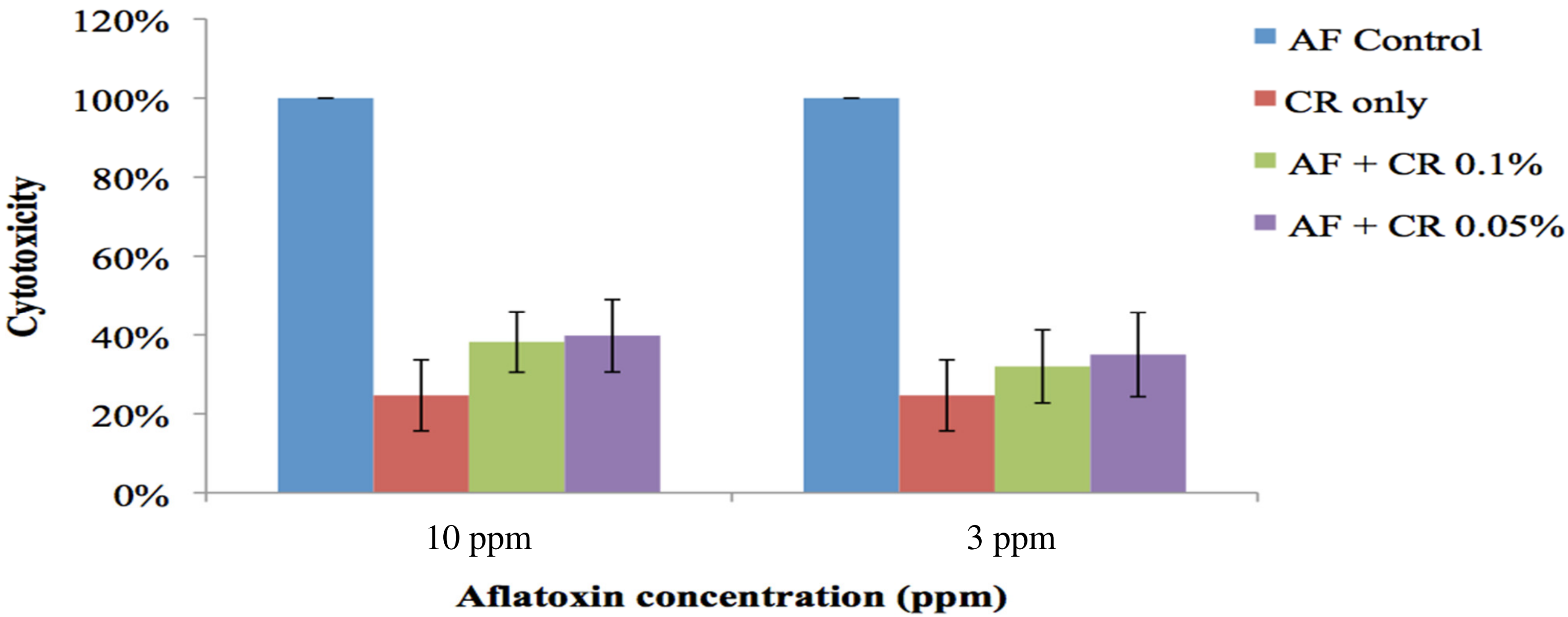
Incubate for 18 hours at 37°C

↓

Cytotoxicity % using LDH assay

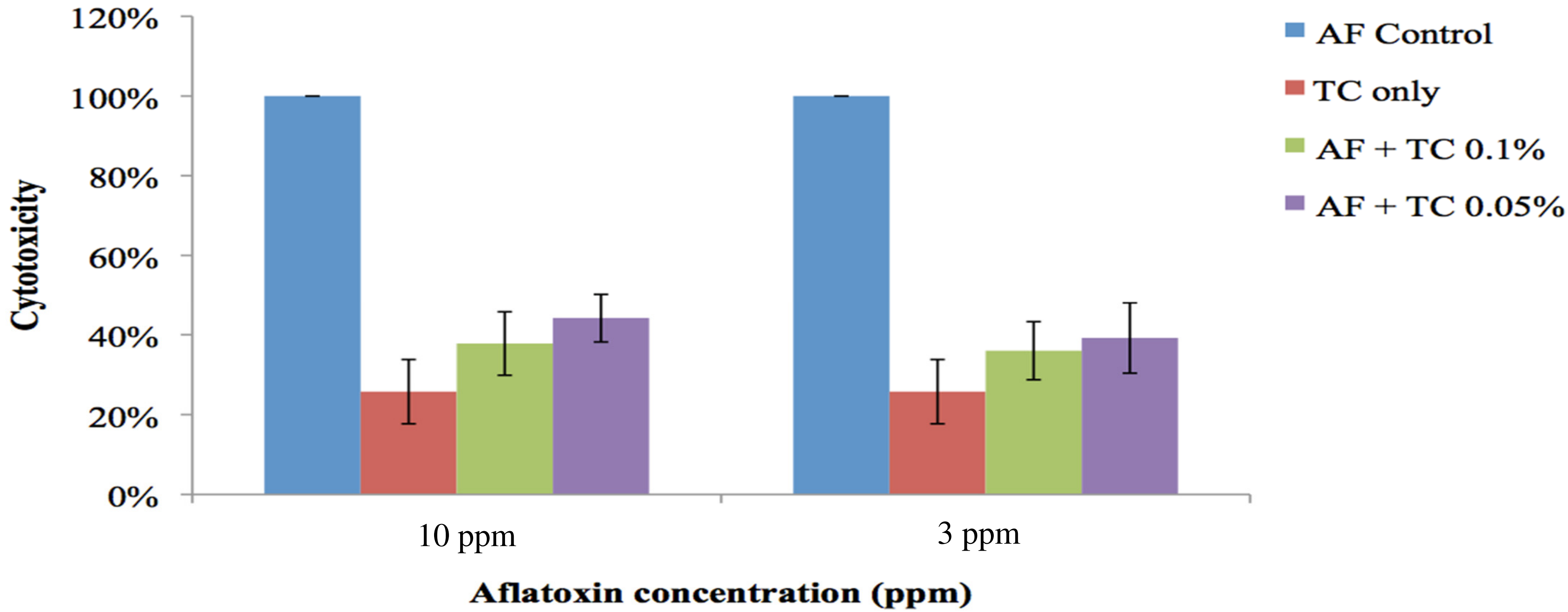
Reddy et al., 2006; Zhou et al., 2006

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

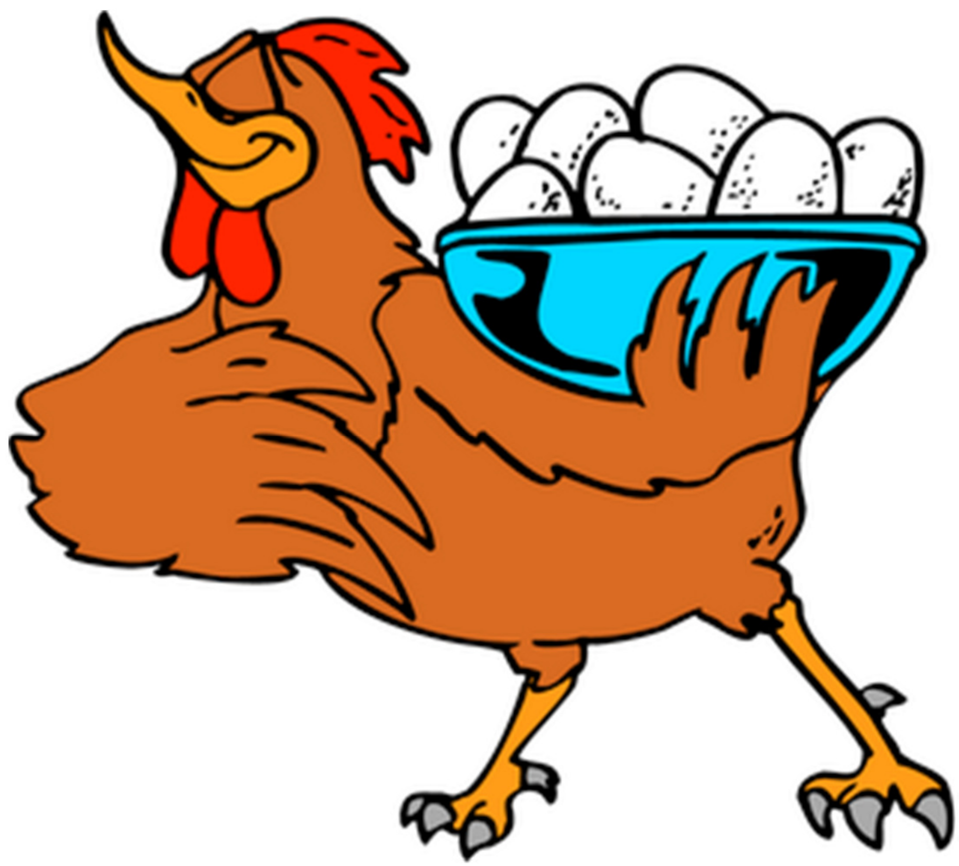
- **CR and TC reduced aflatoxins in chicken feed**
- **CR and TC did not change feed composition**
- **CR and TC significantly decreased aflatoxin-induced cytotoxicity in hepatocytes ($p < 0.05$)**

Concluding remarks

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

- **Field trials in chicken farms**

THANK YOU !



QUESTIONS?