Phytochemicals reduce *Campylobacter jejuni* colonization factors and transcription of virulence genes *in vitro*.

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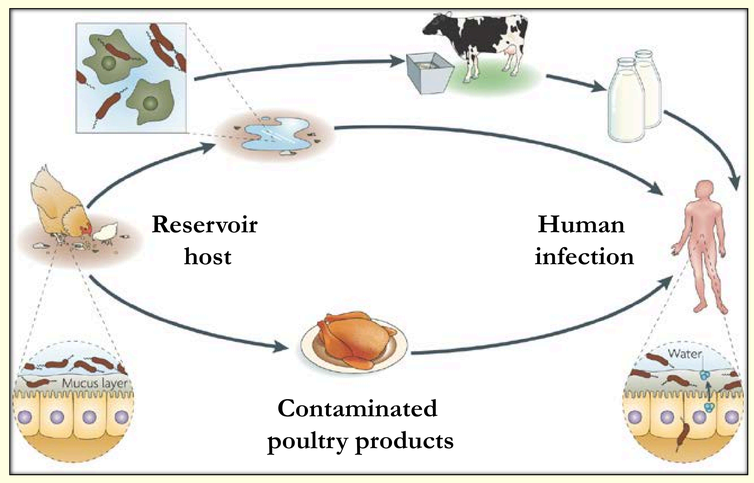
ABSTRACT

Background: The foodborne pathogen *Campylobacter* is the leading cause of bacterial gastroenteritis in humans resulting in an estimated 96 million annual infections globally. In the United States, an estimated 1.3 million cases of Campylobacteriosis occur each year largely due to consumption of contaminated poultry products. Chicken act as the reservoir host for Campylobacter, wherein the pathogen colonizes the intestine especially the ceca thereby leading to contamination of carcass during slaughter. Motility and attachment to intestinal epithelium are the two major factors responsible for Campylobacter colonization in chicken. Reducing the expression of aforementioned factors could potential reduce Campylobacter survival in chickens and risk of subsequent human infections.

Objectives: This study investigated the efficacy of sub-inhibitory concentrations (SICs, concentration not inhibiting bacterial growth) of three, generally regarded as safe (GRAS)-status phytochemicals **(trans-cinnamaldehyde 0.01%, carvacrol 0.002%, eugenol 0.01%)** in reducing the major colonization factors (motility, epithelial adherence) critical for survival of *C. jejuni* in chickens. In addition, the effect of the aforementioned phytochemicals on the expression of critical colonization genes was studied using real-time quantitative PCR. All experiments had duplicate samples and were replicated three times on three strains of *C. jejuni* (Wild type S8, NCTC 11168, 81-176). Data were analyzed using ANOVA with GraphPad ver. 6. Differences between the means were considered significantly different at $P<0.05$.

Results: All phytochemicals reduced *C. jejuni* motility, and adhesion to chicken primary enterocytes ($P<0.05$). Real-time PCR revealed that *trans*-cinnamaldehyde and eugenol reduced the transcription of select *C. jejuni* chicken colonization genes as compared to controls ($P<0.05$). Results suggest that *trans*-cinnamaldehyde, carvacrol, and eugenol could potentially be used to control *Campylobacter* colonization in chickens and reduce the incidence of human foodborne illnesses.

CAMPYLOBACTER ROUTES OF INFECTION[1]

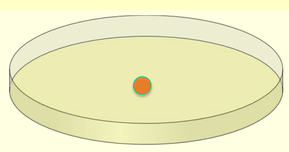


OBJECTIVE: To investigate the efficacy of the phytochemicals *trans*-cinnamaldehyde (0.01%), carvacrol (0.002%), and eugenol (0.01%) in reducing chicken colonization factors and expression of virulence genes in *C. jejuni*

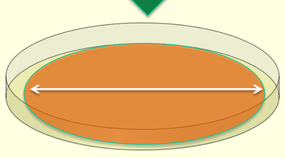
MATERIALS & METHODS

Effect of phytochemicals on *C. jejuni* motility[2] (Fig. 1)

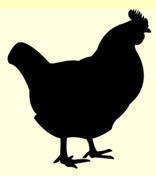
Motility agar (0.3%) — 5 µl *C. jejuni* (~6 log CFU)

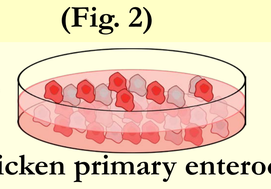


Microaerophilic Incubation (42°C for 24 h)



Effect of phytochemicals on *C. jejuni* adhesion to chicken primary enterocytes[3] (Fig. 2)





Chicken primary enterocytes

C. jejuni (≈6 log CFU) inoculated (MOI 10:1) in presence or absence of phytochemicals followed by enumeration of attached bacteria

Effect of phytochemicals on expression of *C. jejuni* colonization and virulence genes[4] (Fig.3)

C. jejuni (≈6 log CFU/ml) cultured in presence or absence of phytochemicals to mid-log phase (10 h) at 42°C

RNA extraction and cDNA synthesis

Real-time Quantitative PCR

RESULTS

Fig.1. Effect of phytochemicals on *C. jejuni* (wild type-S8) motility

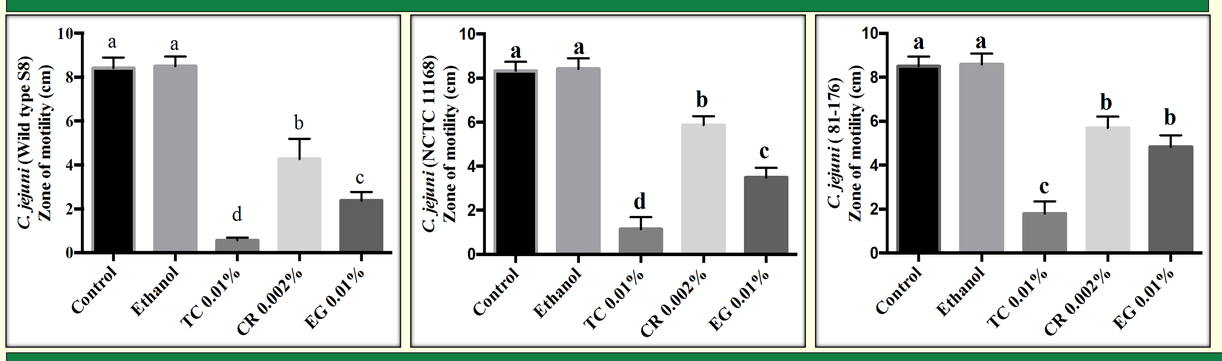


Fig.2. Effect of phytochemicals on *C. jejuni* (wild type-S8) attachment

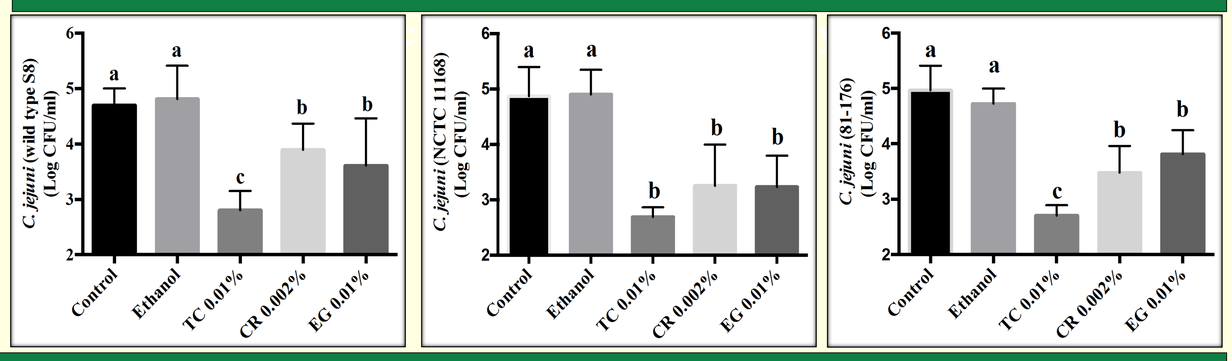
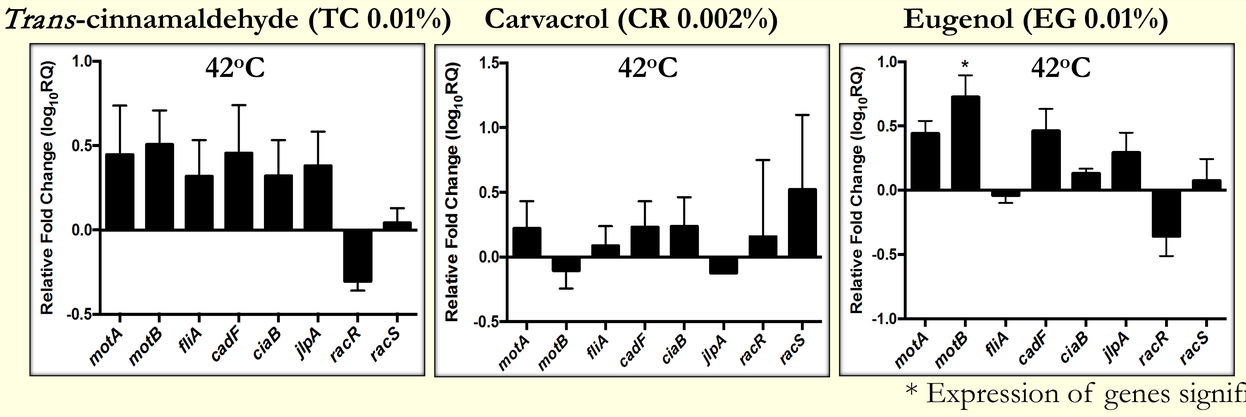


Fig.3. Effect of phytochemicals on expression of *C. jejuni* (wild type-S8) virulence genes at chicken and human body temperatures*



* Expression of genes significantly different from control

SUMMARY OF RESULTS

- All the phytochemical treatments (*trans*-cinnamaldehyde 0.01%, carvacrol 0.002%, eugenol 0.01%) reduced *C. jejuni* motility at 42°C ($p<0.05$).
- All the phytochemical treatments (*trans*-cinnamaldehyde 0.01%, carvacrol 0.002%, eugenol 0.01%) reduced attachment of *C. jejuni* to primary chicken enterocytes ($p<0.05$).
- *Trans*-cinnamaldehyde (0.01%) and eugenol (0.01%) significantly down-regulated the transcription of select *C. jejuni* virulence genes at 37°C ($p<0.05$).

CONCLUSION

- Phytochemicals such as *trans*-cinnamaldehyde, carvacrol, and eugenol can potentially be used as feed supplements to control *C. jejuni* colonization in chickens.
- Follow up mechanistic studies using transcriptomics and microbiome analysis are warranted to elucidate the mechanism(s) of action of phytochemicals and effect on gut microflora in chickens.

REFERENCES

1. Niu C, Gilbert ES. Applied and Environmental Microbiology. 2004 Dec 1;70(12):6951-6. (2) Moroni et al., Applied and Environmental Microbiology. 2006 Nov 1;72(11):6894-901. (3) Upadhyay A et al., International Journal of Food Microbiology. 2012 Jun 15;157(1):88-94. (4) Young et al., Nature Reviews Microbiology. 2007 Sep 1;5(9):665-79. (5) Byrd et al., 2001. Poult. Sci. 80:278-283.