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ISSN 2377-8350 Open Journal http://dx.doi.org/10.17140/AFTNSOJ-1-119

Research

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Volume 1 : Issue 5
Article Ref. #: 1000AFTNSOJ1119

Article History
Received: October 15th, 2015
Accepted: October 21st, 2015
Published: October 27th, 2015

Citation
Arambel HR, Donoghue AN, Arsi K, et al. Chitosan supplementation reduces enteric colonization of *Campylobacter jejuni* in broiler chickens and down-regulates expression of colonization genes. *Adv Food Technol Nutr Sci Open J*. 2015; 1(5): 104-111. doi: 10.17140/AFTNSOJ-1-119

Chitosan Supplementation Reduces Enteric Colonization of *Campylobacter jejuni* in Broiler Chickens and Down-Regulates Expression of Colonization Genes

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ABSTRACT

Campylobacter is one of the leading causes of foodborne bacterial gastroenteritis worldwide, and poultry is considered as the most common source of human infections. *Campylobacter* is prevalent in most poultry flocks and a reduction of *Campylobacter* in poultry would greatly reduce the risk of campylobacteriosis in humans. Unfortunately, efforts to reduce *Campylobacter* in pre-harvest poultry have been met with limited success. Preliminary studies with the natural compound, chitosan, demonstrate its ability to kill *Campylobacter*, *in vitro*. The purpose of this study was to determine the ability of feed supplemented chitosan to reduce enteric *Campylobacter* colonization in broiler chickens. Additionally, the effect of chitosan on expression of *Campylobacter's* chicken colonization genes was investigated using real-time quantitative PCR (RT-qPCR). Because chitosan's antimicrobial properties may vary depending upon its molecular weight, selected doses of three molecular weight chitosans were supplemented in the feed and evaluated for efficacy to reduce *Campylobacter* in chickens. Three replicate trials were conducted, and in each trial, birds were divided into 10 treatments (n=10 birds/treatment) and were fed 0% (controls), 0.25%, 0.5% or 1% (wt./wt.) of a low, medium or high molecular weight chitosan. Birds were fed treated feed for the duration of the study and orally challenged with a four-strain mixture of wild type *C. jejuni* on day 6. On day 15, the ceca samples were collected for enumeration of *Campylobacter*. In all three trials, the 0.5% dose of the medium molecular weight chitosan reduced cecal *Campylobacter* counts ($P<0.05$). RT-qPCR analysis revealed that chitosan down-regulated the expression of chicken colonization genes as compared to control ($P<0.05$). These results suggest that supplementation of chitosan in feed is a potential strategy to reduce the enteric colonization of *Campylobacter* in pre-harvest chickens.

KEYWORDS: *Campylobacter jejuni*; Chitosan; Broiler chickens; Pre-harvest; Colonization gene; Real-time quantitative PCR.

ABBREVIATIONS: RT-qPCR: Real-time quantitative PCR; IBS: Irritable Bowel Syndrome; ReA: Reactive arthritis; IBD: Inflammatory Bowel Disease; BPD: Butterfield's Phosphate Diluent; NCBI: National Center for Biotechnology Information; LMW: Low Molecular Weight; MMW: Medium Molecular Weight; HMW: High Molecular Weight; ECDC: European Centre for Disease Prevention and Control; SIC: Sub-Inhibitory Concentration.

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INTRODUCTION

Campylobacter is one of the most frequently reported food-borne pathogens and causes an estimated 1.3 million infections in the United States annually.[1] While the majority of *Campylobacter* cases result in acute gastroenteritis, infection has also been associated with more severe diseases, including Guillain-Barré syndrome, Reactive arthritis (ReA), Irritable Bowel Syndrome (IBS), and Inflammatory Bowel Disease (IBD).[2] Epidemiological evidence indicates that the most common source for *Campylobacter* infections in humans is due to consumption of poultry products.[3] This is typically caused by the consumption of improperly cooked chicken or cross-contamination from handling raw chicken.[1,4] *Campylobacter* colonization in poultry is common; as many as 90% of US broiler flocks are contaminated with this food-borne pathogen.[5] Therefore, a reduction or elimination of *Campylobacter* in poultry is a research priority to reduce the risk of infection in humans. Many pre-harvest strategies have been evaluated for reducing *Campylobacter* in poultry, such as bio-security, probiotics, competitive exclusion, bacteriocins, bacteriophages, vaccines, and natural compounds, often with limited success.[6] Recently, the natural product chitosan has shown potential to reduce colonization of another food-borne pathogen, *Salmonella* Typhimurium, in pre-harvest poultry[7] and may have application against *Campylobacter*. Chitosan has also shown efficacy against other Gram-negative species, including *Escherichia coli* and *Pseudomonas fluorescens*.[8,9] Chitosan, a natural by-product derived from the deacetylation of chitin, is obtained from crab and shrimp shell waste.[10,11] Chitosan is a potential natural food preservative with broad antimicrobial benefits.[8,12,13] Although the exact mode of action of chitosan is not completely understood, researchers have previously determined that chitosan is capable of interacting with the outer cell membrane of bacterial pathogens, altering its permeability, disrupting cellular physiology and causing cell death.[9,14] To our knowledge, the ability of chitosan to reduce *Campylobacter* colonization in poultry has not been evaluated. The purpose of this study was to determine the efficacy of in-feed supplementation of chitosan on *Campylobacter* colonization in broiler chicks. Young chickens were used in this study because previous results from our laboratory demonstrated that young birds can be used as a reliable model to study *Campylobacter* colonization in market age birds.[15,16]

MATERIALS AND METHODS

Chitosan Materials

Chitosan of molecular weight 50-190 kDa and 190-310 kDa was obtained from Sigma-Aldrich (St. Louis, MO, USA), and 400-600 kDa chitosan was purchased from Spectrum Chemicals (New Brunswick, NJ, USA).

In vitro Susceptibility of *C. jejuni* to Chitosan

Antimicrobial activity of each molecular weight chitosan, low (50-190 kDa), medium (190-310 kDa) and high (400-600 kDa), in a 0.5% (wt./vol.) solution was determined by inoculating each solution with a four-strain mixture of wild-type *C. jejuni*. Preparation of the *Campylobacter* inoculum was done as described previously by Farnell and others.[17] In brief, working stock cultures of the four wild-type strains of *C. jejuni* were obtained by individually inoculating each strain into fresh *Campylobacter* Enrichment Broth (CEB, Acumedia, Neogen Corporation, Lansing, MI, USA) from frozen glycerol stock and successively sub-culturing twice at 42 ºC for 48 h under microaerophilic conditions. Strain mixtures were then combined centrifuged at 3000 * g for 10 minutes and the cell pellet re-suspended in 10 mL Butterfield's Phosphate Diluent (BPD). A 1% stock solution (wt./vol.) of each molecular weight of chitosan was prepared in 50 mM acetic acid as described by Ganan and others.[18] For the experiment, the stock concentration of each of the chitosan solutions and the acetic acid control was diluted 1:1 with an inoculum containing 10^8 CFU/mL of *C. jejuni*, resulting in a final concentration of 0.5% for each chitosan. Sample time points included 0, 2, 4 and 8 h post inoculation. At each time point, an aliquot from the treatments and control was taken and 1:10 serial dilutions were direct plated on Campy Line Agar.[19] The plates were incubated for 48 h at 42 ºC in a microaerophilic atmosphere. Direct enumeration of *Campylobacter* colonies was converted to CFU/mL for each treatment. Each susceptibility assay was repeated in duplicate.

In vivo Susceptibility of *C. jejuni* to Chitosan

Day of hatch Cobb broiler chicks (Siloam Springs, AR, USA) from a local commercial hatchery were utilized for the animal experiments. In each of three replicate trials, 100 chicks per trial were randomly divided into 10 treatments, which consisted of three concentrations (0.25%, 0.5%, or 1% wt./wt.) of each molecular weight chitosan, which was added to the feed and a positive control (0% chitosan). Birds were placed in floor pens and provided feed and water *ad libitum*; treated feed was provided throughout the entire trial.

The *Campylobacter* challenge was prepared as mentioned above. Birds were challenged by oral gavage with 0.25 mL of a four-strain mixture of wild-type *C. jejuni* on day 6, at a concentration of 10^7-10^8 CFU/mL. On day 15, birds were euthanized and the ceca were excised for *Campylobacter* enumeration. Cecal contents were serially diluted 10-fold with BPD and plated on CLA for direct enumeration. Plates were incubated at 42 ºC under microaerophilic conditions for 48 h and enumerated for *Campylobacter* colonies as previously described by our laboratory.[20] All the experiments conducted in this study were approved by the Institutional Animal Care and Use Committee of the University of Arkansas.

Chitosan Solution Preparation and Determination of Sub-Inhibitory Concentration (SIC)

The chitosan solution was prepared as mentioned pre-

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viously.[18] The SIC of chitosan was determined using previously published protocol.[21] Briefly, 24 well polystyrene plates (Costar, Corning, NY, USA) containing CEB (2 mL/well) supplemented with two-fold dilutions of chitosan (0, 0.2, 0.1, 0.05, 0.025, 0.0125 and 0.00625%), were inoculated with ~5.0 log CFU of *C. jejuni* wild strain, followed by incubation at 42 ºC for 24 h. Bacterial growth was determined by culturing on CLA agar plates. The highest concentration of chitosan that did not inhibit *C. jejuni* growth during mid-log (8 h), and stationary phase (24 h) were selected as the SIC for the compound.

RNA Isolation, cDNA Synthesis and Real-time Quantitative PCR

The effect of SIC of chitosan on the expression of *Campylobacter* genes critical for colonization in chicken was investigated using real-time quantitative PCR (RT-qPCR), as described previously.[21] The wild type *C. jejuni* strain was randomly selected from the four strains used in the *in vivo* trials for gene expression analysis. The strain was cultured with or without SIC of chitosan at 42 ºC in CEB to mid-log phase (8 h) and total RNA was extracted using the RN easy Mini kit (Qiagen, Valencia, CA, USA), followed by complementary DNA synthesis (iScript cDNA synthesis kit, Bio-Rad). The cDNA synthesized was used as the template for RT-qPCR. The amplification product was detected using SYBR Green reagent (iQ SYBR Green Supermix, Bio-Rad). The primers for each gene (Table 1) were designed from published GeneBank *C. jejuni* sequences using Primer 3 software National Center for Biotechnology Information (NCBI) and synthesized from IDT DNA. The relative expression of candidate genes was determined using the comparative critical threshold (ΔΔCt) method on a Quant Studio 3 real-time PCR system (Applied Biosytems, Thermo Fisher Scientific, Carlsbad, CA, USA). Data were normalized to the endogenous control (16S rRNA), and the level of expression of target genes between treated and untreated samples were analyzed to study effect of chitosan on expression of each gene. Duplicate samples were used and the assay was repeated three times.

Statistical Analysis

Cecal *Campylobacter* counts were logarithmically transformed before analysis to achieve homogeneity of variance.[22] Analysis of the data was done using the PROC GLM procedure of SAS.[23] Treatment means were partitioned by LSMEANS analysis and probability of $p<0.05$ was required for statistical significance. Data comparisons for the gene expression study were performed using multiple t-test with GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California, USA, www.graphpad.com).

RESULTS

Chitosan *in vitro*

Campylobacter counts were reduced by approximately 1 log at 2 and 4 h when co-incubated with 0.5% for all three molecular weights of chitosan as compared with controls (Table 2). At 8 h, all three chitosan preparations produced a 4.5 to 5 log reduction in counts when compared with controls.

Chitosan *in vivo*

In trial 1, *Campylobacter* counts were reduced in six of the chitosan treatments: 0.25% and 0.5% Low Molecular Weight (LMW), 0.25% and 0.5% Medium Molecular Weight (MMW), 0.25% and 1% High Molecular Weight (HMW), in comparison to the positive control (Table 3). Trial 2 showed a significant reduction of *Campylobacter* by four of the chitosan treatments: 0.5% LMW, 1% LMW, 0.25% MMW, and 0.5% MMW (Table 3). Results from Trial 3 showed a significant reduction of *Campylobacter* by one of the chitosan treatments: 0.5% MMW (Table 3).

Gene with Accession no.	Primer	Sequence (5'- 3')	Gene description
16S-rRNA (NC_002163.1)	Forward Reverse	5'-TGAGGGAGAGGCAGATGGAA-3' 5'-TCGCCTTCGCAATGGGTATT-3'	Ribosomal RNA (housekeeping gene)
cadF (NC_002163.1)	Forward Reverse	5'-CGCGGGTGTAAAATTCCGTC-3' 5'-TCCTTTTTGCCACCAAAACCA-3'	Outer membrane fibronectin-binding protein
jlpA (NC_002163.1)	Forward Reverse	5'-AGCACACAGGGAATCGACAG-3' 5'-TAACGCTTCTGTGGCGTCTT-3'	Surface exposed lipoprotein
ciaB (NC_002163.1)	Forward Reverse	5'-TCTCAGCTCAAGTCGTTCCA-3' 5'-GCCCGCCTTAGAACTTACAA-3'	Invasion antigen protein
fliA (NC_002163.1)	Forward Reverse	5'-AGCTTTCACGCCGTTACGAT-3' 5'-TCTTGCAAAACCCCAGAAGT-3'	Flagella biosynthesis RNA polymerase sigma factor
motA (NC_002163.1)	Forward Reverse	5'-AGCGGGTATTTCAGGTGCTT-3' 5'-CCCCAAGGAGCAAAAAGTGC-3'	Flagellar motor protein
motB (NC_002163.1)	Forward Reverse	5'-AATGCCCAGAATGTCCAGCA-3' 5'-AGTCTGCATAAGGCACAGCC-3'	Flagellar motor protein

Table 1: Primers used for real time quantitative PCR (RT-qPCR) analysis.

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Treatment	Time in hours			
	0	2	4	8
Positive controls	$6.35*10^7$	$8.15*10^7$	$5.45*10^7$	$3.5*10^7$
Low Molecular Weight	$3.42*10^7$	$6.8*10^6$	$1.24*10^6$	$3.0*10^2$
Medium Molecular Weight	$8.55*10^7$	$2.55*10^6$	$1.82*10^6$	$5.5*10^2$
High Molecular Weight	$7.45*10^7$	$2.59*10^6$	$2.00*10^6$	$6.5*10^2$

[1]0.5% concentration of: low molecular weight chitosan is 50-190 kDa; medium molecular weight chitosan is 190-310 kDa; or high molecular weight chitosan is 400-600 kDa, in 50 mM acetic acid.
[2]*Campylobacter* inoculum was added to each chitosan treatment and sampled at 0, 2, 4, and 8 h; samples were plated and enumerated after 48 h incubation.
[3]Values represent average campylobacteriosis counts of two separate replicate trials.

Table 2: The effect of different molecular weight chitosans on growth of *Campylobacter jejuni in vitro*[1,2,3] *Campylobacter* counts, *in vitro*.

	Chitosan dose	Trial 1	Trial 2	Trial 3
Positive controls Control	0%	$8.77\pm.17^{a}$	$7.05\pm.69^{a}$	$8.36\pm.24^{a}$
Low Molecular Weight	0.25%	$7.06\pm.58^{cde}$	$7.1\pm.29^{ab}$	$8.59\pm.20^{a}$
	0.5%	$7.68\pm.27^{bcd}$	3.96 ± 1.02^{c}	$7.88\pm.38^{ab}$
	1.0%	$7.96\pm.15^{abc}$	ND^{d}	$7.76\pm.40^{ab}$
Medium Molecular Weight	0.25%	$6.76\pm.34^{de}$	4.83 ± 1.08^{bc}	$8.47\pm.21^{a}$
	0.5%	$7.4\pm.38^{bcd}$	$3.25\pm.94^{c}$	$7.28\pm.70^{b}$
	1.0%	$8.03\pm.14^{abc}$	$7.45\pm.34^{a}$	$8.57\pm.17^{a}$
High Molecular Weight	0.25%	$7.45\pm.19^{bcd}$	$7.49\pm.31^{a}$	$8.16\pm.29^{ab}$
	0.5%	$8.43\pm.18^{ab}$	$7.8\pm.35^{a}$	$8.34\pm.26^{a}$
	1.0%	$6.3\pm.74^{e}$	$7.31\pm.30^{a}$	$8.51\pm.19^{a}$

[1]Low molecular weight chitosan is 50-190 kDa; medium molecular weight chitosan is 190-310 kDa; high molecular weight chitosan is 400-600 kDa.
[2]ND: non-detectible.
[3]Day-of-hatch birds were fed chick starter treatments of 0.25%, 0.5% or 1% of either low molecular weight, medium molecular weight or high molecular weight chitosan, respectively, for the entire 15-day study; bird were inoculated with *Campylobacter jejuni* mixture on Day 6 and cecal contents were collected on Day 15 for campylobacteriosis enumeration.
[4]Means within columns with no common superscript differ significantly ($p<0.05$).

Table 3: The effect of different concentrations and molecular weight chitosans on cecal *Campylobacter jejuni* counts (means±SEM) in 15-day old broiler chicks during three separate trials[1,2,3,4] *Campylobacter* counts, *in vivo*.

SIC and Gene Expression Analysis

Since MMW chitosan was found to consistently reduce *Campylobacter* counts *in vivo*; we selected MMW chitosan for the gene expression analysis. One of the *C. jejuni* wild strain used in the *in vivo* study was randomly selected for the mechanistic study. Based on growth curve results (data not shown), the SIC of MMW chitosan that did not inhibit *C. jejuni* strain growth as compared to control was 0.0125%. This concentration of MMW chitosan was used for subsequent gene expression analysis. RT-qPCR results (Figure 1) revealed that MMW chitosan significantly reduced the transcription of genes coding for *Campylobacter* motility; namely, *fliA, motA, motB* and adherence (*cadF, jlpA, ciaB*) as compared to control ($P<0.05$). The expression of chicken colonization genes was not significantly affected by acetic acid ($P>0.05$).

DISCUSSION

Preliminary *in vitro* results utilizing a 0.5% dose demonstrate that the three molecular weight chitosan treatments reduce *Campylobacter* counts in comparison to the untreated controls (Table 2). To evaluate the ability of chitosan to reduce enteric *Campylobacter* colonization in chickens, the 0.5% concentration of all three molecular weight chitosans, plus a lower (0.25%) and higher dose (1%) were also evaluated. In the first trial, cecal *Campylobacter* counts were reduced in 6 out of 8 of the treatments (Table 3). When conducted in a second trial, 4 of the 8 treatments were effective; whereas in the third replicate trial, the 0.5% MMW reduced enteric *Campylobacter* counts

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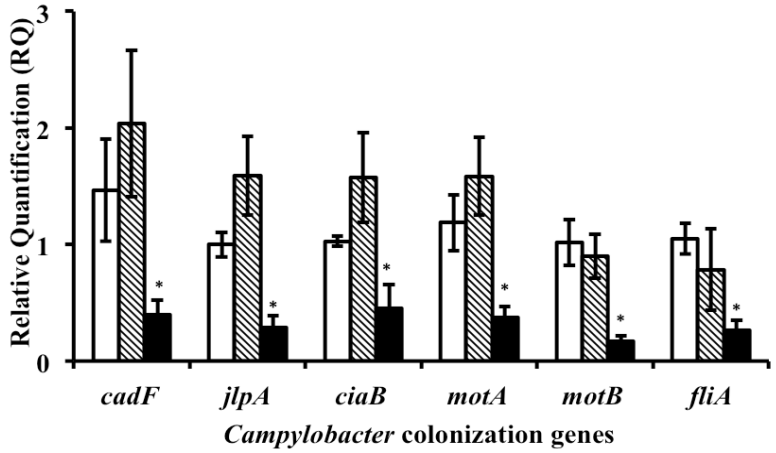

[1]RNA from wild type strain cultured either in the presence or absence (control) of 0.0125% chitosan to mid-log (8 hour) was used for cDNA synthesis and gene-expression analysis. 16S-rRNA was used as endogenous control.
*Means that differ significantly from the control (P<0.05).

Figure 1: Effect of 0.0125% MMW chitosan on the expression of chicken colonization genes (means ±SEM) in *Campylobacter jejuni*.[1]

when compared with controls (Table 3). Although there is variability between replicate trials, the 0.5% MMW chitosan dose consistently reduced *Campylobacter* in all three trials.

To determine the potential mechanism of action of chitosan, we investigated the effect of SICs of MMW chitosan on the expression of critical chicken colonization genes of *Campylobacter*. SICs of antimicrobials, including antibiotics are known to alter pathophysiology of microbes by modulating gene transcription.[21,24-26] In *C. jejuni,* the flagellar biosynthesis gene, *fliA* regulates a large number of genes involved in motility, protein synthesis and colonization.[27] A mutation in *fliA* has been shown to reduce motility and colonization potential in chicken cecum.[28] Similarly, *motA, motB* are critical for flagella motor function and facilitate motility and colonization.[27] *CadF* is another important virulence gene that encodes a 37 kDA outer membrane protein, that along with CiaB and JlpA, promotes adherence to intestinal cells and colonization in the avian intestinal tract.[27,29] We observed that SIC of MMW chitosan significantly decreased the expression of motility genes as well as adherence genes as compared to control (Figure 1), indicating that the anti-colonization effect of chitosan could be potentially mediated through reduced transcription of critical genes.

The importance of replicating results demonstrating a significant reduction in enteric *Campylobacter* counts in pre-harvest poultry cannot be underestimated. Previous research conducted by our laboratory[15,20,30] and others[31-33] have highlighted the variability between trials when evaluating pre-harvest treatments against enteric *Campylobacter*. Because of this inherent variability associated with *Campylobacter* colonization studies, results from a single pre-harvest study may not fully evaluate the consistency or effectiveness of a *Campylobacter* intervention strategy.[34-37]

Feed application of chitosan is a viable application for reducing *Campylobacter* colonization in chickens; however, water application is also a possible option. Unfortunately, chitosan is insoluble in water within the normal pH range.[38,39] This problem can be resolved by mildly acidifying the water, as accomplished in our *in vitro* studies. It is possible this will enhance the efficacy of chitosan as proposed by Qin and co-workers.[39] Acidifying water lines is already being performed in some poultry operations, which can reduce another foodborne pathogen, *Salmonella;*[22] thereby, this aids in the reduction of *Campylobacter* as well in the water lines and during feed withdrawal prior to processing, without altering the gut epithelium.[40,41] Thus, acidifying water in poultry houses could have a number of positive effects on bird health and reduce the potential zoonotic transfer of pathogens to humans. This possibility is currently under investigation.

The use of pre-harvest intervention strategies to reduce *Campylobacter* colonization (e.g., chitosan) can be part of a multifaceted approach to reduce the incidence of this foodborne pathogen. It has been proposed that a 2-log reduction in *Campylobacter* on the chicken carcass could reduce the risk of human campylobacteriosis by up to 30-fold.[42] Perceivably "small" reductions of Campylobacter in chickens could result in large reductions of campylobacteriosis incidences in humans. Olson and colleagues compiled data relevant to the consistent rise of campylobacteriosis incidences from the 1980's through 2006 as occurred in many countries, including Denmark, England, Wales, Norway, Sweden, New Zealand, and Australia, many of which are currently monitored by the European Centre for Disease Prevention and Control (ECDC).[43] In the 2000's, New Zealand focused on poultry as the primary source of *Campylobacter,* and by applying required regulatory implementations, along with the assistance of voluntary interventions, New Zealand saw a 54%

decline in campylobacteriosis incidences in 2008, compared to the period 2002-2006.[44] This decline was associated with a reduction in *Campylobacter* counts in chicken meat.[45] New Zealand's well-documented reduction of campylobacteriosis cases sets precedence for global reduction of *Campylobacter* by focusing intervention strategies on the poultry industry.

In conclusion, enteric *Campylobacter* counts were consistently reduced by in-feed supplementation of 0.5% MMW chitosan in three replicate trials. The use of this chitosan in pre-harvest poultry may be incorporated into a multifaceted strategy to reduce *Campylobacter* counts in chickens. Also, chitosan can be used to further reduce or inhibit *Campylobacters* surviving on the chicken carcass or meat. Further studies are warranted to explore the potential use of chitosan for reducing *Campylobacter* contamination in pre- and post-harvest poultry and the potential mechanism of action through whole transcriptome analysis.

ACKNOWLEDGEMENTS

Funded in part by the USDA-NIFA-OREI 2011-01955.

CONFLICTS OF INTEREST

Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that are suitable.

The authors declare that they have no conflicts of interest.

REFERENCES

1. Centers for Disease Control and Prevention. *Campylobacter* general information. Website: http://www.cdc.gov/nczved/divisions/dfbmd/diseases/campylobacter/ 2014; Accessed September 15, 2015.

2. World Health Organization. The global view of campylobacteriosis. Website: http://apps.who.int/iris/bitstream/10665/80751/1/9789241564601_eng.pdf 2013; Accessed September 15, 2015.

3. Hermans D, Pasmans F, Messens W, et al. Poultry as a host for the zoonotic pathogen *Campylobacter jejuni*. *Vector-Borne and Zoonotic Dis.* 2012; 12(2): 89-98. doi: 10.1089/vbz.2011.0676

4. Sasaki Y, Haruna M, Mori T, et al. Quantitative estimation of *Campylobacter* cross-contamination in carcasses and chicken products at an abattoir. *Food Control.* 2014; 43: 10-17. doi: 10.1016/j.foodcont.2014.02.015

5. Stern NJ, Fedorka-Cray P, Bailey JS, et al. Distribution of *Campylobacter* spp. in selected US poultry production and processing operations. *J. Food Prot*. 2001; 64(11): 1705-1710.

6. Hermans D, Van Deun K, Messens W, et al. Campylobacter control in poultry by current intervention measures ineffective: urgent need for intensified fundamental research. *Vet Microbiol*. 2011; 152(3): 219-228. doi: 10.1016/j.vetmic.2011.03.010

7. Menconi A, Hernandez-Velasco X, Latorre JD, et al. Effect of chitosan as a biological sanitizer for *Salmonella* Typhimurium and aerobic Gram negative spoilage bacteria present on chicken skin. *Int J Poult Sci*. 2013; 12(6): 318-321. doi: 10.3923/ijps.2013.318.321

8. No HK, Park NY, Lee SH, Meyers SP. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *Int J of Food Microbiol*. 2002; 74: 65-72. doi: 10.1016/S0168-1605(01)00717-6

9. Rabea EI, Badawy ME, Stevens CV, Smagghe G, Steurbaut W. Chitosan as animicrobial agent: applications and mode of action. *Biomacromolecules*. 2003; 4(6): 1457-1465. doi: 10.1021/bm034130m

10. No HK, Meyers SP. Preparation and characterization of chitin and chitosan-a review. *J Aquat Food Prod Technol*. 1995; 4(2): 27-52. doi: 10.1300/J030v04n02_03

11. No HK, Meyers SP, Prinyawiwatkul W, Xu Z. Applications of chitosan for improvement of quality and shelf life of foods: a review. *J Food Sci.* 2007; 72: R87-R100. doi: 10.1111/j.1750-3841.2007.00383.x

12. Sagoo S, Board R, Roller S. Chitosan inhibits growth of spoilage micro-organisms in chilled pork products. *Food Microbiol*. 2002; 19(2): 175-182. doi: 10.1006/fmic.2001.0474

13. Beverlya RL, Janes ME, Prinyawiwatkula W, No HK. Edible chitosan films on ready-to-eat roast beef for the control of *Listeria monocytogenes*. *Food Microbiol*. 2008; 25(3): 534-537. doi: 10.1016/j.fm.2007.11.002

14. Helander IM, Nurmiaho-Lassila E-L, Ahvenainen R, Rhoades J, Roller S. Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. *Int J Food Micribiol*. 2001; 71(2): 235-244.

15. Solis de los Santos F, Donoghue AM, Venkitanarayanan K, et al. Therapeutic supplementation of caprylic acid in feed reduces *Campylobacter jejuni* colonization in broiler chicks. *Appl Environ Microbiol*. 2008; 74(14): 4564-4566. doi: 10.1128/AEM.02528-07

16. Solis de los Santos F, Donoghue AM, Venkitanarayanan K, et al. The natural feed additive caprylic acid decreases *Campylo-*

bacter jejuni colonization in market-aged broiler chickens. *Poult Sci.* 2009; 88(1): 61-64. doi: 10.3382/ps.2008-00228

17. Farnell MB, Donoghue AM, Cole K, Reyes-Herrera I, Blore PJ, Donoghue DJ. *Campylobacter* susceptibility to ciprofloxacin and corresponding fluoroquinolone concentrations within the gastrointestinal tracts of chickens. *J Appl Microbiol*. 2005; 99(5): 1043-1050. doi: 10.1111/j.1365-2672.2005.02712.x

18. Ganan M, Carrascosa V, Martínez-Rodríguez AJ. Antimicrobial activity of chitosan against *Campylobacter* spp. and other microorganisms and its mechanism of action. *J. Food Prot*. 2009; 72(8): 1735-1738.

19. Line JE. Development of a selective differential agar for isolation and enumeration of *Campylobacter* spp. *J Food Prot*. 2001; 64(11): 1711-1715.

20. Arsi K, Donoghue AM, Venkitanarayanan K, et al. The efficacy of the natural plant extracts, thymol and carvacrol against *Campylobacter* colonization in broiler chickens. *J Food Safety.* 2014; 34(4): 321-325. doi: 10.1111/jfs.12129

21. Upadhyay A, Johny AK, Amalaradjou MAR, Baskaran SA, Kim KS, Venkitanarayanan K. Plant-derived antimicrobials reduce *Listeria monocytogenes* virulence factors in vitro, and down-regulate expression of virulence genes. *Int. J.Food Microbiol.* 2012; 157(1): 88-94. doi: 10.1016/j.ijfoodmicro.2012.04.018

22. Byrd JA, Anderson RC, Callaway TR, et al. Effect of experimental chlorate product administration in the drinking water on *Salmonella* Typhimurium contamination of broilers. *Poult Sci*. 2003; 82(9): 1403-1406. doi: 10.1093/ps/82.9.1403

23. SAS Institute. SAS/STAT® Users guide: Release 9.2 edition. SAS Institute, Cary, NC, 2002.

24. Goh EB, Yim G, Tsui W, McClure J, Surette MG, Davies J. Transcriptional modulation of bacterial gene expression by sub-inhibitory concentrations of antibiotics. *Proc Natl Acad Sci USA*. 2002; 99(26): 17025-17030.

25. Fonseca AP, Extremina C, Fonseca AF, Sousa JC. Effect of sub-inhibitory concentration of piperacillin/tazobactam on *Pseudomonas aeruginosa*. *J Med Microbiol.* 2004; 53(9): 903-910. doi: 10.1099/jmm.0.45637-0

26. Upadhyay A, Upadhyaya I, Kollanoor-Johny A, Venkitanarayanan, K. Anti-biofilm effect of plant derived antimicrobials on *Listeria monocytogenes*. *Food Microbiol.* 2013; 36(1): 79-89. doi: 10.1016/j.fm.2013.04.010

27. Hermans D, Van Deun K, Martel A, et al. Colonization factors of *Campylobacter jejuni* in the chicken gut. *Vet Res.* 2011; 42(1): 82. doi: 10.1186/1297-9716-42-82

28. Fernando U, Biswas D, Allan B, Willson P, Potter AA. Influence of *Campylobacter jejuni* fliA, rpoN and flgK genes on colonization of the chicken gut. *Int. J Food Microbiol.* 2007; 118(2): 194-200. doi: 10.1016/j.ijfoodmicro.2007.07.038

29. Konkel ME, Gray SA, Kim BJ, Garvis SG, Yoon J. Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the cadF virulence gene and its product. *J. Clin. Microbiol.* 1999; 37(3): 510-517.

30. Metcalf JH, Donoghue AM, Venkitanarayanan K, et al. Water administration of the medium-chain fatty acid caprylic acid produced variable efficacy against enteric *Campylobacter* colonization in broilers. *Poult. Sci*. 2011; 90(2): 494-497. doi: 10.3382/ps.2010-00891

31. Hakkinen M, Schneitz C. Efficacy of a commercial competitive exclusion product against *Campylobacter jejuni*. *Br Poult Sci.* 1999; 40(5): 619-621. doi: 10.1080/00071669986990

32. Hilmarsson H, Thormar H, Thrainsson JH, et al. Effect of glycerol monocaprate (monocaprin) on broiler chickens: An attempt at reducing intestinal *Campylobacter* infection. *Poult Sci.* 2006; 85(4): 588-592. doi: 10.1093/ps/85.4.588

33. Robyn J, Rasschaert G, Hermans D, Pasmans F, Heyndrickx M. Is allicin able to reduce *Campylobacter jejuni* colonization in broilers when added to drinking water? *Poult Sci*. 2013; 92(5): 1408-1418. doi: 10.3382/ps.2012-02863

34. Carvalho CM, Gannon BW, Halfhide DE, et al. The *in vivo* efficacy of two administration routes of a phage cocktail to reduce numbers of *Campylobacter coli* and *Campylobacter jejuni* in chickens. *BMC Microbiol*. 2010; 10(1): 232. doi: 10.1186/1471-2180-10-232

35. Molatová Z, Skrivanova E, Baré J, Houf K, Bruggeman G, Marounek M. Effect of coated and non-coated fatty acid supplementation on broiler chickens experimentally infected with *Campylobacter jejuni*. *J Anim Physiol Anim Nutr*. 2010; 95(6): 701-706. doi: 10.1111/j.1439-0396.2010.01100.x

36. Van Deun K, Haesebrouck F, Van Immerseel F, Ducatelle R, Pasmans F. Short chain fatty acids and lactate as feed additives to control *Campylobacter jejuni* infections in broilers. *Avian Path*. 2008; 37(4): 379-383. doi: 10.1080/03079450802216603

37. Neal-McKinney JM, Lu X, Duong T, et al. Production of organic acids by probiotic Lactobacilli can be used to reduce pathogen load in poultry. *PLoS One*. 2012; 7(9): 1-11. doi: 10.1371/journal.pone.0043928

38. Chandumpai A, Singhpibulporn N, Faroongsarng D, Sorn-

ISSN 2377-8350 Open Journal http://dx.doi.org/10.17140/AFTNSOJ-1-119

prasit P. Preparation and physico-chemical characterization of chitin and chitosan from the pens of the squid species, *Loligo lessoniana* and *Loligo formosana*. *Carbohydrate Polymers*. 2004; 58(4): 467-474. doi: 10.1016/j.carbpol.2004.08.015

39. Qin C, Li H, Xiao Q, Liu Y, Zhu J, Du Y. Water-solubility of chitosan and its antimicrobial activity. *Carbohydrate Polymers*. 2006; 63(3): 367-374. doi: 10.1016/j.carbpol.2005.09.023

40. Byrd JA, Anderson RC, Brewer RL, et al. Effect of lactic acid administration in the drinking water during preslaughter feed withdrawal on *Salmonella* and *Campylobacter* contamination of broilers. *Poult Sci*. 2001; 80(3): 278-283. doi: 10.1093/ps/80.3.278

41. Chaveerach P, Keuzenkamp DA, Lipman LJ, Van Knapen F. Effect of organic acids in drinking water for young broilers on *Campylobacter* infection, volatile fatty acid production, gut microflora and histological cell changes. *Poult Sci*. 2004; 83(3): 330-334. doi: 10.1093/ps/83.3.330

42. Rosenquist H, Nielsen NL, Sommer HM, Norrung B, Christensen BB. Quantitative risk assessment of human campylobacteriosis associated with thermophilic *Campylobacter* species in chickens. *Int J Food Microbiol*. 2003; 83(1): 87-103. doi: 10.1016/S0168-1605(02)00317-3

43. Olson CK, Ethelberg S, van Pelt W, Tauxe RV. Epidemiology of *Campylobacter jejuni* infections in industrialized nations. In: Nachamkin I, Szymanski C, Blaser M, eds. *Campylobacter*. 3rd ed. Washington: ASM Press; 2008: 163-189.

44. Sears A, Baker MG, Wilson N, et al. Marked campylobacteriosis decline after interventions aimed at poultry, New Zealand. *Emerg Infect Dis*. 2001; 17(6): 1007-1015.

45. New Zealand Food Safety Authority. *Campylobacter* risk management strategy, 2013-2014. Website: http://www.foodsafety.govt.nz/elibrary/industry/Campylobacter_Risk-Comprehensive_Aimed.pdf 2013; Accessed September 23, 2015.