Mitigation of Post-processing Pathogen Contamination in Pet foods using Topical Additives

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Foodborne diseases

- Most common foodborne pathogens - *E. coli*, *Salmonella*, norovirus and *Listeria*

- *Salmonella* infections in humans occur from handling contaminated pet foods & treats (Adley et al., 2011; Clark et al., 2001; Finley et al., 2006; Freeman et al., 2013)
Why focus more on pet food safety?

- FDA-Food Safety Modernization Act Act 2011
- Healthy pets - Asymptomatic carriers of *Salmonella* and *E. coli*
- Handlers - Children and elderly
- Prevalence of *Salmonella*-positive dogs and cats in the U.S. is declining
- Less than 1% (3/542) of cats and 2.5% (60/2,422) of dogs feces were tested positive (Reimschuessel et al., 2017)
Pathogens of concern

- *Salmonella* spp.
- *Listeria monocytogenes*
- *Escherichia. coli*
- Fungi
  - *Aspergillus flavus, Fusarium graminearum*
- *Mycobacterium bovis* in cats from commercial raw cat diet (O’Halloran et al., 2019)
Common *Salmonella* isolates in dogs and cats
(Carter and Quinn, 2000)

<table>
<thead>
<tr>
<th><em>Salmonella</em> serovars isolated from dogs in USA</th>
<th><em>Salmonella</em> serovars isolated from cats in USA</th>
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<tr>
<td>S. worthington</td>
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- Most of the *Salmonella* contaminations
  - Pig ears
  - Raw dog food & treats
- Most of the *Listeria* contaminations
  - Raw dog food
- *E. coli* contaminations
  - Fresh meat & raw dog food
Multidrug-Resistant *Salmonella* Infections Linked to Contact with Pig Ear Dog Treats

Investigation Notice

Published on September 5, 2019 at 9:30 AM ET

CDC, public health and regulatory officials in several states, and the U.S. Food and Drug Administration (FDA) are investigating a multistate outbreak of multidrug-resistant *Salmonella* infections linked to contact with pig ear dog treats.

Latest Outbreak Information

- CDC and FDA are advising people not to buy or feed any pig ear dog treats, including any that may already be in homes.
- People can get sick after handling the treats or caring for dogs who ate the treats. Dogs might get sick after eating them.
- 16 ill people have been added to this investigation since the last update on July 31, 2019.
- 143 people infected with the outbreak strains of *Salmonella* have been reported from 35 states.
  - Of 110 ill people with available information, 33 (30%) have been hospitalized. No deaths have been reported.
  - 26 illnesses (20%) are among children younger than 5 years.

At A Glance

- **Reported Cases:** 143
- **States:** 35
- **Hospitalizations:** 33
- **Deaths:** 0
FDA discourages pet owners from feeding frozen/raw pet food to their pets due to *Salmonella* and *Listeria* contamination.

- Canned pet food
  - Botulinum toxicity
- Semi-moist pet food
  - Post-processing mold contamination
- Dry pet food/kibbles
  - Post-processing pathogen contamination (*Salmonella*, *E. coli*)
Pathogen control in pet food

- Dry pet food kibbles
  - Extrusion - heat kill step
- Canned pet food
  - Canning - 121°C and 21 psi
- Semi-moist pet food
  - Cooking/baking
- Raw pet food - Most of the outbreaks and recalls
Residual moisture in transport container of poultry fat does not affect *Salmonella* growth at 48°C (Trinetta et al., 2019)

Lactic acid (0.2%) was able to knock down 3 logs of *Salmonella* in rendered chicken fat system (Kumar et al., 2019)

GRAS plant-derived antimicrobials such as trans-cinnamaldehyde, carvacrol, thymol, eugenol & caprylic acid reduced *S. Schwarzengrund* in dry dog food (Chen et al., 2019)
Potential post-processing route of contamination

- Fats and flavors are commonly coated on dry pet food to increase energy density and to enhance palatability.
- This occurs after the established kill step.
- **Residual water** in bulk fat could be a source of *Salmonella* contamination of pet food.

*Image source: AFB international*
Mitigation strategies

- Thermal processing
  - point in time
- Irradiation
  - Not well accepted by pet owners
- Topical antimicrobials
  - Acidulants with residual control
- Others
  - Ozone, high pressure pasteurization etc.
Topical antimicrobials being evaluated:

- Sodium bisulfate (SBS)
- Organic acids
  - Butyric, lactic & propionic acids
- Inorganic acid
  - Phosphoric acid
- Medium chain fatty acids (MCFA)
  - Caproic (C6), caprylic (C8) & capric (C10) acids
Sodium bisulfate is a known hygroscopic chemical which kills pathogens by its desiccant properties.

Organic acids and MCFAs in their undissociated form penetrate the cytoplasmic membrane, resulting in reduced intracellular pH and disruption of transmembrane proton motive force (Ray & Sandine, 1992).
Current Research Projects
1. SBS and Organic acids as topical additives to mitigate *Salmonella* in chicken fat

Research Note

Assessing the Efficacy of Sodium Bisulfate and Organic Acid Treatments for Control of *Salmonella Typhimurium* in Rendered Chicken Fat Applied to Pet Foods

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ABSTRACT

This study was conducted to evaluate the effects of sodium bisulfate (SBS), lactic acid (LA), phosphoric acid (PA), and
Introduction

- Sodium bisulfate
  - Acidifier
  - Used in animal feed as a palatability enhancer, preservative & anti-bacterial
  - Known to reduce *Salmonella* and *Campylobacter* populations (Line, 2001)
- Organic acids
  - Preservative and antimicrobial
Materials & Methods

- *Salmonella* culture used: S. Typhimurium (ATCC 14028)
- Food matrix: rendered chicken fat/Dry dog food kibble
- Antimicrobials used:
  - Sodium bisulfate (SBS)
  - Butyric acid
  - Lactic acid
  - Propionic acid
  - Phosphoric acid
Objectives

- To determine the minimum inhibitory concentration (MIC) of SBS and organic acids
- To evaluate the effect of these chemicals against *S. Typhimurium* (ATCC 14028) in chicken fat applied to dry dog food kibbles individually as well as in combinations

Hypothesis

- SBS and organic acid topical application reduces *Salmonella* loads in fat-coated dry dog food kibbles
Efficacy testing in chicken fat

45°C/24h

Serial dilution in 0.1% PW

Plating

37°C/24 h

Count
Results
## Minimum inhibitory concentrations of various chemicals

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>MIC (%)</th>
</tr>
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<tbody>
<tr>
<td>Sodium bisulfate</td>
<td>0.50%</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0.50%</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>0.25%</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>0.25%</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>0.25%</td>
</tr>
<tr>
<td>Sodium bisulfate + Butyric acid</td>
<td>0.10% + 0.05%</td>
</tr>
<tr>
<td></td>
<td>0.05% + 0.075%</td>
</tr>
<tr>
<td>Sodium bisulfate + Lactic acid</td>
<td>0.10% + 0.10%</td>
</tr>
<tr>
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<td>0.05% + 0.15%</td>
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<tr>
<td>Sodium bisulfate + Propionic acid</td>
<td>0.10% + 0.05%</td>
</tr>
<tr>
<td></td>
<td>0.05% + 0.075%</td>
</tr>
</tbody>
</table>
Log reduction of *Salmonella* using SBS and organic acids

**Water phase**

- 0.5% SBS
- 0.5% LA
- 0.05% PA
- POS
- Neg

**Fat phase**

- 0.5% SBS
- 0.5% LA
- 0.05% PA
- POS
- Neg
Log reduction of *Salmonella* using SBS + Lactic acid

**Water phase**

**Fat phase**

- **0.1% SBS + 0.1% LA**
- **0.05% SBS + 0.15% LA**
- **POS**
- **NEG**
Log reduction of *Salmonella* using SBS + Propionic acid

**Water phase**

**Fat phase**

[Graphs showing the log reduction of *Salmonella* using SBS + Propionic acid in both water and fat phases.]

- **Water phase**
  - 0.1% SBS + 0.05% ProA
  - 0.05% SBS + 0.075% ProA
  - POS
  - NEG

- **Fat phase**
  - 0.1% SBS + 0.05% ProA
  - 0.05% SBS + 0.075% ProA
  - POS
  - NEG
Log reduction of *Salmonella* using SBS + Butyric acid

**Water phase**

**Fat phase**
Conclusions

- The use of SBS alone or in combination with organic acids is effective in mitigating *Salmonella* in rendered chicken fat.

- The combination of SBS with organic acids has a potential synergistic effect against *Salmonella*. 
2. Medium chain fatty acids (MCFAs) as topical additives to mitigate *Salmonella* Typhimurium in dry pet food kibbles
Introduction

- Medium chain fatty acids (MCFAs) are aliphatic fatty acids with 6-12 carbon atoms
- Palm kernel oil & coconut oil are sources for commercial extraction

\[
\text{Caproic acid, } C_6H_{12}O_2 \quad \text{Caprylic acid, } C_8H_{16}O_2 \quad \text{Capric acid, } C_{10}H_{20}O_2
\]
Beneficial effects of MCFAs

- Prebiotic effect
  - Improves gut health & intestinal epithelial structure
- Lowers blood glucose level
- Lowers cholesterol and atherosclerosis
- Antimicrobial effects
Use of MCFAs in dry dog food

- MCFAs are known to inhibit and eliminate pathogens including *E. coli* and *Salmonella* (Kim & Rhee, 2013; Wang & Johnson, 1992; Marounek et al., 2003; Skřivanová et al., 2004; Molatova et al., 2010)

- They could be an alternative to conventional antimicrobials, to reduce *Salmonella* contamination during post processing (fat & flavor coating) of dry extruded pet foods

- No studies to our knowledge have examined the bactericidal activity of MCFAs when used in combination
Objectives

- To determine the minimum inhibitory concentration (MIC) of MCFAs
- To evaluate the effects of MCFAs against *Salmonella Typhimurium* (ATCC 14028) applied to dry dog food kibbles individually as well as in combinations

Hypothesis

- The application of MCFAs reduce *Salmonella* loads in fat/oil coated dry dog food kibbles
Determination of MIC using a polar solvent

- Broth microdilution with modified Huang (2011) and Kitahara (2004) methods

- Make 10% stock solution of C6/C8/C10:
  - Treatments: 200µl of C6/C8/C10 + 50µl of ethanol + 750µl of SDW* = 1 ml
  - Ethanol control: 200µl of SDW + 50µl of ethanol + 750µl of SDW = 1 ml
  - Positive control
  - Negative control

*SDW = Sterile distilled water
## MCFA dosages used

<table>
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<tr>
<th>MCFA</th>
<th>dose</th>
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<tbody>
<tr>
<td>C6</td>
<td>0.5%</td>
</tr>
<tr>
<td>C8</td>
<td>0.5%</td>
</tr>
<tr>
<td>C10</td>
<td>1.0%</td>
</tr>
<tr>
<td>C6 + C8</td>
<td>0.25 - 0.5%</td>
</tr>
<tr>
<td>C6 + C10</td>
<td>0.25 - 1.0%</td>
</tr>
<tr>
<td>C8 + C10</td>
<td>0.25 - 1.0%</td>
</tr>
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</table>
Efficacy of MCFA on dry pet food kibbles

- Spray 5ml of ~6 log Salmonella
  - 37 °C/30 mins
- 225 ml of BPW

- 25 g of sample in a Whirlpak bag
- Stomach for 2 min
- Dilute and plate on TSA plates
  - 37 °C/24h
- Count and analyze

- Sampling at pre-determined time intervals (0, 1, 2, 3, 4, and 5h)
Results

[Cartoon image showing a person at a table with a dog wearing protective gear, and a speech bubble saying "YES... IT'S SAFE."]
Minimum inhibitory concentration (MIC) was calculated as the lowest concentration of lipid that reduced growth of bacteria by more than 50% (Fischer et al., 2012)

- MIC for C6 and C8 = 0.3125%
- MIC for C10 = 0.625%
Log reduction of *Salmonella* with MCFA over time

C6, C8 & C10

Log (CFU/ml) vs Time (hr)

- 0.5% C6
- 0.5% C8
- 1% C10
- Pos Cont
- Neg cont
Log reduction of *Salmonella* with MCFA over time

![Graph showing log reduction of *Salmonella* with MCFA over time.](image)
Log reduction of *Salmonella* with MCFA over time

[Graph showing log reduction of *Salmonella* with various concentrations of MCFA over time.]
Log reduction of *Salmonella* with MCFA over time

<table>
<thead>
<tr>
<th>Log (CFU/ml)</th>
<th>Time (hr)</th>
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<td>0</td>
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<td>5</td>
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**C8+C10**

- 0.25%C8+1%C10
- 0.25%C8+ 0.5%C10
- 0.5%C8+ 0.5%C10
- 0.25%C8+ 0.75%C10
- 0.5%C8+1.0%C10
- POS
- NEG
Conclusions

- During individual applications, at the given concentration, all 3 MCFAs (C6, C8 & C10) were equally effective in reducing *Salmonella* loads in dry dog food kibbles.

- C6 and C8 were more effective even at low concentrations (0.5%) when compared to C10 (1%).

- Combinations of C6 or C8 with C10 were more effective at reducing *Salmonella* faster, which could be due to potential synergistic action.
Take home messages

- Application of food grade antimicrobials during the coating step in dry pet food production is an optimistic approach to tackle post processing pathogen contamination.
- Use of SBS alone or with lactic acid mitigates *Salmonella* in rendered chicken fat used to coat pet food.
- Use of MCFAs, individually or in combination, reduces *Salmonella* in dry dog food kibbles when coated using oil system.
Acknowledgements

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Pet Food Program Coordinator
Kansas State University
References

Thank you!