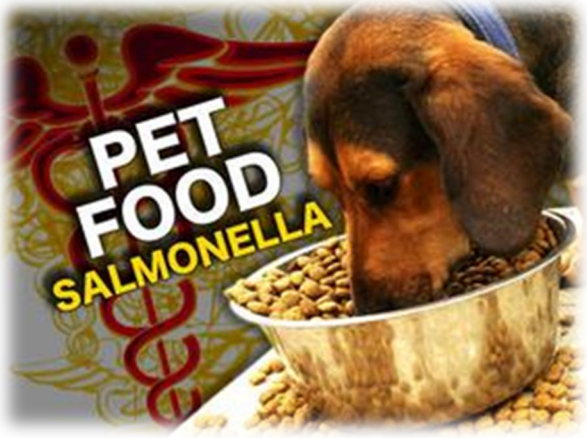
Mitigation of Post-processing Pathogen Contamination in Pet foods using Topical Additives



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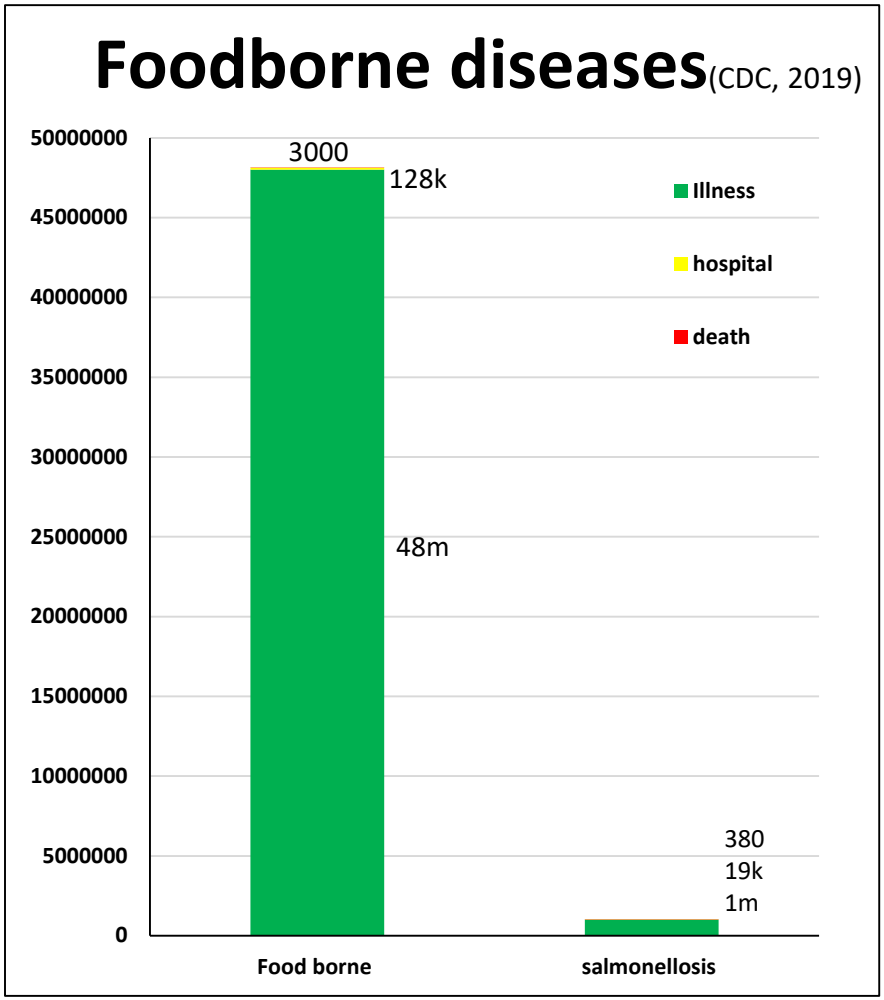
Foodborne diseases

- 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?

- Pet food - $31.7B market in the US (American Pet food association, 2019)
- 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)

Salmonella serovars isolated from dogs in USA

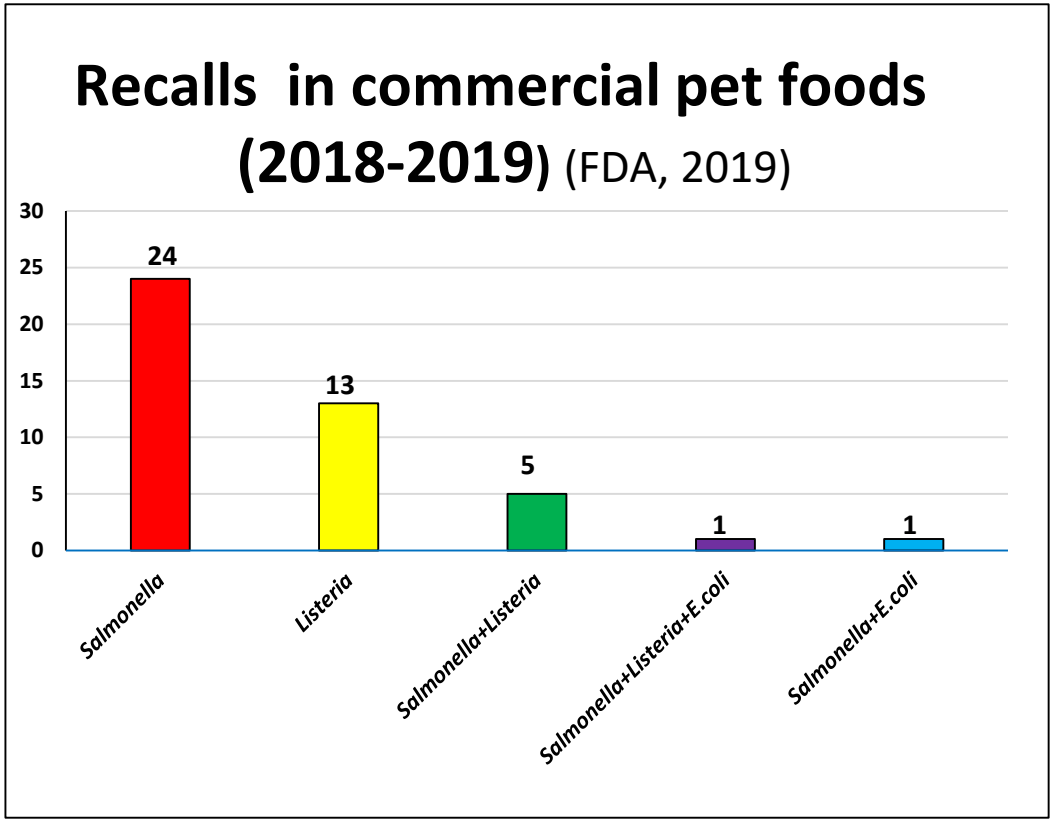
S. anatum	*S. illinois*	*S. newbrunswick*
S. bareilly	*S. infantis*	*S. newington*
S. bonariensis	*S. inverness*	*S. newport*
S. bovismorbificans	*S. javiana*	*S. norwich*
S. bredeney	*S. johannesburg*	*S. oranienburg*
S. budapest	*S. kentucky*	*S. oregon*
S. california	*S. krefeld*	*S. paratyphi B*
S. canoga	*S. litchfield*	*S. pomona*
S. carrau	*S. livingstone*	*S. poona*
S. cerro	*S. lomita*	*S. pullorum*
S. choleraesuis	*S. luciana*	*S. rubislaw*
S. cubana	*S. macallen*	*S. saintpaul*
S. derby	*S. madelia*	*S. sandiego*
S. duval	*S. manhattan*	*S. seftenberg*
S. enteritidis	*S. meleagridis*	*S. tallahassee*
S. florida	*S. memphis*	*S. tennessee*
S. gaminara	*S. miami*	*S. typhimurium*
S. give	*S. minnesota*	*S. urbana*
S. hartford	*S. montevideo*	*S. weslaco*
S. homosassa	*S. muenchen*	*S. worthington*

Salmonella serovars isolated from cats in USA

- *S. anatum*
- *S. bareilly*
- *S. bredeney*
- *S. choleraesuis*
- *S. derby*
- *S. donna*
- *S. enteritidis*
- *S. javiana*
- *S. lomita*
- *S. montevideo*
- *S. pharr*
- *S. poona*
- *S. pullorum*
- *S. sandiego*
- *S. saintjuan*
- *S. typhimurium*
- *S. weslaco*

Recalls in commercial pet foods

- 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*

Outbreak of 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

(CDC, 2019)

Pet food type & pathogens

- 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

Current research on topical additives

- Residual moisture in transport container of poultry fat does not affect *Salmonella* growth at 48^{o}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**

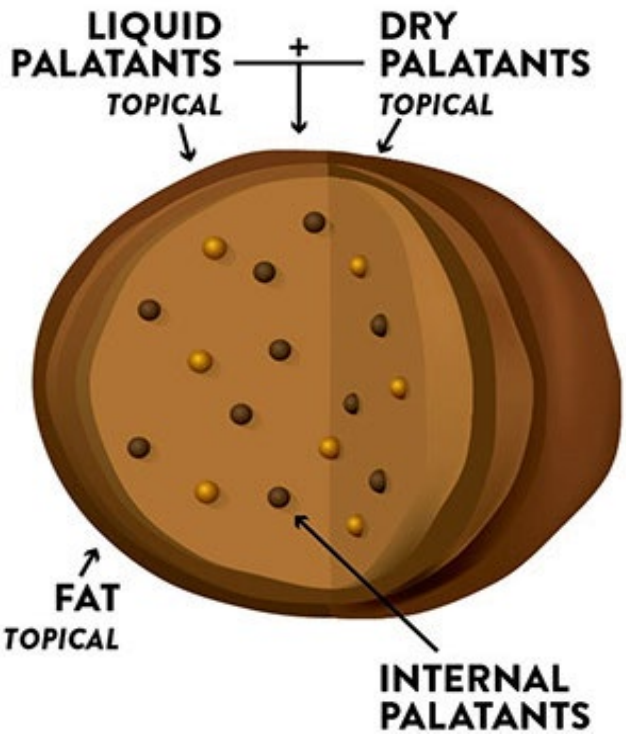


Image source : AFB international

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.

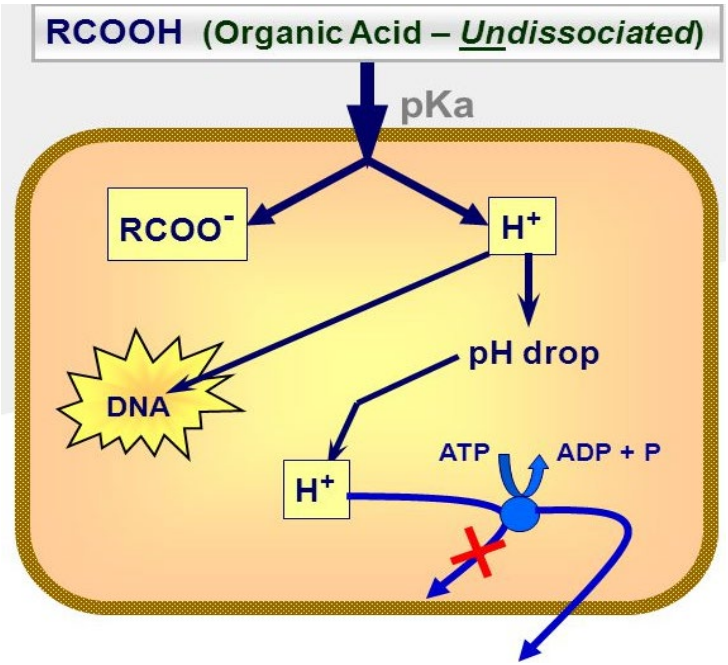
Topical antimicrobials being evaluated:

- 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

Anti-bacterial effects of topical antimicrobials

- 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 trans-membrane proton motive force
(Ray & Sandine, 1992)



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Current Research Projects

1. SBS and Organic acids as topical additives to mitigate *Salmonella* in chicken fat

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https://doi.org/10.4315/0362-028X.JFP-18-560
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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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MS 18-560: Received 24 November 2018/Accepted 8 June 2019/Published Online 0 Month 2019

ABSTRACT

21 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

Sodium bisulfate

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

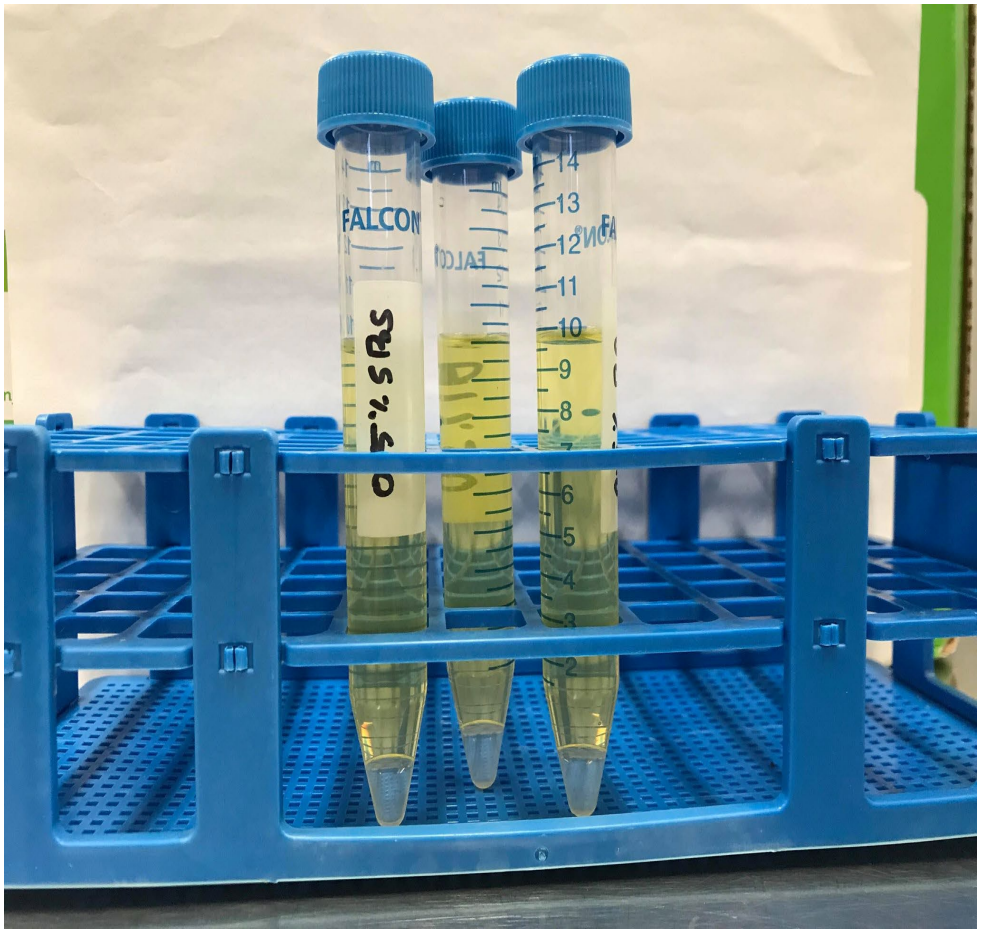
Hypothesis

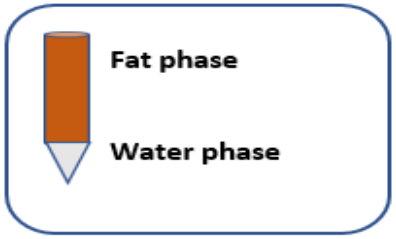
- SBS and organic acid topical application reduces *Salmonella* loads in fat-coated dry dog food kibbles

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

Efficacy testing in chicken fat





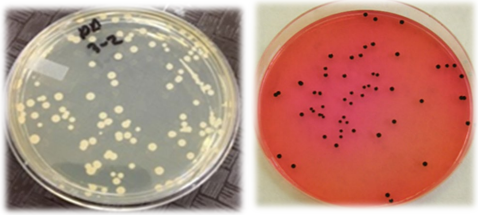
45°C/24h

Serial dilution in 0.1% PW

Plating

37°C/24 h

Count

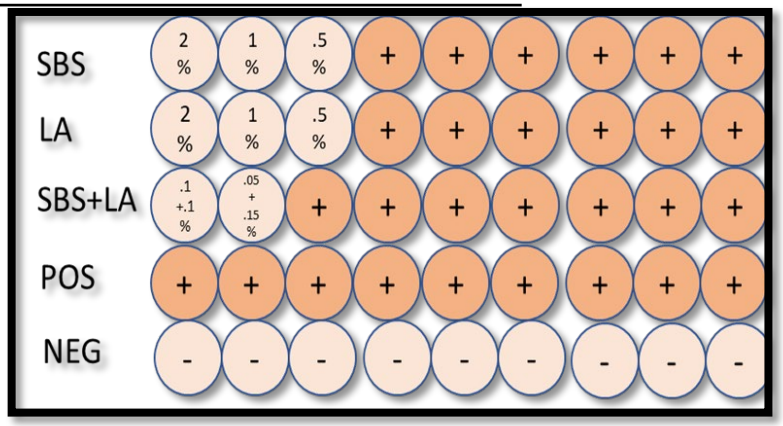


Results

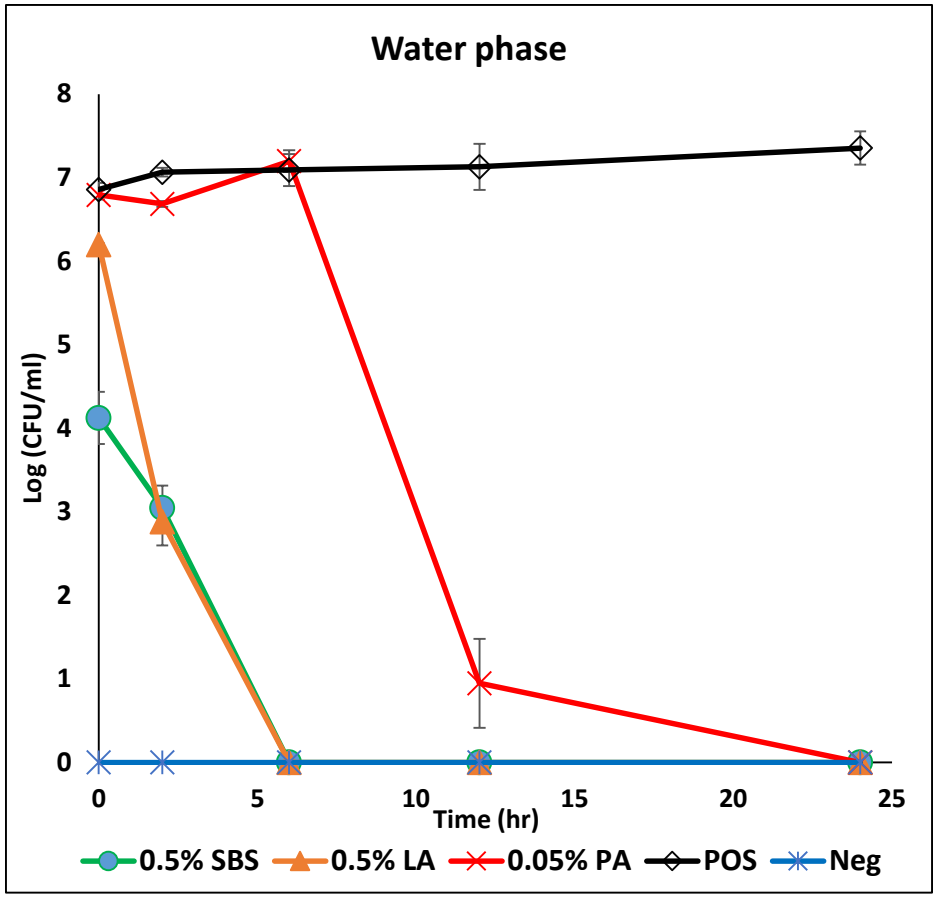
MIC results

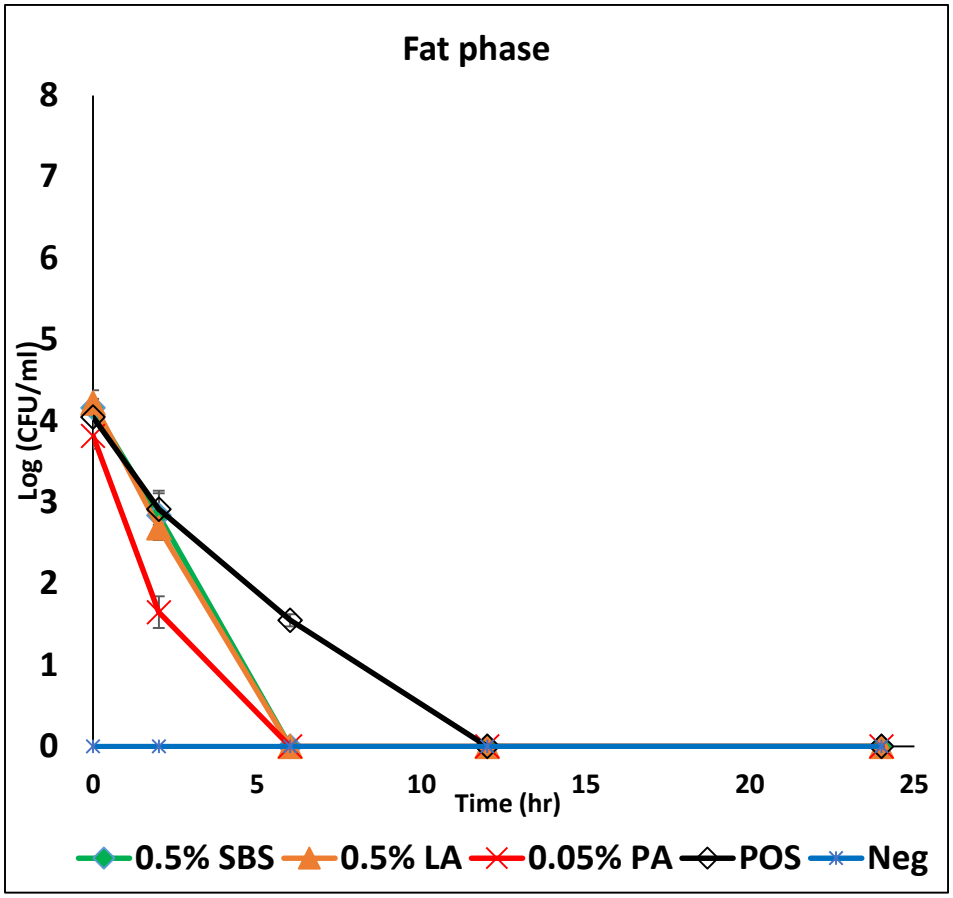
Minimum inhibitory concentrations of various chemicals

Antimicrobials	MIC (%)
Sodium bisulfate	0.50%
Lactic acid	0.50%
Phosphoric acid	0.25%
Propionic acid	0.25%
Butyric acid	0.25%
Sodium bisulfate + Butyric acid	0.10% + 0.05%
	0.05% + 0.075%
Sodium bisulfate + Lactic acid	0.10% + 0.10%
	0.05% + 0.15%
Sodium bisulfate + Propionic acid	0.10% + 0.05%
	0.05% + 0.075%

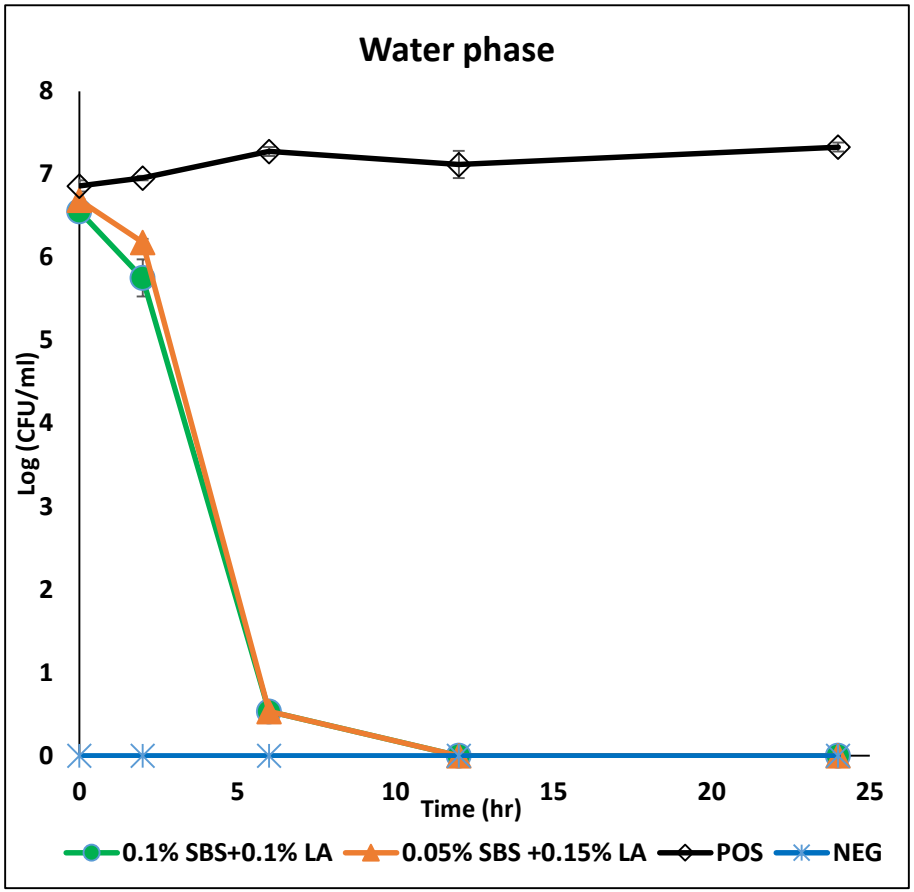


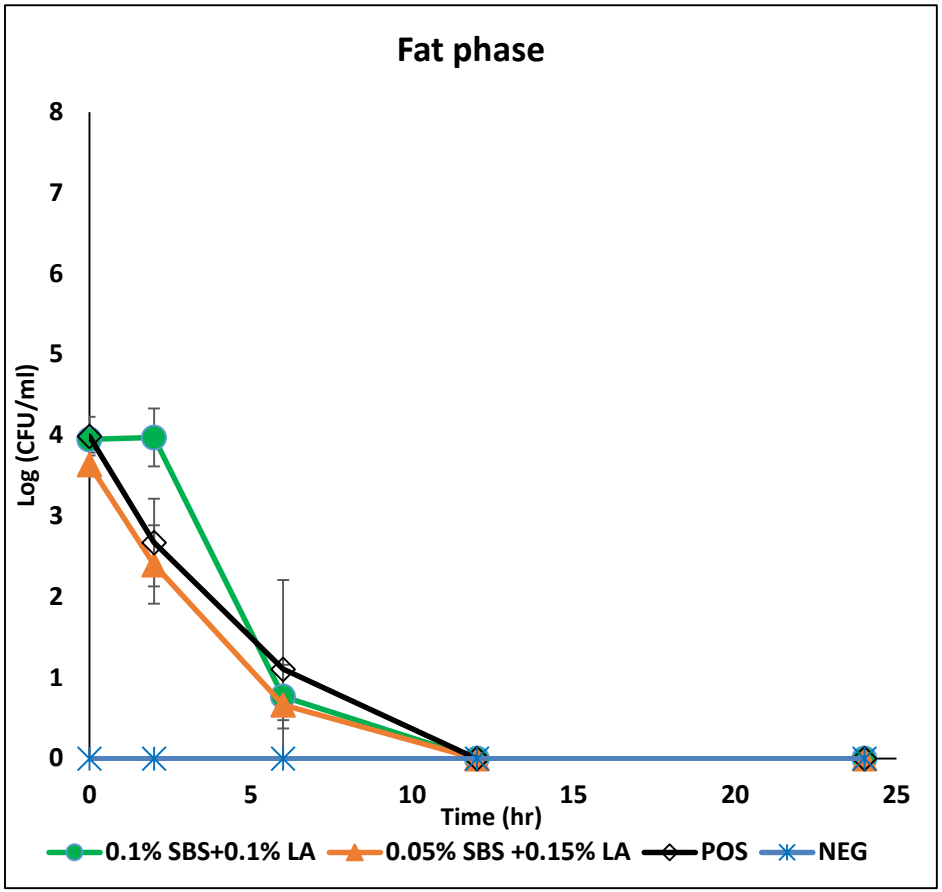
Log reduction of *Salmonella* using SBS and organic acids



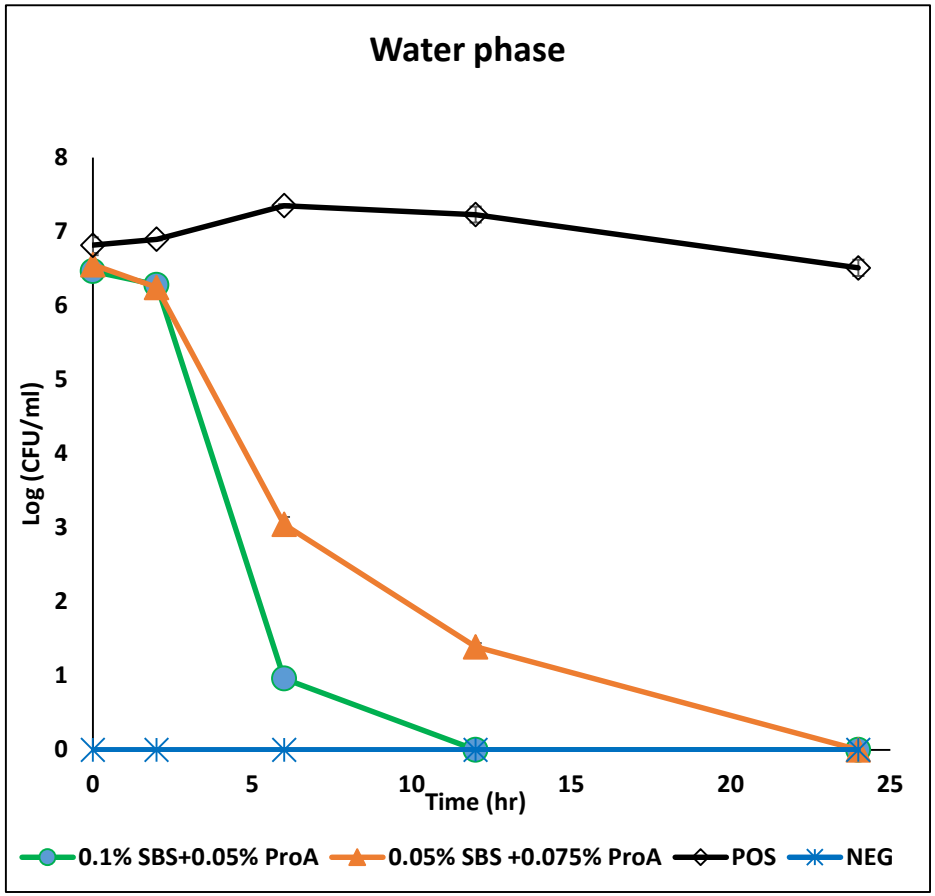


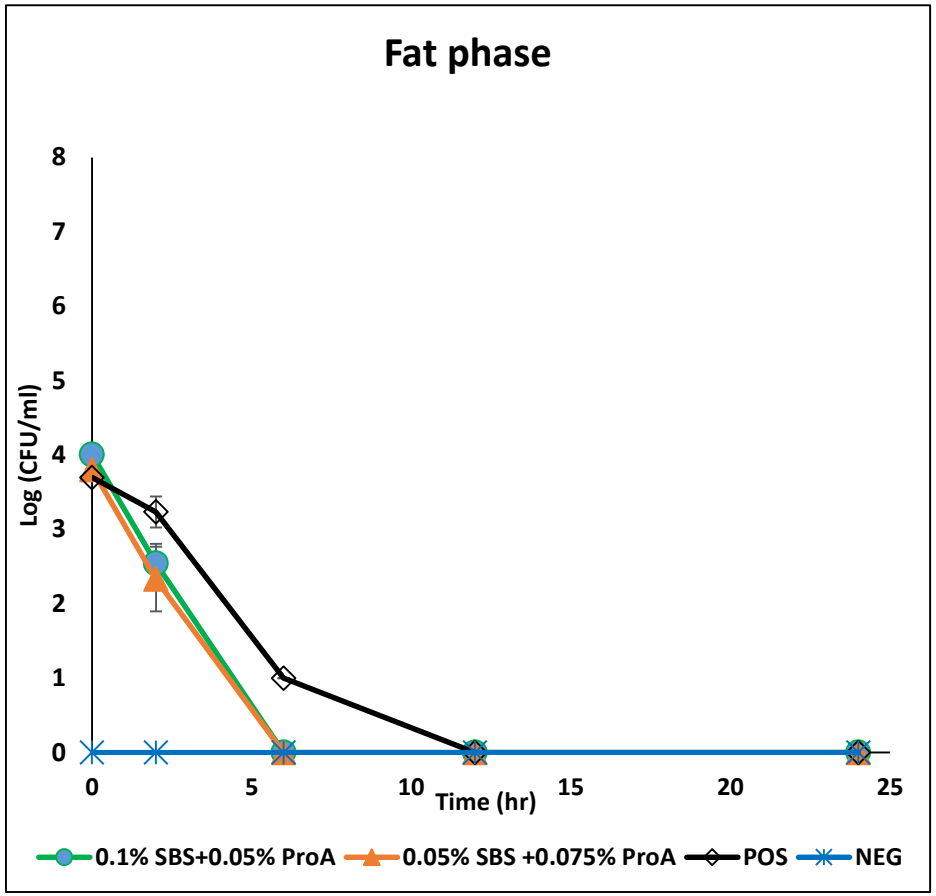
Log reduction of *Salmonella* using SBS + Lactic acid



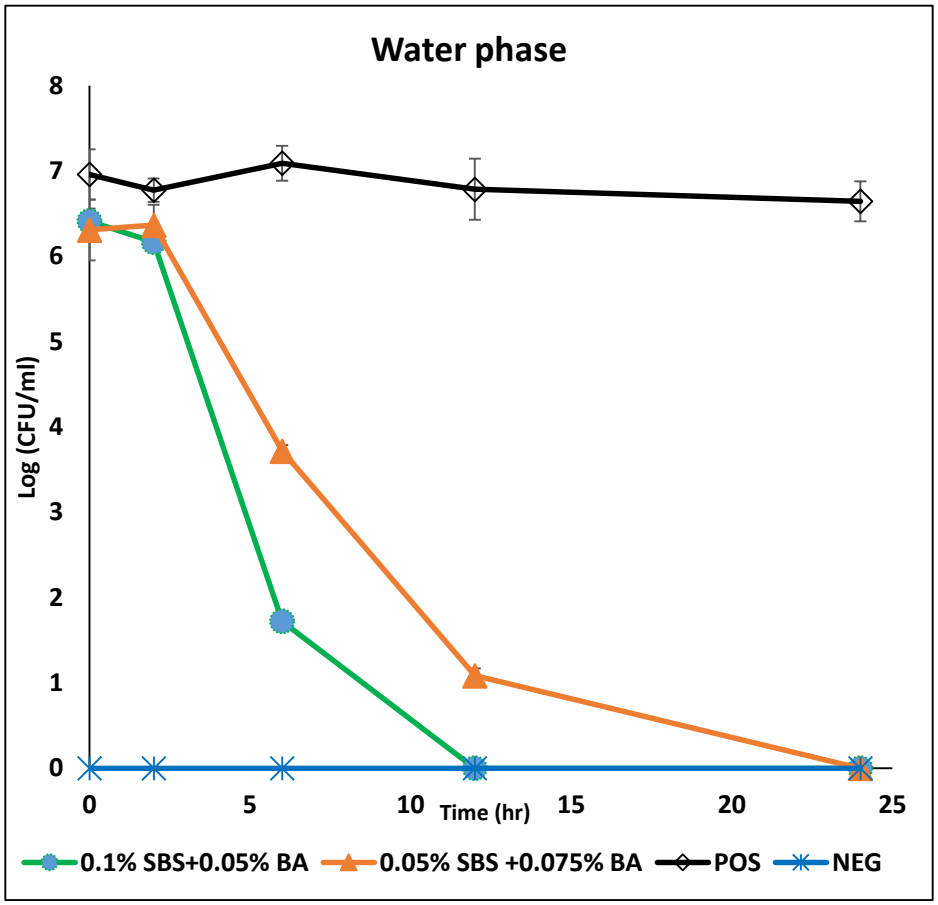


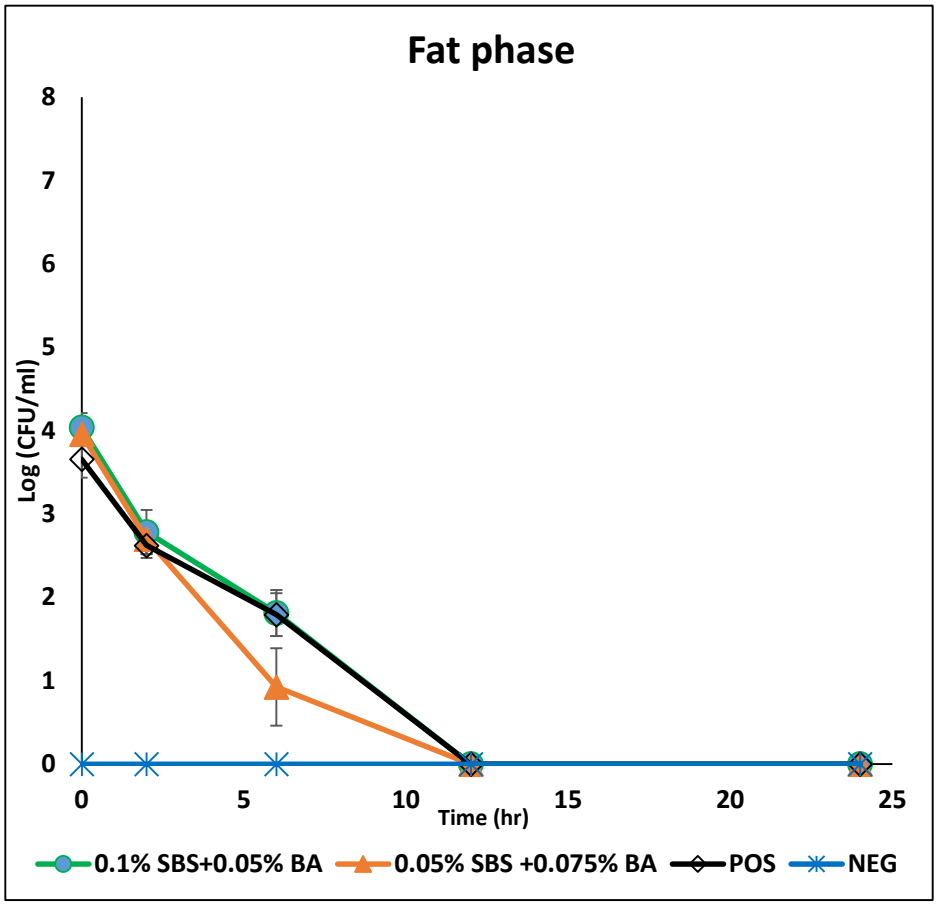
Log reduction of *Salmonella* using SBS + Propionic acid





Log reduction of *Salmonella* using SBS + Butyric acid





Conclusions

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

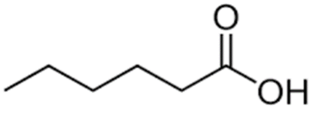
- The combination of SBS with organic acids has a potential synergistic effect against *Salmonella*

2. Medium chain fatty acids (MCFAs) as topical additives to mitigate *Salmonella* Typhimurium in dry pet food kibbles

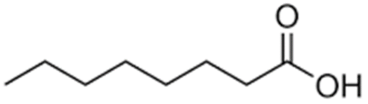
Introduction

- Medium chain fatty acids (MCFAs) are aliphatic fatty acids with 6-12 carbon atoms

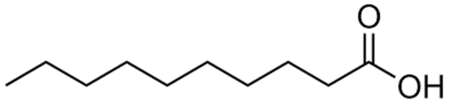
- Palm kernel oil & coconut oil are sources for commercial extraction



Caproic acid, $C_6H_{12}O_2$



Caprylic acid, $C_8H_{16}O_2$



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

Hypothesis

- The application of MCFAs reduce *Salmonella* loads in fat/oil coated dry dog food kibbles

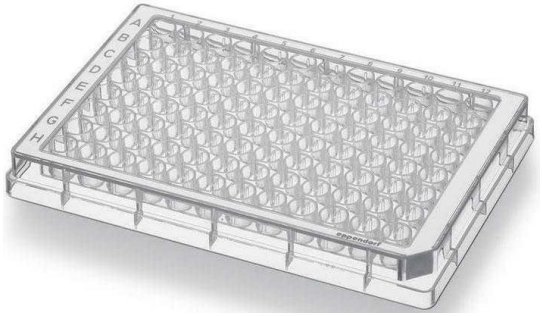
Objectives

- 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

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



Micro titer well plate

MCFA dosages used

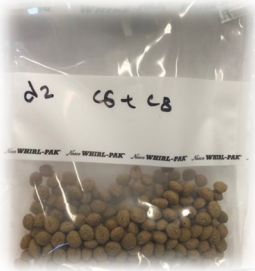
MCFA	dose
C6	0.5%
C8	0.5%
C10	1.0%
C6 + C8	0.25 - 0.5%
C6 + C10	0.25 -1.0%
C8 + C10	0.25 -1.0 %

Efficacy of MCFA on dry pet food kibbles

37 °C/30 mins

Spray 5ml of ~6 log *Salmonella*

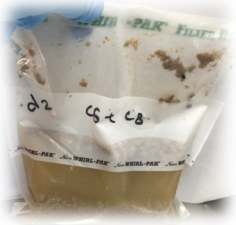
37 °C/30 mins



225 ml of BPW

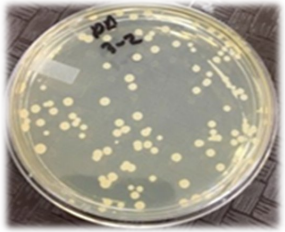
25 g of sample in a Whirlpak bag

Stomach for 2 min



Dilute and plate on TSA plates

37 °C/24h



Count and analyze

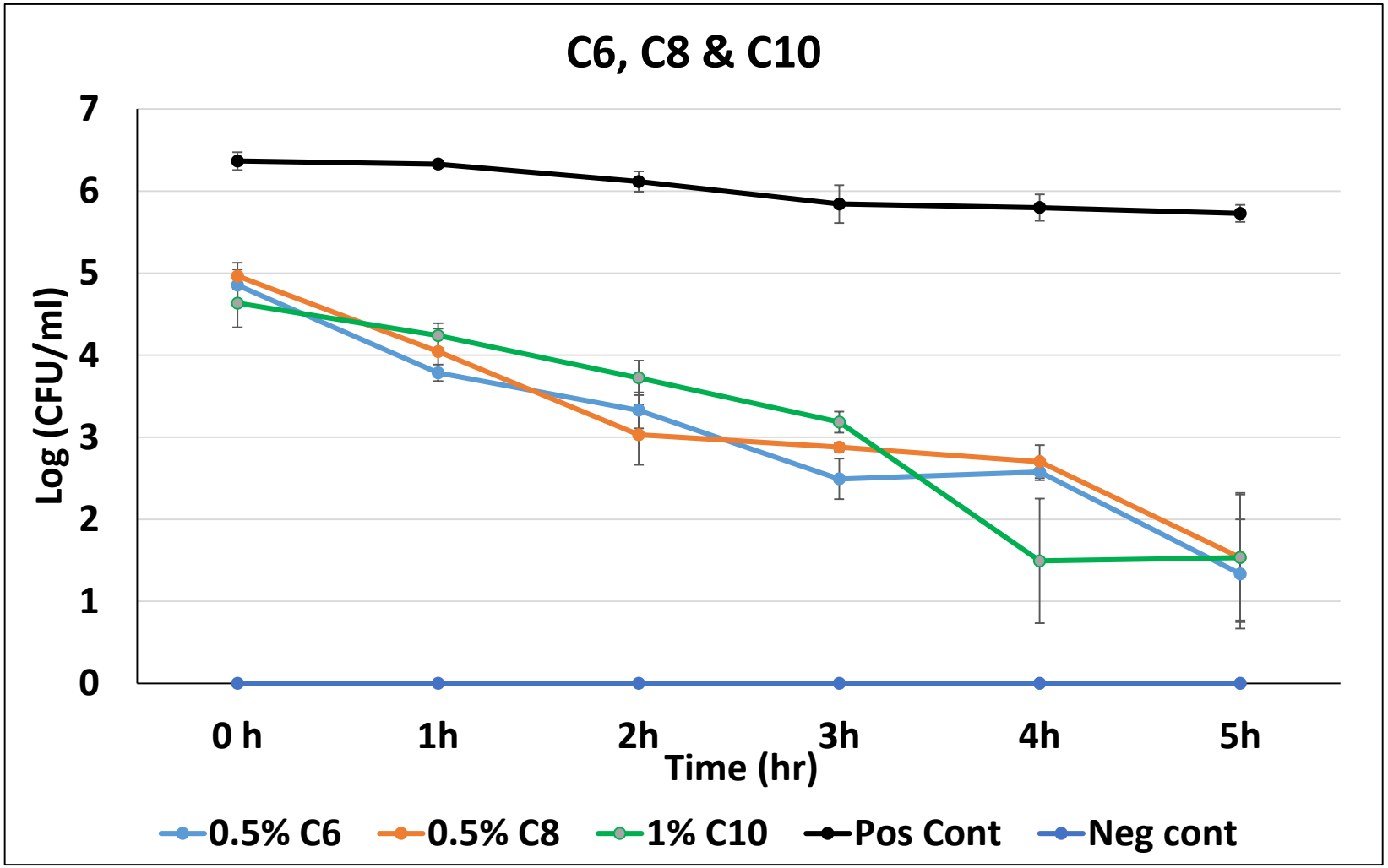
- Sampling at pre-determined time intervals (0, 1, 2, 3, 4, and 5h)

Results

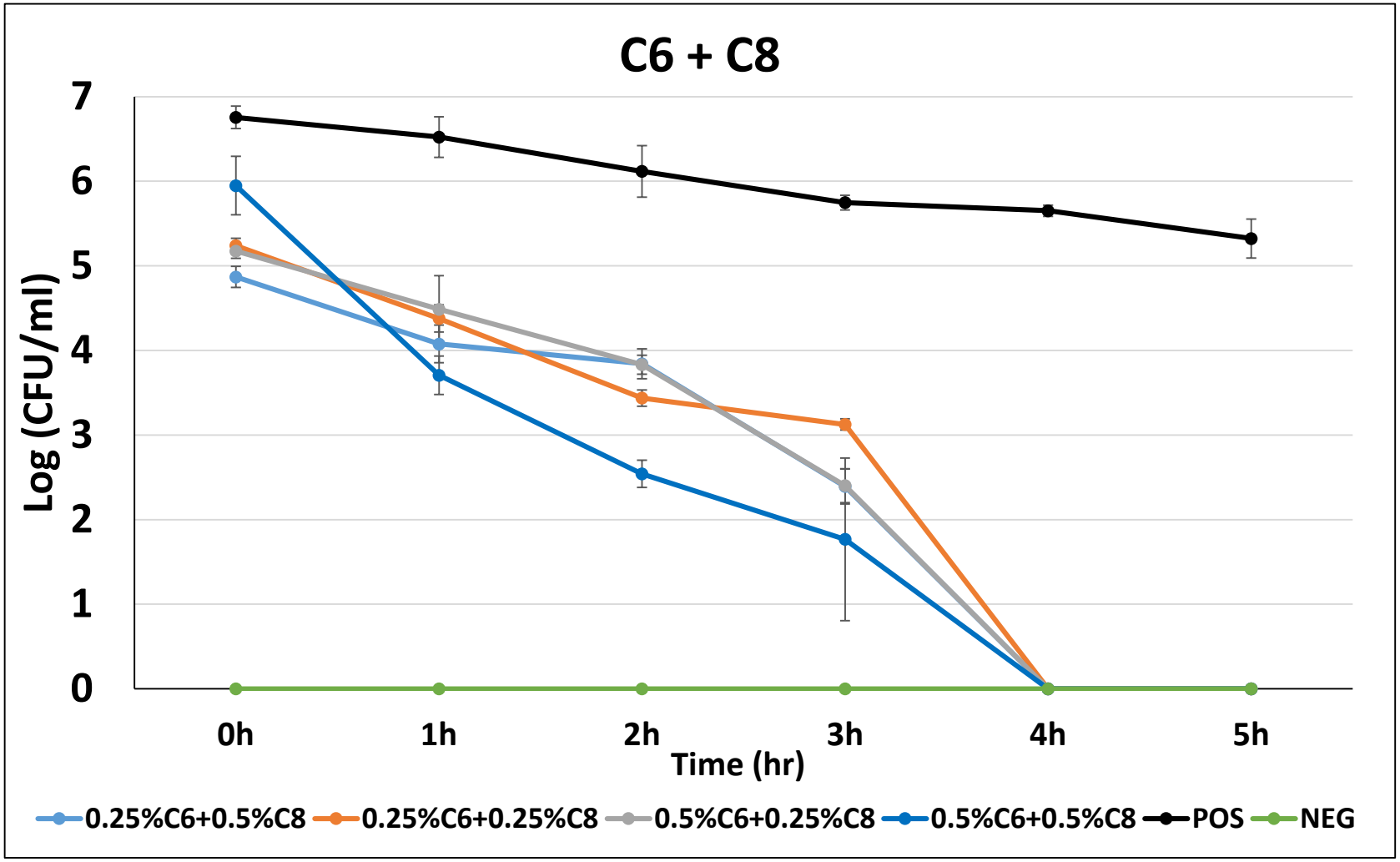
MIC results

- 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%**

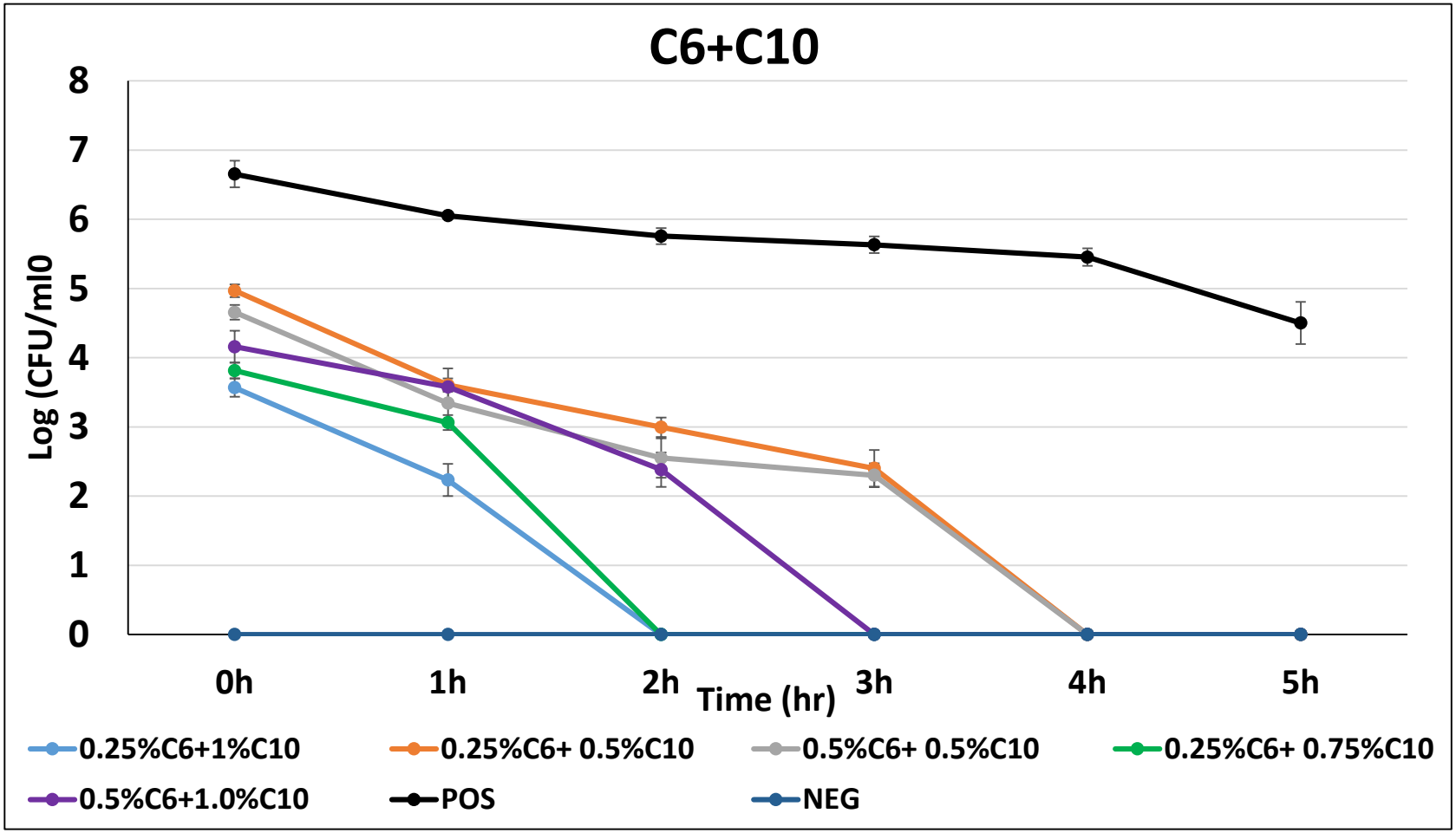
Log reduction of *Salmonella* with MCFA over time



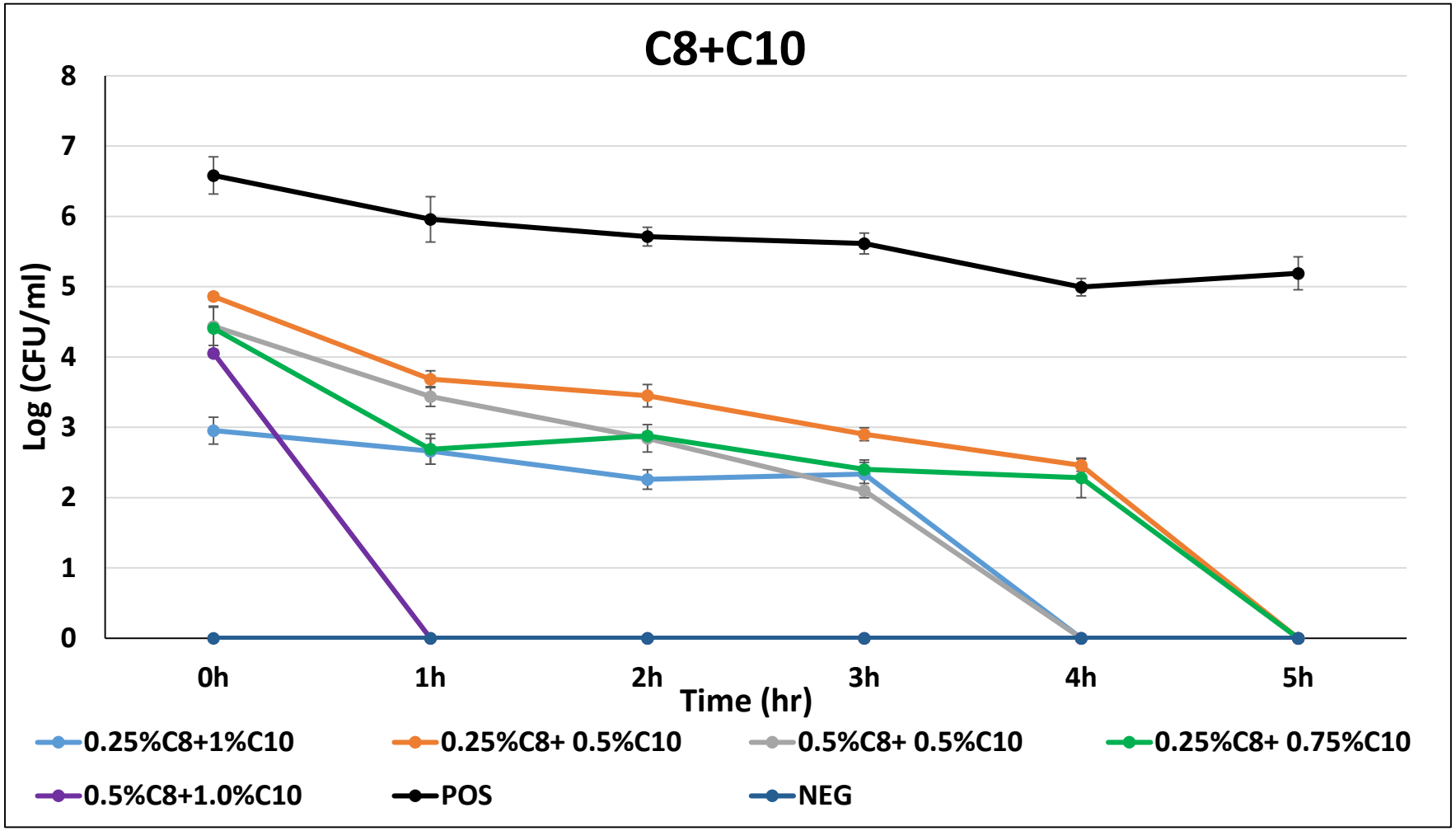
Log reduction of *Salmonella* with MCFA over time



Log reduction of *Salmonella* with MCFA over time



Log reduction of *Salmonella* with MCFA over time



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

Dr. Greg Aldrich
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Pet Food Program Coordinator
Kansas State University

JH JONES-HAMILTON CO.

PMI NUTRITIONAL ADDITIVES

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Thank you!