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**Research Paper**

# Use of Medium Chain Fatty Acids To Mitigate *Salmonella* Typhimurium (ATCC 14028) on Dry Pet Food Kibbles

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## ABSTRACT

This study evaluated the antimicrobial effects of medium chain fatty acids (MCFAs) against *Salmonella* Typhimurium (ATCC 14028) when used on dry dog food kibbles. The MIC of three MCFAs, caproic (C6), caprylic (C8), and capric (C10), was determined using the broth micro- and macrodilution assay technique. Using canola oil as a fat coating, the efficacy of each MCFA was then tested on dry dog food kibbles at 37°C for up to 5 h. The MIC was found to be 0.3125, 0.3125, and 0.625% for C6, C8, and C10, respectively. When the MCFAs were tested on fat-coated dry kibbles, all three MCFAs reduced ($P \leq 0.05$) *Salmonella* levels by >4.5 log after 5 h when the *Salmonella* recovery from a no-treatment control was ~6.4 log. At each evaluation time point, the three treatments were effective in reducing ($P \leq 0.05$) *Salmonella* loads. No countable colonies of *Salmonella* were detected at 4 h when the combination of C6+C8 was used on the kibbles ($P \leq 0.05$), whereas with the C6+C10 combination, the *Salmonella* colonies were not detectable between 2 and 4 h after treatments ($P \leq 0.05$). Different combinations of C8 and C10 caused *Salmonella* to drop to a nondetectable limit (1 CFU/g) between 1 and 5 h after treatment ($P \leq 0.05$). This study suggests that the use of MCFAs during kibble coating may mitigate postprocessing *Salmonella* recontamination on dry dog food kibbles.

## HIGHLIGHTS

- Application of MCFA(s) mitigated postprocessing *Salmonella* contamination in dry dog food kibbles.
- Combinations of MCFAs had synergistic effects against *Salmonella*.
- A palatant may be necessary to mask the aroma or flavor of MCFAs applied to kibbles.

Key words: Dog food kibble; Medium chain fatty acid; *Salmonella*

Postprocessing coating of dry dog food kibbles with fats, oils, and flavoring agents poses a risk of contamination from pathogens such as *Salmonella* and *Escherichia coli*. Because of the risk of *Salmonella* contamination to pet owners and pet food handlers, the U.S. Food and Drug Administration has implemented a zero-tolerance policy for *Salmonella* spp. in pet foods under the 2011 Food Safety Modernization Act. Several pet foods linked to *Salmonella* contamination have been reported in recent years, including a multidrug-resistant *Salmonella* outbreak in 2017 *(9)*. *Salmonella* Schwarzengrund *(7)* and *Salmonella* Infantis *(8)* recalls of pet foods during 2007 and 2012, respectively, were traced back to dry pet food kibbles. Dry pet food constitutes the most commonly sold type of pet food in the world, constituting 75.2% of dog food and 53.9% of cat food categories *(25)*.

Recontamination after processing has been proposed as a potential source of *Salmonella* spp. in final rendered products *(12, 23, 37)*. Fats and oils are commonly coated on dry pet food kibbles. This procedure occurs after the process kill step. There is a potential for *Salmonella* contamination in coating materials. The most likely source of postprocessing contamination of *Salmonella* is from fats, oils, additives (probiotics and enzymes), and flavoring agents that are added after processing or after cooking. A survey sponsored by the Fats and Proteins Research Foundation evaluated raw materials (animal waste tissues), crax (material from cooking and expelling processes), and final rendered products for five pathogenic bacteria, including *Salmonella* spp. *(37)*. The presence of *Salmonella* spp. was found in 84.5% of the raw ingredients sampled, 0% of crax samples sampled, and 26.1% of final rendered products sampled *(37)*. Previous reports of human salmonellosis in the United States from 2006 to 2007 were attributed to *Salmonella* Schwarzengrund, traced back to dry dog food at the Mars Petcare U.S. production facility *(7)*. During further investigation of the manufacturing facility in which the contaminated pet food was produced, a swab collected from the enrobing-flavoring room tested positive for the outbreak

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TABLE 1. *Formulation of the dry kibbles*

| Ingredient | % |
|---|---|
| Chicken meal, low ash | 41.926 |
| Brewers rice | 36.842 |
| Corn | 12.632 |
| Beet pulp | 5.263 |
| Dicalcium phosphate | 1.579 |
| Salt | 0.632 |
| Potassium chloride | 0.632 |
| Trace mineral premix | 0.211 |
| Vitamin premix | 0.158 |
| Choline chloride, 60% | 0.126 |

strain. In the enrobing-flavoring room, materials such as fats and palatability enhancers are sprayed onto the surface of the finished product to enhance palatability *(3)*. Organic acids and acidulants when added to chicken fat had bactericidal effects against *Salmonella (14)*. Based on work by Cochrane et al. *(11),* the application of medium chain fatty acids (MCFAs) might also provide protection against *Salmonella* in a similar manner.

Commercial sources of MCFAs are derived from palm kernel oil and coconut oil. In addition to their role in benefiting gut health *(26)* and weight control *(35),* MCFAs have also been reported to have antibacterial effects against *Salmonella (33)* and *Escherichia coli (32)*. Therefore, MCFAs may be a nutritionally beneficial additives that also mitigate reduced *Salmonella* in fats and oils. Thermal mitigation of *Salmonella* during extrusion offers a point-in-time method for destruction of *Salmonella* but offers no residual antimicrobial activity or protection from recontamination, whereas a topical addition with an antimicrobial agent such as a MCFA might.

The mechanism of antibacterial action of MCFAs may be due to their anionic effect. They are known to act as surfactants and to incorporate into the bacterial cell membranes, potentially causing transient or permanent opening in the membranes *(1, 4, 13)*. Changes in gastrointestinal microbiota caused by MCFAs are also known to reduce the colonization of pathogens in the animal gut *(27)*. Antimicrobial efficacy of MCFAs varies depending on the bacterial species. Batovska et al. *(2)* reported that lauric acid (C12) was the most potent MCFA against gram-positive bacteria such as *Staphylococcus aureus, Streptococcus pyogenes, Clostridium diphtheriae,* and *Listeria monocytogenes,* whereas Van Immerseel et al. *(38)* reported that caproic acid (C6) was the most potent MCFA against *Salmonella* Enteritidis, a gram-negative enteric pathogen. Thus, MCFAs could be an alternative to conventional antimicrobial agents to reduce *Salmonella* contamination during postprocessing (fat and flavor coating) of dry extruded pet foods. We hypothesize that the use of MCFAs as a coating agent on kibbles prevents the postprocessing contamination of *Salmonella* and hence reduces cross-contamination. A comprehensive approach to test individual MCFAs and their combinations against *Salmonella* may elucidate the dosage and proportions necessary to be effective in pet food kibble applications.

## MATERIALS AND METHODS

**Salmonella serotypes and sources of MCFAs and pet food kibbles.** *Salmonella* Typhimurium (ATCC 14028) was maintained in tryptic soy broth (TSB)–glycerol (7:3) at 80°C. Before use, the frozen cultures were streaked on tryptic soy agar (TSA) plates and incubated at 37°C for 24 h. A single colony of this *Salmonella* strain was inoculated into 10 mL of TSB and incubated at 37°C for 18 to 24 h. The MCFAs included caproic acid (C6, 50%), caprylic acid (C8, 50%), and capric acid (C10, 50%) that were provided by the study sponsor (PMI Nutrition, Land O'Lakes, Arden Hills, MN). The uncoated dry dog food kibbles were custom manufactured at Extru-Tech Inc. (Manhattan, KS). The composition of the kibble is presented in Table 1.

**MIC assay.** The MICs of C6, C8, and C10 were determined by the broth micro- and macrodilution assay according to the method described by the Clinical and Laboratory Standards Institute *(10)* in TSB. The MCFAs were prepared in ethanol stock solutions (5% ethanol), and the MIC was tested against *Salmonella* Typhimurium (ATCC 14028). In brief, a 10% working solution of C6, C8, and C10 was added in the microtiter wells. A 100-μL aliquot of bacterial culture (~6 log CFU/mL) was added to each well of the plate containing 100 μL of decreasing concentrations of MCFAs to make a final volume of 200 μL per well. The positive control consisted of *Salmonella* inoculum only (no treatments), the negative control consisted of TSB alone, and an ethanol control consisted of 5% ethanol in TSB. The MIC was defined as the lowest concentration of MCFA that inhibited visible growth of *Salmonella* after 24 h of incubation at 37°C.

***Salmonella* mitigation on dry dog food kibbles.** A 140-g aliquot of dry dog food kibble from each treatment was transferred to a plastic container and autoclave sterilized. For individual MCFAs, a total of five containers were maintained: three for each of the MCFAs (C6, C8, and C10), one for the positive control, and one for the negative control. For the treatment combination study, a total of 16 containers were maintained: four for C6+C8 combinations, five for C6+C8 combinations, and five for C8+C10 combinations, along with one for the positive control and one for the negative control. The MCFAs were applied by uniformly coating the kibbles with canola oil to make a final weight of 150 g in each container. The final concentrations of the MCFAs and MCFA combinations on the kibbles are reported in Table 2. The concentrations of the individual MCFAs used were based on the MICs. For the combination of the MCFAs, the various combinations selected were based on the MICs of the each MCFA. The positive control was *Salmonella* inoculum in TSB without any MCFAs, and the negative control was canola oil only, without MCFAs. The final oil percentage on the kibbles was maintained at ~7%, and the moisture percentage of the kibbles was 7.6% dry basis. After 30 min of the MCFA-oil treatment, a bulk-harvested *Salmonella* culture (~8 log) from an overnight-grown 800-mL TSB broth was concentrated and spray treated on the kibbles. After uniform mixing of the kibbles, the containers were incubated at 37°C. Microbiological analyses were conducted for each of the containers at various predetermined time intervals: 0, 1, 2, 3, 4, and 5 h. From each treatment and control, a 25-g subsample was collected in sterile Whirl-Pak bags (Nasco, Ft. Atkinson, WI) and was mixed in 225 mL of buffered peptone water and stomached for 2 min. The mixtures were serially diluted in 0.1% peptone water and plated on TSA. A total 1-mL volume of the diluent was plated on four TSA plates. The plates were

TABLE 2. *MCFAs used in kibble treatments*

| | Dose (%) | pH |
|---|---|---|
| Individual MCFA | | |
| Caproic acid (C6) | 0.50 | 4.29 |
| Caprylic acid (C8) | 0.50 | 4.47 |
| Capric acid (C10) | 1 | 4.51 |
| MCFA combination | | |
| C6+C8 | 0.25 + 0.5 | 4.62 |
| | 0.25 + 0.25 | 4.50 |
| | 0.5 + 0.25 | 4.48 |
| | 0.5 + 0.5 | 4.49 |
| C6+C10 | 0.25 + 1.0 | 4.67 |
| | 0.25 + 0.5 | 4.59 |
| | 0.5 + 0.5 | 4.61 |
| | 0.25 + 0.75 | 4.68 |
| | 0.5 + 1.0 | 4.66 |
| C8+C10 | 0.25 + 1.0 | 4.74 |
| | 0.25 + 0.5 | 4.70 |
| | 0.5 + 0.5 | 4.69 |
| | 0.25 + 0.75 | 4.64 |
| | 0.5 + 1.0 | 4.70 |
| Control (kibble + canola oil) | | 4.64 |

incubated at 37°C for 24 h and then colonies were counted. The detection limit for the *Salmonella* was <1 CFU/g of the sample.

Data were analyzed using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC) separately for the two studies. The first experiment was a 5 × 6 factorial arrangement of treatments using three chemicals (C6, C8, and C10) and the positive and negative controls, and six sampling intervals. The second experiment was a 16 × 5 factorial arrangement of treatments using 14 chemical combinations and the positive and negative controls, and five sampling intervals. Each treatment consisted of three replicates.

**Palatability study.** Palatability in pets was performed using a two-bowl forced choice evaluation *(19, 28, 34)* at a commercial research kennel (Summit Ridge Farms, Susquehanna, PA). The kennel facility is registered with the U.S. Department of Agriculture No. 23-R-0126 under the Animal Welfare Act and follows the best practices for animal care and use in a humane manner. A total of four combination treatments consisting of 0.25% C6 plus 0.25% C8 (treatment 1), 0.25% C6 plus 0.5% C10 (treatment 2), 0.25% C8 plus 0.5% C10 (treatment 3), and 0.5% C6 plus 1.0% C8 (treatment 4) were surface applied to coat kibbles along with chicken fat (8% final fat coating). The control diet was coated with chicken fat only. Twenty dogs were offered 325 g of food per dog per day for each of 2 days. The four palatability assays consisted of the control fed versus treatment 1, treatment 2, treatment 3, and treatment 4. Each dog was allowed to smell the food and then the bowls were placed simultaneously before the animal for consumption with a positional shift each day. The technician monitored which of the two foods (A and B) had been approached first and from which one the first bite was taken. The bowls were left with the dog for 30 min or until food in one of the bowls was completely consumed. First choice was based on the first bite taken, and consumption was calculated as the ratio of A:B and consumption ratio as A/A+B. First choice was evaluated by a chi-square probability. The consumption of each diet was compared by a Wilcoxon signed rank test and a two-way analysis of variance.

## RESULTS

**MIC assay.** The MICs of caproic (C6) and caprylic (C8) acids were 0.3125% and that of capric acid (C10) was 0.625%. For capric acid, the MIC was determined as the lowest concentration of the lipid that reduced growth of *Salmonella* by >50% *(15)*.

***Salmonella* control on dry dog food kibbles.** When individual MCFAs were tested on fat-coated dry kibbles against *Salmonella,* all three treatments (caproic, caprylic, and capric acids) reduced ($P \leq 0.05$) pathogen levels by ~4.5 log after 5 h of incubation at 37°C (Fig. 1). The *Salmonella* recovery from a no-treatment control was ~6.4 log. At each evaluation time point, all three treatments were effective in reducing ($P \leq 0.05$) *Salmonella* loads. When *Salmonella* reductions were analyzed among different time points, caproic acid caused significant reduction at 1 h compared with 0 h, and the reduction (4.4 log) at 5 h was the highest ($P \leq 0.05$) compared with the other time intervals (Fig. 1). The *Salmonella* reduction by caprylic acid at 2 h was significant compared with 0 h ($P \leq 0.05$). With 4.2-log reduction compared with the positive control, the reduction was the greatest at 5 h (Fig. 1). Capric acid caused

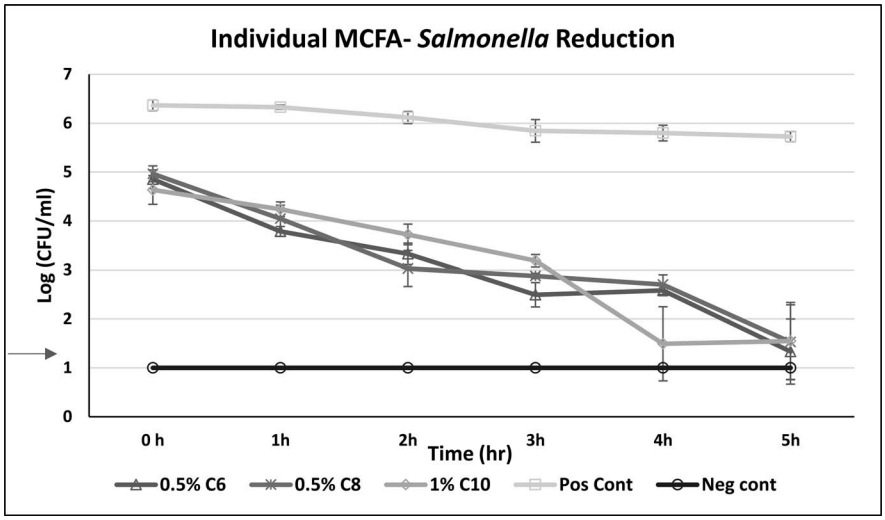


FIGURE 1. *Effects of various medium chain fatty acids (MCFAs) against* Salmonella *Typhimurium (ATCC 14028) evaluated on dry dog food kibbles system at 37°C. Treatments from each phase were plated on TSA plates at different time intervals. C6, caproic acid; C8, caprylic acid; C10, capric acid; Pos Cont, positive control (*Salmonella *only, no antimicrobial agents added); Neg cont, negative control (no* Salmonella, *no antimicrobial agents). The arrow bar indicates the detection limit (1.0 log CFU/g) of sampling using TSA plates.*

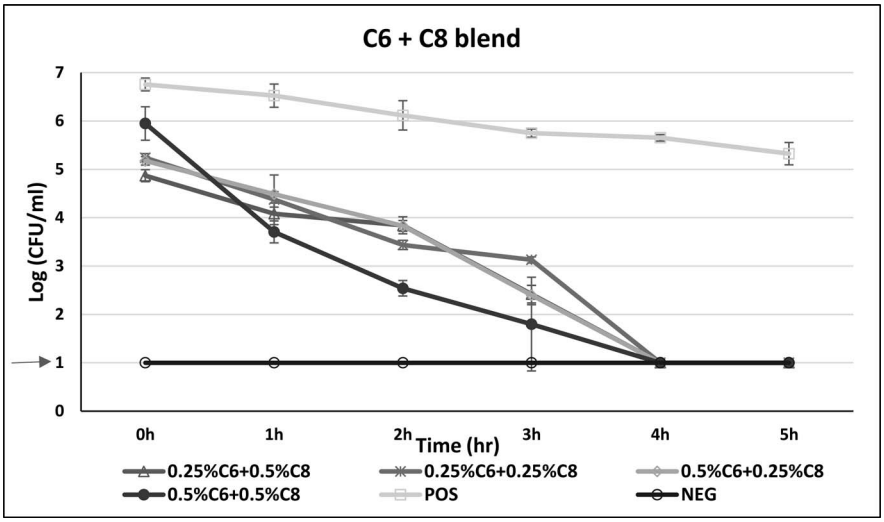


FIGURE 2. *Effects of various combinations of caproic acid (C6) and caprylic acid (C8) against* Salmonella *Typhimurium (ATCC 14028) evaluated on dry dog food kibbles system at 37°C. Treatments from each phase were plated on TSA plates at different time intervals. POS, positive control (*Salmonella *only, no antimicrobial agents added); NEG, negative control (no* Salmonella, *no antimicrobial agents). The arrow bar indicates the detection limit (1.0 log CFU/g) of sampling using TSA plates.*

a reduction ($P \leq 0.05$) of *Salmonella* at 4 and 5 h compared with 0 h, and the reduction was the greatest (4.3 log) at 4 h (Fig. 1). The pH of the food matrix after the application of MCFAs was between 4.3 and 4.5. Across the different time points, recovery of *Salmonella* from the positive control remained constant until 2 h before a decline in the recovery ($P \leq 0.05$). There was a 0.6-log reduction in the *Salmonella* recovery in the untreated control (Fig. 1).

When combinations of different MCFAs were evaluated, all the treatment combinations of C6+C8, C6+C10, and C8+C10 were effective in reducing ($P \leq 0.05$) *Salmonella* loads on kibbles compared with the positive control (Figs. 2 through 4). Various concentrations and their respective pH values are reported in Table 2. All four combinations of C6+C8 (Table 2) reduced ($P \leq 0.05$) *Salmonella* load on kibbles at each time point of the evaluation and achieved a nondetectable level by 4 h. The 0.5% C6 plus 0.5% C8 combination had the greatest reduction from 1 to 3 h (Fig. 2). All five treatment combinations of C6+C10 (Table 2) reduced ($P \leq 0.05$) *Salmonella* loads at each time point. The combinations 0.25% C6 plus 1.0% C10 and 0.25% C6 plus 0.5% C10 reduced *Salmonella* to a nondetectable level at 2 h of treatment, whereas 0.5% C6 plus 1% C10 did so at 3 h, and the remaining two combinations (0.5% C6 plus 0.5% C10 and 0.25% C6 plus 0.5% C10) did so after 4 h of treatment (Fig. 3). At each time point, all five treatment combinations of C8+C10 (Table 2) reduced ($P \leq 0.05$) *Salmonella* on kibble. The combination 0.5% C8 plus 1.0% C10 seemed to be the most effective at reducing *Salmonella* to a nondetectable level (within 1 h), whereas the combinations 0.25% C8 plus 1.0% C10 and 0.5% C8 plus 0.5% C10 decreased the *Salmonella* to a nondetectable level at 4 h, and the rest of the combinations did so by 5 h of incubation (Fig. 4). In the combination study, there was also an ~1.3-log reduction ($P \leq 0.05$) in the pathogen load in the no-treatment control by the end of incubation period (5 h).

The two-bowl palatability consumption of the control diet was greater ($P < 0.05$) than that of all the MCFA-treated diets (average, 2.43:1), with an intake ratio average of 0.676 (Table 3). First choice as an indication of aroma averaged at 2.34:1 ($P < 0.05$) in favor of the control over the MCFA-treated diets.

## DISCUSSION

The individual fatty acids C6, C8, and C10 used in this study were stored at room temperature and had melting

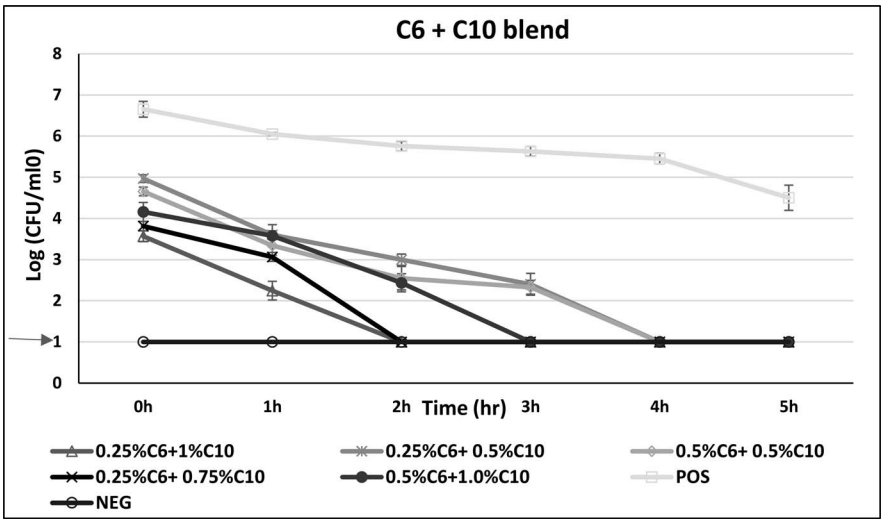


FIGURE 3. *Effects of various combinations of caproic acid (C6) and capric acid (C10) against* Salmonella *Typhimurium (ATCC 14028) evaluated on dry dog food kibbles system at 37°C. Treatments from each phase were plated on TSA plates at different time intervals. POS, positive control (*Salmonella *only, no antimicrobial agents added); NEG, negative control (no* Salmonella, *no antimicrobial agents). The arrow bar indicates the detection limit (1.0 log CFU/g) of sampling using TSA plates.*

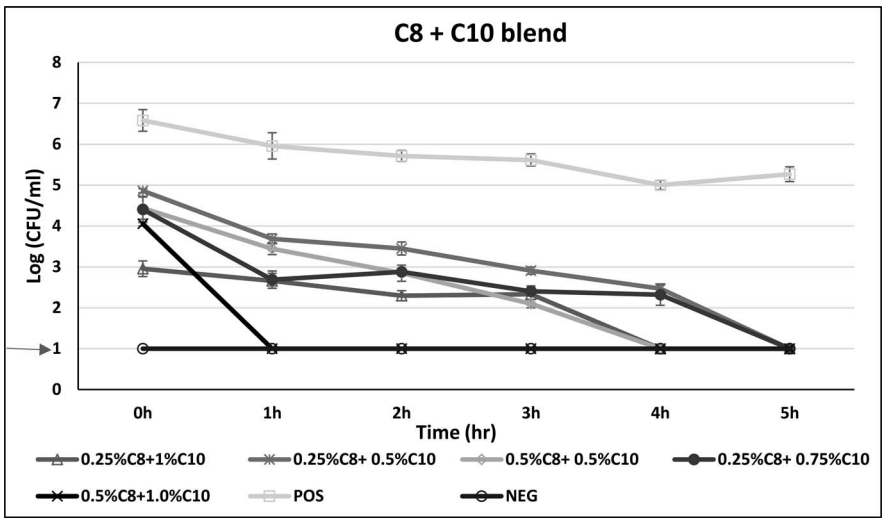


FIGURE 4. *Effects of various combinations of caprylic acid (C8) and capric acid (C10) against* Salmonella *Typhimurium (ATCC 14028) evaluated on dry dog food kibbles system at 37°C. Treatments from each phase were plated on TSA plates at different time intervals. POS, positive control (*Salmonella *only, no antimicrobial agents added); NEG, negative control (no* Salmonella, *no antimicrobial agents). The arrow bar indicates the detection limit (1.0 log CFU/g) of sampling using TSA plates.*

points of –3°C *(6),* 6.5°C, and 31.5°C *(16),* respectively. Therefore, to determine the MIC of capric acid (C10) required additional manipulation by dissolving in ethanol. Given this, the other two MCFAs, caproic (C6) and caprylic (C8) acids, were also dissolved in the same solvent. The MICs of caproic (C6) and caprylic (C8) acids were read as the lowest concentration, causing inhibition of visual growth of bacteria after incubation at 37°C for 24 h, whereas for capric acid (C10) acid, due to its thick turbid nature, we determined the MIC as the lowest concentration of the acid that reduced growth of *Salmonella* Typhimurium by >50% *(15)*. Against *Salmonella* Typhimurium, we determined the MICs for both caproic and caprylic acids to be 0.3125%, whereas that of the capric acid was 0.625%. Kitahara et al. *(24)* determined the MIC of caprylic acid and capric acid to be 0.16 and 0.08%, respectively, against *S. aureus*. These results did not match our finding against *Salmonella* Typhimurium, which could be due to species differences. In another study, the MIC of caproic, caprylic, and capric acids against *Salmonella* Typhimurium was reported as >0.5, 0.3, and 0.5% and against *E. coli* was >0.5, 0.2, and 0.5%, respectively *(31)*. The MIC results of caprylic and capric acids from our study against *Salmonella* closely aligned with these results, whereas that of caproic acid did not (0.3125 versus >0.5%). Nobmann *(29)* reported the MIC of caprylic acid to be >20 mM for both *Salmonella* Typhimurium (ATCC 14028) and *E. coli*. We determined that the MIC of capric acid was higher (two times) than that of caproic and caprylic acids. The better antimicrobial activity of C6 and C8 could be due to their lower pH in solution (5.36 and 5.68, respectively) compared with C10 (6.18). Boyen et al. *(5)* determined the MICs of caproic and caprylic acids against various strains of *Salmonella* Typhimurium to be in the range of 20 to 40 mM at pH 6. The higher MIC of capric acid could be related to the observation that the pH of C10 was higher (6.18) that that of the other two acids. Sylvester et al. *(36)* reported that capric acid (C10) was less potent (1% MIC) compared with caproic (C6) and caprylic acid (C8), with a MIC of 0.4 and 0.5%, respectively. This was similar to the findings of Skřivanová and Marounek *(30),* who reported a lower antibacterial activity of capric acid than caproic and caprylic acids. Throughout the MIC study, the final concentration of ethanol was maintained at ~5%, and the ethanol itself at that concentration did not display any antimicrobial activity. This was also observed by Huang et al. *(18),* who did not notice any significant antibacterial activity, even at 25% concentration of ethanol.

TABLE 3. *Palatability assessment of MCFA-coated dry dog food kibbles relative to the control (0% MCFA) by dogs*

| Diet A vs B | FC of A, $n^a$ | IR of diet A$^b$ |
|---|---|---|
| Control vs treatment 1 | 24 | 0.671 |
| Control vs treatment 2 | 30$^c$ | 0.712 |
| Control vs treatment 3 | 30$^c$ | 0.755 |
| Control vs treatment 4 | 28$^c$ | 0.566 |

[a] First choice (FC) number of first visits to bowl with diet B can be obtained by $40 - n$.
[b] Intake ratio (IR) of diet A = avg of intake (g) of diet A/total intake (g) of diets A+B.
[c] $P$ value is <0.05.

During the production of dry dog food kibbles, extrusion processing serves as a thermal kill step, but it does not protect the food from postprocessing contamination of pathogens such as *Salmonella* and *E. coli*. Fat and flavor ingredients that are coated after extrusion are a potential source of cross-contamination. The use of MCFAs as a coating ingredient on kibbles showed a promising effect to mitigate postprocessing *Salmonella* Typhimurium contamination on kibbles. All three MCFAs when individually applied reduced *Salmonella* Typhimurium loads by 4.2 log after 5 h of incubation. This suggests that the coating of MCFA could provide a residual effect against this pathogen. Caprylic acid added in poultry feed at a concentration between 0.7 and 1% also lowered the colonization of *Salmonella* in broiler chicks *(20)*. The concentrations used were comparable to those of our study (0.5 to 1% of MCFAs as individual chemical applications). By contrast, caprylic acid (C8) was not effective at reducing fecal shedding of *Salmonella* Typhimurium in pigs *(5)*. The antimicrobial effect of the MCFAs was also demonstrated by Kiesel et al. *(21),* who proved that when the MCFAs were coupled with

a nonionic surfactant they could be used as a disinfecting solution.

During the study of MCFA blends, we combined pairs of three MCFAs with each other at multiple concentrations within each combination. C6 and C8 each had the same MIC and reduced *Salmonella* Typhimurium by 4.4 and 4.2 log, respectively, after 5 h. However, when combined, they eliminated *Salmonella* at 4 h of incubation. This suggests a strong synergism between the two chemicals. By contrast, C10 had a higher MIC than C6 or C8 but a similar reduction of *Salmonella* Typhimurium by 4.2 log and surprisingly resulted in an even stronger effect when combined with C6. All five combinations of treatments eliminated *Salmonella* Typhimurium after 4 h of treatments. The treatment combinations with higher C10 levels (0.25% C6 plus 1.0% C10 and 0.25% C6 plus 0.75% C10) and 0.5% C6 plus 1% C10 were more effective at reducing *Salmonella* Typhimurium to a nondetectable level (2 and 3 h, respectively). Similarly, when C8 was combined with C10, the combination containing a higher level of C10 (0.5% C8 plus 1% C10) reduced the *Salmonella* Typhimurium to a nondetectable level within the first hour. This indicated that even though C10 is less potent when used alone, it exerts a very strong antimicrobial effect against *Salmonella* Typhimurium when combined with C6 or C8. Kim and Rhee *(22)* reported a synergistic antibacterial effect of MCFAs and organic acid, and the underlying reason was suspected to be the effect of organic acids to facilitate the entry of MCFAs into the bacterial cells. Because of the similar bactericidal mechanism of all MCFAs, the authors could not explain the precise reason behind this elevated potential of C10. Often, the pH of different treatments might be a consideration; however, we observed that the pH values of all treatments were similar (between 4.5 and 4.7). In summary, MCFA combinations provided a wider spectrum of bacterial control than individual applications. A 0.8% application of C8:C10 mixture (50:50) reduced *E. coli* by 2.9 log within 3 h of incubation at 37°C when applied in pig feed *(11)*. Future research to elucidate the exact mechanism of action and improved efficacy of C6 and C8 when combined with C10 is warranted, along with elucidation of a mechanism. We achieved a better reduction of *Salmonella* Typhimurium with a lower concentration of MCFAs compared with the findings of Cochrane et al. *(11),* who achieved only 3.1-log reduction when using a 2% MCFA (1:1:1) blend in food.

In conclusion, our finding is promising because unlike the use of conventional antimicrobial agents such as organic acids, commercial formaldehyde, or acidulants in feed ingredients, the inclusion of realistic levels of MCFA in pet foods decreased the cross-contamination of *Salmonella* in addition to its known health benefits. This study suggests that the use of MCFAs during kibble coating may mitigate postprocessing *Salmonella* contamination on dry dog food kibbles. In a study by Skřivanová et al. *(32),* rabbits were fed diets containing 0 to 5% C8 and 1% C8+C10 and later challenged with artificial *E. coli* O128 infection. The diets containing MCFAs were significantly better at preventing colibacillosis. Compared with their study, our findings are even more promising because the addition of MCFAs in dog food could help to prevent *Salmonella* and *E. coli* inside the gastrointestinal track of the animals in addition to preventing cross-contamination during production and storage of kibble. Skřivanová and Marounek *(30)* observed that an acidic environment was favorable for MCFAs against the gram-negative bacterium *E. coli*. This is similar to our findings where all of our MCFA-treated feeds were within the pH range 4.3 to 4.7, indicating a better antimicrobial activity against *Salmonella* Typhimurium. Future research to evaluate the effectiveness of MCFAs as antimicrobial agents against other serotypes or a combination of serotypes of *Salmonella* commonly associated with pet foods is suggested. In one important finding, Hovorková et al. *(17)* discovered that MCFAs were significantly bactericidal against gram-positive pathogens such as *L. monocytogenes, Clostridium perfringens,* and *S. aureus,* whereas they did not exert inhibitory effects against gut commensal bacteria. This is very important in terms of maintaining dog colonic microbial ecology when fed MCFAs-added diets. Further research is warranted to look into the effect on the dog microbiome when MCFAs are added to the food. Although effective for *Salmonella* control, coating MCFAs on the dry dog kibbles did not enhance the palatability of the diets.

To summarize, the application of individual or combinations of MCFAs provided control to postprocessing contamination of *Salmonella* Typhimurium on dry dog food kibbles. The combination of MCFAs also had synergistic effects against *Salmonella* Typhimurium. The palatability results suggest that a palatant may be necessary to mask the aroma or flavor of the MCFAs when applied to kibbles.

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