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2021 ANIMAL FEED AND PET FOOD RESEARCH

Application of Encapsulated Lactic Acid to Control the Growth and Multiplication of *Salmonella enterica* in Raw Meat-Based Diets for Dogs

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Summary

Antimicrobial interventions currently being applied to control foodborne pathogens in raw meat-based diets (RMBDs) for dogs are rare and costly, and yet their demand keeps rising. The objective of this study was to determine the antimicrobial efficacy of encapsulated lactic acid challenged against *Salmonella enterica* inoculated in model RMBD. Nutritionally complete model RMBDs were prepared with three levels of encapsulated lactic acid (1.0%, 2.0%, and 3.0%) and formed into approximately 100 g patties. Each treatment was replicated twice, and dilutions were plated in duplicate during microbial analysis. The negative control (NC) and positive control (PC) did not contain any lactic acid. The patties containing lactic acid and the positive control were inoculated with 0.1 mL of three-cocktail serovars of *Salmonella enterica* and refrigerated at 40°F. The negative control was to check for any background *Salmonella* during the study. Microbial analysis by plating serially diluted aliquots of 0.1 mL on XLT4 agar was performed on d 1, 4, 7, 10, 13, 16, 19, and 22. The total log reductions obtained by the encapsulated lactic acid at these levels were 1.0% with a 2.97 Log CFU/patty, 2.0% with a 3.42 Log CFU/patty, and 3.0% with a 3.91 Log CFU/patty reduction. The log reductions were considered significant ($P < 0.05$) at each treatment level as increasing lactic acid concentrations in the patties resulted into more pathogen death compared to the positive control treatment patties that contained no lactic acid. Microbial analysis was completed on multiple days and there was a significant interaction ($P < 0.05$) between time (days) and the different treatment levels as log reductions from each treatment increased over time. In conclusion, encapsulated lactic acid can be used as a more economical antimicrobial intervention in raw meat-based diets for dogs.

Introduction

There is an increasing demand for raw pet food among many pet owners today because raw pet food is perceived as natural and free of synthetic food additives such as preservatives, artificial colors, and flavors. Raw pet food appeals to pet owners due to its similarity to native or ancient diet formats based on prey and canine vitality.[2] Feeding companion animals, like dogs and cats, with raw pet food is on the rise, especially

[1] Animal Nutrition & Health Division, Balchem Corporation, New Hampton, NY.

[2] Lummis, D. (2012). Natural, organic, and eco-friendly pet products in the US, Packaged Facts. Rockville, MD.

in developed countries. Raw diets are mostly referred to as 'Raw meat-based diets' (RMBDs) or 'Biologically appropriate raw food or bones' (BARF).[3,4] Raw diets are often formulated with raw and fresh muscle and organ meats, seafood, eggs, vegetables, tubers, fruits, and sometimes are fortified with micronutrients, including vitamins and minerals.[3] American Pet Products Association (APPA) in 2018 conducted a large survey in the United States that showed at least 3% of dog and 4% of cat owners purchased raw pet food for their pets, while 17% of dog owners purchased raw human food for their dogs.

However, RMBDs do not undergo a kill-step during production and are therefore an ideal product for foodborne pathogen growth and multiplication. This is a health risk to pet owners and their pets. Studies have shown a possibility of transmission of pathogens from the RMBD to humans, or the dogs might shed the pathogens to the environment through their feces, which could potentially be transmitted to humans.[5] The following are examples of pathogens that have been isolated from some commercial RMBDs diets; *Escherichia coli*, *Salmonella* species, *Clostridium* species, *Listeria monocytogenes*, *Yersinia species*, and *Campylobacter*.[6,7]

Organic acids such as lactic acid are recognized by the FDA as GRAS and have been applied on animal and poultry meats as inexpensive antimicrobial interventions, and they have shown some degree of efficacy against foodborne pathogens.[8,9] However, the deployment of organic acids, such as lactic acid, has been limited because higher doses have a significant effect on the sensory and color attributes of the meat or poultry. Using encapsulated organic acids may help increase their applicability, as the acid is slowly released into the product over time, thus leading to insignificant or minor changes in quality while controlling the growth and proliferation of foodborne pathogens in the product. Therefore, the objective of this study was to determine the antimicrobial efficacy of encapsulated lactic acid against *Salmonella enterica* inoculated in model raw meat-based dog food. The secondary objective was to monitor the pH progression in the RMBD upon addition of encapsulated lactic acid.

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Materials and Methods

Bacterial strain and culture conditions

Three serotypes of *Salmonella enterica* (Heidelberg ATCC 8326, Typhimurium ATCC 14802, and Enteritidis ATCC 4931) were obtained from the culture collection in the Feed Toxicology and Microbiology Laboratory at Kansas State University. The cultures were maintained frozen (-112°F) in tryptic soy broth (TSB) (Difco; Becton, Dickinson and Company, Sparks, MD) at a ratio of TSB:Glycerol being 7:3. The frozen cultures were thawed and then transferred to fresh TSB and incubated at 98°F for 24 h. To make a working culture, two consecutive 24-h transfers of each activated stock culture were performed in TSB incubated at 98°F. The inoculum was prepared by centrifuging (5,000 × g, 10 min, 68°F) 10 mL of individual *S. enterica* strains using a Sorvall X1R centrifuge (Thermo Scientific, Waltham, MA). The pelleted cells were then suspended in 10 mL of 0.85% (w/v) NaCl (saline) to obtain a final viable cell concentration of 9 Log CFU/mL. All the suspended cells were then mixed in a 50 mL conical tube to obtain a 3-cocktail mixture of *Salmonella enterica*.

Preparation of raw meat-based pet food

Using an in-house formula, a nutritionally complete model raw pet food was prepared under hygienic conditions to minimize contamination. Turkey, sweet potato, chicken liver, carrots, and apples were ground together to form a batter. The encapsulated lactic acid used in this study was obtained from Balchem Corporation[1] for research purposes. Three levels of encapsulated lactic acid (1.0%, 2.0%, and 3.0%) were added to the batter, whereas the negative and positive controls contained no lactic acid. The meat was mixed and then shaped to form patties of approximately 100 g and each treatment contained 16 patties (8 time-points) since the experiment was replicated twice. Individual patties were stored in stomacher bags with filters and sealed and refrigerated for up to 2 hours until they were ready for inoculation. Prior to inoculation, five patties from the control samples were sampled for any presence of background *Salmonella* and this process continued for the length of the entire study.

Inoculation of raw meat-based pet food

The refrigerated patties with three treatment levels and a positive control were placed in a biohazard hood and inoculated with 0.1 mL of the three serovar *Salmonella enterica* cocktail (*Salmonella* Typhimurium ATCC 14028, *Salmonella* Heidelberg ATCC 8326, and *Salmonella* Enteritidis ATCC 4931) to achieve a final concentration of ~6.0 Log CFU/mL. The negative and positive control patties contained no encapsulated lactic acid, but the positive controls were inoculated while negative controls were not inoculated. The patties were then left for 30 min to allow for pathogen attachment, and then refrigerated at 39°F. Microbial analysis and pH measurement was done at day 1, 4, 7, 10, 13, 16, 19, and 22.

Microbiological analysis

The negative control (NC) samples were routinely analyzed for background salmonella during microbial analysis. One hundred mL of buffered peptone water (BPW, pH 7.2) was added to individual patties that had been stored in whirl pack stomacher bags, which were then pummeled in a stomacher machine at medium speed for 1 min. To enumerate background *Salmonella*, 0.1 mL aliquots were plated on XLT-4 and incubated for 48 h at 98°F. The *Salmonella* survivors on the positive control (PC) and

the other three treatment levels were analyzed at day 1, 4, 7, 10, 13, 16, 19, and 22. The same procedure used for the NC samples was repeated for the other treatments in the study. The colonies were counted after incubation for 48 h at 98°F and then the respective log reductions were calculated. The initial inoculum level was obtained after performing microbial analysis with freshly produced patties that had been inoculated and allowed 30 minutes of attachment. The total log reduction was calculated by subtracting the initial inoculum level from the final Log CFU counts that had been obtained on day 22.

Measurement of pH

The pH of each patty was measured on days 1, 4, 7, 10, 13, 16, 19, and 22 prior to microbial analysis. The pH measurements were obtained using an Apera PH700 Benchtop (Apera Instruments, LLC, Columbus, OH) pH Meter with a stainless-steel electrode, an insertion probe that was used to make 'pokes' on the patties to measure the pH readings.

Statistical analysis

Data were analyzed using JMP Pro version 15 statistical software (SAS Institute, Inc., Cary, NC). This study was a completely randomized design, and one-way Analysis of Variance (ANOVA) was used to determine treatment levels with significant log reductions over the different time points in the study. The Dunnett's test was used to perform multiple comparisons of different treatment levels of encapsulated lactic acid against the positive control that contained no treatment. The pH means of the patties were evaluated for significant differences at a 5% significance between the treatment levels using the Student's t-test.

Results and Discussion

No *Salmonella enterica* was detected on the negative control (NC) samples during the entire duration of the study. The number of *Salmonella enterica* survivors kept decreasing over time and this was a general trend observed in all the treatments. The number of survivors of *Salmonella enterica* inoculated in the treated patties gradually decreased until day 13 when they started decreasing at an increasing rate. This was probably because the encapsulated acid has been fully released into the patty and thus, we observed increased antimicrobial activity. The initial concentration of *Salmonella enterica* serovars on the inoculated raw meat-based patties was ~6.0 Log CFU/patty. The number of *Salmonella enterica* serovars that survived during the duration of the study are presented in Figure 1. The total log reduction of *Salmonella enterica* serovars in the positive control (PC) that contained no lactic acid were 1.18 Log CFU/patty after 22 days. On the 1.0% LA ENC, 2.0% LA ENC, and 3.0% LA ENC, the total log reductions of *Salmonella enterica* after exposure to encapsulated lactic acid were 2.97 Log CFU/patty, 3.42 Log CFU/patty, and 3.91 Log CFU/patty, respectively, after 22 days. Overall, there was a difference in the Log CFU counts observed between the positive control patties and the patties that contained encapsulated lactic acid ($P < 0.05$). Table 1 and Figure 1 shows the total log reductions observed after 22 days. There was a difference ($P < 0.05$) in the log reduction observed when all the treatments were compared against the positive control.

The pH of the patties was measured, and the results were compared to the control patties that did not contain the encapsulated lactic acid (LA ENC). The initial pH of the control patties on day 1 (negative and positive) was 6.24 and 6.28, respectively. In the patties treated with ENC LA at 1.0%, 2.0%, and 3.0%, the pH values on day 1 were 5.94, 5.76, and 5.58, respectively. At the end of the study on day 22, the controls recorded pH values of 5.57 and 5.57, whereas the patties treated with ENC LA at 1.0%, 2.0%, and 3.0%, had pH readings of 5.00, 4.87, and 4.67, respectively. Although, there was a significant difference in the pH ($P < 0.05$) between the controls and the treated patties after 22 days, there was no significant difference ($P > 0.05$) in pH observed between the patties that had been treated with 1.0% LA ENC (w/w), 2.0% LA ENC (w/w), or 3.0% LA ENC (Figure 2, Table 2).

In conclusion, encapsulated lactic acid showed great potential in its application in raw meat-based diets for companion animals. The treatments were effective at controlling the proliferation of *Salmonella enterica* that was artificially inoculated in these raw diets at all levels. The gradual drop in the pH also implies that rapid changes to the sensory and color properties of the product are delayed when using encapsulated organic acids, leading to minor changes in product quality.

Table 1. The total log reduction of *Salmonella enterica* serovars that were inoculated in raw meat-based patties for dogs after 22 days of exposure to different levels of encapsulated lactic acid (LA ENC)

Treatment	Log CFU/patty reduction[1]	SEM
Positive control	1.18[a]	0.1486
1.0% Encapsulated lactic acid	2.97[b]	0.0504
2.0% Encapsulated lactic acid	3.42[c]	0.0883
3.0% Encapsulated lactic acid	3.91[d]	0.1056
Negative control	---	---

[abcd]Means with different superscript (a, b, c, or d) are considered different at $P < 0.05$.
[1]Total log reduction is obtained by subtracting the initial inoculum from the final Log CFU counts that had been obtained on day 22.

Table 2. The final pH values of the raw meat-based patties for dogs at the end of the study on day 22 after being treated with encapsulated lactic acid including the positive and negative controls

Treatment	pH	SEM
Negative control	5.57[A]	0.1321
Positive control	5.57[A]	0.1281
1.0% LA ENC	5.00[B]	0.1161
2.0% LA ENC	4.87[B]	0.1006
3.0% LA ENC	4.67[B]	0.1086

*pH means with different superscripts (A or B) are considered different at $P < 0.05$.
*LA ENC = encapsulated lactic acid.

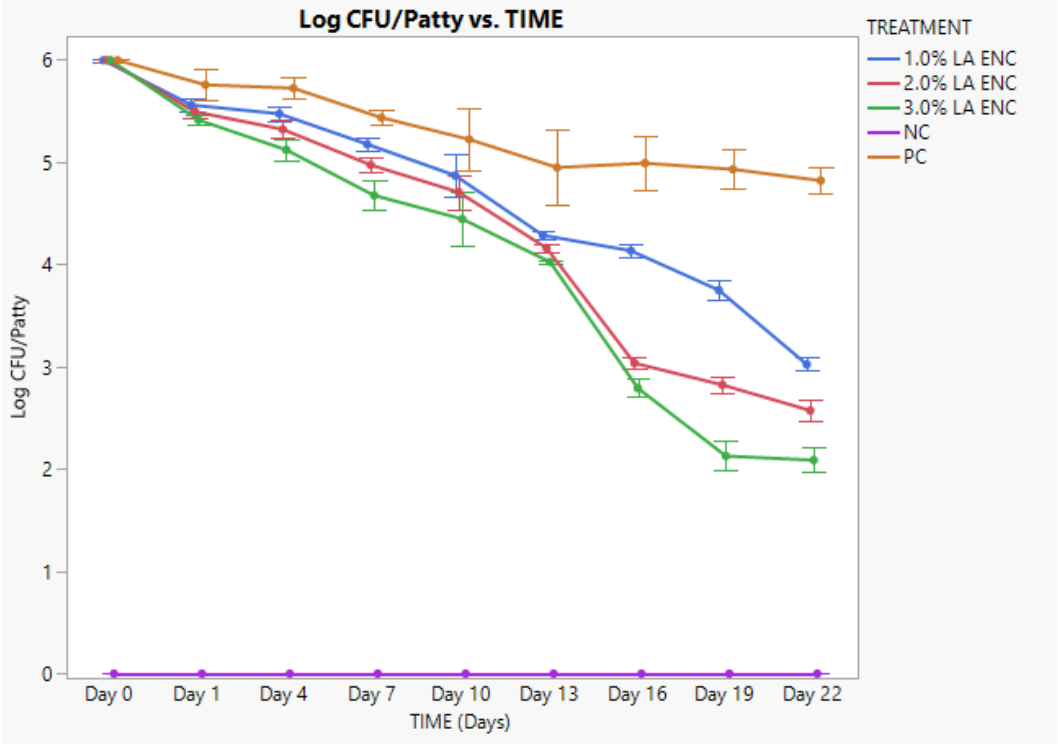

Figure 1. Log CFU/patty survivors of *Salmonella enterica* inoculated in raw meat-based patties for dogs after being exposed to encapsulated lactic acid (LA ENC).

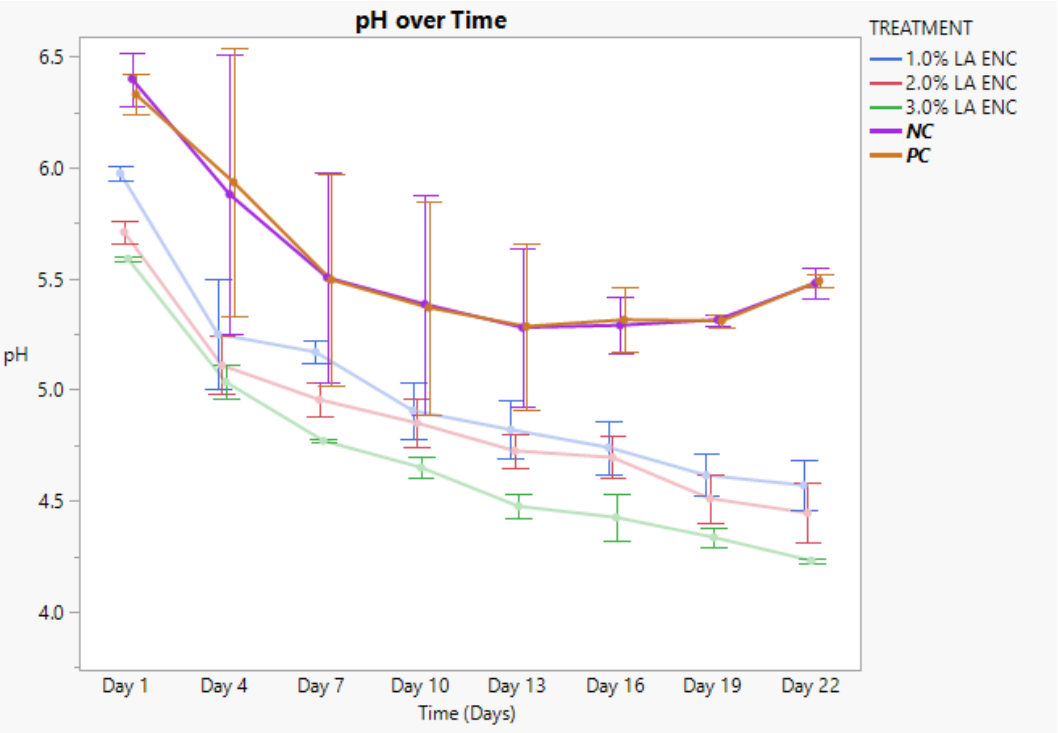

Figure 2. The changes in pH of raw meat-based patties treated with different levels of encapsulated lactic acid (LA ENC) compared with negative and positive control patties without acid during a 22-day period of storage at 39°F.