Executive Summary

Title: Controlling *Listeria monocytogenes* in Natural, Ready-to-Eat Meat and Poultry Products

Investigators: Kathleen Glass and Jeffrey Sindelar

Research Institution: University of Wisconsin-Madison

Submittal Date of Final Report to AMI: August 30, 2010

Objectives:

- To screen a variety of natural flavorings, plant extracts, and microbial fermentation byproducts for antilisterial activity in model uncured and "naturally" cured meat systems (turkey slurries)
- To determine the effect of natural antimicrobial systems on flavor for meat/poultry products
- To compare the antilisterial activity of "natural antimicrobial" systems in naturally cured ham, uncured beef, and uncured deli-style turkey with that for traditional cured ham prepared with lactate-diacetate.

Conclusions: This study identified several commercial ingredients (1.5% lemon/cherry/vinegar blend, 2.0% buffered vinegar and 3.0% cultured cane sugar/vinegar blend) which can be used to inhibit growth of *L. monocytogenes* in natural ham, turkey, and roast beef without significant adverse effect on sensory attributes. The addition of "natural nitrite" through the use of pre-converted vegetable powder enhanced the effect of the antimicrobials tested in ham compared with uncured turkey. Since the antilisterial effect of nitrite is dose dependent, and nitrite levels contributed by preconverted vegetable powder are typically lower than those found in traditionally cured products, naturally cured products logically have reduced microbial inhibition compared with traditional products. Overall, inhibition of *L. monocytogenes* in naturally cured ham supplemented with certain adjunct natural antimicrobials was similar to that of lower salt (1.6-1.8% NaCl), traditionally cured ham with lactate-diacetate blend. However, even the most effective turkey and ham treatments supported a 2-log increase of *L. monocytogenes* within 6 weeks storage at 4°C. It should be noted that listerial growth in beef was significantly delayed compared to the ham and turkey. The additional delay in beef may be attributed to either the relatively small differences in product moisture and pH compared to the other two product types or to other unidentified factors. These data suggest that certain natural growth inhibitors can improve the safety of natural and organic ready-to-eat meat and poultry products, but their efficacy is enhanced in the presence of nitrite, in products with lower moisture and pH, and when stored at strict refrigeration temperatures.

Deliverable: Although the natural ingredients delay growth compared with the No-Antimicrobial Control treatments, *L. monocytogenes* can still grow within 6 weeks storage at 4°C in natural ham and turkey, as well as lower salt, no phosphate traditional ham. Data further emphasize that storage at 4°C or lower is a critical factor in controlling growth of *L. monocytogenes* in ready-to-eat meat and poultry products regardless of antimicrobial addition. These results demonstrate the need to incorporate additional hurdles in natural meat and poultry products as well as when producing reduced-sodium traditional products.

Controlling *Listeria monocytogenes* in Natural, Ready-to-Eat Meat and Poultry Products

Final Report August 30, 2010

Kathleen Glass[a] and Jeffrey Sindelar[b], Principal Investigators
Lindsey McDonnell, Brandon Wanless, Roxanne VonTayson, Megan McGough

University of Wisconsin-Madison
[a]Food Research Institute, 1550 Linden Drive, Madison, WI 53706
[b]Meat Science and Muscle Biology Lab, 1805 Linden Drive, Madison, WI 53706

Phone: 608.263.6935 (KG); 608.262.0555 (JS);
E-mail: kglass@wisc.edu; jsindelar@wisc.edu

Technical Abstract: The objective of this project was to identify ingredients to inhibit growth of *Listeria monocytogenes* in ready-to-eat (RTE), deli-style, meat and poultry products that meet "natural" or organic requirements defined by USDA. Currently, many U.S. manufacturers utilize combinations of the traditional antimicrobials including nitrite, lactate and diacetate to suppress growth of *L. monocytogenes* should it inadvertently recontaminate the product. In recent years, there has been a substantial increase in the demand for both natural and organic, ready-to-eat meat and poultry products. These products contain no added sodium nitrite, which has been identified to greatly enhance the efficacy of lactate-diacetate and other antilisterial ingredients. As a result, additional antimicrobial ingredients need to be identified to ensure the safety of these products during extended refrigerated storage.

The purpose of this study was to 1) screen a variety of natural ingredients for antilisterial activity and 2) validate the antilisterial activity of natural antimicrobial systems found most effective in naturally cured ham, uncured roast beef, and uncured deli-style turkey breast.

Turkey slurries (25% ground turkey breast meat, 2.0% salt, final pH 5.8-6.0) were prepared and included the following treatments: 1) uncured, 2) traditionally cured with 156 ppm sodium nitrite, 3) indirectly cured using celery powder as a source of nitrate and a nitrate-reducing *Staphylococcus carnosus*, and 4) indirectly cured using a pre-converted celery-powder as a natural nitrite source. Nitrite treatments were supplemented with 15 different ingredients, including natural flavorings, plant extracts, and microbial fermentation byproducts. Cooked slurries were inoculated with 3-log CFU/g *Listeria monocytogenes* and stored at 4°C for 4 weeks and duplicate sample per variable enumerated weekly.

Ingredients which prevented growth of *L. monocytogenes* in the screening study for nitrite-containing treatments were used in cooked, uncured deli-style chicken formulations to determine the effect on sensory attributes (appearance, texture, flavor) using an "experienced" panel. The most successful ingredients were then selected for further

evaluation. Three ingredients, 1.5% lemon/cherry/vinegar blend, 2.0% buffered vinegar, and 3.0% cultured cane sugar/vinegar blend, were incorporated into naturally cured ham, uncured roast beef, and uncured deli-style turkey breast. Controls included naturally cured ham, uncured roast beef, and uncured deli-style turkey breast without antimicrobials and a sodium nitrite-cured ham with 2.8% lactate/diacetate. Cooked, sliced product was inoculated with 3-log CFU/g of a 5-strain mixture of *L. monocytogenes*, vacuum packaged, and stored at 4°C for up to 12 weeks. *L. monocytogenes* was enumerated in triplicate samples at 0, 2, 4, 6, 8, 10 and 12 weeks.

A 2-log CFU/g increase in *L. monocytogenes* was observed for ham and turkey without antimicrobials at 2 weeks of storage and at 4 weeks for roast beef. Growth (> 1-log increase) in the traditionally cured ham with lactate/diacetate was delayed until week 6 of sampling. Each of the three antimicrobials delayed growth of *L. monocytogenes* compared to the no antimicrobial containing controls. Compared to the control, the addition of either 1.5% lemon/cherry/vinegar blend or 2.0% buffered vinegar delayed growth for an additional 2 weeks while the addition of 3.0% cultured cane sugar/vinegar blend delayed growth for an additional 4 weeks for both ham and turkey. The greatest delay was observed in uncured roast beef with no *L. monocytogenes* growth detected through 12 weeks at 4°C for all three antimicrobial beef treatments. In addition to the effect of the antimicrobials, delay in the growth of *L. monocytogenes* may also be attributed to differences in product moisture and pH.

These data suggest that natural antimicrobials can improve the safety of natural and organic ready-to-eat meat and poultry products, but their efficacy is enhanced in the presence of nitrite and in products with lower moisture and pH.

Objectives:

- To screen a variety of natural flavorings, plant extracts, and microbial fermentation byproducts for antilisterial activity in model uncured and “naturally” cured meat systems (turkey slurries)
- To determine the effect of natural antimicrobial systems on flavor for meat/poultry products using experienced panelists for informal sensory analysis.
- To compare the antilisterial activity “natural antimicrobial” systems in naturally cured ham, uncured beef, and uncured deli-style turkey with that for traditional cured ham prepared with lactate-diacetate.

Materials and Methods:

The study was divided into two phases, focusing on low-fat products which typically have less antagonistic effect on fat-soluble antimicrobials. An informal sensory evaluation was performed prior to production of products for Phase 2.

Preparation of inocula: *L. monocytogenes* strains Scott A (clinical isolate, serotype 4b), LM 101 (hard salami isolate, serotype 4b), LM 108 (hard salami isolate, serotype 1/2a), LM 310 (goat milk cheese isolate, serotype 4), and V7 (raw milk isolate, serotype 1), were grown individually in 10 ml Trypticase soy broth (BBL, BD Biosciences, Sparks, MD) at 37°C for 18 to 20 h. Cells were harvested by centrifugation (2,500 x *g*, 20 min) and suspended in 4.5 ml 0.1% buffered peptone water (pH 7.2). Equivalent populations of each isolate were combined to provide a five-strain mixture of *L. monocytogenes* to yield target level of 5-log CFU per 100-g package. Populations of each strain and the mixture were verified by plating on Trypticase soy agar and modified Oxford agar (Listeria Selective Agar base, Difco, BD Biosciences, Sparks, MD).

Phase 1 Screening in model meat system: "Natural" antimicrobials evaluated included natural flavorings, organic acid blends, plant extracts, and microbial fermentation byproducts. Fifteen different ingredients (either commercially available or test products near commercialization) were evaluated in turkey slurries for four nitrite treatments: 1) uncured, 2) traditionally cured with 156 ppm of chemical sodium nitrite, 3) indirectly cured using celery powder as a nitrate source and a nitrate-reducing *Staphylococcus carnosus*, and 4) indirectly cured using a pre-converted celery-powder as a natural nitrite source. Ingredients and addition levels are identified in Table 1. Levels were determined from published literature or manufacturer recommendations.

Slurries were prepared using raw ground turkey breast meat (Schlyter et al., 1993). Uncured treatments were supplemented with 2.0% salt and an appropriate concentration of "natural" antimicrobial; final pH was not adjusted but ranged between 5.8-6.1. For one set of "naturally cured" treatments, 0.4% celery powder (source of nitrate; VegStable 502, Florida Food Products), 0.20% cherry powder (source of ascorbic acid; VegStable 515, Florida Food Products), and 0.18% *Staphylococcus carnosus* culture (nitrate-reducing bacteria; CS 299 Bactoferm^{TM}, Chr. Hansen Inc., Gainesville, Fl) were added to the slurry and held 120 minutes at 35-38°C (95-100°F) to allow the starter culture to reduce indigenous nitrate to nitrite prior to cooking (Sindelar et al., 2007a, 2007b). A second set of "naturally cured" treatments utilized commercially available celery powder which had been pre-reduced to yield nitrite (VegStable 504, Florida Food Products) along with cherry powder. The third set of treatments was uncured whereas the fourth set of treatments included 156 ppm sodium nitrite.

In addition, baseline data were collected for the behavior of *L. monocytogenes* in two Control treatments representing traditional cured product were slurries supplemented with 2.8% commercial lactate-diacetate blend, 2.0% NaCl, 547 ppm sodium erythorbate, and 156 ppm sodium nitrite; one control treatment was supplemented with 0.4% STPP whereas the second control treatment did not have phosphate added to mimic natural and organic production practices.

For all treatments, slurries were pasteurized to 74°C to kill background microflora, then rapidly chilled on ice to 4°C. Flasks were inoculated with *L. monocytogenes* to yield 3-log CFU/ml slurry, and dispensed 3 ml per sterile polystyrene tube for incubation at 4°C for up to 4 weeks. Duplicate samples per variable were assayed at 0-time and at 1, 2, 3, and 4 weeks for changes in listerial populations by plating serial dilutions on Modified Oxford agar (35°C, 48 h). Residual nitrites were analyzed as described below on uninoculated cooked samples.

Intermediate Sensory Screening: Small (5 lb) test batches of uncured deli-style chicken were prepared using natural antimicrobials systems for informal sensory evaluation. Products were prepared using the ingredients described in Tables 2 and 3, sliced and presented for sensory evaluation (surface color, aroma, flavor, and overall acceptance) to experienced volunteer panelists from UW-Muscle Biology laboratory and the Food Research Institute. Panel scores used a 9 point hedonic scale where 1= dislike extremely, 9= like extremely. (Protocol SE-2008-0183; Qualified as Exempt from IRB Review (46.101(b)(6)) April 29, 2008).

Color [CIE L*a*b* values (lightness, redness, yellowness, respectively)] was measured on freshly cut surfaces of each sample using a chroma meter (CR-310, 1-cm or 50 mm aperture, illuminant C; Minolta Corp., Osaka, Japan) calibrated with a white plate (L* 97.74, a* -0.06, b* 2.55). The pH values of the finished products were measured on 10% slurries (10 g meat and 90 ml deionized water). Sensory and quality analysis was overseen by PI Sindelar.

Phase 2 Processed Product: Based on the outcome of the slurry study and intermediate sensory screening, three natural antimicrobial systems were chosen for further evaluation in each of three low-fat (<5% fat) RTE products: natural boneless ham, whole muscle roast beef, and deli-style turkey breast. Products were manufactured utilizing typical commercial processing practices. In addition, controls included natural ham (manufactured with preconverted vegetable powder as a nitrite source), uncured turkey, and uncured roast beef without natural antimicrobials plus a traditional nitrite-cured ham with 2.8% lactate-diacetate blend (negative growth control). Products were manufactured in the UW Meat Science and Muscle Biology Laboratory, UW-Madison under the direction of Dr. Jeff Sindelar. A total of 13 formulations (3 antimicrobial systems x 3 products + 4 controls) were tested and each formulation was replicated twice.

Products were formulated with ingredients and levels commonly used in the meat industry. Ham was naturally cured using pre-reduced vegetable powder. Natural boneless ham was manufactured using fresh biceps femoris and semimembranosus with attached semitendinosus muscles trimmed free of external fat. Salt and sugar were held constant for all treatments and control at ingoing injected levels of 2.35% and 1.65%, respectively. Ham brines for all treatments had ingoing injected levels of pre-reduced vegetable juice powder and cherry powder at 0.30% and 0.20%, respectively while the control contained 156 ppm sodium nitrite, 550 ppm sodium erythorbate and an ingoing level of 2.8% sodium

lactate/sodium diacetate. Natural deli-style turkey breast (uncured) treatments and control were manufactured using boneless, skinless turkey breasts. Deli-style turkey breast contained an ingoing level of 1.5% salt, 1.5% dextrose, and treatments included an antimicrobial (n=3). Whole muscle roast beef treatments and control (uncured) were manufactured using closely trimmed cap-off, semimembranosus muscles. Roast beef brines had an ingoing level of 0.50% salt and 0.40% sugar, and treatments included an antimicrobial (n=3). .

For ham manufacture, boneless ham muscles were injected using a multi-needle injector (25% injection), vacuum tumbled for 1 hour, stuffed into fibrous casings, and thermal processed. For roast beef manufacture, inside beef round muscles were injected using a multi-needle injector (20% injection), vacuum tumbled for 20 minutes, placed in shrinkable cooking bags and thermal processed. For deli-style turkey breast manufacture, turkey breast meat was ground, mixed with ingredients, tumbled for 1 hour, stuffed into fibrous casings, and thermal processed. All products were thermal processed in a single truck thermal processing oven (Alkar, Model 450 Mini-Smoker, Alkar Engineering Corp., Lodi, WI,) using schedules typical for each product category. After thermal processing, finished products were chilled at 0-2°C for 10 to 15 hours and be held at temperatures no greater than 4°C for no longer than 72 hours before inoculation.

Cooked products were aseptically removed from the casing and sliced on a sanitized, hand slicer to a thickness of 3 mm and stored at 3-4°C until use. Prepared, chilled products were transported to the Food Research Institute for surface-inoculation. Sliced products were repackaged into gas-impermeable pouches using sterile gloves to create 100 g/pkg sampling units, and inoculated using 0.5 ml of a five-strain mixture of *L. monocytogenes* (Glass et al., 2002, 2007a, 2007b) to yield approximately 5-log CFU/package (3-log CFU/g). Inoculated products were vacuum-packaged, and stored at 4 or 7^{o}C for up to 12 weeks.

Bacterial populations were determined in rinse material obtained after adding 100 ml of sterile Butterfield phosphate buffer to each package and massaging the contents externally by hand for about 3 minutes (Glass and Doyle, 1989; Glass et al., 2002, 2007a, 2007b). Triplicate samples for each variable were assayed at 0-time and after 2, 4, 6, 8, 10, and 12 wks storage for *L. monocytogenes* populations by plating serial dilutions on Modified Oxford agar (35°C, 48 h). In addition, duplicate uninoculated samples were assayed for changes in pH and populations of lactic acid bacteria (APT with bromcresol purple; 25^{o}C, 48-72 h). Testing of a variable was discontinued if listerial growth (>2-log increase) was confirmed in packages tested for two consecutive sampling intervals.

Proximate and chemical analysis: Moisture (5 h, 100°C, vacuum oven method AOAC 950.46), pH (10 g homogenized portion diluted 1:10 in distilled water, pH of slurry measured with Accumet Basic pH meter and Orion 8104 combination electrode, Thermo Fisher Scientific, Waltham, MA), NaCl (measured as % Cl^-, $AgNO_3$ potentiometric titration, Mettler DL22 food and beverage analyzer), nitrite (Colorimetric Method AOAC 973.31),

and water activity (Decagon AquaLab 4TE water activity meter, Pullman, WA) were assayed by the Food Research Institute for triplicate samples of each lot.

Data analysis: The microbiological data reported are average values and standard deviations (log CFU/ml rinse) for triplicate samples and two separate trials for each test formulations (n=6).

Results:
Phase 1 Screening study*:* Residual nitrite levels varied among the various baseline treatments. Analysis revealed an average of 45, 20, and 5 ppm residual nitrite in the cooked/chilled model system for traditional nitrite addition, vegetable powder+starter, and pre-reduced vegetable powder, respectively.

As previously reported, nitrite by itself had an inhibitory effect on *L. monocytogenes* growth in turkey slurries stored at 4°C, but the degree of inhibition was greater under conditions of higher nitrite levels. Populations of *L. monocytogenes* increased 1-log at 1 week in the uncured control without antimicrobials, but no growth observed for the nitrite-containing treatments at the same sampling interval. *L. monocytogenes* increased by 1.0, 1.0 and 1.6 log at 2-weeks for the traditional nitrite, starter, and pre-reduced treatments, respectively. In contrast, screening in the model turkey system confirmed no growth of *L. monocytogenes* in the lactate-diacetate-nitrite controls with or without phosphate during the 4 weeks storage at 4°C (Figure 1).

Subsequently, 15 different ingredients were evaluated under the four nitrite treatments described above. Ingredients, addition levels, and inhibition are identified in Table 1. Six ingredients inhibited growth (< 0.6 log increase) of *L. monocytogenes* during the 4 week study at 4°C regardless of method of nitrite generation. No growth of *L. monocytogenes* was observed in turkey slurries supplemented with 1.5% vinegar/lemon/cherry powder blend, 2.0% buffered vinegar, 2.5% vinegar/lemon juice blend, 2.5% grapefruit/lime/vinegar blend, 500 ppm tea tree oil, or 3.0% cultured cane sugar/vinegar blend with or without nitrite. It is notable that with the exception of the high concentration of tea tree oil, all the other treatments included vinegar (acetic acid) at varying levels.

Additional ingredients inhibited growth of the pathogen when in the presence of nitrite (regardless of source of nitrite) but had no inhibitory effect in the traditional uncured treatment. No growth was observed during the 4-week testing interval in the three nitrite added/generated-treatments supplemented with 0.03% grape seed extract powder, smoke flavor 2, or additional 0.5% cherry powder, but growth of the pathogen in the uncured treatment was similar to the positive growth control without antimicrobials (>4 log increase at the 4-week sampling interval).

Smoke flavor 1, nisin-rosemary blend, cranberry powder, and herb blend, had variable effects. Addition of these ingredients had low or no inhibitory effect in uncured product, and slightly greater effect in traditional cured treatments which contained higher residual nitrite than in "naturally" cured product with reported lower nitrite levels.

A rosemary-tocopheral blend and the green tea extract tested at 0.08 and 0.10%, respectively, did not inhibit pathogen growth compared with the controls without the antimicrobials. Higher concentrations may be effective, but they were not tested further in this study.

Intermediate Sensory Analysis: Eight natural ingredients were further tested for effects on color, aroma, flavor and overall acceptance in uncured deli chicken. Ingredients tested included those which inhibited growth in uncured turkey slurries in Phase 1 (1.5% vinegar/lemon/cherry powder blend, 2% buffered vinegar, 2.5% vinegar/lemon juice blend, 2.5% grapefruit/lime/vinegar blend, and 3.0% cultured cane sugar/vinegar), with the exception of the tea tree oil, which had an obvious pungent odor in the slurries. In addition, several other ingredients which demonstrated antilisterial activity in cured products were tested (0.03% grape seed extract powder, 0.5% cherry powder, and 2.0% cranberry powder). With the exception of 2.0% cranberry powder which had a negative effect on color and flavor, the overall acceptance of the other products were not significantly different than the Control without antimicrobials or from each other (Tables 2, and 3).

Phase 2 Processed Products: Three ingredients which were found to prevent growth of *L. monocytogenes* in uncured turkey slurries in Phase 1, and which were deemed most acceptable utilizing an experienced sensory analysis, were chosen for further testing in meat products (naturally cured ham, uncured turkey, and uncured roast beef). Antimicrobials tested in each meat type included 1.5% lemon/cherry/vinegar blend, 2.0% buffered vinegar and 3.0% cultured cane sugar/vinegar blend. Controls included naturally cured ham, uncured roast beef, and uncured deli-style turkey breast without antimicrobials and a traditional nitrite-cured ham with lactate/diacetate but no phosphate.

L. monocytogenes increased 2-log CFU/g at 2 weeks of storage on both the No-Antimicrobials Control treatments for turkey (Figure 2) and ham (Figure 3) and at 4 weeks for roast beef (Figure 4). Growth (defined as>1-log increase) in the Traditional Cured Ham with lactate/diacetate was delayed until week 6 storage at 4°C. Cooking losses were higher than normal ranging between 13.25 and 15.68%. Higher cook losses can be partially explained from the no addition of phosphates, Because of the greater cooking losses that took place, it is expected that greater than normal amount of salt and lactate-diacetate may have been lost. The final measured salt content was approximately 1.6% compared with the expected 2.0% based on injection rate of 2.35% ingoing. If lactate-diacetate levels were similarly significantly less than the 2.80% target addition, the rate of listerial growth is line with that which is predicted by the OptiForm Listeria Control Model.

Each of the three antimicrobials delayed listerial growth compared to the No Antimicrobial Controls for all three meat types. When products were supplemented with1.5% lemon/cherry/vinegar blend, less than a 1-log increase was noted at 2 and 4 weeks storage at 4°C for turkey and ham, respectively, but greater than a 2-log increase was observed at 4 and 6 weeks for the two products, respectively. A 1- and 2-log increase was observed at 4 and 6 weeks, respectively, for both turkey and ham formulated with 3.0% cultured cane sugar/vinegar blend. The addition of 2.0% buffered vinegar provided the greatest delay, with no growth in either the turkey or ham at 4 weeks, but a 1.8 and 1.0 log increase at 6 weeks for the turkey and ham, respectively. All three antimicrobials were effective in uncured roast beef with no growth of *L. monocytogenes* detected in samples stored 12 weeks at 4°C. The significant inhibition of listerial growth in beef compared to the ham and turkey may be attributed to differences in product moisture and pH. Ham averaged 70% moisture and pH 6.3, turkey 73% moisture and pH 6.2, beef 66% moisture and pH 5.8 (Table 4). As expected, microbial growth was more rapid when products were stored at 7°C than at 4°C (data not shown).

Conclusions: This study identified several ingredients which can be used to inhibit growth of *L. monocytogenes* in natural ham, turkey, and roast beef without significant adverse effect on sensory attributes. Inhibition of *L. monocytogenes* in ham with natural antimicrobials was similar to that of lower salt (1.6-1.8% NaCl), traditionally cured ham with lactate-diacetate blend. However it should be noted that although the ingredients delay growth compared with the No-Antimicrobial Control treatment, *L. monocytogenes* can still grow within 6 weeks storage at 4°C in natural ham and turkey, as well as lower salt traditional ham. These results emphasize the need to incorporate additional hurdles in natural meat and poultry products as well as when producing reduced-sodium traditional products. Formulation and processing must ensure antimicrobials incorporated into the brine are retained at levels which are effective. These data suggest that natural growth inhibitors can enhance the safety of natural and organic ready-to-eat meat and poultry products, but their efficacy is enhanced in the presence of nitrite, in products with lower moisture and pH, and likely in products with higher salt concentrations.

Recommendations for Future Research: This study provided evidence that certain "natural" ingredients are inhibitory to *L. monocytogenes* in ready-to-eat (RTE), deli style, meat and poultry products which may meet "natural" or organic requirements defined by USDA. In order to ensure comparable safety of natural RTE meat and poultry products, future research should identify combinations of "natural nitrite", moisture, pH and salt values to extend the time of growth inhibition for a minimum of eight weeks storage at 4°C in ham and turkey. In addition, we recommend a systematic study to determine what factors in addition to moisture and pH (if any) contribute to the apparent enhance safety of beef vs. turkey. Continued study should be completed to identify other ingredients or processing methods to enhance the safety of reduced-sodium meats while meeting the recommended sodium reduction goals.

Presentations and Publications:

- **Poster:** Controlling *Listeria monocytogenes* in Natural and Organic, Ready-to-Eat Meat and Poultry Products, K.A. Glass, L.M. McDonnell, R. VonTayson, B.J. Wanless, M.M. McGough, J.J. Sindelar, presented at the 2010 Reciprocal Meat Conference.
- **Poster:** Antilisterial Activity of Natural Ingredients in a Model Poultry Product System, KA Glass, LM McDonnell, RR VonTayson, BJ Wanless and JJ Sindelar, presented at the 2010 Annual Meeting of the International Association for Food Protection
- **Manuscript in preparation:** Inhibition of *Listeria monocytogenes* ham, turkey and roast beef by natural ingredients. K.A. Glass, L.M. McDonnell, R. VonTayson, B.J. Wanless, M.M. McGough, J.J. Sindelar

Acknowledgements

We thank Dr. Andy Milkowski for helpful discussion and Russ McMinn, Chris Safranski, Jon Letko, Jennifer Schnacky, Joshua Knackert, Rachael Colpaert and Matthew Terns for technical assistance. Funding was provided by the American Meat Institute Foundation, contributions from the food industry to the Food Research Institute, and the College of Agricultural and Life Sciences.

References:

- Glass, K.A., and M.P. Doyle. 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. Appl. Environ. Microbiol. 55:1565-1569.
- Glass, K. A., D. A. Granberg, A. L. Smith, A. M. McNamara, M. Hardin, J. Mattias, K. Ladwig, and E. A. Johnson. 2002. Inhibition of *Listeria monocytogenes* by sodium diacetate and sodium lactate on wieners and cooked bratwurst. J. Food Prot. 65:116-123.
- Glass, K. A., L. M. McDonnell, R. C. Rassel, and K. L. Zierke. 2007a. Controlling *Listeria monocytogenes* on sliced ham and turkey products using benzoate, propionate, and sorbate. J. Food Prot. 70:2306-2312.
- Glass, K. A., D. Preston, and J. Veesenmeyer. 2007b. Inhibition of *Listeria monocytogenes* in turkey and pork-beef bologna by combinations of sorbate, benzoate, and propionate. J. Food Prot. 70:214-217
- Sindelar, J.J., JC Cordray, JG Sebranek, JA Love, and DU Ahn. 2007a. Effects of varying levels of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of ready-to-eat uncured ham. J. Food Sci. 72(6): S388-S395.
- Sindelar, J.J., JC Cordray, JG Sebranek, JA Love, and DU Ahn. 2007b. Effects of vegetable juice powder concentration and storage time on some chemical and sensory quality attributes of uncured, emulsified cooked sausages. J. Food Sci. 72(5): S324-S332.
- Schlyter, J H, K A Glass, J Loeffelholz, A J Degnan, and J B Luchansky. 1993. Effects of diacetate with nitrite, lactate, and pediocin on viability of *Listeria monocytogenes* in turkey slurries. Int. J. Food Microbiol. 19:271-281.

Table 1. Inhibition of *L. monocytogenes* by natural ingredients in turkey slurries stored at 4°C for 4 weeks (Phase 1, screening study).

	Ingredient	% used
	No growth for cured or uncured through 4 weeks	
1	Buffered Vinegar	2.00%
2	Cultured cane sugar and vinegar blend	3.00%
3	Lemon/cherry/vinegar blend	1.50%
4	Tea Tree oil	0.05%
5	Vinegar/lemon juice blend	2.50%
6	Grapefruit/Lime/Vinegar blend	2.50%
	No growth with cured treatments for 4 weeks	
7	Cherry powder	0.50%
8	Grape Seed Extract powder	0.03%
9	Smoke flavor-2	1.00%
	Variable results (no clear correlation with nitrite)	
10	Cranberry concentrate powder w/MgOH & Ca_3PO4	2.00%
11	Fermentate Nisin-Rosemary blend	0.02%
12	Herb Blend	0.50%
13	Smoke flavor-1	1.00%
	No inhibition at the concentrations used	
14	Green Tea extract	0.10%
15	Rosemary - tocopheral blend	0.08%

TABLE 2: Least squares means for sensory attributes of surface color, aroma, flavor, and overall acceptance for uncured deli-style chicken.

		SENSORY ATTRIBUTES[b]			
Product[a]	Use Level	Surface Color	Aroma	Flavor	Overall Acceptance
Buffered Vinegar	2.0%	6.86[d]	6.00	6.57	6.14[d]
Vinegar/lemon juice blend	2.5%	6.14[d]	6.29	6.29	6.14[d]
Grape seed Extract	0.03%	5.43[d]	5.43	6.14	5.57[d]
Vinegar/lime/grapefruit	2.5%	6.57[d]	6.29	5.86	5.86[d]
Cultured sugar/ vinegar	3.0%	6.43[d]	6.29	7.29[d]	7.29[d]
Cranberry concentrate	2.0%	1.86[e]	5.43	4.86[e]	2.86[e]
Cherry Powder	0.5%	6.57[d]	6.00	7.00	6.57[d]
Control	NA	6.14[d]	6.57	7.00	6.29[d]
Vinegar/lemon/cherry	1.5%	5.29[d]	6.43	6.71	6.43[d]
SEM[c]		0.55	0.42	0.51	0.48

[a] Product = various antimicrobials included in deli-style chicken breast.
[b] SENSORY ATTRIBUTES = Consumer panel scores using a 9 point hedonic scale where 1= dislike extremely, 9= like extremely.
[c] SEM = Standard error of the means
[d-e] Means within same column with different superscripts are different (P<0.05).

TABLE 3: Least squares means for objective color (L*, a*, b*) and finished product pH values for uncured deli style chicken.

		OBJECTIVE COLOR[b]			
Product[a]	Use Level	L*	a*	b*	pH[c]
Buffered Vinegar	2.0%	81.39^{efh}	3.05^{bc}	12.51^{i}	6.11
Vinegar/lemon juice blend	2.5%	82.11^{ef}	2.67^{bc}	12.40^{i}	5.94
Grape seed Extract	0.03%	80.00^{gh}	2.19^{c}	10.93^{e}	6.13
Vinegar/lime/grapefruit	2.5%	81.23^{efh}	2.80^{bc}	12.69^{fg}	5.83
Cultured sugar/ vinegar	3.0%	81.64^{ef}	3.14^{b}	11.51^{i}	5.94
Cranberry concentrate	2.0%	75.76^{d}	4.39^{a}	8.21^{h}	6.10
Cherry Powder	0.5%	81.32^{efh}	2.81^{bc}	14.30^{df}	5.99
Control	NA	82.02^{ef}	2.26^{c}	12.04^{i}	6.00
Vinegar/lemon/cherry	1.5%	79.30^{g}	2.84^{bc}	15.77^{d}	6.10
SEM[j]		0.33	0.18	0.31	0.18

[a] Product = various antimicrobials included in deli-style chicken breast.
[b] Commission International D'Edairerage (CIE) L*a*b* were L* = lightness, a* = redness, and b* = yellowness on a 0-100 white scale.
[c] pH of the finished product (10g sample + 90 g water).
[d] Total pigment and cured pigment (nitrosylhemochrome) analysis.
[j] SEM = Standard error of the means for uncured, no-nitrate/nitrite-added and nitrite-added commercial frankfurter products.
[d-i] Means within same column with different superscripts are different (P<0.05).

Table 4. Analyzed values of moisture[a], salt (NaCl)[b], pH[c], water activity[d] and nitrite[e] in sliced turkey formulations (average for both replicates; n=6).

Formulation	% moisture	% NaCl	pH	A_w	Nitrite (ppm)
Ham Control-No Antimicrobials	70.76	1.57	6.44	0.979	12
Ham - 3.0% Cultured Sugar-Vinegar	71.08	1.57	6.31	0.974	9
Ham - 2.0% Buffered vinegar	70.13	1.64	6.22	0.974	5
Ham - 1.5% Lemon/Cherry/Vinegar	70.15	1.64	6.43	0.975	5
Ham Control-Traditional	70.08	1.57	6.27	0.974	27
Turkey Control No Antimicrobials	74.10	1.19	6.16	0.980	NT[f]
Turkey - 3.0% Cultured Sugar-Vinegar	71.35	1.06	6.04	0.975	NT
Turkey - 2.0% Buffered vinegar	73.41	1.01	6.07	0.978	NT
Turkey - 1.5% Lemon/Cherry/Vinegar	73.85	1.10	6.33	0.977	NT
Beef Control No Antimicrobials	65.12	0.46	5.74	0.985	NT
Beef - 3.0% Cultured Sugar-Vinegar	65.75	0.46	5.69	0.981	NT
Beef - 2.0% Buffered vinegar	66.36	0.50	5.64	0.982	NT
Beef - 1.5% Lemon/Cherry/Vinegar	67.18	0.45	5.93	0.982	NT

[a] Vacuum oven method, 5 h, 100°C (AOAC 934.01, 1990)
[b] Measured as Cl-, Mettler DL22 autotitrator, silver nitrate titration
[c] Measured using a AquaLab 4TE water activity meter, at average of 25˚C
[d] 10g meat with 90ml dH_2O, homogenized and pH taken of slurry using Orion 8104 combination pH electrode and Accumet pH meter
[e] method 973.31 (Nitrites in cured meats, colorimetric method); AOAC 15th edition, 1990
[f] NT, not tested

Figure 1. Growth of *L. monocytogenes* in turkey slurries at 4°C (Baseline study).

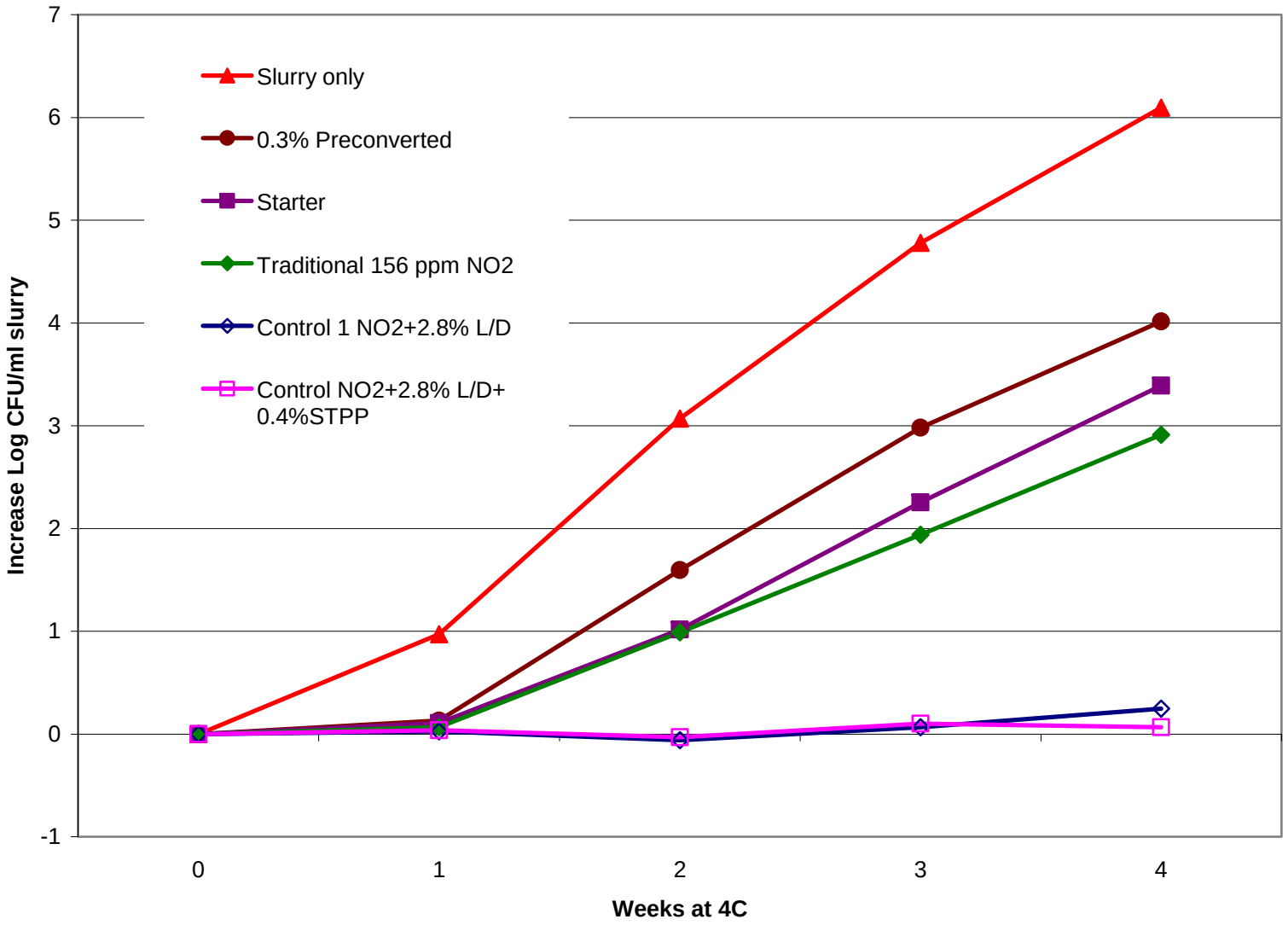

Figure 2. Effect of natural growth inhibitors on *Listeria monocytogenes* on uncured turkey

Averages ± SD reported for duplicate trials (n=6)

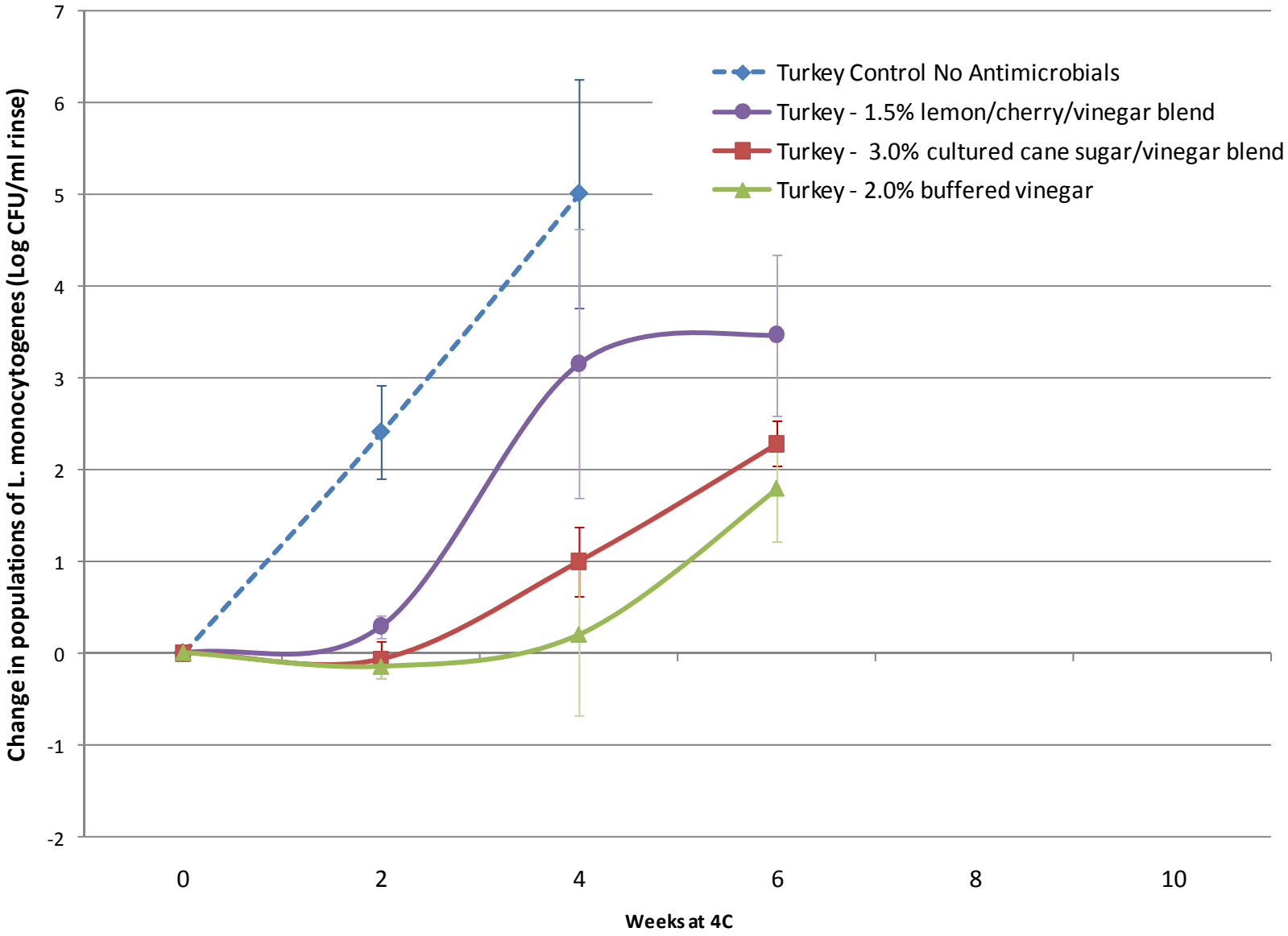

Figure 3. Effect of natural growth inhibitors on *Listeria monocytogenes* on ham

Averages ± SD reported for duplicate trials (n=6)

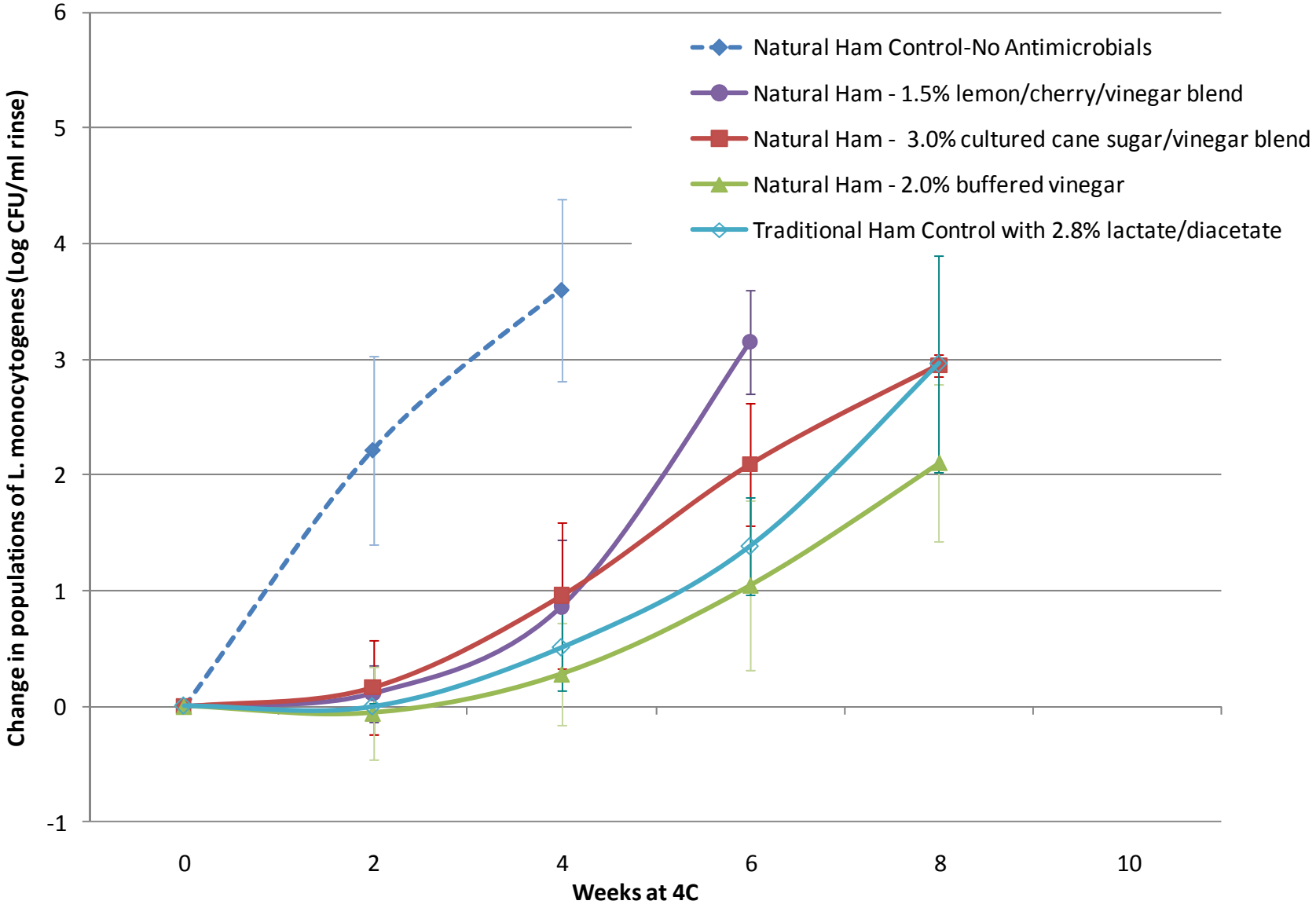

Figure 4. Effect of Natural Growth Inhibitors on *L. monocytogenes* on Uncured Beef
Average reported for duplicate trials (n=6)