Minimum Nitrite Levels Required to Control *Listeria monocytogenes* on Ready-to-Eat Poultry Products Manufactured with Lactate and Diacetate

Kathleen Glass,[a] Lindsey, McDonnell[a], Christopher Sawyer[b], and James Claus[b]

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) 262-0875 (JC)
E-mail: kglass@wisc.edu; clausjr@ansci.wisc.edu
Final Report 31 July 2008

Summary: Lactate and diacetate are more effective in inhibiting *Listeria monocytogenes* in cured ready-to-eat (RTE) meats than in uncured products, but no study has identified the threshold level of nitrite required to prevent *L. monocytogenes* growth in these products. The objective of this project was to compare the antilisterial effect of various nitrite levels in RTE sliced turkey product manufactured with lactate and diacetate. Treatments were manufactured using a central composite design for 4 variables, sodium nitrite, sodium chloride, potassium lactate, and sodium diacetate, with 5 levels for each variable (total 30 runs; center point replicated 6 times). Ranges for antimicrobial testing included 0-120 ppm nitrite, 0.8-3.6% NaCl, 0-3.2% lactate, and 0- 0.24% diacetate. Sliced finished products were surface inoculated with 3-log CFU/g *L. monocytogenes*, vacuum-packaged, stored at 4 or 7°C, and assayed for changes in populations of *L. monocytogenes* for up to 18 and 12 weeks, respectively. Listerial growth (defined as a 1-log increase) was highly variable for samples formulated with 30 and 60 ppm nitrite. Average growth for replicates of the center point treatment formulated with 60 ppm was observed at sampling intervals ranging between 9 and 18 weeks at 4°C. Formulations with similar lactate-diacetate-NaCl combinations but 120 and 0 ppm nitrite supported growth at 13-15 weeks and 4-6 weeks, respectively. Treatments with other combinations of lactate-diacetate-NaCl and 90 ppm nitrite supported growth similar to that which was predicted by the commercial model. Inhibition of *L. monocytogenes* was decreased by 3 to 6 weeks in several treatments with 30 ppm nitrite compared with the predictive model, but the addition of low nitrite levels delayed growth by 3 weeks or more compared to the control without nitrite. Addition of nitrite was the overall controlling factor on cooked product color. In general the cured color was not influenced by the addition of the non-nitrite antimicrobial ingredients. Treatments containing 30 to 120 ppm nitrite did not differ in cooked color. The minimum of 30 ppm nitrite was sufficient to produce a pink cured color in contrast to the absence of pink in the no nitrite control. These results suggest that a minimum 30 ppm nitrite will enhance the antilisterial activity of lactate-diacetate in RTE poultry, but as with other traditional antimicrobials, the effect is concentration dependent.

Specific Objectives of Research Proposal:

- Determine the anti-Listerial effect of various levels of nitrite, lactate and diacetate in a single, model meat system using a turkey bologna-type product

- Determine the effect of various antimicrobial combinations including nitrite on cooked color in turkey bologna-type product

INTRODUCTION

An expert panel convened by the Food and Agriculture Organization of the United Nations and the World Health Organization reported that the best strategy to reduce the rates of listeriosis is to prevent the occurrence of high levels of *L. monocytogenes* in foods at the point of consumption (Buchanan et al., 2004). In practice, the incidence of listeriosis could be reduced by 99% by eliminating higher dose levels (>3.5 log CFU) and by formulating ready-to-eat (RTE) foods to inhibit listerial growth (Buchanan et al., 2004).

The USDA-FSIS 2003 *Final Rule on Control of Listeria monocytogenes in Ready-to-Eat Meat and Poultry Products* addresses the management of risk by mandating that establishments incorporate strategies to control *L. monocytogenes* (Anonymous, 2003). Products produced under Alternative 1 and 2 of the final rule must be processed to eliminate *L. monocytogenes* and/or formulated to limit its growth during its shelf life.

Currently, U.S. manufacturers have incorporated lactate and diacetate into many formulations of RTE products to prevent growth of *L. monocytogenes* in high-moisture, high-pH products and prevent additional outbreaks and recalls (Anonymous, 2001). However, while studies have verified that the addition of lactate and diacetate combinations inhibit growth of *L. monocytogenes* in cured meat and poultry products (with sodium nitrite), these organic acid salts are less effective in uncured products (Glass et al., 2002; Legan et al., 2004; Mdandi and Shelef, 2001; Schlyter et al., 1993; Seman et al., 2002). Two predictive models based on the collaboration by Kraft Foods and Purac, the OptiForm™ Listeria Growth Control Model 2005 and the OptiForm™ Listeria Growth Suppression Model v. 2 (Purac North America, Lincolnshire, IL), focus exclusively on utilization of lactate and diacetate to control *Listeria* in cured products manufactured with at least 100 ppm nitrite; OptiForm™ Listeria Control Model 2007 includes an option for uncured product but the concentration of nitrite for cured product is not defined.

With the understanding that lactate and diacetate work best in cured products, processors are left with few choices to enhance the safety of their products that were traditionally manufactured without nitrite, such as oven-roasted turkey breast. Processors have the option to: 1) Use post-lethality treatments for all uncured products; 2) Use levels of lactate and diacetate at the maximum allowable levels; 3) Research the efficacy of other approved antimicrobials as antilisterial agents; or 4) Add standard levels of sodium nitrite to all ready-to-eat process meat and poultry products.

All of the options listed above have limitations in that they may not reliably guarantee safety or carry with them a negative economic impact. The disadvantage of utilizing a post-lethality treatment alone to control *L. monocytogenes* is that the treated products may be able to support growth of the pathogen should they become recontaminated at retail or in the home; therefore they still have the potential to cause illness. High levels of lactate and diacetate that are inhibitory in high-moisture uncured products usually have a negative effect on sensory properties and may discourage customers from repurchase. While there are several other generally recognized as safe (GRAS) antimicrobials that have antilisterial

properties, such as sorbate, benzoate, or propionate, they are not yet approved for widespread use within formulations and cannot be used in sliced product (Glass et al., 2007a, 2007 b; Islam et al., 2002a, 2002b; Samelis et al., 2001, U.S. Department of Agriculture, 2004). Some manufacturers have added traditional levels of nitrite to certain RTE turkey products that had been previously uncured. However, such products often have a modified flavor profile or the turkey is pink rather than off-white that is typical for roasted turkey.

No published literature has specifically reported the minimum level of nitrite required to affect the growth suppression of *L. monocytogenes* in the presence of lactate and diacetate. However, several studies have demonstrated that low levels of nitrite delay growth of *L. monocytogenes* in a media system by increasing the lag and generation times (Duffy, et al., 1994; McClure et al., 1997; USDA-ARS Pathogen Modeling Program version 7.0; www.arserrc.gov/MFS/PATHOGEN.HTM). For example, the addition of 150 ppm of sodium nitrite to a nutrient broth system (pH 6.0, 1.5% NaCl) doubles the lag and generation times for *L. monocytogenes* (3.77 and 0.66 days, respectively, for 0 ppm nitrite, and 6.81 and 1.14 days, respectively, for 150 ppm nitrite), with intermediate times in the presence of 30 ppm nitrite (4.15 and 0.73 days, lag and generation time, respectively). Other research also suggests that low levels of nitrite may suppress growth of *L. monocytogenes* in the presence of high levels of acetate. Juncher et al. (2000) reported that a combination of 2% lactate + 0.5% acetate prevented pathogen growth in sliced pork saveloy samples with at little as 60 ppm nitrite, and stored at 5 or 10°C for up to 28 days. However, diacetate was not evaluated in the study. The addition of 30 ppm nitrite to chilled slurries after pasteurization enhanced the antimicrobial activity of diacetate and as well as that of sorbate, benzoate, and propionate (Schlyter et al., 1993; Glass et al, 2004), but the combined effect was not determined if nitrite were added before heating.

Nitrite also has an effect on color. In turkey it is known that certain reduced denatured hemochromes (e.g. associated with nicotinamide, form a pink pigment) can develop during refrigerated storage of vacuum package product that has appropriate reducing conditions. Generally, one would expect the cure pink pigment (nitrosylhemochrome) to be seen immediately after thermal processing product that contains adequate nitrite. However, at very low levels, time may be required to allow the meat product to optimize its reducing conditions in order to form/reform nitrosylhemochrome associated with these very low levels of in going nitrite.

Our hypothesis is that nitrite added at levels which are lower than the traditionally-used 156 ppm may have a synergistic or additive antilisterial effect with lactate and diacetate in ready-to-eat meat and poultry products. By evaluating these interactions in a model meat system, we will identify formulation choices to manage the risk of listerial growth in high-moisture, RTE meat and poultry products. The objective of this project is to identify the minimum levels of added sodium nitrite required to suppress growth of *L. monocytogenes* in RTE processed turkey products manufactured with various levels of lactate and diacetate and provide data to assist manufacturers in formulating products that prevent growth of the pathogen. Color measurements will determine if reducing incoming nitrite will result in color more closely resembling uncured RTE turkey product.

MATERIALS AND METHODS

Experimental Design:. Treatments were manufactured using a central composite design for 4 variables, sodium nitrite, sodium chloride, potassium lactate, and sodium diacetate, with 5 levels for each variable (total 30 runs made in 6 sets; each set included four randomized runs and one center point). Ranges for antimicrobials testing included 0-120 ppm nitrite, 0.8-3.6% NaCl, 0-3.2% lactate (calculated on anhydrous basis), and 0-0.24% diacetate (Table 1). Eight different combinations of lactate-diacetate-NaCl were used to compare the effect of 30 vs. 90 ppm nitrite. Replicate center point treatments with 60 ppm were compared similar treatments formulated with 120 or 0 ppm nitrite. The remaining six treatments at the 60 ppm nitrite level evaluated combinations of high / low levels for diacetate, lactate, and NaCl.

Product manufacture: Meat blocks were composed of turkey breast (donated by Kraft-Oscar Mayer, Madison, WI). Upon arrival, turkey was double ground and vacuum bagged in 10 lb quantities and frozen (-1.7°C; -25 °F). Turkey was thawed for two days in a walk-in cooler (4°C) prior to product manufacture. Products were formulated to simulate commercial product with a target moisture value ranging 75-77%. Batter (4.54 kg batch) was prepared to include ground turkey breast, water, sodium chloride, starch (3%, meat block basis), sodium tripolyphosphate (13 g, 0.04% meat block basis), sucrose (0.35%, meat block basis), sodium erythorbate (550 ppm, meat block basis). Quantities of water, sodium chloride, potassium lactate, sodium diacetate powder, and sodium nitrite were adjusted per experimental design.

Ground turkey and all of the non-meat ingredients were processed for 3 minutes (temperature maintained at less than 12.8°C) in a cutter/mixer (Model VCM 12, Stephan Machinery Corp, Columbus, OH) under a full vacuum. Efforts were made to minimize oxidation during processing so that color development associated with the oxidation of nitrite will mimic that for whole turkey breasts. Batter was stuffed into fibrous, moisture-impermeable casings (65 mm, 2 5/8") and steam cooked to an internal temperature of 73.9°C in a batch oven (model: single batch electric oven, Alkar Engineering, Lodi, WI). Products were showered with cold water for 10 minutes prior to being place in a walk-in cooler (4°C) for overnight storage.

The casings were manually removed and product sliced on a sanitized, deli slicer to provide ~10 g per slice, and slices stored at 3-4°C until use. Prepared, chilled products were transferred to the Food Research Institute for surface-inoculation with *L. monocytogenes*. Products were vacuum packaged and used for subsequent color measurements, or used for microbial challenge studies within 48 hours after preparation.

Proximate analysis: Triplicate samples for each treatment were ground and analyzed within 24 hours after slicing for moisture (5 h, 100°C, vacuum oven method, 950.46; AOAC, 2000), pH (1:10 dilution for 10 g homogenized portion, Accumet Basic pH meter and Orion 8104 combination electrode), NaCl (measured at % Cl^-, $AgNO_3$ potentiometric titration, Brinkmann Metrohm autotitrator), and residual nitrite (Colorimetric Method, 973.31, AOAC, 2000). Fat

and protein levels were not analyzed for this study, but were targeted to approximately <3% fat and 18% protein.

Color measurements: CIE L*a*b* values (lightness, redness, yellowness, respectively) were measured on freshly cut surfaces of vacuum packaged samples 3 and 28 days after cook. Measurements were taken using a colorimeter (CR-300, 8-cm aperture, illuminant C; Minolta Corp., Osaka, Japan) calibrated with a white plate (L* 97.74, a* -0.06, b* 2.55). The initial time period established if a pink color was present soon after product manufacture. The second time period determined if a pink color appeared after extended dark storage as a result of low levels of sodium nitrite addition. Color data on cooked bologna-type turkey products were collected after 3 and 28 days storage at 4°C. Duplicate packages per formulation were opened on Day 3 re-vacuum packaged, stored at 4°C, and re-opened on Day 28 for color determination. Six repeated measurements were made on the product from each treatment.

Preparation of inocula: *Listeria monocytogenes* strains Scott A (clinical isolate, serotype 4b), LM 101 (hard salami isolate, 4b), LM 108 (hard salami isolate, 1/2a), LM 310 (goat milk cheese isolate, 4), and V7 (raw milk isolate, 4), were grown individually in 10 ml Trypticase® soy broth (BBL, Cockeysville, 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 (TSA) and Modified Oxford agar (MOX; Oxford Media Agar base, Difco).

Inoculation and testing: For each sampling unit, 10 slices were inoculated by spreading a total of 0.5 ml *L. monocytogenes* mixture over the surfaces using a pipette. Final concentration of *L. monocytogenes* was targeted to yield approximately 5-log CFU per 100 g package (3-log CFU/g). Slices were vacuum-packaged (Multivac AGW, Sepp Haggemuller KG, Wolfertschewenden, Germany) in gas-impermeable pouches (3 mil high barrier EVOH pouches, Deli 1 material, oxygen transmission 2.3 cm^3 per cm^2, 24 h at 24°C; water transmission 7.8 g per cm^2, 24 h at 37.8°C and 90% relative humidity, WinPak, Winnepeg, Manitoba, Canada), and stored at 4 or 7°C for up to 18 and 12 weeks, respectively.

For each treatment, triplicate inoculated samples were assayed for changes in *L. monocytogenes* populations and duplicate uninoculated samples were assayed for changes in lactic acid bacteria and pH. Treatments were assayed at 0-time, and at 3, 6, 9, and 12 weeks storage for 4 and 7°C and additionally at 15 and 18 weeks for storage at 4°C. Testing of a variable was discontinued if listerial growth (>1-log increase) was confirmed for all packages tested for two consecutive sampling intervals.

Bacterial populations were determined in rinse material obtained after adding 100 ml of sterile Butterfield's phosphate buffer to each package and massaging the contents externally by hand for about 3 minutes (Glass and Doyle, 1989; Glass et al., 2002). *L. monocytogenes* was enumerated by surface plating serial (1:10) dilutions of rinse material

on MOX. Select colonies were confirmed as *L. monocytogenes* by Gram-stain, tumbling motility, CAMP test, hemolysis on Trypticase soy agar with sheep blood, and biochemical analysis using MICRO-ID® *Listeria* (Remel, Lenexa, KS).

For uninoculated samples, the pH of homogenized sample was measured as an indicator of LAB growth. Briefly, 10 g was removed from each uninoculated sample and homogenized with 90 ml hot deionized water using a Stomacher. Homogenized sample was allowed to cool to room temperature and the pH measured on the slurry. Changes in populations of lactic acid bacteria were assayed for the remaining uninoculated sample by plating rinse material on APT agar (Difco) with bromcresol purple (25^{o}C, 48-72 h).

Analysis of data:
Formulations chosen were based on a central composite design (Table 1). There were four different antimicrobial ingredients tested, with five levels represented for each antimicrobial (sodium nitrite: 0, 30, 60, 90, 120 ppm; sodium diacetate: 0, 0.06, 0.12, 0.18, 0.24%; potassium lactate: 0, 0.8, 1.6, 2.4, 3.2%; sodium chloride: 0.8, 1.5, 2.2, 2.9, 3.6%). The center point (sodium nitrite, nit: 60 ppm; sodium diacetate, dia: 0.12%; potassium lactate, lac: 1.6; sodium chloride, nacl: 2.2%) was replicated six times. Four different antimicrobial combinations plus a replicate of the center point combination were manufactured in each time block (six blocks).

For microbiological data, colony counts were converted to log CFU/ml rinse. Means and standard deviations for populations of *Listeria monocytogenes* were determined for each temperature, treatment, and sampling interval. Data analysis identified time to an average 1-log increase in listerial populations for each formulation and for individual samples within each formulation. Any negative growth rates were set to 0 for regression analysis of the data to define the growth rate for each formulation. Linear and quadratic effects were analyzed using response surface regression for factors of nitrite, diacetate, lactate, NaCl and week (RSREG procedure, Minitab 14); model was condensed by eliminating all nonsignificant (p>0.05) two-way interactions and second order terms. In addition, data was compared to predicted time for a 1-log increase of *L. monocytogenes* in a 74% moisture product formulated with the specified combinations of lactate, diacetate and salt, and at least 100 ppm nitrite (OptiForm™ Listeria Growth Suppression Model, Purac; see Table 1 for comparison). Data for changes in populations of *L. monocytogenes* at each sampling interval were statistically analyzed among similar lactate-diacetate-NaCl combinations but with different nitrite levels using one-way ANOVA (Minitab 14). Differences with p<0.05 were considered significant.

For color data, linear (nit, dia, lac, nacl) and quadratic (nit^2, dia^2, lac^2, nacl^2, and all other combinations) effects were analyzed using PROC GLM (SAS Institute). To compare various combinations of antimicrobials, the 25 antimicrobial combinations (24 replicated once, center point replicated 6 times) were analyzed using PROC ANOVA. Significance was reported when p<0.10 and means were separated using Tukey's studentized range test (SAS Institute Inc. 2007). To determine the effects of antimicrobial combinations on cooked product color, color data on cooked bologna-type turkey products was analyzed separately by storage day (3 and 28). Separated analysis was performed because packages for each treatment contained both the 3 and 28 day slices. The packages were

opened before color was determined and then re-vacuum packaged, stored, and then opened on 28 day for color determination.

RESULTS AND DISCUSSION

Proximate Analyses: Triplicate samples of each finished ready-to-eat processed turkey product were analyzed for moisture, pH, water activity, NaCl and residual nitrite. Averages (± standard deviation) for the 30 trials were 76.6±0.53% moisture, pH 6.17±0.07, and water activity 0.973±0.005. Protein and fat levels were calculated based on formulation to be 18% and <3% respectively. Analyzed NaCl values were within 0.10% of target salt levels with the exception of Formulations 11, 12, and 20, in which NaCl values were 0.15, 0.3, and 0.14% greater than target, respectively. Analyzed residual nitrite levels after cooking and 48 hours cold storage were typically one-third to one-half of the added sodium nitrite concentration (data not shown).

Control of L. monocytogenes:
Results for growth of *L. monocytogenes* on products stored at 4°C are summarized on Table 2. In general, listerial growth (defined as a 1-log increase) was highly variable for samples formulated with 30 and 60 ppm nitrite. Growth for 60 ppm center point (CP) replicate samples (0.12% diacetate, 1.6% lactate, 2.2% NaCl) was initially observed at sampling intervals ranging typically between 9 and 18 weeks. Comparable lactate-diacetate-NaCl combinations formulated with 120 ppm nitrite supported growth within 13-15 weeks, but within 4-6 weeks for the Control no-nitrite treatment. The time to an average 1-log increase was 12 and 18 weeks for the 60 and 120 ppm nitrite treatments, respectively, compared with 9-12 weeks predicted by the OptiForm model for similar formulations using 100 ppm nitrite. In this study, the log-increase of *L. monocytogenes* at each testing interval was significantly greater ($P<0.05$) in the Control no-nitrite treatments than for either 60 or 120 ppm nitrite treatments. There was no statistical difference between the 60 and 120 ppm nitrite treatments at any testing interval; however, the lack of statistical difference was likely due to the large variation in growth for the 60 ppm CP trials.

Most treatments formulated with 90 ppm nitrite supported growth at a rate similar to that which was predicted by the OptiForm model. The addition of 90 ppm nitrite significantly delayed growth compared to 30 ppm nitrite for treatments with 0.18% diacetate- 2.4% lactate-1.5% NaCl and with 0.06% diacetate- 2.4% lactate-1.5% NaCl (Table 3). However, there was no statistical difference ($P<0.05$) between 30 and 90 ppm nitrite in the time to one-log growth for five of the eight lactate-diacetate-NaCl combinations. Unexpectedly, growth was delayed at 30 ppm compared with 90 ppm nitrite for product formulated with 0.06% diacetate- 2.4% lactate-2.9% NaCl. Additional review of the data confirmed that analyzed moisture, pH, and salt content for the two formulations were similar and confirmed to be within target, nitrite was added at proper concentrations, populations of lactic acid bacteria were comparable throughout the testing interval, and no unusual temperature issues were noted. No factor was identified as an obvious cause for this discrepancy. Data for both formulations was used to construct the limited model.

The limited model generated from this study (Table 4) yielded similar growth patterns for 90 ppm nitrite as predicted by the more extensive OptiForm Model (Table 3). The model

from this study revealed reduced inhibition when products are formulated with lower nitrite levels.

Contour plots generated from Minitab's RSREG procedure illustrate the effect of individual antimicrobials on the growth of *L. monocytogenes* by week (Figure 1). Using mid-point values for remaining factors, increasing NaCl, diacetate, and lactate concentration demonstrate a sustained inhibition of *L. monocytogenes* during storage of turkey with 60 ppm nitrite at 4°C. The shape of the contour plot for nitrite suggests that increasing nitrite levels from 0 to 60 ppm will enhance inhibition of *L. monocytogenes*. Thereafter, as illustrated by the plateau of the curve at concentrations $\geq$70 ppm, analysis suggests that additional increases in nitrite levels will not greatly enhance listerial inhibition. Figure 2 illustrates the interaction between antimicrobials in inhibiting listerial growth and further confirms that nitrite levels as low as 30 ppm have an observable effect in inhibiting *L. monocytogenes*.

As expected, the pathogen grew faster when products were stored at a slight temperature abuse. A 1-log increase was observed in approximately one-third to one-half the time at 7°C compared with samples stored at 4°C (Table 5 and 6). For example, if a 1-log increase was detected at 10-15 weeks for 4°C, similar growth was noted at 4 to 6 weeks for samples stored at 7°C.

Growth of spoilage microflora on uninoculated control samples were sporadic and did not correlate with inhibition of *L. monocytogenes* on comparable inoculated samples (data not shown). Similarly, reduction in pH associated with growth of lactic acid bacteria on control samples did not appear to inhibit *L. monocytogenes* in the treatments reported in this study. Formulations which supported growth of *L. monocytogenes* also supported growth of spoilage microflora.

Effects of Antimicrobials on Cooked Color

Linear and quadratic effect of colors were analyzed for each testing interval, with a significant model effects set to $P<0.10$ (Table 7). Quadratic effects were significant for nitrite for CIE a* on day 3 and CIE b* on day 28. In addition, there was a quadratic effect for lactate for CIE b* on day 28. CIE a* values for no nitrite product and nitrite containing turkey products were similar to those reported by Sammel et al. (2007; CIE a*: 4.9 for no nitrite, 6.0 for 10 ppm nitrite).

The quadratic effect for nitrite on redness (CIE a*) was likely due to an immediate saturation of myoglobin upon nitrite addition. Turkey breast meat is relatively low in myoglobin (0.18 to 0.58 mg/g: Fleming, 1990; Niewiarowicz et al., 1986) and hemoglobin (0.25 to 0.63 mg/g: Babji et al., 1982; Fleming, 1990). in contrast to beef longissimus muscle (3.42 mg/g; Hunt and Hedrick, 1977). Ahn and Maurer (1989) reported that the addition of sodium nitrite to turkey breast meat resulted in increased a* values and nitrosylhemochrome. They also found that the intensity of red color increased up to 10 ppm nitrite at which point redness was not enhanced by up to 50 ppm added nitrite. However, concentrations as low as 1 ppm nitrite were sufficient to significantly increase redness of turkey breast meat. At 30 ppm ingoing nitrite in our study, the residual nitrite analysis determined the presence of 15 ppm, substantiating that nitrite was added and

some of the nitrite was used in the cured color reaction. The only linear effects found were for CIE a* on day 3 for nitrite and NaCl and for CIE b* on day 28 for nitrite, diacetate, and NaCl.

The linear effect of NaCl indicates a decrease in CIE a* as the salt level increases. This may be due to denaturation of myoglobin in the raw state, and thus reduction in the formation of nitrosylhemochrome. A prooxidant effect of salt (Tan, W. and Shele, 2002) may also cause instability of the cured pigment, reducing the redness (CIE a*).

Colorimetric means for the individual combinations of antimicrobial treatments are presented in Table 8. No differences ($P>0.1$) were found in CIE L* on day 3 or day 28. There were no differences in CIE a* among the products formulated with nitrite on either day 3 or day 28. On day 3, the product formulated without nitrite was different ($P<0.05$) than 10 formulations containing nitrite, with some of these in each of the 4 nitrite levels tested (~2 units of CIE a* value are needed to visually detect a difference). Therefore, the no nitrite product visually lack redness compared to the other products. Products formulated to contain nitrite were not different in CIE b*. The product formulated without nitrite was more yellow (CIE b*) on day 3 than Treatment 90-G (90 ppm nitrite, 0.06% diacetate, 0.8% lactate, 2.9% NaCl; CC #27). On day 28, the 0ppm nitrite treatment was more yellow than five of the products formulated with nitrite (Treatments 90-E, 90-G, 60-J, 30-A, 30-G). There was not an obvious relationship between a particular antimicrobial and this difference.

The color analysis shows that addition of nitrite at all tested levels resulted in pink pigment generation (due to nitrosylhemochrome formation). Research (Sammel and Claus, 2007) has shown that at the same ingoing nitrite level, intact turkey breast products have higher redness than in ground products. In addition, even in the absence of nitrite, whole muscle turkey has a much greater likelihood of turning pink, particularly with storage time, because of greater anaerobic and reducing conditions. Therefore, color analysis of minimal nitrite in whole muscle injected turkey was not performed.

CONCLUSIONS

These results suggest that a minimum 30 ppm nitrite will enhance the antilisterial activity of lactate-diacetate in RTE poultry, but as with other traditional antimicrobials, the effect is concentration dependent with adequate activity estimated at $\geq$70 ppm. If products are formulated with combinations of reduced nitrite levels and lactate-diacetate-salt, verification of shelf-life should be completed. Reducing nitrite levels did not have a significant effect on redness in the ground turkey product.

ACKNOWLEDGEMENTS

Thank you to Sandra Olson, Rob Rassel, Lucas Schuette, and Kristine Zierke, for technical assistance in completing laboratory work, and Revis Chmielewski for review of this report. We greatly appreciate the donations of materials by Kraft-Oscar Mayer and Purac, and helpful discussions with Dr. Andy Milkowski, adjunct professor, UW-Madison. This work was funded by the American Meat Institute Foundation, University of Wisconsin-Madison College of Agriculture and Life Sciences, and by contributions from the food industry.

References

1. Ahn DU, Maurer AJ. 1989. Effects of added nitrite, sodium chloride, and phosphate on color, nitrosheme pigment, total pigment, and residual nitrite in oven-roasted turkey breast. Poultry Sci 68:100-106.
2. Anonymous. 2000. Food additives for use in meat and poultry products: sodium diacetate, sodium acetate, sodium lactate and potassium lactate. Fed. Reg. 65:3121-2123.
3. Anonymous. 2001. Summary report: results of interviews regarding lactate and diacetate use in RTE meat and poultry products - May 14, 2001. Office of Policy, Program Development and Evaluation (OPPDE), Food Safety and Inspection Service, US Department of Agriculture. www.fsis.usda.gov/oppde/peis/Reports/OPPDE/Summary_Rpt.pdf
4. Anonymous. 2003. Control of *Listeria monocytogenes* in Ready-to-Eat Meat and Poultry Products; Final Rule. Fed. Reg. 68: 34207-34254.
5. AOAC. 2000. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Gaithersburg, Maryland, USA.
6. Babji, A.S., Froning, G.W., and Ngoka, D.A. 1982. The effect of preslaughter environmental temperature in the presence of electrolyte treatment on turkey meat quality. Poultry Sci. 61:2385-2389.
7. Buchanan, R., R. Lindqvist, T. Ross, M. Smith, E. Todd, and R. Whiting. 2004. Risk assessment of Listeria monocytogenes in ready-to-eat foods – Interpretative summary. Food and Agriculture Organization of the United Nations and the World Health Organization, Geneva. www.who.int/foodsafety/publications/micro/en/mra4.pdf
8. Duffy, L. L., P. B. Vanderlinde, and F. H. Grau. 1994. Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: effects of pH, aw, nitrite and ascorbate. Int. J. Food Microbiol. 23:377-390.
9. Fleming, H.P., Blumer, T.N., and Craig, H.B. 1960. Quantitative estimations of myoglobin and hemoglobin in beef muscle extracts. J. Anim. Sci. 19:1164-1177.
10. Flemming, B.K. 1990. The nature and role of hemoglobin, myoglobin, and cytochrome c in poultry meats. M.S. Thesis. University of Nebraska.

11. 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.

12. 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

13. 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.

14. 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.

15. Glass, K.A., J. Veesenmeyer, L.L. McDonnell, P. Eimerman, and E.A. Johnson. 2004. Control of *Listeria monocytogenes* in wiener and turkey slurries by combinations of antimicrobials J. Food Prot. 67 (Sup. A): 55.

16. Hunt, M.C. and Hedrick, B. 1977. Chemical, physical, and sensory characteristics of bovine muscle from four quality groups. J. Food Sci. 42:716-720.

17. Islam, M., J. Chen, M. P. Doyle, and M. Chinnan. 2002. Control of *Listeria monocytogenes* on turkey frankfurters by generally -recognized-as-safe preservatives. J. Food Prot. 65:1411-1416.

18. Islam, M., J. Chen, M. P. Doyle, and M. Chinnan. 2002. Effect of selected generally recognized as safe preservative sprays on growth of *Listeria monocytogenes* on chicken luncheon meat. J. Food Prot. 65:794-798.

19. Juncher, D., C. S. Vestergaard, J. Soltoft Jensen, C. J. Weber, G. Bertelsen, and L. H. Skibsted. 2000. Effects of chemical hurdles on microbiological and oxidative stability of a cooked cured emulsion type meat product. Meat Sci. 55:483-491.

20. Legan, J. D., D. L. Seman, A. L. Milkowski, J. A. Hirschey, and M. H. Vandeven. 2004. Modeling the growth boundary of *Listeria monocytogenes* in ready-to-eat cooked meat products as a function of the product salt, moisture, potassium lactate, and sodium diacetate concentrations. J. Food Prot. 67:2195-2204.

21. Mbandi, E. and L. A. Shelef. 2002. Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. Int. J. Food Microbiol. 76:191-198.

22. McClure, P. J., A. L. Beaumont, J. P. Sutherland, and T. A. Roberts. 1997. Predictive modeling of growth of *Listeria monocytogenes*. The effects on growth of NaCl, pH, storage temperature and NaNO2. Int. J. Food Microbiol. 34:221-232.

23. Niewiarowicz, A.., Pikul, J., and Czajka, P. 1986. Gehalt an myoglobin und hamoglobin in fleisch verschiedener geflugelarten. Fleischwirtschaft 66:1281-1282.

24. Preston, D. J. Vessenmeyer, E. A. Johnson, and K. A. Glass. 2005. Inhibition of *Listeria monocytogenes* in processed meat and poultry by combinations of sorbate, benzoate, and propionate. J. Food Prot 68 (Sup. A):125

25. Samelis, J., J. N. Sofos, M. L. Kain, J. A. Scanga, K. E. Belk, and G. C. Smith. 2001. Organic acids and their salts as dipping solutions to control *Listeria monocytogenes* inoculated following processing of sliced pork bologna stored at 4 degrees C in vacuum packages. J. Food Prot. 64:1722-1729.

26. Sammel, L.M. and Claus, J.R. 2007. Calcium chloride and tricalcium phosphate effects on the pink color defect in cooked ground and intact turkey breast. Meat Sci. 77(4):492-498.

27. Sammel, L.M., Claus, J.R., Greaser, M. L., and Lucey, J. A. 2007. Identifying constituents of whey protein concentrates that reduce the pink color defect in cooked ground turkey. Meat Sci. 77(4):529-539.

28. SAS Institute Inc. 2007. SAS OnlineDoc® 9.1.3. Cary, NC: SAS Institute Inc.

29. Schlyter, J. H., K. A. Glass, J. Loeffelholz, A. J. Degnan, and J. B. Luchansky. 1993. The effects of diacetate with nitrite, lactate, or pediocin on the viability of *Listeria monocytogenes* in turkey slurries. Int. J. Food Microbiol. 19:271-281.

30. Seman, D. L., A. C. Borger, J. D. Meyer, P. A. Hall, and A. L. Milkowski. 2002. Modeling the growth of *Listeria monocytogenes* in cured ready-to-eat processed meat products by manipulation of sodium chloride, sodium diacetate, potassium lactate, and product moisture content. J. Food Prot. 65:651-658.

31. Tan, W. and Shelef, LA. 2002. Effects of sodium chloride and lactates on chemical and microbiological changes in refrigerated and frozen fresh ground pork. Meat Science 62(1): 27-32

32. U.S. Department of Agriculture. 2004. Preparation and processing operations. 424.23 Prohibited Uses. Code of Federal Regulations, Title 9, Part 424. Food Safety and Inspection Service. Office of the Federal Register, U.S. Government Printing Office, Washington, DC.

Table 1. Formulations (combinations of added nitrite, diacetate, lactate solids, salt) for finely-comminuted cooked turkey product (75.5-77.5% moisture) and weeks to 1-log increase of *L. monocytogenes* when inoculated product is stored at 4°C

CC#	Treat-ment #	Nitrite (ppm)	Diacetate (%)	Lactate (% anhydrous)	NaCl (%)	Week at which average 1-log growth detected	Predicted weeks to 1-log growth	
							Current study[1]	*OptiForm* ® Model[2]
6	120-CP	120	0.12	1.6	2.2	18	10.3	9-12
24	90-A	90	0.18	2.4	2.9	>18	>18	20 to 28
11	90-B	90	0.18	2.4	1.5	>18	>18	16 to 22
13	90-C	90	0.18	0.8	2.9	>18	16.6	8 to 11
30	90-D	90	0.18	0.8	1.5	12	11.9	6 to 9
19	90-E	90	0.06	2.4	2.9	12	16.6	15 to 20
22	90-F	90	0.06	2.4	1.5	18	11.2	11 to16
27	90-G	90	0.06	0.8	2.9	6	5.7	6 to 8
20	90-H	90	0.06	0.8	1.5	6	4.7	5 to 6
5	60-I	60	0.24	1.6	2.2	>18	>18	13-17
8	60-J	60	0.12	3.2	2.2	18	>18	>22
9	60-K	60	0.12	1.6	3.6	>18	17.5	12-16
CP[3]	60-CP	60	0.12	1.6	2.2	12	10.3	9-12
3	60-L	60	0.12	1.6	0.8	12	7.5	7-10
4	60-M	60	0.12	0	2.2	9	6.6	4-6
1	60-N	60	0	1.6	2.2	6	5.7	7-9
14	30-A	30	0.18	2.4	2.9	>18	>18	20-28
26	30-B	30	0.18	2.4	1.5	12	7.8	16-22
29	30-C	30	0.18	0.8	2.9	>18	13.3	8-11
16	30-D	30	0.18	0.8	1.5	6	8.8	6-9
25	30-E	30	0.06	2.4	2.9	18	8.1	15-20
12	30-F	30	0.06	2.4	1.5	6	2.7	11-16
17	30-G	30	0.06	0.8	2.9	6	4.1	6-8
23	30-H	30	0.06	0.8	1.5	6	3.5	5-6
10	0-CP	0	0.12	1.6	2.2	6	<1	9-12

[1] Based on regression model from data collected in this study.
2 Based on OptiForm® Listeria Control Model 2005 for 75% moisture, pH 6.2 product with ≥100 ppm nitrite
[3] Center point included Trials 2, 7, 15, 18, 21, and 28; one center point replicate was manufactured and inoculated for each block

Table 2. Changes in populations of *L. monocytogenes* on bologna-type cooked ground turkey products formulated with various antimicrobials and stored at 4°C for up to 18 weeks.

		Antimicrobial ingredient				***L. monocytogenes* Log CFU/ml rinse[1] At Week**						
CC#	**Treatment**	Nitrite (ppm)	Diacetate (%)	Lactate (%)	NaCl (%)	0	3	6	9	12	15	18
6	120-CP	120	0.12	1.6	2.2	3.17	3.11	3.09	2.87	3.46±0.32	3.95±0.48	**5.47±0.45**
24	90-A	90	0.18	2.4	2.9	2.46	2.48	2.27	2.32	2.17	2.14	2.20
11	90-B	90	0.18	2.4	1.5	2.77	2.40	2.50	2.54	2.67	3.01±0.52	3.56
13	90-C	90	0.18	0.8	2.9	2.54	2.60	2.35	2.51	2.73	3.07±0.66	2.89
30	90-D	90	0.18	0.8	1.5	2.66	2.30	3.02±1.03	2.96	**3.89±0.81**	**5.03±0.45**	Disc.[2]
19	90-E	90	0.06	2.4	2.9	2.58	2.61	2.56	2.74	**3.80±0.60**	**3.32±0.73**	**4.35±0.43**
22	90-F	90	0.06	2.4	1.5	2.46	2.53	2.22	2.25	2.51±0.37	2.80±1.14	**3.87±0.57**
27	90-G	90	0.06	0.8	2.9	2.59	2.60	**3.70**	**5.75**	**5.86**	**Disc.**	**Disc.**
20	90-H	90	0.06	0.8	1.5	2.60	2.87	**4.25 ±0.66**	**5.79±0.43**	**Disc.**	**Disc.**	**Disc.**
5	60-I	60	0.24	1.6	2.2	3.00	2.57	2.63	2.49	2.45	2.69	2.66
8	60-J	60	0.12	3.2	2.2	3.10	3.18	3.15	3.13	3.59±0.52	3.59	3.10
9	60-K	60	0.12	1.6	3.6	3.13	3.11	3.09	3.00	3.86	3.35	3.17
CP[13]	60-CP	60	0.12	1.6	2.2	2.85	2.59	2.77±0.57	**3.33±1.11**	**3.80±1.28**	**3.47±1.02**	**4.54±1.65**
3	60-L	60	0.12	1.6	0.8	3.02	2.51	2.53	2.90±0.55	**4.54**	**Disc.**	**Disc.**
4	60-M	60	0.12	0	2.2	3.00	2.59	3.14	**4.08**	**6.25±0.98**	**Disc.**	**Disc.**
1	60-N	60	0	1.6	2.2	3.02	2.62	**4.03±0.40**	**5.27±0.69**	**Disc.**	**Disc.**	**Disc.**
14	30-A	30	0.18	2.4	2.9	2.80	2.41	2.54	2.45	2.45	2.52	2.14

[1] Average of triplicate samples for each sampling interval; standard deviation <0.3 CFU/ml rinse unless stated
[2] Discontinued testing; consistent growth of *L. monocytogenes* for two consecutive sampling intervals
[3] Treatment: cp= center point used for the center point statistical analysis, replicated once for each set-up date, averages for six replicates, treatment #2,7,15, 18, 21, and 28; triplicate samples per sampling interval

CC#	Treatment	Nitrite (ppm)	Diacetate (%)	Lactate (%)	NaCl (%)	0	3	6	9	12	15	18
26	30-B	30	0.18	2.4	1.5	2.64	2.33	2.23	3.39±0.82	**4.30±0.51**	**5.14**	**Disc.**
29	30-C	30	0.18	0.8	2.9	2.75	2.45	1.66	2.73±0.53	3.06	3.30	3.06 ±0.55
16	30-D	30	0.18	0.8	1.5	2.54	2.86	**3.92 ±0.74**	**3.68±1.05**	**3.61**	**4.31±2.01**	**2.73 ±0.50**
25	30-E	30	0.06	2.4	2.9	2.45	2.40	2.31	2.32	2.33	2.72±0.56	4.37 ±0.45
12	30-F	30	0.06	2.4	1.5	2.87	**3.53±0.40**	**4.89**	**6.30**	**Disc.**	**Disc.**	**Disc.**
17	30-G	30	0.06	0.8	2.9	2.87	2.63	**4.82**	**5.37±0.45**	**Disc.**	**Disc.**	**Disc.**
23	30-H	30	0.06	0.8	1.5	2.55	3.12	**3.95**	**5.48**	**Disc.**	**Disc.**	**Disc.**
10	0-CP	0	0.12	1.6	2.2	3.15	3.78	**5.26**	**6.8 ±0.37**	**Disc.**	**Disc.**	**Disc.**

Table 3. Comparison of time (weeks) to average one log growth of *L. monocytogenes* for 90 vs 30 ppm nitrite at given combination of % diacetate, lactate, and NaCl

Treatment #	**90 ppm (CC#)**	**30 ppm (CC#)**	**% Diacetate**	**% Lactate**	**% NaCl**	**Model** *OptiForm* **(100 ppm)**	**Data** (this study) **90 ppm**	**Data** (this study) **30 ppm**	**Model** (this study) **90 ppm**	**Model** (this study) **30 ppm**
A	24	14	0.18	2.4	2.9	20 to 28	>18	>18	>18	>18
B	11	26	0.18	2.4	1.5	16 to 22	15 to 18	7 to 9[7]	>18	7.8
C	13	29	0.18	0.8	2.9	8 to 11	13 to 15	10 to 12	16.6	13.3
D	30	16	0.18	0.8	1.5	6 to 9	4 to 6	4 to 6	10.8	8.8
E	19	25	0.06	2.4	2.9	15 to 20	10 to 12	16 to 18[8]	16.6	8.1
F	22	12	0.06	2.4	1.5	11 to16	13 to15	4 to 6[9]	11.1	5.7
G	27	17	0.06	0.8	2.9	6 to 8	4 to 6	4 to 6	5.7	4.0
H	20	23	0.06	0.8	1.5	5 to 6	4 to 6	4 to 6	4.7	3.5

Table 4. Regression coefficients of significant factors (P<0.05) for predicting growth of *L. monocytogenes* in ready-to-eat turkey products

Factor	**Coefficient**
Constant	1.01589
Nitrite	-0.024063
Diacetate	-9.55154
Lactate	-0.174259
NaCl	0.0727381
Week	0.489557
Nit*Nit	0.000200551
Nit*Lactate	-0.00726852
Diacetate*Lactate	5.70168
Diacetate*week	-0.969224
Lactate*week	-0.0659987
NaCl*week	-0.0505026

R-Sq = 71.1%

[7] Growth significantly greater for 30 ppm nitrite than 90 ppm for weeks 6 through 18
[8] Growth significantly greater for 90 ppm than for 30 ppm at week 12 only
[9] Growth significantly greater for 30 ppm nitrite than for 90 ppm for weeks 3 through 18

Table 5. Changes in populations of *L. monocytogenes* on bologna-type cooked ground turkey products formulated with various antimicrobials and stored at 7°C for up to 12 weeks.

		Antimicrobial ingredient				***L. monocytogenes* Log CFU/ml rinse[10]**				
CC#	**Treatment**	Nitrite (ppm)	Diacetate (%)	Lactate (%)	NaCl (%)	0-time	3 week	6 week	9 week	12 week
6	120-CP	120	0.12	1.6	2.2	3.17	3.20	**4.90**	**3.65**	**7.63**
24	90-A	90	0.18	2.4	2.9	2.46	2.47	2.22	2.15	2.24
11	90-B	90	0.18	2.4	1.5	2.77	2.67	2.51	2.62±0.34	**3.90**
13	90-C	90	0.18	0.8	2.9	2.54	2.86	**3.89±0.31**	**4.63**	**4.76±1.03**
30	90-D	90	0.18	0.8	1.5	2.66	2.84	**4.49±0.37**	**6.05**	**7.06**
19	90-E	90	0.06	2.4	2.9	2.58	2.37	**3.36±0.59**	**4.24±0.37**	**4.76±0.33**
22	90-F	90	0.06	2.4	1.5	2.46	2.39	**3.83±0.63**	**4.91**	**5.44**
27	90-G	90	0.06	0.8	2.9	2.59	**3.97**	**5.93±0.40**	**6.87**	**Disc.[11]**
20	90-H	90	0.06	0.8	1.5	2.60	**3.84±0.50**	**5.88±0.37**	**6.71**	**Disc.**
5	60-I	60	0.24	1.6	2.2	3.00	2.52	2.55	2.33	3.24±0.50
8	60-J	60	0.12	3.2	2.2	3.10	3.06	3.42	**4.68**	**4.79**
9	60-K	60	0.12	1.6	3.6	3.13	3.04	3.30	**5.11**	**5.49**
CP[12]	60-CP	60	0.12	1.6	2.2	2.85	3.22	**4.56±0.40**	**5.80**	**5.84**
3	60-L	60	0.12	1.6	0.8	3.02	3.46	**5.62±1.20**	**Disc.**	**Disc.**
4	60-M	60	0.12	0	2.2	3.00	**4.27**	**5.93**	**Disc.**	**Disc.**
1	60-N	60	0	1.6	2.2	3.02	**4.78±0.31**	**5.94±0.31**	**Disc.**	**Disc.**
14	30-A	30	0.18	2.4	2.9	2.80	2.69	2.64	2.63	2.54

[10] Mean of triplicate samples for each sampling interval; standard deviation <0.3 CFU/ml rinse unless stated.
[11] Disc., discontinued testing; growth of *L. monocytogenes* observed for two consecutive sampling intervals
[12] Treatment: cp= center point used for the center point statistical analysis, replicated once for each set-up date, averages for six replicates, triplicate samples per sampling interval, treatment #2, 7,15, 18, 21, and 28

Cc#	Treatment	Nitrite (ppm)	Diacetate (%)	Lactate (%)	NaCl (%)	0	3	6	9	12
26	30-B	30	0.18	2.4	1.5	2.64	**3.99±0.34**	**5.32±0.54**	**4.91**	**Disc.**
29	30-C	30	0.18	0.8	2.9	2.75	**3.20±0.33**	**4.56**	**5.25**	**7.03**
16	30-D	30	0.18	0.8	1.5	2.54	**3.74**	**4.61±1.63**	**4.14±0.61**	**5.93±1.20**
25	30-E	30	0.06	2.4	2.9	2.44	2.62	**3.38±0.91**	**4.29±0.80**	**5.45**
12	30-F	30	0.06	2.4	1.5	2.87	2.87	**3.63**	**4.20±0.32**	**5.96±0.38**
17	30-G	30	0.06	0.8	2.9	2.87	**4.70**	**6.53**	**6.98**	**Disc.**
23	30-H	30	0.06	0.8	1.5	2.56	**6.00±1.21**	**6.56**	**7.07**	**Disc.**
10	0-CP	0	0.12	1.6	2.2	3.15	**5.04**	**7.66**	**8.08±0.31**	**Disc.**

Table 6. Comparison of time (weeks) to average 1-log increase for treatments stored at 4 vs. 7°C.

		Weeks to average 1-log increase	
Cc#	**Treatment**	**4°C**	**7°C**
6	120-CP	18	6
24	90-A	>18	>18
11	90-B	>18	12
13	90-C	>18	6
30	90-D	12	6
19	90-E	12	9
22	90-F	18	6
27	90-G	6	3
20	90-H	6	3
5	60-I	>18	>18
8	60-J	18	9
9	60-K	>18	9
3	60-L	12	6
4	60-M	9	3
1	60-N	6	3
2, 7,15, 18, 21, 28	60-CP	12	6
14	30-A	>18	>18
26	30-B	12	3
29	30-C	>18	6
16	30-D	6	3
25	30-E	18	6
12	30-F	6	9
17	30-G	6	3
23	30-H	6	3
10	0-CP	6	3

Table 7. Linear and quadratic effects (F-statistics) of antimicrobials on colorimeter values of cooked bologna-type turkey products.

Model[1], Linear and Quadratic Effects	CIE L*		CIE a*		CIE b*	
	Day 3	Day 28	Day 3	Day 28	Day 3	Day 28
Model	0.215	0.157	0.057	0.156	0.112	0.054
nit	0.232	0.191	0.010	0.127	0.325	0.045
dia	0.364	0.054	0.871	0.463	0.069	0.058
lac	0.016	0.020	0.113	0.078	0.986	0.106
nacl	0.896	0.832	0.010	0.017	0.080	0.074
nit*nit	0.797	0.047	0.003	0.031	0.010	0.005
dia*dia	0.196	0.250	0.244	0.173	0.187	0.947
lac*lac	0.392	0.094	0.414	0.601	0.272	0.056
nacl*nacl	0.209	0.241	0.453	0.200	0.556	0.759
nit*dia	0.603	0.982	0.720	0.665	0.793	0.497
nit*lac	0.499	0.579	0.987	0.746	0.690	0.892
nit*nacl	0.669	0.425	0.283	0.427	0.443	0.626
dia*lac	0.774	0.396	0.864	0.899	0.911	0.112
dia*nacl	0.120	0.033	0.359	0.483	0.071	0.158
lac*nacl	0.526	0.739	0.805	0.527	0.329	0.381

[1]Model: nit=sodium nitrite, dia=diacetate, lac=lactate, nacl=sodium chloride.

Table 8. Linear and quadratic effects (F-statistics) of antimicrobials on colorimeter values of cooked bologna-type turkey products.

Treatment[3]		Antimicrobial ingredient[2]				Day 3			Day 28		
		Nitrite (ppm)	Diacetate (%)	Lactate (%)	NaCl (%)	CIE L*	CIE a*	CIE b*	CIE L*	CIE a*	CIE b*
6	120-CP	120	0.12	1.6	2.2	78.64^{a}	6.43^{a}	6.51^{ab}	78.97^{a}	6.22^{a}	6.57^{ab}
24	90-A	90	0.18	2.4	2.9	78.41^{a}	6.31^{ab}	6.13^{ab}	78.88^{a}	6.03^{a}	5.75^{ab}
11	90-B	90	0.18	2.4	1.5	78.72^{a}	5.89^{ab}	6.23^{ab}	78.78^{a}	5.27^{a}	5.76^{ab}
13	90-C	90	0.18	0.8	2.9	78.76^{a}	5.80^{ab}	6.02^{ab}	78.25^{a}	5.15^{a}	5.82^{ab}
30	90-D	90	0.18	0.8	1.5	77.34^{a}	6.70^{a}	6.08^{ab}	77.31^{a}	6.38^{a}	6.19^{ab}
19	90-E	90	0.06	2.4	2.9	78.53^{a}	5.65^{ab}	5.56^{ab}	77.88^{a}	5.71^{a}	5.42^{b}
22	90-F	90	0.06	2.4	1.5	79.07^{a}	6.45^{a}	6.11^{ab}	79.12^{a}	6.35^{a}	5.96^{ab}
27	90-G	90	0.06	0.8	2.9	76.52^{a}	6.02^{ab}	5.29^{b}	76.55^{a}	5.80^{a}	5.36^{b}
20	90-H	90	0.06	0.8	1.5	78.03^{a}	6.36^{ab}	6.37^{ab}	77.48^{a}	6.62^{a}	6.00^{ab}
5	60-I	60	0.24	1.6	2.2	78.84^{a}	6.70^{a}	6.36^{ab}	78.45^{a}	6.81^{a}	6.65^{ab}
8	60-J	60	0.12	3.2	2.2	78.69^{a}	5.95^{ab}	5.83^{ab}	77.70^{a}	5.56^{a}	5.10^{b}
9	60-K	60	0.12	1.6	3.6	79.90^{a}	5.92^{ab}	6.07^{ab}	79.25^{a}	6.01^{a}	5.96^{ab}
cp	60-CP	60	0.12	1.6	2.2	78.56^{a}	6.19^{ab}	6.07^{ab}	78.23^{a}	5.87^{a}	5.96^{ab}
3	60-L	60	0.12	1.6	0.8	79.28^{a}	6.93^{a}	6.43^{ab}	78.76^{a}	6.92^{a}	6.63^{ab}
4	60-M	60	0.12	0	2.2	78.62^{a}	7.00^{a}	6.35^{ab}	77.72^{a}	6.92^{a}	6.37^{ab}
1	60-N	60	0	1.6	2.2	77.91^{a}	6.52^{a}	5.62^{ab}	77.38^{a}	6.34^{a}	5.88^{ab}
14	30-A	30	0.18	2.4	2.9	78.94^{a}	5.21^{ab}	5.69^{ab}	79.01^{a}	4.97^{a}	5.25^{b}
26	30-B	30	0.18	2.4	1.5	79.65^{a}	6.57^{a}	6.32^{ab}	78.82^{a}	6.44^{a}	6.34^{ab}
29	30-C	30	0.18	0.8	2.9	78.17^{a}	6.13^{ab}	6.30^{ab}	79.10^{a}	5.93^{a}	7.08^{ab}
16	30-D	30	0.18	0.8	1.5	76.89^{a}	6.05^{ab}	5.40^{ab}	77.40^{a}	6.02^{a}	5.69^{ab}
25	30-E	30	0.06	2.4	2.9	79.18^{a}	5.58^{ab}	5.52^{ab}	78.83^{a}	5.53^{a}	5.77^{ab}

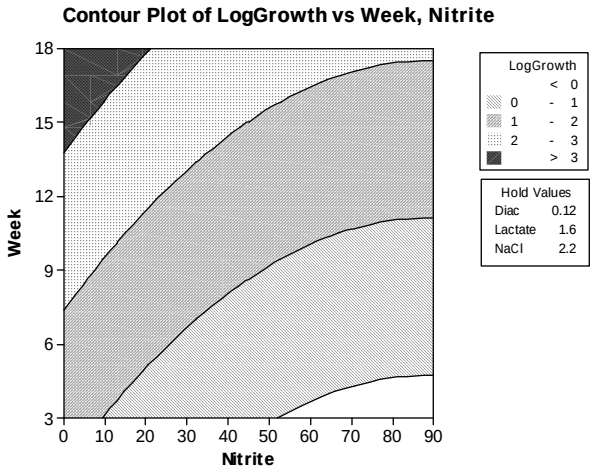
12	30-F	30	0.06	2.4	1.5	78.83[a]	6.47[a]	6.17[ab]	78.45[a]	6.28[a]	5.98[ab]
17	30-G	30	0.06	0.8	2.9	77.48[a]	5.55[ab]	5.43[ab]	76.63[a]	5.69[a]	4.95[b]
23	30-H	30	0.06	0.8	1.5	78.33[a]	6.66[a]	5.81[ab]	78.18[a]	6.74[a]	5.89[ab]
10	0-CP	0	0.12	1.6	2.2	79.47[a]	3.47[b]	7.90[a]	79.87[a]	4.23[a]	8.45[a]

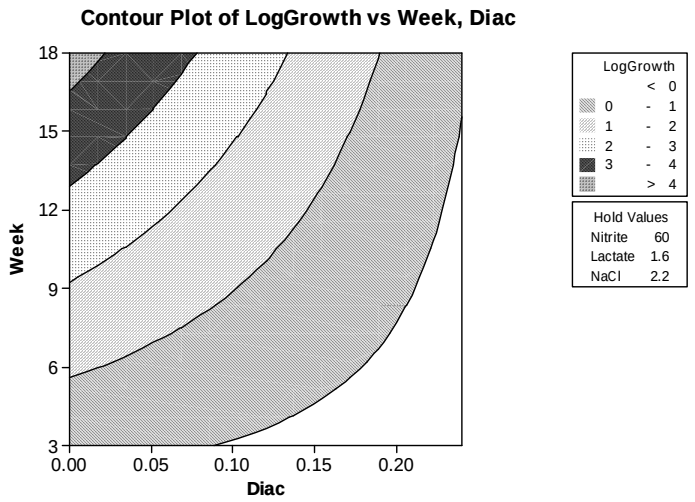
[1]Cooked turkey: produced from ground turkey breasts formulated (meat weight basis) with starch (3%), sodium tripolyphosphate (0.4%), sucrose (0.35%), and sodium erythorbate (550 ppm). Steam cooked (endpoint temperature, 73.9°C) in moisture impermeable casings.
[2]Antimicrobial ingredient: sodium nitrite, sodium diacetate, potassium lactate, sodium chloride~~)~~.

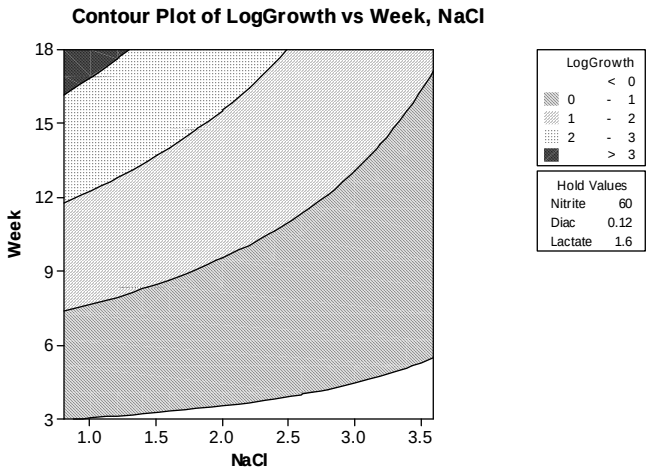
[3]Treatment: cp= center point used for the center point statistical analysis.

.

Figure 1. Contour plots for effect of antimicrobials on growth of *L. monocytogenes* during storage of inoculated turkey product at 4°C for 18 weeks







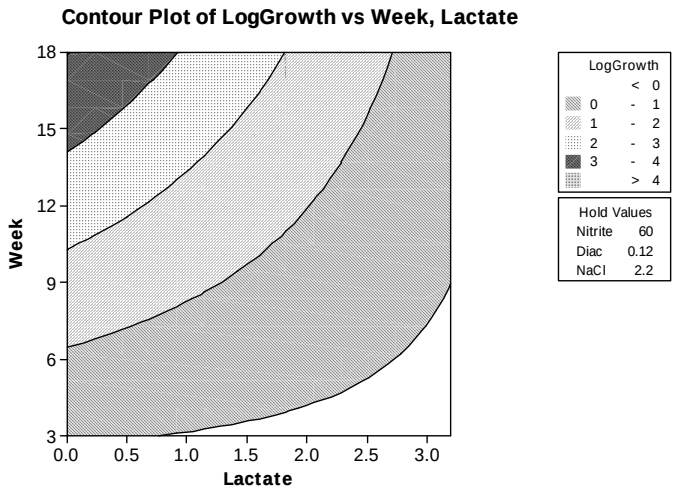


Figure 2. Interaction of antimicrobials to control growth of *L. monocytogenes* on turkey product stored at 4°C for 18 weeks.