Control of Post-Extrusion *Salmonella* Contamination in Dry Pet Food Utilizing *pHresh 10™* (Acidic Calcium Sulfate)

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Executive Summary

This study evaluated the effectiveness of pHresh 10™ (pHresh Technologies, LLC; Sabetha, KS), a food-grade antimicrobial comprised of 10N aqueous acidic calcium sulfate (ACS), for controlling moderate levels of post-extrusion *Salmonella* contamination in a dry, commercially manufactured canine kibble-style pet food. In phase 1, a scenario whereby freshly extruded kibbles might be recontaminated with environmental *Salmonella* between the extruder and end of the drying cabinet was studied. Control (CK; no-ACS/pH_{4h} 5.86) and test (AK; 1.14% ACS/pH_{4h} 5.34) kibbles were extruded and inoculated with ca. 10^5 *Salmonella*/g (five-strain mixture) prior to drying (pH values shown in Fig. 1). Inoculated products were dried to 6.0% moisture using 200°F forced air and were analyzed to determine surviving *Salmonella* populations on days 0, 1, 2 , 6 and 13 of ambient storage. Phase 1 survival data demonstrated similar immediate reductions for CK and AK kibbles; 3.6 and 3.9 log cfu/g, respectively (Fig.2). Dried non-coated stored kibbles showed similar low-level *Salmonella* recoveries of 1.3 and 1.4 logs, respectively, by day 2. There appeared to be a slight advantage for the AK non-coated kibbles by day 6 where more cellular injury [difference between *Salmonella* counts on non-selective (NS) and selective (XLD) agars] was observed; however, both kibble types remained *Salmonella* positive by enrichment detection throughout the thirteen storage days.

Phase 2 of the study was conducted to evaluate the impact of ACS as an ingredient in kibble coating to control potential environmental *Salmonella* recontamination occurring between the enrober and final product packages. CK and AK diets were manufactured and dried at a commercial manufacturing facility. Dried kibbles of each type were coated with a mixture of liquefied choice white grease, and palatability enhancing digest either containing no ACS (C; pH of

control coating 2.81) or 2.4% ACS (A; pH of coating containing ACS 1.09). The pH_{4h} values of the four kibble/coating treatment combinations (Fig. 3) were CK-C (5.79), CK-A (5.73), AK-C (5.27) and AK-K (5.20). Coated kibbles were surface inoculated with the mixed *Salmonella* culture to attain a target of ca. 10^4 cfu/g of product. *Salmonella* recoveries after 1 h of storage were lower for both kibble-types when ACS was added to the coating solution (Fig. 4). CK and AK kibbles coated with C solution demonstrated *Salmonella* levels of 4.0 and 3.7 log cfu/g, respectively, after 1 h storage. CK and AK kibbles coated with A solution exhibited *Salmonella* recoveries of 3.5 and 3.2 logs, respectively. Therefore, a 0.5 log advantage in *Salmonella* reductions on the kibble surfaces was immediately achieved by incorporating ACS into the coating solution.

After storage of coated kibbles for 24 h, there were no major differences observed among the four treatments in the total level of *Salmonella* recovered on non-selective (NS) agar, with 1.6-2.0 logs being detected (Fig. 4). However, the proportion of the recovered *Salmonella* population that was sub-lethally injured was substantially greater for treatments containing ACS in the kibble coating (injured populations were 0.7 and 1.1 log cfu/g for CK-A and AK-A treatments, respectively). By contrast, the injured populations in the CK-C and AK-C treatments were only 0.4 and 0.3 log cfu/g, respectively. After two days of ambient storage, a continued *Salmonella* control advantage was observed for kibbles coated with grease containing ACS. Very slight increases in *Salmonella* levels were detected in CK-C and AK-C products (0.1 and 0.4 logs, respectively) between the 24 and 48 h samplings. During this same period, CK-A and AK-A kibbles showed additional *Salmonella* reductions of 0.1 and 0.4 log cfu/g, respectively). In summary, a total *Salmonella* reduction of 2.4 log cycles was achieved by incorporating ACS into both the kibble and coating formulae within 48 h of storage, whereas, only 1.9 log reductions occurred in the control formulated and coated kibbles.

The most obvious *Salmonella* control effect of ACS in dry extruded canine kibbles was observed after extended storage (6 and 13 days at ambient room temperature). The average *Salmonella* level (three replications) in CK-C product at this time was 1.3 log cfu/g, with the majority (0.9 log) of the detected population being in a non-injured condition (Fig. 4). Kibbles that were coated with ACS-containing grease showed virtually no detection using direct plating on selective XLD agar (CK-A had no detection in 2/3 and AK-A had no detection in 3/3 replications). Most impressively, no *Salmonella* was detected by direct plating on non-selective agar for AK-A kibbles in 2/3 replications. In CK-A kibbles, non-selective agar plating revealed 1.0-1.3 logs of *Salmonella* (mostly injured) in all three replications. All treatments remained *Salmonella* positive after six days of storage when qualitative enrichment analyses (SDI RapidChek® immunoassay) was utilized. **However, after 13 days of storage, the kibbles formulated with ACS and coated with ACS-containing grease mixture (AK-A) demonstrated no detection by direct plating or enrichment, while viable *Salmonella* was detected in all other treatments.** In summary, a total *Salmonella* reduction on the surface of coated canine kibbles of ≥3.3 log cfu/g was achieved over six days of storage of product that contained ACS in both the kibble and coating (AK-A); whereas, a 2.7 log reduction occurred for the control product (CK-C). The magnitude of *Salmonella* reduction for the AK-A treatment was ≥ 4.0 log cycles.

This study (phases 1 and 2) clearly demonstrates the propensity of post-extrusion environmental *Salmonella* to survive at low levels in dry kibble-style pet foods for an extended period. It is unlikely that the recontamination levels used in phases 1 and 2 would be encountered in actual commercial manufacturing; however, a small percentage of surface-inoculated *Salmonella* can

remain during ambient storage. Previous research at the K-State Food Safety & Defense Laboratory (FSDL) has conclusively demonstrated the ability of the pre-conditioning/extrusion process used in pet food manufacturing to eliminate high levels (6.5 log cfu/g) of *Salmonella* that might contaminate raw ingredients of the kibble formulations when defined parameters are met (data not reported). Thus, it is vitally important to control environmental sources of *Salmonella* within the processing plant to reduce the risk of re-contaminating kibbles at very low to moderate levels as they exit the extruder and progress through drying, enrobing, cooling and bin staging to final packaging.

The concept of a secondary (2°) antimicrobial barrier technology has immense merit for dry pet foods since typical commercial manufacturing facilities are culpable to sporadic, infrequent low-level *Salmonella* contamination due to factors such as facility and equipment design, a "high product moisture" phase of processing, use of air to cool dried products, post-extrusion application of topical ingredients (i.e. flavorings) that receive no lethality treatment, and a dry manufacturing stage that is commonly open to the processing environment. The concept of a 2° antimicrobial barrier is widely used in food manufacturing, and approved acidulants are commonly incorporated into formulae to reduce the likelihood of outgrowth of pathogens and/or to provide an extended pathogen reduction effect. Generally, 2° barriers are not expected to provide large magnitudes of lethality towards a target pathogen, as is expected by primary bactericidal technologies (i.e. thermal and chemical pasteurization, irradiation or high pressure processing). In dry pet food manufacturing, the pre-conditioning and extrusion steps offer such a primary pathogen kill step. This study and similar large-scale experiments that have been conducted over the past 24 months at the FSDL show that *pHresh 10 ™* serves as an effective 2° anti-*Salmonella* barrier to help ensure that post-extrusion kibble and/or coating surface contamination would decline significantly during storage and distribution of commercial dry kibble pet foods. Even though actual *Salmonella* reductions were similar for control and ACS containing kibbles through drying, acidifying the kibble enhanced the ability of subsequent grease-based coatings containing ACS to reduce post-enrobing surface contamination.

This study did not determine palatability or nutritional effects of including ACS into pet food formulae, and companies choosing to incorporate any 2° barrier ingredient should evaluate these important aspects within their own product line. *pHresh 10™* was chosen as a likely ingredient for kibble acidulation because it does not negatively impact kibble extrusion characteristics like other food-grade acidulants (according to XIM Group extrusion expert consultants) and products acidified with ACS tend to demonstrate less sensory impact (sourness) compared to other commonly used food acidulants. When scientifically manufacturing dry pet food to control *Salmonella* contamination, coupling a primary and 2° antimicrobial approach, supporting this with stringent sanitation and good manufacturing programs, and verifying the overall process effectiveness through microbial testing and technical audits should result in predictable control of *Salmonella* in finished products.

Figure 1. Average pH values of non-coated kibbles before (wet) and after drying. Kibbles were rehydrated in deionized water and pH was read after 30 min and again after 4 h of stabilization time at room temperature. The 4h pH values are used for discussion in this report.

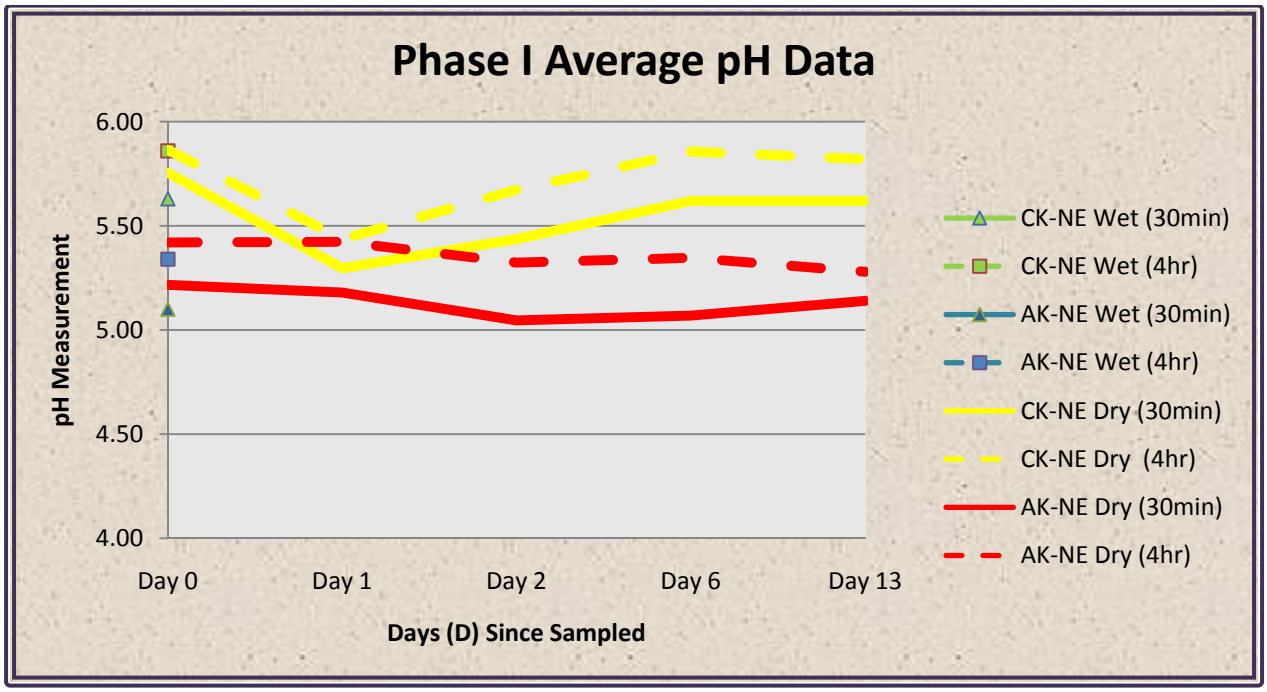

Figure 2. Average *Salmonella* recoveries from non-coated kibbles with and without ACS in the formulation. $^{+}$ indicates that *Salmonella* was detected only by qualitative enrichment. (CK=control kibble, AK= ACS containing kibble, NE=non-enrobed, NS=non-selective agar, XLD=selective agar)

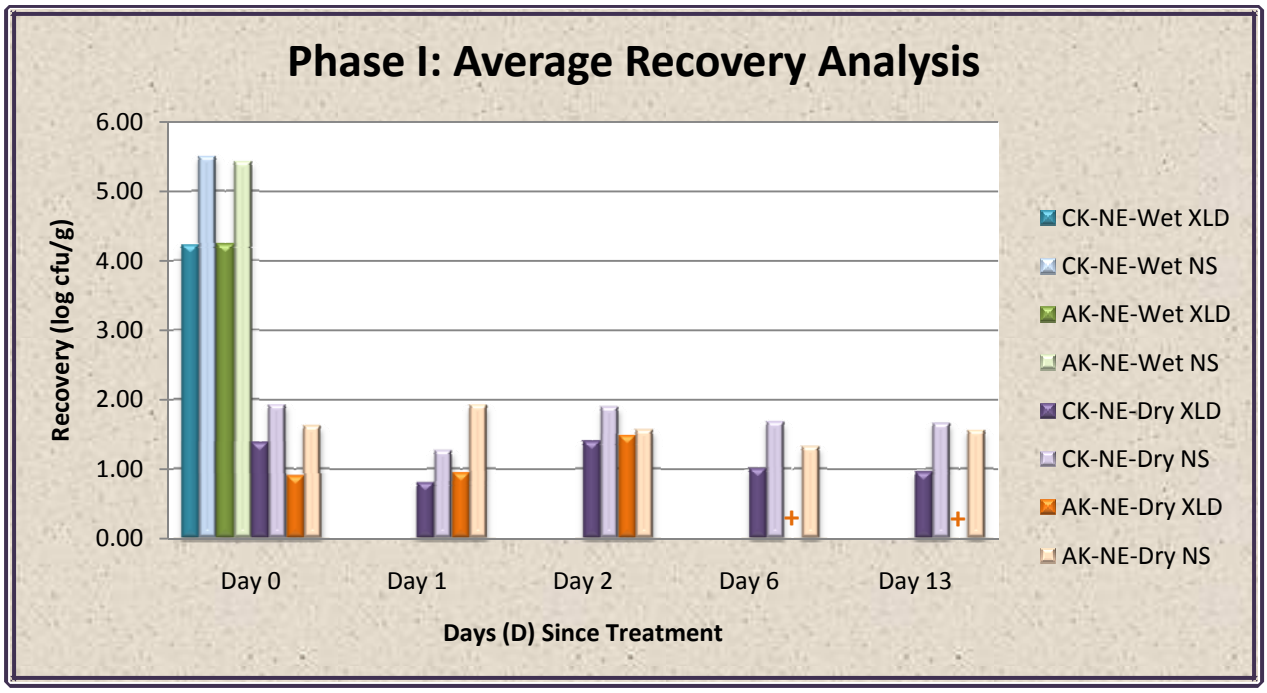

Figure 3. Average pH values of control (CK) and ACS-containing (AK) kibbles coated with choice white grease containing no ACS (C) or with ACS (A). Kibbles were rehydrated in deionized water and pH was read after 30 min and again after 4 h of stabilization time at room temperature. The 4h pH values are used for discussion in this report.

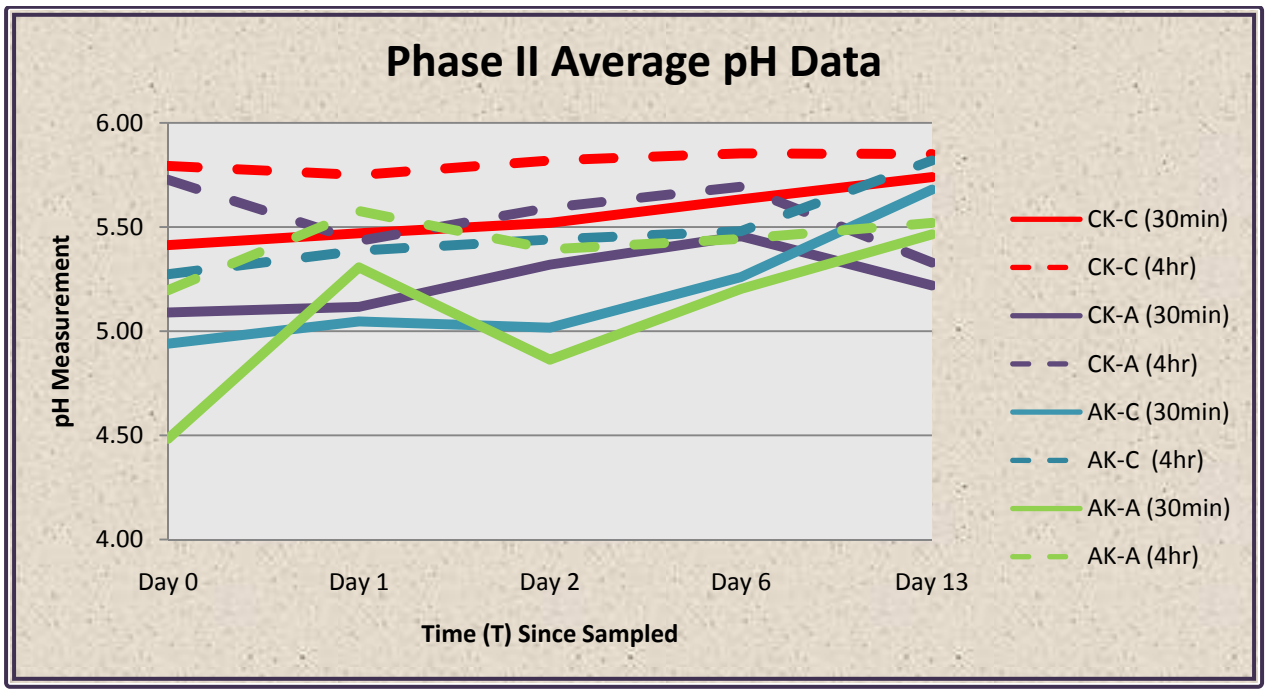

Figure 4. Average *Salmonella* recoveries from non-coated kibbles with and without ACS in the formulation. $^{+}$ indicates that *Salmonella* was detected only by qualitative enrichment. (CK=control kibble, AK= ACS containing kibble, NE=non-enrobed, NS=non-selective agar, XLD=selective agar)