BEEF SAFETY - CURRENT RESEARCH AND SUMMARY OF PROGRESS[1,2]

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Beef and beef products are significant parts of a balanced diet in the U.S. and major parts of the Kansas economy. Therefore, these products must be carefully processed, handled, and monitored for microbial quality to ensure safety for the consumer. KSU Animal Sciences research is designed to accomplish this end and to enhance demand for beef. We have made a major commitment to beef safety research. Moreover, results with beef are generally applicable to other meats.

A major objective of our beef safety research is to develop rapid analytical methods for estimating microbial numbers and species in meat and meat products. To achieve this end, Dr. Fung's group has established a Rapid Methods and Automation in Microbiology Center, which develops effective automated methods for monitoring disease-causing microorganisms. We have established that commercially available laser counter instrumentation is very effective for rapid enumeration of microbes on meat samples. Although other publications point to the effectiveness of this system, it was necessary that we evaluate and perfect the system for beef. Since refining this procedure, Dr. Fung's group has applied the system successfully to several projects related to beef safety and processing. The Center has demonstrated the procedure's ability to analyze for the presence and number of potential pathogens in beef and beef products. The microbial profile of a "typical" restructured, precooked, vacuum-packaged, refrigerated product has been evaluated after storage. This type of baseline evaluation is imperative as we develop value-added processes to produce convenient, precooked, beef products. Furthermore, we are utilizing the Omnispec® reflectance colorimetry method to estimate the number of microbes in meat and are developing procedures to study possible pathogens that might be present in beef. Refinement of these techniques will gave the industry improved ability to monitor and ensure the wholesomeness of beef for the consumer. This is particularly important for Kansas, because it enables our packing industry to rapidly monitor the microbial status of beef products before they are shipped to a variety of distant locations. Product recall is expensive and difficult and must be minimized.

We continue to evaluate dye-containing growth media for isolating bacteria, yeasts, and molds from food samples. A Klebsiella pneumoniae medium was developed to effectively isolate and enumerate this potential pathogen from food and the environment. The Candida albicans medium developed in Dr. Fung's laboratory exhibited 98% sensitivity and 99.5% accuracy in isolating and characterizing this important human yeast pathogen. Another of our dye-containing media can specifically isolate Penicillium and Aspergillus from meat

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and other foods. Dr. Fung's laboratory also developed and evaluated Trypan Blue Agar for the enumeration of yeasts and molds. Rapid methods to detect, isolate, and identify Salmonella, Listeria, Campylobacter, Yersinia, Clostridium, and Staphylococcus, which are bacterial pathogens possibly present in beef and beef products, were also developed.

In work on Clostridium perfringens, a double tube system proved superior to all other systems we tested, and we plan to submit this procedure for AOAC (Association of Official Analytical Chemists) approval in the near future. Furthermore Dr. Fung's double tube system has shown promise in effectively recovering Clostridium sporogenes. Research on control of E. coli 0157:H7 by fermentation in meat systems and antioxidants is in progress.

Development of a system for detecting low numbers of facultative anaerobic, motile, bacterial pathogens by using the oxyrase enzyme along with a unique motility enrichment procedure has been successful, and a patent application has been filed. The first organism successfully tested was Listeria monocytogenes. In the presence of oxyrase, Listeria grows very fast and can be detected in 5 to 7 hours, approximately 4 times faster than with other methods. Confirmation of this system through detection of Listeria in food is in progress.

Monitoring the sanitation and quality of air in meat processing areas is a key to maintaining product sanitation. We have presented research outlining our techniques at the 1989 International Association of Milk and Food Environmental Sanitarians meeting in Kansas City. A very simple catalase swab test can ascertain the sanitary condition of surfaces in meat processing plants. If swabbed surfaces of equipment or product are not "clean", catalase present in the microbes will form bubbles when the swab is placed in a hydrogen peroxide solution. This rapid test may be used as a quick indication of relative cleanliness. A major meat processors association is interested in making this test available to its members. More detailed research is under way.

Drs. Kropf and Hunt have evaluated several raw materials and processing parameters for their influence on the color of ground beef patties precooked to selected internal temperatures. Colored photographs of patties representing a range of raw materials, processing parameters, and a range of final internal temperatures surrounding the critical control temperature for Escherichia coli 0157:H7 and its verotoxin have been prepared. Studies are being designed to verify results and determine the best ways to present and utilize this information in the meat and food service industries. This research is critical to the beef industry because of the importance of ground beef; the need to develop convenient, precooked, "fast food" items; and the common practice of using cooked meat color to evaluate degree of cooking.

Dr. Cunningham's group has studied the effect of various foods and food ingredients on the activity of lysozyme, a naturally occurring antimicrobial enzyme. This knowledge can help ensure the safety of meat and meat products and extend shelflife. Lysozyme activity in the presence of seven basic amino acids increased to more than 100% of its original activity. Glucose increased lysozyme activity to 109.7%. Aspartame, a non-caloric sweetener, lowered lysozyme activity. Ingredients commonly used in processed meats, such as mustard, pepper, paprika, smoke, and curing ingredients, either enhanced lysozyme activity or were only slightly inhibitory. The antimicrobials, potassium sorbate or sorbic acid in a 1% solution, slightly decreased lysozyme activity to 95.2%. The antioxidant, n-propyl gallate (PG) decreased lysozyme activity to 85.7%, but 5% flour (91.3% activity) and 5% cornstarch (96% activity) only decreased lysozyme activity slightly. Lysozyme stability in food and food ingredients was studied on a long-term basis. Lysozyme activity remained high over a 1-yr period in solutions of boric acid, salt, sodium benzoate, sodium benzoate mixed with phosphoric acid, ethanol, and glycerol. The minimum concentration of lysozyme or EDTA

(a product that binds metals) that will control Listeria monocytogenes has been determined. However, lysozyme and EDTA work best in combination.

Selected food acids and their salts and antioxidants, singularly or in combination, are being tested. To date, four acids, five antioxidants, and 11 combinations have been tested against Salmonella enteritidis, Salmonella newport, and Salmonella typhimurium. Acetic and lactic acid used alone inhibited all three types of Salmonella. Of the antioxidants used alone, TBHQ showed the most promise. The other four antioxidants, PG, BHT, BHA, and Ethoxyquin showed little effect when used alone. However, when BHA was combined with acetic or lactic acid, we observed synergistic effects. The combinations of BHA-TBHQ and BHA-PG inhibited the Salmonella serotypes. The antioxidant-antioxidant combinations of BHA-PG and BHA-TBHQ, as well as the antioxidant-acid combinations of BHA-acetic acid and BHA-lactic acid, appear to be the most inhibitory.

Microbes pose a much more important health risk than chemical (i.e., pesticide) residues. However, the consumer perceives chemical residue as the most important factor influencing food safety. Therefore, we must have rapid, reliable techniques to detect chemical residues in meat and be able to address consumer and inspection agency questions about their significance. Dr. Smith is developing techniques to detect multiple organophosphate (OP) pesticide metabolites in animal tissues. His overall objective is to identify the formation of and measure the depletion of major organophosphate (OP) metabolites in meats. To measure these compounds, a method was needed to detect both the parent molecule, the oxidized parent molecule (oxon), and the hydrolysis metabolite.

Preliminary results show that at least 13 common OP's can be separated and detected. This method (high performance liquid diromatography) is simple and straightforward. Results to date with several OP's indicate that their oxons and hydrolysis products can be assayed using the same analytical method, leading to the probability that all CP compounds can be monitored with one procedure. Additional studies are underway, testing an extraction procedure to recover OP's and their metabolites from liver and cooked tissues.

To address questions about the industry's ability to monitor animal health care and maintain animal identity, Drs. Schafer, Simms, and Larson have combined efforts to evaluate identification and drug record-keeping systems for cattle. Over 340 completed questionnaires representing approximately 1.0 million cattle have been received to evaluate identification methods and success rates in Kansas. Approximately 75% of the respondents use brands and 80% use ear tags. Over 70% of the respondents reject the idea of a universal cattle identity system. Even though most questionnaires have been summarized, more are being solicited, and the final report will include that information.

Two companies that sell implantable electronic identification devices have agreed to research trials with their product and negotiations are underway with a third. The original trials will test the ease of administration, animal tolerance, and product features. We are interested in how far away the device can be read, if information can be added to the implants, the features of the peripheral computer programs, and practicality. Following FDA approval, we plan to use up to 150 of each company's devices in calves and follow them through to slaughter. Future tests may include devices that not only identify animals, but also measure indices such as body temperature and respiration rate. The technology for measuring body temperature with an implant device is close to commercial availability. Such a device could allow early detection of fevers and earlier treatment with less medication, leading to a reduction in residue concerns. Electronic identification could also allow automated collection of production, genetic, and health data. For tracing purposes, this semi-permanent identification system would also provide an

accurate history of animal ownership.

Interviews of feedlot managers and employees in Southwest Kansas determined that both microcomputer and manual record-keeping systems are used to keep track of pharmaceutical use. The vast majority of yards depend on custom feeding for their livelihood. Yard managers are acutely aware of economic repercussions to their customers, if residues are detected. Therefore, they are very cautious in following label-prescribed withdrawal times. Microcomputer software packages are available that allow feedlots to monitor such items as treatments, withdrawal times, drug inventory, and animal inventory. Pharmaceuticals represent a significant operating expense for feedlots. Programs that carefully monitor product usage help guarantee proper customer billing, and from a residue avoidance point of view, help assure that all treated animals are recorded.

Employees are aware that all drug inventory must be accounted for with treatment records. Feedlot managers were generally pleased that computer use has been well received by feedlot employees.

Companies selling pharmaceutical treatment software packages were contacted for the names of feedlots using their respective packages. Then, 72 yards in Kansas and Nebraska were surveyed. Survey questions addressed advantages and disadvantages of the packages and evaluated their usefulness as tools to aid in avoiding residues. This survey information is being summarized.

A questionnaire addressing general record-keeping systems used by Kansas feedlot operations has been mailed. Questions are geared to discovering manager's concerns and potential problems with record keeping systems. From the 232 yards surveyed to date, 55 questionnaires have been returned and a resurvey is in progress.

Kansas State University has made significant progress toward its research goals of developing technology for rapid identification of microbial and chemical agents and analyzing the food chain to determine the most effective points at which to prevent contamination. The knowledge gained from this research will continue to enhance beef's role in the American diet.