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INSIDE:

Former UA Researcher Returns
to Bulgaria

NCTR Scientists Explore
Emerging *Salmonella*

Orange Peels and Pulp
Evaluated for Cattle Feed

Travel Grant Awarded to
Doctoral Student

UA Press Releases Food Safety
Consortium Book

Food Protection Workshop
Slated for June at UA

Biological Sciences Student
Probes *Staphylococcus aureus*

M.S. Students at Center
Graduate, Ready for Next Tasks

Food Protection Workshops

Center Presentations,
Publications, Book Chapters

Spring 2010

Vol. 1, No. 2

Milillo's NRI Fellowship Supports *Salmonella* Work



Dr. Sara Milillo

By September, Sara Milillo will complete her two-year fellowship grant at the University of Arkansas Division of Agriculture Center for Food Safety. But after that there will still remain unexplored areas of the *Salmonella* research she is pursuing.

Milillo, a postdoctoral associate, is working under center director Steven Ricke's supervision on research looking at new ways to reduce *Salmonella* contamination of poultry. The research is examining how effective certain antimicrobial treatments are in mitigating *Salmonella* and a genetic analysis of the *Salmonella* that survive the treatments. The project is being supported by a National Research Initiative postdoctoral fellowship grant for \$113,383 from the U.S. Department of Agriculture.

Milillo's two presentations at American Society for Microbiology conferences summarize her work. At the 2009 meeting in Philadelphia, she presented a report on "Efficacy of Organic Acid Salts at Reducing *Salmonella* Typhimurium Populations Grown in BHI or Model Chicken Media at Different Temperatures." The report concluded that combining sodium lactate with thermal treatment achieves a synergistic antimicrobial effect on *Salmonella* Typhimurium grown in nutrient rich media.

At this summer's ASM meeting in San Diego, Milillo will present a paper on "Exposure to Acidified 55°C Solutions of Select Organic Acid Salts Leads to a Loss of *Salmonella* Typhimurium Viability Due in Part to Disruption of the Cell Membrane and Corresponding Cell Damage." The report's data will suggest that a combined thermal acidified sodium propionate treatment may provide an effective antimicrobial treatment for *Salmonella*-

(Continued on page 2)

Center for Food Safety

Spring 2010 Vol. 1, No. 2

Milillo's NRI Fellowship Supports *Salmonella* Work (Continued from page 1)

related foodborne illness cases.

Milillo said the results gained from her work with Ricke are providing “an important contribution to food safety research by serving as a foundation for future examination of *Salmonella* gene regulation in post-harvest environments.” She added that her project, in combination with Ricke’s previous work in *Salmonella* research, is providing “a unique opportunity for making connections between survival and persistence mechanisms used by *Salmonella* during pre- and post-harvest.”

Former UA Researcher Returns to Bulgaria, Pursues Food Safety Efforts



Drs. Steven Ricke and Vesela Chalova-Zhekova

From 2006 to 2009, Vesela Chalova-Zhekova was a post-doctoral associate at the University of Arkansas Division of Agriculture Center for Food Safety, where she worked under the supervision of Center director Steven C. Ricke. Chalova-Zhekova, a native of Bulgaria, earned an M.S. degree in biotechnology in 1994 from the University of Food Technologies in Bulgaria and a Ph.D. in 2005

from Texas A&M University. Ricke was her major advisor at Texas A&M, where she developed her expertise in bacterial physiology and molecular biology and applied it to the potential use of biosensors in poultry nutrition.

At Arkansas, she worked on new projects involving *Salmonella* spp. and *Lactobacilli*. In the process of developing these additional research areas, she expanded her microbiological skills and molecular techniques. Her research focused on the health aspect of the complex interaction between food derivatives (citrus oils, Maillard Reaction Products) and gastrointestinal microorganisms including *Salmonella*, *Lactobacilli*, and *Bifidobacteria*.

Chalova-Zhekova returned to Bulgaria last year and joined the faculty at the University of Food Technologies in Plovdiv, a city in the southern part of the country, where she is an assistant professor of biochemistry. She recently answered questions about her work there.

Center for Food Safety

Spring 2010 Vol. 1, No. 2

Q: Do you teach classes? What topics do you teach? What level of students do you teach?

A: I teach Food Biochemistry for undergraduate students. In addition, starting from the new academic year, I will be teaching Genetically Modified Foods, which is designated for master level students.

Q: What research area did you pursue at the University of Arkansas as a postdoctoral associate?

A: My research area included foodborne pathogens with emphasis on *Escherichia coli* and *Salmonella*.

Q: What research do you pursue at the Bulgarian university?

A: Food safety and the interaction between food-related microorganisms and food matrix will continue to be a part of my research. A new direction is the research on functionality of proteins isolated from sunflower oil cake, which is a byproduct of sunflower oil production in Bulgaria.

Q: Will you be collaborating with the University of Arkansas on any of your future research projects?

A: Collaborating with University of Arkansas would be a pleasure and great opportunity for diversifying research projects, exchanging ideas, and experiencing the high level expertise of the University of Arkansas scientists.

Q: Do you work on research projects with any other European universities?

A: Currently, University of Food Technologies has numerous contracts with universities from EU countries which allow collaboration and mobility of students and teachers. Partnerships with local industrial companies with EU participation also exist. Students graduating from University of Food Technologies obtain an educational degree, which is equivalent to the respective degree obtained in any university in the EU and therefore have the opportunity to be hired by EU food companies when their qualification is needed.

NCTR Scientists Explore Emerging *Salmonella*

Food safety scientists Rajesh Nayak and Steven Foley at the National Center for Toxicological Research (NCTR) in Jefferson, Ark., are focusing on emerging pathogens, such as *Salmonella* Javiana and *Salmonella* Heidelberg. They are collaborating with researchers at the University of Arkansas Division of Agriculture Center for Food Safety, the U.S. Department of Agriculture, other federal Food and Drug Administration centers and state public health laboratories in developing technologies that promote and protect public health.

“Foods in themselves are complex matrices,” said Rajesh Nayak, a research scientist at NCTR, an FDA center. In a talk on March 31 to UA food science faculty and students, Nayak advised that university researchers can help by focusing on technologies that can be useful in the detection, surveillance and control of foodborne pathogens.

Center for Food Safety

Spring 2010 Vol. 1, No. 2



Dr. Rajesh Nayak

NCTR's investigations into *Salmonella* Javiana and *Salmonella* Heidelberg are part of FDA's overall food protection plan, developed in 2007, to address both accidental and deliberate contamination of the nation's food supply. In particular, Nayak said, NCTR looks at the issues surrounding pathogens' resistance to antimicrobials and is developing technologies for rapidly detecting foodborne pathogens.

Salmonella Javiana was one of the most commonly reported serovar to the Centers for Disease Control's PulseNet database. "We wanted to know more about the genetics of this pathogen, so we looked at the demographics, the genetic diversity, the antimicrobial resistance issues and the pathogenicity," Nayak said. They found that the infection rates of this pathogen

occurred mostly in children under age 10, and only a few of these bacterial isolates were resistant to multiple drugs.

Salmonella Heidelberg is the fourth most common serovar to cause salmonellosis in humans and the most common serovar in retail meats. There is also some evidence to suggest that it can colonize in the reproductive tract of laying hens and subsequently contaminate eggs. "Our goal is to characterize the distribution of *Salmonella* Heidelberg populations in turkeys," Nayak explained. "We have analyzed *S.* Heidelberg isolates collected from farm samples, veterinary diagnostics samples, processing plants and retail fresh meats."

Looking at the bigger picture, Nayak said, "Food safety has many unknown variables and there are no simple solutions. There is a need to move from organoleptic to science-based testing methods. We have been doing that successfully with some risk-assessment work and HACCP protocols. We also need to improve our food safety education by partnering with the universities and state public health laboratories."

Foley also spoke about projects that his research group is pursuing at NCTR. He described his work examining the genetics of antimicrobial resistance in *Salmonella* serovars that are commonly detected in food animal species and are sources of human infections. Foley also described the results of DNA sequencing studies of plasmids from multidrug-resistant *Salmonella* Heidelberg isolates. These plasmids were found to contain genes encoding resistance to multiple antibiotics and likely increased virulence in poultry.

Center for Food Safety

Spring 2010 Vol. 1, No. 2

Orange Peels and Pulp Evaluated for Cattle Feed

Research presented at the 2010 Beef Industry Safety Summit claims the essential oils in orange peel and pulp kill *E. coli* O157:H7 and *Salmonella*. These natural byproducts, created by making juice, are now being investigated as a potential feed ingredient for cattle.

The citrus research was one of 13 safety studies funded in 2009 by the National Cattlemen's Beef Association. The project's principal investigators were Steven Ricke and Phil Crandall, both of the University of Arkansas Center for Food Safety, and Todd Callaway and Tom Edrington, both of the U.S. Department of Agriculture Agricultural Research Service in College Station, Texas.

Callaway said dietary orange peel and pulp reduced *Salmonella* populations in the intestinal tract of sheep. Callaway anticipates the next phase of the study to yield similar results for reducing *E. coli* O157:H7.

Salmonella and *E. coli* O157:H7, commonly associated with cattle, are bacterial pathogens that can contaminate beef during processing and are responsible for many diarrheal diseases each year. Reducing pathogen populations in cattle before slaughter may enhance beef safety and reduce human illnesses. However, preharvest intervention strategies must pass governmental approval before implementation, which has slowed the implementation of new interventions.

The research team's approach involves feeding cattle a natural feedstuff, orange peel that is a byproduct of the citrus industry, and is already widely fed to cattle in least cost rations. Orange peel and its associated essential oils have been shown to reduce or eliminate the carriage of pathogens in laboratory studies through a natural antimicrobial effect.

The researchers said the studies are designed to determine if orange peel feeding can reduce the shedding of *E. coli* O157:H7 and *Salmonella* in sheep used as a model of cattle intestinal tracts. This research can demonstrate if orange peel or essential citrus oils can serve as potential non-antibiotic pathogen reduction strategies, helping improve the safety and wholesomeness of beef.

Travel Grant Awarded to Doctoral Student

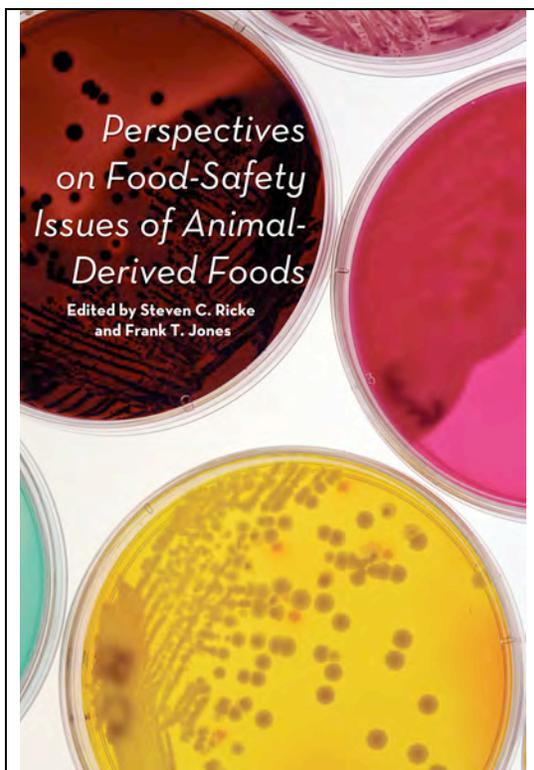
Suwat Saengkerdsub, doctoral student in food science, received a travel grant for \$500 from the American Society for Microbiology. Saengkerdsub is presenting a poster, "Initial Screening of Methionine-Producing Bacteria for Organic Poultry Feed," at ASM's General Meeting in San Diego on May 25. Saengkerdsub will graduate in May 2011.

Center for Food Safety

Spring 2010 Vol. 1, No. 2

UA Press Releases Food Safety Consortium Book of Essays by Leading Researchers

Timely reports on several topics dealing with the food supply comprise the 362-page edition of *Perspectives on Food-Safety Issues of Animal-Derived Foods*, a volume of essays by the nation's leading food safety researchers. The University of Arkansas Press published the book in association with the Food Safety Consortium (FSC).



The FSC is an alliance of researchers at the University of Arkansas, Iowa State University and Kansas State University that was established by Congress in 1988. Its work is funded annually through the U.S. Department of Agriculture. The book includes a brief history of the FSC's founding and its research accomplishments.

“Although great strides have been made by the scientific community in understanding the biology and dissemination of foodborne pathogens, recent media headlines publicizing pathogenic bacterial contamination of foods such as spinach and peanut butter indicate that foodborne disease is still a high-profile issue for the consumer,” said Steven C. Ricke, who holds the Donald W. “Buddy” Wray Chair in Food Safety at the University of Arkansas Division of Agriculture.

Ricke, the FSC's research director at the UA, served as co-editor of the book with Frank T.

Jones, who retired last year as associate director for extension at the UA Center of Excellence for Poultry Science.

The book consists of 24 essays written by researchers at universities, industries and government agencies across the United States. Their work is divided into five categories: preharvest foodborne pathogen ecology and intervention strategies, postharvest foodborne pathogen ecology, rapid methods and detection strategies for foodborne pathogens, antibiotics and antimicrobials in food safety, and emerging issues in food safety.

The book has its origins in an FSC symposium presented in 2006 at which several researchers from the organization's three universities and other institutions delivered oral reports on a variety of food safety issues. In the following years, many of the symposium's

Center for Food Safety

Spring 2010 Vol. 1, No. 2

presenters worked with other researchers to expand their reports into peer-reviewed book chapters that were reviewed by scientific experts in their respective fields and comments were addressed during the revisions and editing of the book chapters.

The book is available for sale at the University of Arkansas bookstore, or through the UA Press at <http://www.uapress.org/titles/fa09/ricke.html> or 800-626-0090.

Biological Sciences Student Probes *Staphylococcus aureus* at Center

Colby Baker, a biological sciences major who wrote her honors thesis in collaboration with the Center for Food Safety, graduated magna cum laude this spring with a bachelor of science degree from the J. William Fulbright College of Arts and Sciences.



Colby Baker (center) at the food science lab with Dr. Steven Ricke (left) and Dr. Arunachalam Muthaiyan.

Baker wrote her thesis on “Use of Orange Essential Oils and Natural Compounds as Novel Antimicrobial Against *Staphylococcus aureus*” under the supervision of Dr. Steven C. Ricke, director of the center who holds the Donald “Buddy” Wray Chair in Food Safety, and Dr. Arunachalam Muthaiyan, a postdoctoral associate at the Center for Food Safety. Baker’s research

was funded by the UA Honors College.

Baker said the goals of her research project were to study the effects of natural compounds, such as orange essential oils and Lauric Arginate on multiple strains of antibiotic resistant *Staphylococcus aureus*. She identified these as potential antimicrobial agents that may be used to reduce the growth rate of the bacterium in foods and/or in the human body.

Her interest in *Staphylococcus aureus* goes back to a staph infection sustained by her grandfather, Don Baker, after he underwent heart bypass surgery. He suffered through 10 months of limited walking when his knee became infected.

Baker, a native of Malvern, has been accepted at the College of Medicine at the University of Arkansas for Medical Sciences in the fall semester.

Center for Food Safety

Spring 2010 Vol. 1, No. 2

M.S. Students at Center Graduate, Ready for Next Tasks



Ellen Van Loo

Two graduate students at the Center for Food Safety – Ellen Van Loo and Sherry Melendez – received their master of science degrees this spring and look forward to the next phase of their careers.

Van Loo, a native of Belgium, earned bachelor's and master's degrees in bioengineering at the University of Ghent in

her home country. She first came to the UA in the fall of 2006 as an exchange student. She returned to Ghent to finish her master's degree in the spring of 2008. She then came back to the UA to pursue a master's degree in food science.

Van Loo's master's thesis is titled "Consumer Perception of Ready-to-Eat Deli Foods and Organic Meat." She said the thesis examined ready-to-eat deli meat's food safety issues related particularly to *Listeria*. The thesis also explored the effect of the frequency of organic meat purchases on consumer attitudes and consumers'



Sherry Melendez

willingness to pay a premium for it.

Van Loo's plans after commencement include a trip home to see her family in Belgium. But she won't be gone long from Fayetteville. She has been hired as a research associate at the Center for Food Safety lab.

Center for Food Safety

Spring 2010 Vol. 1, No. 2

Melendez, a native of Utica, N.Y., holds a bachelor's degree from Cornell University. Her M.S. thesis is titled "*Salmonella enterica* Isolates from Pasture-Raised Broilers Exhibit Antimicrobial Resistance and Class I Integrans."

She noted in the thesis that while considerable foodborne pathogen research has been conducted on conventionally produced broilers and turkeys, few studies have focused on free-range, organic and pastured poultry. Her current surveillance study was designed to isolate, identify and genetically characterize *Salmonella* from the pastured poultry farm and processing environments. "The findings of this study show that *Salmonella* serotypes isolated from pasture-raised poultry are similar to those from conventional poultry " she said.

After graduation, Melendez will join Mars Inc. in Hackerstown, N.J., as a food safety microbiology technician. She will be doing both raw material and finished product testing for the major pathogens (*E. coli*, *Salmonella* and *Listeria*).

Food Protection Workshop Slated for June at UA

Entrepreneurs, small-business operators and younger management employees of larger companies will have the opportunity to learn more about food defense at a workshop set for June 9-10 presented by the University of Arkansas Institute of Food Science and Engineering. The workshop will be held at the Center for Continuing Education in downtown Fayetteville. Topics to be covered include good management practices, quality systems, vulnerability assessments, reportable food registry, GPS traceback, agroterrorism, the Food Safety Modernization Act and the role of the FBI in food defense.

The conference registration fee is \$250 in advance and \$350 at the site. Registration will close when the course is filled. For additional details and to complete the online registration form, see the conference website at

http://www.uark.edu/ua/foodpro/Workshops/Food_Safety_Defense_Workshop.html

Workshops at Center for Food Safety

A) Microbiological Laboratory Logistics and Fundamentals - This workshop will be held on several dates: (May 18-20, June 15-17, July 20-22, Aug. 17-19, Sept. 14-16, Oct. 19-21, 2010).
http://www.uark.edu/ua/foodpro/Workshops/Micro_Lab.html

B) Molecular Biology and Biotechnology; Workshop for Beginners - This workshop will be held on several dates: May 26-27, June 23-24, July 28-29, Aug. 25-26, Sept. 22-23, Oct. 27-

Center for Food Safety

Spring 2010 Vol. 1, No. 2

28, 2010). [http://](http://www.uark.edu/ua/foodpro/Workshops/Molecular-lab.html)

www.uark.edu/ua/foodpro/Workshops/Molecular-lab.html

C) Food and Nutritional Labeling Workshop – This workshop will be held Aug. 4-5, 2010 in Fayetteville AR. http://www.uark.edu/ua/foodpro/Workshops/Food_Labeling_Workshop.html

D) Sensory Evaluation of Foods – This workshop will be held June 7-8, 2010.

http://www.uark.edu/ua/foodpro/Workshops/Sensory_Evaluation_Workshop.html

E) Food Protection Workshop - This workshop will be held June 9-10, 2010. It involves both Food Safety and Food Defense. http://www.uark.edu/ua/foodpro/Workshops/Food_Safety_Defense_Workshop.html

F) RCA Culinary Arts for Food Technologists Courses - Taught at the University of Arkansas: May 10-14, June 14-18 and Sept. 20-24, 2010. More information can be found at the following URL: <http://www.poultryscience.uark.edu/culinary/index.html>

G) GMP, SOPs and HACCP - Taught by Dr. John Marcy and Dr. Rich Linton of Purdue University. The 2010 class will be in Indiana in the Fall. The next Fayetteville class will be May 2011.

H) Advanced HACCP – In conjunction with Bob Galbraith of the HACCP Consulting Group. October 2010. Contact Dr. John Marcy for additional information.

CFS Presentations

Steven C. Ricke - "Issues and Future Direction for Food Safety Research in Food Animals" Dept. of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, April 19, 2010.

Steven C. Ricke - "Natural Microbial Methionine Sources for Organic Poultry Feed" National Organic Standards Board, Heidrick Ag History Center, 1962 Hays Lane, Woodland, CA, April 27, 2010.

Irene Hanning - "Gastrointestinal microbial profiles: the chicken as a model for population shifts impacting host health" Dept. of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, April 19, 2010.

Center for Food Safety

Spring 2010 Vol. 1, No. 2

CFS Publications

O'Bryan, C.A., D.M. Johnson, K. Shores-Ellis, P.G. Crandall, J. A. Marcy, S.C. Seideman, and S.C. Ricke. 2010. Designing an affordable usability test for E-Learning modules. *J. Food Sci. Educ.* 9: 6-10.

Patterson, J.A., V.I. Chalova, R.B. Hespell, and S.C. Ricke. 2010. Dilution rates influence ammonia assimilating enzyme activities and cell parameters of *Selenomonas ruminantium* strain D in continuous (glucose-limited) culture. *J. Appl. Microbiol.* 108: 357-365.

Churi, A., V.I. Chalova, I.B. Zabala-Díaz, C.L. Woodward, and S.C. Ricke. 2010. Increased temperature influences *hilA* gene fusion expression in a *Salmonella* Typhimurium poultry isolate. *Food Biotechnol.* 24: 51-61.

Burr, G., M. Hume, S. Ricke, D. Nisbet, and D. Gatlin III. 2010. In vitro and in vivo evaluation of the prebiotics GroBiotic® - A, inulin, mannanooligosaccharide, and galactooligosaccharide on the digestive microbiota and performance of hybrid striped bass (*Morone chrysops* x *M. saxatilis*), *Microbial Ecol.* 59: 187-198.

Zabala Diaz, I.B., V.I. Chalova, C. A. O'Bryan, P.G. Crandall, and S.C. Ricke. 2010. Effect of soluble maillard reaction products on *cadA* expression in *Salmonella* Typhimurium. *J. Environ. Health.* B45: 162-166.

Milillo, S.R. and S.C. Ricke. 2010. Synergistic reduction of *Salmonella* in a model raw chicken media using a combined thermal and organic acid salt intervention treatment. *J. Food Sci.* 75: M121-M125.

Chalova, V.I., P.G. Crandall, and S.C. Ricke. 2010. Microbial inhibitory and radical scavenging activities of cold-pressed terpeneless Valencia (*Citrus sinensis*) orange oil in different dispersing agents. *J. Sci Food Agric.* 90: 870-876.

Muthaiyan, A. and S.C. Ricke. 2010. Current perspectives on detection of microbial contamination in bioethanol fermentors. *Bioresource Technol.* 101: 5033-5042.

Chalova, V.I., C.A. Froelich, Jr., and S.C. Ricke. 2010. Potential for development of an *Escherichia coli* - based biosensor for assessing bioavailable methionine: A review. *Sensors* 10: 3562-3584.

Center for Food Safety

Spring 2010 Vol. 1, No. 2

CFS Book Chapters

- Kwon, Y.M., D. Bhattacharya and S.C. Ricke. 2010. Genomic approaches to bacterial pathogens using transposon mutagenesis: food - safety applications. pp. 165-176 In: Perspectives on Food Safety Issues of Food Animal Derived Foods, S.C. Ricke and F.T. Jones (eds.) University of Arkansas Press, Fayetteville, AR.
- Nannapaneni, R., O. A. Oryarzabal, S.C. Ricke, and M.G. Johnson. 2010. Fluoroquinolone-resistant *Campylobacter* in raw poultry products. pp. 247-258 In: Perspectives on Food Safety Issues of Food Animal Derived Foods, S.C. Ricke and F.T. Jones (eds.) University of Arkansas Press, Fayetteville, AR.
- Norberg, L.M., J.L. McReynolds, W.-K. Kim, V.I. Chalova, D.J. Nisbet, and S.C. Ricke. 2010. Colonization and pathogenesis of foodborne *Salmonella* in egg - laying hens. pp. 63-84 In: Perspectives on Food Safety Issues of Food Animal Derived Foods, S.C. Ricke and F.T. Jones (eds.) University of Arkansas Press, Fayetteville, AR.
- Lungu, B., S.C. Ricke, and M.G. Johnson. 2010. Occurrence of *Listeria monocytogenes* in raw and ready-to-eat foods and food-processing environments and intervention strategies for control. pp. 129-152 In: Perspectives on Food Safety Issues of Food Animal Derived Foods, S.C. Ricke and F.T. Jones (eds.) University of Arkansas Press, Fayetteville, AR.
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- Sirsat, S.A., A. Muthaiyan, S.E. Dowd, Y.M. Kwon, and S.C. Ricke. 2010. The potential for application of foodborne *Salmonella* gene expression profiling assays in postharvest poultry processing. pp. 195-222 In: Perspectives on Food Safety Issues of Food Animal Derived Foods, S.C. Ricke and F.T. Jones (eds.) University of Arkansas Press, Fayetteville, AR.
- Seideman, S.C. T.R. Callaway, P.G. Crandall, S.C. Ricke and D.J. Nisbet. 2010. Organic beef production: Production and food safety issues. pp. 307-321 In: Perspectives on Food Safety Issues of Food Animal Derived Foods, S.C. Ricke and F.T. Jones (eds.) University of Arkansas Press, Fayetteville, AR.

Center for Food Safety

Spring 2010 Vol. 1, No. 2

Ricke, S.C. 2010. Future prospects for advancing food - safety research in food animals. pp. 335-350 In: Perspectives on Food Safety Issues of Food Animal Derived Foods, S.C. Ricke and F.T. Jones (eds.) University of Arkansas Press, Fayetteville, AR.

Hanning, I., J. Lingbeck, and S.C. Ricke. 2010. Probiotics and heart health: reduction of risk factors associated with cardiovascular disease and complications due to foodborne disease. Pp. 423-439 In: Bioactive Foods in Promoting Health: Probiotics-Prebiotics, R.R. Watson and V.R. Preedy (eds) Elsevier, San Diego, CA.