

# Food Safety

**759 Do dried distillers grains with solubles affect the occurrence of *Salmonella enterica* colonization in pigs?** M. H. Rostagno\*<sup>1</sup>, B. T. Richert<sup>2</sup>, L. V. C. Girao<sup>2</sup>, G. M. Preis<sup>2</sup>, L. J. Lara<sup>2</sup>, A. F. Amaral<sup>2</sup>, A. D. B. Melo<sup>2</sup>, and A. Jones<sup>2</sup>, <sup>1</sup>USDA-ARS, West Lafayette, IN, <sup>2</sup>Purdue University, West Lafayette, IN.

As an alternative to counteract the increased feed costs, dried distillers grains with solubles (DDGS) have been increasingly included in pig diets. Much research has been conducted recently to evaluate growth performance and carcass characteristics associated with feeding DDGS to pigs. However, little is known about the effect of DDGS on the intestinal microbiota, and on the susceptibility to infection or colonization with pathogens. Therefore, 2 experiments were conducted to determine if inclusion of DDGS in the diet of grow-finish pigs affects their susceptibility to or the intestinal levels and shedding of *Salmonella*. In experiment 1, 36 pigs (12 pigs/treatment) were assigned to 3 treatments: Control diet with no DDGS, diet with 20% DDGS, or diet with 40% DDGS. After an adaptation period of 2 wk, each pig was inoculated with *Salmonella* Typhimurium ( $10^4$  cfu) and euthanized after 6 h to determine their susceptibility to the challenge. In experiment 2, 40 pigs (20 pigs/treatment) were assigned to 2 treatments: Control diet with no DDGS or diet with 30% DDGS. After 2 wk, each pig was inoculated with *Salmonella* Typhimurium ( $10^4$  cfu); individual fecal samples were collected during 5 weeks, and pigs were euthanized at 3 and 5 weeks post-challenge to determine intestinal colonization. In experiment 1, no differences among treatments were observed on the susceptibility to *Salmonella* infection. In experiment 2, most pigs shed *Salmonella* at one of the fecal samplings during the study period, with control pigs having a significantly higher cumulative shedding frequency ( $P < 0.05$ ) than pigs receiving the diet with 30% DDGS (80% versus 50%). The overall average shedding level was  $2.2 \log_{10}$  cfu/g of feces, with no difference between treatments ( $P > 0.10$ ). Also, no difference between treatments was found on the frequency or levels of *Salmonella* in intestinal samples collected at 3 or 5 weeks post-challenge. In conclusion, dietary inclusion of DDGS does not alter the susceptibility to or colonization with *Salmonella* of grow-finishing pigs.

**Key Words:** DDGS, *Salmonella*, pig

**760 Characterization of phage-resistant *Escherichia coli* O157:H7.** Y. Hong\*, J. Zhang, Y. Pan, and P. Ebner, Purdue University, West Lafayette, IN.

Phage therapy has great potential as an antimicrobial intervention in both the pre- and post-harvest stages of meat production. However, similar to other antimicrobial therapies, the application of phage therapy can result in rapid development of phage-resistance in targeted bacteria, which could lead to reduction in the efficacy of any phage-based products. Therefore, basic understanding of phage-resistance development in pathogens is critical to evaluate the effect of resistance on phage therapy application and assist in developing efficient and durable phage products. In this study, we isolated 2 phage-resistant strains (PR1, PR2) of *E. coli* O157:H7 after an 18 h co-incubation. Phage resistance was maintained throughout a 4-d subculture period (8 passes), indicating that resistance is stable in vitro in the absence of selection pressure from the phage. While other groups have reported a fitness cost associated with resistance development, no significant growth impairment was observed in resistant strains. The susceptible parent *E. coli* O157:H7 strain and 2 resistant strains had generation times of  $20.9 \pm 1.47$  min,  $19.8 \pm 2.04$  min

(PR1) and  $19.9 \pm 1.21$  min (PR2) and stationary concentrations of  $4.30 \times 10^8$  cfu/mL,  $4.49 \times 10^8$  cfu/mL (PR1), and  $4.52 \times 10^8$  cfu/mL (PR2). Phage resistance in PR1 was, at least in part, the result of adsorption prevention. After a 10-min incubation, 82.1% of inoculating phages failed to adhere to PR1 while only 2.8% of inoculating phages failed to adhere to the susceptible parent strain. In PR2, adsorption did not appear altered, however, there was no phage proliferation indicating a second mechanism for resistance. Phage resistance did not affect adhesion to Caco-2 cells as the parent and resistant strains had recovered adhesion percentages of 2.94%, 2.75% (PR1) and 2.75% (PR2), respectively. These results indicate that the acquisition of resistance comes at little cost to the bacterium under these experimental conditions. Preliminary experiments indicate, however, that phage may overcome resistance in as few as 5 h. Future studies are aimed at more clearly characterizing this phage capacity.

**Key Words:** phage resistance, *E. coli* O157:H7, phage therapy

**761 The effect of phage on the growth of *E. coli* O157:H7 and release of shiga toxins.** J. Zhang\*, K. Walton, Y. Pan, Y. Hong, S. Hayes, and P. Ebner, Purdue University, West Lafayette, IN.

*E. coli* O157:H7 is often associated with cattle and ground beef products and can produce and release powerful shiga toxins. As such, infections with *E. coli* O157:H7 can lead to renal failure and death, especially in the young, elderly or immunocompromised. It was recently shown that antibiotic treatment can lead to increased release of shiga toxins. Here we aimed to determine whether phage-based technologies could limit *E. coli* O157:H7 without the concomitant increase in shiga toxin production and release. We isolated *E. coli* O157:H7 phages from wastewater treatment samples. Based in in vitro growth kinetics and killing efficiencies, we chose 3 phages from this library to develop a treatment cocktail. The eclipse periods for these phages ranged from 14.6 to 23.7 min. The latent periods ranged from 23.9 to 31.5 min. The burst sizes ranged from 4.0 to 89.9 PFU. We then compared the phage cocktail to common antibiotics fosfomycin and ciprofloxacin in terms of bactericidal properties and associated shiga toxin production. Phages were added to log phase growth *E. coli* O157:H7 at an MOI = 0.1, 1 or 10. At 8 h, the concentration of bacteria in phage treated samples was reduced 98.5% (MOI = 0.1), 99.9% (MOI = 1.0) and 99.95% (MOI = 10). Similar treatment with fosfomycin or ciprofloxacin reduced bacterial concentrations by 99.95% and 99.7%, respectively. Phage concentration increased by approximately  $3 \log_{10}$  over the 8 h period regardless of MOI. At 8 h, treatment with fosfomycin and phage (MOI = 1 or 10, but not MOI = 0.1) resulted in higher concentrations of shiga toxin 1 compared with untreated controls. There were no differences among treatments in shiga toxin 2 productions. Thus, phage therapy was similar to fosfomycin and ciprofloxacin in terms of antibacterial activity. Future studies will focus on optimizing phage conditions to maximize bactericidal activity while minimizing the effect on shiga toxin production and release.

**Key Words:** *E. coli* O157:H7, phage, shiga toxin

**762 Arginine and glutamine alleviate the impairment induced by DON stress and enhance immunity in growing pigs.** W. Wang\*<sup>1,2</sup>, L. Wu<sup>2</sup>, T. Zhou<sup>3</sup>, L. Yang<sup>1</sup>, H. Zhang<sup>4</sup>, J. Yin<sup>2</sup>, T. Li<sup>2</sup>, K. Yao<sup>2</sup>, Q. Wang<sup>3</sup>, R. Huang<sup>2</sup>, and Y. Yin<sup>2</sup>, <sup>1</sup>College of Animal Science, South China Agricultural University, Guangzhou, China, <sup>2</sup>Research

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Deoxynivalenol (DON) is a mycotoxin, which reduces feed intake and animal performance, especially in swine. Arginine and glutamine play an important role in swine nutrition. The objective of present study was to investigate the effects of dietary arginine and glutamine supplementation on alleviating the impairment induced by DON stress to enhance immunity in growing pigs. A total of 30, 60-d-old healthy growing pigs (Landrace × Yorkshire) with a mean body weight ( $16.28 \pm 1.54$  Kg) were divided into 5 groups randomly. Before experiment, 3 amino acid groups fed 1.0% arginine (Arg), 1.0% glutamine (Gln) and 0.5% Arg + 0.5% Gln respectively for 21 d for immune-fortification. Control group and toxic group fed diet with 1.64% Ala for isonitrogenous control. After

immune-fortification, the toxic group and amino acid groups fed DON-contaminated diet with the final DON concentration 6mg/kg in diet for 30 d. Amino acid groups continually fed with amino acids supplementation as before. The control group fed with DON-free commercial diet at same time. No big difference between DON group and amino acid groups was observed for the average daily gain (ADG) of pig, the average daily feed intake (ADFI) of amino acid groups were significantly higher than that in toxic group ( $P < 0.01$ ). As to the relative weight of liver, spleen and kidney, there were no significant difference among these groups. For serum biochemistry characters, values of BUN, ALP, ALT and AST in amino acid groups were lower than that in toxic group. GLU and ALB were not different between control and amino acid groups. IGF1, GH and SOD in amino acid groups were significantly higher than that in toxic group ( $P < 0.01$ ). IL-2 and TNF $\alpha$  values in amino acid groups were similar with that in control group, which were significantly lower than that in toxic group ( $P < 0.01$ ). These results showed that dietary arginine and glutamine could alleviate the impairment induced by DON stress and enhance the immune system in growing pigs.

**Key Words:** deoxynivalenol, arginine, growing pig