To the Graduate Council:

I am submitting herewith a dissertation written by Sukanya Rattanatabtimtong entitled "Effects of Egg Yolk Antibodies on Weanling Pigs Challenged with Pathogenic *Salmonella* Typhimurium." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

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EFFECTS OF EGG YOLK ANTIBODIES ON WEANLING PIGS CHALLENGED WITH PATHOGENIC *SALMONELLA* TYPHIMURIUM

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ABSTRACT

An experiment was conducted to determine effects of anti-*Salmonella* egg yolk antibodies (ASEYA) on shedding and antibiotic resistance of *Salmonella enterica* Typhimurium and *E. coli,* growth performance and immunological parameters. Weaned pigs in two replicate trials (n = 132) were randomly assigned to six dietary treatments, including a control diet without additives, apramycin followed by carbadox, oxytetracycline (OXY), egg yolk powder containing ASEYA, egg yolk powder lacking ASEYA, or spray dried plasma protein (SDPP). Treatments were given to pigs on day 3 of the trial and all pigs were intranasally and orally challenged with *Salmonella* Typhimurium containing a nalidixic acid resistance marker on day 7. Fecal samples were collected on day 0, 7, 8, 12, 14, 21, 58, 88, and 118 for detection of *Salmonella* and *E. coli* to determine shedding and antibiotic resistance. Blood samples were collected and rectal temperatures (RT) were measured on day 0, 7, 8, 12, 14, 21, and 28. Blood was analyzed for white blood cell (WBC) counts and serum was analyzed for *Salmonella* antibody and interleukin-1β (IL-1β). The percentage of pigs shedding *Salmonella* was lower ($P<0.05$) for antibiotic treatments compared to other diets; however, resistance was greater ($P<0.05$) in *E. coli* from pigs fed antibiotics. Weight gains did not differ between treatments. Pigs fed OXY had lower RT compared to pigs fed SDPP and ASEYA ($P<0.05$); however, pigs in all groups had higher RT 24 h after challenge ($P<0.05$) and had decreasing RT by day 12. Concentrations of anti-*Salmonella* antibodies and WBC counts did not differ between treatment groups. *Salmonella* antibody concentrations increased in all

groups beginning on day 14 and continued to increase through day 28 ($P<0.05$). IL-1β was generally below detection limits. These studies indicate that in-feed ASEYA may not be effective in controlling shedding of *Salmonella* or improving the performance or health status of pigs challenged with *Salmonella* likely because ASEYA cannot reach invasive *Salmonella* that move through routes outside of the GI tract and/or because ASEYA lose activity as a result of animal digestive processes.

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PART I. INTRODUCTION

Salmonella infections were identified as the most common pathogenic diseases of humans in 2004 by the Foodborne Diseases Active Surveillance Network (FoodNet) (Centers for Disease Control and Prevention, 2005). In the United States, *Salmonella* is estimated to cause 1.4 million human illnesses and approximately of 590 deaths annually (Mead et al., 1999). In 2005, the Economic Research Service estimated that the annual economic cost of human salmonellosis, the illness caused by the *Salmonella* bacterium, is $2.4 billion which includes medical costs due to acute illness, the value of time lost from work due to chronic illness, and the cost of premature death (Economic Research Service, 2006). Infection caused by *Salmonella enterica* subspecies *enterica* serovar Typhimurium can be detected in both humans and animals. In human cases, most *Salmonella* infections occur via foodborne transmission and others through animal contact or environmental spread (Rice et al., 2003; Centers for Disease Control and Prevention, 2001; Besser et al., 2000). In animal cases, especially in swine, *Salmonella* pathogen causes serious enteric and systemic infections, frequently in young pigs (Ekperigin and Nagaraja, 1998). However, farm animals, which carry bacteria in their intestine, are most likely the source for human salmonellosis (Oosterom, 1991). Frequently, *Salmonella* bacteria in pigs are spread among animals prior to slaughter, particularly in the period of holding and transport (Hurd et al., 2002). In this case, *Salmonella* contaminated feces is the major transmission source of the pathogen (Beloeil et al., 2004). *Salmonella* contamination of holding pens, water (Rostagno et al., 2003), vehicle floors, and bedding material during transportation (Rajkowski et al., 1998) as well as animal stress during transportation (Berends et al., 1996) can be additional factors for *Salmonella* spread among pigs. The contaminated environment may also stimulate

Salmonella infection in market pigs and this possibly influences pork safety (Hurd et al., 2001).

Antimicrobial agents are used in animals for treatment of bacterial infection, prevention of disease, control of sick animals in large groups, and for growth promotion (Schwarz et al., 2001). Several studies have demonstrated that the use of antibiotics in food animals can lead to the development of drug resistance and associated risks to humans since resistant bacteria are transferred by the food chain (Van den Bogaard and Stobberingh, 2000) and are indirectly disseminated from animals to man (Mazel and Davies, 1999). Other ways in which humans obtain resistant bacteria are direct personal contact and environmental exposure. Some researchers have studied the risks of antibiotic use in food animals to human health (Phillips et al., 2004). There has been increasing concern by regulating agencies over similar or identical drugs used in humans and animals because of the potential for bacteria to become antibiotic resistant and affect human medicine alternatives, possibly resulting in increased sickness and death (Cox and Popken, 2006).

Currently, antibiotic alternatives are being studied for their roles in improving food safety. Several alternatives to antibiotics in animal feed for improved growth and/or to minimize intestinal infectious disease have been investigated, including spray dried plasma (Bosi et al., 2004), zinc oxide, fumaric acid (Owusu-Asiedu et al., 2003), probiotics (Zeyner and Boldt, 2006), and prebiotics (Tzortzis et al., 2005). A recent novel approach, chicken egg yolk antibodies, has been proposed for passive treatment of bacterial infections in young animals (Wiedemann et al., 1991). Neonatal and early-weaned piglets that experimentally received egg yolk antibodies were protected against

enterotoxigenic *E. coli* infection and had increased weight gains and a high survival rate (Marquardt et al., 1999). Chicken egg yolk antibodies represent an alternative to antibiotic therapies (Wiedemann et al., 1991). Verifying this strategy of egg yolk antibody supplementation, as applied toward control of *Salmonella* infection in pigs, was the emphasis of this investigation.

Research Objectives

This study was designed to determine the effects of in-feed egg yolk powder containing anti-*Salmonella* egg yolk antibodies (ASEYA), egg yolk powder lacking ASEYA, spray dried porcine plasma (SDPP), and antibiotics on colonization and shedding of *Salmonella enterica* Serovar Typhimurium and non-pathogenic *E. coli*, antibiotic resistance in *Salmonella* and naturally occurring *E. coli*, and growth performance and immunological parameters of pigs.

References

Beloeil, P. A., C. Chauvin, K. Proux, F. Madec, P. Fravalo, and A. Alioum. 2004. Impact of the *Salmonella* status of market-age pigs and the pre-slaughter process on *Salmonella* caecal contamination at slaughter. Veterinary Research. 35(5):513-530.

Berends, B. R., H. A. P. Urlings, and J. M. A. Snidjers. 1996. Identification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs. International Journal of Food Microbiology. 30(1-2):37-53.

Besser, T. E., M. Goldoft, L. C. Pritchett, R. Khakhria, D. D. Hancock, and D. H. Rice. 2000. Multiresistant *Salmonella* Typhimurium DT104 infections of humans and domestic animals in the Pacific Northwest of the United States. Epidemiology and Infection. 124(2):193–200.

Bosi, P., L. Casini, A. Finamore, C. Cremokolini, G. Merialdi, P. Trevisi, F. Nobili, and E. Mengheri. 2004. Spray-dried plasma improves growth performance and reduces inflammatory status of weaned pigs challenged with enterotoxigenic *Escherichia coli* K88. Journal of Animal Science. 82(6):1764-1772.

Centers for Disease Control and Prevention. 2001. Outbreaks of multidrug-resistant *Salmonella* Typhimurium associated with veterinary facilities-Idaho, Minnesota, and Washington, 1999. Morbidity Mortality Weekly Report. 50(33):701–704.

Center for Disease Control and Prevention. 2005. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-10 sites, the United States, 2004. Morbidity and Mortality Weekly Report. 54(14):352-356.

Cox, L. A., and D. A. Popken. 2006. Quantifying potential human health impacts of animal antibiotic use: Enrofloxacin and macrolides in chickens. Risk Analysis. 26(1):135-146.

Economic Research Service. 2006. Foodborne illness cost calculator: *Salmonella*. June 13, 2006. Available at http://www.ers.usda.gov/Data/FoodborneIllness/salm_Intro.asp

Ekperigin H. E., and K. V. Nagaraja. 1998. *Salmonella*. Page 17-29 in Microbial Foodborne Pathogens. L. Tollefson, ed. W. B. Saunders Company, Philadelphia, PA.

Hurd, H. S., J. K. Gailey, J. D. McKean, and M. H. Rostagno. 2001. Rapid infection in market-weight swine following exposure to a *Salmonella* Typhimurium-contaminated environment. American Journal of Veterinary Research. 62(8):1194-1197.

Hurd, H. S., J. D. McKean, R. W. Griffith, I. V. Wesley, and M. H. Rostagno. 2002. *Salmonella enterica* infections in market swine with and without transport and holding. Applied and Environmental Microbiology. 68(5):2376-2381.

Marquardt, R. R., L. Z. Jin, J. W. Kim, L. Fang, A. A. Frohlich, and S. K. Baidoo. 1999. Passive protective effect of egg-yolk antibodies against enterotoxigenic *Escherichia coli* K88+ infection in neonatal and early-weaned piglets. FEMS Immunology and Medical Microbiology. 23(4):283-288.

Mazel, D., and J. Davies. 1999. Antibiotic resistance in microbes. Cellular and Molecular Life Science. 56(9-10):742-754.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, and C. Shapiro. 1999. Food-related illness and death in the United States. Emerging Infectious Diseases. 5(5):607–625.

Oosterom, J. 1991. Epidemiological studies and proposed preventive measures in the fight against human salmonellosis. International Journal of Food Microbiology. 12(1):41–51.

Owusu-Asiedu, A., C. M. Nyachoti, and R. R. Marquardt. 2003. Response of early-weaned pigs to an enterotoxigenic *Escherichia coli* (K88) challenge when fed diets containing spray-dried porcine plasma or pea protein isolate plus egg yolk antibody, zinc oxide, fumaric acid, or antibiotic. Journal of Animal Science. 81(7):1790-1798.

Phillips, I., M. Casewell, T. Cox, B. De Groot, C. Friis, R. Jones, C. Nightingale, R. Preston, and J. Waddell. 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. Journal of Antimicrobial Chemotherapy. 53(1):28–52.

Rajkowski, K. T., S. Eblen, and C. Laubauch. 1998. Efficacy of washing and sanitizing trailers used for swine transport in reduction of *Salmonella* and *Escherichia coli*. Journal of Food Protection. 61(1):31-35.

Rice, D. H., D. D. Hancock, P. M. Roozen, M. H. Szymanski, B. C. Scheenstra, K. M. Cady, T. E. Besser, and P. A. Chudek. 2003. Household contamination with *Salmonella enterica*. Emerging Infectious Diseases. 9(1):120–122.

Rostagno, M. H., H. S. Hurd, J. D. McKean, C. J. Ziemer, J. K. Gailey, and R. C. Leite. 2003. Preslaughter holding environment in pork plants is highly contaminated with *Salmonella enterica*. Applied and Environmental Microbiology. 69(8):4489-4494.

Schwarz, S., C. Kehrenberg, and T. R. Walsh. 2001. Use of antimicrobial agents in veterinary medicine and food animal production. International Journal of Antimicrobial Agents. 17(6):431-437.

Tzortzis, G., A. K. Goulas, J. M. Gee, and G. R. Gibson. 2005. A novel galactooligosaccharide mixture increases the bifidobacterial population numbers in a continuous in vitro fermentation system and in the proximal colonic contents of pigs *in vivo*. The Journal of Nutrition. 135(7):1726-1731.

Van den Bogaard, A. E., and E. E. Stobberingh. 2000. Epidemiology of resistance to antibiotic. Links between animals and humans. International Journal of Antimicrobial Agents. 14(4):327-335.

Wiedemann, V., E. Linckh, R. Kuhlmann, P. Schmidt, and U. Losch. 1991. Chicken egg antibodies for prophylaxis and therapy of infectious intestinal diseases. V. In vivo studies on protective effects against *Escherichia coli* diarrhea in pigs. Journal of Veterinary Medicine. 38(4):283-291.

Zeyner, A., and E. Boldt. 2006. Effects of a probiotic *Enterococcus faecium* strain supplemented from birth to weaning on diarrhea patterns and performance of piglets. Journal of Animal Physiology and Animal Nutrition. 90(1-2):25-31.

PART II. LITERATURE REVIEW

Overview of *Salmonella*

Salmonella, recognized as a "zoonotic" pathogen, commonly colonize humans and most animals (Libby et al., 2004). The genus *Salmonella* was named to recognize D. E. Salmon, the American bacteriologist, who in 1880s, identified the bacteria, which caused hog cholera (D'Aoust, 1989). Eventually, *Salmonella enterica* serotype Choleraesuis was named for this infectious bacteria. *Salmonella* bacteria, members of the *Enterobacteriaceae* family, are facultatively anaerobic, non-spore-forming, non-lactose fermenting Gram-negative rods (Ekperigin and Nagaraja, 1998). Their optimal growth temperature is at 37 ºC (Libby et al., 2004). These bacteria have been classified on the basis of the Kauffmann-White scheme, which mostly depends on H (flagella), O (somatic) antigens, and Vi (a capsular polysaccharide) antigens (Libby et al., 2004). To date, almost 2,545 serotypes of *Salmonella* have been identified (Popoff et al., 2004) and *Salmonella* species are recognized as very important foodborne and waterborne organisms, which cause different degrees of disease severity (Bell and Kyriakides, 2002). *Salmonella* have been classified into two species, *Salmonella enterica* and *Salmonella bongori*. There are six subspecies of *Salmonella enterica*, described as *enterica, salamae, arizonae, diarizonae, houtenae,* and *indica.* *Salmonella enterica* subspecies *enterica* is likely to inhabit birds and mammals, and represents approximately 60% of *Salmonella* serotypes (Libby et al., 2004). Within a serotype, plasmid content, biotype and phage type are used to identify the variation of phenotypes and to create sub-types (Ekperigin and Nagaraja, 1998). Phage typing is based on "sensitivity of bacterial cells to the lytic activity of selected bacteriophages". This procedure has been recently applied

to other serotypes of *Salmonella*, including *Salmonella* Typhimurium with the phage type referring to "provisional phage type (PT) and definitive phage type (DT)" (Bell and Kyriakides, 2002). Among *Salmonella* serotypes isolated from human sources, *Salmonella enterica* serovar Typhimurium is frequently reported and normally causes acute gastroenteritis (Lax et al., 1995). *Salmonella* Typhimurium can infect a variety of hosts, whereas several other serotypes, for example, *Salmonella* Choleraesuis, *Salmonella* Typhi, *Salmonella* Dublin and *Salmonella* Pullorum, cause diseases in specific hosts, i.e., pig, human, cattle and poultry, respectively. This distinction has been called "serotype-host specificity", "host adaptation" or "host restriction" (Watson et al., 2000). *Salmonella* primarily occupy the intestinal tract of humans and animals; therefore, they are commonly disseminated into the environment, including soil and water, where they possibly survive for long periods (Bell and Kyriakides, 2002).

Salmonella in Swine

A study of foodborne disease observed that pork was responsible for *Salmonella* outbreaks (Delpech et al., 1998). At the slaughterhouse, pigs are recognized as a source of contamination and transmission which possibly occurs by close contact among animals or exposure to the contaminated environment (Williams and Newwell, 1970; Botteldoorn et al., 2003). Additionally, contaminated pigs may come from a group previously impacted by *Salmonella* outbreaks at the pre-harvest operation (Oliveira et al., 2005). In pigs, *Salmonella* infections are linked to "significant animal suffering and economic impact" and they may be a source of transmission to humans (Berends et al., 1997). *Salmonella* serotypes generally involved in systemic infections in swine salmonellosis are *Salmonella* Choleraesuis and *Salmonella* Typhimurium, but the enteric infection form is

more related to *Salmonella* Typhimurium (Ekperigin and Nagaraja, 1998). In addition, some serotypes may reside for long periods in hosts as contaminated sources and cause infection when stress is induced, including during shipping to the slaughterhouse (Libby et al., 2004). One study shows that *Salmonella* Typhimurium was the most predominant serotype isolated from pig samples (43%) and in environmental samples (33%) from slaughterhouses in the Netherlands (Swanenberg et al., 2001).

Salmonella Pathogenesis

Salmonellosis

With *Salmonella* infection in humans, people develop the symptoms of diarrhea, fever, and abdominal cramps for 12 to 72 h after infection, which lasts 4 to 7 days. Most people recover without treatment. However, in the cases of severe diarrhea, the person needs to be hospitalized. In these patients, the *Salmonella* infection may disperse from the intestines to the blood circulation, and then enter other body sites and, finally, death may occur unless the person is treated properly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to have a severe illness (Centers for Disease Control and Prevention, 2005).

In several countries, the incidence of salmonellosis is high in swine, particularly those in the growing phase (Reed et al., 1986). In animals, sub-clinical infection is usual and many animals may become carriers. Cattle and calves are likely to suffer severe diarrhea and high fever during the *Salmonella* acute infection and high mortality is also observed in calves (Bertoldo, 2006). Salmonellosis in swine exhibits the symptoms in

two forms, a systemic form and an enteric form. This is dependent on several factors, including the *Salmonella* strain, the dose, and the disease resistance of the infected animal (Hirsh, 2004). The incubation period is normally 1-2 days. In the enteric form, the clinical signs of "a watery, yellow, fetid diarrhea" with or without the appearance of blood may last 3 to 7 days and then may occur again one or more times. Other symptoms such as moderate fever and dehydration may be observed. The incidence of mortality is low and occurs only after several days of diarrhea. In the systemic form, additional symptoms can be detected, including "anorexia, fever of 40.5-41.6 °C, cough and dyspnea". Pigs shed *Salmonella* for several months after infection and can become a source of contamination. Swine salmonellosis is commonly found in weaned pigs raised in intensive livestock systems (Ekperigin and Nagaraja, 1998).

Salmonella Infection

The consequences of *Salmonella* infections, including enteric and systemic infections, carrier state, and high mortalities are serious problems, which widely occur in humans and animals (Libby et al., 2004). The severity of infection caused by *Salmonella* Typhimurium depends on several factors including condition of the host species (Morgan et al., 2004). A minimum dose of 10^3 CFU/ml for acute infection involving both intestinal and other tissues, particularly in swine, has been reported by Loynachan and Harris (2005). Animal feed contaminated with *Salmonella* is considered as a significant source of *Salmonella* infection (Berends et al., 1996). After *Salmonella* are ingested, the invasive *Salmonella* are either killed by protective systems, excreted from the body, or survive in the host (Ekperigin and Nagaraja, 1998). If *Salmonella* survive and initiate the

infectious process, salmonellosis occurs (Ekperigin and Nagaraja, 1998). Tsolis and coworkers (1999) indicated that *Salmonella* attach to intestinal epithelial cells before invading and causing systemic infection and diarrhea. However, *Salmonella* pathogens are able to release secretory products, including effector proteins to activate infection (Zhang et al., 2003). *Salmonella* predominantly attach to the portion of the ileum mucosa close to the ileocecal valve. They then move into the body (Salyers and Whitt, 2002) through the sites of penetration called "Peyer's patches, which contain specialized membranous epithelium cells called microfold cells or M cells, in the follicle-associated epithelium that overlay collections of lymphoid cells" (Libby et al., 2004; Jepson and Clark, 2001). In some species, including Rhesus monkeys and the guinea pig, *Salmonella* are likely to interact with villi of enterocytes in contrast to M cells (Salyers and Whitt, 2002). In the early stages of infection, Meyerholz and coworkers (2002) demonstrated that bacterial attachment to M cells was experimentally observed within 5 minutes after inoculation, and bacterial penetration of the apical membrane was detected in M cells, goblet cells, and enterocytes by 10 minutes. A number of adhesion mechanisms including type 1 fimbriae (*fim* genes), plasmid encoded fimbriae (*pef* genes), long polar-fimbriae (*lpf* genes) and thin aggregative fimbriae (*agf* genes) are used by *Salmonella* to facilitate infection (Salyers and Whitt, 2002; Libby et al., 2004). Furthermore, *Salmonella* also have several virulence factors, which play important roles during invasion and inflammation. These include: 1) Type III secretion genes clustered in regions called "*Salmonella* Pathogenicity Islands (SPIs)" (Groisman and Ochman, 1997), described as SPI-1 to SPI-5, which cause membrane ruffling (invasion), penetration of intestinal epithelium (Brown et al., 2005), inflammation and fluid

accumulation, 2) *Salmonella* virulence plasmids that encode genes required for the ability to cause systemic disease (Gulig and Doyle, 1993), 3) lipopolysaccharide (LPS), a part of the outer membrane and an infection factor, which triggers the immune system of host and can cause increased severity (Libby et al., 2004), and 4) flagella which stimulate intestinal invasiveness, polymorphonuclear leukocytes recruitment, and fluid secretion, particularly in infected calves (Schmitt et al., 2001). *Salmonella* can move into membrane-bound endocyte vacuoles via the cell membrane ruffling mechanism which is caused by the interaction with M cell (Francis et al., 1992; Finlay et al., 1991). In addition, internalized invasive *Salmonella* Typhimurium impair the M cells. The alteration of M cells results in a change of gap in the follicle-associated epithelium, which promotes organisms to move through the apical and basolateral surfaces of enterocytes near damaged M cells (Jones et al., 1994). *In vitro* studies simulating the calf model of enterocolitis have shown that during the complex series of host-pathogen interactions causing diarrhea, type III secreted effector proteins, including SipA, SopA, SopB, SopD, and SopE2, are released from *Salmonella* Typhimurium into host cells (Zhang et al., 2002), and are required for the movement of neutrophils, presumably by inducing the production of chemoattractant chemokines in bovine ileal tissue. The phenomenon of increased vascular permeability resulting in mucosal edema is a response to inflammation. The damaged enterocytes cause leakage of fluid from blood and a large infiltration of neutrophils into the intestinal lumen, which can be observed by the excretion of fluids (Zhang et al., 2003).

At the initiation of infection, *Salmonella* Typhimurium activate signal transduction pathways, and then are able to enter into the intestinal epithelium, penetrate

and reside within host cells (Khullar et al., 2003). These pathways are stimulated by *Salmonella* type III secretion proteins, which are released in the host cell and induce various responses (Finlay and Cossart, 1997) and cause cytoskeletal changes (Santos et al., 2003). Mitogen-activated protein kinases and phospholipase C associate with these pathways triggered during infectious process (Pace et al., 1993). Some research has demonstrated that *Salmonella* are able to access Henle-407 cells by mobilizing free calcium inside the cells, producing leukotriene D4 and activating phospholipase A_2 (Pace et al., 1993). Further information about the signals used by *Salmonella* Typhimurium to enter cultured cells comes from work that describes the activation of the epidermal growth factor receptor in response to *Salmonella* Typhimurium in Henle-407 cells (Galan et al., 1992). This is also associated with intermediates of phosphatidylinositol 3-kinase, phospholipase D, and protein kinase C (Procyk et al., 1999). Khullar and coworkers (2003) indicated that greater levels of calcium and inositol 1,4,5-triphosphate inside intestinal cells have been detected in *Salmonella* Typhimurium grown under anaerobic condition in comparison to those grown under aerobic condition. Lee and Falkow (1990) observed that *Salmonella* Typhimurium had more virulence and penetration in the distal ileum, the target site of infection with anaerobic condition (pO_2 of 5-40 mmHg). In the mechanism of signal activation, phosphatidylinositol 4,5-bisphosphate, membrane phospholipids, is hydrolyzed by phospholipase C gamma which results in the production of diacylglycerol and inositol 1,4,5-triphosphate. These components and calcium ions potentially activate protein kinase C (Su et al., 2006), thereby possibly inducing some mechanisms to alter the cell membrane, express genes, or act through metabolic reactions (Kikkawa et al., 1986).

Libby and coworkers (2004) stated that *Salmonella* passed through the thoracic duct and lymphatic systems, then replicated in macrophages residing in inner organs, i.e., lymph nodes, liver, and spleen. A study of *Salmonella* Typhimurium systemic infection in mice demonstrated that these pathogens were detected in visceral organs, including the mesenteric lymph nodes, spleen, liver, Peyer's patches, gall bladder, and cecum during several weeks of acute infectious disease (Monack et al., 2004). They remained in these organs, particularly in macrophages of mesenteric lymph nodes after several months of infection. This is indicated that they were able to disperse in the lymphatic circulation. Furthermore, they mostly appear in the ileocolic lymph nodes and in the ileum. This perhaps leads to *Salmonella* shedding in feces (Vieira-Pinto et al., 2005). In a related study, over 90% of pigs infected with *Salmonella* Typhimurium had the organisms in mesenteric lymph nodes, tonsils, cecum, and feces (Schneider, 2003). To survive systemically, *Salmonella* Typhimurium uses several SPI-1 encoded type III secretion system effectors/translocases (Lawley et al., 2006).

Host Immunity

Salmonella infection is described as intestinal epithelium penetration and survival of bacteria in systemic circulation (Bloom and Boedeker, 1996). In response to the bacterial infection, both innate immunity and adaptive immunity are required to protect the host. Innate immune systems associate with the intestinal epithelium, neutrophils, macrophages, dendritic cells, and natural killer cells, and the adaptive immune mechanisms involve with specific T lymphocytes and B lymphocytes (Mizuno, 2004). The mechanism for innate immune response to the microorganism is as follow. Briefly, the innate immune system recognizes conserved molecular pattern of microorganism,

called “Pathogenic-Associated Molecular Patterns” with the association of germ-line encoded receptors, referred to as “Pattern Recognition Receptors” (Medzhitov and Janeway, 1997). However, the adaptive immune response is activated with the basis of “clonal selection of lymphocytes” expressing receptors with specific recognition (Medzhitov and Janeway, 1998). During the early period of an infection, the roles of phagocytic cells are to control bacterial multiplication and to produce chemical messengers, i.e., cytokines and chemokines which induce and recruit additional cells (Wick, 2004) in order to control the invasive bacteria (Medzhitov and Janeway, 1997). In the initiation of innate immune response, Toll-like receptors (TLRs) are important for the interaction between the microbes and phagocytic cells (Li and Cherayil, 2003), including macrophages, neutrophils and dendritic cells (Wick, 2004). For example, TLR4 interacts with lipopolysaccharide and then activates cellular reactions (Tapping et al., 2000). Additionally, bacterial flagellins, necessary for microbial movement, are able to trigger the host immune system. Pro-inflammatory response and dendritic cells are activated by contact between flagellin and TLR5 (Salazar-Gonzalez and McSorley, 2005). In intestinal infection, distribution of pathogen can efficiently be protected by rapid neutrophil attraction; meanwhile delayed movement of neutrophils during systemic infection cannot inhibit circulating bacteria (Cheminay et al., 2004). To effectively control and eliminate bacteria, adaptive immunity is crucial (Mittrucker and Kaufmann, 2000) which includes cell-mediated immunity and antibody production (Bloom and Boedeker, 1996). During *Salmonella* infection, specific T cells are activated in primary and secondary response and B cells are also induced to produce antibody in response to *Salmonella* antigens (Mittrucker and Kaufmann, 2000). For instance, antibodies and

CD4+ T cells specifically target flagellins of *Salmonella* during infection (Salazar-Gonzalez and McSorley, 2005). In body circulation, specific antibodies, such as immunoglobulin G, produced against *Salmonella*-epitopes, are able to bind and promote migration of bacteria to phagocytic cells (Hirsh, 2004). At the level of local immune responses in intestine, the secretion of immunoglobulin A (IgA), a class of antibodies, can recognize and bind bacteria, thereby inhibiting their colonization or invasion into the intestinal mucosa (Neutra and Kraehenbuhl, 1993). Fever accounts for the common aspect of innate immunity in response to infectious pathogens (Ellis et al., 2005). During acute infectious response, fever is regulated in the brain and activated by pyrogens. Endogenous pyrogens, including interleukin-1β (IL-1β), are produced by host immune cells, i.e., macrophages when triggered with exogenous pyrogens, such as LPS from Gram-negative bacteria (Fabricio et al., 2005). IL-1β activates prostaglandin E_2 synthesis, which induces fever reaction (Sanchez-Alavez et al., 2006). IL-1β circulates to the anterior hypothalamus and activates preoptic neurons to secrete prostaglandin E, which alter the brain thermoregulator to set the higher body temperature (Tortora and Grabowski, 1993).

Salmonella Defensive Mechanisms in Hosts

To move into the small intestine, microorganisms have to survive exposure to the acidic condition in the stomach or the gastric acidic barrier, which varies depending on different feed types ingested (Libby et al., 2004). Under exposure of a low pH environment (pH 3), *Salmonella* have the ability to adapt with an acid resistant response; therefore they can survive in this environment, which includes both inorganic and organic

acids (Garcia-del Portillo et al., 1993; Libby et al., 2004). *Salmonella* exhibit acid-resistant systems between the log phase and stationary phase of growth (Audia et al., 2001; Lee et al., 1995). In the log phase, the system is controlled by the iron uptake regulator (*Fur*) providing a moderate degree of acid tolerance and in the stationary phase the alternate sigma factor (*RpoS*), including *PhoP* and *OmpR* defined as "acid-response regulators" are necessary for the more effective resistance system (Lee et al., 1995; Libby et al., 2004; Hirsh, 2004). Additionally, when the mechanisms and antimicrobial factors of host defense, including "bile salts, intestinal mucus, lysozyme, lactoferrin, organic acids and peristalsis in intestine and normal flora in large intestine" are damaged, *Salmonella* are able to colonize and cause infection (Libby et al., 2004). For further *Salmonella* defensive mechanisms, *Salmonella* have to resist host protective immune systems (Lawley et al., 2006). After adherence, *Salmonella* move into M cells by moving through intestinal barriers (Clark et al., 1994). After reaching the lamina propria of the intestinal cell wall, *Salmonella* confront resident tissue macrophages in M cells. *Salmonella* survive inside the phagocytic cells without being killed (Fields et al., 1986). The ability of *Salmonella* to cause death of the phagocytes by apoptosis and replicate within phagocytic cells is a potential way to initiate infection, to survive and to escape the host immune killing systems (Monack et al., 1996; Lindgren et al., 1996). Additionally, *Salmonella* need to resist both "the oxygen-independent and oxygen-dependent killing processes of phagocytes" in order to survive within intestinal tract and become systemic (Libby et al., 2004). Mostly, *Salmonella* resistance is associated with resistance to oxygen-dependent killing systems (Vazquez-Torres and Fang, 2001). In these killing mechanisms of phagocytes, i.e., neutrophils, eosinophils and mononuclear phagocytes,

the productions of oxygen-dependent metabolites produced by phagocytic cells play important roles in impairing microbial components (Demple and Halbrook, 1983). Phagocytic cells are associated with the mechanism of "respiratory burst" which produces superoxide anion ($\cdot O_2^-$) via the reaction of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and most of this O_2^- reacts with H^+, generating the productions of oxygen and hydrogen peroxide (H_2O_2) (Babior, 1984). The process of "respiratory burst" producing reactive oxygen and derivatives which include superoxide anion: O_2^-, hydrogen peroxide: H_2O_2, and nitrogen species, i.e., peroxynitrite: $ONOO^-$, is the predominant mechanism to kill bacteria (Schlosser-Silverman et al., 2000; Mastroeni et al., 2000; Vazquez-Torres et al., 2000a). Likewise, *Salmonella* are killed by phagocytes with toxic oxygen generated by NADPH oxidase process following phagocytosis (Vazquez-Torres and Fang, 2001). However, under aerobic condition, *Salmonella* have developed various strategies to efficiently overwhelm oxidative killing, which include producing enzymes, i.e., superoxide dismutases, hydroperoxidases, oxidoreductases, repairing systems, suppressing respiratory burst activity and complement binding, inhibiting NADPH oxidase and nitric oxide synthase movement towards the *Salmonella*-containing vacuole (Vazquez-Torres and Fang, 2001; Donne et al., 2005; Knodler and Steele-Mortimer, 2003; Vazquez-Torres et al., 2000b). Mechanisms including limited nutrients, increased production of degradative enzymes, production of antimicrobial peptides, reduced phagosomal pH, and induced defensins are involved in oxygen-independent killing (Finlay and Falkow, 1997; Groisman et al., 1992). To resist the killing systems, there are several *Salmonella* genes associated, such as SPI-2, SPI-3, SPI-4 and SPI-5, that encode for proteins required to survive and grow

inside macrophages, plasmid encoded *Spv*, necessary for the intracellular growth and serum resistance, systemic encoded *PhoQ/PhoP* genes, important for resistance to defensins, and *SlyA* encoded genes, needed for resistance to the products of oxygen dependent killing mechanisms (Hirsh, 2004).

Control and Treatment of Disease

In several countries, controlling strategies at the farm level to diminish *Salmonella* incidence in food animals have been emphasized since *Salmonella* can become a source of human salmonellosis (Fedorka-Cray et al., 1999; Dahl et al., 1997; Letellier et al., 2000). To efficiently control *Salmonella* on the farm, animals need increased pathogen resistance (Letellier et al., 2001). For the farm practices, Berends and coworkers (1996) state that pen hygiene is an important way to control *Salmonella*. Care in the nursery is also a principle management method to treat *Salmonella* intestinal infection (Hirsh, 2004). *Salmonella* bacteria are not commonly found in animal feed (Morrow, 2000). For feeding practices, providing liquid feed and on-farm mixing of feed are good strategies for *Salmonella* protection (Wegener and Baggesen, 1996). In addition, vaccine administration can decrease the severity of infections (Lumsden and Wilkie, 1992). This can significantly lower colonization of tissues and organs and *Salmonella* Typhimurium shedding (Roesler et al., 2004) or prevent clinical porcine salmonellosis. Providing therapeutic compounds including antimicrobial drugs, fluids, electrolytes, and non-steroidal anti-inflammatory drugs can also treat *Salmonella* infection (Ekperigen and Nagaraja, 1998). Since *Salmonella* grow inside the host cells

during infectious disease and survives in phagocytes, the selected drugs for treatment should be intracellularly active in order to increase efficiency of therapy (Hirsh, 2004).

Antibiotic Uses in Agriculture

For animal production in the United States, 18 million pounds of antibiotics are used annually (Roe and Pillai, 2003) and approximately 40% of those are used in animal feed (Prescott, 1997). In Australia, a report shows that over 50% of imported drugs were used in animal feed during 1992-1993 and 1996-1997 (Joint Expert Technical Advisory Committee on Antibiotic Resistance, 1999). In the early 1950s, antimicrobial agents were introduced in animals after drugs were applied for human treatments (McEwen and Fedorka-Cray, 2002). The aims of antibiotics application to animals were to treat unhealthy animals and control infection at therapeutic levels, to prevent diseases in animals (prophylactic use), and to enhance feed efficiency and animal production at growth promoting levels (Barton, 2000; McEwen and Fedorka-Cray, 2002). The phenomenon of growth promotion by antibiotics was first observed in the late 1940s, and was termed "animal protein factor" and later as vitamin B_{12} (Jones and Ricke, 2003). Further investigation revealed that enhancement of growth rate and feed efficiency was noted when animals were administered fungal waste from antibiotic production for the purpose nutrients and a direct growth promoting effect of residual antibiotic was discovered (Prescott, 2006; Jones and Ricke, 2003). Over a half-century, various antibiotics have been added to feed for improving growth and production. During this period, antibiotic uses for growth promotants have significantly increased in animal operations, mainly in industrial farms (Barton, 2000). Antimicrobial agents registered for

use at sub-therapeutic levels are different around the world (World Health Organization, 1997; Joint Expert Technical Advisory Committee on Antibiotic Resistance, 1999). The mode of action of antibiotics used to improve growth is not known with certainty (Commission on Antimicrobial Feed Additives, 1997). However, the effects of these agents are thought to act in several ways including on the beneficial bacteria in intestine, causing pathogen death, decreasing the amount of microbial toxin, diminishing nutrient utilization by microbes, synthesizing vitamins and other growth factors, increasing the nutrient absorption by lowering the enterocyte epithelium thickness, decreasing turnover of intestinal epithelium and reducing intestinal motility (Prescott and Baggot, 1993). Antibiotic uses in animal operation also improve production and increase income benefits (Cromwell, 2002), as well as decrease cost of animal products (Dupont and Steele, 1987).

Classification of Antibiotics

Antibiotics, small molecule compounds, are able to kill or impede microbial growth when applied to an animal orally, intravenously, subcutaneously or intramuscularly (Salyers and Whitt, 2002). Fungi and bacteria are recognized as sources of many antibiotics, in which chemical processes are applied to induce greater activities of antibiotics (Salyers and Whitt, 2002). Chemical properties and modes of action distinguish antimicrobial agents. The classifications of drugs are described by different principles, including the microbial target, chemical structure, and the susceptible microbial ranges (Guardabassi and Courvalin, 2006). In this review, details of only the 3 drug classes used in this trial will be presented.

Tetracyclines

The tetracyclines were discovered in the 1940s and applied in animal production in the early 1950s (Prescott, 2006). The action of the tetracycline family is to stop microbial protein synthesis by blocking aminoacyl-tRNA binding to the ribosome (Chopra and Roberts, 2001). Several reports demonstrate that tetracyclines can interact with different binding sites such as the 30S ribosomal subunit, the 70S ribosomal protein (Goldman et al., 1983), 16S rRNA conserved region (Rasmussen et al., 1991), and the cytoplasmic membrane (Oliva and Chopra, 1992). Tetracyclines are broad-spectrum drugs which act against various microorganisms, including Gram-positive, Gram-negative bacteria, mycoplasma, and rickettsiae (Roberts, 2003). In addition, in some countries, including the United States, tetracyclines are applied to animal feeds for enhancing growth. *Streptomyces* spp. produce several tetracycline derivatives such as chlortetracycline, oxytetracycline, tetracycline, demethylchortetracycline, etc. (Guardabassi and Courvalin, 2006). Tetracyclines are frequently provided to animals (Guardabassi and Courvalin, 2006; Danish Integrated Antimicrobial Resistance Monitoring and Research Program, 2004). Oxytetracycline, a member of tetracycline class, is normally administrated to swine by ingestion (Pijpers et al., 1991).

In 1953, *Shigella dysenteriae*, a bacterium that causes bacterial dysentery, was the first bacterium that demonstrated tetracycline resistance. Subsequently, the prevalence of tetracycline resistant bacteria has been increasingly detected in many types of bacteria (Roberts, 1996). Currently, tetracycline resistant microbes are widely distributed, thereby narrowing the effectiveness of drugs to treat infections caused by bacteria (Jones et al., 1992). Bacteria resistant to tetracyclines use the following mechanisms: 1) controlling

entry of tetracycline into the microbial target sites by reducing uptake and activating tetracycline efflux, 2) changing the ribosomal binding sites to block the attachment of this drug and 3) inducing enzyme synthesis to inactivate tetracyclines (Speer et al., 1992). Generally, a porin channel, which is encoded as *OmpF* genes and located on outer membrane of Gram-negative, is a main entry site of tetracycline into bacterial cell. By lowering expression of this gene, mutant bacteria express some resistance to tetracyclines (Franklin and Snow, 1998). The prevalence of tetracycline resistance and resistant mechanisms has been continuously studied in *E. coli* (Miles et al., 2006; Karami et al., 2006; Bryan et al., 2004), *Salmonella* spp. (Frech and Schwarz, 1999; Frech and Schwarz, 1998; Pasquali et al., 2005) and other types of bacteria (Dzierzanowska-Fangrat et al., 2006; Bertrand et al., 2005; Malhotra-Kumar et al., 2005). Humans, animals, food and the environments are significant sources of tetracycline resistant bacteria. Presumably, non-pathogens from these sources pool antibiotic resistance genes (Roberts, 1996).

Aminoglycosides

Aminoglycoside was first discovered in 1944; after which, the agent was chemically modified to produce more potent drug activity (Kotra et al., 2000). Gentamicin, amikacin, streptomycin, kanamycin, neomycin and apramycin are members of aminoglycoside class produced from *Streptomyces* and *Micromonospora* spp. The drugs in this group are extensively applied to treat diseases in poultry, mammals, and humans with the exception of apramycin, which is only used in animal treatments (Guardabassi and Courvalin, 2006). Apramycin has been widely used in animal practice since 1978. However, the detection of apramycin resistance has been reported in

Klebsiella pneumoniae and *E. coli* isolated from human sources (Johnson et al., 1995). This finding, in contrast to a recent report by Phillips and coworker (2004), indicates that the appearance of resistance gene transfer occurs between bacteria from humans and animals. The effective action of aminoglycosides is to tightly attach to 30S ribosome binding site and cluster 30S initiation complex (30S-mRNA-tRNA), thereby stopping protein synthesis. Additionally, the attachment of this drug activates mRNA misreading which result in lower protein synthesis (Guardabassi and Courvalin, 2006). The actions of these drugs are broad spectrum, efficient against Gram-negative and Gram-positive bacteria, and are less effective under anaerobic condition, i.e., inside the animal cell. Therefore, these drugs may have limited ability to kill intracellular bacteria (Franklin and Snow, 1998).

Bacterial resistance mechanisms to aminoglycosides include reducing access and aggregation of the drug in the bacterial cell and producing several enzymes to modify and inactivate the antibiotics (Shaw et al., 1993). These enzymes include phosphotransferases, acetyltransferases and adenyltransferases which change the chemical properties of aminoglycosides, resulting in failure to access and target the binding sites in bacteria (Smith and Baker, 2002). For an example, apramycin resistance is associated with the enzyme of 3-*N*-aminoglycoside acetyltransferase type IV [AAC(3)IV] and this enzyme also modifies tobramycin and gentamicin which are normally used for infectious therapy in humans (Brau et al., 1984). Aminoglycoside resistance such as apramycin resistance found in bacteria, including *E. coli, Salmonella* spp. has been investigated in animal and human sources (Yates et al., 2004; Mathew et al., 2003; Mathew et al., 1998; Chaslus-Dancla et al., 1991).

Carbadox

Quinoxaline-di-N-oxides are broad range antibiotics, in which two derivatives, carbadox and olaquindox, are commonly included in swine and bovine feeds (Beutin et al., 1981). Carbadox is also recognized as a growth enhancer to improve weight gain and increase feed utilization. However, the use of quinoxalines for promoting growth is uncertain since the basic activity of these drugs, mostly carbadox, is to prevent pig dysentery (Guardabassi and Courvalin, 2006). The action of carbadox is to inhibit deoxyribonucleic acid (DNA) synthesis, and then cause DNA damage in bacterial cell (Suter et al., 1978). Carbadox resistant isolates, particularly among *E. coli*, have been experimentally observed in pigs fed with this type of antibiotic as a feed additive (Spanoghe and Pohl, 1987; Mathew et al., 1998). In the late 1980s, carbadox in animal feeds was prohibited in Australia (Joint Expert Technical Advisory Committee on Antibiotic Resistance, 1999) and in the 1990s, the use of olaquindox and carbadox were also prohibited in the European Union (Burch, 2005) because of carcinogen concerns.

Antibiotic Resistance

The occurrence of antibiotic resistance reduces efficacy of antimicrobial agents applied to treat or prevent diseases (Centers for Disease Control and Prevention, 2006). In the 1960s, antibiotic resistance was detected in bacteria of the *Enterobacteriaceae* family (Prescott, 2006). Presumably, due to extended, incorrect and overuse of antibiotics in human and animal therapy, these may lead to an increased antibiotic resistance in bacteria, including foodborne pathogens (Bronzwaer et al., 2002; McEwen

and Fedorka-Cray, 2002; Shea et al., 2004). Similarly, the extensive uses of antibiotics at sub-therapeutic levels for long periods may potentially cause a crisis of antibiotic resistance in microbes, possible transferred from animals to humans (Kelly et al., 2004; Shea et al., 2004). Typically, sensitive bacteria may mutate to drug resistant strain, or obtain antibiotic resistant encoded genes from other bacteria (Tenover, 2006).

Genetic Acquisition of Antibiotic Resistance

Related resistance genes have been detected in different types of many bacteria, representing the transfer of resistant genes which is described as "horizontal transfer" (Kazimierczak et al., 2006; Winokur et al., 2001; Shoemaker et al., 2001). The obtaining of external genes, encoding resistance to a variety of antibiotic classes is expressed in several phenotypes of antibiotic resistance (McDermott et al., 2003). The exchange of resistant genes occurs in both non-pathogens and pathogens (Sorum and Sunde, 2001; Swartz, 2002). The acquisition of genes by "vertical transfer" is through DNA mutation and mutated genes are then transferred to following generations by sexual mechanisms (Tenover, 2006) while "horizontal transfer" involves the integration of antibiotic encoded genes to plasmid or transposons followed by transfer (Schwarz et al., 2006). Whatever the mechanism of resistant mutation, the sensitive bacteria are killed, but resistant bacteria live and grow (Tenover, 2006). Horizontal gene exchange involves the genetic elements, including transposons, plasmids, gene cassettes, integrons and chromosomal genomic islands which vary in structure, properties, size and spreading method (Hall, 1997; Herrero et al., 2006; Schwarz et al., 2006). Resistance to multiple antibiotics can be obtained via a single genetic occurrence by integrating the resistant genes in mobile elements (McDermott et al., 2003). Plasmids, or "the extra-chromosomal elements", are

basically found in bacteria and carry chemical agent resistance and antibiotic resistance genes (Schwarz et al., 2006). Plasmids normally represent as vectors for other genetic elements (Radstrom et al., 1994). Plasmids can transfer and integrate into plasmids and/or chromosomal DNA of the other bacterial cells and then the encoded genes may steadily replicate from new integrated plasmids and/or integrated chromosomal DNA (Schwarz et al., 2006). Transposons which generally combine specifically and non-specifically to vector and chromosomal DNA have been characterized by size and structure. The smallest transposons consist of transposase genes which associate with insertion and excision of the elements; however larger transposons frequently have one or more additional antibiotic resistance genes (Schwarz et al., 2006). Gene cassettes contain specific recombination sites, described as "59-base elements" (Hall et al., 1999), and genes mainly encoding antibiotic resistance (Fluit and Schmitz, 1999). These elements which express in both Gram-negative and Gram-positive bacteria are able to transfer by site-specific integration in an integron since they lack "replication systems" or "transposition systems" (Fluit and Schmitz, 1999; Schwarz et al., 2006). An integron consists of a "site-specific integration system" that is able to capture and mobilize genes that are set in gene cassettes (Hall and Collis, 1995) and these elements can obtain several genes and express the integrated genes by providing the promoter (Stokes and Hall, 1989). The genetic elements exist in several different forms since integrated genes vary in numbers and characteristics (Stokes and Hall, 1989). Consequently, integrons commonly demonstrate multidrug resistance genes because they carry several distinguishable gene cassettes (Leverstein-van Hall et al., 2003). The basic structure of an integron is composed of two conserved segments, defined as 5' and 3' conserved

regions (Daly and Fanning, 2004; Levesque et al., 1995). The 5' conserved region contains the integrase gene; *intIl*, which is associated with gene cassette integration at a specific site, the promoter; *Pc*, express the cassette genes (Schwarz et al., 2006; Collis and Hall, 1995), and the attachment site; *attI*, responsible for gene cassette integration (Daly and Fanning, 2004). The 3' conserved region has several open reading frames which include *qacEΔ1* gene, "a semi-functional derivative of the quaternary ammonium compounds resistance gene *qacE*", commonly related to sulfonamide resistance gene, *sul1* (Daly and Fanning, 2004). Reports reveal that integrons are widely detected in clinical isolates (Levesque et al., 1995). Several classes of integrons have been identified, with the predominant interest in classes 1, 2 and 3 for antimicrobial resistance spread (Ploy et al., 2000).

In general, the exchange of drug resistant genes between resistant and sensitive bacteria occurs by three processes; transduction, conjugation, or transformation (Tenover, 2006; Wolska, 2003). For each of these processes, transposons may also enable transfer and integration of resistance genes into the plasmids or chromosomal DNA (Tenover, 2006). Transduction process is facilitated by bacteriophage (Barbosa and Levy, 2000; Schwarz et al., 2006), in which transferred DNA is packed, representing the vector for transporting DNA into a recipient cell (Davison, 1999), where it can directly obtain new phage elements (Schwarz et al., 2006). Transduction process may have less efficiency to distribute resistance genes due to the limitation of DNA amounts packed into a vector and specific receptors for phage binding on new host cell surface (Schwarz et al., 2006). Conjugation, or "cell-contact-dependent DNA transfer mechanism" (Davison, 1999), is associated with direct cell contact for the exchange of plasmids or chromosomal DNA

(Barbosa and Levy, 2000). This method is recognized as important for DNA exchange and spreading drug resistance genes (Barbosa and Levy, 2000; Schwarz et al., 2006). Salyers and coworkers (1995) indicate that drug resistant genes contained in conjugative plasmids and transposons are detected in both Gram-positive and Gram-negative pathogens. Transformation is a process of transferring, obtaining, and integrating the naked DNA into recipient cells (Schwarz et al., 2006; Davison, 1999). Generally, the bacterial free DNA from cell lysis mechanism can be either degraded in the environment or obtained by some bacteria (Schwarz et al., 2006).

Antibiotic Resistance Mechanisms

Bacteria are resistant to antibiotics by various mechanisms (Tenover, 2006), which include changes in the bacterial cell wall/membrane to limit drug entry to target sites, activation of efflux mechanism to move drugs out of the cell, mutation of the target site, and synthesis of enzymes to modify or degrade the drugs (McDermott et al., 2003). Porins of Gram-negative bacteria that limit the movement of antibiotic across the outer membrane are an example of restriction entry of the antibiotic to its target. An additional mechanism that can prevent antibiotics from reaching their target is efflux pumps in the cytoplasmic membrane (Tenover, 2006). Modification processes are associated with mutations of genes encoding target proteins. For example, under the exposure of antibiotics that target ribosomal binding site and stop protein synthesis, a bacterium may alter an rRNA target site, thereby not allowing an antibiotic to bind (Schwarz et al., 2006). Hydrolyzation and adding chemical groups to antibiotics are examples of antimicrobial agent inactivation (Schwarz et al., 2006). To destroy drug activity, some enzymes can transfer acetyl, adenyl or phosphoric groups to specific targets of the

antibiotics, meanwhile other enzymes, including β-lactamase, hydrolases and esterases, are able to directly inactivate the drug molecules (Schwarz and Chaslus-Dancla, 2001).

Drug Resistant *Salmonella*

Antimicrobial resistance is present in *Salmonella* isolates from animals and humans (Padungtod and Kaneene, 2006). Gebreyes and Altier (2002) noted that high prevalence and diversity of multidrug resistance are observed in *Salmonella*. The extended use of antibiotics in animals results in the spread of multidrug resistant bacteria (Daly et al., 2000). The appearance of resistance genes on plasmids is recognized as a factor involved in the dissemination and stability of antibiotic resistance in *Salmonella* Typhimurium (Gebreyes and Altier, 2002). In the 1960s multidrug resistance was reported in *Salmonella* (Watanabe and Fukasawa, 1961; Watanabe and Lyang, 1962; Prescott, 2006). The resistance patterns of *Salmonella* related to public health concern are frequently associated with specific phage types. One notable multidrug resistant strain is *Salmonella enterica* subspecies *enterica* serovar Typhimurium definitive type 104 (DT104), basically known to be "pentaresistant", expressing resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline, indicated as the "AmCmStSuTe resistance phenotype" (Gebreyes and Altier, 2002). The first recognition of this *Salmonella* phage type was in the United Kingdom (Threlfall et al., 1996) and this strain has been reported in many parts of the world (Glynn et al., 1998; Lai-King et al., 1999; Baggesen et al., 2000; Wasyl et al., 2006) and from various host species including food animals and pets (Wright et al., 2005; Gebreyes et al., 2006; and Guardabassi et al., 2004) as well as from processed meat products (White et al., 2001). In the United States,

the prevalence of *Salmonella* Typhimurium DT104 increased approximately 33.4% within 16 years (1980-1996) (Glynn et al., 1998) and, recently, these multidrug resistant bacteria have become wide spread (Threlfall et al., 1997; Dechet et al., 2006). Furthermore, the additional multidrug resistance patterns of *Salmonella* Typhimurium have been investigated for phage types involving public health concern, i.e., DT193, DT21, etc. (Gebreyes et al., 2004).

Alternatives to Antibiotics for Animal Growth and Health

The use of antibiotics in animal feeds is increasingly opposed by consumers, thereby forcing livestock producers to treat and prevent *Salmonella* using alternative methods (Gurtler et al., 2004). Probiotics and prebiotics have been reported to contribute to protection or therapy for some diseases (Guarner and Malagelada, 2003). Probiotics, defined as "live microorganisms", beneficially affect health (Nomoto, 2005). The actions of probiotics are unclear, but their roles are possibly to change gut pH, to induce antimicrobial compounds in order to antagonize pathogens, to compete for pathogen adherence and available nutrients, to activate immune cells and to produce lactase enzyme (Parvez et al., 2006). Furthermore, Collington and coworkers (1990) observed that feeding probiotics as an alternative to antibiotics affected the development of digestive enzyme activities (including sucrase, alpha-D-glucohydrolase, and lactase: beta-D-galactoside galactohydrolase and tripeptidase) prior to weaning in pigs. Prebiotics, recognized as "non-digestible food fiber components", promote animal health with the activation of multiplication and roles of microflora in intestine (Nomoto, 2005). An example of a prebiotic is mannanoligosaccharides (MOS), which possibly provide an

alternative to some feed ingredients to increase growth rate in nursery pigs (Davis et al., 2002). MOS may enhance growth by binding to bacterial cell walls in order to prevent attachment to intestinal epithelium (Spring et al., 2000) or activate a direct antibody response to enhance the immune system (Newman and Newman, 2001; O'Quinn et al., 2001). Inclusion of MOS in feed before possibly increases feed utilization, thereby promoting intestinal function (Burkey et al., 2004).

Spray dried porcine plasma (SDPP), identified as a common protein source supplemented to early weaned pig diets in North America, promotes growth performance and feed consumption in weaned pigs (Pierce et al., 2005; Van Dijk et al., 2002a). The mode of action SDPP is uncertain, but it possibly increases appetite in small pigs (Ermer et al., 1994). Furthermore, specific immunoglobulin proteins in SDPP may positively increase growth performance (Owen et al., 1995). Providing SDPP to animals possibly offers a protection against enteric pathogens (Bosi et al., 2004) and has been shown to diminish enterotoxigenic *E. coli* K88 shedding in experimentally weaned pigs (Owusu-Asiedu et al., 2003). Van Dijk and coworkers (2001) indicated that immunoglobulin G and glycoprotein fractions of supplemented SDPP can prevent pathogen adherence which results in the reduction of intestinal infection in post weaning pigs. Further studies demonstrated that in-feed inclusion of SDPP in pigs can suppress basal immune responses compared to those of pigs fed a control diet without SDPP (Touchette et al., 2002), and maintain mucosal responses as well as enhance specific antibody defensive system (Bosi et al., 2004). However, some research indicates that dietary SDPP does not impact intestinal digestive enzyme functions including lactase, sucrase or maltase, small intestinal morphology or disaccharidase activity in early weaned piglets reared under low

infection environment (Van Dijk et al., 2002b). Similarly, in-feed SDPP did not increase survival of piglets challenged with pathogenic *E. coli* (Van Dijk et al., 2002a).

Egg Yolk Antibodies

Oral administration of egg yolk powder containing specific immunoglobulins as passive immunization is an alternative way to prevent and treat intestinal infection (Gurtler et al., 2004). After immunization of laying hens, specific immunoglobulins are produced and transported to the hen egg yolk (Larsson and Carlander, 2003). Immunoglobulin Y (IgY) antibodies mainly deposited in egg yolk, have a similar function to IgG in mammals (Sharma, 1997). Annually, 280 eggs can be collected in a chicken and 100-150 mg of IgY are obtained per yolk, resulting in approximately 40 g of IgY produced from a single chicken (Mine and Kovacs-Nolan, 2002). Gassmann and coworkers (1990) indicate that further advantages producing IgY by immunizing chickens are the very low amount of antigen used to immunize the chicken, duration of IgY production, simple, cheap, and fast method used to purify IgY, and no inflammatory reactions.

Structure and Properties

Eggs natural components have no adverse effect on IgY (Larsson and Carlander, 2003). The basic structure of IgY consists of 2 heavy chains and 2 light chains with a molecular mass of 180 kDa. Both the heavy and light chains contain variable region genes (V), joining region genes (J) and multiple diversity segments (D) that are rearranged by the mechanism of conventional V(D)J recombination. In order to generate

a large diversity of antibody production, poultry use an additional process of DNA recombination, or gene conversion (McCormack and Thompson, 1993). Each heavy chain typically carries one variable and four constant region domains (Warr et al., 1995) and no hinge region, whereas, IgG has three constant domains and a hinge region (Morrison et al., 2002). The surface of the second and third constant domains of IgY (C_H2/C_H3) in heavy chain is recognized by the receptors on developed chicken oocytes necessary for immunoglobulin transport into chicken yolk (Morrison et al., 2002). Noll and coworkers (1982) observed that principle structures of IgG are similar to those of IgY which are that immunoglobulin molecules present in Y-shape with an angle of about 90 degrees between fragment antigen binding (Fab) sites with approximately 10 nm for one Fab length. IgY do not trigger mammalian complement or activate mammalian Fc receptors which may stimulate inflammatory response in the gastrointestinal tract (Carlander et al., 2000). IgY is a more hydrophobic molecule than IgG (Davalos-Pantoja et al., 2000).

Roles of Egg Yolk Antibodies

Yolk antibodies have been investigated for the protection of microbial infections (Carlander et al., 2000). In bacterial disease, the roles of IgY are to neutralize toxins, to enable opsonization, and to modulate the lysis of bacteria via compliment system. For infection caused by viruses, IgY inhibit virus entry into healthy cells, and supports the killing systems of immune cells (Keller and Stiehm, 2000). *In vitro* study of passive immunization against *Salmonella* Enteritidis suggests that anti-fimbriae egg-yolk antibodies possibly diminish bacterial attachment and penetration during the early stages of infection in mice (Peralta et al., 1994). Similarly, anti-fimbriae antibodies from

chicken egg yolk experimentally prevented the binding of *E. coli* K88+ to mucosal receptors from the piglet intestine (Jin et al., 1998). Additionally, a study of adherence of enterotoxigenic *E. coli* on intestinal epithelial surfaces in piglets by using scanning electron microscopy demonstrate that *E. coli* colonized on the surface of intestinal epithelium in control group, but no binding of bacteria was observed in piglets treated with high-titer chicken egg yolk antibodies (Yogoyama et al., 1992). *In vitro* study of chicken egg yolk antibody against *Salmonella* Typhimurium and *Salmonella* Enteritidis demonstrate that the inhibition of *Salmonella* growth by anti-*Salmonella* yolk antibodies was observed in non-solid medium. Further study of antibody adherence activity observed by using immunofluorescence and immunoelectron microscopy revealed that anti-*Salmonella* antibodies were able to attach to the antigens present on the *Salmonella* surface, which led changes of bacterial surface structures (Lee et al., 2002).

Potential Uses to Treat Bacterial Infection in Small Intestine

Oral supplementation of IgY has been used to treat gastrointestinal infectious diseases, such as bovine and human rotaviruses, bovine coronavirus, *Yersinia ruckeri*, enterotoxigenic *Escherichia coli*, *Salmonella* spp., *Edwardsiella tarda*, *Staphylococcus*, and *Pseudomonas* (Mine and Kovacs-Nolan, 2002). After passive ingestion of yolk, the antibodies interact with digestive environments that include a sharp reduction of pH and proteolytic digestive enzymes. Such an environment may degrade or impact the activity of the antibodies. However, Schmidt and coworkers (1989) indicated that globulin fractions or isolated antibodies were less resistant to acidic condition than antibodies in protein rich solutions which included egg or yolk. In addition, in-feed inclusion of additional egg white significantly revealed a protective effect on antibodies from yolk

during the digestive process (Wiedemann et al., 1990). Ikemori and coworkers (1997) also suggest that protein and lipid components naturally found in the yolk, may encapsulate IgY molecules and protect them from degradation. Furthermore, IgY in yolk are by design inherently resistant to digestive processes and may sufficiently offer protection in the target sites of the small intestine. Chicken antibodies are resistant to heat and possibly applied to prevent or to treat intestinal diseases in young animals or humans (Gassmann et al., 1990). However, Chang and coworkers (1999) indicate that under the stability tests with temperature from 70 to 80 °C, activity of IgY was reduced, but with adding high levels of sucrose, maltose, glycerol, or 2% glycine, IgY were efficiently protected.

Related Studies

Chicken egg antibodies can be supplemented to young animals for treatment of intestinal infection diseases (Wiedemann et al., 1991; Marquardt, 2000). Currently, they represent a potential alternative to antibiotic therapies (Girard et al., 2006; Van Nguyen et al., 2006). Marquardt and coworkers (1999) obtained egg yolk antibodies from hens immunized with fimbrial antigens from a strain of enterotoxigenic *E. coli* (ETEC) K88+ and given to newborn (3 day old) and early-weaned (21 day old) piglets challenged with ETEC K88+. It was noted that the incidence of diarrhea was decreased in the 3-day-old piglets one day after providing specific egg yolk antibodies. For protection in early-weaned piglets, feeding egg yolk antibodies resulted in shorter durations of diarrhea, increased weight gains and survival found in all pigs. This study indicates that piglets administered specific egg yolk antibodies were protected against ETEC infections.

Further evidence of the beneficial effects of egg yolk antibodies is provided by Yogoyama and coworkers (1998a), who studied specific chicken egg yolk antibody efficiency to control salmonellosis in mice. Yolk antibodies provided to mice were produced against outer membrane proteins (OMP), lipopolysaccharide (LPS) or flagella (Fla) from *Salmonella* Typhimurium and *Salmonella* Enteritidis. Compared to control group without antibody treatments, higher survival rates were observed in mice provided the antibody treatments. The researchers suggested that mice fed egg yolk antibodies specific for *Salmonella* OMP, LPS, and Fla could possibly be protected from experimental salmonellosis. In another study, Yokoyama and coworker (1998b) provided chicken egg yolk antibodies specific against *Salmonella* Typhimurium or *Salmonella* Dublin to newborn Holstein calves. The results showed that oral supplementation of antibodies provided dose-dependent protection against infections in both *Salmonella* strains. All calves in control group were dead, but calves fed with low-titer antibodies had approximately 40% mortality and calves obtained the highest concentration of antibody showed clinical signs of fever and diarrhea without deaths within 7 to 10 days after inoculation. It was concluded from these studies that specific egg yolk antibodies against *Salmonella* Typhimurium or *Salmonella* Dublin exhibit the protection of mortality from salmonellosis in young calves fed high doses of antibodies.

References

Audia, J. P., C. C. Webb, and J. W. Foster. 2001. Breaking through the acid barrier: An orchestrated response to proton stress by enteric bacteria. International Journal of Medical Microbiology. 291(2):97-106.

Babior, B. M. 1984. The respiratory burst of phagocytes. The Journal of Clinical Investigation. 73(3):599-601.

Baggesen, D. L., D. Sandvang, and F. M. Aarestrup. 2000. Characterization of *Salmonella enterica* serovar Typhimurium DT104 isolated from Denmark and comparison with isolates from Europe and the United States. Journal of Clinical Microbiology. 38(4):1581-1586.

Barbosa, T. M., and S. B. Levy. 2000. The impact of antibiotic use on resistance development and persistence. Drug Resistance Updates. 3(5):303-311.

Barton, M. A. 2000. Antibiotic use in animal feed and its impact on human health. Nutrition Research Review. 13(2):279-299.

Bell, C., and A. Kyriakides. 2002. *Salmonella*: A practical approach to the organism and its control in foods. Blackwell Science Ltd., London. UK.

Berends, B. R., H. A. P. Urlings, and J. M. A. Snidjers. 1996. Identification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs. International Journal of Food Microbiology. 30(1-2):37-53.

Berends, B. R., F. Van Knapen, J. M. Snijers, and D. A. Mossel. 1997. Identification and quantification of risk factors regarding *Salmonella* spp. on pork carcass. International Journal of Food Microbiology. 36(2-3):199-206.

Bertoldo, J. 2006. Zoonotic diseases for man and beast: Salmonellosis. Available online: http://www.nwnyteam.org/AgFocus2006/Mar/Salmonellosis.htm

Bertrand, S., G. Huys, M. Yde, K. D'Haene, F. Tardy, M. Vrints, J. Swings, and J. M. Collard. 2005. Detection and characterization of tet(M) in tetracycline-resistant *Listeria* strains from human and food-processing origins in Belgium and France. Journal of Medical Microbiology. 54(12):1151-1156.

Beutin, L., E. Preller, and B. Kowalski. 1981. Mutagenicity of quindoxin, its metabolites, and two substituted quinoxaline-di-*N*-oxides. Antimicrobial Agents and Chemotherapy. 20(3):336-343.

Bloom, P. D. and E. C. Boedeker. 1996. Mucosal immune responses to intestinal bacterial pathogens. Seminars in Gastrointestinal Disease. 7(3):151-166.

Bosi, P., L. Casini, A. Finamore, C. Cremokolini, G. Merialdi, P. Trevisi, F. Nobili, and E. Mengheri. 2004. Spray-dried plasma improves growth performance and reduces inflammatory status of weaned pigs challenged with enterotoxigenic *Escherichia coli* K88. Journal of Animal Science. 82(6):1764-1772.

Botteldoorn, N., M. Heyndrickx, N. Rijpens, K. Grijspeerdt, and L. Herman. 2003. *Salmonella* on pig carcasses: positive pigs and cross contamination in the slaughterhouse. Journal of Applied Microbiology. 95(5):891-903.

Brau, B., U. Pilz, and W. Piepersberg. 1984. Genes for gentamicin-(3)-N-acetyltransferases III and IV: I. Nucleotide sequence of the AAC(3)-IV gene and possible involvement of an IS140 element in its expression. Molecular and General Genetic. 193(1):179-187.

Bronzwaer, S. L., O. Cars, U. Buchholz, S. Molstad, W. Goettsch, I. K. Veldhuijzen, J. L. Kool, M. J. Sprenger, and J. E. Degener. 2002. A European study on the relationship between antimicrobial use and antimicrobial resistance. Emerging Infectious Diseases. 8(3):278-282.

Brown, N. F., B. A. Vallance, B. K. Coombes, Y. Valdez, B. A. Coburn, and B. B. Finlay. 2005. *Salmonella* pathogenicity island-2 is expressed prior to penetrating the intestine. PLoS Pathogens. 1(3)e32:252-258.

Bryan, A., N. Shapir, and M. J. Sadowsky. 2004. Frequency and distribution of tetracycline resistance genes in genetically diverse, non-selected, and non- clinical *Escherichia coli* strains isolated from diverse human and animal sources. Applied and Environmental Microbiology. 70(4):2503-2507.

Burch, D. 2005. Anticipated effects of the withdrawal of antibiotic growth promoters (AGPs) from pigs in the European Union on 1st January, 2006. The American Association of Swine Veterinarian's Electronic Newsletter, December, 2005. Available online: http://www.octagon-services.co.uk/articles/withdrawalAGP.htm

Burkey, T. E., S. S. Dritz, J. C. Nietfeld, B. J. Johnson, and J. E. Minton. 2004. Effect of dietary mannanoligosaccharide and sodium chlorate on the growth performance, acute-phase response, and bacterial shedding of weaned pigs challenged with *Salmonella enterica* serotype. Journal of Animal Science. 82(2):397-404.

Carlander, D., H. Kollberg, P. E. Wejaker, and A. Larsson. 2000. Peroral immunotherapy with yolk antibodies for the prevention and treatment of enteric infections. Immunologic Research. 21(1):1-6.

Centers for Disease Control and Prevention. 2005. Salmonellosis. Available online: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_g.htm

Center for Disease Control and Prevention. 2006. About antibiotic resistance. Available online: http://www.cdc.gov/drugresistance/community/anitbiotic-resistance.htm

Chang, H. M., R. F. Ou-Yang, Y. T. Chen, and C. C. Chen. 1999. Productivity and some properties of immunoglobulin specific against *Streptococcus mutans* serotype c in chicken egg yolk (IgY). Journal of Agricultural and Food Microbiology. 47(1):61-66.

Chaslus-Dancla, E., P. Pohl, M. Meurisse, M. Marin, and J. P. Lafont. 1991. High genetic homology between plasmids of human and animal origins conferring resistance to the aminoglycosides gentamicin and apramycin. Antimicrobial Agents and Chemotherapy. 35(3):590-593.

Cheminay, C., D. Chakravortty, and M. Hensel. 2004. Role of neutrophils in murine salmonellosis. Infection and Immunity. 72(1):468-477.

Chopra, I., and M. Roberts. 2001. Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and Molecular Biology Reviews. 65(2):232-260.

Clark, M. A., M. A. Jepson, N. L. Simmons, and B. H. Hirst. 1994. Preferential interaction of *Salmonella* Typhimurium with mouse Peyer's patch M cells. Research in Microbiology. 145(7):543-552.

Collington G. K., D. S. Parker, and D. G. Armstrong. 1990. The Influence of inclusion of either an antibiotic or a probiotic in the diet on the development of digestive enzyme activity in the pig. The British Journal of Nutrition. 64(1):59-70.

Collis, C. M., and R. M. Hall. 1995. Expression of antibiotic resistance genes in the integrated cassettes of integrons. Antimicrobial Agents and Chemotherapy. 39(1):155-162.

Commission on Antimicrobial Feed Additives. 1997. Antimicrobial feed additives. Ministry of Agriculture, Stockholm.

Cromwell, G. L. 2002. Why and how antibiotics are used in swine production. Animal Biotechnology. 13(1):7-27.

D' Aoust, J. Y. 1989. *Salmonella*. Page 327-445 in Foodborne Bacterial Pathogens. M. P. Doyle, ed. Marcel Dekker Inc., New York, USA.

Dahl, J., A. Wingstrand, B. Nielsen, and D. L. Baggesen. 1997. Elimination of *Salmonella* Typhimurium infection by the strategic movement of pigs. The Veterinary Record. 140(26):679-681.

Daly, M., J. Buckley, E. Power, C. O'Hare, M. Cormican, B. Cryan, P. G. Wall, and S. Fanning. 2000. Molecular characterization of Irish *Salmonella enterica* Serotype Typhimurium: Detection of class I integrons and assessment of genetic relationships by DNA amplification fingerprinting. Applied and Environmental Microbiology. 66(2):614-619.

Daly, M., and S. Fanning. 2004. Integron analysis and genetic mapping of antimicrobial resistance genes in *Salmonella enterica* serotype Typhimurium. Methods in Molecular Biology. 268:15-32.

Danish Integrated Antimicrobial Resistance Monitoring and Research Program. 2004. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. Danish Veterinary Institute, Copenhagen, Denmark. p. 1-95.

Davalos-Pantoja, L., J. L. Ortega-Vinuesa, D. Bastos-Gonzalez, and R. Hidalgo-Alvarez. 2000. A comparative study between the adsorption of IgY and IgG on latex particles. Journal of Biomaterials Science, Polymer Edition. 11(6):657-673.

Davis, M. E., C. V. Maxwell, D. C. Brown, B. Z. de Rodas, Z. B. Johnson, E. B. Kegley, D. H. Hellwig, and R. A. Dvorak. 2002. Effect of dietary mannan oligosaccharide and (or) pharmacological additions of supplemental copper on growth performance and immunocompetence of weanling and growing/finishing pigs. Journal of Animal Science. 80(11):2887–2894.

Davison, J. 1999. Genetic exchange between bacteria in the environment. Plasmid. 42(2):73-91.

Dechet, A. M., E. Scallan, K. Gensheimer, R. Hoekstra, J. Gunderman-King, J. Lockett, D. Wrigley, W. Chege, J. Sobel, and the Multistate Working Group. 2006. Outbreak of multidrug-resistant *Salmonella enterica* Serotype Typhimurium Definitive Type 104 infection linked to commercial ground beef, Northeastern United States, 2003–2004. Clinical Infectious Diseases. 42(6):747-752

Delpech, V., J. McAnulty, and K. Morgan. 1998. A salmonellosis outbreak linked to internally contaminated pork meat. Australian and New Zealand Journal of Public Health. 22(2):243-246.

Demple, B., and J. Halbrook. 1983. Inducible repair of oxidative DNA damage in *Escherichia coli*. Nature. 304(5925):466-468.

Donne, E., F. Pasmans, F. Boyen, F. Van Immerseel, C. Adriaensen, J. P. Hernalsteens R. Ducatelle, and F. Haesebrouck. 2005. Survival of *Salmonella* serovar Typhimurium inside porcine monocytes is associated with complement binding and suppression of the production of reactive oxygen species. Veterinary Microbiology. 107(3-4):205-214.

Dupont, H. L., and J. H. Steele. 1987. The human health implications of the use of antimicrobial agents in animal feeds. Veterinary Quarterly. 9(4):309-320.

Dzierzanowska-Fangrat, K., K. Semczuk, P. Gorska, S. Giedrys-Kalemba, M. Kochman, A. Samet, S. Tyski, D. Dzierzanowska, and K. Trzcinski. 2006. Evidence for tetracycline resistance determinant tet(M) allele replacement in a *Streptococcus pneumoniae* population of limited geographical origin. International Journal of Antimicrobial Agents. 27(2):159-164.

Ekperigin H. E., and K. V. Nagaraja. 1998. *Salmonella*. Page 17-29 in Microbial Foodborne Pathogens. L. Tollefson, ed. W. B. Saunders Company, Philadelphia, PA.

Ellis, S., A. Mouihate, and Q. J. Pittman. 2005. Early life immune challenge alters innate immune responses to lipopolysaccharide: Implications for host defense as adults. The Federal of American Societies for Experimental Biology Journal. 19(11): 1519-1521.

Ermer, P. M., P. S. Miller, and A. J. Lewis. 1994. Diet preference and meal patterns of weanling pigs offered diets containing either spray-dried porcine plasma or dried skim milk. Journal of Animal Science. 72(6):1548–1554.

Fabricio, A. S., G. A. Rae, P. D'Orlean-Juste, and G. E. Souza. 2005. Endothelin-1 as a central mediator of LPS-induced fever in rats. Brain Research. 1066 (1-2):92-100.

Fedorka-Cray, P. J., J. S. Bailey, N.J. Stern, N. A. Cox, S. R. Ladely, and M. Musgrove. 1999. Mucosal competitive exclusion to reduce *Salmonella* in swine. Journal of Food Protection. 62(12):1376-1380.

Fields, P. I., R. V. Swanson, C. G. Haidaris, and F. Heffron. 1986. Mutants of *Salmonella* Typhimurium that cannot survive within the macrophage are avirulent. Proceedings of the National Academy of Sciences of the United States of America. 83(14):5189-5193.

Finlay, B. B., S. Ruschkowski, and S. Dedhar. 1991. Cytoskeletal rearrangements accompanying *Salmonella* entry into epithelial cells. Journal of Cell Science. 99(2):283-296.

Finlay, B. B., and P. Cossart. 1997. Exploitation of mammalian host cell functions by bacterial pathogens. Science. 276(5313):718-725.

Finlay, B. B., and S. Falkow. 1997. Common themes in microbial pathogenicity revisited. Microbiology and Molecular Biology Reviews. 61(2):136-169.

Fluit, A. C., and F. J. Schmitz. 1999. Class 1 integrons, gene cassettes, mobility, and epidemiology. European Journal of Clinical Microbiology and Infectious Diseases. 18(11):761-770.

Francis, C. L., M. N. Starnbach, and S. Falkow. 1992. Morphological and cytoskeletal changes in epithelial cells occur immediately upon interaction with *Salmonella* Typhimurium grown under low-oxygen conditions. Molecular Immunology. 6(21):3077-3087.

Franklin, T. J., and G. A. Snow. 1998. Biochemistry and Molecular Biology of Antimicrobial Drug Action. 5th ed. Kluwer Academic Publishers. Norwell, MA. p. 1-166.

Frech, G., and S. Schwarz. 1998. Tetracycline resistance in *Salmonella enterica* subsp. *enterica* serovar Dublin. Antimicrobial Agents and Chemotherapy. 42(5):1288-1289.

Frech, G., and S. Schwarz. 1999. Plasmid-encoded tetracycline resistance in *Salmonella enterica* subsp. *enterica* serovars Choleraesuis and Typhimurium: Identification of complete and truncated Tn1721 elements. FEMS Microbiology Letters. 176(1):97-103.

Galan, J. E., J. Pace, and M. J. Hayman. 1992. Involvement of the epidermal growth factor receptor in the invasion of cultured mammalian cells by *Salmonella* Typhimurium. Nature. 357(6379):588-589.

Garcia-del Portillo, F., J. W. Foster, and B. B. Finlay. 1993. Role of acid tolerance response genes in *Salmonella* Typhimurium virulence. Infection and Immunity. 61(10):4489–4492.

Gassmann, M., T. Weiser, P. Thommes, and U. Hubscher. 1990. The chicken egg as a supply of polyclonal antibodies. Schweizer Archiv fur Tierheilkunde. 132(6):289-294.

Gebreyes, W. A., and C. Altier. 2002. Molecular characterization of multidrug-resistant *Salmonella enterica* subsp. *enterica* serovar Typhimurium isolates from swine. Journal of Clinical Microbiology. 40(8): 2813-2822.

Gebreyes, W. A., S. Thakur, P. R. Davies, J. A. Funk, and C. Altier. 2004. Trends in antimicrobial resistance, phage types and integrons among *Salmonella*

serotypes from pigs, 1997–2000. Journal of Antimicrobial Chemotherapy. 53(6): 997-1003.

Gebreyes, W. A., C. Altier, and S. Thakur. 2006. Molecular epidemiology and diversity of *Salmonella* serovar Typhimurium in pigs using phenotypic and genotypic approaches. Epidemiology and Infection. 134(1):187-198.

Girard, F., I. Batisson, G. Martinez, C. Breton, J. Harel, and J. M. Fairbrother. 2006. Use of virulence factor-specific egg yolk-derived immunoglobulins as a promising alternative to antibiotics for prevention of attaching and effacing *Escherichia coli* infections. FEMS Immunology and Medical Microbiology. 46(3):340-350.

Glynn, M. K., C. Bopp, W. Dewitt, P. Dabney, M. Mokhtar, and F. J. Angulo. 1998. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. The New England Journal of Medicine. 338(19):1333-1338.

Groisman, E. A., C. Parralopez, M. Salcedo, C. J. Lipps, and F. Heffron. 1992. Resistance to host antimicrobial peptides is necessary for *Salmonella* virulence. Proceedings of the National Academy of Sciences of the United States of America. 89(24):11939–11943.

Groisman, E. A. and H. Ochman. 1997. How *Salmonella* became a pathogen. Trends in Microbiology. 5(9):343-349.

Goldman, R. F., T. Hasan, C. C. Hall, W. A. Strycharz, and B. S. Cooperman. 1983. Photoincorporation of tetracycline into *Escherichia coli* ribosomes. Identification of the major proteins photolabeled by native tetracycline and tetracycline photoproducts and implications for the inhibitory action of tetracycline on protein synthesis. Biochemistry. 22(2):359-368.

Guardabassi, L., S. Schwarz, and D. H. Lloyd. 2004. Pet animals as reservoirs of antimicrobial-resistant bacteria. Journal of Antimicrobial Chemotherapy. 54(2):321-332.

Guardabassi, L., and P. Courvalin. 2006. Modes of antimicrobial action and mechanisms of bacterial resistance. Page 1-18 in Antimicrobial Resistance in Bacteria of Animal Origin. F. M. Aarestrup, ed. ASM press. Washington D.C.

Guarner, F., and J. R. Malagelada. 2003. Gut flora in health and disease. Lancet 361(9356):512-519.

Gulig, P. A., and T. J. Doyle. 1993. The *Salmonella* Typhimurium virulence plasmid increases the growth rate of *Salmonella*e in mice. Infection and Immunity. 61(2):504-511.

Gurtler M., U. Methner, H. Kobilke, and K. Fehlhaber. 2004. Effect of orally administered egg yolk antibodies on *Salmonella* Enteritidis contamination of hen's eggs. Journal of Veterinary Medicine. Series B, Infectious Diseases and Veterinary Public Health. 51(3): 129-134.

Hall, R. M., and C. M. Collis. 1995. Mobile gene cassettes and integrons: Capture and spread of genes by site-specific recombination. Molecular Microbiology. 15(4):593-600.

Hall, R. M. 1997. Mobile gene cassettes and integrons: Moving antibiotic resistance genes in gram-negative bacteria. Ciba Foundation Symposium. 207:192-202.

Hall, R. M., C. M. Collis, M. J. Kim, S. R. Partridge, G. D. Recchia, and H. W. Stokes. 1999. Mobile gene cassettes and integrons in evolution. Annals of the New York Academy of Sciences. 870:68-80.

Herrero, A., M. R. Rodicio, M. A. Gonzalez-Hevia, and M. C. Mendoza. 2006. Molecular epidemiology of emergent multidrug-resistant *Salmonella enterica* serotype Typhimurium strains carrying the virulence resistance plasmid pUO-StVR2. The Journal of Antimicrobial Chemotherapy. 57(1):39-45.

Hirsh, D. C. 2004. *Enterobacteriaceae*: *Salmonella*. Page 69-74 in 2nd Veterinary Microbiology. D. C. Hirsh, N. J. MacLachlan, and R. L. Walker, ed. Blackwell Publishing, Oxford, UK.

Ikemori, Y., M. Ohta, K. Umeda, F. C. Icatlo Jr., M. Kuroki, H. Yokayama, and Y. Kodama. 1997. Passive protection of neonatal calves against bovine coronavirus-induced diarrhea by administration of egg yolk or colostrum antibody powder. Veterinary Microbiology. 58(2-4):105-111.

Jepson, M. A., and M. A. Clark. 2001. The role of M cells in *Salmonella* infection. Microbes and Infection. 3(14-15):1183-1190.

Jin, L. Z., S. K. Baidoo, R. R. Marquardt, and A. A. Frohlich. 1998. *In vitro* inhibition of adhesion of enterotoxigenic *Escherichia coli* K88 to piglet intestinal mucus by egg-yolk antibodies. FEMS Immunology and Medical Microbiology. 21(4):313-321.

Johnson, A. P., M. Malde, N. Woodford, R. J. Cunney, and E. G. Smyth. 1995. Urinary isolates of apramycin-resistant *Escherichia coli* and *Klebsiella pneumoniae* from Dublin. Epidemiology and Infection. 114(1):105-112.

Jones, B. D., N. Ghori, and S. Falkow. 1994. *Salmonella* Typhimurium initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. The Journal of Experimental Medicine. 180(1):15-23.

Jones, C. S., D. J. Osborne, and J. Stanley. 1992. Enterobacterial tetracycline resistance in relation to plasmid incompatibility. Molecular and Cellular Probes. 6(4):313-317.

Jones, F. T., and S. C. Ricke. 2003. Observations on the history of the development of antimicrobials and their use in poultry feed. Poultry Science. 82(4):613-617.

Joint Expert Technical Advisory Committee on Antibiotic Resistance. 1999. The Use of Antibiotics in Food-producing animals: Antibiotic-resistant Bacteria in Animal and Humans. Commonwealth of Australia, Canberra, Australia.

Karami, N., F. Nowrouzian, I. Adlerberth, and A. E. Wold. 2006. Tetracycline resistance in *Escherichia coli* and persistence in the infantile colonic microbiota. Antimicrobial Agents and Chemotherapy. 50(1):156-161.

Kazimierczak, K. A., H. J. Flint, and K. P. Scott. 2006. Comparative analysis of sequences flanking tet(W) resistance genes in multiple species of gut bacteria. Antimicrobial Agents and Chemotherapy. 50(8):2632-2639.

Keller, M. A., and E. R. Stiehm. 2000. Passive immunity in prevention and treatment of infectious disease. Clinical Microbiology Reviews. 13(4):602-614.

Kelly L., D. L. Smith, E. L. Snary, J. A. Johnson, A. D. Harris, M. Wooldridge, and J. G. Jr. Morris. 2004. Animal growth promoters: To ban or not to ban? A risk assessment approach. International Journal of Antimicrobial Agents. 24(3): 205-212.

Khullar, M., R. D. Singh, M. Smriti, and N. K. Ganguly. 2003. Anaerobiosis-induced virulence of *Salmonella enterica* subsp. *enterica* serovar Typhimurium: Role of phospholipase C gamma signaling cascade. Journal of Medical Microbiology. 52(9):741-745.

Kikkawa, U., T. Kitano, N. Saito, A. Kishimoto, K. Taniyama, C. Tanaka, and Y. Nishizuka. 1986. Role of protein kinase C in calcium-mediated signal transduction. Ciba Foundation Symposium. 122:197-211.

Knodler, L. A., and O. Steele-Mortimer. 2003. Taking possession: Biogenesis of the *Salmonella*-containing vacuole. Traffic. 4(9):587–599.

Kotra, L. P., J. Haddad, and S. Mobashery. 2000. Aminoglycosides: Perspectives on mechanisms of action and resistance and strategies to counter resistance. Antimicrobial Agents and Chemotherapy. 44(12):3249-3256.

Lai-King, N. G., M. R. Mulvey, I. Martin, G. A. Peters, and W. Johnson**.** 1999. Genetic characterization of antimicrobial resistance in Canadian isolates of

Salmonella serovar Typhimurium DT104. Antimicrobial Agents and Chemotherapy. 43(12)**:**3018-3021.

Larsson, A., and D. Carlander. 2003. Oral immunotherapy with yolk antibodies to prevent infections in humans and animals. Upsala Journal of Medical Sciences. 108(2):129-140.

Lawley, T. D., K. Chan, L. J. Thompson, C. C. Kim, G. R. Govoni, and D. M. Monack. 2006. Genome-wide screen for *Salmonella* genes required for long-term systemic infection of the mouse. PLoS Pathogens. 2(2):87-100 (e11).

Lax, A. J., P. A. Barrow, P. W. Jones, and T. S. Wallis. 1995. Current perspectives in salmonellosis. British Veterinary Journal. 151(4):351-377.

Lee, C. A., and S. Falkow. 1990. The ability of *Salmonella* to enter mammalian cells is affected by bacterial growth state. Proceedings of the National Academy of Sciences of the United States of America. 87(11):4304–4308.

Lee, E. N., H. H. Sunwoo, K. Menninen, and J. S. Sim. 2002. In vitro studies of chicken egg yolk antibody (IgY) against *Salmonella* Enteritidis and *Salmonella* Typhimurium. Poultry Science. 81(5):632-641.

Lee, I. S., J. Lin, H. K. Hall, B. Bearson, and J. W. Foster. 1995. The stationary-phase sigma factor sigma S (RpoS) is required for a sustained acid tolerance response in virulent *Salmonella* Typhimurium. Molecular Microbiology. 17(1):155-167.

Letellier, A., S. Messier, L. Lessard, and S. Quessy. 2000. Assessment of various treatments to reduce carriage of *Salmonella* in swine. Canadian Journal of Veterinary Research. 64(1):27-31.

Letellier, A., S. Messier, L. Lessard, S. Chenier, and S. Quessy. 2001. Host response to various treatments to reduce *Salmonella* Infections in swine. Canadian Journal of Veterinary Research. 65(3):168-172.

Leverstein-van Hall, M. A., H. E. M. Blok, A. R. T. Donders, A. Paauw, A. C. Fluit, and J. Verhoef. 2003. Multidrug resistance among *Enterobacteriaceae* is strongly associated with the presence of integrons and is independent of species or isolate origin. The Journal of Infectious Diseases. 187(2):251-259.

Levesque, C., L. Piche, C. Larose, and P. H. Roy. 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrobial Agents and Chemotherapy. 39(1):185-191.

Li, Q., and B. J. Cherayil. 2003. Roll of Toll-like receptor 4 in macrophage activation and tolerance during *Salmonella enterica* serovar Typhimurium infection. Infection and Immunity. 71(9):4873-4882.

Libby, S. J., T. A. Halsey, C. Altier, J. Potter, and C. L. Gyles. 2004. *Salmonella*. Page 143-167 in Pathogenesis of Bacterial Infections in Animals. C. L. Gyles, J. F. Prescott, J. G. Songer, and C. O. Thoen, ed. Blackwell Publishing. Oxford, UK.

Lindgren, S. W., I. Stojiljkovic, and F. Heffron. 1996. Macrophage killing is an essential virulence mechanism of *Salmonella* Typhimurium. Proceedings of the National Academy of Sciences of the United States of America. 93(9):4197-4201.

Loynachan, A. T., and D. L. Harris. 2005. Dose determination for acute *Salmonella* infection in pigs. Applied and Environmental Microbiology. 71(5):2753-2755.

Lumsden, J. S., and B. N. Wilkie. 1992. Immune response of pigs to parenteral vaccination with an aromatic-dependent mutant of *Salmonella* Typhimurium. Canadian Journal of Veterinary Research. 56(4):296-302.

Malhotra-Kumar, S., C. Lammens, J. Piessens, and H. Goossens. 2005. Multiplex PCR for simultaneous detection of macrolide and tetracycline resistance determinants in streptococci. Antimicrobial Agents and Chemotherapy. 49(11):4798-4800.

Marquardt, R. R., L. Z. Jin, J. W. Kim, L. Fang, A. A. Frohlich, and S. K. Baidoo. 1999. Passive protective effect of egg-yolk antibodies against enterotoxigenic *Escherichia coli* K88+ infection in neonatal and early-weaned piglets. FEMS Immunology and Medical Microbiology. 23(4):283-288.

Marquardt, R. R. 2000. Control of intestinal diseases in pigs by feeding specific chicken egg antibodies. Page 289-299 in Egg Nutrition and Biotechnology. J. S. Sim, S. Nakai, and W. Guenter, ed. CABI Publishing, NY.

Mastroeni, P., A. Vazquez-Torres, F. C. Fang, Y. Xu, S. Khan, C. E. Hormaeche, and G. Dougan. 2000. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. Part II: Effects on microbial proliferation and host survival in vivo. The Journal of Experimental Medicine. 192(2):237-248.

Mathew, A. G., W. G. Upchurch, and S. E. Chattin. 1998. Incidence of antibiotic resistance in fecal *Escherichia coli* isolated from commercial swine farms. Journal of Animal Science. 76(2):429-434.

Mathew, A. G., D. B. Arnett, P. Cullen, and P. D. Ebner. 2003. Characterization of resistance patterns and detection of apramycin resistance genes in *Escherichia coli* isolated from swine exposed to various environmental conditions. International Journal of Food Microbiology. 89(1):11-20.

McCormack, W. T., and C. B. Thompson. 1993. Special features of the development of the chicken humoral immune system. Research in Immunology. 144(6-7):467–475.

McEwen, S. A., and P. J. Fedorka-Cray. 2002. Antimicrobial use and resistance in animals. Clinical Infectious Diseases. 34(Supplement 3)**:**S93-S106.

McDermott, P. F., R. D. Walker, and D. G. White. 2003. Antimicrobial: Modes of action and mechanisms of resistance. International Journal of Toxicology. 22(2):135-143.

Medzhitov, R., and C. A. Janeway Jr. 1997. Innate immunity: The virtues of a nonclonal system of recognition. Cell. 91(3):295-298.

Medzhitov, R., and C. A. Janeway Jr. 1998. Innate immune recognition and control of adaptive immune responses. Seminar in Immunology. 10(5):351-353.

Meyerholz, D. K., T. J. Stabel, M. R. Ackermann, S. A. Carlson, B. D. Jones, and J. Ohlenz. 2002. Early epithelial invasion by *Salmonella enterica* serovar Tphimurium DT104 in the swine ileum. Veterinary Pathology. 39(6):712-720.

Miles, T. D., W. McLaughlin, and P. D. Brown. 2006. Antimicrobial resistance of *Escherichia coli* isolates from broiler chickens and humans. BMC Veterinary Research. 2(7):1-9.

Mine, Y., and J. Kovacs-Nolan. 2002. Chicken egg yolk antibodies as therapeutics in enteric infectious disease: A review. Journal of Medicinal Food. 5(3):159-169.

Mittrucker, H. W., and S. H. Kaufmann. 2000. Immune response to infection with *Salmonella* Typhimurium in mice. Journal of Leukocyte Biology. 67(4):457-463.

Mizuno, Y. 2004. Host defense mechanisms against *Salmonella* infection. Japanese Journal of Clinical Immunology. 27(6):367-372.

Monack, D. M., B. Raupach, A. E. Hromockyj, and S. Falkow. 1996. *Salmonella* Typhimurium invasion induces apoptosis in infected macrophages. Proceedings of the National Academy of Sciences of the United States of America. 93(18):9833-9838.

Monack, D. M., D. M. Bouley, and S. Falkow. 2004. *Salmonella* Typhimurium persists within macrophages in the mesenteric lymph nodes of chronically infected Nramp1$^{+/+}$ mice and can be reactivated by IFNgamma neutralization. The Journal of Experimental Medicine. 199(2):231–241.

Morgan, E., J. D. Campbell, S. C. Rowe, J. Bispham, M. P. Stevens, A. J. Bowen, P. A. Barrow, D. J. Maskell, and T. S. Wallis. 2004. Identification of host-specific colonization factors of *Salmonella enterica* serovar Typhimurium. Molecular Microbiology. 54(4):994-1010.

Morrison, S. L., M. S. Mohammed, L. A. Wims, R. Trinh, and R. Etches. 2002. Sequences in antibody molecules important for receptor-mediated transport into the chicken egg yolk. Molecular Immunology. 38(8):619-625.

Morrow, M. 2000. *Salmonella* as a foodborne pathogen. Swine News. 24(1):1-4. Available online: http://mark.asci.ncsu.edu/Swine_News/2001/sn_v2401.htm

Neutra, M. R., and J. P. Kraehenbuhl. 1993. The role of transepithelial transport by M cells in microbial invasion and host defense. Journal of Cell Science. 17(supplement):209-215.

Newman, K. E., and M. C. Newman. 2001. Evaluation of mannan oligosaccharide on the microflora and immunoglobulin status of sows and piglet performance. Journal of Animal Science. 79(Supplement 1):189.

Noll, F., G. Lutsch, and H. Bielka. 1982. Structure of IgG and IgY molecules in ribosome-antibody complexes as studied by electron microscopy. Immunology Letters. 4(3):117-123.

Nomoto, K. 2005. Prevention of infections by probiotics. Journal of Bioscience and Bioengineering. 100(6):583-592.

Oliva, B., and I. Chopra. 1992. Tet determinants provide poor protection against some tetracycline: Further evidence for division of tetracyclines into two classes. Antimicrobial Agents and Chemotherapy. 36(4):876-878.

Oliveira C. J., L. F. Carvalho, S. A. Fernandes, A. T. Tavechio, and F. J. Jr. Domingues. 2005. Prevalence of pigs infected by *Salmonella* Typhimurium at slaughter after an enterocolitis outbreak. International Journal of food Microbiology. 105(2):267-271.

O'Quinn, P. R., D. W. Funderburke, and G. W. Tibetts. 2001. Effects of dietary supplementation with mannan oligosaccharides on sow and litter performance in a commercial production system. Journal of Animal Science. 79(Supplement 1):212.

Owen, K. Q., J. L. Nelssen, R. D. Goodband, M. D. Tokach, K. G. Friesen, B. T. Ricert, J. W. Smith, and L. E. Russell. 1995. Effects of various fractions of spray-dried porcine plasma on performance of early-weaned pigs. Journal of Animal Science. 73(Supplement 1):81.

Owusu-Asiedu, A., C. M. Nyachoti, S. K. Baidoo, R. R. Marquardt, and X. Yang. 2003. Response of early-weaned pigs to an enterotoxigenic *Escherichia coli* (K88) challenge when fed diets containing spray-dried porcine plasma or pea protein isolate plus egg yolk antibody. Journal of Animal Science. 81(7):1781–1789.

Pace, J., M. J. Hayman, and J. E. Galan. 1993. Signal transduction and invasion of epithelial cells by *S.* Typhimurium. Cell. 72:505-514.

Padungtod, P., and J. B. Kaneene. 2006. *Salmonella* in food animals and humans in northern Thailand. International Journal of Food Microbiology. 108(3):346-354.

Parvez, S., K. A. Malik, S. Ah Kang, and H. Y. Kim. 2006. Probiotics and their fermented food products are beneficial for health. Journal of Applied Microbiology. 100(6):1171-1185.

Pasquali, F., C. Kehrenberg, G. Manfreda, and S. Schwarz. 2005. Physical linkage of Tn3 and part of Tn1721 in a tetracycline and ampicillin resistance plasmid from *Salmonella* Typhimurium. Journal of Antimicrobial Chemotherapy. 55(4):562-565.

Peralta, R. C., H. Yokoyama, Y. Ikemori, M. Kuroki, and Y. Kodama. 1994. Passive immunization against experimental salmonellosis in mice by orally administered hen egg-yolk antibodies specific for 14-kDa fimbriae of *Salmonella* Enteritidis. Journal of Medical Microbiology. 41(1):29-35.

Phillips, I., M. Casewell, and T. Cox. 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. Journal of Antimicrobial Chemotherapy. 53(1):28-52.

Pierce, J. L., G. L. Cromwell, M. D. Lindemann, L. E. Russell, and E. M. Weaver. 2005. Effects of spray-dried animal plasma and immunoglobulins on performance of early weaned pigs. Journal of Animal Science. 83(12):2876-2885.

Pijpers, A., E. J. Schoevers, N. Haagsma, and J. H. M. Verheijden. 1991. Plasma levels of oxytetracycline, doxycycline, and minocycline in pigs after oral administration in feed. Journal of Animal Science. 69(11):4512-4522.

Ploy, M. C., T. Lambert, J. P. Couty, and F. Denis. 2000. Integrons: An antibiotic resistance gene capture and expression system. Clinical Chemistry and Laboratory Medicine. 38(6):483-487.

Popoff, M. Y., J. Bockemuhl, and L. L. Gheesling. 2004. Supplement 2002 (no. 46) to the Kauffmann-White scheme. Research in Microbiology. 155(7):568-570.

Prescott, J. F. 2006. History of antimicrobial usage in agriculture: An overview. Page 19-27 in Antimicrobial Resistance in Bacteria of Animal Origin. F. M. Aarestrup, ed. ASM press. Washington, D.C.

Prescott, J. F. 1997. Antibiotics: Miracle drugs or pig food? The Canadian Veterinary Journal. 38(12):763-766.

Prescott, J. F., and J. D. Baggot. 1993. Antimicrobial Therapy in Veterinary Medicine, 2nd ed. p. 564-565. Iowa State University Press, Ames, IA.

Procyk, K. J., P. Kovarik, A. V. Gabain, and M. Baccarini. 1999. *Salmonella* Typhimurium and lipopolysaccharide stimulate extracellularly regulated kinase activation in macrophages by a mechanism involving phosphatidylinositol 3-Kinase and phospholipase D as novel intermediates. Infection and Immunity. 67(3):1011-1017.

Radstrom, P., O. Skold, G. Swedberg, J. Flensburg, P. H. Roy, and L. Sundstrom. 1994. Transposon Tn5090 of plasmid R751, which carries an integron, is related to Tn7, Mu, and the retroelements. Journal of Bacteriology. 176(11):3257-3268.

Rasmussen, B., H. F. Noller, G. Daubresse, B. Oliva, Z. Misulovic, D. M. Rothstein, G. A. Ellestad, Y. Gluzman, F. P. Tally, and I. Chopra. 1991. Molecular basis of tetracycline action: Identification of analogs whose primary target is not the bacterial ribosome. Antimicrobial Agents and Chemotherapy. 35(11):2306-2311.

Reed, W. M., H. J. Olander, and H. L. Thacker. 1986. Studies on the pathogenesis of *Salmonella* Typhimurium and *S. choleraesuis* var *kunzendorf* Infection in weanling pigs. American Journal of Veterinary Research. 47(1):75-83.

Roberts, M. C. 1996. Tetracycline resistance determinants: Mechanisms of action, regulation of expression, genetic mobility, and distribution. FEMS Microbiology Reviews. 19(1):1-24.

Roberts, M. C. 2003. Tetracycline therapy: Update. Clinical Infectious Diseases. 36(4):462-467.

Roe, M. T., and S. D. Pillai. 2003. Monitoring and identifying antibiotic resistance mechanisms in bacteria. Poultry Science. 82(4):622-626.

Roesler U., H. Marg, I. Schroder, S. Mauer, T. Arnold, J. Lehmann, U. Truyen, and A. Hensel. 2004. Oral vaccination of pigs with an invasive gyrA-cpxA-rpoB *Salmonella* Typhimurium mutant. Vaccine. 23(5):595-603.

Salazar-Gonzalez, R. M., and S. J. McSorley. 2005. *Salmonella* flagellin, a microbial target of the innate and adaptive immune system. Immunology Letters. 101(2):117-122.

Salyers, A. A., N. B. Shoemaker, A. M. Stevens, and L. Y. Li. 1995. Conjugative transposons: An unusual and diverse set of integrated gene transfer elements. Microbiological Reviews. 54(4):579-590.

Salyers, A. A., and D. D. Whitt. 2002. Bacterial Pathogenesis: A Molecular Approach 2nd. ASM Press, Washington D.C. p. 1-539.

Sanchez-Alavez, M., I. V. Tabarean, M. M. Behrens, and T. Bartfai. 2006. Ceramide mediates the rapid phase of febrile response to IL-1β. Proceedings of the National Academy of Sciences of the United States of America. 103(8):2904-2908.

Santos, R. L., R. M. Tsolis, A. J. Baumler, and L. G. Adams. 2003. Pathogenesis of *Salmonella*-induced enteritis. Brazilian Journal of Medical and Biological Research. 36(1):3-12.

Schlosser-Silverman, E., M. Elgrably-Weiss, I. Rosenshine, R. Kohen, and S. Altuvia. 2000. Characterization of *Escherichia coli* DNA lesions generated within J774 macrophages. Journal of Bacteriology. 182(18):5225-5230.

Schlundt, J. 2002. New directions in foodborne disease prevention. International Journal of Food Microbiology. 78(1-2):3-17.

Schmidt, P., V. Wiedemann, R. Kuhlmann, R. Wanke, E. Linckh, and U. Losch. 1989. Chicken egg antibodies for prophylaxis and therapy of infectious intestinal diseases. II. *In vitro* studies on gastric and enteric digestion of egg yolk antibodies specific against pathogenic *Escherichia coli* strains. Journal of Veterinary Medicine. 36(8):619-628.

Schmitt, C. S., J. S. Ikeda, S. C. Darnell, P. R. Watson, J. Bispham, T. S. Wallis, D. L. Weinstein, E. S. Metcalf, and A. D. O'Brien. 2001. Absence of all components of the flagella export and synthesis machinery differentially alters virulence of *Salmonella enterica* serovar Typhimurium in models of typhoid fever, survival in macrophages, tissue culture invasiveness, and calf enterocolitis. Infection and Immunity. 69(9):5619-5625.

Schneider, P. 2003. The why's and how's of eliminating clinical *Salmonella*. London Swine Conference-Maintaining Your Competitive Edge, 9-10 April, 2003. p. 9-16.

Schwarz, S., and E. Chaslus-Dancla. 2001. Use of antimicrobials in veterinary medicine and mechanisms of resistance. Veterinary Research. 32(3-4):201-225.

Schwarz, S., A. Cloeckaert, and M. C. Roberts. 2006. Mechanical and spread of bacterial resistance to antimicrobial agents. Page 73-98 in Antimicrobial

Resistance in Bacteria of Animal Origin. F. M. Aarestrup, ed. ASM press. Washington, D.C.

Sharma, J. M. 1997. The structure and function of the avian immune system. Acta Veterinaria Hungarica. 45(3):229-238.

Shaw, K. J., P. N. Rather, R. S. Hare, and G. H. Miller. 1993. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiology and Molecular Biology Reviews. 57(1):138-163.

Shea, K. M., American Academy of Pediatrics Committee on Environmental Health, and American Academy of Pediatrics Committee on Infectious Disease. 2004. Nontherapeutic use of antimicrobial agents in animal agriculture: Implications for pediatrics. Pediatrics. 114(3):862-868.

Shoemaker, K. B., H. Vlamakis, K. Hayes, and A. A. Salyers. 2001. Evidence for extensive resistance gene transfer among Bacteroides spp. and among Bacteroides and other genera in the human colon. Applied and Environmental Microbiology. 67(2):561-568.

Smith, C. A., and E. N. Baker. 2002. Aminoglycoside antibiotic resistance by enzymatic deactivation. Current drug targets. Infectious Disorder. 2(2):143-160.

Sorum, H., and M. Sunde. 2001. Resistance to antibiotic in the normal flora of animals. Veterinary Research. 32(3-4):227-241.

Spanoghe, P., and P. Pohl. 1987. Comparison between the influence of carbadox and that of traditional antibiotics on the resistance of enterobacteria in pigs. Annals of Veterinary Research. 18(3):249-254.

Speer B. S., N. B. Shoemaker, and A. A. Salyers. 1992. Bacterial resistance to tetracycline: Mechanisms, transfer, and clinical significance. Clinical Microbiology Review. 5(4):987-399.

Spring, P., C. Wenk, K. A. Dawson, and K. E. Newman. 2000. The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of *Salmonella*-challenged broiler chicks. Poultry Science. 79:205-211.

Stokes, H. W., and R. M. Hall. 1989. Novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: Integrons. Molecular Microbiology. 3(12):1669-1683.

Su, Y. C., D. Lu, X. D. Tan, A. R. Dong, H. Y. Tian, S. Q. Luo, and Q. K. Deng. 2006. Mathematical model of phosphatidylinositol-4,5-biphosphate hydrolysis mediated by epidermal growth factor receptor generating diacylglycerol. Journal of Biotechnology. 124(3):574-591.

Suter, W., A. Rosselet, and F. Knudel. 1978. Mode of action of quindoxin and substituted quinoxaline-di-N-oxides on *Escherichia coli.* Antimicrobial Agents and Chemotherapy. 13(5):770-783.

Swanenburg, M., H. A. Urlings, J. M. Snijders, D. A. Keuzenkamp, and F. Van Knapen. 2001. *Salmonella* in slaughter pigs: Prevalence, serotypes and critical control points during slaughter in two slaughterhouses. International Journal of Food Microbiology. 70(3):243-254.

Swartz, M. N. 2002. Human diseases caused by foodborne pathogens of animal origin. Clinical Infectious Diseases. 34(Supplement 3): S111-S122.

Tapping, R. I., S. Akashi, K. Miyake, P. J. Godowski, and P. S. Tobias. 2000. Toll-like receptor 4, but not toll-like receptor 2, is a signaling receptor for *Escherichia* and *Salmonella* lipopolysaccharides. Journal of Immunology. 165(10):5780- 5787.

Tenover, F. C. 2006. Mechanisms of antimicrobial resistance in bacteria. The American Journal of Medicine. 119(6):S3-S10.

Threlfall, E. J., J. A. Frost, and L. R. Ward. 1996. Increasing spectrum of resistance in multiresistant *Salmonella* Typhimurium. Lancet. 347(9007): 1053-1054.

Threlfall, E. J., L. R. Ward, and B. Rowe. 1997. Increasing incidence of resistance to trimethoprim and ciprofloxacin in epidemic *Salmonella* Typhimurium DT104 in England and Wales. Eurosurveillance. 2(11):8-84.

Tsolis, R. M., L. G. Adam, T. A. Ficht, and A. J. Baumler. 1999. Contribution of *Salmonella* Typhimurium virulence factors to diarrheal disease in calves. Infection and Immunity. 67(9):4879-4885.

Tortora, G. J., and S. R. Grabowski. 1993. Principles of Anatomy and Physiology, 7th ed. HarperCollins Publishers, New York, NY. p. 856.

Touchette, K. J., J. A. Carroll, G. L. Allee, R. L. Matteri, C. J. Dyer, L. A. Beausang, and M. E. Zannelli. 2002. Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: I. Effects on the immune axis of weaned pigs. Journal of Animal Science. 80(2):494–501.

Van Dijk, A. J., P. M. M. Enthoven, S. G. C. Van den Hoven, M. M. M. H. Van Laarhoven, T. A. Niewold, M. J. A. Nabuurs, and A. C. Beynen. 2002a. The

effect of dietary spray-dried porcine plasma on clinical response in weaned piglets challenged with a pathogenic *Escherichia coli*. Veterinary Microbiology. 84(3):207-218.

Van Dijk, A. J., T. A. Niewold, M. J. Nabuurs, J. Van Hees, P. De Bot, N. Stockhofe-Zurwieden, M. Ubbink-Blanksma, and A. C. Beynen. 2002b. Small intestinal morphology and disaccharidase activities in early-weaned piglets fed a diet containing spray-dried porcine plasma. Journal of Veterinary Medicine. Series A, Physiology, Pathology, Clinical Medicine. 49(2):81–86.

Van Dijk, A. J., H. Everts, M. J. A. Nabuurs, R. J. C. F. Margry, and A. C. Beynen. 2001. Growth performance of weanling pigs fed spray-dried animal plasma: A review. Livestock Production Science. 68:263-274.

Van Nguyen, S., K. Umeda, H. Yokoyama, Y. Tohya, and Y. Kodama. 2006. Passive protection of dogs against clinical disease due to canine parvovirus-2 by specific antibody from chicken egg yolk. Canadian Journal of Veterinary Research. 70(1):62-64.

Vazquez-Torres, A., Y. S. Xu, C. J. Jones, D. W. Holden, S. M. Lucia, M. C. Dinauer, P. Mastroeni, and F.C. Fang. 2000a. *Salmonella* pathogenicity island 2-dependent evasion of the phagocyte NADPH oxidase. Science. 287(5458):1655-1658.

Vazquez-Torres, A., J. Jones-Carson, P. Mastroeni, H. Ischiropoulos, and F. C. Fang. 2000b. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. I. Effects on microbial killing by activated peritoneal macrophages in vitro. The Journal of Experimental Medicine. 192(2): 227–236.

Vazquez-Torres, A., and F. C. Fang. 2001. *Salmonella* evasion of the NADPH phagocyte oxidase. Microbes and Infection. 3(14-15):1313-1320.

Vieira-Pinto, M., P. Temudo, and C. Martins. 2005. Occurrence of *Salmonella* in the ileum, ileocolic lymph nodes, tonsils, mandibular lymph nodes and carcasses of pigs slaughtered for consumption. Journal of Veterinary Medicine. Series B, Infectious Diseases and Veterinary Public Health. 52(10):476-481.

Warr, G. W., K. E. Magor, and D. A. Higgins. 1995. IgY: Clues to the origins of modern antibodies. Immunology Today. 16(8):392-398.

Wasyl, D., D. Sandvage, M. N. Skov, and D. L. Baggensen. 2006. Epidemiological characteristics of *Salmonella* Typhimurium isolated from animals and feed in Poland. Epidemiology and Infection. 134(1):179-185.

Watanabe, T., and T. Fukasawa. 1961. Episome-mediated transfer of drug resistance in *Enterobacteriacae* III. Transduction of resistance. Journal of Bacteriology. 82(2):202-209.

Watanabe, T., and K. W. Lyang. 1962. Episome-mediated transfer of drug resistance in *Enterobacteriacae* V. spontaneous segregation and recombination of resistance factors in *Salmonella* Typhimurium. Journal of Bacteriology. 84(3):422-430.

Watson, P. R., and S. M. Paulin, P. W. Jones, and T. S. Wallis. 2000. Interaction of *Salmonella* serotypes with porcine macrophages in vitro does not correlate with virulence. Microbiology. 146(7): 1639-1649.

Wegener, H. C., and D. L. Baggesen. 1996. Investigation of an outbreak of human salmonellosis caused by *Salmonella enterica spp. enterica* serovar Infantis by pulsed field gel electrophoresis. International Journal of Food Microbiology 32(1-2):125-131.

White, D. G., S. Zhao, R. Sudler, S. Ayers, S. Friedman, S. Chen, P. F. McDermott, D. D. Wagner, and J. Meng. 2001. The isolation of antibiotic-resistant *Salmonella* from retail ground meats. The New England Journal of Medicine. 345(16):1147-1154.

Wick, M. J. 2004. Living in the danger zone: Innate immunity to *Salmonella*. Current Opinion in Microbiology. 7(1):51-57.

Wiedemann, V., E. Linckh, R. Kuhlmann, P. Schmidt, and U. Losch. 1991. Chicken egg antibodies for prophylaxis and therapy of infectious intestinal diseases. V. In vivo studies on protective effects against *Escherichia coli* diarrhea in pigs. Journal of Veterinary Medicine. 38(4):283-291.

Wiedemann, V., R. Kuhlmann, P. Schmidt, W. Erhardt, and U. Losch. 1990. Chicken egg antibodies for prophylaxis and therapy of infectious intestinal diseases. III. In vivo tenacity test in piglets with artificial jejunal fistula. Journal of Veterinary of Medicine. 37(3):163-172.

Williams, L. P. Jr., and K. W. Newwell. 1970. *Salmonella* excretion in joy-riding pigs. American Journal of Public Health and the Nation's Health. 60(5):926-929.

Winokur, P. L., D. L. Vonstein, L. J. Hoffman, E. K. Uhlenhopp, and G. V. Doern. 2001. Evidence for transfer of CMY-2 AmpC beta-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. Antimicrobial Agents and Chemotherapy. 45(10):2716-2722.

Wright, J. G., L. A. Tengelsen, K. E. Smith, J. B. Bender, R. K. Frank, J. H. Grendon, D. H. Rice, A. M. B. Thiessen, C. J. Gilbertson, S. Sivapalasingam, T. J.

Barrett, T. E. Besser, D. D. Hancock, and F. J. Angulo. 2005. Multidrug-resistant *Salmonella* Typhimurium in four animal facilities. Emerging Infectious Diseases. 11(8):1235-1241.

Wolska, K. I. 2003. Horizontal DNA transfer between bacteria in the environment. Acta Microbiologica Polonica. 52(3):233-243.

World Health Organization. 1997. The medical impact of antimicrobial use in food animals. Report of a WHO meeting, 13-17 October 1997, Berlin, Germany.

Yates, C. M., M. C. Pearce, M. E. Woolhouse, and S. G. Amyes. 2004. High frequency transfer and horizontal spread of apramycin resistance in calf fecal *Escherichia coli*. The Journal of Antimicrobial Chemotherapy. 54(2):534-537.

Yokoyama, H., R. C. Peralta, R. Diaz, S. Sendo, Y. Ikemori, and Y. Kodama. 1992. Passive protective effect of chicken egg yolk immunoglobulins against experimental enterotoxigenic *Escherichia coli* infection in neonatal piglets. Infection and Immunity. 60(3):998-1007.

Yokoyama, H., K. Umeda, R. C. Peralta, T. Hashi, F. C. Jr. Icatlo, M. Kuroki, Y. Ikemori, and Y. Kodama. 1998a. Oral passive immunization against experimental salmonellosis in mice using chicken egg yolk antibodies specific for *Salmonella* Enteritidis and *Salmonella* Typhimurium. Vaccine. 16(4):388-393.

Yokoyama, H., R. C. Peralta, K. Umeda, T. Hashi, F. C. Jr. Icatlo, M. Kuroki, Y. Ikemori, and Y. Kodama. 1998b. Prevention of fatal salmonellosis in neonatal calves, using orally administered chicken egg yolk *Salmonella*-specific antibodies. American Journal of Veterinary Research. 59(4):416-420.

Zhang, S., R. L. Santos, R. M. Tsolis, S. Stender, W. Hardt, A. J. Bäumler, and L. G. Adams. 2002. The *Salmonella enterica* serotype Typhimurium effector proteins SipA, SopA, SopB, SopD, and SopE2 act in concert to induce diarrhea in calves. Infection and Immunity. 70(7): 3843–3855.

Zhang, S., R. A. Kingsley, R. L. Santos, H. Andrews-Polymenis, M. Raffatellu, J. Figueiredo, J. Nunes, R. M. Tsolis, L. G. Adams, and A. J. Baumler. 2003. Molecular pathogenesis of *Salmonella enterica* serotype Typhimurium-induced diarrhea. Infection and Immunity. 71(1):1-12.

PART III. MATERIALS AND METHODS

Pigs, Housing, and Treatment Design

In two replicate trials, 132 weaned pigs (approximately 21 days old) which had never been exposed to antibiotics were received from The University of Tennessee Martin Swine Research Station, Martin, TN and transported to the Johnson Animal Research and Teaching Unit (JARTU), The University of Tennessee, Knoxville, TN. For this experiment, all pigs were housed and managed under properly controlled conditions and all animal procedures and experimental protocols were approved by The University of Tennessee Institutional Animal Care and Use Committee (IACUC).

Salmonella enterica serovar Typhimurium (798) 4232 (National Animal Disease Center, Ames, Iowa) was used for the bacterial challenge. This strain was identified by a nalidixic acid resistance marker (Ebner and Mathew, 2000; Cullen et al., 2002; Mathew et al., 2002). *Salmonella* inocula were generated by culturing from stock on XLT4 agar (Difco, Sparks, MD) containing 50 μg/ml of nalidixic acid (Sigma, St. Louis, MO) at 37°C overnight. The following day, *Salmonella* colonies were picked from plates and grown in 2 flasks containing 200 ml Luria-Bertani (LB) Broth (10 g Bacto-tryptone, 5 g yeast extract, 10 g NaCl / 1 liter H_2O) and supplemented with 50 μg/ml of nalidixic acid (Sigma, St. Louis, MO) for 18 h at 37°C in incubator. Before challenge, *Salmonella* cultures from 2 flasks were mixed and the cell concentration in broth was obtained by enumeration using serial dilution and XLT4 plus nalidixic acid (50 μg/ml) agar plating. *Salmonella* with final concentration of 7.95×10^7 colony forming unit (CFU)/ml for the first replicate trial and 1.90×10^8 CFU/ml for the second replicate trial were used for oral (3 ml) and intranasal (2 ml per nostril) challenge to all pigs on day 7 of trial.

Pigs were randomly assigned to separate treatment rooms with controlled lighting regimen (12 h of light and 12 h of dark). The treatments are shown in Table 1 (All tables are located in the Appendix). Feed treatments were given to weaned pigs on day 3 of trial. All treatment pigs had feed and water access *ad libitum*. Feed and treatment dietary compositions were formulated according to the recommendations of National Research Council Nutrient Requirements of Swine (1998) for the starter phase I for 14 days, starter phase II after 14 days until pigs reached 20 kg and feeder/grower phase III after 20 kg until 35 kg, as listed in Table 2, 3, and 4, respectively. After reaching 35 kg (approximated day 35), pigs in all treatment groups were continuously fed a control diet for the rest of the study. Egg yolk powder containing *Salmonella* antibodies was provided by R. R. Marquardt and C. M. Nyachoti at University of Manitoba, Winnipeg, Manitoba, Canada. Briefly, the procedure for producing specific egg yolk antibodies involves immunizing laying hens with antigens isolated from *Salmonella* Typhimurium, including lipopolysaccharide, fimbrial proteins, and outer membrane proteins. Immunized chicken eggs were cracked and egg yolks were separated from whole eggs by straining. After removing yolks from egg whites, egg yolks were filtered to clear the rest of egg white and transferred into a funnel attached with filter paper to collect the yolk contents. The yolk contents were adjusted to a pH of 5.0 by diluting (1:9, v/v) with acidified double distilled water and kept frozen at -20°C for 24 h. On the following day, yolk contents were thawed and centrifuged at 10000×g for 30 minutes at 15°C. The supernatant liquid was filtered twice with filter paper (Marquardt et al., 1999). The egg yolk antibody powder was obtained by freeze-drying via a rapid vacuum freezing process and then stored at 4°C until shipping.

In each treatment room, pigs were raised in nursery crates (4'x 4') for 3 weeks, and subsequently, moved to finishing pens (8' x 8'). Biosecurity regulations were followed for the whole period of the study. Cleanliness and sanitation in all rooms were maintained prior to the study and throughout the study. The pen and floor in each room were cleaned 3 times per week. Separate feed containers and scoops were used for each room. Disposable coveralls (Fisher Scientific, Suwanee, GA), plastic boots (Nasco, Ft Atkinson, WI), and latex gloves (Fisher Scientific, Pittsburgh, PA) were used during cleaning, feeding and collecting samples from pigs. Coveralls and gloves were changed between rooms. Before entry and after exiting each room, personnel used footbaths containing chlorhexidine diacetate disinfectant (Nolvasan® solution; Fort Dodge Animal Health, Fort Dodge, IA). All pigs were transported to The University of Tennessee, Plateau Research and Education Center, Crossville, TN on day 89 of the experiment. Pigs were housed in concrete pens, which were in a conventional swine facility.

Sampling Plan and Bacterial Analysis

For each pig, a fecal sample was taken from the rectum by using a sterile swab (Fisherbrand Dacron, Houston, TX) prior to treatment application (day 0 of the experiment), before challenge with the pathogenic *Salmonella* strain (day 7) and on days 8, 12, 14, 21, 28, 58, 88 and 118 for the isolation of *Salmonella* Typhimurium and *E. coli*. Each sample swab was kept in an individual sterile glass tube (16x150 mm; Fisher Scientific, Pittsburgh, PA) on ice and then transported to the laboratory for bacterial analysis.

After samples were brought to the laboratory, each fecal swab was streaked onto individual lactose MacConkey agar (Difco, Sparks, MD) and all inoculated plates were placed in the incubator for 24 h at 37°C for growing of *E. coli*. After streaking, the tip of the same swab was cut and placed in 2 ml of Nutrient Broth (Becton Dickinson, Sparks, MD), and then broth was mixed using vortex (VORTEX-GENIE™, Scientific industries, Inc., Bohemia, NY). To enrich for *Salmonella* Typhimurium, the swab tip and 1 ml of broth were transferred to a stomacher bag filled with 80 ml of tetrathionate broth (Difco) and this bag was completely sealed, then, incubated at 42°C for 24 h. On the following day, *Salmonella* tetrathionate culture was streaked onto XLT4 agar containing 50 μg/ml nalidixic acid using a wire loop and all inoculated plates were placed in the incubator for 24 h at 37°C.

Four individual colonies for each bacteria type isolated from each sample were analyzed for antibiotic resistance. Microbial shedding data were generated based on determination of *E. coli* and *Salmonella*-positive samples.

Minimum Inhibitory Concentration Analysis

A total of 4,763 *E. coli* colonies and 2,139 *Salmonella* Typhimurium colonies from fecal samples were analyzed for resistance to apramycin sulfate (Sigma, St. Louis, MO), tetracycline (Sigma), and carbadox (Trek Diagnostic Systems, Inc., Westlake, OH) via the minimum inhibitory concentration (MIC) broth dilution method (National Committee for Clinical Laboratory Standard, 2002). Briefly, each *E. coli* and *Salmonella* Typhimurium isolate cultured on individual MacConkey agar and XLT4 agar, respectively, was picked using a wire loop and transferred to a sterile glass tube (16 x 150

mm; Fisher Scientific, Pittsburgh, PA) filled with 5 ml of Mueller-Hinton II cation-adjusted Broth (Becton, Dickinson and Company, Sparks, MD). Glass tubes containing inoculated broth were incubated with shaking at 37°C. The cell concentration adjusted to 0.5 McFarland units (10^8 CFU/ml) (National Committee for Clinical Laboratory Standards, 2002) by colorimeter (BioMerieux Vitex, Inc., Hazelwood, MO). For each bacterial isolate sample, when the culture concentration was appropriately adjusted, 25.3 μl of prepared bacterial culture was added to 2.5 ml of diluted mixture of Mueller-Hinton II cation-adjusted Broth and sterile water at a ratio of 1:10. The mixture (50 μl) of the diluted Mueller-Hinton II cation-adjusted Broth and adjusted bacterial culture was added to each well within a column of microtiter plate for antibiotic resistance analysis. At the end, mixed bacteria concentration was approximately 5 x 10^5 CFU/ml (National Committee for Clinical Laboratory Standards, 2002).

For each microtiter plate used for the MIC analysis, the first to the eleventh columns were used for the bacterial sample analysis and the twelfth column was set for the control bacteria (American Type Culture Collection # 25922 *E. coli*; *Salmonella* Typhimurium (798) 4232, USDA, Animal Disease Control Center, Ames, Iowa). Apramycin and tetracycline microtiter plates were prepared in the laboratory by producing two-fold serial dilutions from 0 to 128 μg/ml in all columns. Carbadox microtiter plates were prepared for the same serial dilutions (Trek Diagnostic Systems, Inc., Westlake, OH) and freeze-dried and sealed for shipping. At room temperature, 50 μl of diluted bacterial cultures were added to each well of the antibiotic microtiter plates, then covered with Para film (American National Can™, Menasha, WI) and incubated for

18-22 h at 37°C. Antibiotic resistance was determined by observing bacterial growth. The lowest concentration of antibiotic that the turbidity or a deposited spot of cells was not detected in the well was recorded as minimum inhibitory concentration (MIC). The MIC breakpoints for each antibiotic test in both *E. coli* and *Salmonella* Typhimurium are presented in Table 5 (Danish Integrated Antimicrobial Resistance Monitoring and Research Program, 1999; National Antimicrobial Resistance Monitoring System-Enteric Bacteria, 2001; National Committee for Clinical Laboratory Standards. 2002).

Weight, Rectal Temperature Measurements and Blood Collection

On days 0, 7, 8, 12, 14, 28, 58, and 88 of the experiment, pigs were weighed on a digital scale for performance analysis. Rectal temperatures of twelve pigs in two replicate trials randomly chosen in each treatment were monitored by digital thermometer (MABIS Healthcare Inc., Lake Forest, IL) and blood samples were taken from those same pigs for immunological analysis on days 0, 7, 8, 12, 14 and 28. Eight milliliters of blood were withdrawn from the jugular vein using an 18-gauge 1.5 inch needle (Sherwood Medical Company, St. Louis, MO) attached to a vacuum tube syringe filled with glass beads (Sarstedt, Inc., Newton, NC), after which approximately 2 ml of blood was transferred to a small sterile tube coated with EDTA, anti-coagulant, (Vacutainer®PLUS, Franklin Lakes, NJ) for white blood cell analysis (Vet Count IIIB, Mallinckrodt, Phillipsburg, NJ). The remainder of the blood was kept in vacuum tube syringes. All blood samples were placed on ice for transport to the laboratory. Serum was separated from blood samples in the vacuum tubes by the centrifugation at 260 × g

for 15 minutes and each serum sample was approximately divided in half and saved in 3 Eppendorf microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA). Serum samples were kept in the freezer at -20 ºC for later analysis of swine interleukin-1β and porcine *Salmonella* antibody concentrations.

Swine Interleukin-1β Analysis

Serum concentration of interleukin-1β (IL-1β) was analyzed using a commercially available Enzyme Linked Immuno-Sorbent Assay kit (ELISA kit) specific for porcine IL-1β (Biosource International Inc., Camarillo, CA). Analyses were conducted as described by the manufacturer with the minimum dose sensitivity of <15 pg/ml. A monoclonal antibody specific for IL-1β was coated onto the wells of 96-well microtiter plate by the manufacturer. The standard curve with range from 0 to 2500 pg/ml was generated by adding 100 µl of standards to microtiter wells and adding standard diluent buffer to the well set for 0 pg/ml. Wells reserved for the Chromogen Substrate blank were left empty. For each well reserved for swine serum sample, 50 µl of standard diluent buffer were added followed by 50 µl of sample for the dilution of 1:2, then the plate was tapped gently on the side to mix the solution, covered with a plate cover and incubated for 2 h at 37°C. After incubation, solution in the wells was thoroughly aspirated and discarded. The plate was washed 4 times as follows. One hundred microliters of Biotinylated Polyclonal Anti-IL-1β (Biotin Conjugate) solution were added into each well except the well reserved for the Chromogen Substrate blank, and then the plate was tapped on the side to mix, covered with plate cover and incubated for 1 h at room temperature. Following incubation, the solution in the wells was

thoroughly aspirated and discarded and the plate was washed. One hundred microliters of Streptavidin-Peroxidase HRP working solution were added to each well except the wells for the Chromogen blank, and then the plate was covered and incubated for 30 minutes at room temperature. The solution in the wells was thoroughly aspirated and discarded and the plate was washed. At each of above steps, the plate was completely washed 4 times by wash buffer. One hundred microliters of Stabilized Chromogen substrate were added to each well. The plate was incubated for 20 minutes at room temperature in the dark, and then 100 μl of stop solution were added to each well, and the plate was gently tapped to mix. The solution in the wells was read against a Chromogen substrate blank, which included 100 μl each of Stabilized Chromogen substrate and stop solution at 450 nm by microplate reader (BioTek Instruments, Inc., Winooski, VT). The plate was read within 2 h after adding the stop solution. The results of swine IL-1β concentration were generated by reading optical density values of samples against standard concentration and then multiplied by 2.

Porcine *Salmonella* Antibody Analysis

Serum concentration of *Salmonella* antibody was analyzed using a commercial ELISA kit specific for porcine *Salmonella* antibody (IDEXX Laboratory, Westbrook, ME). Ninety-six well microtiter plates were coated with *Salmonella* antigen (lipopolysaccharide antigen) at the manufacturer. Serum samples used for this procedure were diluted twenty-fold (1:20) by mixing 15 μl of sample and 285 μl of sample diluent. One hundred microliters each of undiluted negative control, undiluted positive control, and diluted sample were added into the appropriate wells and incubated at room

temperature for 30 minutes. The solution was discarded from the wells and the plate was washed. After washing, 100 µl of Anti-Porcine: Horseradish Peroxidase Conjugate was added into each well, after which plates were incubated for 30 minutes at room temperature. Unbound conjugate was aspirated and the plate was washed. One hundred microliters of TMB substrate solution was added in each well and incubated at room temperature for 15 minutes. Following incubation, 100 µl of stop solution were added into each well to stop the reaction. The absorbance values were measured related to the blank with air and recorded in absorbance values at 650 nm. The presence of *Salmonella* antibody in the serum sample is interpreted by relating absorbance values at 650 nm of unknown sample to the positive control mean by calculating the sample to positive control (S/P) ratio as shown in following formulation:

$$\text{S/P ratio} = \frac{\text{Absorbance of sample read at 650 nm} - \text{Average negative control}}{\text{Average positive control} - \text{Average negative control}}$$

Statistical Design and Analysis

The statistical model for growth performance, immunological data, shedding and antibiotic resistance (tetracycline, apramycin, and carbadox) for *Salmonella* Typhimurium and *E. coli* consisted of a randomized block design with replication and repeated measures with individual pig as the experimental unit, and blocking on trial replications. Data were analyzed using the Mixed Procedure (SAS, 2002) to determine the effects of treatments, days, and interactions of treatment by day. Least squares means were analyzed and compared using least significant difference at P = 0.05. Shedding and antibiotic resistance data were converted to binary indicators (i.e., if bacterial numbers

were > 0 then = 1, otherwise = 0 for shedding analysis, and if MIC of tetracycline was ≥16 then = 1; otherwise = 0 for resistance analysis). The resistance threshold MIC was different for each antibiotic. Correlations among *Salmonella* shedding, growth, rectal temperature and immunological data were analyzed using Proc CORR (SAS, 2002).

References

Cullen, P., A. G. Mathew. R. Clift, and S. Chattin. 2002. Effects of management and environmental conditions on antibiotic resistance in bacteria associated with swine. Annual Report. Department of Animal Science, The University of Tennessee, Knoxville, TN.

Danish Integrated Antimicrobial Resistance Monitoring and Research Program. 1999. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. Danish Veterinary Institute, Copenhagen, Denmark. p. 1-54.

Ebner, P. D., and A. G. Mathew. 2000. Effects of antibiotic regimens on the fecal shedding patterns of pigs infected with *Salmonella* Typhimurium. Journal of Food Protection. 63(6):709-714.

Marquardt, R. R., L. Z. Jin, J. W. Kim, L. Fang, A. A. Frohlich, and S. K. Baidoo. 1999. Passive protective effect of egg-yolk antibodies against enterotoxigenic *Escherichia coli* K88+ infection in neonatal and early-weaned piglets. FEMS Immunology and Medical Microbiology. 23:283-288.

Mathew A. G., F. Jackson, and A. M. Saxton. 2002. Effects of antibiotic regimens on resistance of *Escherichia coli* and *Salmonella* serovar Typhimurium in swine. Journal of Swine Health and Production. 10(1):7-13.

National Antimicrobial Resistance Monitoring System—Enteric Bacteria (NARMS-EB). 2001. Veterinary Isolates Final Report. US Food and Drug Administration, Center for Veterinary Medicine. Washington, D.C.

National Committee for Clinical Laboratory Standards. 2002. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals 2nd ed. 19(11): 1–86.

National Research Council. 1998. Nutrient Requirements of Swine 10th ed. National Academy Press, Washington, D.C.

SAS. 2002. SAS/STAT Users Guide (Version 9.1). SAS Institute Inc., Cary, NC.

PART IV. RESULTS

Bacterial Shedding

Salmonella Typhimurium were detected from pigs in all groups 24 h after they were challenged (Table 6). The percentage of pigs shedding *Salmonella* across all treatments was highest on day 8 with declining levels between day 12 and day 118 of the experiment ($P<0.05$). Significant treatment effects were noted ($P<0.0001$). Throughout the study period, pig groups receiving the antibiotics apramycin, carbadox, and oxytetracycline, showed a lower incidence of *Salmonella* shedding compared to pigs in non-antibiotic groups and the control group ($P<0.05$). There were no differences in pigs shedding *Salmonella* across all experimental days among groups that received egg yolk powder containing ASEYA, egg yolk powder lacking ASEYA, or spray dried porcine plasma compared to those receiving control diets.

All pigs were observed to shed *E. coli* at the beginning of the study and before treatments were applied. A high percentage of pigs also shed this organism throughout the period of the study (Table 7). Slightly decreased percentages of pigs shedding *E. coli* across all treatments were noted on days 21, 58, and 118 of the experiment compared to those observed from day 0 to day 12 of the experiment ($P<0.05$). There were no differences in the overall treatment effects across all days on pig shedding *E. coli*; however, treatment × day interactions were noted ($P<0.0001$).

Bacterial Antibiotic Resistance

Less than seven percent of 2,139 *Salmonella* Typhimurium isolates were found to be resistant to tetracycline, apramycin, and carbadox. On day 0 and day 7 of the

experiment, no *Salmonella* in any group were available for resistance measurements. Resistance to tetracycline was only noted in *Salmonella* from pigs fed with diets containing oxytetracycline. However, *Salmonella* from pigs fed apramycin did not demonstrate resistance to apramycin. Apramycin resistance was noted in a few isolates from pigs fed spray dried plasma, although no significant treatment effects were noted. *Salmonella* resistant to carbadox were noted in all treatment groups except for pigs fed with egg yolk powder containing ASEYA. However, the dietary treatments did not affect the percentages of *Salmonella* Typhimurium isolates resistant to any of the test antibiotics.

Escherichia coli isolated from pig fecal samples were resistant to tetracycline, apramycin, and carbadox (Tables 8-10). Treatment effects, day effects, and treatment × day interaction effects were noted. A significant percentage of *E. coli* was resistant to tetracycline at the beginning of the experiment (Table 8). On day 12, the average percentage of isolates resistant to tetracycline from all groups declined from that observed on days 0, 7, and 8 ($P<0.05$). The lower percentages of tetracycline resistant isolates persisted from day 12 to day 118. Across all days, the highest percentage of tetracycline resistant *E. coli* was noted in pigs fed oxytetracycline ($P<0.05$). However, the lowest percentage of tetracycline resistant *E. coli* was noted in pigs fed egg yolk powder containing ASEYA ($P<0.05$). The percentages of tetracycline resistant *E. coli* from pigs fed egg yolk powder lacking ASEYA and SDPP were lower than those from pigs fed oxytetracycline ($P<0.05$), but indistinguishable from those from pigs fed control diets or apramycin and carbadox. Similarly, across all days, the highest percentages of apramycin resistant *E. coli* were observed in pigs fed apramycin ($P<0.05$) (Table 9). In

that group, higher percentages of apramycin resistant *E. coli* were noted beginning on day 7, and remained higher in that group until day 21, compared to pigs fed other diets, including egg yolk powder containing ASEYA, egg yolk powder lacking ASEYA, SDPP, oxytetracycline, and control diets. However, two weeks after withdrawal of apramycin (on day 28 of the experiment), the percentages of *E. coli* resistant to apramycin decreased by 50% compared to day 21, and steadily declined until the end of the experiment. Greater percentages of carbadox resistant *E. coli* were noted from pigs fed carbadox compared to pigs in the control group, pigs fed egg yolk powder containing ASEYA and pigs fed egg yolk powder lacking ASEYA ($P<0.05$) (Table 10). On day 28 and day 58 of the experiment, higher percentages of *E. coli* resistant to carbodox were observed in the pigs fed with carbadox compared to pigs fed egg yolk powder with ASEYA, egg yolk powder without ASEYA, SDPP, oxytetracycline, and control diets.

Growth Performance

No treatment effects were observed for body weight or weight gain throughout the course of the study (Table 11). However, day effects ($P<0.0001$) and day × treatment effects ($P<0.01$) were noted. The body weights of pigs increased from the beginning to the end of the study ($P<0.05$) for all treatments. On day 14 and 21 of trial, pigs fed control diets had lower body weights compared to pigs fed apramycin and carbadox. On day 28 of trial, lower body weights were observed in pigs fed control diets compared to pigs fed apramycin and carbadox, and SDPP. On day 58 of trial, body weights of pigs fed apramycin and carbadox, oxytetracycline, and SDPP were greater than body weights

of pigs fed control diets. The body weights of pigs fed egg yolk powder containing ASEYA were not different from pigs fed control diets on days 0, 7, 8, 12, 14, 21, 28, 58 and 88 of the experiment.

Rectal Temperature

Treatment effects ($P<0.05$), day effects ($P<0.0001$) and day × treatment effects ($P<0.0001$) were noted for rectal temperatures (Table 12). Mean rectal temperatures increased ($P<0.05$) across all treatment groups one day following challenge with *Salmonella* Typhimurium. Decreased rectal temperatures were observed by day 12 (5 days post-challenge) and maintained through day 28 of trial. Overall, pigs fed diets with oxytetracycline had significantly lower rectal temperatures compared to pigs fed spray dried porcine plasma and egg yolk powder containing ASEYA ($P<0.05$), but were not statistically different from pigs fed apramycin and carbadox, egg yolk powder lacking ASEYA, or the control diet.

Immunological Values

White Blood Cell Counts

White blood cell counts of pigs across all diets were variable over the course of the study (Table 13). No treatment effects on the numbers of white blood cells were observed. However, day effects ($P<0.0001$) and day × treatment effects ($P<0.01$) were noted for white blood cell counts. The numbers of white blood cells of pigs across all groups were increased from day 7 to day 28 compared to those at the initiation of the

experiment and were highest (P<0.05) 7 days after challenge with *Salmonella* Typhimurium, then dropped to pre-challenge levels (day 7) approximately 2 weeks following the challenge (day 21 of the experiment). On day 14 of trial, when the greatest levels of white blood cell were reached, pigs fed control diet, egg yolk powder containing ASEYA, egg yolk powder lacking ASEYA, and SDPP had significantly higher numbers of white blood cells compared to pigs fed apramycin and oxytetracycline.

Porcine *Salmonella* Antibody

Salmonella antibody responses of pigs as indicated by the S/P ratio did not differ among the dietary treatments (Table 14); however, day effects were detected (P<0.0001), indicating that the porcine *Salmonella* antibody varied over time. The levels of anti-*Salmonella* antibody of pigs across all groups increased beginning on day 14 and continued to increase through day 28, or 3 weeks after they were challenged (P<0.05).

Swine Interleukin-1β

Serum IL-1β concentrations were not detected in most pigs regardless of treatments during the course of the study. However, five pigs in the first replicate trial had very low IL-1β responses. These included a pig fed oxytetracycline on day 12 (5.33 pg/ml), 2 pigs fed egg yolk powder containing ASEYA on day 12 (3.67 and 4.69 pg/ml), a pig fed control diet on day 21 (3.74 pg/ml) and a pig fed control diet on day 28 (2.97 pg/ml). These 5 pigs also shed *Salmonella* Typhimurium.

Relationships among *Salmonella* Shedding, Growth Performance, Rectal Temperature and Immunological Values

The correlations among all variables were evaluated from day 0 to day 28 of the experiment. More specifically, *Salmonella* shedding was unrelated to body weight, rectal temperature, porcine *Salmonella* antibody, white blood cell counts, or swine IL-1β concentration. In addition, body weights were not correlated to rectal temperature, or immunological variables, such as IL-1β concentrations, white blood cell counts or antibody levels. Rectal temperatures also were not related to porcine *Salmonella* antibody levels, white blood cell counts, and swine IL-1β concentrations. Among immunological values, no relationships were observed in this analysis.

PART V. DISCUSSION

The present study investigated the effects of in-feed egg yolk powder containing specific antibodies against lipopolysaccharide, fimbrial proteins, and outer membrane protein antigens isolated from *Salmonella* Typhimurium on bacterial colonization, bacterial antibiotic resistance, growth performance, rectal temperature and immunological response in weaned pigs challenged with *Salmonella* Typhimurium.

Bacterial Shedding

Salmonella Typhimurium were not detected in pigs at the initiation of the experiment and prior to inoculation with *Salmonella* Typhimurium, indicating that pigs were likely free of this organism and that their health status was maintained during shipping and holding prior to the initiation of the experiments. *Salmonella* Typhimurium were detected in pig fecal samples 24 h after they were challenged orally and intranasally. The relatively short time between inoculation with the challenge organism and detection in the feces may be explained by the ability of *Salmonella* Typhimurium to invade the internal organs, including tonsils, lung, and intestinal tract, and rapidly disseminate through the lymph system, ultimately reaching the ileocolic lymph nodes, and becoming present in the feces (Fedorka-Cray et al., 1995; Wood et al., 1989).

The present study indicates a lower incidence of *Salmonella* Typhimurium shedding in pigs fed antibiotics, including oxytetracycline, apramycin, and carbadox. These findings support the results of earlier studies in our laboratory, in which pigs supplemented with apramycin and oxytetracycline had lower *Salmonella* shedding (Ebner and Mathew, 2000). Additionally, another study, focusing on the influence of oxytetracycline on colonization of *Salmonella* Typhimurium in animals, found that use of

oxytetracycline and neomycin resulted in a decrease in the percentage of pigs carrying *Salmonella* Typhimurium (Evangelisti et al., 1975).

In contrast to the use of antibiotics, our results indicate that in-feed inclusion of egg yolk powder containing specific antibodies is not effective in reducing shedding of *Salmonella* Typhimurium by weaned pigs. The incidence of *Salmonella* shedding in our study is similar to that of a study by Letellier and coworkers (1999), indicating that feeding specific egg yolk antibodies against *Salmonella* Typhimurium did not diminish colonization of this bacterium in mesenteric lymph nodes and feces in early weaned pigs (12 days old). This is in contrast to the findings that such products can help reduce enterotoxigenic *E. coli* in pigs (Imberechts et al., 1997: Owusu-Asiedu et al., 2003). We suspect that the invasive nature of *Salmonella* Typhimurium may result in a systemic infection, whereby organisms bypass the gut and move through vascular and/or lymphatic routes directly into colon, thus contaminating feces just prior to excretion. This possible mechanism of natural *Salmonella* infection may be an obstacle to the potential effects of in-feed antibodies, as noted by Jin and coworkers (1998) that such egg yolk products introduce specific antibodies to neutralize bacterial surfaces and inhibit adherence of bacterial antigens to the mucosal target sites. Therefore, naturally invasive *Salmonella* would differ from intestinal *E. coli*, including enterotoxigenic forms, which do not invade peripheral tissues or cause systemic infections. Organisms, such as *E. coli*, would likely be affected to a greater degree by specific egg yolk antibodies, directly providing resistance to bacterial attachment (Yokoyama et al., 1992). An additional explanation for the lack of effect of ASEYA is that an alteration of immunoglobulin (IgY) may have

occurred during digestion in the alimentary tract of the pig, thereby resulting in unsuccessful recognition of *Salmonella* (Letellier et al., 1999).

We also observed that the percentage of *Salmonella* shedding in pigs fed SDPP was not different from pigs fed the control diet. Similarly, Van Dijk and coworkers (2002) reported that fecal shedding of pathogenic *E. coli* O139:K82 detected from 19-day-old weaned pigs fed 8% SDPP and inoculated with *E. coli* O139:K82 did not differ from weaned pigs fed control diets.

In our study, pigs across all treatment groups had an approximately 60% decrease in *Salmonella* shedding 51 days post-inoculation compared to those one day post-inoculation. Inexplicitly, on day 88 of trial, *Salmonella* shedding across all treatments slightly increased.

In our study, *Salmonella* Typhimurium bacteria were detected from day 8 to day 118 of the experiment or from 1 day to 111 days after challenge. A previous study in our lab reported that pigs shed *Salmonella* until 64 days post-inoculation (Cullen et al., 2002). This indicates that the recent study shows longer shedding duration. In our study, pigs in all groups also shed *E. coli* from day 0 to day 118 of the experiment because *E. coli* bacteria are commonly found in the intestinal tract of animals. Even though pigs were transported to a conventional-type facility on day 89 of the experiment before samples were collected on day 118, no effects on *Salmonella* shedding were observed.

Antibiotic Resistance

In our study, antibiotic resistance of *E. coli* and *Salmonella* Typhimurium were analyzed via a minimum inhibitory concentration (MIC) broth dilution method. A

significant number of *E. coli* isolates demonstrated resistance to the test antibiotics, whereas far fewer *Salmonella* demonstrated resistance to those same antibiotics. Our results agree with those of Edrington et al. (2001), who reported that the increased prevalence of antimicrobial resistant *Salmonella* was not detected in market swine supplemented with carbadox and apramycin. This may be due to the fact that *Salmonella* may not be exposed to a sufficient degree to produce resistant phenotypes in the gut lumen. Therefore, the organisms did not adapt to the antibiotics, or they did not have adequate residence time in the gut to acquire antibiotic resistance because they are highly invasive (Jensen et al., 1998). Additionally, Spanoghe and Pohl (1987) indicate that *Salmonella* have a poor ability to obtain carbadox resistance from other sources.

We observed that a high percentage of *E. coli* isolated in this study was resistant to tetracycline prior to application of oxytetracycline. One possible reason is that pigs may receive resistant organisms from other sources, such as the environment in which pigs were placed before weaning or during moving to the experimental study unit (Hinton et al., 1985). Langlois and coworkers (1988) also observed that pigs in the weanling stage obtained from a farm, which was not exposed to antibiotics, had a high percentage of fecal coliforms resistant to tetracycline compared to pigs at other growth stages, which came from the same farm. This may indicate that animal age is a possible factor in presence of resistant isolates.

Our results illustrate that feeding apramycin had the most pronounced effect on the development of apramycin resistance. Similarly, Kim and coworkers (2005) reported a high percentage of apramycin resistant non-pathogenic *E. coli* in piglets supplemented with apramycin. This may support the findings of Gellin et al. (1989), who noted that

feeding sub-therapeutic and therapeutic levels of antibiotic to pigs results in a high proportion of antibiotic resistance in fecal bacteria. Dawson and coworkers (1984) also indicated an increase in drug resistance may be impacted by other factors which include "livestock history", "cross-contamination" and "farm environment".

Our antibiotic resistance results demonstrate that in-feed antibiotics impact naturally occurring *E. coli*, which reside in pig intestinal tract, and then develop resistance to antibiotics. Tetracycline resistant *E. coli* bacteria were detected across all treatments in a high proportion of isolates (60-88 %) from day 0 to day 118 of the experiment. The lowest percentage of tetracycline resistant *E. coli* was observed across all groups on day 58 of the experiment. These results indicate that tetracycline resistant *E. coli* were persistent in the intestine for a long duration.

In contrast to tetracycline resistance, *E. coli* isolated from pigs fed apramycin were resistant to apramycin 4 days after treatment was applied but not before. Over 80% of apramycin resistant *E. coli* isolates were observed from day 8 to day 21 of trial. The percentage of apramycin resistant *E. coli* was dropped by half after approximately 2 weeks after apramycin was withdrawn from pig feed. Less than 13% apramycin resistant *E. coli* were observed on days 58, 88, and 118 of trial. These observations indicate that apramycin resistant *E. coli* quickly decreased after drug was withdrawn.

The appearance of carbadox resistant *E. coli* was observed approximately 2 weeks after carbadox was used in the feed (day 28 of trial). Carbadox resistant *E. coli* isolates were slightly detected approximately 53 days after carbadox was removed (day 88 of trial). These results indicate that *E. coli* bacteria are resistant to carbadox when they are under exposure to this drug. Kim and coworkers (2005) also indicated that non-

pathogenic *E. coli* isolated from pigs is "drug-dependent" as they observed that piglets supplemented with apramycin, carbadox, and chlortetracycline had an increase in antibiotic resistant non-pathogenic *E. coli* in response to those three antibiotics.

The present study shows that feeding of egg yolk powder containing ASEYA did not affect the prevalence of antibiotic resistance of either *Salmonella* Typhimurium or *E. coli* isolates. Some researchers propose that chicken egg yolk antibodies could be used as an alternative to antibiotics (Wiedemann et al., 1991; Girard et al., 2006; Larsson and Carlander, 2003).

Growth Performance and Health

In this study, growth performance of pigs was measured in terms of body weight over the course of study. Body weights across all days were not different among weaned pigs challenged with *Salmonella* Typhimurium fed any of the diets, including the diet containing egg yolk powder with *Salmonella* antibodies. Pig body weights increased over time in all groups, as expected. Differences in growth rates also were not observed in a separate study involving 10 day old weaned pigs challenged with F18 enterotoxigenic *E. coli* and fed dietary spray dried animal plasma, spray dried porcine plasma, and chicken egg yolk antibodies containing specific antibodies or egg-yolk powder lacking specific antibodies from day 0 to 14 post-weaning (Owusu-Asiedu et al., 2002). In contrast, Yokoyama and coworkers (1997) reported greater weight gains in weaned pigs at 28-days of age inoculated with virulent F18+ *E. coli* and supplemented with anti-F18 fimbriae egg yolk antibodies at highest level (1:50). This research suggested that feeding egg yolk antibodies specific to F18 fimbriae could efficiently

diminish infection severity, thereby resulting in less weight loss after challenge. Also in contrast to our results, Ding and coworkers (2006a) observed that after inoculation with K88 *E. coli*, piglets supplemented with olaquindox and cyadox, derivatives of carbadox, showed improved growth performance, average daily gain and feed conversion ratio. A study also indicated that the greatest average growth rates were observed in calves treated with oxytetracycline after inoculation of *Salmonella* Typhimurium (Remillard et al., 1981).

Although final body weights and mean weights did not differ among treatments, this may be due to the length of time each diet treatment was applied. Therefore, we evaluated body weights at each time period. Our results indicate that dietary treatments differently affect body weights at each time point. In our study, we observed that pigs fed apramycin and carbadox had higher body weights compared to pigs fed the control diets on days 14, 21, 28 and 58 of trial. Similarly, pigs fed oxytetracycline had greater body weights than control pigs on day 58. A recent study by Kim et al. (2005) also showed that piglets fed apramycin, carbadox, and chlortetracycline had an increase in weight gains compared to pigs fed the control diets. Pigs supplemented with SDPP in our study had higher body weights than the control group on days 28 and 58 of trial. This finding is related to the investigation reported by Pierce and coworkers (2005), who indicated that feeding porcine plasma increased growth performance 7 days after weaning. The increase in growth performance observed by feeding porcine plasma may be associated with immunoglobulin molecules in the plasma product. In addition, the current study could also indicate that *Salmonella* infection triggered by such a challenge may not be severe enough to affect growth performance.

In this investigation, most pigs developed evidence of infection, including watery diarrhea and fever, 24 h following inoculation with *Salmonella* Typhimurium. Cote et al. (2004) noted that clinical signs associated with salmonellosis in pigs include "yellowish-diarrhea, fever, prostration, and/or mortality". We observed average higher rectal temperatures in all treatment groups of 40.07 °C 24 h post-inoculation, which is similar to what has been reported for pigs exhibiting signs of systemic salmonellosis (Pfizer Animal Health, 2006). That report indicates rectal temperatures as high as 40.5 °C to 41.6 °C after 24 to 48 h incubation following infection. In the current study, increased rectal temperatures on day 8 of trial were greater than reported by Bollen and coworkers (2000), who indicated that normal body temperature of swine is 38-39°C. Similarly, work by Balaji and coworkers (2000), showed that pigs (35 days old) orally challenged with 3×10^9 CFU of *Salmonella* Typhimurium had an increase in rectal temperatures by 12 h and had the highest rectal temperature at 42 h compared to control pigs. Higher rectal temperatures of challenged pigs were maintained through 5 days after challenge. Another study also showed that weaned pigs (24 days old) had increased rectal temperatures to a maximum (~ 40.7 °C) 2 days after pigs orally challenged with 6×10^9 CFU of *Salmonella* Typhimurium and decreased rectal temperatures (~ 40.2 °C) were detected 3 days after *Salmonella* inoculation. Rectal temperatures of challenged pigs (~39.9 °C) were not different from control pigs (~39.8 °C) 4 days after challenge (Turner et al., 2002). Our study shows that rectal temperatures of pigs in all treatments decreased by day 12 of trial or 5 days after challenge. However, daily rectal temperatures were not

measured from day 8 to day 12, so variations in rectal temperatures during this period cannot be determined.

In our study, rectal temperatures in pigs fed oxytetracycline were lower relative to pigs fed spray dried porcine plasma and egg yolk powder containing ASEYA. A previous study of calves challenged with *Salmonella* Typhimurium, showed that therapeutic dosages of oxytetracycline resulted in a more rapid decline of body temperature following challenge (Remillard et al., 1981). Therefore, it is possible that sub-therapeutic levels of antibiotics may reduce the numbers of *Salmonella* which infect the host, thus limiting the rise of body temperature. In our study, significant differences were observed but the range was only 0.5 °C which suggests biological responses were similar 24 h after challenge.

In our study, rectal temperatures of pigs fed egg yolk powder containing ASEYA were not different from pigs fed the control diet, apramycin and carbadox, egg yolk powder without ASEYA and SDPP. In contrast to our results, Yokoyama and coworkers (1998a) indicated that direct oral supplementation of isolated specific egg yolk antibodies against outer membrane protein, lipopolysaccharide, and flagella antigens of either *Salmonella* Typhimurium or *Salmonella* Enteritidis in solution for 3 days, 3 times per day, can diminish and protect against the virulence of *Salmonella* antigens in mice. These researchers further stated that, in animal studies, some factors including "challenge dose", "host susceptibility", and "bacterial pathogenicity" may influence the consequences of bacterial colonization even though specific antibodies have the same degree of activity to block bacterial attachment to gut epithelium.

In our study, only one pig from the control group in the second replicate trial died 2 weeks after inoculation. However, no other mortalities occurred in pigs fed antibiotics, egg yolk powder containing ASEYA, egg yolk powder without ASEYA, and SDPP. In contrast to our study, Van Dijk and coworkers (2002) reported that feeding 8% SDPP in weaned pigs (19 days old) challenged with pathogenic *E. coli* was not efficient to prevent mortality compared to weaned pigs fed control feed. However, other studies showed similar outcomes when compared to our study. Specific egg yolk antibody supplementation of calves by Yokoyama and coworkers (1998b) showed that 7 days post-inoculation no mortalities occurred in newborn calves orally inoculated with 10^{11} CFU *Salmonella* Typhimurium and fed commercial milk containing specific egg yolk antibodies against *Salmonella* Typhimurium at the highest concentration (1:1000). Whereas no survival was observed in calves fed similar egg antibodies at very low concentrations (1:<10). In the same study, researchers also found a longer onset period of fever in calves receiving specific egg yolk antibodies at high concentrations (1:1000) compared to calves supplemented at the lowest concentration (1:<10). Presumably, the numbers of bacteria required to cause infection by attaching to the target enterocytes may be lowered by specific yolk antibodies and reduce the level of infection and subsequent change in temperature.

In comparison between our study and the investigation by Yokoyama et al. (1998b) which observed a significant effect of *Salmonella* egg yolk antibodies on disease severity in calves, other than species differences, these researchers inoculated the infective *Salmonella* dose orally whereas we infected pigs orally and nasally. The inoculated routes may impact the consequences of egg yolk antibody used for control of

intestinal disease since egg yolk antibody potentially active at gut level. Furthermore, differences of digestive tract conditions between newborn calves from previous study and weaned pigs from our study may affect control of *Salmonella* infection when egg yolk antibodies were provided to animals. Findlay (1998) indicated that development of enzymatic activities and intestinal functions rely on animal ages and feed types. Newborn calves have incompletely developed gastrointestinal functions, particularly acid conditions in abomasums (Moran, 2002). Therefore, providing IgY to newborn calves may allow immunoglobulin molecules to pass through the digestive mechanisms in the stomach and remain active at intestinal sites. However, pigs in the weaning stage have better development of the digestive system, i.e., higher digestive enzyme secretions and activities in the gastrointestinal tract and greater acidic conditions in the stomach because weaned pigs start non-milk containing diets (Efird et al., 1982; Makkink et al., 1994). Thus, feeding egg yolk antibodies to weaned pigs, IgY may be digested during the digestive processes, resulting in IgY losing activity before immunoglobulin molecules reach the intestine. Another possible reason for observed differences is that calves received specific egg yolk antibodies which were directly diluted with milk, whereas weaned pigs in our study obtained egg yolk powder containing ASEYA mixed in dry feed. Specific yolk antibodies might be affected during feed mixing. In addition, these researchers directly fed individual calves with milk containing specific egg yolk antibodies, in contrast to our study where we fed pigs in a group setting. As a result, all pigs might not have received egg yolk powder containing ASEYA equally.

Immunological Response

Factors that may impact the host response to *Salmonella* infection, as indicated by Mastroeni (2002), are "bacterial virulence", "infectious dose" and "immunological status of host". These factors may be responsible for different disease severity and influence various consequences of systemic infection by *Salmonella*. In this study, we challenged weaned pigs with *Salmonella* concentration of 7.95×10^7 and 1.90×10^8 CFU/ml for the first and second replicate trials, respectively, which were higher than suggested dose of *Salmonella* Typhimurium ($>10^3$ CFU/ml), which causes acute infection in young pigs (Loynachan and Harris, 2005).

In our study, weaned pigs were orally and nasally challenged with *Salmonella* Typhimurium to simulate the conditions of gastrointestinal and systemic infection caused by *Salmonella*. After inoculation, the body develops an immune response to *Salmonella* infection (Mizuno, 2004). Our results indicate that predominant responses in pigs from all groups following challenge were increased white blood cell counts (1-7 days post-inoculation) and *Salmonella* antibody titers (7-21 days post-inoculation). The increase in white blood cells was similar to that of Wick (2004), who determined the increase in white blood cells was due to increase macrophages, neutrophils, dendritic cells, natural killer cells, and killer T cells following *Salmonella* infection. However, Turner and coworkers (2002) observed that *Salmonella* Typhimurium challenge did not significantly impact total serum immunoglobulin G (IgG) in 24-days-old pigs; although an increasing trend of IgG was observed over the course of study (0-14 days post-challenge). Steinbach and coworkers (2003) also reported an increase in specific serum

immunoglobulin G levels of pigs (14-70 days old) 7 days after oral *Salmonella* Typhimurium infection and continued to elevate after 14 and 21 days of infection (the end of study). Gray and coworkers (1995) also reported that pigs (10-14 days old) challenged with *Salmonella* Choleraesuis had an increase in specific serum immunoglobulin G from week 2 to week 12 after *Salmonella* challenge by intranasal and gastric routes. In our study, if we analyze serum antibody a longer period in pigs infected with *Salmonella*, greater increases may be observed.

In the present study, mean *Salmonella* antibody responses across all days were similar among pigs for all treatment groups. Dietary treatments also did not affect white blood cell counts among all groups of pigs. The varying numbers of white blood cells observed through this study were in the normal range of 6.3-21.1 10^3/ml (Bollen et al., 2000), but average numbers of white blood cells tended to be greater 7 days post-challenge in pigs fed non-medicated diets compared to those receiving antibiotics. These data may indicate that antibiotic diminishes the numbers of *Salmonella* Typhimurium in the intestine, possibly lowering immune induction by *Salmonella* pathogens as observed in studies examining the use of olaquindox and cyadox in controlling *E. coli* infection (Ding et al., 2006b).

Throughout the duration of this study, interleukin-1β (IL-1β) was detected in only 5 pigs and those levels were low with the range of 3 to 5 pg/ml. In response to bacterial infection, interleukin-1 has been associated with fever, increasing lymphocyte responses, and inducing acute-phase responses (Curfs et al., 1997). Dinarello (1994) also has reported that several systemic effects are induced by interleukin-1 as "femtomolar". In our study, only one of 5 pigs (control group) in which IL-1β was detected had fever

(40.22 °C) and *Salmonella* shedding on day 21 of trial. Our result showed low serum IL-1β after *Salmonella* challenge. However, the assay we used for IL-1β analysis has sensitivity to detect ~15 pg/ml or approximately 882 pM which is higher than the potential active range which can be "femtomolar". Therefore, this assay is not sensitive enough to detect very low levels of IL-1β in pigs. In our study, we analyzed IL-1β 24 h after challenge. In general, an increase in IL-1β has been reported 6-12 h after lipopolysaccharide activation of endothelial cells *in vitro* (McGuire et al., 2005).

The relatively low level of systemic immune responses observed in our study may be linked to local immune protection in the intestinal tract. Wijburg and coworkers (2006) indicate that *Salmonella* Typhimurium bacteria were bound by non-specific secretory immunoglobulin A stimulated by microflora in the gut mucosa of mice, thereby minimizing the ability of invasive bacteria to invade and activate systemic responses. Additionally, Lorenz and Newberry (2004) reported that increased specific immunoglobulin A in gut mucosa can protect challenged *Salmonella* Typhimurium at intestinal level. During penetration by *Salmonella* Typhimurium, local cytokines and chemokines, such as IL-8 and CCL20, in the alimentary tract of pigs are secreted to recruit neutrophils and dendritic cells, respectively, for control of invasive pathogens (Skjolaas et al., 2006).

Feeding of anti-*Salmonella* egg yolk antibodies did not affect measured immune responses in weaned pigs inoculated with *Salmonella* Typhimurium. This is possibly explained by natural invasion of gut epithelial cells by *Salmonella* (Sansonetti, 2002), thereby escaping adherence to specific yolk antibodies in the gut lumen. In addition, the

stability of antibodies in egg yolk may be less in the pig gastrointestinal tract due to digestive environments and mechanisms; thus, antibodies would be less able to bind *Salmonella* bacteria before they invade the gut epithelium cells (Letellier et al., 1999).

In this study, we observed no correlation among *Salmonella* shedding, body weight, rectal temperature, *Salmonella* antibody, white blood cell counts, and IL-1β. The lack of effect of *Salmonella* challenge on growth performance may be because shedding bacteria may not have infected the body or may have infected the pig less severely. Rectal temperatures, *Salmonella* antibody, white blood cell counts, and IL-1β also are independent. This is possibly explained that, after challenge, *Salmonella* bacteria get into the host body through oral and nasal routes, reside in the body, and are excreted in feces (*Salmonella* shedding). By the oral challenge, *Salmonella* bacteria pass through the digestive tract until they reach the intestinal target and disseminate through inner systems as indicated by Wood et al. (1989). By the intranasal inoculation, *Salmonella* bacteria are able to penetrate the tonsils and lung and circulate through the lymphatic system toward internal organs, i.e., ileum, cecum, colon, spleen, liver (Fedorka-Cray et al., 1995). When *Salmonella* bacteria are in the intestinal tract, systemic circulation or inner organs, they may not cause infection; on the other hand, pigs may only carry *Salmonella* in body and then shed in feces. Ekperigin and Nagaraja (1998) indicated that *Salmonella* bacteria, which do not cause infection, possibly colonize in intestinal tract, mingle with normal flora, and excrete in feces. Pigs that obtain *Salmonella* may be carriers of *Salmonella* after they recover from infection (Ekperigin and Nagaraja, 1998) or most pigs just carry *Salmonella* with healthy appearance (Cote et al., 2004). These

carrier pigs are sources of *Salmonella* contamination on farms, slaughter environments, or pork products.

The outcomes of controlling shedding, health, infectious incidence and immune response by providing specific chicken egg yolk antibodies against *Salmonella* antigens in this study were different from previous studies (Yokoyama et al., 1998a; Yokoyama et al., 1999b). These may vary depending on host species, animal ages, gastrointestinal structures and functions of hosts, antibody application method, and duration of antibody supplementation. Additionally, some reports show that specific yolk antibody dose is important for efficient use to inhibit bacterial attachment and control animal mortality (Jin et al., 1998; Yokoyama et al., 1998b). Uses of specific egg yolk antibodies against *Salmonella* antigens in protection of *Salmonella* infectious disease and prevention bacterial shedding are limited in weaned pigs experimentally inoculated with *Salmonella* by intranasal and oral routes, whereas antibiotics are effective to control *Salmonella* shedding and incidence of *Salmonella* infection. However, use of in-feed antibiotics promotes the ability of non-pathogenic *E. coli* in the intestine to resistant antibiotics, resulting in large public health concerns.

References

Balaji, R., K. J. Wright, C. M. Hill, S. S. Dritz, E. L. Knoppel, and J. E. Minton. 2000. Acute phase responses of pigs challenged orally with *Salmonella* Typhimurium. Journal of Animal Science. 78(7):1885-1891.

Bollen, P. J. A., A. K. Hansen, and H. J. Rasmussen. 2000. Important biological features Page 1-15 in The Laboratory Swine. M. A. Suckow, ed. CRC Press LLC, Boca Raton, FL.

Cote, S., A. Letellier, L. Lessard, and S. Quessy. 2004. Distribution of *Salmonella* in tissues following natural and experimental infection in pigs. Canadian Journal of Veterinary Research. 68(4):241-248.

Cullen, P., A. G. Mathew. R. Clift, and S. Chattin. 2002. Effects of management and environmental conditions on antibiotic resistance in bacteria associated with swine. Annual Report. Department of Animal Science, The University of Tennessee, Knoxville, TN.

Curfs, J. H., J. F. Meis, and J. A. Hoogkamp-Korstanje. 1997. A primer on cytokines: Sources, receptors, effects, and inducers. Clinical Microbiology Reviews. 10(4):742-780.

Dawson, K. A., B. E. Langlois, T. S. Stahly, and G. L. Cromwell. 1984. Antibiotic resistance in anaerobic and coliform bacteria from the intestinal tract of swine fed therapeutic and subtherapeutic concentrations of chlortetracycline. Journal of Animal Science. 58(1):123-131.

Dinarello, C. A. 1994. The interleukin-1 family: 10 years of discovery. The FASEB Journal. 8(15):1314-1325.

Ding, M. X., Z. H. Yuan, Y. L. Wang, H. L. Zhu, and S. X. Fan. 2006a. Olaquindox and cyadox stimulate growth and decrease intestinal mucosal immunity of piglets orally inoculated with *Escherichia coli*. Journal of Animal Physiology and Animal Nutrition. 90(5-6):238-243.

Ding, M. X., Y. L. Wang, H. L. Zhu, and Z. H. Yuan. 2006b. Effects of cyadox and olaquindox on intestinal mucosal immunity and on fecal shedding of *Escherichia coli* in piglets. Journal of Animal Science. 84(9):2367-2373.

Ebner, P. D., and A. G. Mathew. 2000. Effects of antibiotic regimens on the fecal shedding patterns of pigs infected with *Salmonella* Typhimurium. Journal of Food Protection. 63(6):709-714.

Edrington, T. S., R. B. Harvey, L. A. Farrington, and D. J. Nisbet. 2001. Evaluation of subtherapeutic use of the antibiotics apramycin and carbadox on the

prevalence of antimicrobial-resistant *Salmonella* infection in swine. Journal of Food Protection. 64(12):2067-2070.

Efird, R. C., W. D. Armstrong, and D. L. Herman. 1982. The development of digestive capacity in young pigs: Effects of weaning regimen and dietary treatment. Journal of Animal Science. 55(6):1370-1379.

Ekperigin H. E., and K. V. Nagaraja. 1998. *Salmonella*. Page 17-29 in Microbial Foodborne Pathogens. L. Tollefson, ed. W. B. Saunders Company, Philadelphia, PA.

Evangelisti, D. G., A. R. English, A. E. Girard, J. E. Lynch, and I. A. Solomons. 1975. Influence of subtherapeutic levels of oxytetracycline on *Salmonella* Typhimurium in swine, calves, and chickens. Antimicrobial Agents and Chemotherapy. 8(6):664-672.

Fedorka-Cray, P. J., L. C. Kelley, T. J. Stabel, J. T. Gray, and J. A. Laufer. 1995. Alternate routes of invasion may affect pathogenesis of *Salmonella* Typhimurium in swine. Infection and Immunity. 63(7):2658-2664.

Findlay, A. L. R. 1998. The developing gastro-intestinal system. Available online: http://www.chu.cam.ac.uk/~ALRF/gidevel.htm

Gellin, G., B. E. Langlois, K. A. Dawson, and D. K. Aaron. 1989. Antibiotic resistance of Gram negative enteric bacteria from pigs in three herds with different histories of antibiotic exposure. Applied and Environmental Microbiology. 55(9):2287-2292.

Girard, F., I. Batisson, G. Martinez, C. Breton, J. Harel, and J. M. Fairbrother. 2006. Use of virulence factor-specific egg yolk-derived immunoglobulins as a promising alternative to antibiotics for prevention of attaching and effacing *Escherichia coli* infections. FEMS Immunology and Medical Microbiology. 46(3):340-350.

Gray, J. T., P. J. Fedorka-Cray, T. J. Stabel, and M. R. Ackermann. 1995. Influence of inoculation route on the carrier state of *Salmonella* Choleraesuis in swine. Veterinary Microbiology. 47(1-2):43-59.

Hinton, M., D. J. Hampson, E. Hampson, and A. H. Linton. 1985. The effects of oxytetracycline on the intestinal *Escherichia coli* flora of newly weaned pigs. The Journal of Hygiene. 95(1):77-85.

Imberechts, H., P. Deprez, E. Van Driessche, and P. Pohl. 1997. Chicken egg yolk antibodies against F18ab fimbriae of *Escherichia coli* inhibit shedding of F18

positive *E. coli* by experimentally infected pigs. Veterinary Microbiology. 54(3-4):329-341.

Jensen, V. B., J. T. Harty, and B. D. Jones. 1998. Interactions of the invasive pathogens *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Shigella flexneri* with M cells and murine Peyer's patches. Infection and Immunity. 66(8):3758-3766.

Jin, L. Z., S. K. Baidoo, R. R. Marquardt, and A. A. Frohlich. 1998. *In vitro* inhibition of adhesion of enterotoxigenic *Escherichia coli* K88 to piglet intestinal mucus by egg-yolk antibodies. FEMS Immunology and Medical Microbiology. 21(4):313-321.

Kim, L. M., J. T. Gray, B. G. Harmon, R. D. Jones, and P. J. Fedorka-Cray. 2005. Susceptibility of *Escherichia coli* from growing piglets receiving antimicrobial feed additives. Foodborne Pathogens and Disease. 2(4):304-316.

Langlois, B. E., K. A. Dawson, I. Leak, and D. K. Aaron. 1988. Effect of age and housing location on antibiotic resistance of fecal coliforms from pigs in a non-antibiotic-exposed herd. Applied and Environmental Microbiology. 54(6):1341-1344.

Larsson, A., and D. Carlander. 2003. Oral immunotherapy with yolk antibodies to prevent infections in humans and animals. Upsala Journal of Medical Sciences. 108(2):129-140.

Letellier, A., S. Messier, L. Lessard, and S. Quessy. 1999. Assessment of different treatments to reduce *Salmonella* in swine. Proceedings of the 3rd International Symposium on the Epidemiology and Control of *Salmonella* in Pork, Washington D. C. August 5-7, 1999. p. 321-325.

Lorenz, R. G., and R. D. Newberry. 2004. Isolated lymphoid follicles can function as sites for induction of mucosal immune responses. Annals of the New York Academy of Sciences. 1029:44-57.

Loynachan, A. T., and D. L. Harris. 2005. Dose determination for acute *Salmonella* infection in pigs. Applied and Environmental Microbiology. 71(5):2753-2755.

Makkink, C. A., P. J. Berntsen, B. M. op den Kamp, B. Kemp, and M. W. Verstegen. 1994. Gastric protein breakdown and pancreatic enzyme activities in response to two different dietary protein sources in newly weaned pigs. Journal of Animal Science. 72(11):2843-2850.

Mastroeni, P. 2002. Immunity to systemic *Salmonella* infection. Current Molecular Medicine. 2(4):393-406.

McGuire, T. R., W. J. Trickler, L. Smith, E. B. Hoie, and D. W. Miller. 2005. Release of TNF-alpha and IL-1beta from porcine brain endothelium corresponds to the

pyrogenic potential of three marketed formulations of amphotericin. Inflammation Research. 54(9):375-379.

Mizuno, Y. 2004. Host defense mechanisms against *Salmonella* infection. Japanese Journal of Clinical Immunology. 27(6):367-372.

Moran, J. 2002. Calf rearing: A practical guide 2nd ed. CSIRO Publishing. Collingwood Victoria, Australia. p. 1-14

Owusu-Asiedu, A., S. K. Baidoo, C. M. Nyachoti, and R. R. Marquardt. 2002. Response of early-weaned pigs to spray-dried porcine or animal plasma-based diets supplemented with egg-yolk antibodies against enterotoxigenic *Escherichia coli*. Journal of Animal Science. 80(11):2895-2903.

Owusu-Asiedu, A., C. M. Nyachoti, and R. R. Marquardt. 2003. Response of early-weaned pigs to an enterotoxigenic *Escherichia coli* (K88) challenge when fed diets containing spray-dried porcine plasma or pea protein isolate plus egg yolk antibody, zinc oxide, fumaric acid, or antibiotic. Journal of Animal Science. 81(7):1790-1798.

Pfizer Animal Health. 2006. *Salmonella*. Available online: http://www.pfizerah.com/health.asp?country=US&species=SW&lang=EN&drug=NX&t=2328&key=1936&type=3&sec

Pierce, J. L., G. L. Cromwell, M. D. Lindemann, L. E. Russell, and E. M. Weaver. 2005. Effects of spray-dried animal plasma and immunoglobulins on performance of early weaned pigs. Journal of Animal Science. 83(12):2876-2885.

Remillard, R. L., D. E. Johnson, and J. S. Avens. 1981. Subtherapeutic tetracycline effects on recovery patterns of calves after *Salmonella* Typhimurium challenge. Journal of Dairy Science. 64(3):454-458.

Sansonetti, P. 2002. Host-pathogen interaction: The seduction of molecular cross talk. Gut. 50(Supplement 3):iii 2-8.

Skjolaas, K. A., T. E. Burkey, S. S. Dritz, and J. E. Minton. 2006. Effects of *Salmonella* enterica serovars Typhimurium (ST) and Choleraesuis (SC) on chemokine and cytokine expression in swine ileum and jejunal epithelial cells. Veterinary Immunology and Immunopathology. 111(3-4):199-209.

Spanoghe, P., and P. Pohl. 1987. Comparison between the influence of carbadox and that of traditional antibiotics on the resistance of enterobacteria in pigs. Annals of Veterinary Research. 18(3):249-254.

Steinbach, G., U. Methner, S. Springer, T. Linder, and H. J. Selbitz. 2003. The humeral immune response of swine after experimental infection with *Salmonella* Typhimurium. Berliner und Münchener tierärztliche Wochenschrift. 116(3-4):124-129.

Turner, J. L., S. S. Dritz, J. J. Higgins, and J. E. Minton. 2002. Effects of Ascophyllum nodosum extract on growth performance and immune function of young pigs challenged with *Salmonella* Typhimurium. Journal of Animal Science. 80(7):1947-1953.

Van Dijk, A. J., P. M. M. Enthoven, S. G. C. Van den Hoven, M. M. M. H. Van Laarhoven, T. A. Niewold, M. J. A. Nabuurs, and A. C. Beynen. 2002. The effect of dietary spray-dried porcine plasma on clinical response in weaned piglets challenged with a pathogenic *Escherichia coli*. Veterinary Microbiology. 84(3):207-218.

Wick, M. J. 2004. Living in the danger zone: Innate immunity to *Salmonella*. Current Opinion in Microbiology. 7(1):51-57.

Wiedemann, V., E. Linckh, R. Kuhlmann, P. Schmidt, and U. Losch. 1991. Chicken egg antibodies for prophylaxis and therapy of infectious intestinal diseases. V. In vivo studies on protective effects against *Escherichia coli* diarrhea in pigs. Journal of Veterinary Medicine. 38(4):283-291.

Wijburg, O. L., T. K. Uren, K. Simpfendorfer, F. E. Johansen, P. Brandtzaeg, and R. A. Strugnell. 2006. Innate secretory antibodies protect against natural *Salmonella* Typhimurium infection. The Journal of Experimental Medicine. 203(1):21-26.

Wood, R. L., A. Pospischil, and R. Rose. 1989. Distribution of persistent *Salmonella* Typhimurium infection in internal organs of swine. American Journal of Veterinary Research. 50(7):1015-1021.

Yokoyama, H., R. C. Peralta, R. Diaz, S. Sendo, Y. Ikemori, and Y. Kodama. 1992. Passive protective effect of chicken egg yolk immunoglobulins against experimental enterotoxigenic *Escherichia coli* infection in neonatal piglets. Infection and Immunity. 60(3):998-1007.

Yokoyama, H., T. Hashi, K. Umeda, F. C. Icatlo Jr., M. Kuroki, Y. Ikemori, and Y. Kodama. 1997. Effect of oral egg antibody in experimental F18+ *Escherichia coli* infected in weaned pigs. Journal of Veterinary and Medical Science. 59(10):917-921.

Yokoyama, H., K. Umeda, R. C. Peralta, T. Hashi, F. C. Jr. Icatlo, M. Kuroki, Y. Ikemori, and Y. Kodama. 1998a. Oral passive immunization against experimental salmonellosis in mice using chicken egg yolk antibodies specific for *Salmonella* Enteritidis and *S*. Typhimurium. Vaccine. 16(4):388-393.

Yokoyama, H., R. C. Peralta, K. Umeda, T. Hashi, F. C. Jr. Icatlo, M. Kuroki, Y. Ikemori, and Y. Kodama. 1998b. Prevention of fatal salmonellosis in neonatal calves, using orally administered chicken egg yolk *Salmonella*-specific antibodies. American Journal of Veterinary Research. 59(4):416-420.

PART VI. CONCLUSIONS AND IMPLICATIONS

Feeding egg yolk powder containing anti-*Salmonella* egg yolk antibody to weaned pigs orally and nasally challenged with *Salmonella* Typhimurium was not effective in controlling *Salmonella* shedding, did not affect growth performance, and did not provide obvious health benefits. Our current work indicates little benefit of anti-*Salmonella* egg yolk antibody in reducing risks of *Salmonella* in pigs which result in contamination of pork products via shedding in market animals. In contrast, antibiotic regimens appear to offer some benefits in reducing shedding and controlling infection in young pigs. However, in-feed inclusion of anti-*Salmonella* egg yolk antibody did not appear to add additional risks associated with antibiotic resistant bacteria. Further studies that include confirmation of anti-*Salmonella* egg yolk antibody stability in gastrointestinal tract, possible uses in mature animals, antibody stability or loss during feed mill processing and storage, and applications for large quantity use in commercial animal production will be necessary.

APPENDIX

Table 1. Treatments applied to pig groups (n=22 pigs/treatment in 2 replicate trials)

Treatment	Description
Control (C)	Control diet formulated at or above 1998 NRC recommendations.
Apramycin[1] and Carbadox[2] (APR+CB)	Control treatment plus apramycin at 150 g/ton for 14 days, followed by carbadox at 25 g/ton until pigs reached 35 kg.
Oxytetracycline[3] (OXY)	Control treatment plus oxytetracycline at 100 g/ton of feed until pigs reached 35 kg.
Anti-*Salmonella* egg yolk antibodies[4] (ASEYA)	Control treatment plus egg yolk powder with antibodies specific to *Salmonella* Typhimurium at a level of 6 kg/ton of feed for 14 days
Egg yolk antibodies without ASEYA[5] (EY)	Control treatment plus egg yolk powder without antibodies specific to *Salmonella* Typhimurium at a level of 6 kg/ton of feed for 14 days
Spray dried porcine[6] plasma (SDPP)	Control treatment plus spray dried porcine plasma at a level of 3 % of feed for 14 days

[1]Apramycin (Apralan G 20; Elanco Animal Health, Greenfield, IN)
[2]Carbadox (Madcadox 10; Phibro Animal Health, Ridgefield Park, NJ)
[3]Oxytetracycline (Phoenix Scientific, Inc., St. Joseph, MO)
[4]Dried egg yolk powder (University of Manitoba, Winnipeg, Canada)
[5]Spray dried pasteurized egg yolk powder (Primera Foods, Cameron, WI)
[6]Spray dried porcine plasma (AP920; American Protein Corp, Ankeny, IA)

Table 2. Composition of treatment diets (Phase I[1]: for 14 days)

	Diet[2]					
Item	C	APR+CB	OXY	ASEYA	EY	SDPP
Ingredients, %						
Corn	55.70	55.68	55.70	55.70	55.70	58.20
Soy Bean Meal, 48%	33.65	33.65	33.70	33.05	33.05	28.30
Fish meal	5.00	5.00	5.00	5.00	5.00	5.00
Salt (NaCl)	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin/Mineral Premix[3]	2.50	2.50	2.50	2.50	2.50	2.50
Animal Fat[4]	3.00	3.00	3.00	3.00	3.00	3.00
APR	-	0.015	-	-	-	-
OXY	-	-	0.01	-	-	-
ASEYA	-	-	-	0.60	-	-
EY	-	-	-	-	0.60	-
SDPP	-	-	-	-	-	3.00
Calculated nutrient composition						
Crude protein, %	23.50	23.50	23.50	23.40	23.40	23.40
ME, Kcal/kg	3,428.3	3,427.6	3,427.8	3,407.8	3,407.8	3,442.3
Calcium, %	1.01	1.01	1.01	1.01	1.01	1.0
Total phosphorus, %	0.73	0.73	0.73	0.73	0.73	0.75
Available P[5], %	0.43	0.43	0.43	0.43	0.43	0.48
Lysine, %	1.40	1.40	1.40	1.40	1.40	1.45

[1]Based on 23.7% crude protein, 1.35% lysine (total basis) (NRC, 1998)
[2]C = Control diet without antibiotics or egg yolk products
APR+CB = Control diet + apramycin followed by carbadox
OXY = Control diet + oxytetracycline
ASEYA = Control diet + egg yolk powder containing anti-*Salmonella* antibody
EY = Control diet + egg yolk powder without anti-*Salmonella* antibody
SDPP = Control diet + spray dried porcine plasma
[3]Co-op Swine Base Mix "50" (Tennessee Farmers Cooperative, LaVergne, TN)
[4]Co-op FAT MIX "30" (Tennessee Cooperative, LaVergne, TN)
[5]Available phosphorus

Table 3. Composition of treatment diets (Phase II[1]: after 14 days until 20 kg)

	Diet[2]					
Item	C	APR+CB	OXY	ASEYA	EY	SDPP
Ingredient, %						
Corn	60.85	60.85	60.84	60.85	60.85	58.20
Soy Bean Meal, 48%	28.50	28.50	28.50	28.50	28.50	28.30
Fish meal	5.00	5.00	5.00	5.00	5.00	5.00
Salt (NaCl)	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin/Mineral Premix[3]	2.50	2.50	2.50	2.50	2.50	2.50
Animal Fat[4]	3.00	3.00	3.00	3.00	3.00	3.00
CB	-	0.003	-	-	-	-
OXY	-	-	0.01	-	-	-
Calculated nutrient composition						
Crude protein, %	21.50	21.50	21.50	21.40	21.40	21.40
ME, Kcal/kg	3,427.6	3,427.6	3,427.6	3,427.6	3,427.6	3,427.6
Calcium, %	1.00	1.00	1.00	1.00	1.00	1.00
Total phosphorus, %	0.71	0.71	0.71	0.71	0.71	0.71
Available P[5], %	0.43	0.43	0.43	0.43	0.43	0.43
Lysine, %	1.26	1.26	1.26	1.26	1.26	1.26

[1]Based on 20.9% crude protein, 1.15% lysine (total basis) (NRC, 1998)
[2]C = Control diet without antibiotics or egg yolk products
APR+CB = Control diet + apramycin followed by carbadox
OXY = Control diet + oxytetracycline
ASEYA = Control diet + egg yolk powder containing anti-*Salmonella* antibody
EY = Control diet + egg yolk powder without anti-*Salmonella* antibody
SDPP = Control diet + spray dried porcine plasma
[3]Co-op Swine Base Mix "50" (Tennessee Farmers Cooperative, LaVergne,TN)
[4]Co-op FAT MIX "30" (Tennessee Cooperative, LaVergne, TN)
[5]Available phosphorus

Table 4. Composition of treatment diets (Phase III[1]: from 20 kg until 35 kg)

	Diet[2]					
Item	C	APR+CB	OXY	ASEYA	EY	SDPP
Ingredient, %						
Corn	66.35	66.34	66.34	66.35	66.35	66.35
Soy Bean Meal, 48%	24.00	24.00	24.00	24.00	24.00	24.00
Fish meal	4.00	4.00	4.00	4.00	4.00	4.00
Salt (NaCl)	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin/Mineral Premix[3]	2.50	2.50	2.50	2.50	2.50	2.50
Animal Fat[4]	3.00	3.00	3.00	3.00	3.00	3.00
CB	-	0.003	-	-	-	-
OXY	-	-	0.01	-	-	-
Calculated nutrient composition						
Crude protein, %	19.20	19.20	19.20	19.20	19.20	19.20
ME, Kcal/kg	3,426.7	3,426.7	3,426.7	3,426.7	3,426.7	3,426.7
Calcium, %	0.94	0.94	0.94	0.94	0.94	0.94
Total phosphorus, %	0.67	0.67	0.67	0.67	0.67	0.67
Available P[5], %	0.39	0.39	0.39	0.39	0.39	0.39
Lysine, %	1.09	1.09	1.09	1.09	1.09	1.09

[1]Based on 18.0% crude protein, 0.95% lysine (NRC, 1998)
[2]C = Control diet without antibiotics or egg yolk products
APR+CB = Control diet + apramycin followed by carbadox
OXY = Control diet + oxytetracycline
ASEYA = Control diet + egg yolk powder containing anti-*Salmonella* antibody
EY = Control diet + egg yolk powder without anti-*Salmonella* antibody
SDPP = Control diet + spray dried porcine plasma
[3]Co-op Swine Base Mix "50" (Tennessee Farmers Cooperative, LaVergne, TN)
[4]Co-op FAT MIX "30" (Tennessee Cooperative, LaVergne, TN)
[5]Available phosphorus

Table 5. MIC Breakpoints for antibiotics and antibiotic dilution ranges for *Salmonella enterica* Typhimurium and *E. coli*

	Bacterial types			
	E. coli		*Salmonella* Typhimurium	
Antibiotic types	Breakpoint, μg/ml	Dilution range, μg/ml	Breakpoint, μg/ml	Dilution range, μg/ml
Tetracycline	≥16[1,2]	0-128	≥16[1,2]	0-128
Apramycin	≥32[2]	0-128	≥32[2]	0-128
Carbadox	≥64[3]	0-128	≥64[3]	0-128

[1]National Committee for Clinical Laboratory Standards, 2002
[2]National Antimicrobial Resistance Monitoring System—Enteric Bacteria, 2001
[3]Danish Integrated Antimicrobial Resistance Monitoring and Research Program, 1999

Table 6. Effect of dietary treatments on various days on percentage of pigs shedding *Salmonella* Typhimurium[1]

Day[3]	Treatment[2]						
	C	APR+CB	OXY	ASEYA	EY	SDPP	Day Mean
0	0	0	0	0	0	0	0[g]
7[4]	0	0	0	0	0	0	0[g]
8	100	77.27	86.36	100	100	100	93.94[a]
12	95.45	27.27	63.64	86.36	95.45	100	78.03[b]
14	86.36	22.73	36.36	86.36	100	100	71.97[b]
21	77.27	40.91	54.55	59.09	63.64	59.09	59.09[c]
28	59.09	40.91	22.73	59.09	50.00	54.55	47.73[d]
58	13.64	27.27	13.64	31.82	4.54	0	15.15[f]
88	31.82	45.45	27.27	36.36	40.91	22.73	34.09[e]
118	27.27	27.27	0	0	9.09	4.54	11.36[f]
Treatment Mean	61.36[x]	38.64[y]	38.07[y]	57.39[x]	57.95[x]	55.11[x]	

[a-g]Means within a column without a common superscript letter differ (P<0.05).
[x-y]Means within a row without a common superscript letter differ (P<0.05).
[1]Data are least squares means from two replicate trials (n = 22 pigs/treatment).
[2]C = Control diet without antibiotics or egg yolk products
APR+CB = Control diet + apramycin followed by carbadox
OXY = Control diet + oxytetracycline
ASEYA = Control diet + egg yolk powder containing anti-*Salmonella* antibody
EY = Control diet + egg yolk powder without anti-*Salmonella* antibody
SDPP = Control diet + spray dried porcine plasma
[3]Days of experiment.
[4]All pigs were challenged with *Salmonella* Typhimurium on day 7.
Treatment effects (P<0.0001).
Day effects (P<0.0001).
Treatment × Day effects (P<0.0001).
SEM = 4.65, 4.78 and 8.90 for treatment, day, and interaction means, respectively.

Table 7. Effect of dietary treatments on various days on percentage of pigs shedding *E. coli*[1]

Day[3]	Treatment[2]						Day Mean
	C	APR+CB	OXY	ASEYA	EY	SDPP	
0	100	100	100	100	100	100	100[a]
7[4]	100	81.82	100	100	95.45	100	96.21[ab]
8	95.45	90.91	100	100	95.45	95.45	96.21[ab]
12	100	95.45	95.45	95.45	100	100	97.73[ab]
14	90.91	100	95.45	81.82	100	100	94.70[abc]
21	95.45	100	77.27	77.27	86.36	95.45	88.64[d]
28	95.45	90.91	86.36	100	90.91	100	93.94[bc]
58	95.45	77.27	90.91	68.18	100	90.91	87.12[d]
88	95.45	90.91	100	100	100	95.45	96.97[ab]
118	90.91	90.91	100	95.45	81.82	81.82	90.15[cd]
Treatment Mean	95.91	91.82	94.55	91.82	95.00	95.91	

[a-d] Means within a column without a common superscript letter differ (P<0.05).
[1]Data are least squares means from two replicate trials (n = 22 pigs/treatment).
[2]C = Control diet without antibiotics or egg yolk products
APR+CB = Control diet + apramycin followed by carbadox
OXY = Control diet + oxytetracycline
ASEYA = Control diet + egg yolk powder containing anti-*Salmonella* antibody
EY = Control diet + egg yolk powder without anti-*Salmonella* antibody
SDPP = Control diet + spray dried porcine plasma
[3]Days of experiment.
[4]All pigs were challenged with *Salmonella* Typhimurium on day 7.
Day effects (P<0.0001)
Treatment × Day effects (P<0.0001)
SEM = 2.53, 2.63 and 5.14 for treatment, day, and interaction means, respectively.

Table 8. Effect of dietary treatments on various days on tetracycline resistance of *E. coli* (%) isolated from pigs[1]

Day[3]	Treatment[2]						
	C	APR+CB	OXY	ASEYA	EY	SDPP	Day Mean
0	97.73	87.50	97.73	76.14	85.23	82.95	87.88[a]
7[4]	76.14	71.59	93.18	79.55	82.95	71.59	79.17[b]
8	65.91	82.95	97.73	79.55	84.09	75.00	80.87[ab]
12	65.91	75.00	94.32	43.18	87.50	59.09	70.83[c]
14	57.95	72.73	93.18	34.09	80.63	71.59	68.37[cd]
21	70.45	78.41	77.27	39.77	72.73	65.91	67.42[cd]
28	68.18	53.41	80.68	65.91	52.27	57.95	63.07[cd]
58	71.59	37.50	78.41	23.86	86.36	63.64	60.23[d]
88	68.18	65.91	89.77	61.36	45.45	48.86	63.26[cd]
118	59.09	53.41	85.23	65.91	75.00	48.86	64.58[cd]
Treatment Mean	70.11[xy]	67.84[xy]	88.75[w]	56.93[z]	75.23[x]	64.55[y]	

[a-d]Means within a column without a common superscript letter differ (P<0.05).
[w-z]Means within a row without a common superscript letter differ (P<0.05).
[1]Data are least squares means from two replicate trials with a total of 4,763 isolates.
[2]C = Control diet without antibiotics or egg yolk products
APR+CB = Control diet + apramycin followed by carbadox
OXY = Control diet + oxytetracycline
ASEYA = Control diet + egg yolk powder containing anti-*Salmonella* antibody
EY = Control diet + egg yolk powder without anti-*Salmonella* antibody
SDPP = Control diet + spray dried porcine plasma
[3]Days of experiment.
[4]All pigs were challenged with *Salmonella* Typhimurium on day 7.
Treatment effects (P<0.0001).
Day effects (P<0.0001).
Treatment × Day effects (P<0.0001).
SEM = 6.98, 7.11 and 9.75 for treatment, day, and interaction means, respectively.

Table 9. Effect of dietary treatments on various days on apramycin resistance of *E. coli* (%) isolated from pigs[1]

Day[3]	Treatment[2]						
	C	APR+CB	OXY	ASEYA	EY	SDPP	Day Mean
0	6.82	0	5.68	0	1.14	0	2.27[d]
7[4]	1.14	55.68	3.41	6.82	11.36	3.41	13.64[a]
8	0	84.09	2.27	2.27	0	1.14	14.96[a]
12	1.14	85.23	0	0	0	0	14.39[a]
14	0	86.36	4.54	2.27	0	0	15.53[a]
21	2.27	84.09	1.14	2.27	3.41	5.68	16.48[a]
28	0	40.91	3.41	0	0	1.14	7.58[bc]
58	3.41	12.50	12.50	9.10	14.77	3.41	9.28[b]
88	5.68	7.96	3.41	7.96	1.14	5.68	5.30[cd]
118	2.27	5.68	4.54	4.54	10.23	9.10	6.06[bc]
Treatment Mean	2.27[y]	46.25[x]	4.09[y]	3.52[y]	4.20[y]	2.95[y]	

[a-d]Means within a column without a common superscript letter differ (P<0.05).
[x-y]Means within a row without a common superscript letter differ (P<0.05).
[1]Data are least squares means from two replicate trials with a total of 4,763 isolates.
[2]C = Control diet without antibiotics or egg yolk products
APR+CB = Control diet + apramycin followed by carbadox
OXY = Control diet + oxytetracycline
ASEYA = Control diet + egg yolk powder containing anti-*Salmonella* antibody
EY = Control diet + egg yolk powder without anti-*Salmonella* antibody
SDPP = Control diet + spray dried porcine plasma
[3]Days of experiment.
[4]All pigs were challenged with *Salmonella* Typhimurium on day 7.
Treatment effects (P<0.0001).
Day effects (P<0.0001).
Treatment × Day effects (P<0.0001).
SEM = 2.97, 3.01 and 4.24 for treatment, day, and interaction means, respectively.

Table 10. Effect of dietary treatments on various days on carbadox resistance of *E. coli* (%) isolated from pigs[1]

Day[3]	Treatment[2]						Day Mean
	C	APR+CB	OXY	ASEYA	EY	SDPP	
0	0	0	6.82	0	2.27	0	1.52^{bc}
7[4]	0	0	2.27	1.14	2.27	4.54	1.71^{bc}
8	0	0	0	0	0	2.27	0.38^{c}
12	0	0	0	0	0	0	0^{c}
14	0	0	0	1.14	1.14	1.14	0.57^{bc}
21	0	0	2.27	0	0	7.96	1.71^{bc}
28	0	10.23	3.41	1.14	0	0	2.46^{b}
58	0	20.45	5.68	1.14	0	1.14	4.73^{a}
88	0	2.27	0	0	1.14	0	0.57^{bc}
118	0	0	0	0	1.14	2.27	0.57^{bc}
Treatment Mean	0^{z}	3.29^{w}	2.04^{wx}	0.45^{yz}	0.79^{xyz}	1.93^{wxy}	

[a-c]Means within a column without a common superscript letter differ (P<0.05).
[w-z]Means within a row without a common superscript letter differ (P<0.05).
[1]Data are least squares means from two replicate trials with a total of 4,763 isolates.
[2]C = Control diet without antibiotics or egg yolk products
APR+CB = Control diet + apramycin followed by carbadox
OXY = Control diet + oxytetracycline
ASEYA = Control diet + egg yolk powder containing anti-*Salmonella* antibody
EY = Control diet + egg yolk powder without anti-*Salmonella* antibody
SDPP = Control diet + spray dried porcine plasma
[3]Days of experiment.
[4]All pigs were challenged with *Salmonella* Typhimurium on day 7.
Treatment effects (P<0.001).
Day effects (P<0.0001).
Treatment × Day effects (P<0.0001).
SEM = 1.05, 1.13 and 1.91 for treatment, day, and interaction means, respectively.

Table 11. Effect of dietary treatments on various days on body weights (kg) in pigs challenged with *Salmonella* Typhimurium[1]

Day[3]	Treatment[2]						
	C	APR+CB	OXY	ASEYA	EY	SDPP	Day Mean
0	7.88	8.83	8.26	8.91	9.14	9.30	8.72[h]
7[4]	12.26	13.65	12.68	13.27	13.43	13.83	13.19[g]
8	12.50	14.04	13.15	13.45	13.65	13.88	13.45[g]
12	13.51	16.51	14.59	13.33	14.81	15.02	14.63[f]
14	14.73	18.10	17.13	16.24	16.65	17.05	16.65[e]
21	19.56	23.60	22.09	20.83	21.43	21.82	21.55[d]
28	24.38	29.50	27.58	26.94	27.27	27.96	27.27[c]
58	48.98	53.74	52.37	51.11	51.67	52.98	51.81[b]
88	80.13	82.52	78.06	81.38	82.50	79.93	80.75[a]
Treatment Mean	25.99	28.94	27.32	27.27	27.84	27.97	

[a-h]Means within a column without a common superscript letter differ (P<0.05).
[1]Data are least squares means from two replicate trials (n = 22 pigs/treatment).
[2]C = Control diet without antibiotics or egg yolk products
APR+CB = Control diet + apramycin followed by carbadox
OXY = Control diet + oxytetracycline
ASEYA = Control diet + egg yolk powder containing anti-*Salmonella* antibody
EY = Control diet + egg yolk powder without anti-*Salmonella* antibody
SDPP = Control diet + spray dried porcine plasma
[3]Days of experiment.
[4]All pigs were challenged with *Salmonella* Typhimurium on day 7.
Day effects (P<0.0001).
Treatment × Day effects (P<0.01)
SEM = 0.93, 0.49 and 1.16 for treatment, day, and interaction means, respectively.

Table 12. Effect of dietary treatments on various days on rectal temperature (°C) in pigs challenged with *Salmonella* Typhimurium[1]

Day[3]	Treatment[2]						
	C	APR+CB	OXY	ASEYA	EY	SDPP	Day Mean
0	39.56	39.25	39.50	39.73	39.83	39.69	39.60^{bc}
7[4]	39.77	39.71	39.43	39.74	39.56	39.79	39.67^{b}
8	40.10	39.85	39.99	40.08	40.10	40.30	40.07^{a}
12	39.45	39.52	39.32	39.46	39.59	39.73	39.51^{c}
14	39.33	39.75	39.57	39.66	39.49	39.63	39.57^{bc}
21	39.76	39.71	39.48	39.56	39.42	39.63	39.59^{bc}
28	39.50	39.50	39.47	39.72	39.64	39.63	39.58^{bc}
Treatment Mean	39.64^{yz}	39.61^{yz}	39.54^{z}	39.71^{xy}	39.66^{xyz}	39.77^{x}	

[a-c]Means within a column without a common superscript letter differ (P<0.05).
[x-z]Means within a row without a common superscript letter differ (P<0.05).
[1]Data are least squares means from two replicate trials (n = 12 pigs/treatment).
[2]C = Control diet without antibiotics or egg yolk products
APR+CB = Control diet + apramycin followed by carbadox
OXY = Control diet + oxytetracycline
ASEYA = Control diet + egg yolk powder containing anti-*Salmonella* antibody
EY = Control diet + egg yolk powder without anti-*Salmonella* antibody
SDPP = Control diet + spray dried porcine plasma
[3]Days of experiment.
[4]All pigs were challenged with *Salmonella* Typhimurium on day 7.
Treatment effects (P<0.05).
Day effects (P<0.0001).
Treatment × Day effects (P<0.0001).
SEM = 0.069, 0.065 and 0.104 for treatment, day, and interaction means, respectively.

Table 13. Effect of dietary treatments on various days on white blood cell counts ($\times 10^3$/ml) in pigs challenged with *Salmonella* Typhimurium[1]

Day[3]	Treatment[2]						
	C	APR+CB	OXY	ASEYA	EY	SDPP	Day Mean
0	13.04	11.17	12.63	12.20	12.95	11.94	12.32[e]
7[4]	16.73	15.54	16.79	15.82	15.79	14.26	15.82[cd]
8	16.74	19.39	19.63	19.68	16.91	16.53	18.14[ab]
12	21.12	15.31	16.77	17.98	20.21	19.31	18.45[a]
14	21.03	16.96	14.74	20.83	20.10	21.11	19.13[a]
21	19.06	13.20	15.77	18.32	17.36	17.39	16.85[bc]
28	19.74	10.76	12.74	15.11	15.08	17.78	15.20[d]
Treatment Mean	18.21	14.61	15.58	17.13	16.91	16.90	

[a-e]Means within a column without a common superscript letter differ (P<0.05).
[1]Data are least squares means from two replicate trials (n = 12 pigs/treatment).
[2]C = Control diet without antibiotics or egg yolk products
APR+CB = Control diet + apramycin followed by carbadox
OXY = Control diet + oxytetracycline
ASEYA = Control diet + egg yolk powder containing anti-*Salmonella* antibody
EY = Control diet + egg yolk powder without anti-*Salmonella* antibody
SDPP = Control diet + spray dried porcine plasma
[3]Days of experiment.
[4]All pigs were challenged with *Salmonella* Typhimurium on day 7.
Day effects (P<0.0001).
Treatment × Day effects (P<0.01).
SEM = 2.06, 1.98 and 2.35 for treatment, day, and interaction means, respectively.

Table 14. Effect of dietary treatments on various days on serum *Salmonella* antibody in pigs challenged with *Salmonella* Typhimurium[1]

Day[3]	Treatment[2]						
	C	APR+CB	OXY	ASEYA	EY	SDPP	Day Mean
0	0.022	0.053	0.029	0.034	0.063	0.060	0.043[d]
7[4]	0.008	0.026	0.008	0.009	0.018	0.019	0.015[d]
8	0.009	0.021	0.005	0.013	0.013	0.022	0.014[d]
12	0.034	0.017	0.016	0.063	0.018	0.027	0.029[d]
14	0.158	0.068	0.165	0.226	0.067	0.092	0.129[c]
21	0.255	0.183	0.274	0.429	0.184	0.177	0.250[b]
28	0.824	0.370	0.530	0.636	0.485	0.493	0.556[a]
Treatment Mean	0.187	0.105	0.147	0.202	0.121	0.127	

[a-d]Means within a column without a common superscript letter differ (P<0.05).
[1]Data are least squares means of S/P ratio from two replicate trials (n = 12 pigs/treatment)
[2]C = Control diet without antibiotics or egg yolk products
APR+CB = Control diet + apramycin followed by carbadox
OXY = Control diet + oxytetracycline
ASEYA = Control diet + egg yolk powder containing anti-*Salmonella* antibody
EY = Control diet + egg yolk powder without anti-*Salmonella* antibody
SDPP = Control diet + spray dried porcine plasma
[3]Days of experiment.
[4]All pigs were challenged with *Salmonella* Typhimurium on day 7.
Day effects (P<0.0001).
SEM = 0.064, 0.041 and 0.086 for treatment, day, and interaction means, respectively.

VITA

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