ON FARM PATHOGENS AND INTERVENTION STRATEGIES

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The control of *Salmonella enteritis* (SE) has been reported as a priority matter by the poultry industry (Todd, 1980). In working towards the ultimate goal of SE eradication, several important problems must be solved. More information about the interactions between the pathogen and its host is required. It is important to know the pathogenesis of salmonellae infections in animals and people, the size of the infective dose, and the circumstances leading to the multiplication of the organisms, transmission (human-to-human, animal-to-human, and animal-to-animal), the ecology of the salmonellae in the intestinal tract, and salmonellae interactions with other enteric (cecal) organisms. Implicit here is knowledge of the significance of the genetic variability of salmonellae and in particular, the effects of antibiotics and other drugs in the organism's environment. Current studies conducted in our laboratory have indicated that exposure to normal chicken enteric flora may be an efficient means of protection from these enteric pathogens. Realization of the importance of normal flora in the exclusion of salmonellae in poultry may provide for immediate benefits to the poultry industry since the misuse of antibiotics may be a critical factor in allowing the establishment of salmonellae infections (Corrier *et al*., 1992; Manning, 1991;).

As with other species of the paratyphoid salmonellae, SE does not appear to be host specific. Sources of infection for poultry may include humans, wild birds, contaminated feed, rodents and perhaps insects (Todd, 1980). Because of the potential public health concerns and mounting regulatory pressure on poultry producers, prevention of SE infection is becoming increasingly important (Todd, 1980). The need for SE prevention is required, not only to avoid the potential introduction of more virulent strains of SE, such as phage type 4 (common in parts of Europe) into U.S. domestic poultry, but also because it would serve as a good model for prevention of other enteric pathogens in avian as well as mammalian species.

Salmonellosis: Seriousness of the Problem

Several investigators have reported salmonellosis as one of the most important communicable disease problems in the United States with an estimated of 2,000,000 human cases annually (Todd, 1980). In addition to the pain and suffering it causes, this disease is responsible for substantial costs in the form of medical care, hospitalization, and lost income through absence from work (Todd, 1980). Additionally, salmonellosis also causes substantial losses to livestock and poultry industries through death of young animals, decrease milk and egg production, costly testing and control programs, and reduced value of contaminated products (Todd, 1980). The food processing industry spends huge sums for testing and control programs, for remodeling plants and equipment, and for recall of contaminated products that are inadvertently placed on the market (St. Louis *et al*., 1988). Investigators have considered the cost of salmonellosis to the American economy to be at least $300 million annually and probably more (Todd, 1980).

As primarily a food-borne disease, salmonellosis is a potential threat to every resident of the United States; the prevention and control requires the attention of the entire food-production, food-processing, food-service industries, and public health workers, as well as regulatory agencies, physicians, veterinarians, hospital employees, laboratory personnel, and consumers (Todd, 1980).

Salmonellosis is an infection caused by bacteria of the genus *Salmonella*. Their native habitat is the intestinal tract of animals, including man, but they are easily spread to other environments where they may survive and even multiply (Todd, 1980). When ingested by a susceptible host, the salmonellae can either cause a variety of disease syndromes or they may simply multiply without eliciting clinical signs of disease. Such replication results in "Asymptomatic carriers" which can spread the infectious agent just as effectively as an individual who is clinically ill.

Salmonellae infections may be divided into three groups on the based on their host predilections:

1. Primarily adapted to man. This group includes *Salmonella typhi* and a few serotypes of *S. enteritis* that are rarely found in animals other than man. Infections are characterized by a prolonged incubation period (10 to 20 days or more), generalized disease with bloodstream invasion and a tendency to produce carriers and to become endemic.

2. Primarily adapted to particular animal hosts. Included in this group are several important pathogens of domestic animals, such as *Salmonella choleraesuis* and serotypes of *S. enteritis*, *S. pullorum*, *S. gallinarum*, *S. dublin*, and *S. abortusequi*. Most of these organisms may cause gastroenteritis in man, which may be quite severe, particularly in children.

3. Unadapted. This group includes some 1,300 distinct serotypes of *S. enteritis* that attack man and other animals with equal facility and with no evident host preference. In man, the disease typically consists of gastroenteritis beginning 6 to 24 hours after ingestion of the organisms.

Because the large number of antigenically distinct serotypes, some investigators have postulated that salmonellae possess a degree of "genetic plasticity" (Rao, *et al*., 1987). It is known that salmonellae exhibit the usual mechanisms of genetic recombination; e.g., transduction, phage conversion, conjugation, and they readily give rise to mutants as usually do other members of the Enterobacteriaceae family (Rao, *et al*., 1987).

The observation that multiple drug resistance can be transferred to salmonellae from *Escherichia coli* and possibly other intestinal microorganisms has raised questions about the safety of feeding antibiotics to domestic animals for the stimulation of growth. The potential hazard of this practice has been demonstrated by observations that:

1. Antibiotic therapy in hospitals is associated with greatly increased incidence of antibiotic resistance among salmonellae isolated from patients (Todd, 1980).

2. Prophylactic and therapeutic use of antibiotics has led to enhanced susceptibility to *S. enteritis* in Leghorn chicks, thus, there is evidence that widespread use of antibiotics may increase the drug resistance of the *Salmonella* population in selected environments and, in some cases, increases the susceptibility to salmonellae infections (Manning, 1991).

INTERVENTION STRATEGIES

Gastrointestinal (GI) microflora consist of hundreds of different types of microorganisms and are a biologically important component of the body. At the beginning of this century, Metchnikoff (Metchnikoff, E., 1907) observed that the consumption of fermented milks had beneficial effects associated with the autodigestion of lactose. Through a process of fermentation, the metabolites of these complex microbes have varying consequences on host health. Proteolytic species, for example, produce toxic compounds, whereas metabolism of shortchain fatty acids may result in an increased energy yield (Tissier, H., 1905). Modern perspectives on consumption of fermented milk supplements (e.g., yogurt, fluid milk) are aimed at consumer well-being using products enriched with acid bacteria (*Lactobacilli*), particularly *Bifidobacteria*, that normally inhabit the intestinal tract of human infants and adults. A probiotic, defined as a live microbial food supplement, benefits the host by improving its intestinal microbial balance (Gibson, G.R. *et al*, 1995). In contrast, a prebiotic is a nondigestible nutritional compound [e.g., transgalactosylated and fructooligosaccharides (FOS)] that selectively stimulates the growth of endogenous lactic acid bacteria and *Bifidobacteria* to improve the health of the host (Gibson, G.R. *et al*, 1995). The concept of synbiotics has been proposed recently to characterize colonic foods with prebiotic and probiotic properties as health enhancing functional foods (Gibson, G.R. and Roberfroid, M.B., 1995). Biotechnological advances with these health supplements require more detailed knowledge of the roles that enteric feeding and the microenvironment play in host defense than is currently available. Harnessing the biotechnology of biotic supplements introduces new perspectives that require scientific investigation of interactions with genomic, biochemical, cellular, and physiologic functions that promote human and animal health as well as disease prevention.

Impact of Diet on Microbial Communities of the Gastrointestinal Tract

The gastrointestinal bacterial community is as metabolically powerful as any "organ" in the animal body (Gibson, G.R. and Roberfroid, M.B., 1995). The gastrointestinal walls are the interface between this massive bacterial organ and the healthy, sterile tissues such as blood. If the immunological or other animal defense mechanisms fail, bacteria readily penetrate tissues rich in utilizable nutrients and cause infections. Chemical compounds and biomolecules on either side of the interface interact continuously and maintain the delicate balance, allowing nutrient absorption, but keeping bacteria out. Bacteria in the gastrointestinal tract derive most of their energy for reproduction and growth from dietary compounds, which are either resistant to attack by digestive fluids or absorbed so slowly that bacteria can successfully compete for them (Hentges, D., 1992). Since bacterial species have different substrate preferences and growth requirements, the chemical composition of the digesta largely determines the composition of the microbial community in the gastrointestinal tract. The bacterial community at a given time point, therefore reflects the fitness of the bacterial species to the chemical and physical environment and capabilities to compete against other bacterial residents and the defense system of the host (Tissier, H., 1905). By dietary means it is possible to affect the competitiveness of harmful and non-harmful bacteria by changing gut dynamics. Specific species can be selected for by certain feed ingredients, which escape digestion by the host, but are readily available for the metabolic machinery of the target microbes. Products belonging to this group include synbiotic products (Gibson, G.R. and Roberfroid, M.B., 1995).

Functional effects of probiotics and prebiotics

Research and development of synbiotic products have been increasingly focused on evidence of functional benefits including resistance to infection, antibacterial activity (Nava, G., 2001), and improved immune status (Calderón, L., 1996). Health claims associated with probiotic supplements include prevention of diarrhea ((A)Duffy, L.C. *et al*, 1994; Yolken, R.H. *et al*, 1994) and colitis (Favier, C. *et al*, 1997), antitumorigenic effects (Takano, T. *et al*, 1985) and cholesterol reduction (Tahri, K., *et al*, 1995). Balanced colonic microflora and immunostimulation are major functional effects attributed to the consumption of probiotics. The concept of prebiotics is relatively new; it developed in response to the notion that nondigestible food ingredients (e.g. nondigestible oligosaccharides) are selectively fermented by one or more bacteria known to have positive effects on gut physiology. Bacteria fed by a preferential food substrate have a proliferative advantage over other bacteria. Prebiotics selectively modify the colonic microbiota and can potentially modulate lipid metabolism. Gastrointestinal functional effects of prebiotics have been shown almost exclusively for fructooligosaccharides (derived from chicory). Chicory FOS (chiFOS) are prebiotics that have a bifidogenic effect (Roberfroid, M.B., 1996). This class of nondigestible oligosaccharides resists hydrolysis, reaches the colon intact, and as short-chained oligosaccharides is extensively fermented in the colon by resident symbiotic anaerobic bacteria. The fermentability and bifidogenic effect of FOS have been confirmed with *in vitro* (Wang, X. and Gibson, G.R., 1993). The results have verified increases in short-chain fatty acids, lower pH, and proliferation of *Bifidobacteria* sp. in the colon and fecal flora. All of these functional effects are of interest and deserve further study. However, functional food science demands rigorous scientific investigation of biotic products in humans and animals to validate their importance in human health. Evidence from a few rigorously designed prospective cohorts and randomized double-blinded studies tentatively indicate that a few well-characterized probiotic strains have documented health-promoting effects (Lee, Y.-K. and Salminen, S., 1995).

Perspectives on probiotic applications

Lactic acid bacteria traditionally used in the production of fermented foods constitute roughly 25% of human dietary compounds. The application of probiotic organisms has been extended to uses outside of human nutrition, including animal production and plant protective in agriculture (Abe, F. *et al*, 1995). Current perspectives on biotechnical applications of probiotic products require further *in vitro* and *in vivo* investigation to evaluate the safety of using wild-type organisms or those obtained by genetic engineering. Selection of the best adapted and safely performed laboratory strains depend on: (1) selection of strains with demonstrated probiotic effects; (2) evidence of health-promoting effects (e.g., production of essential amino acids, antitumor activity, and vitamins); and (3) strains with food protective activities (growth inhibition of food spoilage or poisoning bacteria) (Havenaar, R. *et al*, 1992). Successful probiotic bacteria are generally identified by additional strain properties including the ability to survive gastric conditions, colonize the intestine, and adhere to intestinal epithelium (Beachey, E.H., 1981). The promising use of probiotic supplements and the composition of complex biological preparations may increasingly rely on gene engineering for the treatment dysbacteriosis of various etiologies in the future. Modern genetic techniques will include advanced typing procedures that are based on genetic tagging (16SrRNA, 16S DNA) with oligonucleotides (Lane, D. J. *et al*,1985).

Mechanisms of action from fermenting bacteria

Mechanisms by which probiotic and prebiotic supplements affect the microecology of the intestinal tract are not well studied, but at least four mechanisms of action have been observed: (1) antibacterial agents (bacteriocins) that are produced and secreted by probiotic organisms may have an inhibitory effect on controlling pathogenic microflora; (2) human breast milk may alter bacterial antagonism for essential nutrients and impede overgrowth of aerobes; (3) stimulation of immune responses (e.g., antibody titers, macrophage activity, T cells, and interferon) may suppress potential pathogens; and (4) specific competition for adhesion receptors to gut epithelium may allow lactic acid bacteria and *Bifidobacteria* to occupy the niche normally required by toxin-producing organisms for colonization (Wilson, K.H. and Perini, F., 1988). The various fermenting substrates for specific lactic acid bacteria and *Bifidobacteria* differ in their metabolic potential but in general include the following: (1) production of lactic acid and lesser amounts of acetic and formic acids; (2) production of antimicrobial bacteriocins and fatty acids; and (3) reduction in toxin-producing organisms and bacterial translocation (Dubos, R. *et al*, 1963). The mechanisms behind specific benefits include: (1) strengthening of the gut mucosal barrier; (2) gut microflora modification; (3) adherence to intestinal mucosa with an ability to prevent adherence of pathogen proliferation; (4) modification of dietary proteins by the intestinal microflora; (5) modification of bacterial enzyme activity; and (6) influence on gut mucosal permeability (Goldin, B. and Gorbach, S.L., 1984). The intestinal epithelium acts as a natural barrier to the movement of pathogenic bacteria, antigens, and toxic substances from the gut lumen. When either the normal microflora or epithelial cells are disturbed by dietary antigens, pathogens, chemicals, or radiation, defects in the barrier mechanisms can occur. Altered permeability further facilitates the invasion of pathogens and other harmful substances (Isolauri, E., 1995).

Intestinal barrier function

Most foreign antigens are excluded by the intestinal mucosal barrier, which serves as an important organ of host defense. In addition to its barrier function, the intestinal mucosa is sufficient for assimilating antigens via specialized antigen transport mechanisms in the gut epithelium, particularly in Peyers patches (Berg, R.D., 1985). In the healthy host, pericellular leakage of macromolecules is not allowed because intact intercellular tight junctions maintain the macromolecular barrier (Deitch, E.A., 1990). Even in normal physiologic conditions, a small percentage of antigens bypass the defense barrier and are absorbed across the epithelial layer via lysosomal processing of the protein (Salminen, S. *et al*, 1996). The integrity of the defense barrier is necessary to prevent uncontrolled antigen transport. Recently, investigators have determined that nearly 90% of dietary antigen handling evokes a tolerogenic hyporesponsiveness in postnatal development of infants and children. However, in preterm infants intestinal permeability can be dramatically increased postnatally (Beach, R. C. *et al*, 1982). The increased uptake of dietary and other environmental antigens to the immature gut microvillus membrane may lead to an aberrant immune response and sensitization to the immature gut defense barrier. The intestinal microflora affect gut permeability, offering a rationale for the successful use of fermenting anaerobic bacteria to prevent aberrant antigen transport that can traverse the mucosal barrier.

Recent investigations with probiotics

The potential of lactic acid bacteria for the production of safe and wholesome foods has been the subject of several recent reviews (Hammes, W.P. and Tichaczek, P.S., 1994). The human intestinal flora strain *Lactobacillus* GG (ATCC 53103) has been shown to promote local antigen specific immune responses, alleviate intestinal inflammation, and perhaps act in the treatment of food allergy (Majamaa, H. and Isolauri, E., 1997). Several studies have shown that the oral administration of *Bifidobacterium* or *Lactobacillus* organisms improved birth weight gain and piglet mortality in calves and piglets fed probiotic supplements compared with control animals (Abe, F. *et al*, 1995). Other recent model systems that use transgenic and germ-free mice have demonstrated that colonizing bacteria on the small bowel villus can send signals for the maturation of its epithelial cells (Gordon, J.I. *et al*, 1997). In breast-fed babies *Bifidobacteria*, *Lactobacilli*, and *Staphylococci* are the predominant organisms in the feces, whereas in formula-fed babies coliforms, enterococci, and Bacteroides predominate. The mechanisms responsible for microbiotic differences in breast and formula infants appear to be related to the acid-based properties of the formula and immunologic proteins including lactoferrin, oligosaccharides, and secretory immunoglobulin A (IgA) (Newt, C. *et al*, 1980). Several investigators (Duffy, L.C. *et al*, 1994) have demonstrated in *in vivo* animal models and human studies that rotavirus-specific *Bifidobacterium* strains protect against rotavirus-induced diarrhea by reducing rotavirus antigen and stimulating RV-specific IgA antibody. We have been able to publish several articles in which we used probiotics based on defined and undefined cultures and we have proved a significant reduction of *Salmonella Enteritis, S. Gallinarum and S. Tiphymurium* organ invasion in chicken models (Corrier, D.E. *et al*, 1992; Kogut, M.H., 1994; Nisbet, B. *et al*, 1996; Tellez, G.I. *et al*, 2001).

Recent investigations with prebiotics

Our laboratories have reported several publications to explain some of the endogenous factors and mechanisms which confer resistance to *Salmonella enteritis* (SE) infectivity in the intestinal tract of Leghorn chicks (Téllez, G. *et al*, 1993a,b,c; Tellez, G., *et al.* 1994). The effects of 14 or 19 days of 10% dietary lactose administration on SE infection, and pH, histological, morphometric and organic acid changes of the ceca. In both trials, the observed lactose-induced resistance to SE organ invasion following 14 days ($P < 0.001$) or 19 days ($P < 0.005$) was associated not only with an increase in organic acid concentration and acidic cecal pH ($P < 0.05$), but with measurable morphologic and immunohistochemical changes of the cecal mucosa as well ($P < 0.05$)(a). The effects of 14 or 19 days of 18 ppm dietary capsaicin administration on SE infection, and histological, morphometric and pH changes of the ceca. In both trials, the observed capsaicin-induced resistance to SE organ invasion ($P < 0.05$) was associated with measurable pH and morphometric changes of the cecal mucosa ($P < 0.05$) due to the presence of inflammatory cells in lamina propria (Téllez, I.G. *et al*. 1993abc). Both enzyme (bacterial phytase) and prebiotic (*Aspergillus sp.*) have been used to improve broiler performance but the specific mechanism of action is not well known. We conducted a trial to determine the effects of adding 0.2% prebiotic, 0.04% enzyme and the combination on body weight (BW), gastrointestinal transit time (GTT) and pH changes in crop and cecum of the broiler chick. All of the sorghum-soybean diets were isocaloric and isonitrogeneous and consisted of four diets with two replicates of 30 birds each (n = 240). The four experimental diets were: 1). Control S/S diet; 2). Control plus prebiotic; 3). Control plus enzyme; and 4). Control plus prebiotic and enzyme. On days 1, 9, 16 and 27, ten chicks from each diet were weighed. GTT was evaluated on days 15 and 26 using ten chicks from each diet. Crop and cecal content pH were performed on days 10 and 20 using ten chicks from each diet. There were no significant differences ($P>0.05$) between diets for body weight. The dietary effect was not significant for the GTT measurements at day 15 (results: 135.20[a], 168.60[a], 152.40[a] and 152.20[a] minutes for diet one through four, respectively). The dietary effect upon GTT at day 26 was significantly longer ($P<0.05$) for the prebiotic diet (results: 161.9[b], 193.20[a], 187.20[ab] and 178.80[ab] minutes for diet one through four, respectively). The cecum pHs were modified ($P<0.05$) at days 10 by the enzyme diet and the prebiotic diets (results: 6.06[a], 5.79[ab], 5.40[b] and 5.82[ab] pH units for diet one through four, respectively). The prebiotic and enzyme may function as a bacterial substrate that modify the intestinal microflora resulting in changes in the intestinal physiology and bacterial metabolite production. This may be very critical in neonatal poultry (Nava *et* al., 2001. In another study, the addition of enzyme (bacterial phytase) and prebiotic (*Aspergillus sp.*) to the broiler chick diet improve gastrointestinal microbial activity causing an increase of fatty volatile acid (VFA). The increase of VFA has been related to the nutrient absorption process. A trial was conducted to determine the effects of adding 0.2% prebiotic, 0.04% enzyme and the combination on the intestinal villi size and blood chemistries of the broiler chick. This experiment employed a completely randomized design. All of the sorghum plus soybean (S/S) diets were isocaloric and isonitrogeneous and consisted of four diets with two replicates of 30 birds each (n = 240). The four experimental diets were: 1). Control S/S diet; 2). Control plus prebiotic; 3). Control plus enzyme; and 4). Control plus prebiotic and enzyme. On day 10 and 20, ten serum samples per experimental diet were analyzed for glucose, calcium, phosphorus and alkaline phosphatase. From the same chicks samples of the ileum and cecum were fixed in 10% formaline and subsequently microscopically evaluated for morphological changes. There were no significant dietary effects ($P>0.05$) on the blood parameters analyzed. The intestinal villi size of the ileum and cecum of the 10 day-old broiler chick demonstrated no dietary effect ($P>0.05$). The intestinal villi size of the ileum of the 20 day-old broiler chick demonstrated significant dietary effect ($P<0.05$) due to the prebiotic addition (results: 392.4[b],

768.0^{a}, $505.^{b}$ 2 and 411.6^{b} microns for diet one through four, respectively). There were no significant dietary differences (P>0.05) in the length of the cecum villi in the 20 day-old chick (results: 233.40^{a}, 249.60^{a}, 199.20^{a} and 233.40^{a} microns for diet one through four, respectively). The prebiotic supplementation in neonatal broiler chick diets may improve the intestinal microflora activity and bacterial metabolite production (VFA) as related to the increase of 20 day-old ileum villi length ((B)Nava, G. *et al*., 2001). Finally, we conducted an experiment in neonatal chicks with the same prebiotics. In the mature broiler the intestinal microflora (IM) is active in reducing the potential for many intestinal diseases. In the neonatal broiler chick, the IM is not sufficiently developed to offer sufficient protection from potential pathogenic invasion. Both enzyme (bacterial phytase) and prebiotic (*Aspergillus sp.*) have been used to improve the IM of the broiler and enhance performance. A trial was conducted to determine the effects of adding 0.2% prebiotic, 0.04% enzyme and the combination on *Salmonella enteritis* (SE) organ invasion in the broiler chick. This experiment employed a completely randomized design. All of the sorghum plus soybean (S/S) diets were isocaloric and isonitrogeneous and consisted of four diets with two replicates of 30 birds each (n = 240). The four experimental diets were: 1). Control S/S diet; 2). Control plus prebiotic; 3). Control plus enzyme; and 4). Control plus prebiotic and enzyme. On day 9 and 19, ten broiler chicks from each diet were challenged with 10^{8} CFU of SE. 24 hours post challenge liver and spleen tissues were collected aseptically and incubated for 24 hours at 37 C in tetrathionate enrichment broth. After incubation, the broth was streaked onto brilliant green agar plates, incubated for an additional 24 hours at 37 C, and examined for the presence of lactose-negative, SE colonies. The percentage of cecal tonsils invasion for SE at days 10 and 20 were virtually 100% confirming uniform inoculation with SE. The effects of the dietary additions (prebiotic and enzyme) significantly reduced subsequent liver and spleen SE invasion at days 10 and 20. The SE invasion of liver and spleen tissues in the 10 day-old chick was: 100, 55^{**}, $60^{**}$and 80^{*} percent for diet one through four, respectively (*P<0.05, **P<0.01). The SE invasion of liver and spleen tissues in the 10 day-old chick was: 80, 40^{**}, $45^{**}$and 65^{*} percent for diet one through four, respectively (*P<0.05, **P<0.01). The dietary addition of both the prebiotic and the enzyme demonstrated similar effects to “probiotics” in the improvement of the IM maturity to resist SE organ invasion in broiler chicks.

Translocation of microorganisms from the GI tract

The transit of viable bacteria through the intestinal epithelium into the lamina propria, and consequently to the mesenteric lymph nodes, peritoneal cavity, and other previously sterile tissues, is called bacterial translocation (BT). Bacterial translocation has been associated with increased coliform adherence to cecal epithelium in rats by using biochemical fingerprinting methods (Kullen, M.J. *et al*, 1997). Not all cecal coliforms adhered to the epithelium during catabolic stress, implying that for translocation to occur bacterial properties other than adhesion are needed (Duffy, L.C. *et al*, 1997).

Several studies conducted in our group, have shown that the effect of prophylactic treatment of chickens with the soluble products from Con A-stimulated SE-immune T-cells can decrease SE infection, associated with histological and morphometric changes. In these experiments, the resistance to SE organ infectivity was associated with a significant increase in lamina propria thickness based on morphometric analysis ($P < 0.05$). This increase appears to be due to a marked infiltration of inflammatory cells, indicating that the protective effect of prophylactic administration of lymphokines, prepared from Con A-activated SE-immune T-cells, against a challenge infection with SE is associated with measurable and morphometric changes of the cecal mucosa ((B)Téllez, I.G. *et al,* 1993). In other studies, we have been able to prove the important role of those inflammatory cells, particularly heterophils (Kogut, M.H. *et al*, 1994). Comparable measurable and morphometric changes were associated with the significant decrease in SE organ infectivity ($P < 0.05$) following the administration of either low doses of *Eimeria tenella* (ET) or high doses of the heterologous *Eimeria adenoeides* (EA). In both trials, a significant correlation, ($r = -0.98$) for ET or ($r = -0.99$) for EA, between organ invasion and lamina propria thickness was observed, suggesting that the thicker the lamina propria, due to the presence of inflammatory cells, the less SE organ invasion occurs ((A)Téllez, I.G. *et al,* 1992).

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