

**Water-Soluble Vitamin E and Sodium Salicylate (Uni-Sol®)
Increase the Resistance of Turkeys
to *E. coli* Respiratory Infection**

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Water-Soluble Vitamin E and Sodium Salicylate (Uni-Sol®)¹ Increase the Resistance of Turkeys to *E. coli* Respiratory Infection

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INTRODUCTION

Regulatory pressures to limit antibiotic use in livestock and recent international marketing agreements that prohibit treating poultry with antibiotics have limited the disease-fighting tools available to poultry and livestock producers. There is a need to evaluate potential antibiotic alternatives to improve disease resistance in high intensity food animal production. Nutritional and non-nutritional approaches to counteract the debilitating effects of stress and infection may provide producers with useful alternatives to antibiotics. USDA Agricultural Research Service recognizes that improving the disease resistance of animals grown without antibiotics can benefit the animals' health, potentially increasing production efficiency and food safety. USDA ARS research initiatives are increasingly aimed at guarding food safety, and improving disease resistance in food animals is a key strategy.

Injured or infected tissues respond with a cascade of events that produce the prostaglandins responsible for immune suppression and the pain, fever, and edema associated with inflammation (Lees and May, 1992). An objective of using prostaglandin inhibitors that are antiinflammatory is to selectively improve circulating immunity while reducing the negative effects of local inflammation. The present study compares the successful prostaglandin inhibitors vitamin E and sodium salicylate, two tools that show promise for decreasing disease incidence and mortality during an infectious challenge.

Vitamin E has been recognized as an immunomodulator that confers improved disease resistance to poultry and livestock primarily through increases in antibody response (Leshchinsky and Klasing, 2001), and phagocytosis (Likoff et al., 1981). The effects of vitamin E on the immune response are not only related to its general function as an antioxidant but perhaps more to its specific ability to regulate arachidonic acid metabolism and reduce prostaglandin

synthesis. Since the infectious process itself increases prostaglandin levels, and high levels of prostaglandins are associated with immunosuppression, supplemental vitamin E may help to mitigate the disease process.

Others have used similar approaches to change fat metabolism and reduce immunosuppressive prostaglandins. Korver and Klasing (1997), and Korver et al. (1998) reported improved antibody responses when feeding omega-3 fatty acids from fish oil. The oil shifts fat metabolism away from arachidonic acid and its pro-inflammatory eicosanoid end products.

Although feeding diets rich in vitamin E or alternate fats can improve antibody titers and macrophage function, these approaches have not been widely adopted. Perhaps because they can be expensive to employ full-time, and intensive confinement feeding limits the responsiveness of special feeds made for occasional use. Supplying a prostaglandin inhibitor through the drinking water can be a much more responsive way to deliver the agent to animals when needed.

Another way to reduce prostaglandins is to use non-steroid anti-inflammatory agents (NSAIA). NSAIA block cyclooxygenase (prostaglandin synthetase) and keep the enzyme from converting arachidonic acid to prostaglandins and the harmful eicosanoid end products.

The most widely recognized NSAIA are the various members of the salicylate family. Traditionally, salicylates are administered at low doses and are consigned to roles as antipyretics and analgesics. The dose used for anti-prostaglandin activity (Likoff et al., 1981, and Stiles et al., 1990) is two to three times that necessary to reduce fever and pain in livestock (Plumb, 1995). Some salicylates are more suited for convenient administration through the drinking water. Aspirin, the best known salicylate, is only slightly soluble in water, making it unsuited for uniform distribution in drinking water systems at high concentrations. We chose to use sodium salicylate in part because it and aspirin have comparable therapeutic effects (excepting acetylation in

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platelets), anti-inflammatory properties, and potencies (Osol et al., 1973; and USP, 1990). Because sodium salicylate is over 300 fold more water-soluble than aspirin (Budavari et al., 1989), and was previously determined to be very stable in aqueous solutions, it was intuitively more appropriate as a NSAIA to inhibit prostaglandins in livestock and poultry via the drinking water.

MATERIALS AND METHODS

The present experiment was designed to evaluate potential antibiotic alternatives to improve disease resistance in high intensity poultry production. An infectious challenge model was used to examine the efficacy of inhibiting prostaglandins with doses of sodium salicylate (Uni-Sol[®]) and vitamin E in the drinking water of turkeys. Colisepticemia is one of the most costly diseases to affect turkeys and often requires antibiotic treatment. The respiratory challenge model in this research was designed to induce colisepticemia, and was a modification of the osteomyelitis model established by Huff et al. (1998).

The drinking water of male turkey poults was non-supplemented, or supplemented with either vitamin E at 14 IU/lb/day, Uni-Sol[®] at 25 mg of sodium salicylate/lb/day, or a combination of both. Fresh solutions were prepared each day. There were 220 birds in each treatment housed in four replicate floor pens on wood shavings. At 5 weeks of age, birds in half of the pens were challenged with an air sac inoculation of approximately 10 cfu of a non-motile, serotype O2 avian pathogenic *E. coli*. Water treatment commenced 5 days before challenge and continued for 2 weeks after challenge, when birds were bled and necropsied.

RESULTS AND DISCUSSION

The respiratory challenge succeeded in inducing colisepticemia in the poults. As expected from previous experience with the model, the *E. coli* challenge significantly increased mortality, air sacculitis, bacterial isolation from liver, and other measures of infection. Vitamin E and Uni-Sol[®] both eliminated mortality, air sacculitis, and the incidence of *E. coli* in livers of challenged birds (Figures 1-3). While vitamin E and Uni-Sol[®] ameliorated these conditions for infected birds and normalized them back to unchallenged levels, combining the two treatments did not protect the poults as well as either product alone. The literature is not

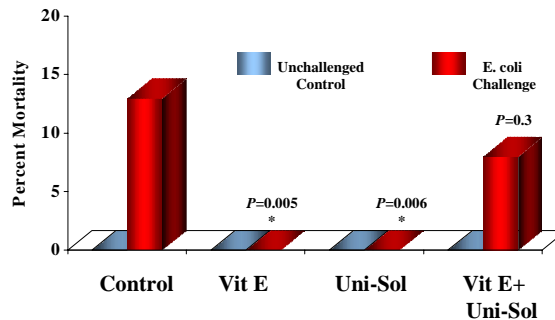


Figure 1. Percent mortality of 7-week-old turkeys supplemented with vitamin E, Uni-Sol[®], or a combination and challenged with *E. coli*. Both vitamin E and Uni-Sol[®], but not the combination, decreased mortality of *E. coli* challenged birds relative to the challenged control.

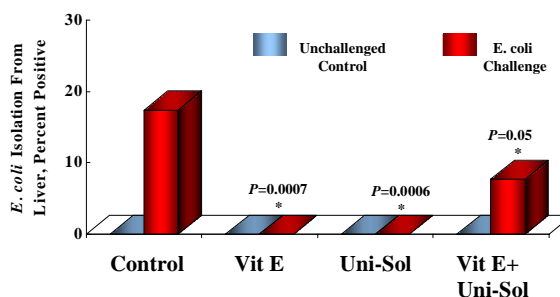


Figure 2. Isolation of *E. coli* from livers of 7-week-old turkeys supplemented with vitamin E, Uni-Sol[®], or a combination and challenged with *E. coli*. All treatments decreased the isolation of *E. coli* from livers of challenged birds.

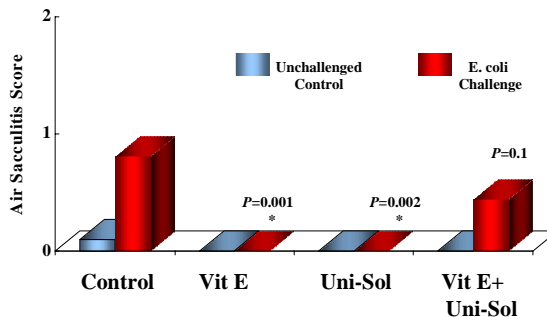


Figure 3. Air sacculitis scores of 7-week-old turkeys supplemented with vitamin E, Uni-Sol[®], or a combination and challenged with *E. coli*. Both vitamin E and Uni-Sol[®], but not the combination, decreased air sacculitis scores of *E. coli* challenged birds.

without similar contradictions. Leshchinsky and Klasing (2001) noted that moderate increases in vitamin E in chicken diets increased antibodies, but significant deterioration of antibodies occurred with higher doses of vitamin E. They found that 7-day antibody titers to SRBC in chickens increased with vitamin E level up to an optimum corresponding to 60 IU/kg diet. Increasing the anti-inflammatory vitamin up to 110 IU/kg decreased antibodies by half, and half again at 210 IU/kg. This antibody titer decay was not consistent among all antigens. Likoff et al. (1981) also reduced prostaglandins with a combination of anti-inflammatory agents to improve chicken survival to an *E. coli* challenge. They noted that a combination of vitamin E and aspirin unexpectedly increased prostaglandins in spleen homogenates, but this antagonism was not evident in bursal tissue. They partly attributed the differences to greater diversity in spleen cell types than is found in bursas.

The experimentally induced colisepticemia lowered the birds' body weight (Figure 4) and greatly increased the relative bursa, spleen, liver, and heart weight (Figures 5-9). Uni-Sol[®], vitamin E, and the combination all made the birds more resilient. The treatments all prevented the body weight loss associated with the infection, and also the relative enlargement of the organs. Vitamin E and Uni-Sol[®] both lowered the body weight of unchallenged controls, while the combination did not.

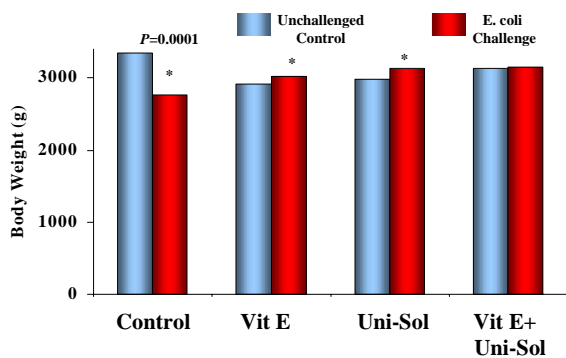


Figure 4. Body weights of 7-week-old turkeys supplemented with vitamin E, Uni-Sol[®], or a combination and challenged with *E. coli*. All treatments prevented the body weight decrease due to *E. coli* infection. However, vit E alone ($P=0.04$) and Uni-Sol[®] alone ($P=0.01$), but not the combination decreased the body weight of unchallenged treated birds relative to unchallenged controls.

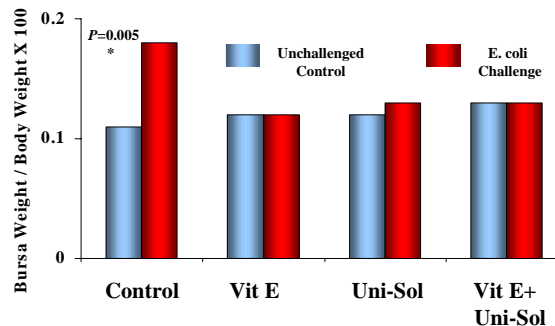


Figure 5. Bursa weights (as a percent of body weight) of 7-week-old turkeys supplemented with vitamin E, Uni-Sol[®], or a combination and challenged with *E. coli*. All treatments protected the bursa from the effects of *E. coli* challenge.

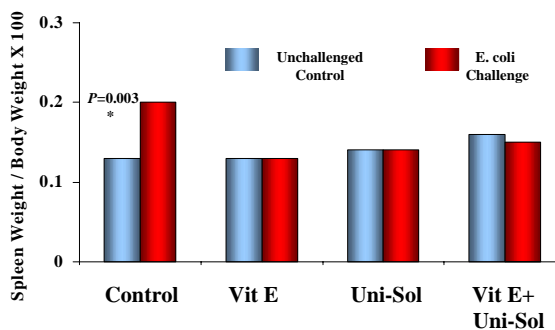


Figure 6. Spleen weights (as a percent of body weight) of 7-week-old turkeys supplemented with vitamin E, Uni-Sol[®], or a combination and challenged with *E. coli*. All treatments protected the spleen from the effects of *E. coli* challenge.

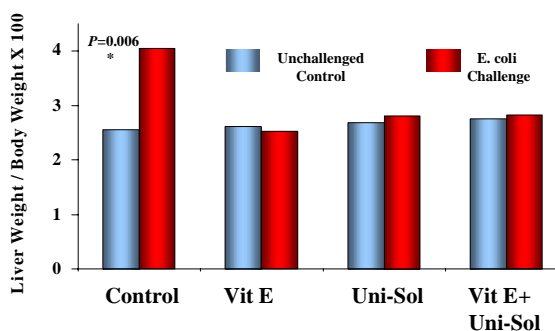


Figure 7. Liver weights (as a percent of body weight) of 7-week-old turkeys supplemented with vitamin E, Uni-Sol[®], or a combination and challenged with *E. coli*. All treatments protected the liver from the effects of *E. coli* challenge.

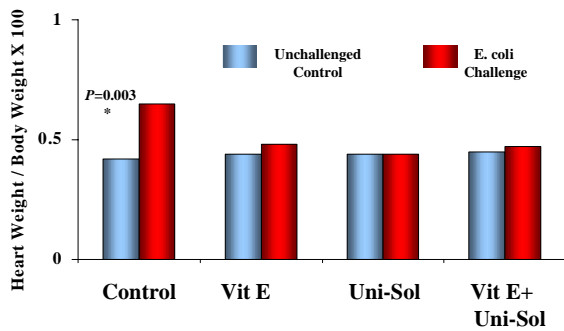


Figure 8. Heart weights (as a percent of body weight) of 7-week-old turkeys supplemented with vitamin E, Uni-Sol[®], or a combination and challenged with *E. coli*. All treatments protected the heart from the effects of *E. coli* challenge.

Uni-Sol[®] used independently or in combination with vitamin E increased the total leukocyte count (Figure 9) and the number and percent of lymphocytes (Figure 10). Uni-Sol[®] alone achieved a reduction in the heterophyl/lymphocyte ratio (data not shown). Consequently, the greater number of total white blood cells was also richer in the lymphocyte fraction that recognizes antigens, becomes memory cells, and produces antibodies. This infers that the Uni-Sol[®] treated birds had a more robust response to the infection than controls or those receiving vitamin E alone, and the response was biased more toward humoral immunity.

Conversely, vitamin E alone failed to increase total leukocytes at the sampling time we selected, and it increased the heterophyl/lymphocyte ratio. The improved disease resistance it obviously provided seems to have been shifted more toward phagocytosis than antibody response. The mechanisms for the improved immunity from vitamin E are increasingly attributed to its intracellular interruption of arachidonic acid metabolism (Leshchinsky and Klasing, 2001). As with salicylates, vitamin E interrupting arachidonic acid metabolism would directly inhibit prostaglandin production, but perhaps not to the same degree.

The direct increase in the treated birds' blood-borne defenses, the accompanying improvement in mortality, air sacculitis and organ weight, and complete clearance of the infectious organism from the turkey's livers is promising. Moreover, one would ordinarily expect local inflammation in organs to deteriorate the body weight of the birds. Body weight deteriorated in the controls, but not in the treated poult. A key goal of prostaglandin

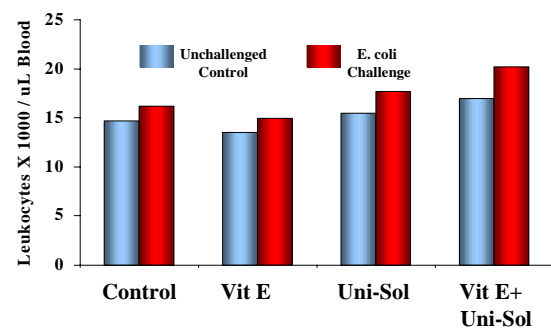


Figure 9. Total leukocyte counts of 7-week-old turkeys supplemented with vitamin E, Uni-Sol[®], or a combination and challenged with *E. coli*. Uni-Sol[®] alone ($P=0.01$) or in combination with vitamin E ($P=0.03$) increased total leukocyte counts over the control.

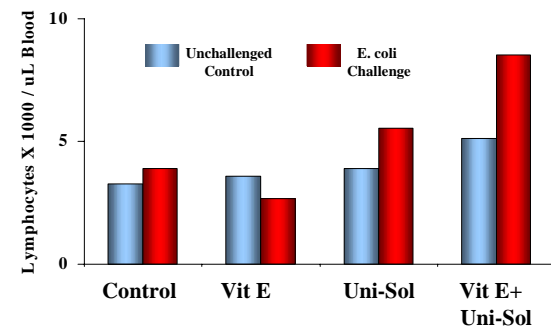


Figure 10. Total lymphocyte counts of 7-week-old turkeys supplemented with vitamin E, Uni-Sol[®], or a combination and challenged with *E. coli*. Uni-Sol[®] alone ($P=0.01$) or in combination with vitamin E ($P=0.03$) increased total lymphocyte counts over the control.

management has been to improve systemic immunity while at the same time arresting the inflammation that robs muscle growth and feed conversion. Our work demonstrates that treating turkey poults with Uni-Sol[®] and vitamin E prior to and during an infectious challenge can guard against the debilitating effects of stress and infection. Incorporating useful alternatives to antibiotics can benefit the animals' health, potentially increasing production efficiency and food safety.

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