The Role of Dietary Vitamin E in Experimental *Listeria monocytogenes* Infections in Turkeys

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Summary and Implications

The recovery of *L. monocytogenes* from intestine and tissues of vitamin E treated turkeys was generally lower than that of control diet turkeys. The reason for the observed difference in the clearance of *L. monocytogenes* among diet treatments could be related to changes in immune responses after vitamin E treatment. Flow cytometric analysis indicated that CD_4^+ and CD_8^+ lymphocytes were elevated at 6- and 8- DPI in infected turkeys given 200 IU vitamin E. Taken together, these data suggest that vitamin E may stimulate host defenses, which may augment clearance of *L. monocytogenes*.

Introduction

L. monocytogenes is a major human bacterial foodborne pathogen which annually accounts for ~ 2,500 cases (meningitis, encephalitis, sepsis, fetal death, prematurity) and 504 deaths at a loss of ~$200 million. Nearly 90% of all reported cases result in hospitalization. Sporadic human cases of listeriosis have been epidemiologically linked to the consumption of undercooked poultry products. In contrast to young birds, adults may not be reservoirs of *L. monocytogenes*, but may be transiently colonized by consuming contaminated feed or water. Thus, *L. monocytogenes* may enter the packing plant at low levels in the intestine of birds, survive in biofilms, and ultimately contribute to both environmental and product contamination.

Dietary supplementation of vitamin E may stimulate the immune responses of live turkeys and thus enhance gut clearance of potential human foodborne pathogens. This may subsequently reduce the contamination of carcasses at slaughter as well as during processing and contribute to a decrease in human bacterial foodborne illness. In chickens, vitamin E supplement increased the number of lymphocytes in the bursa and the thymus gland and stimulated the proliferation and differentiation of T cells. In broilers, vitamin E increased the percentage of mature $CD_4^+CD_8^-$ T helper cells in the thymus and spleen but did not alter the percentage of thymic and splenic B cells and macrophages in total immune cell. Vitamin E enhanced immunity of birds to *E. coli* infection, coccidiosis, infectious bursal disease, and Newcastle disease and altered cytokine expression in broilers. Besides improving immune responses, dietary vitamin E also improves meat quality. The current study was designed primarily to assess the effectiveness of dietary vitamin E in accelerating the gut clearance of adult turkeys experimentally infected with *L. monocytogenes*.

Materials and Methods

Two experiments were conducted to evaluate the effect of dietary vitamin E in turkeys experimentally infected with *Listeria monocytogenes*. In Experiment I, 1-d-old turkeys (n=90) were assigned to one of three dietary treatments (0,100 or 200 IU vitamin E). In Experiment II, 1-day old turkeys (n=70) were fed diets containing either 0 or 200 IU vitamin E. In both experiments I and II, after 6 weeks on the experimental diet, turkeys were orally inoculated with *L. monocytogenes* (~ 10^9 CFU). To monitor infection status, cloacal swabs were taken on selected days post inoculation (DPI). In both experiment, *L. monocytogenes* was detected more frequently in cloacal swabs of control diet turkeys than that in turkeys assigned to the vitamin E diet groups. In Experiment II, *L. monocytogenes* was not recovered in cloacal swabs of birds fed 200 IU vitamin E at 6-DPI, yet was cultured in control birds at 8-DPI.

Results

At the time of experimental inoculation with *L. monocytogenes* (week 6), serum vitamin E levels in both the In Experiment I, pre-inoculation cloacal swabs were negative for *L. monocytogenes*. At 1-DPI, *L. monocytogenes* was detected in cloacal swabs of turkeys (91.7%) fed the control diet (0 IU vitamin E), as well as birds fed 100 IU (87.5%) and 200 IU (68%) vitamin E (Table 3). On 4-DPI, *L. monocytogenes* was detected in swabs of control diet turkeys (8.3 %), while only one bird each was positive in the100 IU (4.2 %) and 200 IU (4 %) treatment groups. On day 5 PI, three control birds (12.5%) were positive, in contrast to none of the turkeys in the 100 IU and 200 IU groups. In Experiment II, dietary vitamin E (200 IU) has similar role in the clearance of *L. monocytogenes* in turkeys, as indicated by cloacal swabs (Table 2). At all days of post infection sampling, *L. monocytogenes* was recovered more frequently from cloacal swabs of turkeys fed the control diet than in turkeys fed 200 IU vitamin E. At 6-DPI, *L. monocytogenes* was not detected in the cloacal swabs of birds fed 200 IU vitamin E, whereas clearance was achieved by 10^-DPI in birds fed the control diet (0 IU).

In experiment I, *L. monocytogenes* was recovered more often in the caeca and distal of control diet turkeys *versus*

vitamin E-treated birds at 5 PI, but *L. monocytogenes* was not recovered from the intestine and tissue samples of turkeys in all diet treatment at 8-DPI and after that. For Experiment II, as summarized in Table 3, there were fewer tissue samples positive for *Listeria* in vitamin E treated birds at 2,4,6PI. After at 8 PI and after that both diet treated turkeys (control and vitamin E) are all negative to *Listeria Monocytogenes*.

As summarized in Figure 1, for experimentally infected birds, $CD_4^+CD_8^-$ populations of turkeys fed 200 IU vitamin E were markedly enhanced at 6-, 8-, 10-, and 31-DPI when compared to infected turkeys fed control diets (Figure 1a). At 6- and 8- DPI, the $CD_4^-CD_8^+$ lymphocytes were significantly higher in infected turkeys given 200 IU vitamin E than that in infected turkeys on control diets (Figure 1b). $CD_4^+CD_8^+$ double positive lymphocytes of experimentally infected turkeys on 200 IU vitamin E diet were also markedly elevated at 6- and 8- DPI when compared to infected birds on the control diet (Figure 1c).

Discussion

The impact of dietary vitamin E on both gut colonization as well as on CD_4^+ and CD_8^+ lymphocyte populations was evaluated in turkeys experimentally infected with *L. monocytogenes*. Dietary vitamin E linearly increased the serum vitamin E content. Turkeys received dietary vitamin E had lower recovery of *L. monocytogenes* after inoculation in both experiment I and II, based on both cloacal swabs and tissues culture results. The enhanced *L. monocytogenes* clearance in birds receiving vitamin E could be related to enhanced immune responses. Therefore, the lymphocytes of infected turkeys in both diets were analyzed. In this current study, dietary vitamin E (200 IU) was associated with significant elevation of $CD_4^+CD_8^-$ (6-, 8-, and 31- DPI) and $CD_4^-CD_8^+$ lymphocytes (6- and 8-DPI) in *Listeria*-infected turkeys, when compared with infected turkeys on control diets. At 6- and 8-DPI, $CD_4^-CD_8^+$ populations were elevated in infected birds fed 200 IU vitamin E. This was in concert with the functions of CD_4^+ cells, which respond to exogenous antigens by synthesizing IL-2 that in turn activates CD_8^+ cells.

In summary, this is the first attempt to correlate lymphocyte subpopulation dynamics with dietary vitamin E and subsequent clearance of acute listeirosis in any animal species, including turkeys. The results of this pilot study suggested that dietary vitamin E enhanced CD_4^+ and CD_8^+ populations may alter the clearance of *L. monocytogenes* in even transiently colonized adult birds. Thus, as a general immune potentiator, vitamin E may be an attractive alternative to the on-farm use of antimicrobials and may ultimately contribute to the reduction of *Listeria* contamination of the final poultry product.

Acknowledgement

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Table 1. Serum vitamin E levels (μg/ml) in turkeys fed 0, 100, 200 IU of vitamin E (Experiment I and II)

Days post infection	0 IU	100 IU	200 IU
Experiment I			
5	0.11±0.29c	2.08±0.33ab	2.88±0.29a
8	0.16±0.20c	1.51±0.20b	3.10±0.20a
11	0.09±0.22c	1.75±0.22b	3.88±0.22a
14	0.15±0.21c	1.89±0.21b	3.67±0.22a
Experiment II			
0	0.10±0.09b		3.42±0.24a
2	0.10±0.08b		3.31±0.39a
4	0.12±0.04b		3.19±0.29a
6	0.09±0.06b		3.41±0.35a

Four to five birds were analyzed at each sampling point for each dietary regimen.

Table 2. Recovery of *L. monocytogenes* from cloacal swabs of experimentally infected turkeys

Diet	Number positive (% positive)			
Experiment I				
	1st day	4th day	5th day	6th day
0 IU	22 of 24 (91.7%)	2 of 24 (8.3%)	3 of 24 (12.5%)	1 of 19 (5.3%)
100 IU	21of 24 (87.5%)	1 of 24 (4.2%)	0 of 24 (0%)	0 of 19 (0%)
200 IU	17 of 25 (68%)	1 of 25 (4.0%)	0 of 25(0%)	0 of 19 (0%)

Experiment II							
	1st day (n=30)	2nd day (n=30)	3rd day (n=25)	4th day (n=25)	6th day (n=20)	8th day (n=15)	10th day (n=10)
0 IU	26 (86.7%)	16 (53.3%)	6 (24%)	5 (20%)	1 (5%)	2 (13.3%)	0 (0%)
200 IU	24 (80.0%)	13 (43.3%)	4 (16%)	1 (4%)	0 (0%)	0 (0%)	0 (0%)

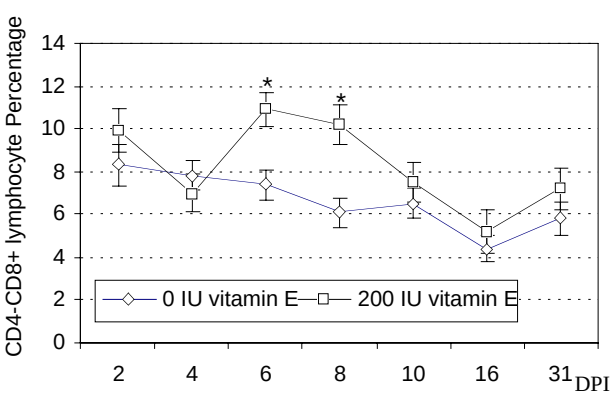
*positive cloacal swab at day 1 PI

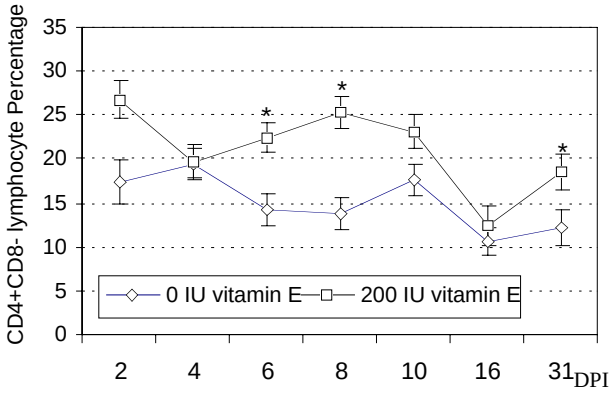
Table 3. Recovery of *L. monocytogenes* from tissues for Experiment II

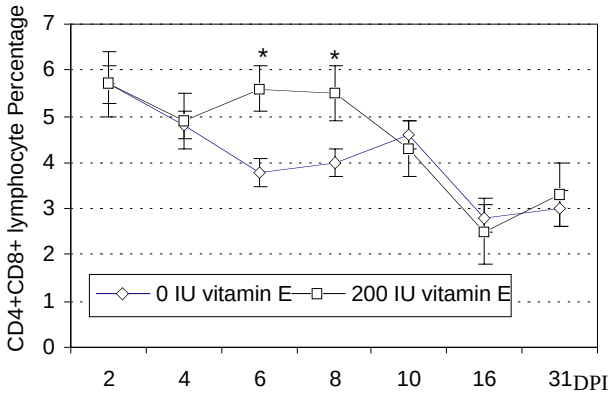
				Small intestine		
Diet	Liver*	Spleen	Caecal	Prox	Distal	Large Intestine
2 days						
0 IU	20%	0%	60%	40%	60%	40%
200 IU	40%	0%	60%	20%	20%	0%
4 days						
0 IU	0%	0%	60%	20%	0%	0%
200 IU	0%	0%	60%	0%	0%	0%
6 days						
0 IU	20%	0%	0%	0%	0%	40%
200 IU	0%	0%	0%	0%	0%	20%

*Percentage of infected birds for each group, n=5.

Figure 1. The lymphocyte populations of turkeys fed vitamin E.







" *" indicated the significant difference between 0 IU and 200 IU vitamin E treatments