CHANGES IN BACTERIAL COUNTS OF VACUUM-PACKAGED BEEF FROM ANIMALS FED HIGH VITAMIN E

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Background

Delay of oxidation reactions and meat color deterioration through dietary use of vitamin E during animal growth has been evaluated in recent years (Faustman *et al.*, 1989a,b; Cabedo *et al.*, 1998). Higher levels of vitamin E in muscle have been shown to improve the color stability of resulting meat Although the positive effects of vitamin E supplementation on meat color shelf-life have been widely studied, there is limited published work on whether supplementing diets with increased levels of vitamin E affects bacterial growth on the resulting meat. Without treatments that prolong acceptable product appearance, consumers may discard spoiled product and avoid potential pathogens; while, by extending the visual shelf-life of a product, and if the pathogens develop during the extended shelf-life, consumers may use product that looks acceptable but may be toxic.

Objectives

Cabedo *et al.* (1998) evaluated microbial growth and color shelf-life of beef patties during storage at 4°C and 12°. This study was designed to determine changes in spoilage, as well as pathogenic, bacteria on vacuum-packaged beef steaks stored at 4°C, from animals fed diets containing 0, 1000 or 2000 IU of added vitamin E.

Methods

Steaks of approximately 100 g each were cut from shoulder clod muscles to be used in the study. The steaks originated from Angus x Hereford x Salers steers, coming from grass pasture, that were fed a finishing diet, for 100 days, consisting of: 36% corn silage, 60% corn and 4% supplement (54% soybean meal, 17.9% corn, 21.7% limestone, 2% dicalcium phosphate, 3% urea, 0.6% vitamin A, 0.2% trace minerals, 0.5% monensin and 0.1% sulfur). Three different doses of supplemental vitamin E were fed daily with the finishing ration. Rovimixin E-50%-SD (Hoffman-La Roche, Inc., Nutley, NJ), which is composed of α-tocopheryl acetate, was added to pulverized corn in such a way that the consumption of 227 g of corn screenings per day supplied 0, 1000 or 2000 I.U. of vitamin E per animal per day. The steers were slaughtered at 19 to 21 months of age. Individual steaks from animals fed each level of dietary vitamin E supplementation were inoculated with 0.5 mL of an *Escherichia coli* O157:H7 inoculum, 0.5 mL of a *Listeria monocytogenes* inoculum, or 0.5 mL of sterile peptone water. The cultures were prepared and inoculated as indicated by Cabedo *et al.* (1998). Inoculated steaks were individually vacuum packaged (-0.85 bar) in nylon vacuum pouches (17.7 cm x 20.5 cm) and stored at 4°C. Triplicate steaks were removed from vacuum packages and sampled at 20 day intervals for 80 days.

For analysis, samples were blended in sterile 0.1% peptone water, in a stomacher (Stomacher Lab-Blender 400, Tekmar® Company, Cincinnati, OH) and appropriate decimal dilutions were spread-plated on plate count agar (PCA) (Difco Laboratories, Detroit, MI), lithium chloride-phenylethanol-moxalactam agar (LPM) (Difco), MacConkey agar (Difco) with 1% sorbitol (Sigma Chemical Co., St. Louis, MO) (SMAC), and pour-plated in violet red bile agar (VRBA) (Difco), to determine total plate counts after incubating PCA plates for 48 hr at ambient (22°C) temperature; *L. monocytogenes* counts after incubating LPM plates for 48 hr at 35°C; sorbitol-negative counts (including *E. coli* O157:H7), and total presumptive coliform counts after incubating SMAC plates and VRBA plates for 24 hr at 35°C. *Listeria monocytogenes* colonies from LPM agar plates were confirmed by testing them for xylose and rhamnose fermentation and catalase activity, following the procedures of Lachica (1990). One sorbitol-negative colony from each SMAC plate was tested for the presence of the O157 antigen through the agglutination of the O157 antiserum (Difco). Presumptive coliform colonies from VRBA plates were confirmed in brilliant green lactose bile broth (BGLB) (Difco). Those colonies producing gas from lactose in BGLB were considered coliforms (Hitchins *et al.*, 1992).

Appropriate decimal dilutions of samples inoculated with *E. coli* O157:H7 were also tested in lauryl sulfate tryptose broth (LST) (Difco) to determine coliforms *E. coli* and *E. coli* O157:H7 most probable number (MPN) with the BioControl ColiComplete® method (BioControl Systems Inc., Bothell, WA) after incubating for 48 hr at 35°C. Sets of three tubes per dilution, and three to five dilutions per sample, were used to determine the MPN. Those tubes in which the ColiComplete® disc turned blue after incubation were considered positive for coliforms, and those tubes with blue disc and fluorescence under a 366 nm wavelength ultraviolet light were considered positive for *E. coli*. Positive tubes for *E. coli* were streaked on MacConkey + 1% sorbitol. Tubes showing sorbitol-negative growth in MacConkey agar + 1% sorbitol were considered positive for *E. coli* O157:H7.

The pH of the samples that were blended for microbiological analyses was determined at each sampling time with an Orion Model 610 pH meter (Orion Research Inc., Boston, MA). Vitamin E content of the patties was analyzed by the College of Veterinary Medicine, Oregon State University (Corvallis, OR) by extraction of the sample after saponification and subsequent analysis by HPLC (Craig *et al.*, 1992).

Analysis of variance was performed on each type of bacterial count and on pH values utilizing the general linear models procedure of SAS (SAS Institute, 1990). When differences were significant ($P<0.05$), means were separated with the least significant difference (LSD) value.

Results and Discussion

Meat from animals fed 1000 or 2000 IU of vitamin E had significantly ($P\leq0.05$) higher amounts (6.12 – 7.77 μg/g) of vitamin E than meat from control animals (2.50 μg/g). Total plate counts of bacteria on vacuum-packaged steaks showed approximately a 6 log increase during 80 days of storage at 4°C (Table 1). There was no significant effect of dietary vitamin E supplementation on the rate of increase of total numbers of bacteria. Also, there was no significant ($P>0.05$) effect of the level of dietary vitamin E supplementation on the pH of the vacuum-packaged steaks, which ranged between 5.68 to 5.92 at time zero and 5.49 to 5.54 after 80 days of storage. As expected, there was no increase in total coliforms (data not shown) or sorbitol-negative organisms (Table 4) in vacuum-packaged steaks stored for 80 days at 4°C, regardless of the level of dietary vitamin E supplementation. Most probable number counts (MPN) of total coliforms, *E. coli* and *E. coli* O157:H7 on steaks also were not different ($P>0.05$) among levels of dietary vitamin E supplementation (Table 3). *Listeria monocytogenes* counts (Table 4) increased approximately 1.5 - 2.0 logs in

steaks from all levels of dietary vitamin E supplementation. Initially (at day 20), counts were numerically higher for control steaks, but at day 40 counts were similar in control and treatment groups. However, at 60 days of storage, numbers of *L. monocytogenes* were lower ($P<0.05$) on steaks from animals fed 1000 I.U. of supplemental vitamin E. Presumptive *Listeria* colonies isolated on LPM agar from uninoculated steaks were not confirmed as *L. monocytogenes*.

Conclusions

In general, different levels of vitamin E in the diets of the animals had only small effects on the rate of bacterial growth on beef steaks. At 4°C, there was no change in numbers of coliform bacteria or *E. coli* O157:H7 on vacuum-packaged steaks from cattle subjected to the three levels of vitamin E supplementation. There was no effect of level of dietary vitamin E supplementation on the total plate counts of vacuum-packaged steaks stored at 4°C. However, *L. monocytogenes* counts increased in steaks stored under vacuum at 4°C, regardless of vitamin E supplementation.

References

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Table 1. Mean (SD) total plate counts (log CFU/g) from uninoculated vacuum-packaged beef steaks from animals fed different levels of supplemental vitamin E and stored for 80 days at 4°C.

Vitamin E supplementation (IU)	0 days	20 days	40 days	60 days	80 days
0	2.2^{a} (0.5)	5.4^{a} (1.7)	6.9^{a} (1.0)	7.0^{a} (0.8)	8.0^{a} (0.2)
1,000	2.2^{a} (0.4)	6.8^{b} (0.1)	6.2^{a} (1.1)	6.6^{a} (0.6)	8.0^{a} (0.4)
2,000	2.0^{a} (0.3)	6.6^{b} (0.1)	6.7^{a} (0.7)	6.8^{a} (0.3)	7.5^{a} (0.2)

^{abc}Means in the same column followed by a common superscript letter are not different ($P>0.05$).

Table 2. Sorbitol-negative mean (SD) colony counts (log CFU/g) from vacuum-packaged beef steaks from animals fed different levels of supplemental vitamin E, inoculated with *E. coli* O157:H7 and stored for 80 days at 4°C.

Vitamin E supplementation (IU)	0 days	20 days	40 days	60 days	80 days
0	3.8^{a} (0.2)	3.2^{a} (0.1)	2.8^{a} (0.7)	3.1^{a} (0.1)	2.9^{a} (0.2)
1,000	3.8^{a} (0.0)	3.2^{a} (0.1)	3.2^{a} (0.1)	2.9^{a} (0.2)	2.7^{a} (0.2)
2,000	3.8^{a} (0.1)	3.1^{a} (0.0)	3.1^{a} (0.1)	3.0^{a} (0.4)	2.9^{a} (0.2)

^{abc}Means in the same column followed by a common superscript letter are not different ($P>0.05$).

Table 3. Mean (SD) Enterobacteriaceae counts (log MPN/g; LST broth plus ColiComplete®) from vacuum-packaged steaks from animals fed different levels of supplemental vitamin E and stored for 80 days at 4°C.

Enterobacteria	Level of dietary vitamin E supplementation (IU)	0	40	80
Coliforms	0	>3.0	3.6^{a} (0.4)	3.0^{a} (0.6)
	1,000	>3.0	3.5^{a} (0.2)	2.7^{a} (0.1)
	2,000	>3.0	3.0^{a} (0.7)	2.9^{a} (0.6)
E. coli	0	>3.0	2.1^{a} (0.1)	3.0^{a} (0.6)
	1,000	>3.0	3.0^{a} (0.6)	2.7^{a} (0.1)
	2,000	>3.0	2.5^{a} (0.2)	2.8^{a} (0.7)
E. coli O157:H7	0	>3.0	3.7^{a} (0.4)	3.0^{a} (0.6)
	1,000	>3.0	3.4^{a} (0.0)	2.7^{a} (0.1)
	2,000	>3.0	2.9^{a} (0.6)	2.9^{a} (0.6)

^{abc}Means in the same column grouping followed by a common superscript letter are not different ($P>0.05$).

Table 4. Mean (SD) *Listeria monocytogenes* counts (log CFU/g) from vacuum-packaged steaks from animals fed different levels of supplemental vitamin E and stored for 80 days at 4°C.

Vitamin E supplementation (IU)	0 days	20 days	40 days	60 days	80 days
0	4.0^{a} (0.1)	6.0^{a} (0.4)	5.2^{a} (0.3)	6.0^{a} (0.1)	5.2^{a} (1.2)
1,000	3.9^{a} (0.1)	4.9^{ab} (0.7)	5.5^{a} (1.1)	2.9^{b} (0.2)	3.8^{a} (2.4)
2,000	3.9^{a} (0.1)	4.0^{a} (0.9)	3.7^{a} (0.7)	5.6^{a} (0.6)	3.6^{a} (1.8)

^{abc}Means in the same column followed by a common superscript letter are not different ($P>0.05$).