

PELIMINARY STUDIES OF BACTERIAL CHALLENGE AND ANTIBIOTIC TREATMENT RELATED TO BEEF DISCOLORATION

W.K.M. CHAN¹, S.T. JOO¹, K. HAKKARAINEN², C. FAUSTMAN¹, D.M. SCHAEFER³, K.K. SCHELLER³ AND Q. LIU³¹Dept. of Animal Science, Univ. of Connecticut, Storrs, CT 06269, USA; ²Meat Science Dept., Univ. of Helsinki, 00710 Helsinki, Finland; ³Dept. of Meat and Animal Science, Univ. of Wisconsin, Madison, WI 53706.**KEYWORDS:** bacterial, discoloration, vitamin E supplementation, beef

BACKGROUND: Oxymyoglobin oxidation results in beef discoloration and is affected by many factors. While consumers frequently associate beef discoloration with bacterial growth, the actual role of bacteria in mediating oxymyoglobin oxidation remains unclear. Lin et al. (1977) found that total bacterial load in ground beef was unrelated to pigment oxidation. Other researchers (Bala et al., 1977; Robach and Costilow, 1961) maintained that bacterial growth enhanced beef discoloration. Dietary vitamin E supplementation improves beef color stability without affecting total microbial load (Chan et al., 1995). A concern exists that vitamin E supplementation may potentially interfere with bacterial-mediated discoloration if a cause-and-effect relationship between bacterial growth and beef discoloration exists.

OBJECTIVE: The objective of this study was to investigate the role of bacteria in discoloration of beef from control and vitamin E supplemented steers.

METHODS: All cattle were provided diets (ad libitum) consisting of 90% corn-plus-supplement/10% corn silage.

CHALLENGE STUDY Six Holstein steers per treatment were supplemented with dl- α -tocopheryl acetate at a dosage of 0 (E-0), 500 (E-500) and 2000 (E-2000) mg/steer/day for 126 days. Actual daily intakes of supplemental vitamin E were 67.3 (E-0), 380.1 (E-500) and 1447.4 (E-2000) IU/steer/day. Steers were slaughtered and strip loins were removed at 24 hr postmortem, vacuum-packed and stored at 4°C. At 28 days postmortem, the longissimus lumborum (LL) was dissected from the loin and slices (1 cm thick) prepared. Beef cores (12 cm² x 1 cm thick) were removed from the slices. Within each vitamin E treatment, half of the beef cores were inoculated with 0.1 mL of a fluorescent pseudomonad culture (10⁸ cells/mL; treated) (Faustman et al., 1990) and the remaining cores with 0.1 mL sterile peptone water (0.1%; control). Beef cores were then placed onto fiberboard trays, overwrapped with oxygen-permeable PVC film and stored at 4°C. Surface metmyoglobin accumulation (Steward et al., 1965) and microbial load (Steinbrugge and Maxcy, 1988) were determined on days 0, 1, 2 and 3. α -Tocopherol concentrations were analyzed according to Arnold et al. (1993).

ANTIBIOTIC STUDY Three Holstein steers per treatment were supplemented with dl- α -tocopheryl acetate at a dosage of 0 (E-0) or 2000 (E-2000) mg/steer/day for 126 days in each of two trials. Steers were slaughtered and strip loins removed at 24 hr postmortem, vacuum-packed and stored at 4°C. Strip loins were aged for 7 days in trial 1, and 28 days in trial 2. Beef cores of longissimus lumborum were prepared as described above and assigned to control or antibiotic treatments. Control beef cores were dipped in 0.1% sterile peptone water for 1 min. while antibiotic-treated beef cores were dipped in sterile antibiotic solution (streptomycin 1 mg/mL and tetracycline 1 mg/mL) for 1 min (treated). The control and treated beef cores were then placed onto sterile fiberboard trays and overwrapped with oxygen-permeable PVC film. Beef cores were temperature abused at 25°C for 24 hr to stimulate microbial growth and subsequently stored at 4°C. Metmyoglobin accumulation and total microbial loads were analyzed on days 0, 1, 3, 5 and 7.

RESULTS AND DISCUSSION:

CHALLENGE STUDY Inoculation of beef cores surfaces with the Pseudomonad culture (10⁶ cells/cm²) resulted in approximately 1 log higher microbial load than control samples on day 3 ($P < 0.05$; Table 1). Metmyoglobin percentage was not different in Pseudomonad-inoculated and control samples on days 0, 1 and 2 ($P > 0.05$, data not shown). On day 3 of 4°C storage, Pseudomonad-inoculated beef cores of E-0, E-500 and E-2000 had 4.0, 3.3 and 4.0 folds more metmyoglobin than their non-inoculated counterparts, respectively (Table 1). This suggested that microbial growth may enhance oxymyoglobin oxidation on beef cores. The exact mechanism of bacterial-mediated discoloration is unclear; reduction in oxygen tension (O'Keefe and Hood, 1982) or accelerated lipolysis (Bala et al., 1977) caused by bacterial growth have been suggested. Microbial loads were the same for E-0, E-500 and E-2000 beef cores ($P > 0.05$), while α -tocopherol concentrations followed the order E-2000 > E-500 > E-0 ($P < 0.05$; Table 1). E-500 and E-2000 treated beef cores showed lower surface metmyoglobin accumulation, respectively, than E-0 treated beef cores (Table 1).

ANTIBIOTIC STUDY Initial microbial loads were the same for control and antibiotic-treated beef cores ($P > 0.05$; Fig. 1b and 2b). After temperature abuse at 25°C for 24 hr and storage at 4°C, the total microbial loads in antibiotic-treated beef cores were 10² to 10³ CFU/cm² lower than controls ($P < 0.05$; Fig. 1b and 2b). In trial 1, metmyoglobin accumulation was not different between control and antibiotic-treated beef cores within each vitamin E treatment (E-0 or E-2000) for 5 days storage ($P < 0.05$; Fig. 1a). On day 7, control beef cores had more metmyoglobin than their antibiotic-treated counterparts ($P < 0.05$; Fig. 1a), though bacterial loads were not different on that day. In trial 2, a higher metmyoglobin percentage was found in control than in antibiotic-treated beef cores within each vitamin E treatment (E-0 and E-2000) during storage at 4°C for 5 days ($P < 0.05$; Fig. 2a). Control and antibiotic-treated beef cores showed a difference in metmyoglobin percentage earlier in trial 2 (day 1) than in trial 1 (day 5) (Fig. 1a and 2a). Beef cores in trial 2 had approximately 2 log higher initial microbial load than those in trial 1 (Fig. 1b and 2b), probably due to differences in initial contamination and/or aging period of the strip loins. These results suggested that a higher microbial load in controls compared with antibiotic-treated beef cores may be responsible for the higher metmyoglobin percentage in beef cores. However, antibiotic treatments may interfere with metmyoglobin formation by a mechanism that did not involve bacterial loads; the mechanism was initiated earlier in trial 2 than in trial 1. Further research is necessary to clarify the role of bacteria in discoloration of beef.

CONCLUSION:

1. Vitamin E supplementation did not affect microbial load and decreased oxymyoglobin oxidation in LL beef cores stored at 4°C.
2. Increasing microbial load by inoculation of a Pseudomonad culture increased oxymyoglobin oxidation in E-0, E-500 and E-2000 LL beef cores during 3 days storage at 4°C.
3. Decreasing microbial load by antibiotic treatment decreased oxymyoglobin oxidation in E-0 and E-2000 LL beef cores compared with controls during 7 days storage at 4°C.

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Table 1. Surface metmyoglobin%, microbial load and α -tocopherol concentrations of beef from cattle supplemented with vitamin E at levels of 0 (E-0), 500 (E-500) and 2000 (E-2000) mg/steer/day. Beef samples were inoculated with a fluorescent pseudomonad culture (10^6 cells/cm²) or sterile peptone water and stored at 4°C for 3 days.

		Surface MetMb (%)	Total Microbial Load (log CFU/cm ²)	α -Tocopherol (μ g/g muscle)
No Pseudomonad Inoculation	E-0	16.4±2.60 ^b	6.5±0.14 ^a	1.1±0.10 ^a
	E-500	11.1±2.80 ^b	6.3±0.15 ^a	2.7±0.28 ^b
	E-2000	6.40±1.80 ^a	6.2±0.07 ^a	5.7±0.43 ^c
With Pseudomonad Inoculation	E-0	66.0±11.2 ^d	7.3±0.21 ^b	
	E-500	36.8±8.20 ^c	7.1±0.21 ^b	
	E-2000	25.4±9.00 ^c	7.0±0.21 ^b	

^{a-c} Means \pm SE with different superscripts within the same column are different ($P < 0.05$).

Fig. 1. Surface metmyoglobin (1a) and aerobic plate count (1b) of beef longissimus lumborum muscle samples (aged 7 days; trial 1) obtained from cattle supplemented with vitamin E at levels of 0 (E-0) and 2000 (E-2000) mg/head/day. Beef samples were treated with peptone water (0.1%; C) or antibiotic solution (streptomycin a mg/mL and tetracycline 1 mg/mL; T) for 1 min, placed at 25°C for 24 hr and subsequently stored at 4°C.

Fig. 2. Surface metmyoglobin (2a) and aerobic plate count (2b) of beef longissimus lumborum muscle samples (aged 28 days; trial 2) obtained from cattle supplemented with vitamin E at levels of 0 (E-0) and 2000 (E-2000) mg/head/day. Beef samples were treated with peptone water (0.1%; C) or antibiotic solution (streptomycin a mg/mL and tetracycline 1 mg/mL; T) for 1 min, placed at 25°C for 24 hr and subsequently stored at 4°C.

