Effect of transport stress on respiratory disease, serum antioxidant status, and serum concentrations of lipid peroxidation biomarkers in beef cattle

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Objective—To determine the effect of transportation stress on serum concentrations of oxidative stress biomarkers of calves.

Animals—105 crossbred beef steer calves (mean [± SD] body weight, 207 ± 21.2 kg).

Procedure—Calves were assembled at 1 location in Tennessee, and pretransit (day –3) blood samples were collected. Calves were allotted randomly by body weight into 2 groups. Calves were transported 1,930 miles to a feedlot in Texas, and 1 group received tilmicosin phosphate (33 µg/kg, SC) upon arrival. Calves were weighed and blood samples collected on the day of arrival (day 1) and on days 15, 22, and 28. Calves were scored daily for signs of bovine respiratory disease (BRD). Serum total antioxidant capacity (TACA) and serum malondialdehyde (MDA) concentrations were determined.

Results—Transportation stress significantly decreased mean serum TACA concentrations (from 147 ± 31.2 U/mL to 133 ± 20.1 U/mL) and significantly increased serum MDA concentrations (from 10.9 ± 18.3 µg/mL to 30.2 ± 50.5 µg/mL). Calves that died had a 43% increase in serum MDA concentration on day 1, compared with calves that lived (42.2 ± 67.0 µg/mL vs 29.4 ± 49.4 µg/mL, respectively). Calves that had ≥ 3 episodes of BRD had 2-fold higher serum MDA concentrations on day 1 than healthy calves. Tilmicosin-treated calves had a 20.8% significantly greater average daily gain and significantly greater serum TACA concentration than nontreated calves on day 28.

Conclusions and Clinical Relevance—Transportation stress increases serum concentrations of oxidative stress biomarkers that are related to episodes of BRD and mortality in calves. (*Am J Vet Res* 2004;65:860–864)

There is a growing interest in the role of oxidative stress in diseases of feeder cattle (ie, cattle that need further feeding prior to slaughter) because of the link between the antioxidant defense and immune systems of humans and nonruminants.[1-3] **Bovine respiratory disease (BRD)** complex still represents the main cause of morbidity and mortality of feedlot cattle, with substantial annual economic losses resulting from decreased feed efficiency and increased therapeutic costs, as well as lower final body weight, average daily gain, carcass weight, and standard USDA grades.[4,5] To date, a decrease in serum antioxidant status and an increase in lipid peroxidation have not been identified as etiologic factors in BRD. However, accumulating circumstantial evidence is consistent with their involvement.[a,b] Acute confinement of calves has been reported to decrease serum ascorbic acid (vitamin C) concentrations, which is consistent with its depletion by reaction with reactive oxygen species.[6] Calves purchased in Arkansas that were fed a receiving diet (ie, a diet fed to calves upon arrival to a feedlot) before transit for 42 days and then transported to Texas had a decrease in mean plasma ascorbic acid concentrations from 2.67 to 0.16mM, with some calves with plasma concentrations below detectable limits.[c] Supplementation of diets with antioxidant vitamin E (800 to 1,600 IU/calf/d) produced a 12% to 27% decrease in the incidence of BRD in feeder calves and improved their performance, as measured by average daily gain.[d] Although performance of beef cattle was not affected by supplemental vitamin E (1,140 IU/animal/d), a linear increase was observed in serum IgG titers against ovalbumin challenge 21 days after cattle had been fed a receiving diet.[7]

Oxidative stress occurs when the generation of reactive metabolites of oxygen exceeds their safe detoxification by antioxidant mechanisms.[1,2] Oxidative stress can contribute to the onset of periparturient disorders in dairy cattle.[8] It has also been reported that cows exposed to moderate heat stress

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(rectal temperature, 39.5 ± 0.2°C) as a result of a high summer temperature-humidity index[9] (daily mean ± SD humidity index, 73.2 ± 2.5) had higher erythrocyte superoxide dismutase, glutathione peroxidase activity, intracellular thiols, and thiobarbituric acid reactant substances, compared with cows that calved in the spring; these findings provide evidence of oxidative stress in lactating dairy cows during the hot summer months in tropical climates.[10] However, other researchers reported no effect of heat stress on plasma concentration of vitamin E and β-carotene or on muscle content of thiobarbituric acid reactant substances of dairy cows.[11] The immature antioxidant defense system of calves during the neonatal period could make them susceptible to oxidative stress.[12] We propose that stressors such as marketing (through an auction barn) and transportation to the feedlot precipitate oxidative stress in cattle, which reduces the antioxidant defense capacity and increases total body lipid peroxidation, resulting in the susceptibility of cattle to BRD at the feedlot. Therefore, the objective of the study reported here was to determine the effect of transportation stress on serum **total antioxidant capacity** (**TACA**; oxidative stress biomarker) and **malondialdehyde** (**MDA**; lipid peroxidation biomarker) concentrations in steer calves that were either treated or not treated with tilmicosin phosphate upon arrival at the feedlot. These biomarkers could be useful to assess the risk of developing BRD in cattle at the feedlot.

Materials and Methods

Animal purchasing—One hundred five crossbred steer calves (mean [± SD] body weight, 207 ± 21.2 kg) were purchased from 3 locations in eastern Tennessee. Farms of origin of calves were unknown. All calves were assembled at an order buyer barn (ie, a facility where cattle are purchased for customers in the meat packing industry), weighed individually, and ear tagged with unique identification numbers. Rectal temperatures were measured, and calves were vaccinated against infectious bovine rhinotracheitis, parainfluenza virus,[e] and clostridial disease (ie, *Clostridium chauvei*, *C septicum, C novyi* Type B, *C sordelli, C perfringens* Types C and D, *and C novyi* Type D).[f] Calves were also treated for internal and external parasites.[g] All drugs were used according to label directions. Blood samples were obtained from calves by jugular venipuncture by use of sterile techniques. Serum samples obtained from blood samples were frozen and used later to determine serum TACA and MDA concentrations. Cattle were transported by truck and trailer for approximately 1,930 km (19 hours and 40 minutes) from Tennessee to the Texas A&M University beef cattle research facility in Bushland, Tex. The time required from the day of purchasing and processing to the day of arrival at the research facility was 3 days. Prior to shipping and in addition to all other vaccines received, 50% of calves were also vaccinated against *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*).

Animal housing and treatments—Upon arrival (day 1) at the feedlot, calves were housed in outdoor pens without cover and offered clean water and hay. On day 1 and throughout the rest of the experiment (28 days total), calves were fed a typical pelleted receiving diet formulated to meet or exceed the growing nutrient requirements of feeder steer calves.[13] The diet consisted of 45% roughage, 55% concentrate, and 13.5% crude protein. Throughout the study, feed and water were available on an ad libitum basis. Calves that were vaccinated against *M haemolytica* were weighed (day 1), sorted by weight from largest to smallest, and allotted into the following 2 groups that were used to evaluate the effects of tilmicosin on serum TACA concentrations: 1) treatment-group calves that received tilmicosin (33 µg/kg, SC)[h] and 2) control-group calves that did not receive tilmicosin. Thus, a 2 × 2 factorial arrangement of pretransit vaccination and treatment with tilmicosin was used in this study. Throughout the study, calves were visually evaluated daily at 8 AM for nasal or ocular discharge, anorexia, and depression.[4] When 2 or more of these signs were observed, rectal temperatures were measured and used to confirm morbidity. A calf was considered morbid if the rectal temperature was ≥ 40°C. Morbid calves from the treatment group were treated with tilmicosin[h] when first identified as sick and as not having received tilmicosin at the time of arrival. Morbid calves from the control group were treated with oxytetracycline[i] or ceftiofur.[j] All drugs were used according to labeled directions. Calves were weighed, rectal temperature was measured, and blood was obtained on day –3 (in Tennessee) and on days 1, 15, 22, and 28 (in Texas) after transit. Only blood samples obtained on days -3 and 1 were used for MDA assays. All blood samples were centrifuged at 3,000 × g for 20 minutes in a refrigerated centrifuge, and the harvested serum was frozen at < –40°C until subsequent TACA and MDA analyses. From the time blood samples were collected and centrifuged and serum was obtained, samples were kept away from light to minimize oxidation.

TACA assay—Concentrations of TACA were determined on serum obtained from blood samples collected before (day –3) and after (days 1, 15, 22, and 28) transit; serum TACA concentration represents the total reductant capacity of blood. For this analysis, a previously used method[14] with some modification was used. A 40-µL aliquot of serum (diluted if too concentrated for TACA detection) was added to a 12 × 75-mm test tube with 40 µL of cumene hydroperoxide solution, 120 µL of methanol, and 200 µL of chemiluminescent reagent. Methanol was used as a positive control and prepared exactly the same as the serum samples. After providing a vortex, the tube was put in a luminometer.[k] Chemiluminescent light was recorded 9 times in 3 minutes (10 s/count). The TACA units were calculated in the serum or control according to the following inhibitory slope of the sample:

$$1 \text{ unit} = (\text{Log } I_o/I) \times 100/30$$

where I_o = chemiluminescence count of control and I = chemiluminescence count of serum. Results are presented as units per milliliter.

Malondialdehyde assay—Serum MDA concentrations were analyzed on the basis of a previously used method[15] with some modifications. Serum samples were incubated with an equal volume of 0.1M perchloric acid for 15 minutes at room temperature (approx 24°C). After centrifugation, 400 mL of supernatant from the sample was mixed thoroughly with 20 mL of 15mM 2,4-dinitrophenylhydrazine in 2N HCl (10:1, vol/vol) for 20 minutes (derivatization). The formed hydrazone of MDA was extracted with pentane, dried under gas nitrogen, and reconstituted with 100 mL of the high-pressure liquid chromatography mobile phase. Samples were automatically injected into a high-pressure liquid chromatography-UV light system,[l] which was run by use of a software program.[m] Samples were eluted through a C18 column (4 µm; 4.8 × 100 mm)[n] that was guarded by use of an insert[o] with 49% aqueous acetonitrile at a flow rate of 1 mL/min. A detector[p] that was set at 280 to 380 mm was used for measurement of MDA. Results are presented as micrograms per milliliter.

Statistical analysis—Data were analyzed in 2 stages to determine the following: 1) the effects of transportation stress and vaccination against *M haemolytica* on serum TACA and MDA concentrations on day –3 (in Tennessee) and day 1 (in Texas), and 2) the effects of transportation stress, antibiotic (tilmicosin) treatment, time (days), and vaccination against *M haemolytica* on average daily gain, rectal temperature, and serum TACA concentrations. Data were analyzed by use of mixed-model procedure[16,q] for a completely randomized design with repeated measures. Fixed effects included in the model were transportation stress, post-transit treatment with tilmicosin, time (days at the feedlot after transit), and all corresponding interactions. The random factor was a steer calf within the pen. The model of choice for these data was the spatial power for covariance structures, which was used to fit a matrix to the time error variance and covariance. The spatial power model type specification included unequal spacing for day variance and covariance. Degrees of freedom were calculated by use of specifications of Kenward and Rogers.[16] When a significant ($P < 0.05$) difference was found, as indicated by results of the F test, least-square means procedures were used to separate the means. For variables of serum concentrations and rectal temperature, pretransit values were included in the analysis as covariates for the feedlot data analysis.

Results

Animal health and average daily gain—Of 105 calves, 10 (9.5%) died of acute BRD, including 3 (2.85%) that died before arrival. Pretransit vaccination against *M haemolytica* had no significant ($P = 0.351$) effect on average daily gain, rectal temperature, or serum TACA and MDA concentrations, nor was there a significant ($P = 0.211$) vaccination interaction with post-transit tilmicosin treatment. Transportation stress significantly ($P = 0.030$) decreased the mean (± SD) body weight of all steer calves (from 207 ± 21.2 kg to 196 ± 11.4 kg) by 5.3%. Pretransit (day –3) rectal temperature was negatively correlated with post-transit body weight ($R = –0.24$; $P = 0.033$) measured on arrival (day 1). Post-transit (day 1) rectal temperature positively correlated with the number of episodes of BRD ($R = 0.24$; $P = 0.026$). On days 1 and 15 after transit, all calves had a significantly ($P = 0.001$) higher rectal temperature, compared with the pretransit rectal temperature. During the entire study, treatment with tilmicosin significantly ($P = 0.017$) increased the mean average daily gain of calves by 20.8%, compared with that of control-group calves (1.16 ± 0.60 kg/d vs 0.96 ± 0.88 kg/d, respectively).

Serum TACA—Transportation stress significantly ($P = 0.024$) decreased mean serum TACA concentrations of all steer calves (from 147 ± 31.2 U/mL to 133 ± 20.1 U/mL). Pretransit (day –3) serum TACA concentrations were correlated ($R = 0.74$; $P = 0.049$) with post-transit (day 1) concentrations. However, post-transit (day 1) serum TACA concentrations were negatively correlated ($R = –0.17$), but not significantly ($P = 0.108$), with serum MDA concentrations measured at the same time. Calves that eventually died had serum TACA concentrations before transit (day –3) that were positively correlated ($R = 0.74$; $P = 0.002$) with values measured upon arrival at the feedlot (day 1).

No significant ($P = 0.940$) tilmicosin treatment by time (day) interaction was found for serum TACA concentrations measured on days 1, 15, 22, and 28. Also, treatment with tilmicosin had no significant ($P = 0.753$) effect on serum TACA concentrations except on day 28. However, as calves spent more time at the feedlot, serum TACA concentrations continued to decrease up to and including day 28 ($P = 0.001$; **Fig 1**). At the feedlot, mean serum TACA concentrations on day 1 were not significantly ($P = 0.231$) different from mean serum TACA concentrations measured on day 15 (133 ± 20.0 U/mL vs 126 ± 20.5 U/mL, respectively). However, on days 22 and 28 after transit, serum TACA concentrations were significantly ($P = 0.001$) lower than serum TACA concentrations measured before transit (day –3) or on day 1 after transit. On day 28, serum TACA concentrations were still 19.6% lower than pretransit values.

Serum lipid peroxidation—A 3-fold increase ($P < 0.001$) in mean serum MDA concentrations of calves was observed after transportation (day 1), compared with values obtained at the order buyer barn on day –3 (30.2 ± 50.5 mg/mL vs 10.9 ± 10.3 mg/mL, respectively). Consequently, a positive correlation was observed between pretransit and post-transit serum MDA concentrations ($R = 0.51$; $P < 0.001$). More importantly, when serum MDA concentrations on day –3 of calves that survived and calves that eventually died at the feedlot were compared, the mean serum MDA concentration of calves that died significantly ($P = 0.001$) increased 1.44-fold, compared with calves that survived (24.3 ± 24.5 µg/mL vs 9.95 ± 17.5 µg/mL, respectively). Calves that died had serum MDA concentrations on day –3 (before transit) that were positively correlated ($R = 0.85$; $P = 0.001$) with values measured upon arrival at the feedlot (day 1). Also, serum MDA concentrations measured before transit (day –3) of calves that lived were positively correlated ($R = 0.46$; $P < 0.001$) with serum MDA concentrations measured on day 1 after transit. Consequently, calves that died had a 43% greater mean serum MDA concentration on day 1 after transit, compared with calves that lived (42.2 ± 67.2 µg/mL vs 29.4 ± 49.4 µg/mL, respectively).

Post-transit (day 1) serum MDA concentrations of healthy calves (ie, those with 0 episodes of BRD) and calves that had 1 or 2 episodes of BRD during the entire

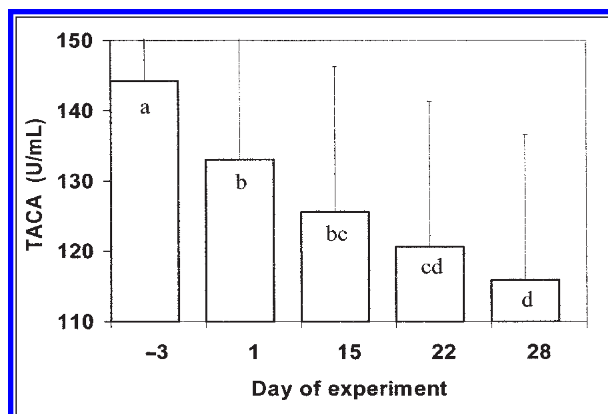

Figure 1—Mean (± SD) serum total antioxidant capacity (TACA) concentrations versus time (days) in 105 transported beef steer calves from 3 days before transport (day –3) until days 1 to 28 after transport.[a,b,c,d] Values with different superscript letters differ significantly ($P < 0.001$) from each other.

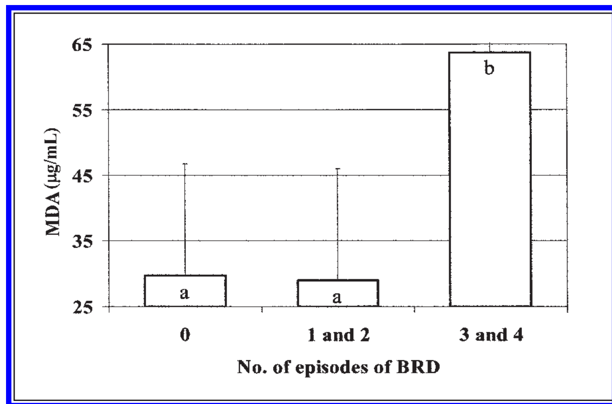

Figure 2—Mean (± SD) post-transit (day 1) serum malondialdehyde (MDA) concentrations versus 0 (18 calves), 1 and 2 (70 calves), or 3 and 4 (7 calves) episodes of bovine respiratory disease (BRD) in 95 transported beef steer calves. [a,b]Values with different superscript letters differ significantly ($P < 0.001$) from each other.

trial were not significantly ($P = 0.781$; **Fig 2**) different. However, calves that had ≥ 3 episodes of BRD had 2-fold higher serum MDA concentrations on arrival (day 1) than healthy calves. Three calves died before arrival at the feedlot; thus, no post-transit serum TACA or MDA concentrations were measured in these calves. Remaining calves (n = 7) that died had a mean post-transit serum MDA concentration of 41.03 ± 67.0 µg/mL. Death of these calves occurred within 7 days after transit.

Discussion

In a report[17] on 2 studies on recieving diets in beef steer calves, treatment with tilmicosin did not significantly ($P = 0.060$) affect average daily gain. However, 1 study[18] on receiving diets did report an increase in average daily gain of calves that had been treated with tilmicosin phosphate,on a mass medication basis or based on rectal temperature. In our study, average daily gain of calves treated with tilmicosin was significantly ($P = 0.017$) greater than that of control-group calves. The difference between our study and the other 2 previous studies is that the calves in our study were vaccinated against *M haemolytica*.

Serum TACA concentration is a measurement of the reductant capacity or capability of the body. Some of the prominent reductants or antioxidants involved in the antioxidant defense system include retinols (vitamin A_1), ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), glutathione (oxidized and reduced glutathione or glutathione peroxidase), superoxide dismutase, catalase, and uric acid.[1-3] Transportation stress significantly decreased serum TACA concentrations of calves in our study. This decrease is a reflection of the reductant capability of the whole antioxidant defense system, with the assumption that all of the antioxidant mechanisms are synergistic.[1,4] Nutritional stress has been shown to be prevalent in calves that have been transported long distances from auction barns to the feedlot.[5,a] Because some of the antioxidants are nutrients, nutritional and environmental stressors could result in their depletion during marketing and transportation of cattle. Thus, a positive correlation was observed between the pretransit and post-transit antioxidant capacity measurements, suggesting that calves with less antioxidant capacity after the marketing process often arrive at the feedlot with even lower antioxidant capacity. A decrease in antioxidant concentrations could result in a decrease in the ability of calves to detoxify reactive metabolites or reactive oxygen species produced by cells during aerobic metabolism.[1]

By the end of our study, serum TACA concentrations had decreased by 12.9% from values measured at arrival (day 1) and 19.6% from the time calves were loaded at the order buyer barn. This continuous and precipitous decrease in serum TACA concentrations indicated that the environmental factors associated with marketing, relocation, crowding, and other factors produced oxidative stress that could increase the susceptibility of market-stressed cattle to pro-oxidant stressors, resulting in oxidative stress. The relationship between oxidative stress and the immune system has been reported in humans and nonruminants.[3,4] In our study, calves that had 3 or more episodes of BRD or died had proportionately greater serum MDA concentrations at arrival to the feedlot than healthy cattle, indicating that a relationship exists between cellular lipid peroxidation and health of cattle purchased from an auction barn and transported cattle.

In our study, the observation that serum TACA concentrations on day 28 were still lower than serum TACA concentrations measured either before transit or at arrival might be a sign of the venerability of the antioxidant defense system in transported calves that have been at the feedlot for ≥ 28 days. This state of venerability might partly explain the unexpected outbreak of diseases in transported cattle in commercial feedlots.

Malondialdehyde is 1 of several end products formed through the peroxidation of primary and secondary lipid products.[19] Malondialdehyde has been used as a biomarker of lipid peroxidation in nonruminants and humans. However, to date, MDA has not been shown to be a putative risk factor for BRD in cattle. In our study, a 3-fold increase was found in serum MDA concentrations, resulting from the transportation of calves to the feedlot, suggesting possible oxidative damage to cellular lipids. Furthermore, calves that died had a 1.44-fold increase in serum MDA concentrations before they were loaded on the truck, compared with calves that survived. These results suggest that transportation stress exacerbated marketing and nutritional stress, resulting in lipid peroxidation and lower antioxidant capacity. Consequently, the risk of BRD was increased as evidenced by the doubling of serum MDA concentrations in calves that had ≥ 3 episodes of BRD, compared with healthy calves.

Lipid peroxidation of biomembranes can affect the structure and function of membranes in several important ways by decreasing the relative content of polyunsaturated fatty acids, forming lipid peroxides that may stimulate or inhibit the membrane and protein conformation in the membrane, oxidizing thiols that may affect enzyme activities in the membrane, decreasing lipid fluidity in the membrane, and liberating breakdown products from the site of lipid peroxidation.[20] Because of the potential effects of lipid per-

oxidation on tissue types (eg, adipocytes),[19] it would be interesting and economically relevant to find out whether serum MDA concentrations are related to carcass characteristics of cattle, especially in cattle that have been exposed to extensive environmental and biological stressors.

The use of serum TACA and MDA concentrations to access the oxidative stress status of transported cattle in our study is 1 of several previously reported methods[10-12] used to determine oxidative stress status. For example, in 1 study,[10] oxidative status was measured in lactating dairy cows by determining oxidative markers in plasma (glutathione peroxidase activity, thiol groups, reactive oxygen metabolites, and thiobarbituric acid reactant substances) and erythrocytes (glutathione peroxidase activity, intracellular thiols, superoxide dismutase, and thiobarbituric acid reactant substances). This approach basically quantified the activity of individual antioxidant species or the presence of tissue damage byproducts (lipid peroxides) in the blood. In another study,[12] thiobarbituric acid reactant substances were used to measure lipid peroxides in the serum of cows and their neonatal calves along with the antioxidative activity of calf serum by measuring superoxide-scavenging activities, ferroxidase activities, and the concentration of bilirubin-associated albumin. Results of this approach revealed target molecules involved in antioxidative activity and byproducts of tissue damage (lipid peroxides). Other researchers[11,a-c] selectively measured plasma key antioxidants (α-tocopherol, β-carotene, ascorbic acid, vitamin E, and uric acid) and used them as biomarkers of oxidative stress status.

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[a]Chirase NK, Greene LW, Purdy CW, et al. Effect of environmental stressors on ADG, serum retinol and α-tocopherol concentrations, and incidence of bovine respiratory disease of feeder steers (abstr). *J Anim Sci* 2001;79(suppl 1):188.

[b]Chirase NK, Greene LW, Purdy CW, et al. Influence of dietary antioxidant vitamins on performance of feeder steers exposed to simulated feedyard dust (abstr). *J Anim Sci* 2001;79(suppl 1):188.

[c]McBride KW, Greene LW, Chirase NK, et al. The effects of ethoxyquin on performance and antioxidants status of feedlot steers (abstr). *J Anim Sci* 2001;79(suppl 1):285.

[d]Hutcheson DP, Cole NA. Vitamin E and selenium for yearling feedlot cattle (abstr). *Fed Proc* 1985;44:549.

[e]Reliant, Rhone Merieux Inc, Athens, Ga.

[f]Electroid, Mallinckrodt Veterinary Inc, Mundelein, Ill.

[g]Ivomec, Merck & Co Inc, Rahway, NJ.

[h]Micotil 300, Elanco Animal Health, Indianapolis, Ind.

[i]Pfizer Inc, New York, NY.

[j]Pharmacia & Upjohn Animal Health, Kalamazoo, Mich.

[k]Lumicounter BG-1 Bacterial Systems, GEM Biomedical Inc, New York, NY.

[l]Waters 600s controller and 616 pump with the 717 autoinjector (4°C), Waters, Milford, Mass.

[m]Waters Millennium 2010 software package, Waters, Milford, Mass.

[n]Waters Nova Pak cartridge, Waters, Milford, Mass.

[o]Waters Nova Pak C18 Guard-Pak insert, Waters, Milford, Mass.

[p]Waters 996 photodiode array, Waters, Milford, Mass.

[q]PC SAS 2000, SAS Institute Inc, Cary, NC.

References

1. Sies H, Stahl W, Sundquist AR. Antioxidant functions of vitamins. *Ann N Y Acad Sci* 1992;669:7–20.
2. Jacob RA. The integrated antioxidant system. *Nutr Res* 1995;15:755–766.
3. Wu D, Meydani SN. Antioxidants and immune function. In: Papas MA, ed. Antioxidants status, diet, nutrition, and health. Washington, DC: CRC Press Inc, 1999;371–400.
4. Edwards A. Respiratory diseases of feedlot cattle in central USA. *Bovine Pract* 1996;30:5–7.
5. Gardner BA, Dolezal HG, Bryant LK, et al. Health of finishing steers: effects on performance, carcass traits and meat tenderness. *J Anim Sci* 1999;77:3168–3175.
6. Mason GL, Friedlander LG, Richards AB, et al. Physiopathological features of acute confinement stress in calves. In: *Bovine respiratory disease: a symposium*. College Station, Tex: Texas A&M University Press, 1984;495–496.
7. Rivera JD, Duff GC, Galyean ML, et al. Effects of supplemental vitamin E on performance, health, and humoral immune response of beef cattle. *J Anim Sci* 2002;80:933–941.
8. Miller JK, Brzezinska-Slebodzinska E, Madsen FC. Oxidative stress, antioxidants, and animal function. *J Dairy Sci* 1993;2812–2823.
9. Ingraham RH, Stanley RW, Wagner WC. Seasonal effects of tropical climate on shaded and nonshaded cows as measured by rectal temperature, adrenal cortex hormones, thyroid hormone, and milk production. *Am J Vet Res* 1979;40:1792–1797.
10. Bernabucci U, Ronchi B, Lacetera N, et al. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *J Dairy Sci* 2002;85:2173–2179.
11. Trout JP, McDowell LR, Hansen PJ. Characteristics of the estrous cycle and antioxidant status of lactating Holstein cows exposed to heat stress. *J Dairy Sci* 1998;81:1244–1250.
12. Inanami O, Shiga A, Okada K, et al. Lipid peroxides and antioxidants in serum of neonatal calves. *Am J Vet Res* 1999;60:452–457.
13. National Research Council. *Nutrient Requirements of beef cattle*. 7th ed. Washington, DC: National Academy Press, 1996.
14. Hirayama O, Takagi M, Hukumoto K, et al. Evaluation of antioxidant activity by chemiluminescence. *Anal Biochem* 1997;247:237–241.
15. Bagchi D, Bagchi M, Hassoun E, et al. Effects of carbon tetrachloride, menadione, and paraquat on the urinary excretion of malondialdehyde, formaldehyde, and acetaldehyde, and acetone in rats. *J Biochem Toxicol* 1993;8:101–106.
16. Kenward MG, Rogers JH. *Small sample inference for fixed effects from restricted maximum likelihood.* Institute of Mathmatics and Statistics, University of Kent, Canterbury, UK 1997;53(3):983–987.
17. Galyean ML, Gunter SA, Malcolm-Callis KJ. Effects of arrival medication with tilmicosin phosphate on health and performance of newly received beef cattle. *J Anim Sci* 1995;73:1219–1226.
18. Duff GC, Walker DA, Malcolm-Callis KJ, et al. Effect of preshipping vs arrival medication with tilmicosin phosphate and feeding chlortetracycline on health and performance of newly received beef cattle. *J Anim Sci* 2000;78:267–273.
19. Halliwell B, Gutteridge JMC. *Free radicals in biology and medicine*. 2nd ed. Oxford, England: Clarendon Press, 1989;88–106.
20. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci U S A* 1993;90:7915–7922.