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.


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 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. 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. 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. 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. 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 0.16 mM, with some calves with plasma concentrations below detectable limits. 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. 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.

Oxidative stress occurs when the generation of reactive metabolites of oxygen exceeds their safe detoxification by antioxidant mechanisms. Oxidative stress can contribute to the onset of periparturient disorders in dairy cattle. It has also been reported that cows exposed to moderate heat stress...
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 nutrient requirements of feeder steer calves. 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) and 2) control-group calves that did not receive tilmicosin. Thus, a 2 x 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. 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 control group were treated with tilmicosin 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 or ceftriaxone. 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 X g for 20 minutes in a refrigerated centrifuge, and the harvested serum was frozen at –4°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 reducible capacity of blood. For this analysis, a previously used method with some modification was used. A 40-µL aliquot of serum (diluted if too concentrated for TACA detection) was added to a 12 X 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 lumimeter. Chemiluminescent light was recorded 9 times in 3 minutes (10 s/count). The TACA units were calculated in the serum sample:

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1 \text{ unit} = (\log I/I_0) \times 100/30
\]

where \(I_0\) = 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 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, which was run by use of a software program. Samples were eluted through a C18 column (4 mm; 4.8 X 100 mm) that was guarded by use of an insert with 49% aqueous acetonitrile at a flow rate of 1 mL/min. A detector that was set at 280 to 380 nm 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 procedure66* 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 degrees of freedom for day variance and covariance. Degrees of freedom were calculated by use of specifications of Kenward and Rogers.6 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 (2.8%) that died before arrival. Pretransport vaccination against M haemolytica had no significant (P = 0.331) 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%. Pretransport (day –3) rectal temperature was negatively correlated with post-transit body weight (R = –0.24; P = 0.033) measured on arrival (day 1). Posttransport (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 pretransport 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). Pretransport (day –3) serum TACA concentrations were correlated (R = 0.74; P = 0.049) with posttransport (day 1) concentrations. However, posttransport (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 pretransport 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 pretransport and posttransport 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).

Posttransport (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](image-url)
Discussion

A study17 on recieving diets in beef steer calves, treatment with tilmicosin did not significantly ($P = 0.060$) affect average daily gain. However, 1 study18 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), ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), glutathione (oxidized and reduced glutathione or glutathione peroxidase), superoxide dismutase, catalase, and uric acid.15,16 Transportaion 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.17 Nutritional stress has been shown to be prevalent in calves that have been transported long distances from auction barns to the feedlot.18 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.19 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 vulnerability of the antioxidant defense system in transported calves that have been at the feedlot for ≥28 days. This state of vulnerability 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.20 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.21 Because of the potential effects of lipid per-

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. Values with different superscript letters differ significantly ($P < 0.001$) from each other.

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oxidation on tissue types (eg, adipocytes),\textsuperscript{18} 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\textsuperscript{10-12} used to determine oxidative stress status. For example, in 1 study,\textsuperscript{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,\textsuperscript{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\textsuperscript{13-15} 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.


\textsuperscript{20}Reliant, Rhone Merteix Inc, Athens, Ga.

\textsuperscript{21}Electroid, Mallinckrodt Veterinary Inc, Mundelein, Ill.

\textsuperscript{22}Itron, Merck & Co Inc, Rahway, NJ.

\textsuperscript{23}Microlit 300, Elanco Animal Health, Indianapolis, Ind.

\textsuperscript{24}Pfizer Inc, New York, NY.

\textsuperscript{25}Pharmacia & Upjohn Animal Health, Kalamazoo, Mich.

\textsuperscript{26}Lumicounter BG-1 Bacterial Systems, GEM Biomedical Inc, New York, NY.

\textsuperscript{27}Waters 600s controller and 616 pump with the 717 autoinjector (4°C), Waters, Milford, Mass.

\textsuperscript{28}Waters Nova Pak C18 Guard-Pak insert, Waters, Milford, Mass.

\textsuperscript{29}Waters 996 photodiode array, Waters, Milford, Mass.

\textsuperscript{30}PC SAS 2000, SAS Institute Inc, Cary, NC.

\textbf{References}


