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 the 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 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 ± 670 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.1,2 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.3 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.4-6 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.7 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.8 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.9 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.10 Oxidative stress occurs when the generation of reactive metabolites of oxygen exceeds their safe detoxification by antioxidant mechanisms.11 Oxidative stress can contribute to the onset of parturient disorders in dairy cattle.12 It has also been reported that cows exposed to moderate heat stress...
(rectal temperature, 39.5 ± 0.2°C) as a result of a high summer temperature-humidity index 3 (daily mean of 7D humidity index, 73.2 ± 2.5) had higher erythrocyte superoxide dismutase, glutathione peroxidase activity, intracellular thiols, and thiobarbimetric 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. 5 However, other researchers reported no effect of heat stress on plasma concentration of vitamin E and β-carotene or on muscle content of thiobarbituric acids of dairy cows. 6 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 arrangement of pretransit vaccination and treatment with tilimicosin was used in this study. Throughout the study, calves were visually evaluated daily at 8 AM for nasal or ocular discharge, anorexia, and depression. 7 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 oxytetracycline 8 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 9 or ceftiofur. 10 All drugs were used according to label 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 < –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 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 sample. After providing a vortex, the tube was put in a luminometer. 5 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:

\[
1 \text{ unit} = \left( \log I_0/I \right) \times 100/50
\]

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 40 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. 41 Samples were eluted through a C18 column (4 mm; 4.8 x 100 mm) 3 that was guarded by use of an insert 4 with 49% aqueous acetonitrile at a flow rate of 1 mL/min. A detector 5 that was set at 280 to 380 nm was used for measurement of MDA. Results are presented as micrograms per milliliter.

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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 procedure16 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. 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 ($P = 0.331$) 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 ($\pm$ SD) body weight of all steer calves (from 207 $\pm$ 21.2 kg to 196 $\pm$ 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 $\pm$ 0.60 kg/d vs 0.96 $\pm$ 0.88 kg/d, respectively).

Serum TACA—Transportation stress significantly ($P = 0.024$) decreased mean serum TACA concentrations of all steer calves (from 147 $\pm$ 31.2 U/mL to 133 $\pm$ 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 ($133 \pm 20.0$ U/mL vs $126 \pm 20.5$ 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 ($30.2 \pm 50.5$ mg/mL vs $10.9 \pm 10.3$ 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 ($24.3 \pm 24.5$ µg/mL vs $9.95 \pm 17.5$ 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 ($42.2 \pm 67.2$ µg/mL vs $29.4 \pm 49.4$ 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](image-url)
after transit.

µtransit serum MDA concentration of 41.03

Remaining calves (n = 7) that died had a mean post-

or MDA concentrations were measured in these calves.

arrival at the feedlot; thus, no post-transit serum TACA

(day 1) than healthy calves. Three calves died before

2-fold higher serum MDA concentrations on arrival

However, calves that had

significant (P = 0.017) greater than that of control-group calves.

Mean (± SD) post-transit (day 1) serum malondialde-

hyde (MDA) concentrations versus 0 (18 calves), 1 and 2 (70

calves), or 3 and 4 (7 calves) episodes of bovine respiratory dis-

ease (BRD) in 95 transported beef steer calves. 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 report17 on 2 studies on receiving diets in beef

steer calves, treatment with tilmicosin did not signifi-

antly (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 pre-

vious studies is that the calves in our study were vacci-

nated against M haemolytica.

Serum TACA concentration is a measurement of the

redundant capacity or capability of the body. Some of the

prominent redutors 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 glu-

tathione or glutathione peroxidase), superoxide dismu-

tase, catalase, and uric acid.10 Transportion stress signifi-

cantly decreased serum TACA concentrations in calves

in our study. This decrease is a reflection of the redundant

capability of the whole antioxidant defense system, with

the assumption that all of the antioxidant mechanisms are

synergistic.11 Nutritional stress has been shown to be

prevalent in calves that have been transported long dis-

tances from auction barns to the feedlot.12 Because some of

the antioxidants are nutrients, nutritional and environ-

mental 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 antiox-

idant capacity. A decrease in antioxidant concentrations

could result in a decrease in the ability of calves to detoxi-

fy reactive metabolites or reactive oxygen species pro-

duced by cells during aerobic metabolism.13

By the end of our study, serum TACA concentrations

did not significantly 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

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duced by cells during aerobic metabolism.13

By the end of our study, serum TACA concentrations
oxidation on tissue types (e.g., 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{11} 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).


References
