Measurement of Exhaled Nitric Oxide and Exhaled Carbon Dioxide in the Breath of Calves upon Arrival and During a 42-Day Receiving Period

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Introduction

Endogenous nitric oxide (NO) is produced in many tissues from the amino acid L-arginine by a group of nitric oxide synthase enzymes. The inducible form of this enzyme has been identified in lung tissue from calves that succumbed to pneumonia. *In vitro* studies have measured NO derivatives from alveolar macrophages, stimulated with lipopolysaccharide and respiratory pathogens. The objective of this study was to measure exhaled nitric oxide (eNO) and exhaled carbon dioxide (CO_2) in steers upon arrival, and during a 42-day receiving trial, using tunable diode laser absorption spectroscopy (TDLAS).

Materials and Methods

Cattle and Experimental Design. Three hundred ninety-five steer and bull calves (mean initial body weight (BW) averaged across loads = 481.9 ± 49.4 lb [218.6 ± 22.4 kg]) were delivered to the Willard Sparks Beef Research Center from central Oklahoma auction markets in September 2005 for a 42-day study trial. Approximately one hour after arrival, calves were individually weighed and tagged with a sequential numbered ear tag. A whole blood sample for serum haptoglobin (Hp) analysis and a breath sample for eNO and CO_2 determination were collected from each calf. Thirty-six (loads 1-3) or 72 (load 4) hours following arrival, steers were individually weighed, administered a viral respiratory vaccine (Bovishield Gold 5, Pfizer Animal Health, Exton, PA), clostridial bacterin/toxoid (Ultrachoice 7, Pfizer Animal Health, Exton, PA) and an anthelmintic (Ivomec Plus, Merial, Duluth, GA); castration and dehorning was done as necessary. Cattle were blocked by weight and allocated into 24 pens. Throughout the trial, cattle were fed a 60% concentrate ration twice daily that was formulated to meet or exceed NRC (1996) nutrient requirements. Each morning, cattle were evaluated by trained personnel for signs of BRD including depression, lack of fill compared with pen mates cough, altered gait or stance, ocular or nasal discharge, or general physical weakness. Calves with clinical signs of BRD and a rectal temperature greater than 40°C were considered morbid and treated with an antimicrobial according to a standard treatment protocol. All animals treated for BRD had whole blood and breath collected for serum Hp and eNO and CO_2 analysis respectively. In addition, one clinically normal and previously untreated calf was selected daily during the first 28 days of the trial from one half of the pens using a predetermined order to serve as a control for blood and breath comparisons. Cattle were weighed on days 15, 29 and 43 of the trial.

Breath Analysis—Breath was simultaneously analyzed for eNO and CO_2 using TDLAS (Ekips Breathmeter, Ekips Technologies, Norman, OK). Briefly, a custom-made mask (Trudell Medical International, ON, Canada) equipped with one-way flutter valves was placed over the calf's muzzle while restrained in the chute. The mask was equipped with carbon dioxide (Capnostat 5, Respironics, Inc, Carlsbad, CA) and differential pressure sensors which engaged a sampling valve based on CO_2 level and a drop in peak exhalation pressure. This system enabled standardized side-stream breath sampling across a wide-range of bovine exhalation patterns, ensured that a relatively consistent sample of lower airway breath was captured for analysis and minimized sampling of ambient air. Typically, twenty exhalations were required, which took approximately 20-40 seconds. Breath analysis included a background subtraction technique, in which the sample was compared with NO scrubbed air (Triple Blend, Purafil, Inc, Doraville, GA) in order to limit the low frequency background noise in the measured spectra and allow for the detection of the low concentrations (< 1 ppb) of nitric oxide present in healthy cattle.

Haptoglobin Analysis—Samples collected for se-

rum Hp analysis were allowed to clot overnight at 39°F (4°C), centrifuged, and stored at 14°F (-10°C) until laboratory analysis could be conducted. Serum Hp concentrations were determined using bovine serum haptoglobin radial immunodiffusion kits (code No. P0105-1, Cardiotech Svcs, Inc, Louisville, KY).

Statistical Analysis—Data were analyzed using the Mixed Procedure of SAS (SAS Institute, Cary, NC). Individual animal was the experimental unit. For arrival data (BW, ADG, eNO, CO_2 and Hp) the number of treatments for BRD (0, 1, >1) was a fixed effect. Load and weight block were random effects. For data collected when animals were treated for clinical signs of BRD, treatment number (control, first, second or third treatment) was a fixed effect with load and block random effects. Control data were contrasted with treatment data for all response variables. Data from breath tests were eliminated from the analysis if eNO concentrations were above 1.7 ppb, indicating contamination from ambient air, the sampling valve was open for less than five seconds or greater than 12 seconds, or measured CO_2 concentrations fell below 0.5%.

Results

Upon arrival, mean eNO was 313.9 ± 415.2 ppt and mean CO_2 was 2.64 ± 0.94%. During the trial, 56.0% of steers were never treated for signs of BRD (Trt 0), 33.9% of steers were treated only once (Trt 1), and 10.1% were treated more than once (Trt>1). One steer died from BRD, and two steers died from reasons unrelated to the study. There were no differences in arrival eNO between steers that never received treatment for BRD and those that did ($P = 0.76$). However, CO_2 was less ($P = 0.03$) for Trt 0 (2.30%) than Trt 1 and Trt>1 (average = 2.50%). Arrival Hp did not differ between number of eventual treatments ($P = 0.38$). Day 43 BW and ADG were greatest for Trt 0 (599.9 lb [272.7 kg]; 2.90 lb[1.32 kg]/d), intermediate for Trt1 (582.3 lb [264.1 kg]; 2.57lb[1.17 kg]/d), and least for Trt>1 (552.8 lb [250.7 kg]; 2.05 lb[0.93 kg]/d; $P < 0.0001$).

For data collected daily from treated and control animals, rectal temperature was greater for the average of the treated animals (104.6°F; 40.3°C) than controls (102.9°F; 39.4°C, $P < 0.0001$). Serum Hp was also greater for the average of treated vs. control cattle ($P < 0.01$). In contrast to arrival day data, control steers had higher ($P < 0.0001$) CO_2 than steers treated for BRD. Least squares means for eNO values were 368.3 ppt for control steers, 564.5 ppt for steers receiving their first treatment, 368.3 ppt for steers receiving a second treatment and 462.2 ppt for steers treated a third time. The F statistic for the main eNO model was significant ($P < 0.05$); however, the contrast between control and treated steers was not significant ($P = 0.29$). When means were separated by least significant difference, eNO from control vs.steers treated the first time were different ($P < 0.01$). The inability to detect other differences in eNO may have resulted from the small number of animals that were treated more than one time.

Significance

Calves treated for BRD had higher CO_2 values upon arrival than calves never treated for BRD, but at time of treatment these values were lower than controls. Numerical trends suggested that eNO might be useful in diagnosing BRD. However, the fact that the eNO values were near the lower detection limits of current instrumentation and occasional contamination by high ambient NO decreased the accuracy of the measurement. Further improvements in technology and more research are needed.