MEAN PULMONARY ARTERIAL PRESSURE IN BEEF CALVES IS POSITIVELY ASSOCIATED WITH MIXED VENOUS CO2 TENSION AND OXYGEN EXTRACTION RATIO WHEN CONTROLLING FOR ALVEOLAR OXYGEN TENSION

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ABSTRACT: Chronic alveolar hypoxia is a known risk factor for bovine pulmonary hypertension (BPH). The recent development of portable blood-gas analyzers makes it possible to perform blood-gas analysis on large numbers of animals in field settings. Fast growing beef calves have a high O_2 demand at a time when their lungs are functionally immature. The effects of mismatch between O_2 demand and O_2 delivery on cardiopulmonary pathophysiology are unknown. Adequacy of O_2 delivery relative to demand can be estimated from the oxygen extraction ratio (OER), which is the absolute difference in oxyhemoglobin saturation between arterial and mixed venous blood divided by arterial oxyhemoglobin saturation. We hypothesized that OER is positively associated with mean pulmonary arterial pressure (PAP). Arterial and mixed venous blood-gas tensions and calculations derived from these indices were evaluated for associations with mean PAP. A total of 122 Angus calves were randomly sampled from 2 herds on 2 occasions at 2,731m (herd A, n = 64) and 2,166m (herd B, n =58) above sea level. Pulmonary pressures were measured using a fluid-filled catheter. Mixed venous blood and arterial blood were collected from the pulmonary and coccygeal arteries, respectively. Generalized estimating equations were used to account for repeated measures. Herd, age of calf and alveolar O_2 tension were included as fixed effects. Mixed venous CO_2 tension ($P < 0.001$) and OER ($P = 0.01$) were positively associated with mean PAP when controlling for herd ($P = 0.99$), alveolar O_2 tension ($P = 0.002$) and age ($P < 0.001$). A calf with an OER of 0.45 has a mean PAP 7.6 ± 1.6 mmHg higher than an calf with an OER of 0.1 when controlling for herd (B), age (200 days), alveolar O_2 tension (70 mmHg) and mixed venous CO_2 tension (50 mmHg). In conclusion, calves with a high O_2 demand relative to O_2 delivery are at increased risk of BPH. We speculate that continued selection of cattle for metabolically expensive traits, such as fast growth, without concurrent selection for physiologic traits associated with O_2 delivery is predicted to increase the incidence of BPH.

Key words: arterial blood-gas, beef calves, oxygen extraction, pulmonary arterial pressure

Introduction

Bovine pulmonary hypertension (**BPH**) is historically considered to be a disease of high altitude environments (Rhodes, 2005). The problem was first reported to occur almost 100 years ago (Glover and Newsom, 1915). It was reported to occur at altitudes over 2,134m (7,000ft) (Hecht et al., 1962). Medial hypertrophy of pulmonary arterioles was found to occur in response to chronic low alveolar O_2 tension (Jaenke and Alexander, 1973). Consequently, the reduction in vessel lumen diameter increases vascular resistance and pulmonary arterial pressure (**PAP**). Cardiac failure and death may ensue. This traditional model of BPH pathogenesis does not adequately explain the occurrence of BPH at moderate altitudes. For example, BPH occurrence in yearling Holsteins at 1,600m (5,249 ft.) (Malherbe et al., 2012). Recent studies prove the pathogenesis to be more complex than previous thought (Frid et al., 2006; Lammers et al., 2008; Stenmark et al., 2006). It has been speculated that cattle are susceptible to BPH because of their small cardiopulmonary system relative to their basal O_2 requirements (Veit and Farrell, 1978). The oxygen extraction ratio (**OER**) is the ratio of oxygen consumption to delivery and is typically 0.2-0.3 (McLellan and Walsh, 2004). An increase in OER above 0.3 represents an inability of the cardiopulmonary system to delivery sufficient O_2 to meet metabolic demands. We hypothesized that OER is positively associated with mean pulmonary arterial pressure (PAP) irrespective of alveolar O_2 tension. Arterial and mixed venous blood-gas tensions and calculations derived from these indices were evaluated for associations with mean PAP.

Materials and Methods

The Colorado State University Animal Care and Use Committee approved of the animal handling and sampling procedures prior to sample collection.

Study site

Calves from one herd in southern Wyoming (Herd A) and one herd in south-west Colorado (Herd B) were studied on 2 occasions (Table 1). Calves consisted predominantly of Aberdeen Angus genetics.

Table 1. Herd, altitude, date of sampling, number of calves sampled and age

Herd	Altitude, m	Date	n[1]	Mean age ± SD, days
A	2,166	07/31/2012	60	124.0 ± 18.2
		10/01/2012	65	186.7 ± 17.6
B	2,731	06/21/2012	58	85.9 ± 6.7
		10/10/2012	51	197.2 ± 6.6

[1] number of calves sampled

The dams of calves studied were given a pre-breeding and pre-calving vaccination offering protection against *Bovine herpesvirus 1* (infectious bovine rhinotracheitis [IBR]), BVDV, *Bovine respiratory syncytial virus* (BRSV), and *Bovine parainfluenza virus 3* (BPIV-3). Calves were vaccinated against the same respiratory pathogens at 4-8 weeks of age and 2-4 weeks prior to weaning. Both herds used a modified-live vaccine on both cows and calves. Calves on both ranches were administered a killed vaccine at 4-8 weeks of age offering protection against: *Cl. chauvoei, Cl. septicum, Cl. novyi, Cl. sordellii* and *Cl. perfringens* Type C and D. Vaccines were given according the manufacturers instructions.

In both herds ear notch samples are routinely collected from all calves kept as replacement heifers for *Bovine viral diarrhea virus* (BVDV) enzyme-linked immunosorbent assay testing. No calves persistently infected with BVDV have been detected in these herds to date. Communal grazing does not occur. Salt licks and mineral blocks are provided year-round. A hormonal growth promotant containing 100 mg progesterone and 10 mg estradiol benzoate was administered to both heifer and steer calves in herd B when they were approximately 8 weeks old.

Sample collection

A full description of the equipment, materials and facilities required for PAP testing is provided elsewhere (Holt and Callan, 2007). In brief, a large bore needle is inserted into the jugular vein. Flexible catheter tubing is then fed through the needle, down through the right atrium, into the right ventricle, and then into the pulmonary artery. A pressure transducer connects the saline-filled catheter to an oscilloscope. The position of the catheter is determined from the pressure waveform on the oscilloscope. The jugular vein, right atrium, right ventricle and pulmonary artery have distinct pressure waveforms.

After the measurement of PAP the catheter was disconnected from the transducer. In order to collect mixed venous blood from the pulmonary artery saline within the catheter was suctioned out using a 12 ml syringe. Approximately, 2 - 2.5 ml of blood was collected in a 3 ml syringe for all of the blood-gas analyses performed. Blood was collected from the coccygeal artery using a 22 gauge, 2.54 cm (1") hypodermic needle. The bovine coccygeal artery is a suitable source for blood-gas analysis (Collie, 1991; Nagy et al., 2002). Arterial blood unlike venous blood can fill a heparinized syringe without applying suction. Therefore, minimal, if any, negative pressure was applied to the syringe chamber by drawing on the plunger when obtaining a sample. Syringes were heparinized with approximately 0.25 ml of sodium heparin (1,000 IU/ml). The plunger of each syringe was pulled back to the 3 ml mark coating the inner chamber surface with heparin. Heparin was then expelled so that only the needle hub contained heparin. Collection of blood up to the 2 ml mark results in dilution of the blood sample with sodium heparin (1,000 IU/ml) by < 5 % (Hopper et al., 2005). Blood dilution of < 10 % is sufficient to minimize pre-analytic error (Hutchison et al., 1983). The sample was discarded if during collection the flow of arterial blood was interrupted. Air bubbles within the blood were immediately expelled and the first several drops of blood discarded before analysis. Blood-gas analysis was performed using a handheld analyser (VetScan i-STAT 1, Abaxis, Union City, CA, USA) within 3 minutes of the blood draw. Results were automatically stored under the animal identification number. A temperature 'correction' algorithm was used to adjust blood-gas tensions according to rectal temperature (CLSI, 2001). Variables evaluated for association with mean PAP included: pH, pO_2, pCO_2, oxyhemoglobin saturation (**$sHbO_2$**) and L-lactate. Alveolar O_2 tension was estimated from the alveolar gas equation (Fenn et al., 1946). The OER was calculated as absolute difference in oxyhemoglobin saturation between arterial and mixed venous blood divided by arterial oxyhemoglobin saturation.

Statistical Procedures

Statistical analyses were performed using STATA version 12 (Stata Corporation, College Station, Texas, USA). Generalized estimating equations were used to account for the repeated measures (Liang and Zeger, 1986; Zeger and Liang, 1986). An exchangeable correlation structure was used. Mean PAP was positively skewed and so was transformed into a normal distribution. Herd was included as a fixed effect to account for clustering. Age was forced into the model to account for functional maturity of the cardio-pulmonary system (Lekeux et al., 1984). Alveolar O_2 tension (**p_AO_2**) was forced into the model to account for the vasoconstrictive effect of alveolar hypoxia (Sylvester et al., 2012). Arterial and mixed venous blood-gas variables (pH, pCO_2, pO_2 and $sHbO_2$), OER and L-lactate were screened for association with mean PAP while controlling for herd, age and p_AO_2. All variables with an association ($P \leq 0.25$) were included in a backwards elimination model. A type 1 significance level of 0.05 was used for the final model. Two-way interactions between all variables in the final model were evaluated.

Results

Mixed venous CO_2 tension ($P < 0.001$) and OER ($P = 0.01$) were positively associated with mean PAP when controlling for herd ($P = 0.99$), p_AO_2 ($P = 0.002$) and age ($P < 0.001$). A calf with an OER of 0.45 has a mean PAP 7.6 ± 1.6 mmHg higher than an calf with an OER of 0.1 when controlling for herd (B), age (200 days), alveolar O_2 tension (70 mmHg) and mixed venous CO_2 tension (50 mmHg).

Discussion

Striking parallels can be drawn between BPH pulmonary hypertension of broiler chickens. Mean PAP is positively associated growth rate in beef calves (Shirley et al., 2008) and broiler chickens (Peacock et al., 1989). Pulmonary hypertension was first reported to occur in both calves and broilers at high altitude (Cueva et al., 1974; Glover and Newsom, 1915). It is now reported to occur in herds at moderate altitude (Malherbe et al., 2012) and flocks at sea level (Peacock et al., 1990). Pulmonary hypertension in broilers is the product of a physiological imbalance. Broilers have inadequate pulmonary (Wideman et al., 2007) and/or cardiac capacity (Olkowski et al., 2005) to deliver sufficient O_2 to meet requirements. It has been suggested that the small cardiopulmonary capacity of cattle relative to O_2 requirements is a risk factor for BPH (Veit and Farrell, 1978). Growth of broilers is positively associated with OER and mixed venous CO_2 tension (Olkowski et al., 2005). Here, we provide evidence that the same relationship exists for beef calves. High O_2 demand relative to delivery is a risk factor for BPH.
The parallels between BPH and pulmonary hypertension in broilers warrant further investigation.

Implications

We speculate that continued selection of cattle for metabolically expensive traits, such as fast growth, without concurrent selection for physiologic traits associated with O_2 delivery will increase the incidence of BPH. Pulmonary hypertension is estimated to cost the broiler industry $1 billion per year (Currie, 1999). The cost of BPH to the cattle industry is likely to be considerable given that BPH is not a disease exclusive to high altitude and current mitigation strategies, although beneficial, are not solving the problem (Neary, 2013).

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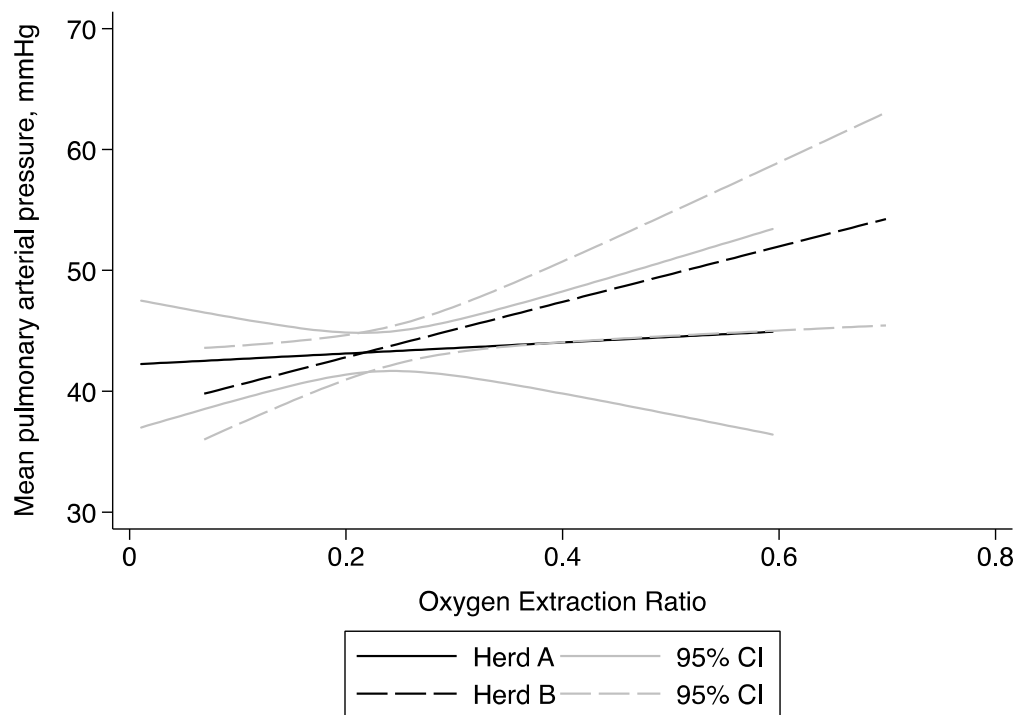
Figure 1. Mean pulmonary arterial pressure (mmHg) by herd and oxygen extraction ratio. Regression lines are provided with 95% confidence intervals (95% CI) of the mean. Calves in herd A and herd B were tested at altitudes of 2,166m and 2,731m, respectively.