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Angus calves born and raised at high altitude adapt to hypobaric hypoxia by increasing alveolar ventilation rate but not hematocrit[1]

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ABSTRACT: The objective of this study was to evaluate the effect of altitude on arterial blood-gases and hematocrit in Angus-based calves. It was hypothesized that alveolar ventilation rate, as indicated by arterial pCO_2, would increase with altitude but hematocrit would not. Five Angus-based herds (n = 30 to 80 per cohort) located at 105 m, 1,470 m, 2,010 m, 2,170 m, and 2,730 m above sea level were enrolled in this prospective cohort study. A portable analyzer measured blood-gas tensions in coccygeal arterial blood. Calves at 1,470 m, 2,170 m, and 2,730 m were sampled twice, at approximately 4 mo and 7 mo of age. Calves at 105 m and 2,010 m were sampled once, at 7 or 4 mo of age, respectively. Linear regression analyses were used to determine the fixed effect of herd (a proxy for altitude) on the 4 outcome variables pCO_2, pO_2, pH, and hematocrit, while controlling for age and sex. As hypothesized, alveolar ventilation rate increased with altitude ($P <$ 0.001). Hematocrit, however, did not show a clear association with altitude except for an increase from 105 m to ≥ 1,470 m ($P < 0.001$). Arterial pO_2 decreased significantly with increasing altitude in calves at 4 mo and 7 mo of age ($P < 0.001$). The adjusted mean values of the 4 variables studied were similar at 4 and 7 mo of age for all of the herds studied. This indicates that suckling calves show minimal respiratory or erythrocytic adaptation to hypoxemia and hypocapnia with increasing age, regardless of altitude. We propose that the lack of an erythrocytic response in hypoxemic calves born and raised at high altitude prevents a deleterious increase in viscous resistance and, consequently, pulmonary arterial pressure. This physiological response, or lack thereof, may be a survival adaptation in a species predisposed to hypoxia-induced pulmonary hypertension.

Key words: beef production, cattle, erythrocyte, respiratory disease

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INTRODUCTION

It has been said that cattle are prone to hypoxemia, or low arterial O_2 tension, because they have small lungs relative to their oxygen requirements (Veit and Farrell, 1978; Kainer and Will, 1981). This means hypobaric hypoxia, or low oxygen tension due to low atmospheric pressure, presents a major physiological challenge for cattle located in the Rocky Mountain region of the U.S. This is particularly true for calves; they are more prone to hypoxemia than older cattle because their lungs are functionally immature (Lekeux et al., 1984; Neary et al., 2014)

Most mammals respond to chronic hypoxemia by increasing hematocrit and alveolar ventilation rate. This allows animals to maintain a normal blood oxygen content in spite of a reduction in arterial

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oxygen tension (McNab, 2002). We recently reported, however, that Angus calves at an altitude of 2,730 m had a hematocrit of just 36% (Neary et al., 2013a), a value comparable to similarly-aged Holstein calves at sea level (Donawick and Baue, 1968; Lumsden et al., 1980). Unfortunately, the majority of prior studies were conducted on a variety of breeds at sea level (Donawick and Baue, 1968; Lekeux et al., 1984; Nagy et al., 2003); consequently, little is known about the effect of altitude on bovine arterial blood-gases and hematocrit. The purposes of this study were, therefore, to evaluate the effect of altitude on arterial blood-gases and hematocrit in Angus-based calves, the predominant breed used in Rocky Mountain beef production. We hypothesized that alveolar ventilation rate would increase with increasing altitude, but hematocrit would not significantly vary with altitude despite increasing hypoxemia and respiratory alkalosis.

MATERIALS AND METHODS

Study Overview

A prospective study was conducted on 5 cohorts of suckling calves from herds located at altitudes ranging from 105 m to 2,730 m. Arterial blood-gas analyses were performed twice in 3 of the cohorts and once in 2 of the cohorts. There were 4 variables of interest: pCO_2, pO_2, pH, and hematocrit. Adjusted means were obtained for calves in each herd at 4 and 7 mo of age. This study received approval from the Colorado State University Animal Care and Use Committee prior to the sampling or handling of any animals (Protocol ID 09–1524A).

Study Sites and Cattle

Five Angus-based herds were enrolled: 3 herds located in Colorado (at altitudes of 1,470 m, 2,010 m, and 2,730 m), 1 herd located in southern Wyoming (altitude 2,170 m), and 1 herd located on the Central Coast, California (altitude 105 m). All calves were born and raised within 300 m of the altitude in which they were sampled. Calves were clustered into 5 cohorts for statistical analyses. These cohorts were sampled in 2011, 2012, or 2013 (Table 1). Arterial blood-gas analyses were performed twice in 3 of the cohorts, at approximately 4 and 7 mo of age. The same calves were sampled twice; however, 2 calves at 1,470 m and 7 calves at 2,730 m were missing ear tag identification at the time of the second sampling and, consequently, were not sampled a second time (Table 1). Calves were sampled just once in 2 of the cohorts, at approximately 4 or 7 mo of age. In the initial sampling for all cohorts, every second calf that entered into the chute was sampled.

Table 1. The altitude, date of sampling, number of calves sampled, sex, and mean age of the 5 herds studied[1]

Altitude, m	Date	Number sampled[2]				Mean age ± SD, d
		H	B	S	T	
105	06/03/13	20	0	24	44	233 ± 29
1,470	07/01/11	17	13	0	30	131 ± 16
	10/27/11	17	11	0	28	250 ± 16
2,010	09/27/11	17	0	13	30	126 ± 13
2,170	07/31/12	0	55	5	60	124 ± 18
	10/01/12	0	55	5	60	187 ± 18
2,730	06/27/11	44	11	25	80	91 ± 7
	10/12/11	40	10	23	73	199 ± 7

[1]Calves that died between test periods were removed from the study. Calves missing at random at the second test, due to missing ear tag identification, for example, remained in the study.

[2]H = heifers, B = bulls, S = steers, T = total.

Sample Collection

Approximately 2.5 mL of arterial blood was collected in a 3-mL syringe. 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). 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. The sample was discarded if during collection the flow of arterial blood was disrupted. Air bubbles within the blood were immediately expelled and the first several drops of blood discarded before analysis.

Blood-Gas Analyses

Blood-gas analyses were performed using a handheld analyzer (VetScan i-STAT 1; Abaxis, Union City, CA). Each analysis required 95 µL of blood to be placed on a single-use, disposable cartridge (CG8+; Abbott Point of Care, Princeton, NJ). The cartridge measured arterial oxygen (p_aO_2) and carbon dioxide (p_aCO_2) tensions, pH, and hematocrit. A temperature "correction" algorithm was used to standardize blood-gas tensions and pH to 37°C (CLSI, 2001). A study of the iSTAT portable analyzer and a conventional clinical analyzer reported strong positive correlations for hematocrit ($r = 0.92$), pH ($r = 0.98$), p_aO_2 ($r = 0.86$), and p_aCO_2 ($r = 0.98$) in cattle (Peiró et al., 2010).

Statistical Analyses

Statistical analyses were performed using STATA version 12 (Stata Corporation, College Station, TX).

Statistics are presented as age-adjusted mean point estimates and Bonferroni-corrected 95% confidence intervals stratified by the altitude and age, 4 or 7 mo, of the calves.

Linear regression analyses were used to determine the fixed effect of herd (a proxy for altitude) on the 4 outcome variables of interest: pCO_2, pO_2, pH, and hematocrit, while controlling for age and sex (heifer, bull, steer). Sex was included in all models to prevent potential confounding. Variance inflation factors were < 5 indicating that the models were not adversely affected by multicollinearity. Two-way interactions were evaluated between herd and age in all models. The interaction was removed from the model if it was not statistically significant.

The results of the linear regression analyses were age-adjusted so that valid between-herd comparisons could be made; consequently, adjusted means were obtained for calves at 4 and 7 mo of age while controlling for sex. Bonferroni adjustment was applied to the *P*-values of the 8 analyses performed (*P*-value × 8) so that the family-wise error rate was 0.05. The 8 linear regression analyses included the 4 outcome variables pCO_2, pO_2, pH, and hematocrit, at the 2 ages studied, 4 and 7 mo old. Bonferroni-corrected confidence intervals were obtained by using $t_{0.025/8}$ instead of $t_{0.025}$ when calculating standard errors. This ensured that all confidence intervals simultaneously contain their target parameters with 95% confidence.

Calves in the herds at 2,010 m and 105 m were sampled only once, at approximately 4 mo and 7 mo of age, respectively. Calves in the 3 other herds at 1,470 m, 2,170 m, and 2,730 m were sampled twice, at approximately 4 mo and 7 mo of age (Table 1). Consequently, the herd at 105 m was omitted from the linear regression analyses when determining the adjusted mean values for the outcome variables at 4 mo of age, and the herd at 2,010 m was omitted when determining the adjusted mean values for the outcome variables at 7 mo of age.

RESULTS

Overview

Hematocrit was greater in calves at altitudes ≥ 1,470 m than it was at sea level (altitude 105 m), but there was no clear association between hematocrit and altitudes ≥ 1,470 m. There was, however, a clear positive association between altitude and both hypoxemia (p_aO_2) and hypocapnia (low p_aCO_2). The adjusted mean values of the 4 variables studied were similar at 4 and 7 mo of age for all of the herds studied, indicating that suckling calves show minimal respiratory or erythrocytic adaptation to hypoxemia and hypocapnia with increasing age, regardless of altitude.

Arterial Hypoxemia and Hypocapnia with Increasing Altitude

Calves showed greater respiratory alkalosis and hypoxemia with increasing altitude. Arterial pCO_2 decreased significantly with increasing altitude in calves at 4 mo ($P < 0.001$) and 7 mo of age ($P < 0.001$) when controlling for sex (Fig. 1A). At 4 mo of age, p_aCO_2 values were approximately 4 mm Hg lower in the higher-altitude herds. At 7 mo of age, p_aCO_2 was greatest at 105 m and decreased with increasing altitude.

Despite the increase in alveolar ventilation rate with increasing altitude, calves became increasingly hypoxemic (Fig. 1B). Arterial pO_2 decreased significantly with increasing altitude in calves at 4 mo and 7 mo of age ($P < 0.001$) when controlling for sex. Like p_aCO_2, p_aO_2 differed little between ages 4 and 7 mo.

Hematocrit

Hematocrit varied significantly with altitude at 4 mo ($P < 0.001$) and 7 mo ($P < 0.001$) of age when controlling for sex, but there was no clear association with altitude. Hematocrit was greater in 7 mo old calves at altitudes ≥ 1,470 m than sea level (altitude 105 m); however, at altitudes ≥ 1,470 m there was no clear association between hematocrit and altitude in calves at either 4 or 7 mo of age (Fig. 1D).

Arterial pH

Arterial pH significantly varied between herds at 7 mo ($P < 0.001$) but not 4 mo ($P = 0.33$) of age when controlling for sex (Fig. 1C).

DISCUSSION

The findings of this study indicate that calves adapt to high-altitude hypoxia by increasing their alveolar ventilation rate, as indicated by a decrease in p_aCO_2. Hematocrit, conversely, did not show a dynamic relationship with altitudes ≥ 1,470 m. Despite the increase in ventilation rate with altitude, calves became increasingly hypoxemic. These physiological responses, or lack thereof, may influence the susceptibility of suckling calves at high altitudes to diseases such as "summer pneumonia" and cor pulmonale (Neary et al., 2013b).

Mammals native to low altitudes, unlike those native to high altitudes, typically respond to high-altitude exposure by increasing hematocrit (McNab, 2002). An increase in hematocrit, however, has the deleterious effect of increasing blood viscosity and, consequently, pulmonary arterial pressure. Cattle with a mean pulmonary arterial pressures greater than 49 mm Hg are considered to be at high risk for right heart

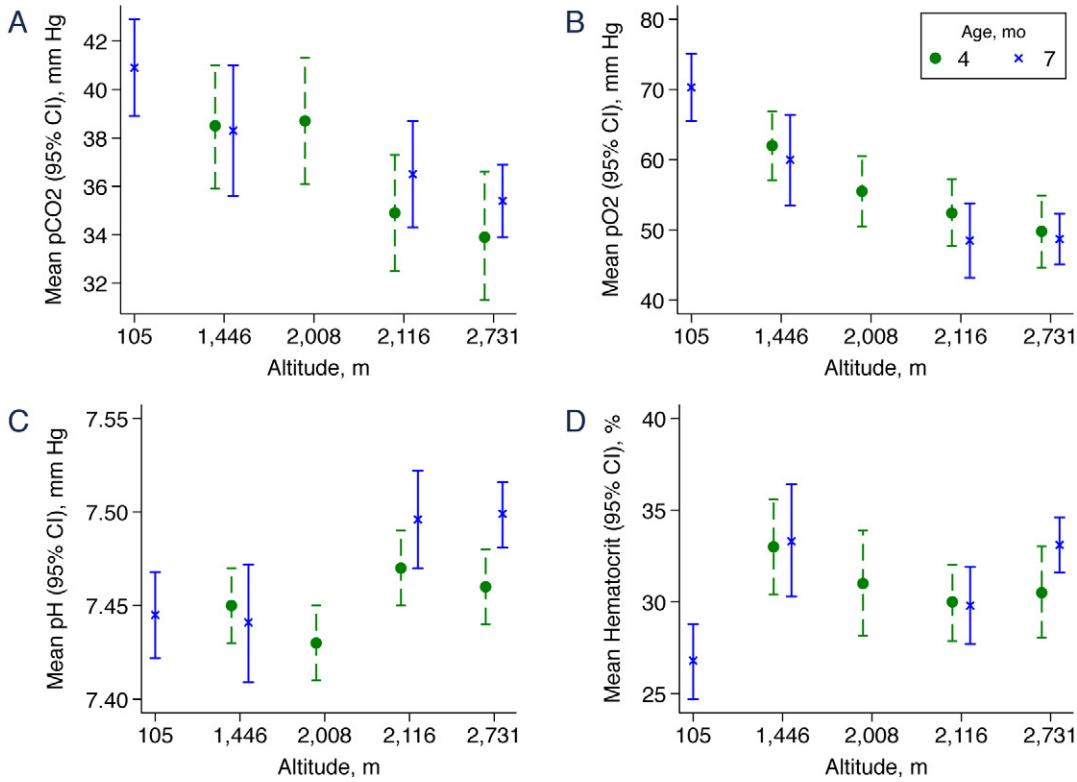

Figure 1. Adjusted point estimates and Bonferroni-corrected 95% confidence intervals of the mean for arterial carbon dioxide tensions (pCO2) (A), oxygen tensions (pO2) (B), pH (C), and hematocrit (D) for calves aged 4 mo (dot and dashed, green line) and 7 mo (cross and solid, blue line) old located on ranches at 105 m, 1,446 m, 2,008 m, 2,170 m, and 2,731 m above sea level while controlling for sex. The probability that all the Bonferroni-corrected intervals contain their target parameters simultaneously is 95% or greater.

failure (Holt and Callan, 2007). This may explain why early studies of cor pulmonale, or right heart failure due to pulmonary hypertension, reported that affected animals had significantly greater hematocrit than healthy herd-mates (Glover and Newsom, 1915; Hecht et al., 1962). Through natural selection, the genetics underlying this unfavorable erythrocytic response to hypobaric hypoxia are likely to have been removed from the herd over multiple generations.

Evidence for a possible genetic adaptation to hypobaric hypoxia was recently provided by a study of Angus cattle: cattle with a double variant *EPAS1* (HIF2α) in the oxygen degradation domain of *EPAS1* had significantly greater mean pulmonary arterial pressures than cattle without the gain of function mutation (Newman et al., 2015). Under hypoxic conditions, the EPAS1 transcription factor is released leading to angiogenesis and erythropoiesis (Tissot van Patot and Gassmann, 2011); consequently, the greater mean pulmonary arterial pressure in cattle with the *EPAS1* gain of function mutation (Newman et al., 2015) may have been attributable to a greater hematocrit, or viscous resistance to blood flow. Genetic selection for low hematocrits is not unique to the Rocky Mountain region: a study of native Ethiopian cattle reported mean hematocrits of just 29% and 34% at altitudes ranging from 550 m to 3,500 m, respectively (Wuletaw et al., 2011). It appears, therefore, that the lack of an erythrocytic response may be a survival adaptation in a species predisposed to hypoxia-induced pulmonary hypertension.

Other physiological adaptations to high altitude are less apparent in our study. Calves were hypoxemic regardless of altitude. This is because cattle have a greater alveolar-arterial oxygen gradient, or lower arterial oxygen tension for a given ventilation rate, than most other mammals (Kainer and Will, 1981). So, for any given atmospheric oxygen tension and ventilation rate, cattle will have lower p_aO_2 than other mammals. In a previous study, we found that calves responded poorly to supplemental oxygen, suggesting that the hypoxemia in calves was due to a low ventilation-perfusion mismatch (Neary, 2013). It is currently unclear as to the health consequences of such profound hypoxemia. A variety of systemic ill effects, such as cardiovascular impairment and systemic inflammation, are feasible (Agustí et al., 2003) and warrant further investigation.

Interestingly, the respiratory alkalosis observed in our study, particularly in the 2 herds located at altitudes ≥ 2,116 m, was greater than previously reported (Nagy et al., 2003). Futhermore, the alkaline conditions may have predisposed the calves to dystrophic calcification

of the vasculature (Chan et al., 2002). Mineralization of the mainstem pulmonary artery, aorta, spleen, and kidney that we previously observed in calves that died on high-altitude ranches with lesions consistent with cor pulmonale (Neary et al., 2013b). The increase in pH observed with age was likely due to a transition from a predominantly milk-based diet to a forage diet (Nagy et al., 2003).

The possibility that altitude was confounded with genetics was minimized by only studying Angus-based calves. Further, all calves were managed in a manner typical for beef cattle herds in this region of the Rocky Mountains; consequently, other ranch-specific confounding factors such as management and (or) nutrition were unlikely. Therefore, and in conclusion, in the herds studied, suckling Angus calves did not show an erythrocytic response to high altitude as would be expected if calves native to low altitude were exposed to hypobaric hypoxic conditions. This likely confers survival advantages by preventing an increase in arterial resistance in a species that is susceptible to hypoxia-induced pulmonary hypertension. Instead, calves responded to increasing altitude by increasing ventilation rate. Despite this, calves became increasingly hypoxemic with altitude. We speculate that such profound hypoxemia has, as yet unrecognized, adverse effects on both the individual animals and the beef production industry, as a whole, in the Rocky Mountain region of the U.S.

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