DISSERTATION

EPIDEMIOLOGICAL, PHYSIOLOGICAL AND GENETIC RISK FACTORS ASSOCIATED WITH CONGESTIVE HEART FAILURE AND MEAN PULMONARY ARTERIAL PRESSURE IN CATTLE

Submitted by

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ABSTRACT

EPIDEMIOLOGICAL, PHYSIOLOGICAL AND GENETIC RISK FACTORS ASSOCIATED WITH CONGESTIVE HEART FAILURE AND MEAN PULMONARY ARTERIAL PRESSURE IN CATTLE

Congestive heart failure, secondary to pulmonary hypertension, has historically been considered a disease associated with high altitude exposure. The disease was first reported to occur at altitudes over 2,440 m (8,000 ft.) and so became known as “high altitude disease”. One common clinical sign due to congestive heart failure in cattle is swelling of the brisket. Consequently, the disease also became known as “brisket disease”.

In more recent years, congestive heart failure has been reported to occur in both beef and dairy cattle at a more moderate altitude of 1,600 m. Anecdotal reports from cattle producers in Nebraska, Colorado and Texas suggest that the incidence of congestive heart failure may be increasing. This suggests that bovine congestive heart failure is not strictly a disease of high altitude exposure.

Anatomical studies of cattle indicate that cattle have a smaller lung volume and alveolar surface area available for gas exchange than mammals with similar body masses and oxygen requirements. This may be because selection for increased growth rate, and other traits of high production, increases metabolic oxygen demand. The overarching hypothesis of this doctoral dissertation was that congestive heart failure secondary to pulmonary hypertension is not strictly a disease of high altitude but, a multifactorial disease, that is also associated with physiological traits that increase metabolic oxygen demand relative to oxygen supply via the cardiopulmonary system. The goal of this

doctoral dissertation was to identify epidemiological, physiological and genetic risk factors associated with congestive heart failure and increased mean pulmonary arterial pressure in cattle.

The results of this dissertation indicate that pulmonary arterial pressures of cattle are substantially higher than other mammalian species. Among pre-weaned calves, mean pulmonary arterial pressures increased significantly with age even at the moderate altitude of 1,470 m. As hypothesized, high oxygen demand relative to supply was positively associated with mean pulmonary arterial pressure in both pre-weaned calves at high altitude (2,170 m) and feedlot cattle at moderate altitudes (1,300 m). A study of 10 Canadian feedlots indicated that the risk of congestive heart failure increased from the year 2000 to the year 2012. The risk of congestive heart failure increased more than the underlying change in the risk of digestive disorders. Death from congestive heart failure occurred throughout the feeding period but typically occurred late in the feeding period, which makes this disease particularly costly to the feedlot industry. Treatment for respiratory disease was a significant risk factor for CHF. Increased growth rate and increased feed efficiency were risk factors for increased mean pulmonary arterial pressure in cattle. Mean pulmonary arterial pressures were significantly higher at the end of the confined feeding period at moderate altitude (1,300 m) than in pre-weaned calves at high altitude (2,170 m). Growth promotion through a steroid implant containing estradiol and trenbolone acetate did not significantly increase mean pulmonary arterial pressure as hypothesized. However, diastolic pulmonary arterial pressure was significantly lower than non-implanted controls, which suggests that one or both of these steroid hormones has cardio-pulmonary protective effects. Genome-wide association analyses of mean

pulmonary arterial pressure and traits physiologically associated with mean pulmonary arterial pressures among calves at 4 and 6 months of age did not identify any concordant single nucleotide polymorphisms (SNPs). However, multiple SNPs were identified to be associated with mean and systolic pulmonary arterial pressures that have been associated with pulmonary hypertension in humans or have a plausible biological role in the development of pulmonary hypertension. In conclusion, the results of these investigations provide evidence to suggest that congestive heart failure of cattle is a multifactorial disease that is exacerbated by high altitude exposure.

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CHAPTER 1: INTRODUCTION

Background

Historically congestive heart failure, secondary to pulmonary hypertension, was only considered to be problematic at high altitude (> 2,130 m) (Glover and Newsom, 1915; Hecht et al., 1962). Pulmonary hypertension, and death from congestive heart failure secondary to pulmonary hypertension, is still problematic in calves at high altitude (Neary et al., 2013a; Neary et al., 2013b). More notably, congestive heart failure secondary to pulmonary hypertension has also been reported to occur at more moderate altitudes (Hull and Anderson, 1978; Jensen et al., 1976; Malherbe et al., 2012; Pringle et al., 1991).

Anatomically, cattle have a substantially smaller lung volume and alveolar surface area (Veit and Farrell, 1978) than mammals of a similar body mass and oxygen requirement. Functional maturity of the pulmonary system is not achieved until approximately 1-year of age in Holstein calves (Lekeux et al., 1984; Neary et al., 2014a). Therefore, hypobaric hypoxia of high altitude may exacerbate any physiological inadequacies, if present, in meeting the high oxygen requirements associated with the growth and development of young calves. A study conducted at altitudes over 2,130 m revealed that pre-weaned calves were markedly hypoxic despite high alveolar ventilation rates, as indicated by hypocapnia (Neary et al., 2013a). The calves also showed evidence of compromised oxygen delivery to peripheral tissues, as indicated by L-lactate concentrations over 1.5 mmol/L (Neary et al., 2013a).

Goals and objectives

Studies of bovine cardio-pulmonary anatomy and physiology in combination with anecdotal reports of congestive heart failure occurring at moderate altitudes and genetic selection for increased productivity suggest that a physiological imbalance between oxygen supply and demand may be a leading risk factor for congestive heart failure secondary to pulmonary hypertension. The overarching hypothesis of this doctoral dissertation was that bovine pulmonary hypertension is not only a disease of high altitude but also a disease of high production; high altitude exacerbates any underlying physiological imbalance between oxygen supply, via the cardio-pulmonary system, and metabolic oxygen demand. The rationale being that domestic cattle have little cardio-pulmonary reserve, which limits their ability to respond to physiological stressors that either increase oxygen demand, such as rapid growth or, decrease oxygen supply, such as high altitude hypobaric hypoxia, or both.

In humans a positive diagnosis of pulmonary hypertension is made when mean pulmonary arterial pressure is over 25 mm Hg at rest and over 30 mm Hg during exercise. There are no criteria in place for establishing a positive diagnosis of pulmonary hypertension in cattle. Mean pulmonary arterial pressure in cattle varies with age, altitude, environmental conditions, diet and health-status (Holt and Callan, 2007). Therefore, in the investigations that follow, pulmonary hypertension was not defined based on a fixed mean pulmonary arterial pressure; rather, it was more loosely defined to mean that an individual had a mean pulmonary arterial pressure that was higher than the mean value of that individual's contemporaries within the cohort studied. Further studies, outside the realm of

this dissertation, are required to define breed-specific reference intervals for mean, systolic and diastolic pulmonary arterial pressures.

It should also be pointed out that pulmonary hypertension is not synonymous with congestive heart failure. Congestive heart failure is not an inevitable consequence of high mean pulmonary arterial pressure. There are multiple factors that influence an individual's susceptibility to heart failure secondary to pulmonary hypertension. Such factors include: how acutely the increase in pulmonary arterial pressure occurred; whether there is regurgitation of blood through the tricuspid valve; how compliant the pulmonary arterial walls are; and, if there is one or more concurrent illnesses. Again, further studies outside of this dissertation are needed to evaluate the prognostic value of pulmonary arterial pressure measurement in cattle. For simplicity, and for the purpose of this dissertation, an animal with a higher mean pulmonary arterial pressure than another animal within the cohort studied was considered to be at greater risk of developing congestive heart failure.

The goal of this doctoral study was to identify epidemiological, physiological and genetic risk factors for bovine pulmonary hypertension. This was achieved through the following objectives:

1. **An epidemiological study of congestive heart failure in US and Canadian feedlots.** It was hypothesized that the risk of congestive heart failure has significantly increased over time. The rationale being that genetic selection practices within the beef industry for increased rate of growth have increased metabolic oxygen demand relative to cardio-pulmonary capacity. If there has been a progressive reduction in bovine cardio-pulmonary reserve it could manifest as a

progressive increase in the incidence of CHF. Select risk factors for congestive heart failure relative to digestive disorders were also evaluated.

2. **A study of calf cardio-pulmonary physiology in association with altitude.** It was hypothesized that calves located at higher altitude would show a greater increase in mean pulmonary arterial pressure (mPAP) with age than calves located at lower altitudes.
3. **An evaluation of pulmonary arterial pressures with age; from calfhood into the confined feeding period.** It was hypothesized that mPAP would increase during the feeding period and, that the calves with the highest mPAP pre-weaning would have the highest mPAP at the end of the confined feeding period.
4. **A study to determine if inadequate oxygen delivery, as indicated by increased systemic oxygen extraction fraction (sOEF), is associated with mPAP and increased odds of mortality in calves at high altitude.** It was hypothesized that sOEF would be positively associated with mPAP and increased odds of mortality.
5. **A study to determine if inadequate oxygen delivery, as indicated by the sOEF, is associated with mPAP in feedlot cattle at moderate altitude.** It was hypothesized that sOEF would be positively associated with mPAP. The rationale being that high oxygen demand relative to supply is not only a risk factor for congestive heart failure among calves at high altitude but is also a risk factor for congestive heart failure among feedlot cattle at moderate altitude.
6. **A study to determine if the rate of body mass gain is associated with mPAP and sOEF in pre-weaned calves at high altitude (altitude 2,170 m) and in cattle during the confined feeding period (altitude 1,300 m).** It was hypothesized that

growth rate would be positively associated with mean pulmonary arterial pressure and sOEF. The rationale being that rapidly growing animals have a high oxygen demand, which mediates an increase in cardiac output. Systemic OEF increases when the increase in cardiac output associated with an increase in oxygen demand is insufficient to meet oxygen requirements.

7. **A study to determine if growth promotion induced by a steroid implant increased the risk of pulmonary hypertension in feedlot cattle.** It was hypothesized that feedlot steers administered a growth hormone implant would develop a higher mPAP and sOEF than non-implanted control steers.
8. **A genome-wide association study of pulmonary arterial pressure and traits associated with mPAP in calves.** It was hypothesized that SNPs associated with mPAP would also be associated with physiological variables found to be physiologically associated with mPAP.

CHAPTER 2: BOVINE PULMONARY HYPERTENSION: A REVIEW OF THE EPIDEMIOLOGY, GENETICS AND PATHOPHYSIOLOGY

In 1913 in South Park, Colorado two CSU researchers, George Glover and Isaac Newsom, set out to investigate a strange new disease of beef cattle. This was the first investigation of bovine congestive heart failure (CHF) secondary to pulmonary hypertension (BPH) in history. One hundred years later, CHF is still a problematic disease for cattle producers, perhaps to an even greater degree. The goal of this chapter was to provide an up to date comprehensive review of the epidemiology, genetics and pathophysiology of the disease. It is not an exhaustive description of all studies undertaken on this subject. The main findings and ideas that have been advanced have been cited as parsimoniously as possible. For a more comparative review of the physiology of hypoxic pulmonary hypertension, see Rhodes (2005).

EPIDEMIOLOGY OF BOVINE PULMONARY HYPERTENSION

The incidence and risk factors for CHF among cattle at high-altitude

There are few studies of the epidemiology of CHF. The first report of CHF contained perhaps the most detailed information regarding the epidemiology of the disease (Glover and Newsom, 1915). At the time of that publication CHF was estimated to cause an annual death loss of 1 to 2 % of all cattle at altitudes over 2,440 m in Colorado, USA. The authors had not seen the disease below an altitude of 2,130 m and it was not commonly recognized in calves because the clinical signs were similar to respiratory diseases, such as diphtheria.

In addition, calves did not always present with edema of the brisket region. Producers were advised to take affected animals down to an altitude of 2,130 to 2,440 m in order to promote recovery: the effect being to "strengthen the heart or lessen its work". There was early evidence of a genetic predisposition to CHF as the authors reported that the progeny of bulls from low altitude were particularly susceptible. In order to minimize cardiac workload the authors suggested that exertion of cattle at high altitude should be minimized.

Approximately 35 years later the incidence of BPH was estimated to be 0.5 to 2.0 % of animals at altitudes over 2,130 m although this varied from year to year and from herd to herd; in some herds, the incidence was reported to reach as high as 10 % (Alexander and Jensen, 1959; Hecht et al., 1962). The authors reported that CHF primarily occurred from fall to spring with calves less than 1-year old most at risk.

The effect of climate on the incidence of BPH

Climate is reported to affect the incidence of CHF; the disease being more problematic in wet pastures than dry (Hecht et al., 1959; Hull and Anderson, 1978) and during wet, cold summers and cold winters (Glover and Newsom, 1915). Cold temperatures may affect pulmonary arterial and venous vasoconstriction (see below). There is currently no explanation for the relationship between wet pasture and increased incidence of CHF. However, tryptophan in lush pastures is a plausible risk factor (see Discussion chapter).

Trends over time

Historically, CHF was considered to be problematic at altitudes over 2,130 m (Glover and Newsom, 1915; Hecht et al., 1962). However, it has since been reported to occur at lower altitudes among: feedlot cattle at 1,600 m above sea-level (Jensen et al., 1976); dairy calves at 1,600 m and 289 m above sea-level (Malherbe et al., 2012; Pringle et al., 1991); and, among cattle pastured in moist areas (Hull and Anderson, 1978).

BPH among feedlot cattle

There are even fewer studies of the epidemiology of CHF in feedlot cattle. In 1974, a study conducted across 4 feedlots located at an altitude of 1,600 m reported the attack risk of CHF to be 2.85 cases per 10,000 yearling cattle entering the feedlot (Jensen et al., 1976). Cases of CHF were also reported to occur predominantly during the last half of the feeding period (Jensen et al., 1976), which would make CHF a particularly costly disease. Other risk factors reported to increase the risk of CHF include: previous mountain grazing, hypoventilation and rapid rate of growth (Jensen et al., 1976).

BPH among dairy cattle

A study conducted from 2007 to 2011 of Holstein heifers at a heifer-raising facility and 2 dairies located at the modest altitude of 1,600 m reported that CHF secondary to pulmonary hypertension was the second leading cause of death loss, behind respiratory disease (Malherbe et al., 2012). Congestive heart failure accounted for 22 % of all deaths over a 7-year period at the heifer-raising facility but no risk factors were identified (Malherbe et al., 2012). Congestive heart failure has also been reported at a much lower

altitude in 5 to 6-month old dairy calves on one farm in Tennessee (Pringle et al., 1991). Again, no risk factor was identified.

PATHOPHYSIOLOGY OF BOVINE PULMONARY HYPERTENSION

The first study of bovine CHF, published in 1915, concluded that some cattle do not have sufficient cardiac reserve to meet the demands of high altitude resulting in "exhaustion of the heart" (Glover and Newsom, 1915). However, recovery could be achieved be taking affected animals down to a lower altitude. Interestingly, they report that one yearling calf brought down to lower altitude was taken back to the original altitude 1 month after recovering; 7 months later the calf was reported to be in perfect health. This suggests that in addition to a genotype by environment interaction, there may also be a third variable of importance: age. When correcting for carcass weight, heart weights of healthy cattle slaughtered in Denver, Colorado were on average 0.40 kg heavier than the heart weights of cattle raised and slaughtered at sea level, supporting their notion that cardiac workload increased with increasing altitude (Glover and Newsom, 1918).

Pulmonary hypertension as a cause of right-sided congestive heart failure

A study conducted at 1,400 m above sea-level reported that calves from 3-months to 1-year of age with BPH in the acute phase (n= 27) had a lower cardiac output than healthy controls (n = 16) (Hecht et al., 1962). It was also found that calves with "brisket disease" or "high altitude disease", as CHF became known, had higher mean pulmonary arterial pressures (mPAP) and higher atrial pressures, both left and right, than control animals

(Hecht et al., 1962). Therefore, it became apparent that the clinical signs and gross pathology of right-sided congestive heart failure associated with "brisket disease" was secondary to pulmonary hypertension (Blake, 1965). The correlation (r = 0.91) of mPAP in calves following acute and chronic exposure to a simulated altitude of 4,572 m (Will et al., 1975b) suggested a common pathophysiology, whether the hypoxia-induced pulmonary hypertension was acute or chronic in nature.

Hypoxia, hypoventilation and mean pulmonary arterial pressure

The positive association between mPAP and altitude was first reported by Donald Will (Will et al., 1962). Twenty Hereford steers of uniform weight (300 kg) and breeding were obtained from one ranch at 1,100 m and then taken to high altitude (3,050 m, n = 10) or moderate altitude (1,525 m, n = 10). Those taken to high altitude showed a significant increase in mPAP but they did not show an increase in minute ventilation, cardiac output or hematocrit relative to the steers that remained at moderate altitude. These findings indicate a failure to adapt to the hypoxic conditions of high altitude and may explain why, over a 6 month period, the weight gain of the steers maintained at the moderate altitude (102 kg) was over twice that of the steers taken to high altitude (45 kg). Robert Grover also reported that following exposure to high altitude cattle had an increased mPAP, were hypoxemic due to a failure to maintain effective ventilation and they showed poor weight gain (Grover et al., 1963; Grover and Reeves, 1962). The potential role of hypoventilation in BPH was confirmed by Bisgard and Vogel (1971); hypoventilation induced by excision of carotid bodies resulted in increased mPAP.

Interestingly, an increase in effective ventilation was observed in BPH 'resistant' cows (n = 4) during pregnancy and when exposed to acute hypoxia but not in BPH 'susceptible' cows (n = 4)(Moore et al., 1979). Hypoxia during the fetal and perinatal period alters development of the pulmonary vasculature (Gao and Raj, 2011; Papamatheakis et al., 2013). Therefore, inadequate maternal ventilation may have detrimental consequences on the development of the pulmonary vasculature of the in-utero calf.

Conversely, hyperventilation may also have a deleterious effect on pulmonary arterial pressure. In vitro studies, that used pulmonary tissue isolated from sheep and cats, suggested that chronic alkalosis may, for an unknown reason, result in a more pronounced pulmonary hypoxic vasoconstrictive response (Gordon et al., 1993).

The increase in mPAP observed to occur on exposure to high altitude was moderately reduced by administration of 100 % oxygen (Will et al., 1962). Within 5-minutes of the supplemental oxygen being removed mPAP returned to baseline values. The rapidity with which changes in mPAP were brought about by oxygen administration was indicative of hypoxia-induced pulmonary vasoconstriction. This phenomenon was first reported to occur in the cat (Von Euler and Liljestrand, 1946).

The primary site of bovine pulmonary hypertension development

Pulmonary arteriographic studies showed that "pruning" of the distal pulmonary arteries occurred in association with BPH (Alexander and Jensen, 1963b). It was suggested by the authors that this vascular remodeling could cause increased pre-capillary resistance to flow thereby increasing mPAP. As a result of this finding an "emphasis was placed upon the small pulmonary arteries in subsequent histologic investigations". Medial hypertrophy

of the pulmonary arterioles was identified to be the most consistent lesion, found in 20 of the 24 calves that died from CHF (Alexander and Jensen, 1963c). However, the next most consistent lesion type was intimal fibroelastosis of the elastic arteries, found in 16 of the 24 calves. Intimal lesions, mineralization of the intima and media, adventitial proliferation and thrombosis were more commonly observed in the elastic arteries than either muscular arteries or arterioles. Therefore, although the most consistent vascular lesion involved the small pulmonary arteries, lesions were apparent throughout the pulmonary vascular tree.

Perhaps the strongest evidence for the small pulmonary arteries being the primary site of pulmonary hypertension development was that medial hypertrophy of these arteries, as determined by the ratio of the area of the media to the area of the intima plus the internal elastic membrane, was highly correlated with mPAP ($r = 0.98$) (Alexander and Jensen, 1963d). More supporting evidence for the small pulmonary arteries as the primary site of BPH development came from the discovery that the medial thickness of small pulmonary arteries from 7 species at 1,600 m was correlated with pulmonary arterial pressure ($r = 0.88$) and right ventricular hypertrophy ($r = 0.97$) following chronic exposure to a simulated altitude of 4,500 m (Tucker et al., 1975). The authors concluded, “the amount of pulmonary vascular smooth muscle inherent within each species may determine the response of each species to high-altitude exposure”. This may be true but it should be pointed out that the species with the highest mPAP and pulmonary medial hypertrophy at 4,500 m (calves and pigs) also had the highest mPAP and greatest medial hypertrophy at 1,600 m. So, whether medial hypertrophy of small pulmonary arteries was a cause or consequence of increased mPAP remains to be determined. Medial hypertrophy may reflect

work hypertrophy due to increased vasomotor tone (Naeye, 1961) or occur in response to altered hemodynamics, such as increased pulmonary flow (Wagenvoort et al., 1969).

Jaenke and Alexander (1973) exposed 7 Hereford cattle, born and raised at 1,524m, to a simulated altitude of 4,572m. A gradual increase in mPAP was recorded but marked 'contracture' of medial smooth muscle cells and endothelial cells was not evident until days 32 to 36. This 'contracture' was only evident at the very distal arterial vasculature. However, only the intermediate to distal pulmonary arteries were examined. The temporal discordance between mPAP and medial hypertrophy apparent in this study suggests that the early increase in mPAP was not due to vascular remodeling in the small pulmonary arteries.

Hypoxia and post-natal pulmonary development

Neonatal hypoxia, like antenatal hypoxia, may be detrimental to the development of the pulmonary vasculature. Studies of calves exposed from birth to an altitude of 3,355 m for several months found: mPAP to increase over 70 mm Hg after just 2 weeks; formation of smaller diameter pulmonary arteries; and, a more pronounced hypoxic pulmonary vasoconstrictor response than control calves maintained at an altitude of 305 m (Reeves and Leathers, 1967). In contrast, low altitude calves (altitude 305 m) showed a drop in mPAP, a decrease in the smooth muscle content of pulmonary arteries, an increase in the diameter of larger pulmonary arteries and an increase in the number of small pulmonary arteries that could be identified on radiographs (Reeves and Leathers, 1967). Another study of healthy cattle at sea-level found that after 1 year of age the decrease in thickness of the media relative to the external diameter of the pulmonary artery reached its minimum

of approximately 11 % (Wagenvoort and Wagenvoort, 1969). This, perhaps by no coincidence, is the approximate age at which the functional maturity of the cardio-pulmonary system is optimal (Lekeux et al., 1984; Neary et al., 2014a).

There is also some evidence to suggest that chronic hypoxia is detrimental to the development of the pulmonary airways. Lung airflow resistance was increased in neonatal calves after 2 weeks of exposure to a simulated altitude of 4,500m due to increased fibrous and smooth muscle content in the bronchioles and large airways (Inscore et al., 1991).

Hypoxia and myocardial depression

There is some evidence to suggest that a reduction in myocardial function occurs in calves exposed to hypoxic conditions. The stroke index (volume of blood ejected from the heart in one cardiac cycle relative to body surface area) of Holstein calves exposed to an altitude of 3,400 m decreased substantially but recovered back towards normal after administration of 100 % oxygen (Will, 1975). A reduction in cardiac index (cardiac output per minute relative to body surface area) was also demonstrated in Holstein calves at 2 and 4 weeks of exposure to a simulated altitude of 3,400 m (Ruiz et al., 1973).

Pulmonary vein involvement

Marked medial muscularization of both pulmonary arteries and veins down to vessels of 20 μm in diameter is present in cattle (Alexander, 1965). The pulmonary veins, unlike arteries, have a sphincter-like appearance (Figures 2.1 and 2.2) (Alexander and Jensen, 1963a).

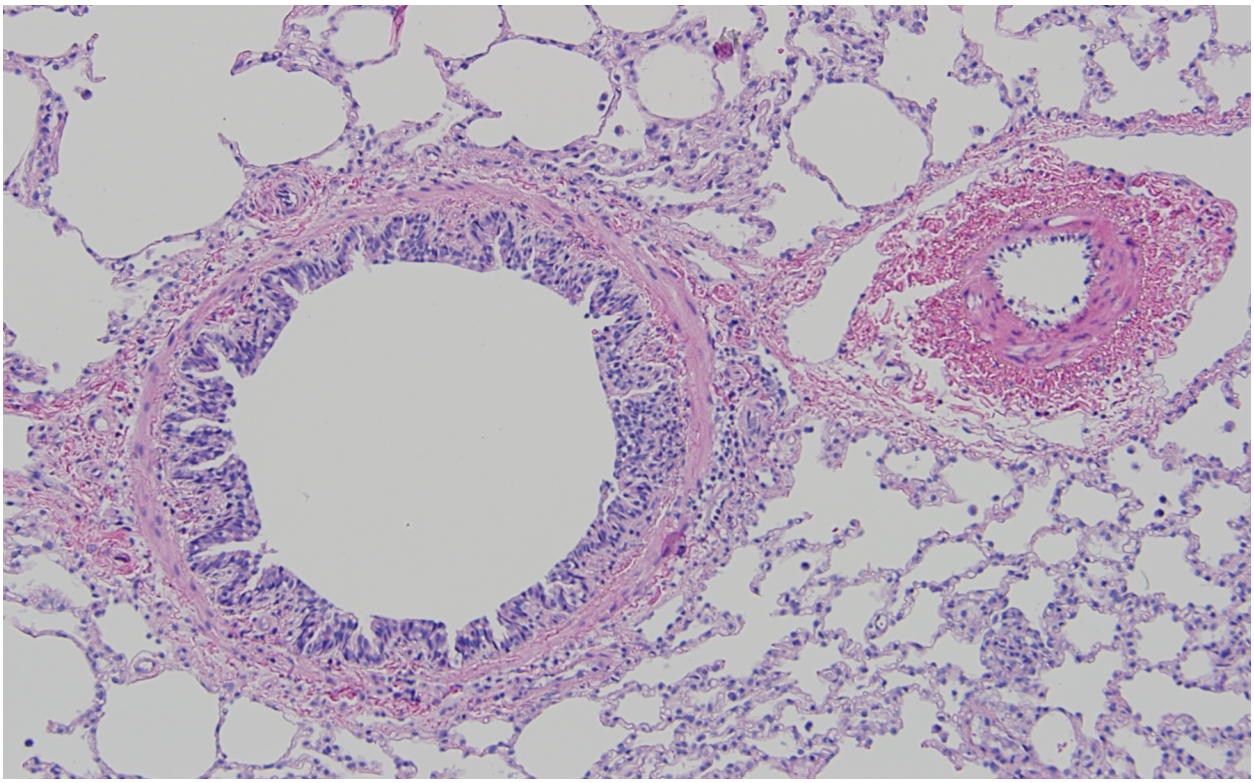

Figure 2.1: Cross-section of a pulmonary artery (right) and vein (left) from a healthy feedlot steer with a mean pulmonary arterial pressure of 44 mm Hg at an altitude of 1,440 m. Magnification: 10-fold

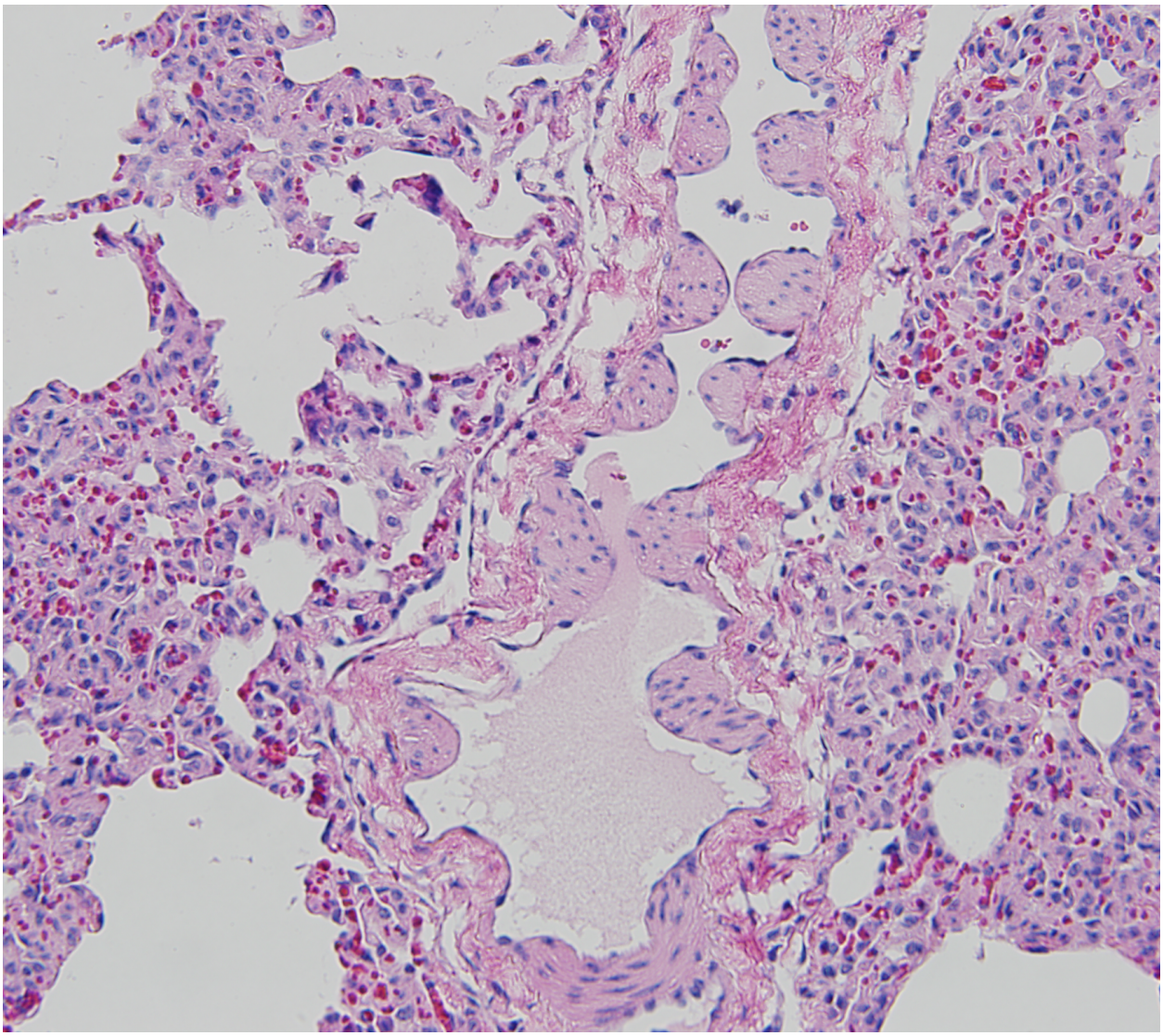

Figure 2.2: Longitudinal-section of a pulmonary vein obtained from a healthy feedlot steer with a mean pulmonary arterial pressure of 67 mm Hg at an altitude of 1,440 m. Magnification: 20-fold

Kuida et al. (1963) provided evidence that some cattle with CHF had increased resistance downstream of the pulmonary bed; these cattle had pulmonary arterial wedge pressures that were over 6 mm Hg greater than left ventricular end-diastolic pressure. The authors of this study suggested that pulmonary venoconstriction or left ventricular failure might be contributing to the development of BPH.

Both hypoxia and cold exposure may lead to pulmonary venoconstriction. In pigs, alveolar hypoxia produced significant pulmonary venoconstriction although the magnitude was not as great as arterial constriction (Nelin et al., 1994). Hereford calves exposed to cold temperatures (- 2 to 1 °C) for 48 hours at both 1,524 m and 3,048 m showed a significant increase in mPAP, pulmonary arterial wedge pressure and pulmonary vascular resistance but no change in cardiac output (Busch et al., 1985). The interpretation of these results indicates that vasoconstriction of both pulmonary arteries and veins were contributing to pulmonary arterial pressure. The effect of altitude and cold exposure on pulmonary arterial pressure was additive. Minute ventilation and p_aO_2 fell on cold exposure but p_aCO_2 increased. Oxygen administration to restore p_aO_2 to control values partially restored pulmonary vascular resistance and mPAP to control values, which suggested that cold-induced alveolar hypoventilation was partly responsible for the pulmonary hypertensive response.

Medial hypertrophy of pulmonary veins was evident in some humans resident at high altitude (Wagenvoort and Wagenvoort, 1982). It seems likely that the pulmonary venous system and left-ventricular function may play a greater role in the development of pulmonary hypertension than is currently appreciated.

Aberrant cardiopulmonary hemodynamics and pulmonary hypertension

It has been known for over 50 years that high pulmonary arterial flow rates may be sufficient to induce pulmonary hypertension in neonatal calves (Vogel et al., 1963). However, only recently has it become apparent that aberrant hemodynamics and vascular injury may contribute to the initiation and development of BPH (Botney, 1999). The

afterload on the right ventricle is composed of two components: hydraulic resistance primarily attributable to the pulmonary arterioles and hydraulic capacitance, a dynamic load, attributable to the large, elastic pulmonary arteries, which act as conduits between the right ventricle and sites of gaseous exchange. In neonatal calves exposed to hypoxia, the large, elastic pulmonary arteries were found, in an ex vivo study, to increase in stiffness due to elastin-based extracellular matrix remodeling (Lammers et al., 2008). Stiffening of the large, elastic pulmonary arteries has multiple adverse consequences: a greater afterload that is independent of the distal vascular resistance; increased pulsatility and energy transfer to the small pulmonary arteries creating a high-stress environment; and, reduced flow efficiency due to a reduction in the Windkessel effect (For a review of hemodynamics in pulmonary hypertension see (Lammers et al., 2012)). Normal pulmonary pulse pressures in healthy humans are approximately 17 mm Hg (Figure 2.3).

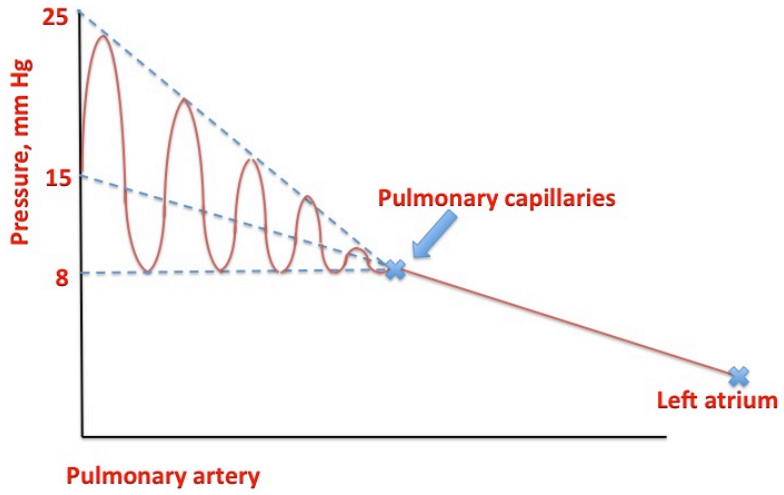

Figure 2.3: In humans, systolic, mean and diastolic pulmonary arterial pressures average 25, 15 and 8 mm Hg, respectively. From left to right, the x-axis represents the flow of blood from the pulmonary artery to the left atrium. Mean pressures in the left atrium and pulmonary veins average 2 mm Hg. Diagram adapted from Guyton (2006)

The result of pathological flow arriving in the distal pulmonary vasculature is an exacerbation of small vessel dysfunction. An in vitro study found that pathologically high and low shear stress resulted in a reduction in the release of vasodilators (nitric oxide, prostacyclin) and increased release of vasoconstrictors (endothelin, thromboxane) from pulmonary arterial endothelial and smooth muscle cells isolated from neonatal calves (Li et al., 2009b). Shear stress is the frictional force imposed on a vessel wall caused by blood flowing rapidly through a stationary vessel lumen. The expression of 2 smooth muscle contractile proteins (α-SM-actin and SM-MHC) was significantly increased at high shear stress (≥ 90 dynes/cm^2) compared to physiological shear stress (20 dynes/cm^2). Increased pulsatility due to upstream vascular stiffening has also been shown to increase

inflammatory gene expression and cell proliferation in the downstream endothelial cells (Li et al., 2009a).

The consequences of proximal vascular stiffening of the pulmonary artery may be particularly severe in cattle as pulmonary blood flow is reported to be markedly pulsatile relative to other mammalian species (Weekley and Veit, 1995). It has also been suggested that the density of the bovine pulmonary capillary bed is less than other mammalian species (Epling, 1964). Therefore, there may be less dampening of the pressure wave due to decreased, or low dispersion of flow among the micro-circulation.

Toxin-induced

Several reports have indicated that CHF may be toxin induced. Holstein bull calves fed Locoweed (*Oxytropis serica* and *Astragalus lentiginosus*), which contains swainsonine, an indolizidine alkaloid toxin, at 3,090 m were at greater risk of developing CHF secondary to BPH than control calves (James et al., 1991). The risk of Locoweed ingestion is magnified for calves. In addition to ingesting swainsonine directly from the plant, which commonly grows on high altitude pastures, the toxin also accumulates in milk (James and Hartley, 1977). The pathogenesis of swainsonine toxicity is unclear.

Ingestion of larkspur (*Delphinium* spp.) can cause neuromuscular paralysis through the action of diterpenoid alkaloids (Pfister et al., 1999). Respiratory distress and rumen bloat, which may both result from intoxication may cause sufficient pulmonary hypertension to result in congestive heart failure.

Water sulfate concentration in yearling steers was found to be positively correlated with mPAP (Loneragan et al., 2005). It is likely that ruminal hydrogen sulfide gas is inhaled

since ruminants inhale a substantial proportion of eructated gas (Dougherty et al., 1962). Hydrogen sulfide may play a role in the oxygen sensing mechanism of pulmonary arteries. Under hypoxic conditions pulmonary metabolism of H_2S is reduced resulting in increased pulmonary H_2S levels, which may be responsible for mediating the vasoconstrictive response to hypoxia (Olson et al., 2009). Therefore, water with high sulfate content may result in the inhalation of hydrogen sulfide gas, which may be a mediator of the hypoxia-induced vasoconstrictive response of the muscular pulmonary arteries.

Reactive oxygen species

Reactive oxygen species are being increasingly implicated in chronic hypoxic pulmonary hypertension (for a review see (Nozik-Grayck and Stenmark, 2007)). The source of reactive oxygen species production is not known but there is accumulating evidence that there are various isoforms of vascular NADPH oxidases, which may be activated by tissue injury and hypoxia and act as a major source of superoxide radicals (Keaney, 2005).

Cold-induced pulmonary hypertension

Cold temperatures have been associated with an increase in mPAP (Busch et al., 1985; Will et al., 1978). At an ambient temperature of 25 °C cattle significantly decreased cardiac output and increased systemic and pulmonary vascular resistances following cooling of the skin but there was no change in alveolar ventilation, as measured by arterial blood-gases (McMurtry et al., 1975). This indicated that the increase in vascular resistance

occurring in response to skin cooling was independent of changes in alveolar oxygen tension.

Gender differences

Female animals have a smaller increase in pulmonary arterial pressure following exposure to chronic hypoxia (Burton et al., 1968; McMurtry et al., 1973; Rabinovitch et al., 1981). However, the gender effect appears to be age-dependent: the significant difference was only apparent in the adult rats, which implies that a hormonal influence in post-pubertal animals may be responsible (Rabinovitch et al., 1981). Female cattle appear to have lower mPAP than males. At 6-months of age, at an altitude of 2,730 m, the mPAP of heifers was approximately 4 mm Hg lower than steer calves although a statistical difference was not detected (Neary et al., 2013a). In that study, husbandry was uniform for heifers and steers. However, the relationship between gender and mPAP may have been confounded by other sex-related traits, such as growth rate.

Recovery from bovine pulmonary hypertension

Cattle can recover from BPH when moved to lower altitude (Glover and Newsom, 1915). In one study, cattle with clinical signs of CHF were moved to a moderate altitude of 1,600 m and serial mPAP measurements and lung biopsies of small pulmonary arteries obtained (Alexander et al., 1965). For the first 6 weeks, the mPAP and the ratio of the tunica media to intima in small pulmonary arteries remained at high altitude levels. However, after 12 weeks at the lower altitude, both mPAP and the ratio of media to intima were significantly reduced. This suggested that vascular lesions associated with pulmonary

hypertension may be reversible. However, the ratio of the tunica media to tunica intima accounts for only a limited part of the vascular remodeling process that occurs in association with pulmonary hypertension. Further studies are needed to determine if pulmonary remodeling is reversible.

GENETICS OF BOVINE PULMONARY HYPERTENSION

The hypoxic pulmonary vasoconstrictive response of cattle (*Bos taurus*) is notably greater than other mammalian species (Tucker et al., 1975). However, within the *Bos taurus* species and within breeds there exists marked variability in the pulmonary pressor response to high altitude exposure (Alexander et al., 1960). Genetics and epi-genetic regulation of gene expression likely has a leading role in determining susceptibility to BPH.

Genotype by environment interaction and bovine pulmonary hypertension

The first evidence of a genotype by environment interaction was provided by Alexander et al. (1960). They took 20 yearling Hereford steers from one ranch at an elevation of 1,100m in eastern Colorado and exposed them to an altitude of either 1,524 m (n = 10) or 3,048 m (n = 10). The mPAP of control steers maintained at an altitude of 1,524 m for 6 months remained consistent at approximately 28 mm Hg. However, the mPAP of the high altitude steers was not uniform: 4 steers, that developed severe pulmonary hypertension 6 months later, had significantly higher mPAP values after 7 weeks at 3,048 m than the 6 steers that subsequently developed mild pulmonary hypertension 6 months

later. The results of this study indicated that genetic predisposition to pulmonary hypertension is not phenotypically apparent at all altitudes.

Evidence for a genotype by environment interaction is also provided by Weir et al. (1974). Two lines of Hereford cattle selected to be either resistant or susceptible to BPH showed physiological differences after 2 weeks at 3,420 m above sea level but not at the original altitude of 1,500 m (Weir et al., 1974). In response to an increase in altitude, susceptible cattle (n = 7) showed a significantly greater increase in mPAP, total pulmonary vascular resistance and hematocrit relative to resistant cattle (n = 5). Susceptible cattle also showed a greater increase in cardiac output suggestive of a greater hypoxic physiological stimulus but a statistically significant difference was not detected.

Similar results were reported one year later. Calves born to susceptible animals showed a greater increase in mPAP when chronically exposed to 3,048 m than calves born to resistant parents (Will et al., 1975a). Calves born to susceptible parents showed a greater increase in mPAP when exposed to acute hypoxia than calves born to resistant parents at both 5 and 9 months of age (Will et al., 1975b). The change in mPAP in response to acute hypoxia was highly correlated with the change in mPAP after 18 days at 4,572 m when 1 year old. This suggested that the genetic risk factors for a profound vasopressor response to acute hypoxia were also responsible for increasing pulmonary arterial pressure in response to chronic hypoxia. However, at the moderate altitude of 1,524 m the mPAP values of BPH susceptible and resistant cattle at 5, 9 and 12-months of age did not differ, once again indicating a genotype by environment interaction.

Native Simien cattle of Ethiopia were reported to have mPAP of only 32.5 ± 5.4 mmHg (n = 32) at an altitude of 3,500m (Wuletaw et al., 2011). One very notable phenotypic characteristic of these cattle is their small mature body weight.

Pulmonary arterial pressure and growth traits

Selection for growth may have the unfavorable consequence of increasing mPAP. Direct genetic correlations between PAP and both body mass at birth and weaning have been estimated to be approximately 0.5 (Levalley, 1978; Shirley et al., 2008). No evidence of maternal genetic effects on the mPAP of offspring has been found (Shirley et al., 2008). Darling and Holt (1999) reported that the correlation between sire PAP and son PAP (r = 0.2) in their dataset of 966 calves differed from the correlation between sire PAP and daughter PAP (r = -0.01). The authors suggested this to be due to an abnormality in the Y-chromosome causing variable penetrance of an autosomal gene.

Comparative studies within the Bos family

Within the Bos family pulmonary hypertension may be a problem unique to the *Bos taurus* species. *Bos indicus,* or zebu, cattle are thought to be resistant (Hecht et al., 1962). However, anecdotal reports suggest that *Bos indicus* cattle may also be susceptible. Historically, CHF has been more often reported in beef breeds than dairy breeds. Beef cattle production, unlike dairy cattle production, is possible on the mountainous terrain commonly associated with high altitude. Therefore, relatively more beef cattle are exposed to high altitude than dairy cattle. This likely explains the greater occurrence of CHF in beef cattle relative to dairy cattle. Holstein dairy cattle are highly susceptible to CHF secondary

to pulmonary hypertension (Malherbe et al., 2012; Stenmark et al., 1987) and are commonly used as an animal model of human pulmonary hypertension (Lammers et al., 2008; Zuckerman et al., 1992).

The germplasm evaluation program conducted at the U. S. Meat Animal Research Center, Clay Center, NE has evaluated divergence among breeds of beef cattle for over 30 years. The results show that breed differences have not remained static over time. In fact, breed convergence has occurred on traits such as yearling weight, weaning weight and mature cow size (Cundiff et al., 2004). This may mean that the risk of congestive heart failure among breeds may have also converged over time.

Weir et al. (1974) suggested that susceptibility to pulmonary hypertension is genetically transmitted in an autosomal dominant manner. This mechanism of transmission was supported by breeding experiments of cattle (*Bos taurus*) and yaks (*Bos grunniens*) (Anand et al., 1986), close family members adapted to life at high altitude. Dzos (n = 6), which are progeny of cow x yak crosses, had mPAPs closer to yak than cattle. Stols (n = 7), progeny of female dzo x *B. taurus* bull crosses, had divergent mPAP values suggestive of simple Mendelian inheritance. The authors suggested a single autosomal dominant genetic transmission of mPAP, but given the limited number of study animals it would be prudent to interpret such results with caution.

Yaks have much reduced muscularization of the tunica media of the small pulmonary arteries than cattle (Durmowicz et al., 1993; Tucker et al., 1975). Unlike cattle, yaks do not demonstrate increased hemodynamic forces under hypoxic challenge and so may lack a stimulus for BPH lesion development. If so, hemodynamic forces, rather than hypoxia per se, may be the principle stimulus for cell wall proliferation and extracellular

matrix production (Durmowicz et al., 1993). Other adaptations of yaks to hypoxic environments include: a shorter, wider trachea, a larger thoracic capacity and a larger heart and lungs than cattle of equivalent body mass (Zhang et al., 2000).

Molecular genetics

Alleleic association analyses of single nucleotide polymorphisms (SNPs) of 10 cattle with severe pulmonary hypertension and 10 cattle with 'low' was performed using a 10 k SNP array (Newman et al., 2011). Due to the low power of the study, no SNPs were found to be statistically associated with mPAP. However, a follow up study of 3 genes (Myosin heavy chain 15, NADH flavoprotein 2 and FK Binding Protein 1A) identified by Newman et al. (2011) to be associated with mPAP, that may plausibly be associated with pulmonary hypertension, were further evaluated in a cohort of 166 yearling bulls (Neary et al., 2014b). It was found that the T allele (rs29016420) of myosin heavy chain 15 gene was linked to lower mPAP in a dominant manner. Interestingly, the allelic frequency of the T allele at the same loci in a sample of 24 Himalayan yaks (*Bos grunniens*) was found to be 100 %.

Gene expression

Transcriptome analysis of peripheral blood mononuclear cells identified respiratory disease to be the top disease process associated with mPAP (Newman et al., 2011). Genes associated with cell-signaling, immune and endothelial cell functions were differentially expressed according to mPAP. The findings of Frid et al. (2006) also indicate extensive involvement of the immune system in the development of bovine pulmonary hypertension. Similarly, in humans (Pullamsetti et al., 2011) and broiler chickens (Wideman et al., 2013),

the immune system is also reported to play a critical role in the etiology and progression of pulmonary hypertension.

Comparative studies

Similar to cattle, pulmonary hypertension in broiler chickens has also been estimated to be moderately to highly heritable (Lubritz et al., 1995; Pavlidis et al., 2007). Various modes of inheritance have been proposed from a few major genes to polygenic (Wideman et al., 2013). One study, starting from baseline with a commercial pedigree flock, found that extremes in incidence of ascites were obtained after 8 generations of selection for pulmonary hypertension susceptibility (ascites incidence 95.1 %) and after 9 generations of selection for pulmonary hypertension resistance (ascites incidence 7.1 %) when reared at a simulated altitude of 2,900 m (Pavlidis et al., 2007). These results suggest that a few major genes are responsible for a large component of the variation seen in the ascites phenotype. An additional finding from the same study was that broilers in the line selected for resistance to pulmonary hypertension were substantially lighter at 42 days of age than the broilers in the line selected for susceptibility to pulmonary hypertension (Pavlidis et al., 2007).

CHAPTER 3: THE RISK OF, AND RISK FACTORS FOR, CONGESTIVE HEART FAILURE IN NORTH AMERICAN FEEDLOTS

INTRODUCTION

Anecdotal reports suggest that the incidence of right-sided congestive heart failure (CHF) in feedlot cattle is increasing. In cattle, pulmonary hypertension is the primary cause of right-sided CHF (Blake, 1965; Will et al., 1975). Historically, CHF was considered to be problematic at altitudes over 2,130 m (Glover and Newsom, 1915; Hecht et al., 1962), but it has since been reported to occur at lower altitudes. In 1974, a study conducted across 4 feedlots located at an altitude of 1,600 m reported the attack risk of CHF to be 2.85 cases per 10,000 cattle entering the feedlot (Jensen et al., 1976). CHF cases were also reported to occur predominantly during the last half of the feeding period (Jensen et al., 1976), which makes CHF a particularly costly disease.

The purposes of this study were to determine: if the risk of CHF among fed-cattle has increased over time; the distribution of CHF mortality through the feeding period; and, to evaluate respiratory disease treatment, season of placement and gender as risk factors for CHF. The first objective was to characterize the risk of CHF across 10 Canadian feedlots every 4 years from the year 2000 to the year 2012. The second objective was to compare the risk of CHF in 2012 among 10 Canadian and among 5 U.S. feedlots. The third objective was to evaluate risk factors for CHF using death from digestive disorders as a control. It has been reported that respiratory disease is a risk factor for CHF (Holt and Callan, 2007). However, to what extent respiratory disease increases the risk of CHF has not been

previously reported. Treatment for respiratory disease was one of the risk factors evaluated in our study.

MATERIALS AND METHODS

Study overview

Data from 10 Canadian feedlots and 5 U.S. feedlots were obtained for this investigation. Data from the Canadian feedlots were obtained for the years 2000, 2004 and 2008 and 2012. Data from the U.S. feedlots were obtained for the year 2012. The number of animals entering a given feedlot within a calendar year was stratified by season of placement, age, gender and risk of undifferentiated fever/respiratory disease (Table 3.1). A cause of death was determined for every animal that died within the feedlots studied. The number of deaths due to CHF and digestive disorders was also stratified (Table 3.2). From these data, the risk of CHF was determined every 4 years from the year 2000 to the year 2012. Additionally, individual animal records were obtained from animals that died of CHF or a digestive disorder (ruminal bloat, enteritis, peritonitis or intestinal disorder). This information included the number of days from entry into feedlot to post-mortem evaluation and, treatment history for respiratory disease. Risk factors for CHF were evaluated using death from a digestive disorder as a control group. Digestive disorders were considered to be an appropriate control because of the minimal risk of confounding due to a common pathogenesis.

Study population

Feedlot Health Management Services (Okotoks, AB, Canada) provided data for this study. The study population consisted of cattle placed in 10 feedlots located in western Canada during the years 2000, 2004 and 2008 and 2012 and cattle placed in 5 feedlots in the western United States during the year 2012. These feedlots were chosen based on the availability of the data required for the purpose of this study. These data were obtainable from the Canadian feedlots going back to the year 2000 and from the U.S. feedlots going back to the year 2012. The procurement and management of cattle within the feedlots studied was typical of those practices used at large commercial cattle feedlots in western Canada and U.S. Black-hided Angus-based cattle were the predominant breeds of cattle in both Canada and the U.S

The same standardized health and production procedures were used across all feedlots as per the protocols developed by veterinarians at Feedlot Health Management Services. In brief, at arrival, all cattle received an ear tag with a unique identification number, a growth implant, a topical avermectin anthelmintic and vaccines against bacterial and viral agents of respiratory disease. Cattle determined to have a high risk of developing a fever of unknown origin (undifferentiated fever) or respiratory disease at the time of arrival were administered a parenteral antibiotic as a prophylactic or metaphylactic treatment. The risk of developing undifferentiated fever/bovine respiratory disease for each group of feedlot animals was determined by feedlot personnel using standardized risk profiles that were based on factors such as age class (calf versus yearling), body weight (often a proxy for age), procurement method (sale barn versus private treaty), amount of commingling before and after arrival, and previous vaccination and management history.

Cattle were provided with provided with water and feed *ad libitum*. The feed offered was typical of a commercial feedlot operation and was formulated using guidance from The National Research Council (2000).

Data collection

The population at risk of CHF was the number of cattle placed within the feedlots studied during a calendar year and was stratified according to the variables listed in Table 3.1. The cattle placed within that year were followed until completion of the feeding period. The buyers and sellers of the animals categorized cattle as calves or yearlings during the normal processes of commerce. Subsequently, feedlot personnel recorded this classification on an individual animal-based computer system.

Table 3.1: The population at risk, the number of cattle placed in a given feedlot within a given year, was stratified according to the variables listed

Variable	Category
Feedlot	15 feedlots identified by a unique number
Placement year	2000, 2004, 2008 and 2012
Season of placement	January 1st to April 30th
	May 1st to August 31st
	September 1st to December 31st
Age	Calf or yearling
Gender	Male or female
Risk of undifferentiated fever/ respiratory disease	High or low

Diagnosis of disease

Cattle health was evaluated daily by trained feedlot personnel. Cattle showing evidence of disease were treated under the supervision of a veterinarian according to

protocols developed by Feedlot Health Management Services. Cattle that died while in the feedlot were examined post-mortem by a veterinarian or trained feedlot personnel. The primary cause of death was recorded according to the criteria in table 3.2. There could only be one cause of death. If a specific cause of death could not be determined but peritonitis was evident then a diagnosis of peritonitis was recorded. Individual animal information from cattle that died of congestive heart failure or a digestive disorder within the feedlots during the years studied was included in the dataset. Digestive disorders included: ruminal bloat, enteritis, intestinal disorders and peritonitis (Table 3.2). Cattle that died of digestive disorders served as a control group for identifying risk factors associated with death from CHF. In addition to the information provided in table 3.1 the individual animal information included: treatment for respiratory disease (yes/no), two or more treatments for respiratory disease (yes/no) and the number of days from entry into the feedlot to post-mortem examination.

Table 3.2: The post-mortem lesions used for determining cause of death. One or more of the lesions listed may have been sufficient to determine the cause of death. Cattle did not have to demonstrate evidence of all of the lesions listed. Peritonitis was diagnosed when evident and a specific cause of death was not determined

Category	Cause of death	Post-mortem lesions
Case	Congestive heart failure	Brisket and ventral edema; hydroperitoneum; hydrothorax and secondary atelectasis; hepatomegaly and chronic passive congestion; intestinal and mesenteric edema; hydropericardium; right-ventricular hypertrophy and dilation
Control (Digestive disorder)	Ruminal bloat	Underinflated lungs; anterior carcass congestion; posterior carcass pallor; edema of subcutaneous tissue and facial planes of hind limbs; rumen distended with gas; small, pale liver; small, pale heart
	Enteritis	Hyperemia and edema of intestinal muscosa; fibrinous mucosa; luminal hemorrhage; dark, fluid-filled intestine; diffuse or segmented;
	Intestinal disorder	Intussusception; mesenteric rent; intestinal parasitism; lodged trichobezoar; stricture; intestinal torsion/volvulus
	Peritonitis (specific cause of death cannot be determined)	Hydroperitoneum; fibrin deposition; adhesions; local or diffuse

Statistical analyses

Statistical analyses were performed using STATA version 12 (Stata Corporation, College Station, Texas, USA). A descriptive analysis of the number of cattle entering the feedlots during the calendar years studied was performed. The number of cattle that died

from CHF and digestive disorders (bloat, enteritis, intestinal disorder and peritonitis) was summated across all feedlots for all of the years studied. The mean ± standard deviation of the number of days to death from arrival at the feedlot was calculated for CHF and each digestive disorder.

Risk factors for CHF were evaluated separately for U.S. and Canadian cattle. The primary risk factor of interest was year of placement, which was evaluated using the Canadian dataset only. Other risk factors of interest were season of placement, risk category, gender, age and feedlot effects. These risk factors were evaluated separately for U.S. and Canadian populations. The number of cattle entering the feedlots during the study periods was the exposure of interest. The duration of exposure was not available for all animals therefore, attack risk per 1,000 cattle entering the feedlot and attack risk ratio were the outcome measures of interest rather than incidence risk and incidence risk ratio. Feedlot effects were controlled in the analyses as fixed-effects so that separate CHF attack risk estimates could be obtained for each feedlot. Likelihood ratio tests were performed to determine the statistical significance of the following categorical variables: season of placement, year of placement and feedlot effects. A zero-inflated negative binomial model was used. A Poisson model was not suitable as a likelihood ratio test of the over-dispersion parameter alpha = 0 was statistically significant ($p < 0.001$). This indicates that the mean is not equal to the variance, which is an assumption of the Poisson model. An excess of zero counts, as indicated by a Vuong statistic of 7.9, meant that a zero-inflated negative binomial model was superior to a standard negative binomial model ($p < 0.001$).

Linear regression was used to compare the attack risk of CHF between U.S. and Canadian feedlots in the year 2012. The outcome of interest was risk of CHF per 1,000

cattle entering the feedlot. The explanatory variable of interest was country when controlling for season of placement, risk category, gender, age and feedlot effects. The least squares mean estimate of the risk of CHF and 95 % confidence interval (95 % CI) were calculated for all feedlots when controlling for country, season of placement, risk category, gender and age.

The third objective was to identify risk factors associated with mortality due to CHF (cases) using mortality due to digestive disorders as a baseline reference (control). Digestive disorders were considered to be an appropriate control because of the minimal risk of confounding due to a common pathogenesis. The etiologies of the various digestive disorders were likely to be distinct from the etiology of CHF. Separate logistic regression models were evaluated for Canadian and U.S. feedlots. Only cattle that died of either CHF or a digestive disorder were included in the logistic regression model. Year of placement (Canadian model only), season of placement, feedlot, gender, risk of undifferentiated fever/respiratory and treatment for bovine respiratory disease were included in the model as explanatory variables. Animals were categorized according to the number of treatments for BRD received: no treatment, 1 treatment or at least 2 treatments.

RESULTS

Cattle numbers and feedlot altitudes

During the 4 calendar years studied a total of 1.28 million cattle entered into the Canadian feedlots (Table 3.3). In 2012, a total of 273,319 cattle entered the 5 U.S. feedlots studied. Feedlots in Canada were located at altitudes ranging from 657 m (Feedlot 10) to

1,145 m (Feedlot 14). Feedlots in the U.S. were located at altitudes ranging from 596 m (Feedlot 185) to 1,282 m (Feedlot 171).

Table 3.3: The total number of cattle entering a feedlot within a calendar year

Country	Feedlot	Altitude, m	Year 2000	2004	2008	2012	Total all years
Canada	1	1,006	30,933	29,107	64,926	44,740	169,706
	3	837	3,453	3,045	1,738	2,646	10,882
	5	1,018	20,933	23,682	25,538	13,368	83,521
	6	934	63,817	60,212	104,364	57,247	285,640
	9	917	23,281	34,761	51,394	23,597	133,033
	10	657	55,489	53,865	60,071	27,735	197,160
	14	1,145	9,858	9,716	6,464	5,252	31,290
	19	887	2,779	1,529	2,234	1,984	8,526
	20	1,102	65,582	60,148	86,947	49,380	262,057
	31	1,005	10,941	25,613	48,016	17,809	102,379
	Total		287,066	301,678	451,692	243,758	1,284,194
USA	169	1,161	-	-	-	91,088	-
	170	1,242	-	-	-	34,736	-
	171	1,282	-	-	-	44,164	-
	174	1,142	-	-	-	28,590	-
	185	596	-	-	-	74,741	-
	Total					273,319	273,319

Time from feedlot entry to death for CHF and digestive disorders

Death from CHF generally occurred later in the feeding period than death from digestive disorders (Table 3.4). However, deaths from CHF and digestive disorders occurred throughout the feeding period. Death from CHF occurred throughout the feeding period but tended to occur approximately 5 months after arrival (Table 3.4). Death from bloat occurred throughout the feeding period but tended to occur approximately 4 months post-arrival. Ruminal bloat accounted for 72 % and 83 % of deaths due to digestive disorders in Canadian and US feedlots, respectively.

Table 3.4: The days on feed to postmortem for congestive heart failure (CHF) and digestive disorders and the total number of cases in Canada for all years (2000, 2004, 2008 and 2012) and in the USA in 2012

		Days in feedlot, mean ± SD		
Disease	n	Canada	n	USA
CHF	537	137 ± 79	261	137 ± 71
Bloat	2,324	129 ± 72	954	121 ± 83
Enteritis	398	59 ± 59	29	88 ± 61
Intestinal disorder	174	91 ± 73	8	118 ± 75
Peritonitis	314	99 ± 78	148	149 ± 106

Risk factors for time from feedlot entry to death from CHF (days on feed) in Canadian feedlots

Only age ($p < 0.001$) and gender ($p = 0.03$) were significantly associated with days from feedlot entry to death from CHF when controlling for season of placement ($p = 0.72$), risk category ($p = 0.97$), feedlot effects ($p < 0.001$) and year of placement ($p < 0.001$). On average, yearlings died 26 days (95 % CI: 34, 17 days) earlier in the feeding period than calves when controlling for gender, season and year of placement, and feedlot effects. On average, males died 6 days (95 % CI: 11, 1 days) earlier than females.

Risk of CHF from the year 2000 to the year 2012

The risk of CHF differed among years, with a trend to increase from 2000 to 2012, in Canadian feedlots when controlling for season of placement, risk category, gender, age and feedlot effects (Table 3.5). The odds of death from CHF relative to digestive disorders also differed significantly in these years with an increasing trend from the year 2000 (Table 3.5). The mean attack risk for digestive disorders was almost 5 times higher than the attack risk for CHF in U.S. feedlots (Risk of digestive disorder = 4.66 per 1,000 cattle, 95 % CI = 3.61, 5.72; Risk of CHF = 1.04 per 1,000 cattle, 95 % CI= 0.82, 1.26).

Table 3.5: The estimated attack risk of congestive heart failure (CHF) and digestive disorders (DD) per 1,000 cattle entering the feedlot by year of placement while controlling for age, risk of undifferentiated fever and respiratory disease, gender and season of placement; and, the odds of CHF relative to the year 2000 using digestive disorders as a baseline reference while controlling for age, risk of undifferentiated fever and respiratory disease, gender, season of placement and the number of treatments for respiratory disease (0, 1 or ≥ 2)

		Attack risk per 1,000 cattle			OR of CHF mortality relative to the year 2000		
Year	Disease	Mean	95 % CI	p-value	Odds Ratio	95 % CI	p-value
2000	CHF	0.27	0.18, 0.37	ref.	1.00		ref.
	DD	1.75	1.32, 2.19				
2004	CHF	0.37	0.26, 0.47	0.18	1.60	1.13, 2.28	0.009
	DD	1.77	1.40, 2.15				
2008	CHF	0.61	0.45, 0.78	< 0.001	2.60	1.88, 3.61	< 0.001
	DD	2.32	1.82, 2.81				
2012	CHF	0.52	0.37, 0.67	0.003	1.44	1.02, 2.04	0.04
	DD	2.82	2.13, 3.50				

Risk ratio of CHF for U.S. and Canadian feedlots by season of placement, risk category, age and gender

Season of placement was significantly associated with CHF risk in Canadian feedlots ($p = 0.005$) and tended to be associated with CHF risk in U.S. feedlots ($p = 0.10$) when controlling for year of placement, feedlot, risk category, gender and age (Table 3.6). Cattle placed in feedlots from May 1st to August 31st or from September 1st to December 31st had a greater risk of CHF than cattle placed in feedlots from January 1st to April 30th. Yearling cattle had a lower risk of CHF than calves in Canadian feedlots but a higher risk of CHF than calves in U.S. feedlots (Table 3.6). High-risk cattle and male cattle were at significantly higher risk of CHF in U.S. feedlots but not in Canadian feedlots (Table 3.6). The risk of CHF was not significantly different between U.S. and Canadian feedlots ($p = 0.17$).

Table 3.6: The attack risk ratio for CHF in U.S. and Canadian feedlots for season of placement, risk category, age and gender

Factor	Level	Canada (all years) Attack risk ratio	p-value	USA (2012) Attack risk ratio	p-value
Season of placement	Jan. 1st to April 30th	Ref.	0.005	Ref.	0.10
	May 1st to Aug. 31st	1.62 (1.00, 2.61)		1.58 (0.97, 2.56)	
	Sept. 1st to Dec. 31st	2.10 (1.35, 3.26)		1.62 (1.02, 2.57)	
Risk	Low	Ref.		Ref.	
	High	1.20 (0.76, 1.90)	0.44	2.89 (1.62, 5.13)	< 0.001
Age	Calf	Ref.		Ref.	
	Yearling	0.40 (0.26, 0.61)	< 0.001	2.38 (1.35, 4.17)	0.003
Gender	Female	Ref.		Ref.	
	Male	1.19 (0.90, 1.58)	0.23	2.21 (1.53, 3.19)	< 0.001

Risk of CHF among feedlots in 2012

There were differences in the risk of CHF among feedlots in both the U.S. and Canada ($p < 0.001$) (Figures 3.1 and 3.2). Among the Canadian feedlots, Feedlot 6 had the highest risk of CHF (Risk = 0.83 per 1,000 cattle, 95 % CI = 0.57, 1.10) and Feedlot 19 had the lowest risk of CHF (Risk = 0.08 per 1,000 cattle, 95 % CI = 0, 0.20). Canadian feedlots with an increased risk of CHF relative Feedlot 19 did not have significantly greater odds of death from CHF relative to death from digestive disorders (Figure 3.1). This indicates that feedlots that had a higher death loss from CHF also had a higher death loss from digestive disorders.

Feedlot 171 had the highest risk of CHF among U.S. feedlots (Risk = 1.73 per 1,000 cattle, 95 % CI = 1.10, 2.35). Feedlot 185 had the lowest risk of CHF among U.S. feedlots (Risk = 0.42 per 1,000 cattle, 95 % CI = 0.18 0.66). Feedlots in the U.S. with an increased risk of CHF relative to Feedlot 185 had significantly greater odds of death from CHF relative to death from digestive disorders ($p < 0.001$) (Figure 3.2). This indicates that U.S. feedlots

with an increased risk of CHF had an increased risk of CHF relative to death from digestive disorders.

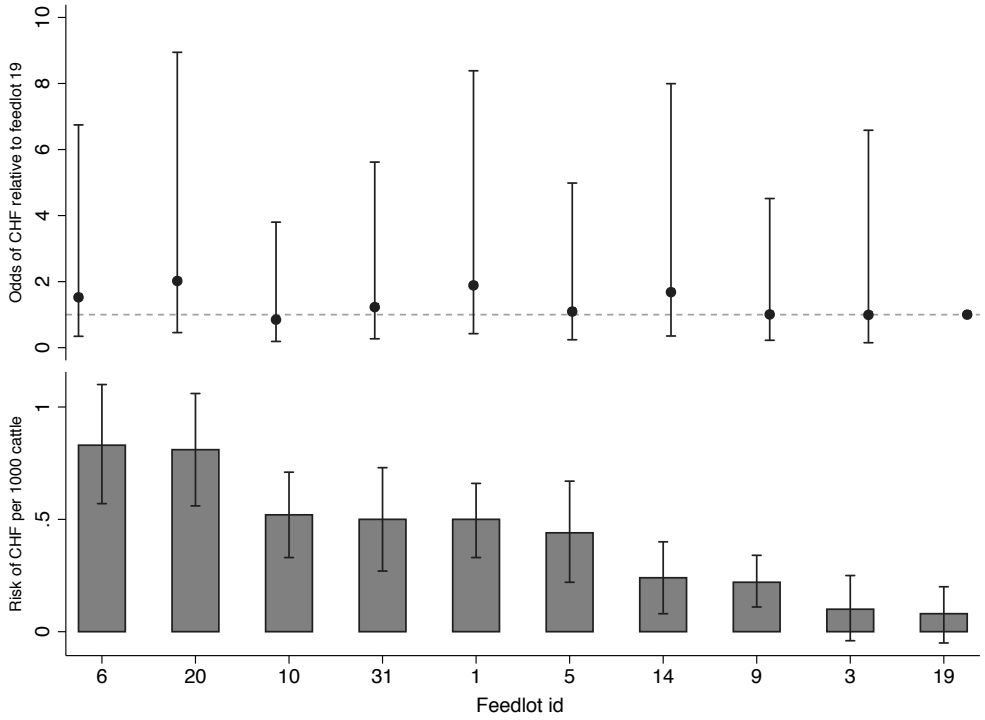

Figure 3.1: Least squares mean estimate of the attack risk of CHF per 1,000 cattle entering Canadian feedlots in 2012 when controlling for country, season of placement, risk category, gender and age (bottom graph); and, the odds of CHF relative to the Canadian (Feedlot 19) with the lowest attack risk in 2012 (top graph)

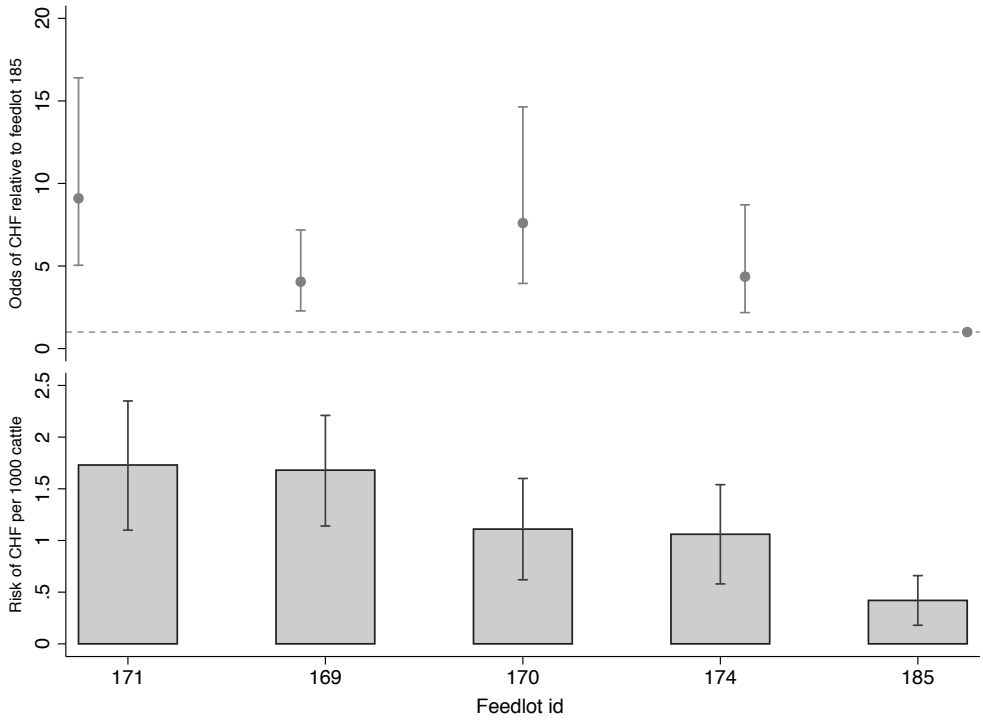

Figure 3.2: Least squares mean estimate of the attack risk of CHF per 1,000 cattle entering U.S. feedlots in 2012 when controlling for country, season of placement, risk category, gender and age (bottom graph); and, the odds of CHF relative to the U.S. (Feedlot 185) feedlot with the lowest attack risk in 2012 (top graph)

The least squares means risk of CHF appeared to increase in association with the altitude at which the feedlot was located in both U.S. and Canadian feedlots when controlling for all other explanatory variables (Figures 3.3 and 3.4). An increased risk of CHF reflected an increased risk of CHF relative to death from digestive disorders. The odds of death from CHF relative digestive disorders appeared to show a positive association with increasing altitude when using the Canadian (Feedlot 10, altitude 657 m) (Figure 3.3) and U.S. (Feedlot 185, altitude 596 m) (Figure 3.4) feedlots located at the lowest altitudes as baseline references.

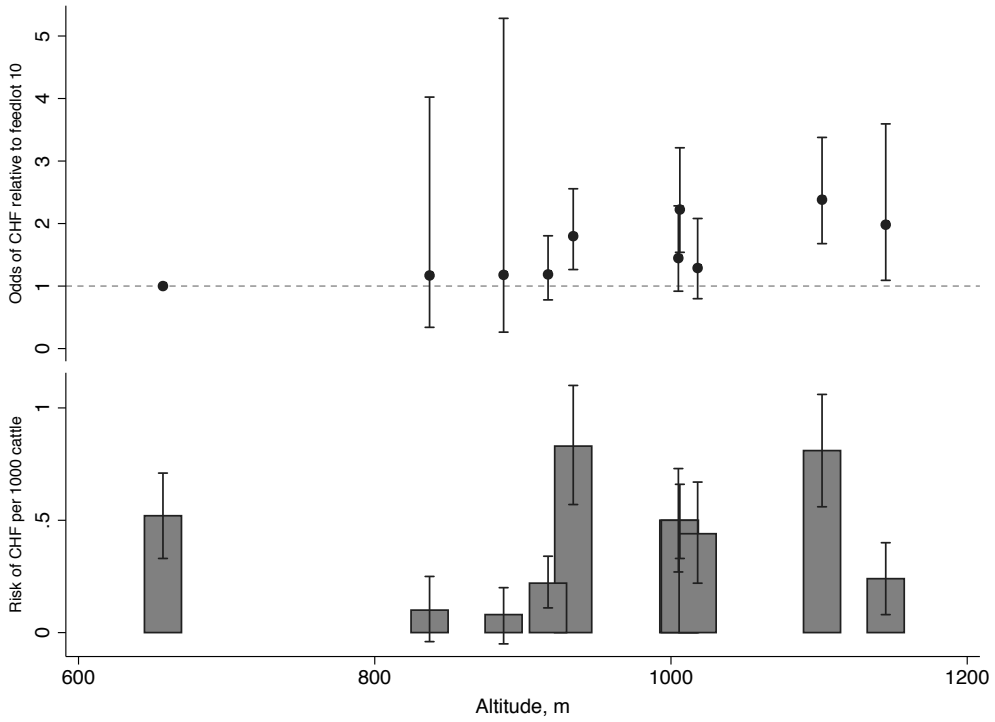

Figure 3.3: Least squares mean attack risk of CHF per 1,000 cattle entering Canadian feedlots in 2012 when controlling for country, season of placement, risk category, gender and age (bottom graph); and, the odds of CHF relative to the Canadian feedlot (Feedlot 10, altitude 657 m) located at the lowest altitudes in 2012 (top graph)

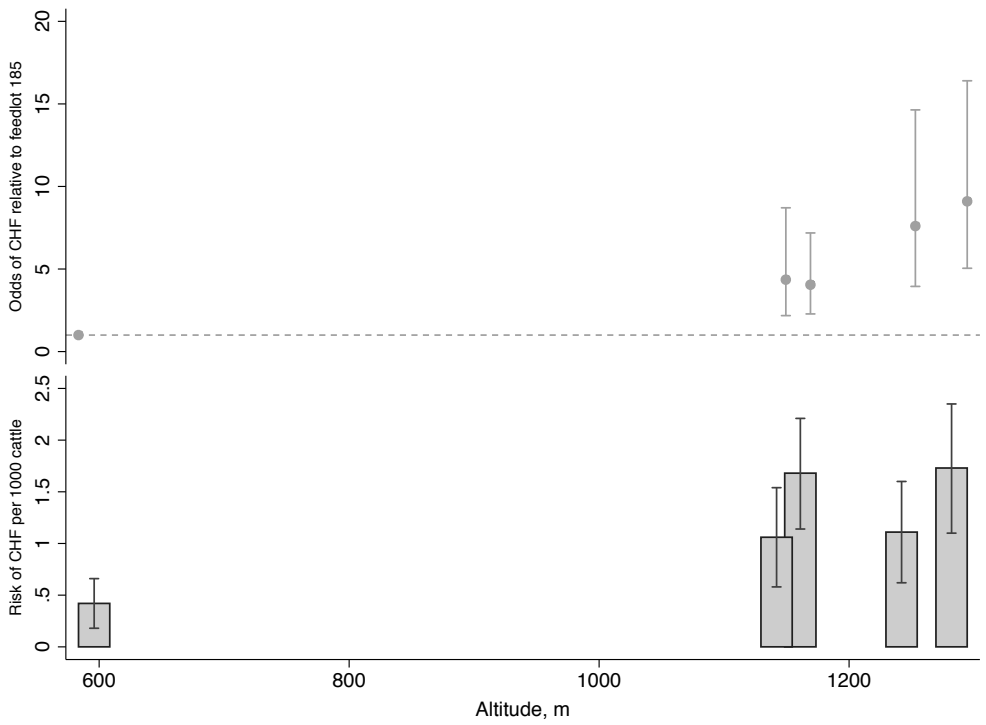

Figure 3.4: Least squares mean attack risk of CHF per 1,000 cattle entering U.S. feedlots in 2012 when controlling for country, season of placement, risk category, gender and age (bottom graph); and, the odds of CHF relative to the U.S. feedlot (Feedlot 185, altitude 596 m) located at the lowest altitudes in 2012 (top graph)

Risk factors for CHF relative to digestive disorders

Cattle entering U.S. and Canadian feedlots from January 1st to April 30th had significantly lower odds of death from CHF relative to digestive disease than cattle entering feedlots at any other time of year when controlling for year of placement, risk, age, gender and the number of times treated for BRD ($p < 0.001$) (Table 3.7). High-risk cattle had significantly lower odds of CHF than low-risk cattle in both U.S. and Canadian feedlots. Age was not associated with the odds of CHF. The odds of CHF were significantly higher in male cattle in U.S. feedlots only. Treatment for BRD significantly increased the odds of CHF ($p < 0.001$).

Table 3.7: The odds of CHF to digestive disorders in Canadian and U.S. feedlots while controlling for year of placement

		Canada (all years)				USA (2012)			
Variable	Canada	Odds Ratio	95 % CI		p-value	Odds Ratio	95 % CI		p-value
Season of	Jan. - April	1.00			< 0.001	1.00			< 0.001
placement	May - Aug.	1.97	1.35	2.89		1.56	1.05	2.33	
	Sep. – Dec.	1.72	1.24	2.39		2.31	1.58	3.37	
Risk	Low	1.00			ref.	1.00			ref.
	High	0.47	0.33	0.65	< 0.001	0.59	0.35	1.00	0.049
Age	Calves	1.00			ref.	1.00			ref.
	Yearlings	0.83	0.60	1.15	0.25	1.29	0.78	2.13	0.32
Gender	Females	1.00			ref.	1.00			ref.
	Males	0.98	0.79	1.20	0.83	1.38	1.01	1.90	0.045
Times	0	1.00			ref.	1.00			ref.
treated for	1	1.98	1.62	2.42	< 0.001	2.27	1.56	3.30	< 0.001
BRD	≥ 2	2.25	1.38	3.69	0.001	4.59	3.13	6.74	< 0.001

DISCUSSION

This study showed that across 10 Canadian feedlots the risk of congestive heart failure (CHF) increased from the year 2000 to the year 2012. From the year 2000 to the year 2008, the risk of CHF doubled. The risk of CHF increased more than the underlying change in the risk of digestive disorders. Death from CHF occurred throughout the feeding period but typically occurred late in the feeding period, which makes this disease particularly costly to the feedlot industry. Treatment for respiratory disease was a significant risk factor for CHF.

The large number of animals in this study permitted the detection of a change in the risk of a relatively rare event over time. Unfortunately, a limitation of this study is that the change could only be evaluated over a 12-year period in Canadian feedlots. We cannot make inferences regarding the risk of CHF in U.S. feedlots over the same time period. In

1974, a study of 4 U.S. feedlots located at an altitude of 1,600 m reported the attack risk of CHF to be 2.85 cases per 10,000 cattle entering the feedlot (Jensen et al., 1976). In our study of 5 U.S. feedlots in 2012, the risk of CHF ranged from 4.2 per 10,000 cattle (feedlot altitude = 596 m) to 17.3 cattle per 10,000 cattle (feedlot altitude = 1,282 m). This suggests that the risk of CHF in U.S. feedlots increased considerably over a 40-year period. However, direct comparisons cannot be made between the previous study and ours, as there are multiple factors that could account for the differing results.

In cattle, right-sided CHF is historically considered to be a disease of high altitude production (Glover and Newsom, 1915; Hecht et al., 1962). The goal of this study was to address producer concerns that the risk of CHF in feedlot cattle is increasing. The results of our study support these observations. Across 10 Canadian feedlots the mean attack risk of CHF increased significantly over a 12-year period. The odds of CHF relative to digestive disease also increased significantly over the same 12-year period. This indicates that the attack risk of CHF increased significantly more so than any underlying change in the attack risk of digestive disorders. Therefore, it is unlikely that the increase in CHF attack risk is simply a reflection of an overall increase in all causes of mortality among feedlot cattle.

Season of feedlot arrival was significantly associated with attack risk of CHF in both Canadian and U.S. cattle populations. Cattle entering feedlots from May 1st to December 31st had significantly higher odds of CHF than cattle placed in feedlots from January 1st to April 31st. A previous study reported CHF mortality to be greatest in the fall and winter than the spring and summer (Jensen et al., 1976). Environmental temperature differences among seasons may account for these findings. Cold-induced constriction of both the pulmonary

arterial and venous system may increase mean pulmonary arterial pressure (Busch et al., 1985) and therefore, increase the risk of CHF.

There were significant differences in the odds of CHF relative to digestive disorders among feedlots in both Canada and the U.S. Altitude was a feedlot-level effect, which means that the effect of altitude on the odds of CHF could not be determined. However, there appeared to be a positive relationship between the altitude at which a feedlot was located and the odds of CHF relative to digestive disorders. This positive relationship was apparent in both U.S. and Canadian feedlots. Until recently, congestive heart failure was considered to be only problematic at altitudes over 2,130 m (Glover and Newsom, 1915; Hecht et al., 1962). Therefore, it is perhaps no surprise that a feedlot operation established in Colorado at an altitude of 2,300 m partly failed because of the excessive number of CHF cases (Jensen et al., 1976). However, a particularly surprising finding of our study was that CHF occurred with any frequency at all in feedlots located at altitudes less than 1,000 m.

Death from CHF tended to occur later in the feeding period than digestive disorders although, both occurred throughout the feeding period. This makes CHF a particularly costly feedlot disease. It is unclear why CHF is particularly problematic in the late feeding period. Physiological studies of feedlot cattle indicate that mean pulmonary arterial pressure is significantly higher in the late-feeding period than the early-feeding period (Chapter 5). It is unclear why this occurs but it likely accounts for the predominant occurrence of CHF in the late feeding period.

CHAPTER 4: PULMONARY ARTERIAL PRESSURES IN CALVES AT 4 DIFFERENT ALTITUDES

INTRODUCTION

Historically, congestive heart failure (CHF) was considered to be only problematic at altitudes over 2,130 m (Glover and Newsom, 1915; Hecht et al., 1962). The incidence of CHF was estimated to be 0.5 % to 2.0 % at altitudes over 2,130 m (Alexander and Jensen, 1959; Hecht et al., 1962). However, incidence varied from year to year and from herd to herd; in some herds, the incidence was reported to reach as high as 10 % with the greatest mortality occurring in calves less than one year of age (Alexander and Jensen, 1959; Hecht et al., 1962).

Mean pulmonary arterial pressure (mPAP) has been estimated to be a moderately heritable trait (Shirley et al., 2008). Therefore, high altitude producers can reduce the risk of calf mortality due to right-sided CHF secondary to pulmonary hypertension by breeding bulls known to have a 'low' mPAP at high altitude (Holt and Callan, 2007). Mean PAP in healthy beef calves selected for low mPAP increased from 34 mm Hg at 1 month of age and an altitude of 2,410 m to approximately 50 mm Hg at 6 months of age and an altitude of 2,730 m (Neary et al., 2013a). This suggested that vascular remodeling may be occurring in calves in response to chronic hypoxic exposure.

The goal of this study was to evaluate if similar changes in pulmonary arterial pressures occur with age at lower altitudes. The first objective was to evaluate changes in pulmonary arterial pressures in beef calves in association with age and altitude. The second objective was to determine if pulmonary arterial pressures are responsive to

supplemental oxygen provided via a mask. We hypothesized that calves located at higher altitude would show a greater increase in mPAP with age than calves located at lower altitudes. We also hypothesized that a reduction in mPAP in response to supplemental oxygen would be age dependent; younger calves showing a greater response than older calves.

MATERIALS AND METHODS

Study overview

The pulmonary arterial pressures (mean, systolic and diastolic) of calves in five cohorts were evaluated from four herds located at altitudes ranging from 1,470 m to 2,730 m. Two cohorts of calves were evaluated on one herd over two consecutive years. All cohorts except one were evaluated twice in the same year. After the collection of an initial pulmonary arterial pressure measurement and a blood sample from the coccygeal artery, a random sample of calves were supplemented with oxygen (100 %) for 5 minutes via a face-mask. After the 5th minute, oxygen administration was continued until a second pulmonary arterial pressure measurement and arterial blood sample were collected. Response to oxygen supplementation may allow differentiation among various causes of hypoxemia. 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

Four herds were enrolled in this study: 3 herds located in Colorado (A, B and D) and 1 herd located in southern Wyoming (C). Herds B and C were commercial cow-calf operations managed by Colorado State University. Herd A was a privately owned seedstock operation and Herd D a privately owned commercial cow-calf operation. Calves were sampled in 2011 or 2012 (Table 4.1). For statistical analyses calves were clustered according to herd. Herd D formed 2 clusters: calves born and sampled in 2011 (D(i)) and calves born and sampled in 2012 D(ii). The calves in D(i) and D(ii) were born to primiparous heifers. The calves studied in herds A, B and C were born to primiparous and multiparous cows. Only male calves in Herd C were sampled due to herd management practices. Every second calf into the chute was sampled.

Table 4.1: The altitude, date of sampling, number of calves sampled by gender, mean age and genetic composition of the 4 herds studied

Herd	Altitude, m	Date	Number sampled				Mean age ± SD, days	Genetic makeup of calves
			H[1]	B[2]	S[3]	T[4]		
A	1,470	07/01/11	17	13	0	30	131 ± 16	Black Aberdeen Angus
		10/27/11	17	11	0	28	250 ± 16	
B	2,010	09/27/11	17	0	13	30	126 ± 13	Red Aberdeen Angus and Hereford composite cows x Red Aberdeen Angus bulls
C	2,170	07/31/12	0	55	5	60	124 ± 18	Black and Red Aberdeen
		10/01/12	0	55	5	60	187 ± 18	Angus
D(i)	2,730	06/27/11	44	11	25	80	91 ± 7	Red Aberdeen Angus,
		10/12/11	40	10	23	73	199 ± 7	Hereford, Gelbvieh and
D(ii)		06/21/12	30	0	28	58	86 ± 7	Simmental composites
		10/10/12	27	0	24	51	197 ± 7	

[1] H – The number of heifer calves sampled
[2] B – The number of bull calves sampled
[3] S – The number of steer calves sampled
[4] T – The total 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]), *Bovine viral diarrhea virus* (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. In 2012, calves in Herd D (cohort Dii) were given a subcutaneous mineral supplement containing chelated manganese, zinc, selenium and copper (Multimin® 90, Fort Collins, CO) and an intra-nasal vaccine offering protection against BRSV, IBR and PI3 (Inforce™ 3, Zoetis, Madison, NJ) at the time of the first test.

Herds tested negative for BVDV by ELISA. Communal grazing with neighboring herds does not occur. Mineral supplements were provided year round. A hormonal growth promotant (Synovex C, Zoetis, Madison, NJ) containing 100 mg progesterone and 10 mg estradiol benzoate was administered to both heifer and steer calves in Herd D in both 2011 and 2012 when they were approximately 8 weeks old.

Herds A and B did not breed bulls with mPAP greater than 45 mm Hg at 1,470 m and 2,010 m, respectively. Approximately half of calves in Herd C were sired by bulls with a mPAP < 40 mm Hg at 2,170 m. Bulls that had an unknown mPAP sired the remaining calves. Approximately half of the calves were born to cows that were bred by artificial insemination using semen from sires with an unknown mPAP. Use of artificial insemination is a common practice for many cow-calf producers in the western U.S. This allows producers to bring new genetics into the herd from diverse geographic, and potentially low altitude, locations. Calves in Herd D were progeny of bulls with a mPAP < 42 mm Hg at 2,440 m.

The majority of calves were tested on 2 occasions approximately 3 months apart. Repeat measures were accounted for in the statistical analyses. Calves known to have died between the testing periods were removed from the data set. Calves missing at random on the second test day, due to lost ear tag identification for example, remained in the data set. Due to herd management practices calves in Herd B were tested only once.

Pulmonary arterial pressure testing

A full description of the equipment, materials and facilities required for pulmonary arterial pressure testing is provided by Holt and Callan (2007). In brief, a 12 gauge needle was inserted into the jugular vein. Flexible saline-filled catheter tubing was then fed through the needle, down through the right atrium, into the right ventricle, and then into the pulmonary artery. A pressure transducer connected the catheter to an oscilloscope. The position of the catheter was determined from the pressure waveform on the oscilloscope. The jugular vein, right atrium, right ventricle and pulmonary artery have distinct pressure waveforms (Holt and Callan, 2007). Mean, systolic and diastolic pressures were recorded after the pulmonary pressure waveforms had stabilized.

Oxygen supplementation

Response to oxygen supplementation may allow differentiation between ventilation-perfusion mismatch and shunting as a cause of hypoxemia (Bach, 2008). Animals with ventilation-perfusion mismatch typically have a marked increase in arterial oxygen tension (p_aO_2) in response to supplemental oxygen. An animal with right-left shunt shows minimal improvement in p_aO_2, if at all.

After the collection of an initial PAP measurement and an arterial blood sample, a rubber-sealed mask was placed over the nares and jaw of randomly selected calves for 5 minutes. Oxygen (100 %) was provided at 10 L/min so that 40 to 60 % of the inspired air was composed of oxygen. After the 5th minute, oxygen administration was continued until a second PAP measurement and arterial blood sample were collected. The procedure was performed on 26 calves in Herd A (n = 5), Herd B (n = 7) and Herd D(i) (n = 14): 19 calves had both PAP and arterial blood-gas measured, 5 calves had just arterial blood-gas measured and 2 calves had just PAP measured.

Statistical analyses

Statistical analyses were performed using STATA version 12 (Stata Corporation, College Station, Texas, USA). Descriptive analyses of calf age and mean, systolic and diastolic pulmonary arterial pressure were performed for all cohorts at tests 1 and 2.

The first objective was to evaluate changes in pulmonary arterial pressures in beef calves in association with age and altitude. Generalized estimating regression equations were used to account for the repeated measures (Liang and Zeger, 1986; Zeger and Liang, 1986). The outcome variables assessed included mean (mPAP), systolic (sPAP) and diastolic (dPAP) pulmonary arterial pressure. Explanatory variables included age (days), gender (heifer, steer or bull) and herd. An exchangeable correlation structure was used.

Pairwise correlation analyses were performed to evaluate the relationship of PAP between tests. Calves in Herd B were tested only once therefore, pairwise analyses were only performed on Herds A, C and D. Changes in PAP (mean, systolic and diastolic) and blood-gas tensions (p_aCO_2 and p_aO_2) in response to oxygen supplementation were

evaluated using paired t-tests (dPAP, p_aCO_2 and p_aO_2) or matched-pairs Wilcoxon signed rank tests (mPAP and sPAP) depending on the distribution of the data. A Bonferroni correction was applied to statistical p-values to account for multiple testing.

RESULTS

Mean pulmonary arterial pressure increased in association with increasing altitude and increasing age (Table 4.2).

Table 4.2: Age and pulmonary arterial pressures (mean ± SD) according to herd and test

		Herd				
Variable	Test	A	B	C	Di	Dii
Altitude, m		1,470	2,010	2,170	2,730	2,730
Age,	1	131 ± 16	126 ± 13	124 ± 18	91 ± 7	86 ± 7
days	2	250 ± 16	No test	187 ± 18	199 ± 7	197 ± 7
mPAP, [1]	1	36.4 ± 4.9	39.0 ± 3.9	41.4 ± 7.9	46.5 ± 10.7	40.5 ± 6.5
mm Hg	2	38.7 ± 3.1	No test	43.9 ± 9.2	50.1 ± 14.6	47.5 ± 8.4
sPAP, [2]	1	58.3 ± 8.7	64.7 ± 10.6	57.3 ± 9.6	68.6 ± 14.4	61.6 ± 8.5
mm Hg	2	60.9 ± 5.7	No test	62.0 ± 9.1	69.4 ± 13.4	63.5 ± 10.0
dPAP, [3]	1	15.3 ± 6.0	16.3 ± 6.0	26.5 ± 7.0	24.0 ± 8.8	22.3 ± 7.1
mm Hg	2	13.4 ± 5.9	No test	26.6 ± 6.3	29.2 ± 11.1	30.1 ± 8.9

[1] Mean pulmonary arterial pressures
[2] Systolic pulmonary arterial pressure
[3] Diastolic pulmonary arterial pressure

For every 1 day increment in age, mPAP increased by 0.045 mm Hg (95 % CI = 0.032, 0.058) when controlling for herd and gender ($p < 0.001$) (Figure 4.1). There was variation of mPAP among herds ($p < 0.001$). Relative to Herd A, the mPAP of calves in Herds B, C, Di and Dii was 4.4 mm Hg (95 % CI = -0.4, 9.1; $p = 0.07$), 6.9 mm Hg (95 % CI =

3.0, 10.9; p = 0.001), 13.1 mm Hg (95 % CI = 9.5, 16.7; p < 0.001) and 8.9 mm Hg (95 % CI = 4.9, 12.8; p < 0.001), respectively. Relative to Herd B, the mPAP of calves in Herds C, Di and Dii was 2.6 mm Hg (95 % CI = -2.4, 7.5; p = 0.31), 8.7 mm Hg (95 % CI = 4.9, 12.5; p < 0.001) and 4.5 mm Hg (95 % CI = 0.6, 8.4; p = 0.025) higher, respectively. Relative to Herd C, calves in Herds Di and Dii had mPAP that were 6.1 mm Hg (95 % CI = 2.4, 9.9; p = 0.001) and 1.9 (95 % CI = -2.3, 6.2; p = 0.38) mm Hg higher, respectively. Calf gender (heifer, steer or bull) was not associated with mPAP when controlling for herd and age (p = 0.93). There was no interaction between herd and age (p = 0.11).

The lowest mPAP recorded was 31 mm Hg (Herd A, 07/01/11) and the highest mPAP recorded was 124 mm Hg (Herd D(i), 06/27/11). The latter calf showed moderate distension of the jugular vein but no other clinical signs of ill health.

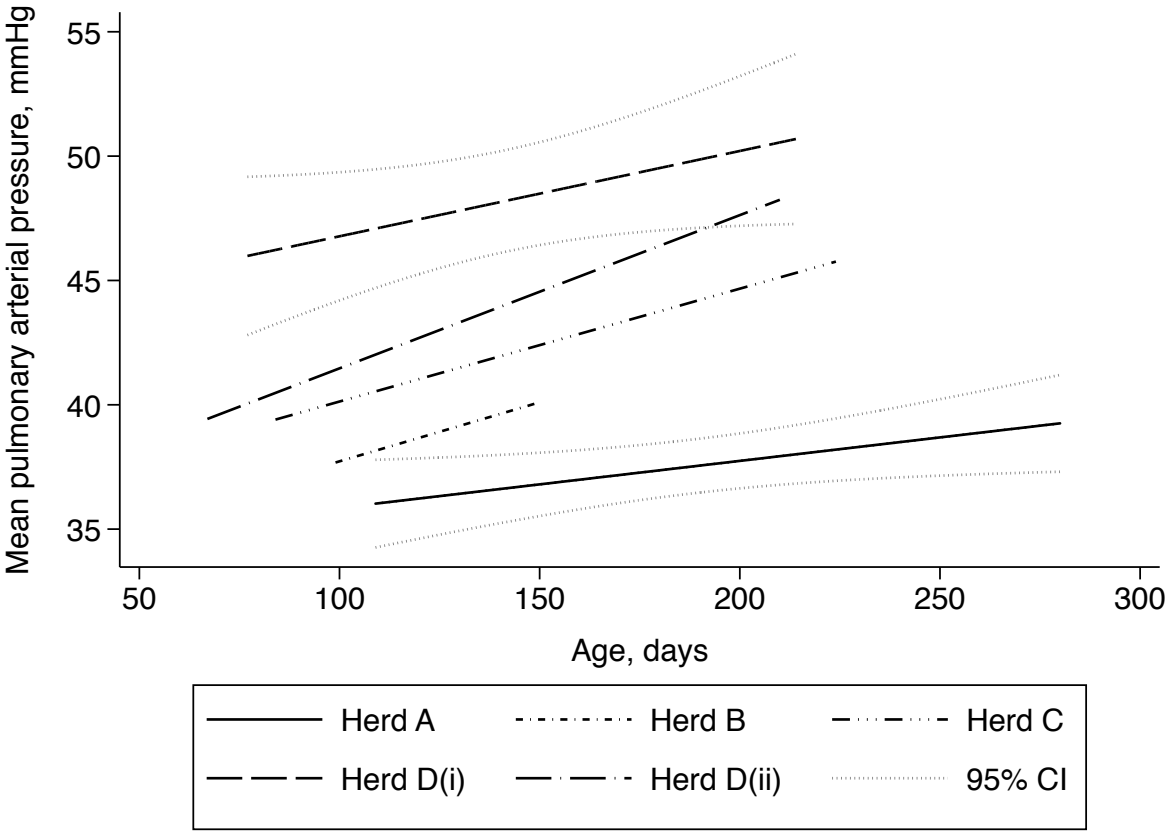

Figure 4.1: Mean pulmonary arterial pressure (mm Hg) by age (days) and herd. Calves in Herds A, B, C and D were sampled at 1,466 m, 2,008 m, 2,166 m and 2,731 m, respectively. Confidence intervals (95%) are only provided for herds A and D(i) for clarity

Pairwise correlation of PAP was weak in Herd A (Table 4.3). Correlation coefficients were highest for mPAP followed by dPAP and sPAP (Table 4.3).

Table 4.3: Pairwise correlation coefficients for pulmonary arterial pressure. Calves in Herd B were tested only once. Therefore, pairwise correlation analyses were not available

Herd	mPAP Correlation coefficient	p-value	sPAP Correlation coefficient	p-value	dPAP Correlation coefficient	p-value
A	0.14	0.48	-0.19	0.33	0.25	0.20
C	0.87	< 0.001	0.23	0.08	0.40	0.002
Di	0.60	< 0.001	0.35	0.004	0.58	< 0.001
Dii	0.61	< 0.001	0.26	0.07	0.49	< 0.001

For every 1 day increment in age sPAP increased by 0.026 mm Hg (95 % CI = 0.007, 0.044) when controlling for herd and gender (p = 0.006) (Figure 2). Herd was significantly associated with sPAP (p < 0.001). Relative to calves in Herd A the sPAP of calves in Herds B, C, Di and Dii was 6.7 mm Hg (95 % CI = 1.3, 12.1; p = 0.014), 1.7 mm Hg (95 % CI = -2.6, 5.9; p = 0.44), 10.7 mm Hg (95 % CI = 6.9, 14.6; p < 0.001) and 4.3 mm Hg (95 % CI = 0.07, 8.6; p = 0.046) higher, respectively. There tended to be an interaction between herd and age (p = 0.08). Calf gender (heifer, steer or bull) was not associated with sPAP when controlling for herd and age (p = 0.99).

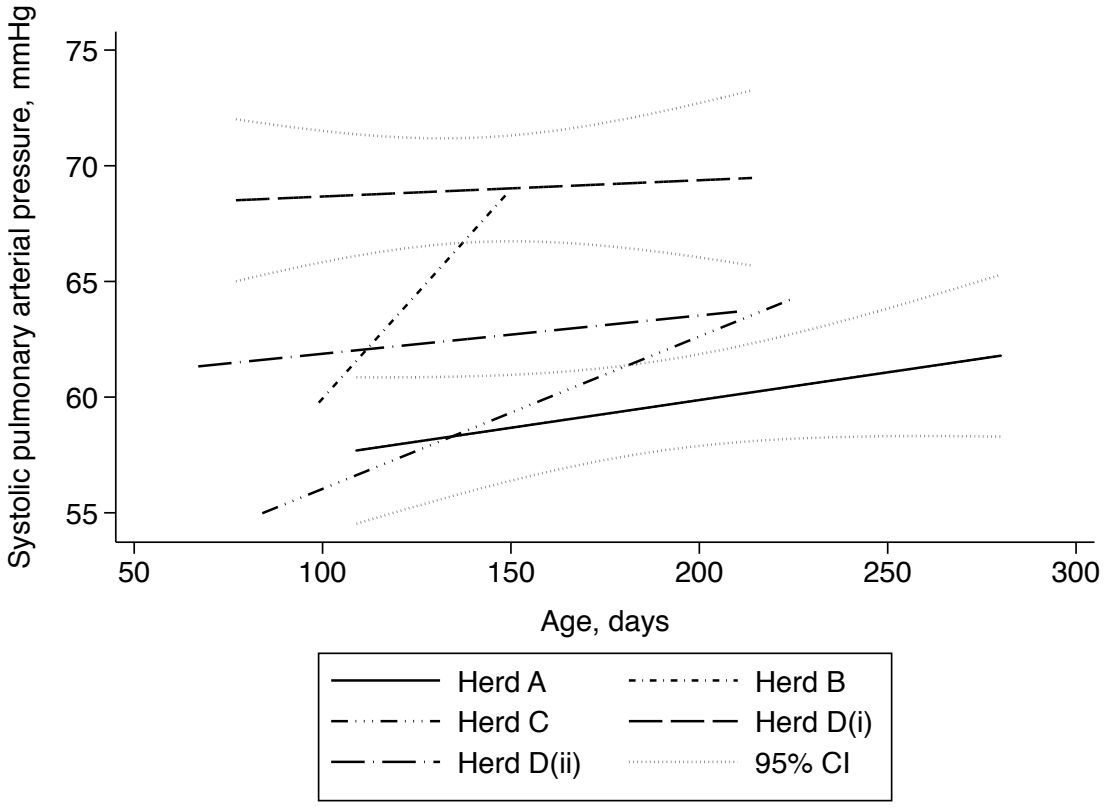

Figure 4.2: Systolic pulmonary arterial pressure (mm Hg) by age (days) and herd. Calves in herds A, B, C and D were sampled at 1,466 m, 2,008 m, 2,166 m and 2,731 m, respectively. Confidence intervals (95%) are only provided for Herds A and D(i) for clarity

Herd ($p < 0.001$) and age ($p < 0.001$) were significantly associated with dPAP. There was a significant interaction between herd and age ($p < 0.001$). There was no significant difference in dPAP between Herds A and B when controlling for age ($p = 0.97$). Herds C ($p < 0.001$), Di ($p < 0.001$) and Dii ($p < 0.001$) had significantly higher dPAP than Herd A when controlling for age and gender. When controlling for gender, dPAP in Herd A ($p = 0.22$) and Herd B ($p = 0.47$) did not change in association with age. Relative to Herd A, dPAP in Herd C tended to increase by 0.037 mm Hg per day (95 % CI = -0.004, 0.078; $p = 0.08$). Relative to Herd A, dPAP increased by 0.068 mm Hg per day (95 % CI = 0.037, 0.010; $p < 0.001$) in Herd Di and 0.087 mm Hg per day (95 % CI = 0.055, 0.120; $p < 0.001$) in Herd Dii when controlling for gender (Figure 4.3).

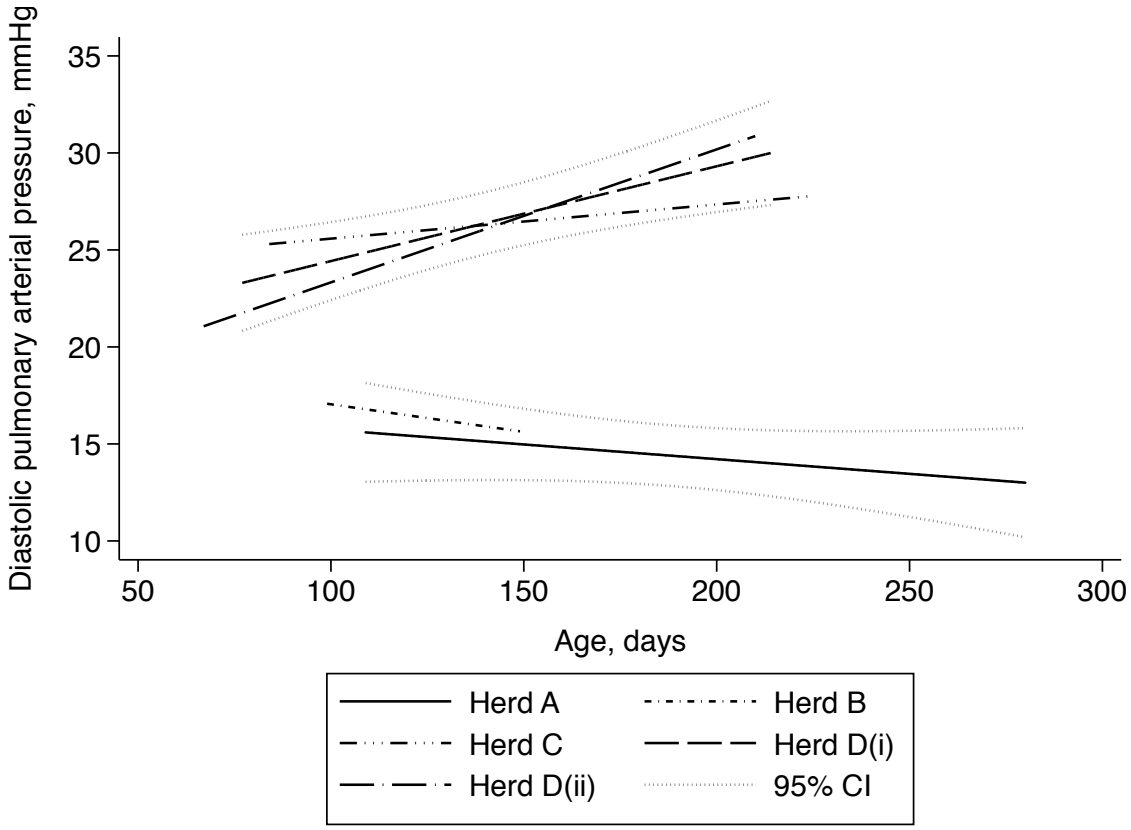

Figure 4.3: Diastolic pulmonary arterial pressure (mm Hg) by age (days) and herd. Calves in herds A, B, C and D were sampled at 1,466 m, 2,008 m, 2,166 m and 2,731 m, respectively. Confidence intervals (95%) are only provided for Herds A and D(i) for clarity

Changes in arterial blood-gas parameters and pulmonary arterial pressures in association with oxygen supplementation

The calves supplemented with oxygen ranged in age from 79 to 274 days old and had an average age of 165.0 ± 63.0 days (mean ± SD). Oxygen supplementation did not significantly reduce mPAP, sPAP or dPAP ($p > 0.5$) (Table 4.4). This suggested that acute hypoxia-induced pulmonary arterial vasoconstriction was not responsible for the pulmonary pressure values recorded. Change in mPAP in response to oxygen supplementation was not age dependent ($p = 0.67$). There was no significant change in p_aCO_2 after Bonferroni correction ($p = 0.20$). Arterial oxygen tension increased significantly

after oxygen supplementation ($p < 0.001$) (Table 4.4). However, the increase in arterial oxygen tension was small relative to the expected increase in oxygen tension following oxygen supplementation. This indicates that the arterial hypoxemia was due to ventilation-perfusion mismatching or intrapulmonary shunting, or both.

Table 4.4: Pulmonary arterial pressures and arterial blood-gas tensions pre and post-oxygen supplementation

		Oxygen supplementation status		
Parameter	n[3]	Pre-O_2	Post-O_2	p-value
Mean PAP,[4] mm Hg	21	(32, 37, 41, 45, 50)[1]	(32, 34, 39, 44, 52)[1]	> 0.5
Systole, mm Hg	21	(52, 57, 61, 64, 77)[1]	(50, 56, 60, 70, 80)[1]	> 0.5
Diastole, mm Hg	21	20.3 ± 7.0 [2]	20.3 ± 6.2 [2]	> 0.5
p_aCO_2,[5] mm Hg	24	39.4 ± 6.3 [2]	43.8 ± 10.7 [2]	0.20
p_aO_2,[6] mm Hg	24	57.8 ± 11.5 [2]	86.5 ± 33.9 [2]	<0.001

[1] (Minimum, Lower quartile, Median, Upper quartile, Maximum)
[2] Mean ± standard deviation
[3] Number of calves tested
[4] Pulmonary arterial pressure
[5] Arterial CO_2 tension
[6] Arterial O_2 tension

DISCUSSION

The results of this study showed that mPAP values were high in pre-weaned beef calves even at the modest altitude of 1,470 m. Mean pulmonary arterial pressures increased in association with age and altitude. Pulmonary arterial pressures did not change in response to oxygen supplementation, which suggested that pulmonary vascular remodeling may be occurring in calves even at altitudes as low as 1,470 m.

A limitation of this study is that confounding at the herd-level may have occurred. Differences in pulmonary arterial pressure among herds were likely attributable to herd

management factors, climate and genetics and altitude. In order to minimize confounding due to genetic differences, the herds were predominantly composed of British breeds of cattle. Seasonal changes in climate likely confounded the relationship between age and PAP. Further studies are required to evaluate the change in PAP with age in calves born at various times of the year.

The rate at which mPAP increased with age did not vary among herds. In other words, in the herds studied, the effect of age on mPAP was independent of altitude. Ventilation rate increased with age in calves at altitudes over 2,400 m (Neary et al., 2013a). Therefore, the positive association between mPAP and age is likely attributed to physiological factors other than alveolar hypoxia that are association with growth or functional maturation of the cardio-pulmonary system.

Systolic PAP also varied among herds but there was no clear association between the altitude at which a herd was located and sPAP. The pairwise correlation coefficient for sPAP was low for all herds. It is plausible that stressors, such as animal handling, more acutely influenced sPAP than mPAP and dPAP. This idea is supported by the finding that mPAP had the highest pairwise correlation coefficient across all of the herds. This is not surprising given that mPAP is a product of the steady-state resistance associated with the small pulmonary arteries. Pairwise correlation coefficients for dPAP were moderately strong. This suggested that dPAP is less susceptible to acute fluctuations than sPAP. Pairwise correlations for all PAP variables were non-significant in Herd A. This suggests that hypoxic exposure may be required to bring about more permanent structural changes within the cardiovascular system.

The progressive increase in diastolic PAP that occurred in association with increasing age in calves located at higher altitudes is intriguing. Diastolic PAP was found to be correlated with left atrial pressure (Fowler et al., 1952). Increased left atrial pressure may result from impaired left ventricular function due to right ventricular enlargement secondary to pulmonary hypertension causing a reduction in left ventricular stroke volume and left ventricular end-diastolic volume (Louie et al., 1995). Chronically elevated mPAP may be causing progressive left ventricular insufficiency in calves within the higher altitude herds. Alternatively, left ventricular insufficiency may be occurring for reasons other than right ventricular enlargement. It has been suggested that pulmonary venoconstriction or left ventricular failure may be contributing to BPH (Kuida et al., 1963). In humans, metabolic syndrome (a collection of risk factors that increases risk of heart disease) may predispose to pulmonary venous hypertension (Robbins et al., 2009).

Alexander et al. (1960) reported that the administration of oxygen to yearling steers via a mask transiently reduced mPAP but the mPAP value observed was still substantially higher than expected for cattle at low altitude. Based on these findings the authors concluded that the bovine species is particularly sensitive to a hypoxic environment (Alexander et al., 1960). In our study, we did not observe a significant reduction of mPAP following oxygen administration, irrespective of age. It was hypothesized than young calves would show a greater reduction in mPAP in response to oxygen supplementation than older calves. The rationale being that younger calves have had less time for pulmonary vascular remodelling to occur. The results of this study suggest that vascular remodelling may be occurring from a young age. Arterial oxygen tension also increased less than expected. This small increase in arterial oxygen tension, relative to the expected increase,

was suggestive of a ventilation-perfusion mismatch or intrapulmonary shunting. The proportion of pulmonary arterial blood that is shunted away from areas of gas exchange is reported to be correlated with mPAP (Cruz et al., 1979).

CHAPTER 5: CHANGES IN PULMONARY ARTERIAL PRESSURE WITH AGE

INTRODUCTION

Historically the risk of congestive heart failure has been only problematic at high altitude (Glover and Newsom, 1915; Hecht et al., 1962). However, it is a misconception that the disease is only problematic at high altitude today (Chapter 3). The incidence of CHF appears to have increased, irrespective of altitude (Chapter 3).

There is a need to characterize how pulmonary arterial pressures change through the confined feeding period. We have previously reported that mean pulmonary arterial pressure (mPAP) increased with age in pre-weaned calves at altitudes over 1,470 m (Chapter 4, (Neary et al., 2013a)). The available evidence indicates that mean pulmonary arterial pressure likely increases during the confined feeding period. Feedlot postmortem data suggests that the majority of deaths from congestive heart failure secondary to pulmonary hypertension occur in the late feeding period (Chapter 3, (Jensen et al., 1976)).

The goal of this study was to characterize the changes in pulmonary arterial pressure from calfhood into the confined feeding period. It was hypothesized that mPAP would increase during the feeding period and that the calves with the highest mPAP pre-weaning would have the highest mPAP at the end of the confined feeding period. Right atrial pressure and pulmonary arteriolar wedge pressure were also measured in the late feeding period in order to better characterize the pathophysiology of pulmonary hypertension.

MATERIALS AND METHODS

Study overview

A cohort of calves was followed from 4 months of age to 18 months of age in order to determine whether the cattle with the high mPAP pre-weaning were also most likely to have the highest mPAP during the late feeding period. Pulmonary arterial pressures were obtained from male calves up to a maximum of 4 occasions: twice pre-weaning at an altitude of 2,170 m and twice during the feeding period at altitudes of 1,560 m and 1,300 m. 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 12-3513A).

Study sites

The study population consisted of calves from the Beef Improvement Center, Colorado State University (Saratoga, Wyoming). The calves were born at 2,170 m. Due to herd management practices only male calves were studied. Approximately 50 % of the calves studied were progeny of bulls with a mPAP < 40 mm Hg at 2,170 m above sea-level. The other 50 % of the calves were progeny of industry relevant bulls of an unknown mPAP. Heifers with low mPAP were retained as herd replacements each year. Therefore, the maternal line has also been selected for 'low' mPAP.

A cohort of 60 calves was randomly selected from among the male calves in the herd for pulmonary arterial pressure (PAP) testing at 4 months of age. Every second calf into the chute was sampled. The same cohort of calves was sampled again at 6 months of age (Table 5.1). Calves were weaned in mid-October, 2012. At 6 months of age (test 2) all male calves

in the herd were pulmonary arterial pressure tested for breeding management purposes. Therefore, in addition to the 60 calves in the original cohort mPAP values were obtained from an additional 93 steers; in total, 152 mPAP measurements were obtained from calves at test 2. Systolic and diastolic PAP values were only recorded from the original cohort of 60 calves at tests 1 and 2.

Some of the bull calves sampled at test 2 were sold prior to test 3 (n = 30). After test 2, all remaining bull calves were castrated and moved with the remaining steers to the Eastern Colorado Research Center (ECRC), Colorado State University (Akron, CO) located at an altitude of 1,300 m in mid-December (n = 123). On April 12th, 2013 half of the steers (n = 61) were transported to the Agricultural Research, Development and Education Center (ARDEC), Colorado State University (Fort Collins, CO) located at an altitude of 1,560 m. Prior to returning to ECRC these steers were sampled (Test 3, 5/2/13). The other half of the steers (n = 62) was sampled at the ECRC (Test 4, 5/15/13) approximately 1 week prior to being transported to ARDEC for feed intake and efficiency assessment. These calves returned to ECRC in mid-August. All steers remained at the ECRC until completion of the feeding period.

The steers were approximately 13 months of age at tests 3 and 4. The final test was performed at the ECRC, approximately 2 to 4 weeks prior to slaughter when the steers were approximately 19 months of age. One steer died from pneumonia between test 3 and 5. Another steer was sold due to ill-thrift and poor growth. Two steers escaped from the chute prior to sampling. Therefore, in total 119 steers were sampled.

Table 5.1: The date, altitude, age and number of calves sampled according to test

Test	Altitude, m	Date	Number sampled			Mean age ± SD, days
			B[1]	S[2]	T[3]	
1	2,170	07/31/12	55	5	60	124 ± 18
2	2,170	10/01/12	55	5[4]	60[4]	185 ± 19
3	1,560	5/2/13	0	61	61	398 ± 16
4	1,300	5/15/13	0	62	62	405 ± 19
5	1,300	10/8/13	0	119	119	554 ± 17

[1] B – The number of bull calves sampled
[2] S – The number of steer calves sampled
[3] T – The total number of calves sampled
[4] mPAP measured on an additional 93 steers for breeding management purposes. Therefore total at test 2 was 153 (mPAP only) & 60 (sPAP and dPAP).

The dams of calves studied were given a pre-breeding and pre-calving vaccination offering protection against *Bovine herpesvirus 1* (infectious bovine rhinotracheitis [IBR]), *Bovine viral diarrhea virus* (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. A record was kept of calf health and any treatments administered.

Pulmonary arterial pressure testing

A full description of the equipment, materials and facilities required for pulmonary arterial pressure testing is provided by Holt and Callan (2007). In brief, a large bore needle was inserted into the jugular vein. Flexible saline-filled catheter tubing was then fed through the needle, down through the right atrium, into the right ventricle, and then into the pulmonary artery. A pressure transducer connected the 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 distinct pressure

waveforms (Holt and Callan, 2007). Right atrial and pulmonary arteriolar wedge pressures were obtained from a random sub-sample of steers during the late-feeding period. Pulmonary arteriolar wedge pressures were obtained by advancing the catheter until the tip became lodged in an arteriolar vessel of sufficiently small diameter to occlude forward flow of blood. The pulmonary arteriolar wedge pressure has a waveform characteristic of a venous pressure trace with 'a' and 'v' waves produced by left-sided physiologic events, which allows it to be distinguished from the pulmonary arterial pressure waveform (Vest and Heupler, 2013). Wedging of the catheter was occasionally verified by aspirating blood through the catheter and checking that the blood-gas tensions were consistent with arterial blood. Mean, systolic and diastolic pressures were recorded after the pulmonary pressure waveforms had stabilized.

Statistical analyses

Statistical analyses were performed using STATA version 12 (Stata Corporation, College Station, Texas, USA). Statistics are provided as mean and 95 % confidence interval (95 % CI) of the mean unless otherwise indicated.

The first objective was to determine if PAP changed in association with age. Generalized estimating regression equations were used to account for any repeated measures (Liang and Zeger, 1986; Zeger and Liang, 1986). Generalized estimating equations are robust to missing observations. An exchangeable correlation structure was used. The outcome variables assessed included: mean (mPAP), systolic (sPAP) and diastolic (dPAP) pulmonary arterial pressure; and pulse pressure. Explanatory variables included

test (1 to 5) and sex (steer or bull). Test was used as an explanatory variable rather than age because age was confounded by multiple variables such as altitude and diet.

Pairwise correlation analyses were performed between all test periods in order to evaluate the relationship of PAP between tests.

RESULTS

Mean pulmonary arterial pressure (mPAP) varied among tests ($p < 0.001$) and between bulls and steers ($p = 0.01$). Mean pulmonary arterial pressures increased with age during the pre-weaning period and during the confined feeding period at moderate altitude (Figure 5.1).

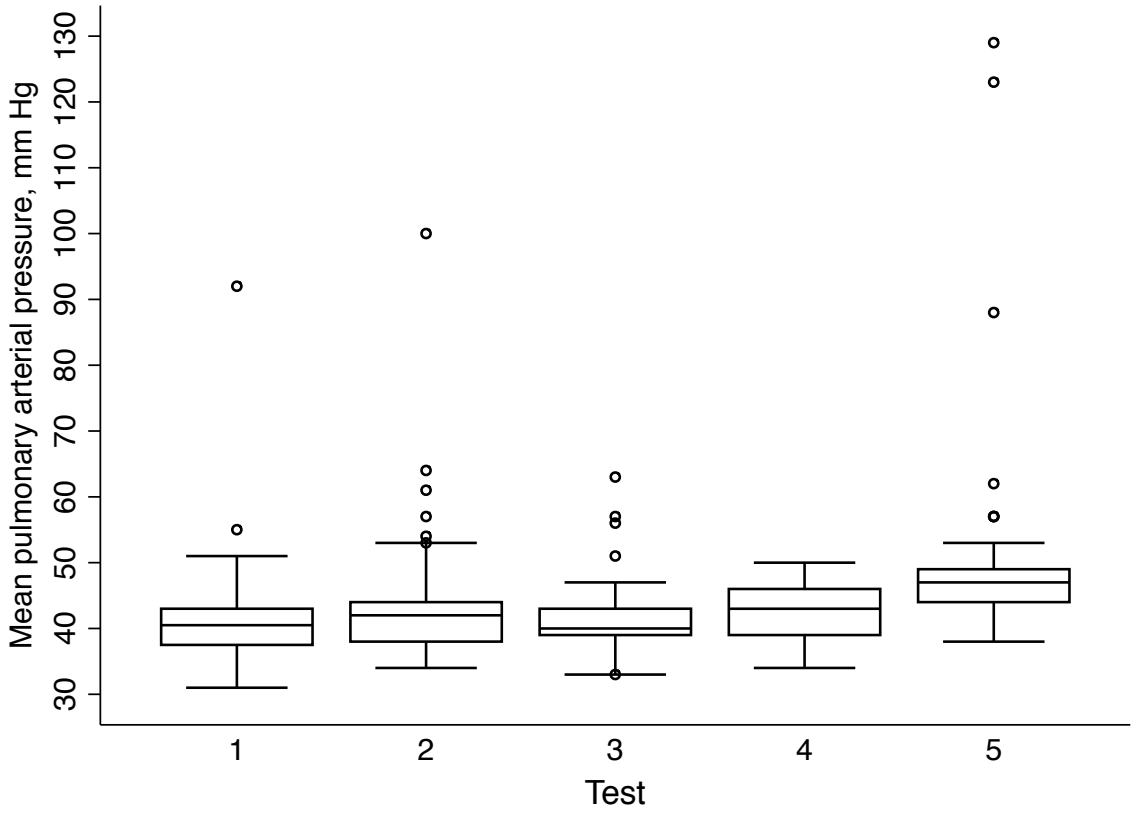

Figure 5.1: Box plot of mean pulmonary arterial pressure against test for all calves. Tests were performed at 2,170 m (tests 1 and 2), 1,560 m (test 3) and 1,300 m (tests 4 and 5) above sea-level. Hollow circles represent outliers (observations 1.5 times the interquartile range away from the mean)

Mean PAP was highest at test 5 (Table 5.2). The mPAP of bulls was 3.2 mm Hg higher than steers (95 % CI = 0.7, 5.7; p = 0.01). The significant increase in pulse pressure (systolic pressure minus diastolic pressure) from test 1 to test 5 indicates that cardiac stroke volume increased, pulmonary artery compliance decreased, or both (Table 5.2).

Table 5.2: Mean and 95 % confidence interval of the mean for pulmonary arterial pressures of calves by test

	Test				
Variable	1	2	3	4	5
Altitude, m	2,170	2,170	1,560	1,300	1,300
mPAP, mm Hg	38.5[a] (35.9, 41.1)	41.3[b, c] (39.6, 43.0)	39.9[a, c] (37.8, 42.1)	43.1[b, c] (40.2, 45.9)	49.4[d] (47.2, 51.6)
sPAP, mm Hg	55.6[a] (51.0, 60.1)	60.4[b] (55.8, 65.0)	65.9[c] (62.8, 69.1)	69.1[c] (64.8, 73.3)	81.0[d] (77.7, 84.2)
dPAP, mm Hg	23.9[a] (20.4, 27.3)	24.2[a] (20.7, 27.8)	18.7[b] (16.3, 21.1)	16.3[b] (13.1, 19.6)	19.1[b] (16.7, 21.6)
Pulse pressure, mm Hg	30.9[a] (22.4, 39.4)	36.0[b] (27.5, 44.5)	47.2[c] (44.2, 50.1)	53.4[d] (50.5, 56.3)	63.2[e] (61.0, 65.3)

Within a row, values without a common superscript differ $p < 0.05$

Calves with the highest mPAP at weaning (test 2) and early in the feeding period (test 3) tended to have the highest mPAP in the late confined feeding period (Figure 5.2). Pairwise correlation coefficients for mPAP were significant between tests 1 and 2, tests 3 and 5, tests 2 and 5 and tests 2 and 3 (Table 5.3). A single outlier with a mPAP of 123 mm Hg at test 5 reduced the pairwise correlation between tests 2 and 5 from 0.67 ($p < 0.001$) to 0.45 ($p < 0.001$) (Figure 5.2).

Table 5.3: Pairwise correlation coefficients, p-values and the number of pairwise comparisons for mean pulmonary arterial pressure

Test period	1	2	3	4	5
1	1.00				
2	0.87 (p < 0.001) n = 60	1.00			
3	0.22 (p = 0.38) n = 18	0.34 (p = 0.007) n = 60	1.00		
4	-0.47 (p = 0.20) n = 9	0.01 (p = 0.94) n = 30	n = 0	1.00	
5	0.29 (p = 0.26) n = 17	0.45 (p < 0.001) n = 56	0.84 (p < 0.001) n = 29	0.32 (p = 0.09) n = 29	1.00

Calves that had the highest mPAP at tests 2 and 3 tended to have the highest mPAP at test 5 (Figure 5.2).

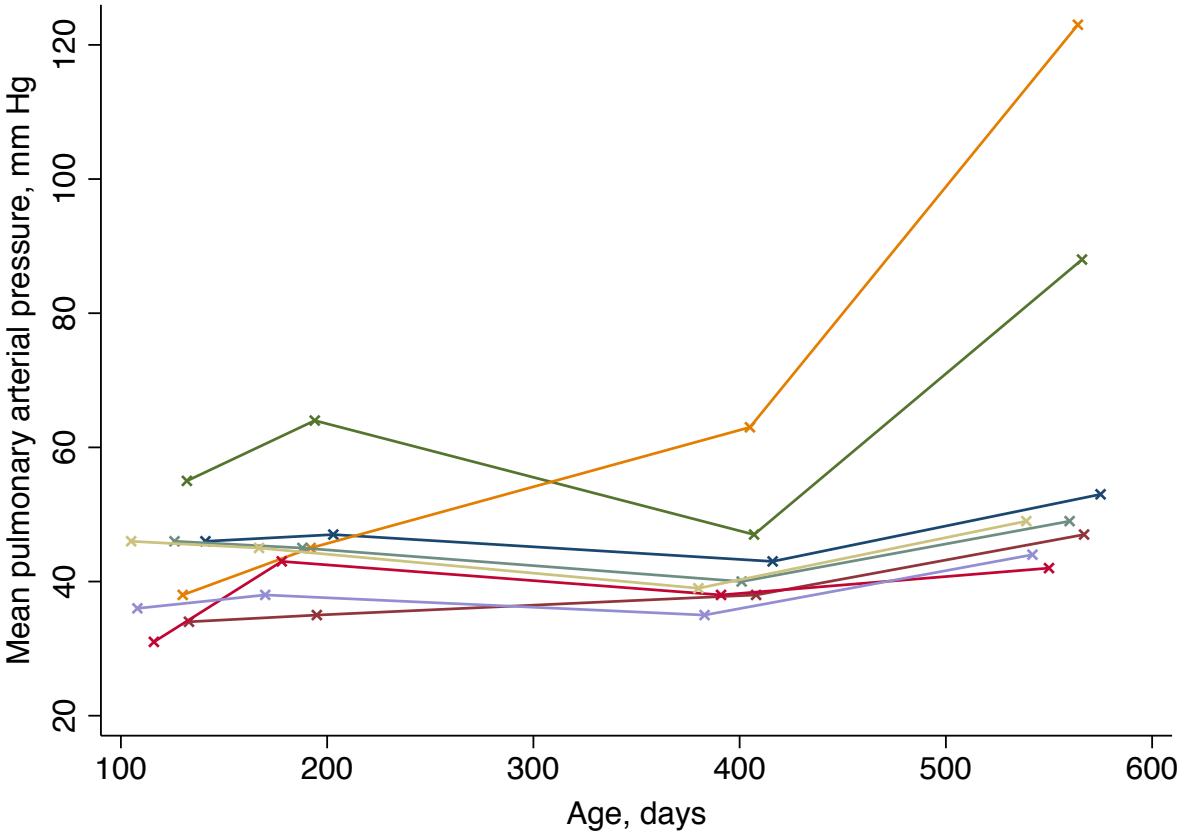

Figure 5.2: Line graph of mean pulmonary arterial pressure against age for all calves that were tested at tests 1, 2, 3 and 5. Calves that were not tested at all 4 of these time points are not shown. Each line represents one animal. Tests were performed at 2,170 m (tests 1 and 2), 1,560 m (test 3) and 1,300 m (test 5) above sea-level

Mean pulmonary arterial pressure at test 4 was not significantly correlated with mPAP at test 5 even though the tests were performed at the same altitude using the same facility (Figure 5.3).

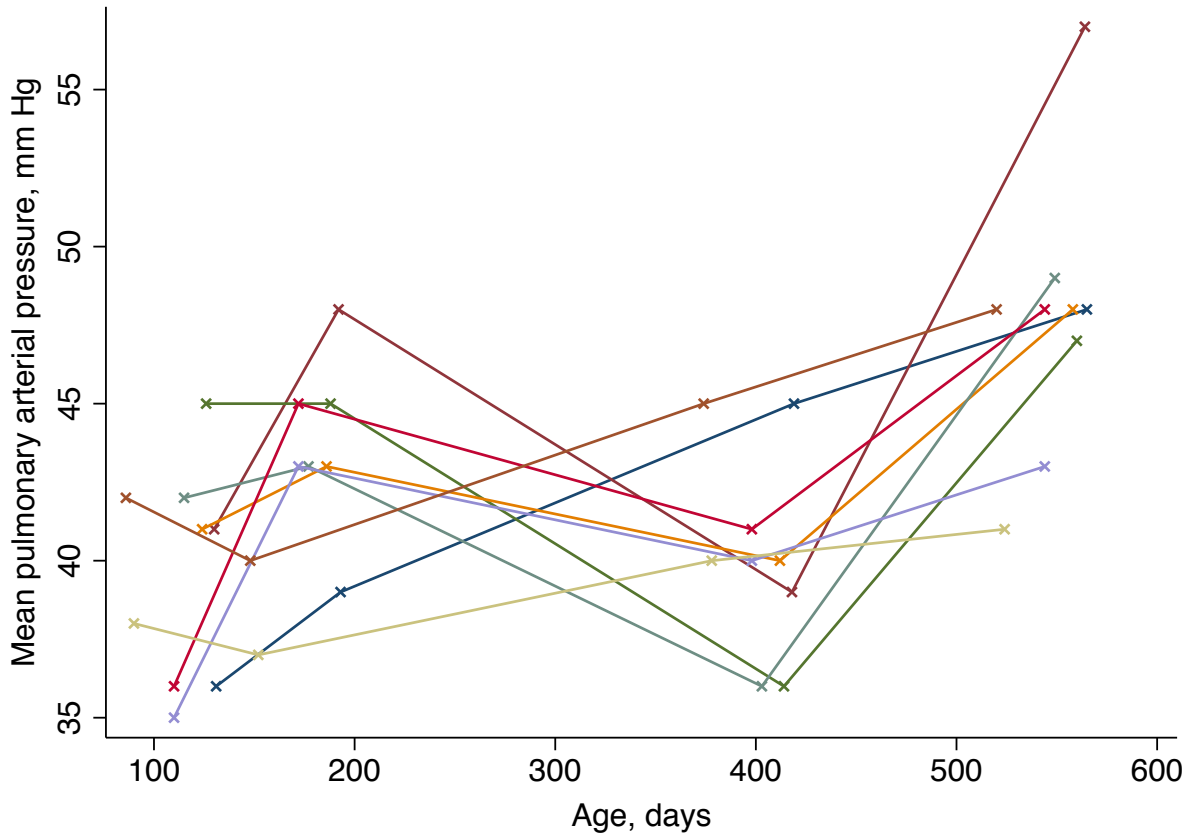

Figure 5.3: Line graph of mean pulmonary arterial pressure against age for all calves that were tested at tests 1, 2, 4 and 5. Calves that were not tested at all 4 of these time points are not shown. Each line represents one animal. Tests were performed at 2,170 m (tests 1 and 2) and 1,300 m (tests 4 and 5) above sea-level

Systolic pulmonary arterial pressure (sPAP) varied among tests ($p < 0.001$) but not between bulls and steers ($p = 0.36$). There was a progressive increase in sPAP from test 1 to test 5 (Figure 5.4, Table 5.2).

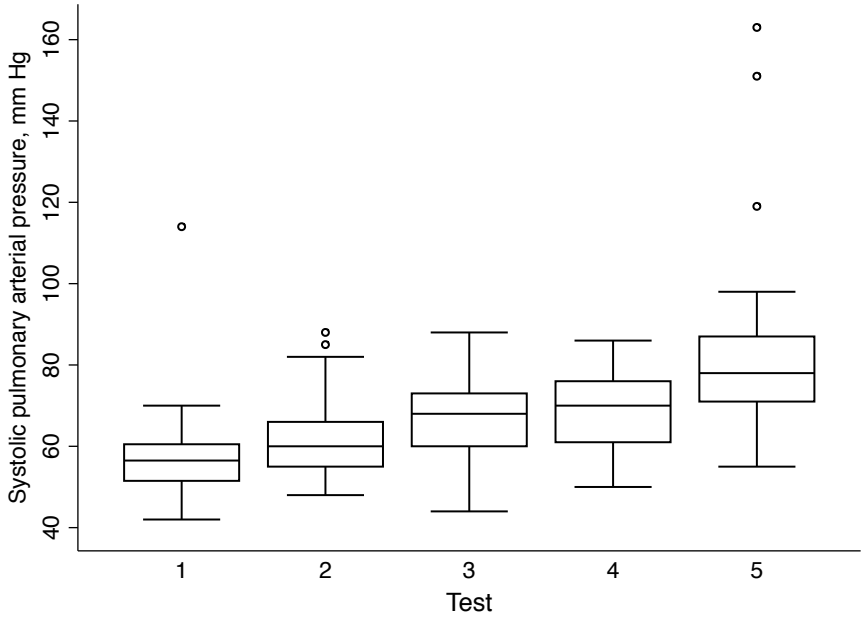

Figure 5.4: Box plot of systolic pulmonary arterial pressure against test for all calves. Tests were performed at 2,170 m (tests 1 and 2), 1,560 m (test 3) and 1,300 m (tests 4 and 5) above sea-level. Hollow circles represent outliers (observations 1.5 times the interquartile range away from the mean)

There were no significant pairwise correlations of sPAP between tests after Bonferroni correction (Table 5.4).

Table 5.4: Pairwise correlation coefficients, p-values and the number of pairwise comparisons for systolic pulmonary arterial pressure

Test period (Age, months)	1 (4 months)	2 (6 months)	3 (13 months)	4 (13 months)	5 (19 months)
1	1.00				
2	0.23 (p = 0.08) n = 60	1.00			
3	0.23 (p = 0.36) n = 18	0.20 (p = 0.43) n = 18	1.00		
4	-0.30 (p = 0.43) n = 9	-0.29 (p = 0.45) n = 9	n = 0	1.00	
5	0.24 (p = 0.34) n = 17	0.14 (p = 0.60) n = 17	0.46 (p = 0.01) n = 29	0.38 (p = 0.04) n = 29	1.00

In general, sPAP was not predictive of sPAP at a subsequent test (Figures 5.5 and 5.6).

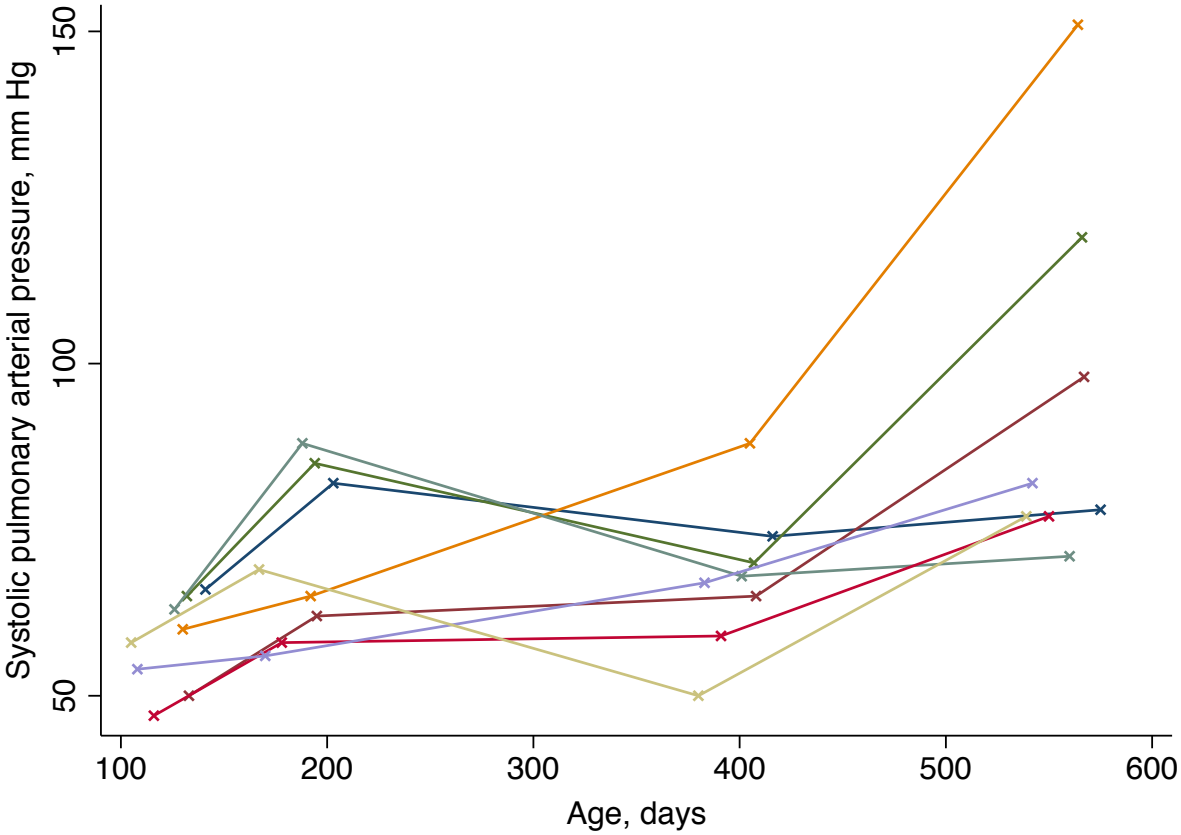

Figure 5.5: Line graph of systolic pulmonary arterial pressure against age for calves tested at tests 1, 2, 3 and 5. Calves that were not tested at all 4 of these time points are not shown. Each line represents a different animal. Tests were performed at 2,170 m (tests 1 and 2), 1,560 m (test 3) and 1,300 m (test 5) above sea-level

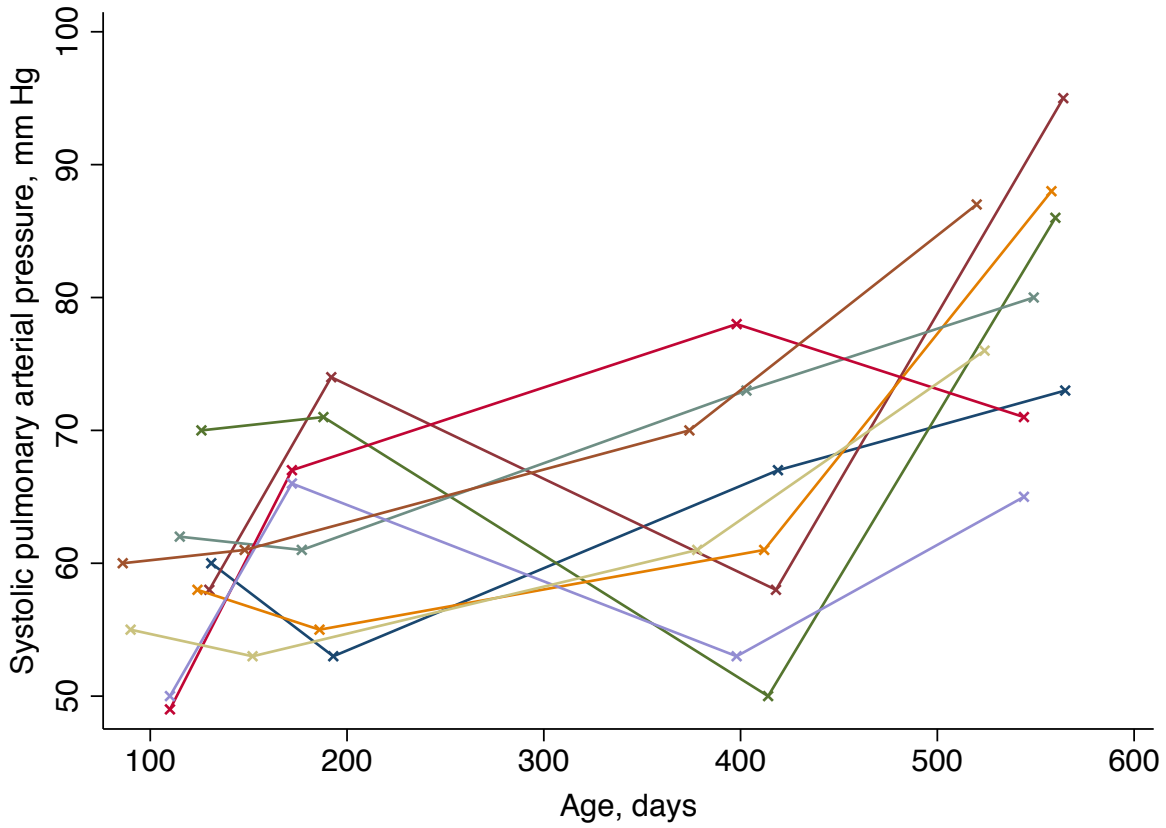

Figure 5.6: Line graph of systolic pulmonary arterial pressure against age for calves tested at tests 1, 2, 4 and 5. Calves that were not tested at all 4 of these time points are not shown. Each line represents a different animal. Tests were performed at 2,170 m (tests 1 and 2) and 1,300 m (tests 4 and 5) above sea-level

Diastolic pulmonary arterial pressure (dPAP) significantly varied among tests ($p < 0.001$) and tended to differ between bulls and steers ($p = 0.08$). Diastolic pressures were significantly lower during the confined feeding period (tests 3 to 5) than during the pre-weaning period (tests 1 and 2) ($p = 0.04$) (Figure 5.7). However, some individuals did not follow the overall pattern (Figures 5.8 and 5.9). Diastolic PAP did not differ between tests 1 and 2 ($p = 0.84$) or among tests 3 to 5 ($p = 0.14$). The dPAP of bulls tended to be 2.9 ± 1.6 mm Hg higher than steers ($p = 0.08$).

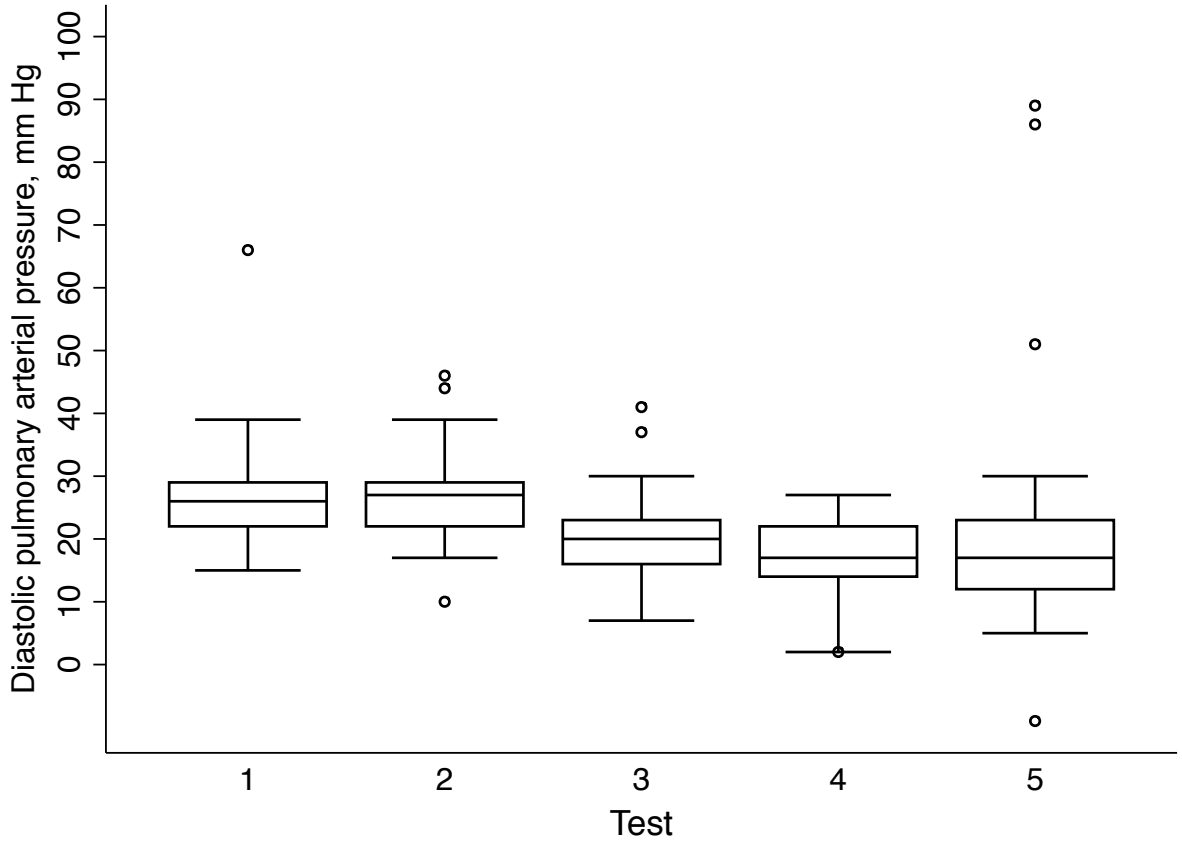

Figure 5.7: Box plot of diastolic pulmonary arterial pressure against test for all calves. Each line represents one animal. Tests were performed at 2,170 m (tests 1 and 2), 1,560 m (test 3) and 1,300 m (tests 4 and 5) above sea-level. Hollow circles represent outliers (observations 1.5 times the interquartile range away from the mean)

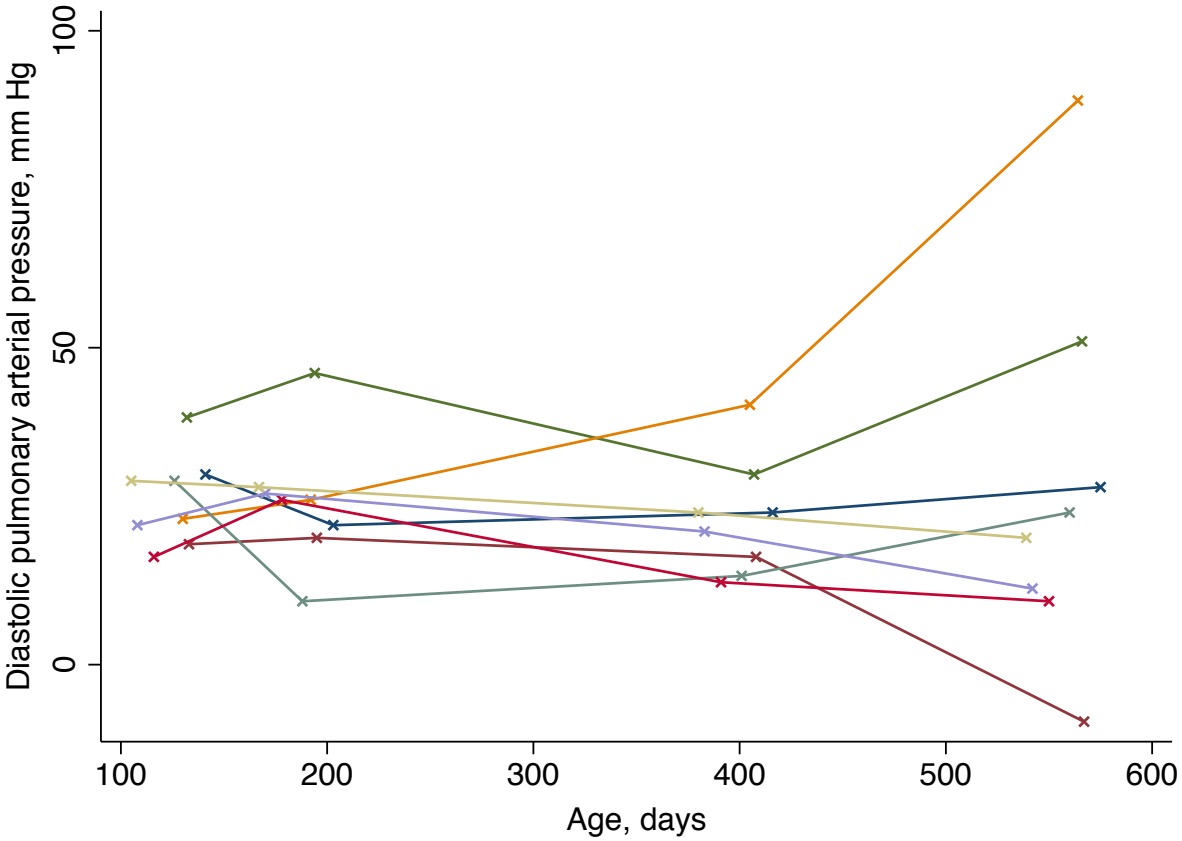

Figure 5.8: Line graph of diastolic pulmonary arterial pressure against age for calves tested at tests 1, 2, 3 and 5. Calves that were not tested at all 4 of these time points are not shown. Each line represents a different animal. Tests were performed at 2,170 m (tests 1 and 2), 1,560 m (test 3) and 1,300 m (test 5) above sea-level

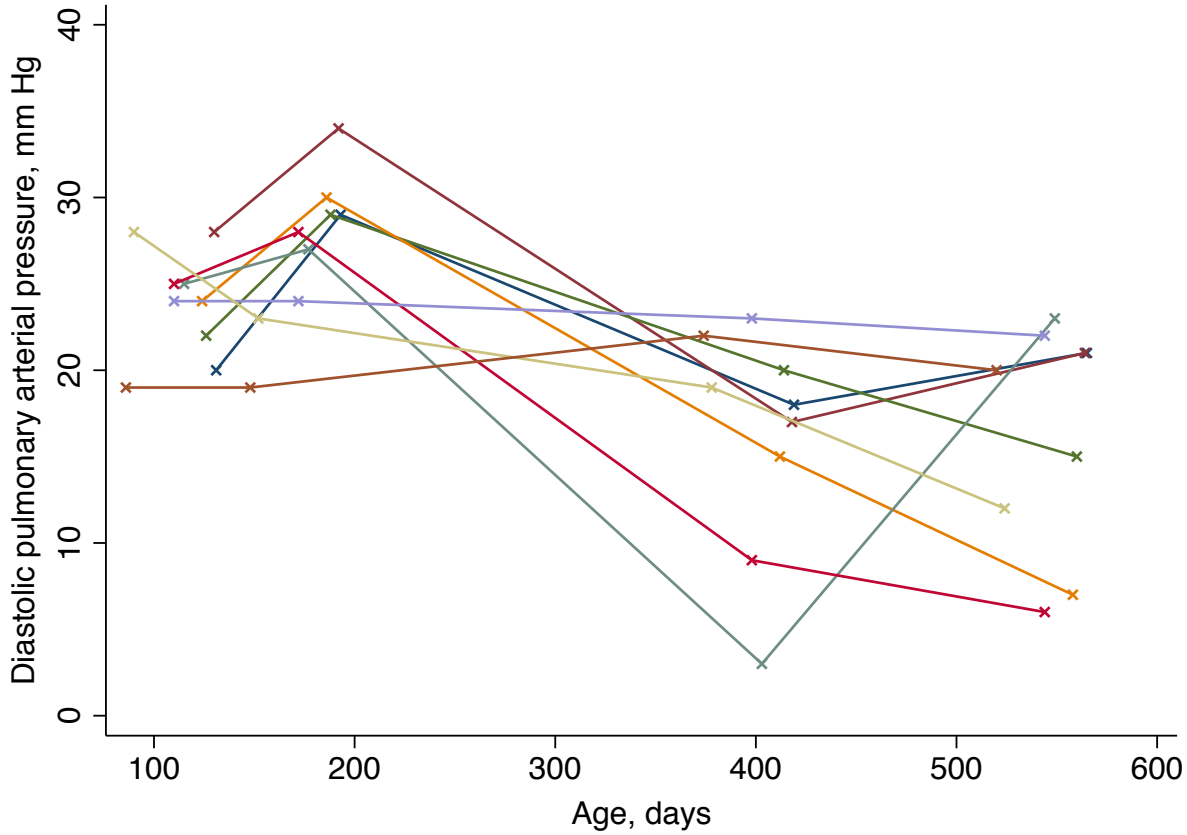

Figure 5.9: Line graph of diastolic pulmonary arterial pressure against age for calves tested at tests 1, 2, 4 and 5. Calves that were not tested at all 4 of these time points are not shown. Each line represents a different animal. Tests were performed at 2,170 m (tests 1 and 2) and 1,300 m (tests 4 and 5) above sea-level

Pairwise correlation coefficients for dPAP were significant between tests 1 and 2 and between tests 3 and 5 (Table 5.5).

Table 5.5: Pairwise correlation coefficients, p-values and the number of pairwise comparisons for diastolic pulmonary arterial pressure

Test period (Age, months)	1 (4 months)	2 (6 months)	3 (13 months)	4 (13 months)	5 (19 months)
1	1.00				
2	0.40 (p = 0.001) n = 60	1.00			
3	0.12 (p = 0.63) n = 18	0.30 (p = 0.23) n = 18	1.00		
4	-0.31 (p = 0.42) n = 9	-0.32 (p = 0.41) n = 9	n = 0	1.00	
5	0.34 (p = 0.17) n = 17	0.25 (p = 0.34) n = 17	0.64 (p < 0.001) n = 29	-0.07 (p = 0.71) n = 29	1.00

Mean right atrial pressure and mean pulmonary arterial wedge pressure in cattle at the end of the feeding period were high (Table 5.6). Right atrial pressure, also known as central venous pressure, may increase due to inadequacy of forward flow out of the right atrium and ventricle into the pulmonary artery or an increased rate of venous return. A pulmonary arteriolar wedge pressure is an indirect measure of left atrial pressure; it may increase in response to increased cardio-vascular volume and or inadequate left ventricular function (Vest and Heupler, 2013).

Table 5.6: Mean and 95 % confidence interval of the mean for right atrial pressure and pulmonary arterial wedge pressures in cattle at the end of the feeding period (test 5)

Pressure	Right atrial pressure (n = 53), mm Hg	Pulmonary arterial wedge pressure (n = 36), mm Hg
Mean	26.5 (24.7, 28.4)	25.9 (24.6, 27.2)
Systolic	80.8 (76.4, 85.3)	51.7 (48.1, 55.2)
Diastolic	3.0 (-1.5, 7.5)	4.0 (2.0, 6.1)

DISCUSSION

As hypothesized, mPAP increased during the feeding period and that the calves with the highest mPAP at the time of weaning tended to have highest mPAP at the end of the feeding period. Mean pulmonary arterial pressures were, on average, higher at the end of the feeding period at 1,300 m above sea-level than they were at 2,170 m above sea-level. The increase in mPAP through the feeding period likely explains why death from congestive heart failure typically occurs in the late feeding period (Chapter 3, (Jensen et al., 1976)).

A major limitation of this study is that only one cohort of cattle from one ranch was followed. The change in pulmonary arterial pressures with age may be different for cattle born and raised at an altitude lower than the feedlot to which they are moved. The post-natal development of pulmonary and bronchial arteries may differ among calves according to altitude (Reeves and Leathers, 1967). This may affect subsequent susceptibility to pulmonary hypertension and right-sided congestive heart failure later in life. It has been suggested that cattle born and raised at high-altitude are more susceptible to congestive heart failure in the feedlot than cattle entering the feedlot from lower altitudes (Jensen et al., 1976).

Interestingly, the calves with the highest mPAP at 6-months of age and 2,170 m above sea-level were most likely to have the highest mPAP 12-months later in the late feeding period at 1,300 m above sea-level. This indicates that the risk factors for right-sided congestive heart failure of calves at high-altitude, or 'high altitude disease' as it has been commonly known, and congestive heart failure of feedlot cattle may be shared. Cattle

are believed to be particularly susceptible to alveolar hypoxia-induced pulmonary hypertension because of their muscular small pulmonary arteries (Tucker et al., 1975). However, alveolar hypoxia alone does not adequately explain why congestive heart failure has been reported in cattle at moderate to low altitudes (Chapter 3, (Jensen et al., 1976; Malherbe et al., 2012). Alternatively, cattle that had high mPAP earlier in life at high-altitude may have pulmonary lesions that predisposed them to have a high mPAP late in the feeding period. Previous mountain grazing has been suggested as a risk factor for congestive heart failure in feedlot cattle (Jensen et al., 1976).

Systolic pulmonary arterial pressure increased with age, regardless of altitude. This is suggestive of pulmonary vascular remodeling. In humans, a small but significant increase of sPAP with age has been attributed to an increase in pulmonary vascular resistance, a decrease in left ventricular compliance with age and vascular stiffening (Lam et al., 2009; McQuillan et al., 2001). However, age alone is unlikely to explain the increase in sPAP reported in our study given that the oldest animals studied were approximately 18-months of age. In humans, obesity has been associated with an increase in cardiac output and decrease in left ventricular function (de Divitiis et al., 1981), which may explain the positive association between body mass index and sPAP (McQuillan et al., 2001). Humans are categorized as obese when body fat exceeds 32 % in women and 25 % in men. In mature feedlot cattle, body fat is approximately 36 % of empty body weight (live weight minus the weight of digesta) (Owens et al., 1995). Excess weight gain and a sedentary lifestyle may be risk factors for pulmonary hypertension that are common to both humans and feedlot cattle.

The mPAP, central venous pressure and pulmonary arteriolar wedge pressures obtained in our study are considerably higher than those obtained in previous studies of cattle (Amory et al., 1992; Doyle et al., 1960; Reeves et al., 1962) and other mammalian species such as domestic cats, dogs and horses. The mean central venous pressure in unsedated domestic cats, dogs and horses have been estimated to be between 3 and 7 mm Hg (Chow et al., 2006; Norton et al., 2011). Pulmonary wedge in healthy dogs and horses at rest are approximately 14 mm Hg (Chaliki et al., 2002; Gehlen et al., 2006). The results obtained from our study suggest that pulmonary venous hypertension due to left-ventricular insufficiency may be contributing to pulmonary arterial hypertension.

CHAPTER 6: SYSTEMIC OXYGEN EXTRACTION IS POSITIVELY ASSOCIATED WITH MEAN PULMONARY ARTERIAL PRESSURE AND THE ODDS OF CALF MORTALITY AT HIGH ALTITUDE

INTRODUCTION

Congestive heart failure secondary to bovine pulmonary hypertension (BPH) is more commonly known as 'high altitude disease' because it was first reported to occur at altitudes over 2,440 m (Glover and Newsom, 1915). The susceptibility of cattle (*Bos taurus*) to hypoxia-induced pulmonary hypertension has been attributed to their highly muscular small pulmonary arteries (Tucker et al., 1975). However, it has also been suggested that the small cardio-pulmonary system of domestic cattle relative to body size and oxygen requirements may be a risk factor for pulmonary hypertension (Veit and Farrell, 1978). It is reported that functional maturity of the bovine cardio-pulmonary system is not achieved until approximately 1 year of age (Lekeux et al., 1984). Therefore, if the bovine cardio-pulmonary system is under-sized relative to oxygen requirements it is likely to manifest in beef calves exposed to the hypoxic conditions of high altitude.

Adequacy of oxygen delivery can be determined from calculation of the systemic oxygen extraction fraction (sOEF); this requires measurement of arterial and mixed venous blood oxygen content. The sOEF is the proportion of oxygen within arterial blood that is utilized by the peripheral tissues before returning to the lungs for re-oxygenation. Cardiac output increases in response to increased oxygen consumption until an individual's cardiac reserve (maximum cardiac output minus cardiac output at rest) is reached. At this point,

sOEF must increase in order to maintain aerobic metabolism (McLellan and Walsh, 2004).

The goal of this study was to determine if sOEF is associated with mean pulmonary arterial pressure (mPAP) and increased odds of mortality in calves at high altitude. The objective of this study was to evaluate mPAP and arterial and mixed venous arterial blood-gas tensions of calves on two ranches at different altitudes. We hypothesized that sOEF would be positively associated with mPAP and increased odds of mortality.

MATERIALS AND METHODS

Study overview

Approximately 60 calves in 2 herds at altitudes over 2,100 m were sampled twice between 3 and 7 months of age. On each occasion, mPAP and arterial and mixed venous arterial blood-gas tensions were measured. Pulmonary arterial pressure and blood-gas variables were evaluated for differences between tests. Oxygen tension, CO_2 tension, sOEF and L-lactate were evaluated for association with the outcome variable mPAP. The odds of calf mortality between tests 1 and 2 were determined in association with mPAP and sOEF at test 1. The CSU Animal Care and Use Committee approved the study protocol before initiation of the investigation.

Study herd

A cohort of 60 calves was randomly selected from all male calves in Herd A and a cohort of 58 calves randomly selected from calves of both genders in Herd B (Table 6.1). Every second calf into the chute was sampled. The calves in Herd A consisted of bulls and

steers. Calves in Herd B consisted of heifers and steers. The cohorts were studied on 2 occasions approximately 2 months (Herd A) and 4 months (Herd B) apart. Approximately half of calves in Herd A were sired by bulls with a mPAP < 40 mm Hg at an altitude of 2,166 m. The remaining calves were progeny of sires that had an unknown mPAP. Calves in Herd B were sired by bulls with mPAP < 42 mm Hg at an altitude of 2,440 m. Between tests 1 and 2 calves in Herd A and in Herd B had access to land ranging in elevation from 2,160 m to 2,900 m above sea-level and from 2,731 m to 3,500 m above sea level, respectively. All bull calves in Herd A had their PAP measured during the second testing period for breeding management purposes unrelated to this study. Of these bull calves that were not part of the original cohort 5 had high mPAP (53 to 64 mm Hg) and were included in the dataset for evaluation of the relationship between sOEF and mPAP only.

Table 6.1: Date, age, altitude, breed and number of calves according to herd and test

Herd	Test	Number sampled				Date	Altitude,	Mean age	Breed
		H[1]	B[2]	S[3]	T[4]	sampled	m	± SD, days	
A	1	0	55	5	60	07/31/12	2,166	124 ± 18	Black and Red Angus
	2	0	60	5	65	10/01/12		186 ± 18	
B	1	30	0	28	58	06/21/12	2,731	86 ± 7	Red Angus, Hereford,
	2	27	0	24	51	10/10/12		197 ± 6	Gelbvieh and Simmental composites

[1] H – The number of heifer calves sampled
[2] B – The number of bull calves sampled
[3] S – The number of steer calves sampled
[4] T- The total 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]), *Bovine viral diarrhea virus* (BVDV), *Bovine respiratory syncytial virus* (BRSV), and *Bovine parainfluenza virus 3* (BPIV-3). Calves were vaccinated against the same respiratory

pathogens at 4 to 8 weeks of age and 2 to 4 weeks prior to weaning. Both herds have tested negative for BVDV by ELISA. Mineral supplements were provided year round. A hormonal growth promotant (Synovex C, Zoetis, Madison, NJ) containing 100 mg progesterone and 10 mg estradiol benzoate was administered to both heifer and steer calves in Herd B 1 month prior to test 1 when calves were approximately 8 weeks old.

Pulmonary arterial pressure measurement

A full description of the equipment, materials and facilities required for pulmonary arterial pressure testing is provided by Holt and Callan (2007). In brief, a large bore needle was inserted into the jugular vein. Flexible saline-filled catheter tubing was then fed through the needle, down through the right atrium, into the right ventricle, and then into the pulmonary artery. A pressure transducer connected the catheter to an oscilloscope. The position of the catheter was determined from the pressure waveform on the oscilloscope. The jugular vein, right atrium, right ventricle and pulmonary artery have distinct pressure waveforms (Holt and Callan, 2007). Mean, systolic and diastolic pressures were recorded after the pulmonary pressure waveforms had stabilized.

Blood-gas analysis

Approximately, 2.5 ml of 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). Mixed venous blood was collected from the pulmonary artery via the catheter used for pulmonary arterial pressure measurement. 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 interrupted. Air bubbles within the blood were immediately expelled and the first several drops of blood discarded before analysis. A temperature 'correction' algorithm was used to adjust blood-gas tensions according to rectal temperature (CLSI, 2001).

Blood-gas analysis was performed using a handheld analyzer (VetScan i-STAT 1, Abaxis, Union City, CA, USA). Hematocrit, pH, pCO_2 and pO_2 were determined from arterial blood. L-Lactate, pH, pCO_2 and pO_2 were determined from mixed venous blood. Oxyhemoglobin saturation of arterial and mixed venous blood was calculated from measured pO_2 and pH and from HCO_3 calculated from measured pCO_2 and pH. The calculation method is available from the manufacturer (Abbott, 2011) or from the corresponding author on request. A limitation of this calculation is that it assumes normal affinity of hemoglobin for oxygen. It does not account for erythrocyte 2,3-diphosphoglycerate concentrations. However, for comparative purposes and therefore, the purpose of this study, the calculation is valid.

Systemic Oxygen Extraction Fraction

The systemic oxygen extraction fraction (sOEF) is the systemic oxygen consumption (VO_2) expressed as a fraction of systemic oxygen delivery (DO_2). Normal systemic OEF is approximately 0.2 (Dellinger, 2002; Leach and Treacher, 2002; McLellan and Walsh, 2004). This means that on average, approximately 4 times more oxygen is delivered to the microcirculation than is consumed. However, this varies by tissue type: at rest the mean

OEF of cardiac and skeletal muscle have been estimated to be 68 % and 25 %, respectively (Binak et al., 1967). As demand for oxygen increases (VO_2) or delivery of oxygen decreases (DO_2) the sOEF must increase in order to maintain aerobic metabolism. Inadequate cardiac output, a reduction in DO_2, results in sOEF to exceed 0.3, or 30 % (Olkowski et al., 2005; Rady et al., 1994). Systemic OEF is calculated using the following formula:

$$sOEF = \frac{((s_aHbO_2 \times Hb \times 1.39) + (0.003 \times p_aO_2)) - ((s_{mv}HbO_2 \times Hb \times 1.39) + (0.003 \times p_{mv}O_2))}{((s_aHbO_2 \times Hb \times 1.39) + (0.003 \times p_aO_2))}$$

Where s_aHbO_2 and s_aHbO_2 are arterial and mixed venous oxyhemoglobin saturation (%), respectively; Hb is hemoglobin concentration (g/L); and, p_aO_2 and $p_{mv}O_2$ are arterial and mixed venous oxygen tensions (mm Hg).

Calf mortality

Calves were considered dead if their dams were accounted for at test 2 but the calves were not. If both calves and their dams were unaccounted they were considered missing. It is plausible that a calf unaccounted at test 2 was missing even if the calf's dam was accounted. However, mothering ability is a trait of high importance in the selection of range cattle. Therefore, calf abandonment as an explanation for unaccounted calves was unlikely.

Statistical analyses

Statistical analyses were performed using STATA version 12 (Stata Corporation, College Station, Texas, USA). Summary statistics are provided as mean ± standard deviation (SD). Regression model statistics are provided as mean ± standard error (SE). Generalized estimating regression equations were used to account for the repeated measures (Liang and Zeger, 1986; Zeger and Liang, 1986). An exchangeable correlation structure was used. Generalized estimating regression equations are robust to missing observations.

Statistically significant differences between tests in the blood-gas variables reported in table 6.2 were evaluated using generalized estimating equations while controlling for gender. Differences between herds were not evaluated for several reasons including age differences at tests 1 and 2 and the potential for genetic confounding. A Bonferroni correction was applied in order to achieve family-wise type I error risk of 0.05.

The following explanatory variables were evaluated for association with the outcome variable mPAP: pO_2 and pCO_2, L-lactate and sOEF. For the first 2 variables (pO_2 and pCO_2) both arterial and mixed venous forms were evaluated. All variables were evaluated individually for association with the dependent variable mPAP when controlling for ranch, age and gender. Variables achieving a statistical significance of < 0.20 were included in the full regression model. Backwards step-wise regression was then performed until all variables in the final model had a statistical significance ≤ 0.05 when controlling for the covariates herd, age and gender. The odds of calf mortality in association with mPAP and sOEF were determined by 2 separate logistic regression models while controlling for herd as a fixed effect.

RESULTS

Descriptive observations

Systemic OEF did not differ between tests in either cohort (Table 6.2).

Table 6.2: Mean and standard deviation of pulmonary pressures, systemic oxygen extraction fraction, hematocrit and arterial and mixed venous blood-gas variables according to herd and test period

Variable	Herd A				Herd B			
	n	Test 1	n	Test 2	n	Test 1	n	Test 2[5]
sOEF,[1, 2] %	59	23.5 ± 5.0	55	22.4 ± 5.9	55	25.8 ± 9.2	49	23.6 ± 6.7
Mean PAP,[3] mm Hg	60	41.4 ± 7.9	60	43.9 ± 9.2**	57	40.5 ± 6.5	51	47.5 ± 8.4**
Systolic PAP,[3] mm Hg	60	57.3 ± 9.6	60	62.0 ± 9.1**	57	61.6 ± 8.5	51	63.5 ± 10.0
Diastolic PAP,[3] mm Hg	60	26.5 ± 6.7	60	26.6 ± 6.3	57	22.3 ± 7.1	51	30.1 ± 8.9**
L-lactate, mmol/L	59	1.8 ± 1.2	58	1.05 ± 0.9**	54	2.9 ± 2.0	50	2.6 ± 2.5
$sHbO_2$,[1, 4] %	60	90.2 ± 7.1	60	87.3 ± 5.0	55	88.8 ± 6.3	51	88.6 ± 4.4
pH_a	60	7.47 ± 0.04	60	7.49 ± 0.03**	55	7.42 ± 0.05	51	7.50 ± 0.06**
pH_{mv}	60	7.40 ± 0.03	59	7.44 ± 0.03**	53	7.35 ± 0.05	50	7.43 ± 0.07**
p_aO_2, mm Hg	60	57.2 ± 8.0	60	50.4 ± 7.1**	55	56.3 ± 9.0	51	51.8 ± 7.6
$p_{mv}O_2$, mm Hg	60	36.8 ± 2.7	59	33.9 ± 5.4**	54	36.3 ± 4.5	49	34.9 ± 3.8
p_aCO_2, mm Hg	60	35.2 ± 4.4	60	36.3 ± 2.9	55	35.5 ± 3.6	51	34.0 ± 3.3
$p_{mv}CO_2$, mm Hg	60	43.7 ± 3.9	58	40.3 ± 3.8**	53	40.1 ± 5.2	50	40.7 ± 4.1
Hematocrit, %	60	29.8 ± 3.7	60	30.9 ± 2.7	55	34.7 ± 3.5	51	31.9 ± 3.0**

** Statistically significant difference between tests after Bonferroni correction ($p \leq 0.0019$)
[1] Calculated value
[2] Systemic oxygen extraction fraction
[3] Pulmonary arterial pressure
[4] Arterial oxyhemoglobin saturation
[5] 7 calves died between tests 1 and 2 in Herd B

Seven percent (4 of 59 calves) and 11 % (6 of 55 calves) of calves in Herd A had a sOEF > 30 % at tests 1 and 2, respectively. Twenty-five percent (15 of 55 calves) and 14 % (7 of 49) of calves in Herd B had a sOEF > 30 % at tests 1 and 2, respectively (Figure 6.1).

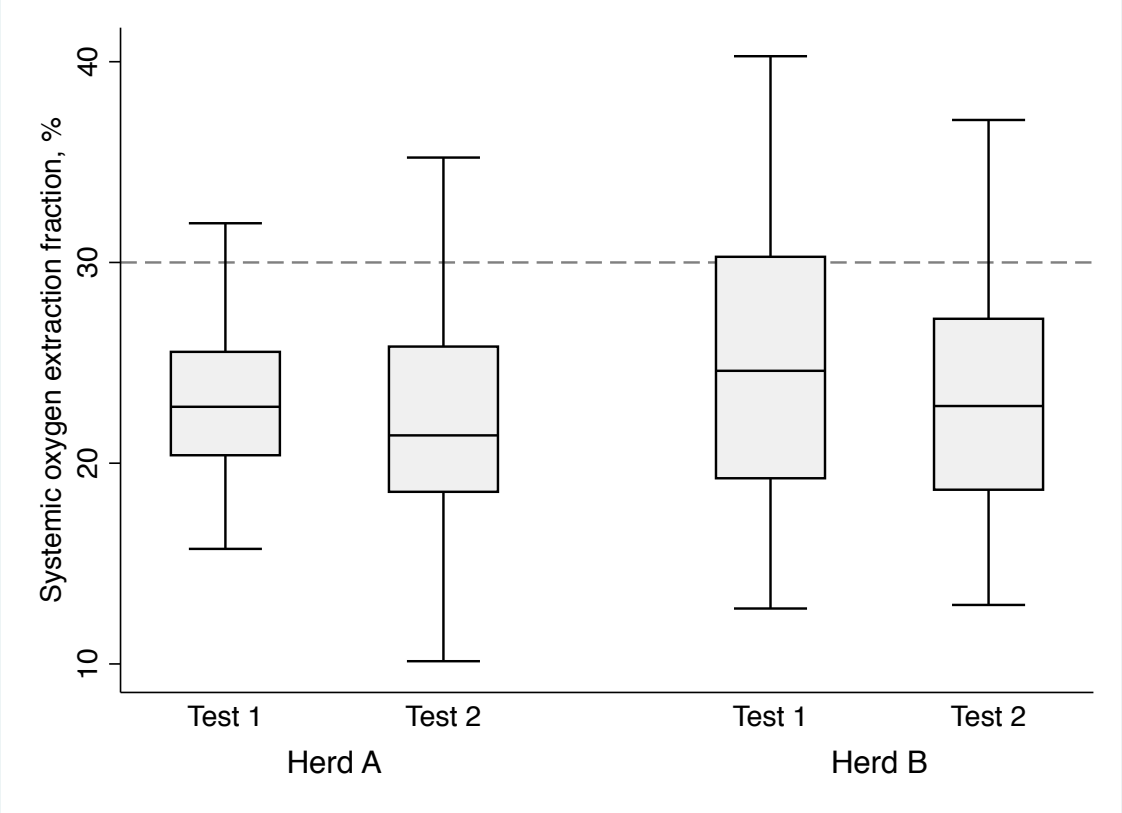

Figure 6.1: Box plot of systemic oxygen extraction fraction according to test and herd. The dashed horizontal line at 30 % indicates the upper extent of systemic oxygen extraction fraction of healthy mammalian subjects at rest

Mean pulmonary arterial pressure increased by 2.5 mm Hg from test 1 to test 2 in Herd A (95 % CI = 1.3, 3.7; $p < 0.001$) (Figure 6.2) and did not differ between bulls steers ($p = 0.18$). Mean pulmonary arterial pressure at test 1 did not differ between heifers and steers in Herd B ($p = 0.20$). The mPAP of heifers increased by 5.7 mm Hg (95 % CI = 2.8, 8.6; $p < 0.001$) and the mPAP of steers by 11.8 mm Hg, from test 1 to test 2 (95 % CI = 7.7,

15.9; p < 0.001). The increase in mPAP between test 1 and 2 was significantly greater in steers than heifers (p = 0.002).

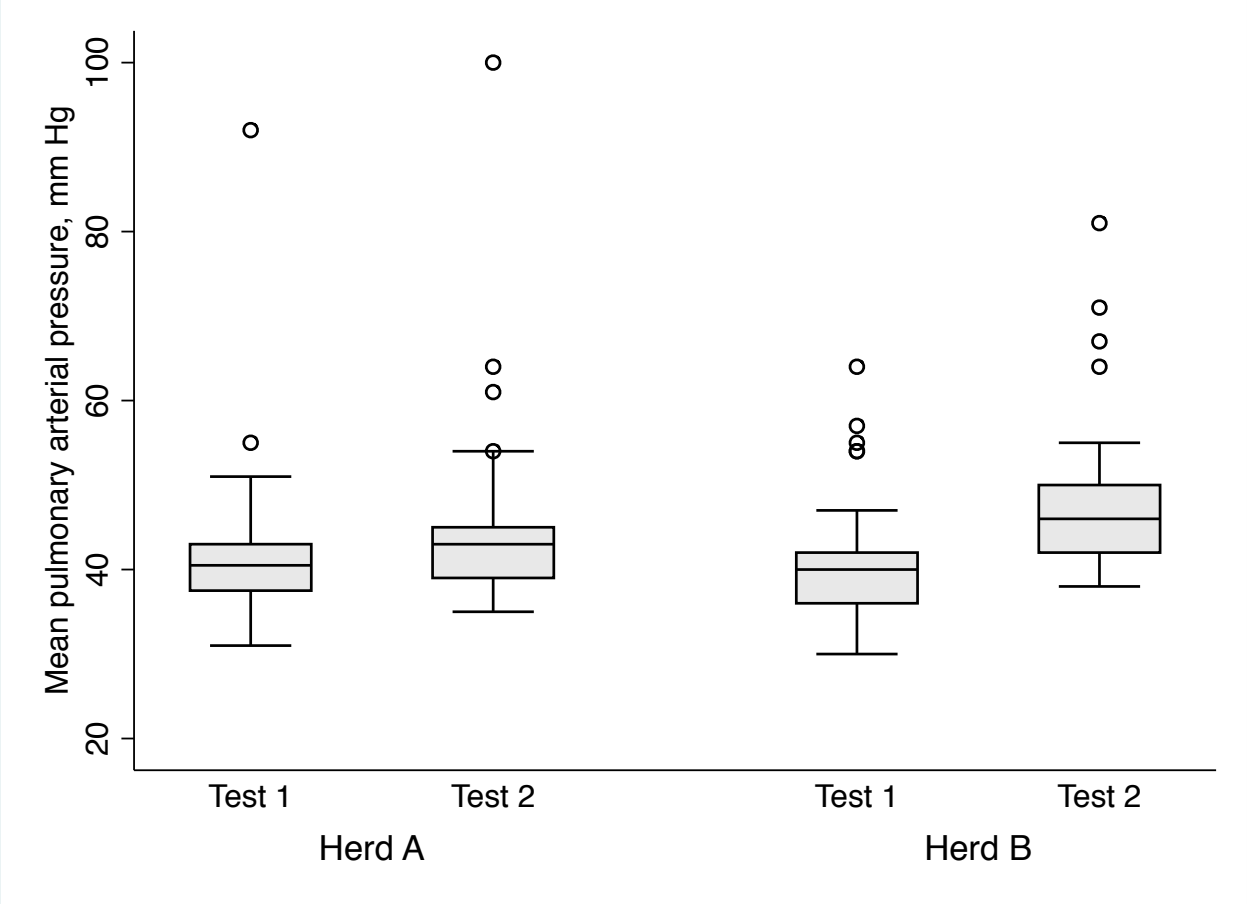

Figure 6.2: Box plot of mean pulmonary arterial pressures in calves according to test and herd. Outliers, represented by hollow circles, are individual observations that are 1.5 times the interquartile range away from the median

Systolic PAP in Herd A was 4.8 mm Hg higher at test 2 than test 1 (95 % CI = 1.7, 7.9; p = 0.026) but did not differ between tests in Herd B. Diastolic PAP was 8.0 mm Hg higher at test 2 than test 1 in Herd B (95 % CI = 5.6, 10.4; p < 0.001). Diastolic pulmonary arterial pressure did not differ between tests in Herd A.

Analytes associated with mPAP

The variables sOEF ($p < 0.001$), $p_{mv}CO_2$ ($p = 0.001$) and p_aCO_2 ($p = 0.047$) were positively associated with mPAP (Table 6.3). L-lactate was negatively associated with mPAP ($p = 0.053$). Pairwise correlations of p_aCO_2 with $p_{mv}CO_2$ ($r = 0.30$, $p < 0.001$), sOEF with $p_{mv}CO_2$ ($r = 0.18$, $p = 0.008$), sOEF with p_aCO_2 ($r = -0.29$, $p < 0.001$) and sOEF with L-lactate ($r = 0.21$, $p = 0.001$) were statistically significant but weak.

Bulls had significantly higher mPAP values than heifers ($p = 0.04$) but did not have significantly higher mPAP values than steers ($p = 0.11$). Calves in Herd B had significantly higher mPAP values than calves in Herd A when controlling for the other explanatory variables ($p = 0.01$).

Table 6.3: Blood-gas analytes that were associated with mean pulmonary arterial pressure

Variable	Coefficient, mm Hg	95 % CI	p-value
p_aCO_2,[1] mm Hg	0.26	0.00, 0.52	0.047
$p_{mv}CO_2$,[2] mm Hg	0.31	0.12, 0.50	0.001
sOEF,[3] %	0.22	0.10, 0.34	< 0.001
L-lactate, mmol/L	- 0.55	- 1.12, 0.01	0.053
Herd B versus A	9.3	1.9, 16.7	0.01
Steers versus heifers	3.0	-0.7, 6.8	0.11
Bulls versus heifers	8.5	0.6, 16.5	0.04
Age, days	0.07	0.06, 0.08	< 0.001
Intercept	-2.3	-16.1, 11.5	0.75

[1] Arterial CO_2 tension
[2] Mixed venous CO_2 tension
[3] Systemic oxygen extraction fraction

Odds of mortality

The risk of mortality between tests 1 and 2 was 0 % in Herd A and 12 % (7 of 58 calves) in Herd B. Pulmonary arterial pressures and mixed venous samples were obtained from 6 of the 7 calves. In one calf the catheter could not be advanced into the right atrium so neither a pulmonary arterial pressure measurement nor a mixed venous blood sample was obtained. High pressure in the right atrium as a result of pulmonary hypertension was the most likely reason for this.

For every 1 mm Hg increase in mPAP, the odds of mortality increased by 1.19 (95 % CI = 1.03, 1.35; p = 0.007) when controlling for herd. For every 1 unit increase in sOEF, the odds of mortality increased by 1.10 (95 % CI = 1.00, 1.20; p = 0.05) when controlling for herd. Calves that died between tests 1 and 2 had a significantly higher mPAP (p < 0.001), systolic PAP (p = 0.018) and sOEF (p = 0.048) than calves that survived. Mixed venous pCO_2 tended to be higher in calves that died (Table 6.4).

Table 6.4: Comparison of test 1 analytes of calves in Herd B according to survival status to test 2

Variable	Survived		Died		p-value
	n	Mean ± SD	n	Mean ± SD	
mPAP, mm Hg	51	39.5 ± 5.1	6	48.8 ± 11.1	< 0.001
sPAP, mm Hg	51	60.5 ± 7.3	6	71.0 ± 12.2	0.018
dPAP, mm Hg	51	21.9 ± 6.0	6	25.2 ± 13.9	> 0.99
sOEF, %	49	24.6 ± 6.9	6	35.0 ± 18.6	0.048
p_aCO_2, mm Hg	48	35.2 ± 3.5	7	38.0 ± 3.6	0.29
$p_{mv}CO_2$, mm Hg	48	39.5 ± 4.7	6	45.5 ± 7.3	0.084

DISCUSSION

Our results support the theory that inadequate cardiac reserve may be a risk factor for bovine pulmonary hypertension (BPH); systemic oxygen extraction fraction was positively associated with mPAP and the odds of calf mortality. Mean sOEF values were above 20 % in both herds and at both test and there was considerable variation about the mean values; in Herd B 25 % of the calves had a sOEF greater than 30 % at test 1. This indicates that a substantial proportion of calves had high metabolic oxygen requirements relative to oxygen delivery.

A limitation of our study is that cattle in both study herds had been selected for adaptation to high altitude hypoxia by breeding for low mPAP. The results may be different for herds that have recently been introduced to high altitude that have not been as extensively selected for low mPAP. If mPAP and sOEF are genetically correlated traits selection for lower mPAP would also reduce sOEF. This remains to be determined. Another limitation is that the values reported in this study may be influenced by factors other than altitude such as genetics, nutrition and management factors. This is a limitation of many field-based studies. However, a strength of our study is that calves were studied on commercial operations under natural-settings. Therefore, although the ability to make inferences to other cattle populations are limited we can be confident that the physiological values reported are valid for the herds studied.

A further limitation of this study was that sOEF is an indicator of low-output failure; cardiac output is insufficient to meet metabolic oxygen demand. However, pulmonary hypertension of cattle is believed to predispose cattle to right-sided congestive heart

failure. Further studies are necessary to evaluate the roles of the left and right ventricles in the development of heart failure secondary to pulmonary hypertension in cattle.

The first study of congestive heart failure in cattle concluded that the disease only occurred at altitudes over 2,440 m because some cattle do not have sufficient cardiac reserve to meet the demands of high altitude leading to "…exhaustion of the heart" (Glover and Newsom, 1915). The results of our study suggest that Glover and Newsom may have been closer to the truth than previously appreciated. Historically, BPH has been largely considered a disease of the small pulmonary arteries caused by a maladaptive vascular remodeling response to alveolar hypoxia (Alexander and Jensen, 1963b; Tucker et al., 1975). Cattle and pigs are thought to be particularly susceptible to pulmonary hypertension because they have muscular pulmonary arteries (Tucker et al., 1975). Broiler chickens also have muscular pulmonary arteries (Sillau and Montalvo, 1982) and are highly susceptible to pulmonary hypertension but their susceptibility has been attributed to inadequate cardiac output to support the higher metabolic oxygen demand associated with rapid growth (Olkowski et al., 2005). An under-sized cardio-pulmonary system relative to oxygen requirements has been suggested as a cause of BPH (Veit and Farrell, 1978). The goal of this study was to determine if a high oxygen requirement relative to oxygen delivery, determined through calculation of sOEF, is associated with mean pulmonary arterial pressure (mPAP) and increased odds of mortality.

Cattle are not the only highly selected species. Pigs (Chen et al., 2002) and poultry (Havenstein et al., 2003) have also been highly selected for meat production and, perhaps by no coincidence, are also highly susceptible to hypoxia-induced pulmonary hypertension (Tucker et al., 1975; Wideman et al., 2013). Systemic OEF is reported to be over 30 % in

pigs (Eriksson et al., 1996; Plochl et al., 1999; Wang et al., 2005) and fast-growing broiler chickens (Olkowski et al., 2005). However, in more athletic species such as horses and dogs reports of sOEF are closer to 20 % (Cambier et al., 2008; Haskins et al., 2005; Kitagawa et al., 1995). Genetic selection for production performance in livestock may have increased metabolic oxygen demand relative to cardio-pulmonary capacity. The result is inadequate oxygen delivery, which may be accentuated by the increased demand placed on the cardio-pulmonary system at high altitude.

Increased growth rate is genetically (Levalley, 1978; Shirley et al., 2008) and phenotypically (Jensen et al., 1976) correlated with increased mPAP in cattle. Similarly, growth rate is correlated with mPAP in broiler chickens (Peacock et al., 1989). We have also found that growth rate from birth to weaning is significantly associated with sOEF in calves (Chapter 8). Fast-growing chickens (sOEF ≈ 0.4) and chickens with congestive heart failure (sOEF ≈ 0.56) had a significantly higher sOEF than slower-growing chickens (sOEF ≈ 0.16) (Olkowski et al., 2005).

Relative hypoventilation, as indicated by increasing arterial pCO_2, was positively associated with mPAP in our study. Hypoventilation has been previously reported as a risk factor for BPH in feedlot cattle (Jensen et al., 1976). Cows 'resistant' to BPH are reported to show a greater increase in effective ventilation when exposed to acute hypoxia than BPH 'susceptible' cows (Moore et al., 1979). Alveolar ventilation was found to be less effective in steers than humans at an altitude of 1,500 m (Grover and Reeves, 1962) and only transiently increased in association with an increase in altitude (Grover et al., 1963; Grover and Reeves, 1962). Even when administered pure oxygen arterial oxygen tension in cattle was substantially less than that of other mammalian species (Kainer and Will, 1981).

Therefore, cattle may be more at risk of alveolar hypoxia than other mammalian species for any given altitude.

A reduction in effective ventilation under hypoxic conditions increases the risk of myocardial depression, a reduction in myocardial function. A drop in the rate of systemic oxygen delivery is most deleterious to organs with a high oxygen extraction fraction, such as the myocardium (Binak et al., 1967), resulting in impaired function. Myocardial function, determined by stroke index, was transiently reduced in Holstein calves at an altitude of 3,400 m, but recovered back towards normal after administration of 100 % O_2 (Will, 1975). Myocardial depression was also demonstrated in Holstein calves at 2 and 4 weeks of exposure to a simulated altitude of 3,400 m (Ruiz et al., 1973).

In addition to arterial pCO_2, mixed venous pCO_2 was also positively associated with mPAP. Broiler chickens that are predisposed to develop pulmonary hypertension are also reported to have higher arterial and venous CO_2 content than chickens that are not predisposed to develop pulmonary hypertension (Olkowski et al., 1999). Carbon dioxide may have pulmonary pressor effects independent of oxygen tension (Rothe et al., 1985).

Interestingly, heifers had significantly lower mPAP than bull calves. This may be related to the cardio-protective action of the female gender hormone estrogen in pulmonary hypertension (Tofovic, 2010). Calves (heifers and steers) in Herd B were given a hormonal growth implant containing estradiol prior to test 1. Therefore, the true gender differences in mPAP may be greater than we report. A steroidal growth hormone implant containing trenbolone acetate and estradiol significantly reduced mPAP in feedlot cattle (Chapter 9). This finding is not unique to cattle. Male broiler chickens raised at an altitude

of 3,300 m until 4 weeks old had significantly greater muscular hypertrophy of the pulmonary arteries than females (Sillau and Montalvo, 1982).

Calves that died in the interval between tests had significantly higher mPAP and sOEF than calves that survived. A diagnostic investigation of calf mortality recently conducted on this ranch attributed mortality to BPH and pneumonia (Neary et al., 2013b). The mortality risk in our study (12 %, Herd B) is similar to the risk of mortality of 10 % found in the previous study (Neary et al., 2013b). Combined our findings suggest that inadequate cardiac reserve to meet oxygen requirements may be a risk factor for cardio-pulmonary diseases and mortality in calves raised at high altitude. Genetic selection for energetically expensive traits such as increased growth rate may be contributing to diseases of the cardio-pulmonary system, particularly at higher altitudes.

CHAPTER 7: SYSTEMIC TISSUE OXYGEN EXTRACTION AND MEAN PULMONARY ARTERIAL PRESSURE IN FEEDLOT CATTLE

INTRODUCTION

We have previously reported that systemic oxygen extraction is positively associated with mean pulmonary arterial pressure (mPAP) in pre-weaned calves at high altitude (Chapter 6). This indicates that high metabolic oxygen demand relative to oxygen delivery may be a risk factor for pulmonary hypertension. We have also reported that mPAP increased throughout the confined feeding period in feedlot steers located at the moderate altitude of 1,300 m (Chapter 5).

The purpose of this study was to determine if metabolic oxygen requirement relative to oxygen delivery, as indicated by systemic oxygen extraction fraction (sOEF), is associated with mPAP in feedlot cattle. The first objective was to evaluate if the following variables are association with mPAP: pO_2 and pCO_2, L-lactate and sOEF. These variables were chosen because hypoxemia and inadequate oxygen delivery have been associated with pulmonary hypertension in broiler chickens (Olkowski et al., 2005; Olkowski et al., 1999; Peacock et al., 1990) and, may have a role in the development of pulmonary hypertension in cattle. We hypothesized that sOEF would be positively associated with mPAP. The second objective was to determine if steers with high values of mPAP or sOEF in the early feeding period tended to have high mPAP or sOEF in the late feeding period.

MATERIALS AND METHODS

Study overview

A cohort of steers (n = 121) was followed through the feeding period from approximately 13 months of age to 18.5 months of age. Baseline pulmonary arterial pressures, arterial and mixed-venous blood gas tensions and body masses were obtained from half of the steers on 5/2/13 at an altitude of 1,560 m and the remainder of the steers on 5/15/13 at an altitude of 1,300 m. Approximately, 2 to 4 weeks prior to slaughter the steers were re-tested at an altitude of 1,300 m. Systemic oxygen extraction fraction was calculated from arterial and mixed-venous blood oxygen content. The study received approval from the Colorado State University Animal Care and Use Committee prior to the sampling or handling of any animals (Protocol ID: 12-3513A).

Study site

The study population consisted of calves from the Beef Improvement Center, Colorado State University (Saratoga, Wyoming). Due to herd management practices only male calves were studied. The steers were born and raised at 2,170 m above sea-level. Approximately, 50 % of the calves studied were progeny of bulls with a mPAP < 40 mm Hg at 2,170 m above sea-level. The other 50 % of the calves were progeny of industry relevant bulls of an unknown mPAP. Heifers with low mPAP were retained as herd replacements each year. Therefore, the maternal line has also been selected for 'low' mPAP.

The steers were weaned in mid-October and moved to the Eastern Colorado Research Center (ECRC), Colorado State University (Akron, CO) located at an altitude of

1,300 m in mid-December (n = 123). On April 12th, 2013 half of the steers (n = 61) were transported to the Agricultural Research, Development and Education Center (ARDEC), Colorado State University (Fort Collins, CO) located at an altitude of 1,560 m. Prior to returning to ECRC these steers were sampled (Test 1, 5/2/13). The other half of the steers (n = 62) was sampled at the ECRC (Test 2, 5/15/13) approximately 1 week prior to being transported to ARDEC for feed intake and efficiency assessment. These calves returned to ECRC in mid-August. All steers remained at the ECRC until completion of the feeding period.

The steers were approximately 13 months of age at tests 1 and 2. The final test was performed at the ECRC, approximately 2 to 4 weeks prior to slaughter when the steers were approximately 19 months of age. One steer died from pneumonia between test 1 and 3. Another steer was sold due to ill-thrift and poor growth. Two steers escaped from the chute during sampling. Therefore, in total 119 steers were sampled (Table 7.1).

Trenbolone acetate and estradiol implant

For the purpose of another research study half of the steers were randomly selected to receive a growth-promoting hormone containing trenbolone acetate (200 mg) and estradiol (40 mg) (Revalor-XS®, Merck Animal Health, Summit, NJ). The effect of the implant on mPAP and oxygen extraction was controlled for in the statistical analyses. The implant consists of 10 pellets that disintegrate at differing rates providing a slow-release of hormone that is reported to increase the rate of weight gain and improve feed efficiency for up to 200 days in steers fed in confinement for slaughter.

The implant was inserted under the skin on the posterior aspect of the left ear below the midline approximately mid-way along the length of the ear. The implantation site was cleaned with a chlorhexidine solution prior implantation.

Health

Calves were vaccinated against the respiratory pathogens *Bovine herpesvirus 1* (infectious bovine rhinotracheitis [IBR]), *Bovine viral diarrhea virus* (BVDV), *Bovine respiratory syncytial virus* (BRSV), and *Bovine parainfluenza virus 3* (BPIV-3) at 4-8 weeks of age and 2-4 weeks prior to weaning. A record was kept of calf health and any treatments administered.

Pulmonary arterial pressure measurement

A full description of the equipment, materials and facilities required for pulmonary arterial pressure testing is provided by Holt and Callan (2007). In brief, a large bore needle was inserted into the jugular vein. Flexible saline-filled catheter tubing was then fed through the needle, down through the right atrium, into the right ventricle, and then into the pulmonary artery. A pressure transducer connected the catheter to an oscilloscope. The position of the catheter was determined from the pressure waveform on the oscilloscope. The jugular vein, right atrium, right ventricle and pulmonary artery have distinct pressure waveforms (Holt and Callan, 2007). Mean, systolic and diastolic pressures were recorded after the pulmonary pressure waveforms had stabilized.

Blood-gas analysis

Approximately, 2.5 ml of 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). Mixed venous blood was collected from the pulmonary artery via the catheter used for pulmonary arterial pressure measurement. 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 interrupted. Air bubbles within the blood were immediately expelled and the first several drops of blood discarded before analysis. A temperature 'correction' algorithm was used to adjust blood-gas tensions according to rectal temperature (CLSI, 2001).

Blood-gas analysis was performed using a handheld analyzer (VetScan i-STAT 1, Abaxis, Union City, CA, USA). Hematocrit, pH, pCO_2 and pO_2 were determined from arterial blood. L-Lactate, pH, pCO_2 and pO_2 were determined from mixed venous blood. Oxyhemoglobin saturation of arterial and mixed venous blood was calculated from measured pO_2 and pH and from HCO_3 calculated from measured pCO_2 and pH. The calculation method is available from the manufacturer (Abbott, 2011) or from the corresponding author on request. A limitation of this calculation is that it assumes normal affinity of hemoglobin for oxygen. It does not account for erythrocyte 2,3-diphosphoglycerate concentrations. However, for comparative purposes and therefore, the purpose of this study, the calculation is valid.

Systemic Oxygen Extraction Fraction

The systemic oxygen extraction fraction (sOEF) is the systemic oxygen consumption (V_{O_2}) expressed as a fraction of systemic oxygen delivery (D_{O_2}). Normal systemic OEF is approximately 0.2 (Dellinger, 2002; Leach and Treacher, 2002; McLellan and Walsh, 2004). This means that, on average, approximately 4 times more oxygen is delivered to the microcirculation than is consumed. However, this varies by tissue type: at rest the mean OEF of cardiac and skeletal muscle have been estimated to be 68 % and 25 %, respectively (Binak et al., 1967). As demand for oxygen increases (V_{O_2}) or delivery of oxygen decreases (D_{O_2}) the OEF must increase in order to maintain aerobic metabolism. Cardiopulmonary insufficiency, a reduction in D_{O_2}, results in sOEF to exceed 0.3, or 30 % (Olkowski et al., 2005; Rady et al., 1994). Systemic OEF is calculated using the following formula:

$$sOEF = \frac{((s_aHbO_2 \times Hb \times 1.39) + (0.003 \times p_aO_2)) - ((s_{mv}HbO_2 \times Hb \times 1.39) + (0.003 \times p_{mv}O_2))}{((s_aHbO_2 \times Hb \times 1.39) + (0.003 \times p_aO_2))}$$

Where s_aHbO_2 and s_aHbO_2 are arterial and mixed venous oxyhemoglobin saturation (%), respectively; Hb is hemoglobin concentration (g/L); and, p_aO_2 and $p_{mv}O_2$ are arterial and mixed venous oxygen tensions (mm Hg).

Statistical analyses

Statistical analyses were performed using STATA version 12 (Stata Corporation, College Station, Texas, USA). Summary statistics are provided as mean ± standard deviation (SD). Regression model statistics are provided as mean ± standard error (SE).

Generalized estimating equations (GEE) were used to evaluate differences in the physiological parameters measured among tests (Table 7.1). Generalized estimating

equations were used in order to account for the repeated measures (Liang and Zeger, 1986; Zeger and Liang, 1986). An exchangeable correlation structure was used. Generalized estimating regression equations are robust to missing observations.

Pairwise correlation analyses were performed between all tests for mPAP and sOEF in order to determine if steers with high values of mPAP or sOEF in the early feeding period (tests 1 and 2) tended to have high mPAP or sOEF in the late feeding period (test 3), respectively.

The following explanatory variables were evaluated for association with the outcome variable mPAP: pO_2 and pCO_2, L-lactate and sOEF. A GEE was used to account for the repeated measures. The explanatory variables pH, pO_2 and pCO_2 were evaluated from both arterial and mixed venous blood. All variables were evaluated individually for association with mPAP when controlling for age, gender, growth-implant status, altitude and clustering by pen. Steers were grouped into 4 pens. Pens were controlled for in the analyses as fixed effects. Variables achieving a statistical significance of < 0.20 were included in the full regression model. Backwards step-wise regression was then performed until all variables in the final model had a statistical significance ≤ 0.05 when controlling for age, gender and clustering by pen.

RESULTS

Descriptive statistics

Mean PAP and sPAP were significantly higher at test 3 than either test 1 or 2 (Table 7.1). Systemic oxygen extraction and L-lactate were significantly lower at test 3 than either

test 1 or 2 (Table 7.1). The steers also showed a reduction in alveolar ventilation with age as indicated by a significant increase in arterial CO_2 tension from tests 1 and 2 to test 3 (Table 7.1). Therefore, relative to the early feeding period the provision of oxygen to peripheral tissues was improved in the late feeding period. A large proportion of steers showed evidence of inadequate cardiac reserve to meet oxygen demands at rest, particularly in the early feeding period. At tests 1 through 3 five (26 %), 10 (53 %) and 9 (8 %) steers had a sOEF greater than 30 %, respectively. Diastolic pulmonary arterial pressure was significantly higher at test 1 than either test 2 or 3.

Table 7.1: Physiological variables according to test (mean ± SD)

Variable	n	Test 1	n	Test 2	n	Test 3
Altitude, m		1,560		1,300		1,300
Age, days	59	398 ± 16	62	406 ± 20	120	554 ± 17
Body mass, Kg	61	414 ± 31	62	384 ± 37	119	625 ± 62
Mean PAP,[3] mm Hg	61	41.3 ± 5.2[a]	62	42.2 ± 4.0[a]	119	48.0 ± 11.9[b]
Systolic PAP,[3] mm Hg	61	66.7 ± 9.2[a]	62	69.0 ± 8.8[a]	119	80.3 ± 15.3[b]
Diastolic PAP,[3] mm Hg	61	19.5 ± 5.9[a]	62	15.7 ± 6.4[b]	119	17.1 ± 12.4[b]
Pulse pressure, mm Hg	61	47.2 ± 10.7[a]	62	53.4 ± 12.1[b]	119	63.2 ± 14.6[c]
sOEF,[1, 2] %	19	26.4 ± 7.5[a]	19	29.0 ± 6.7[a]	117	21.8 ± 5.9[b]
L-lactate, mmol/L	45	2.6 ± 1.7 [a]	39	3.3 ± 2.1[a]	118	1.8 ± 1.4[b]
$sHbO_2$,[1, 4] %	60	94.6 ± 3.3	62	94.7 ± 5.1	117	94.8 ± 1.9
pH_a	60	7.47 ± 0.04	62	7.45 ± 0.05	117	7.48 ± 0.04
pH_{mv}	18	7.41 ± 0.03	19	7.38 ± 0.06	119	7.43 ± 0.04
p_aO_2, mm Hg	60	72.3 ± 11.0	62	75.2 ± 11.7	117	71.4 ± 7.4
$p_{mv}O_2$, mm Hg	18	37.2 ± 3.9	19	38.5 ± 8.4	119	39.1 ± 3.2
p_aCO_2, mm Hg	60	36.8 ± 3.7[a]	62	35.2 ±3.1[a]	117	39.3 ± 4.8[b]
$p_{mv}CO_2$, mm Hg	18	45.4 ± 4.2	19	44.3 ± 5.2	119	44.5 ± 4.6
Hematocrit, %	32	31.2 ± 2.4	40	31.1 ± 2.2	117	31.2 ± 2.6

Variables within rows that do not have a common superscript statistically differ ($p < 0.05$)
[1] Calculated value
[2] Systemic oxygen extraction fraction
[3] Pulmonary arterial pressure

The concentration of L-lactate was generally high (Table 7.1) and was weakly correlated with sOEF ($r = 0.37$, $p < 0.001$) (Figure 7.1). Pairwise correlation of lactate concentration between tests 1 and 2 ($r = 0.47$, $p = 0.001$) and tests 2 and 3 ($r = 0.51$, $p = 0.002$) was positive and moderately strong. Pairwise correlation of mPAP concentration between tests 1 and 2 ($r = 0.73$, $p < 0.001$) and tests 2 and 3 ($r = 0.30$, $p = 0.02$) was

positive and moderately strong. Systemic oxygen extraction was not correlated between tests.

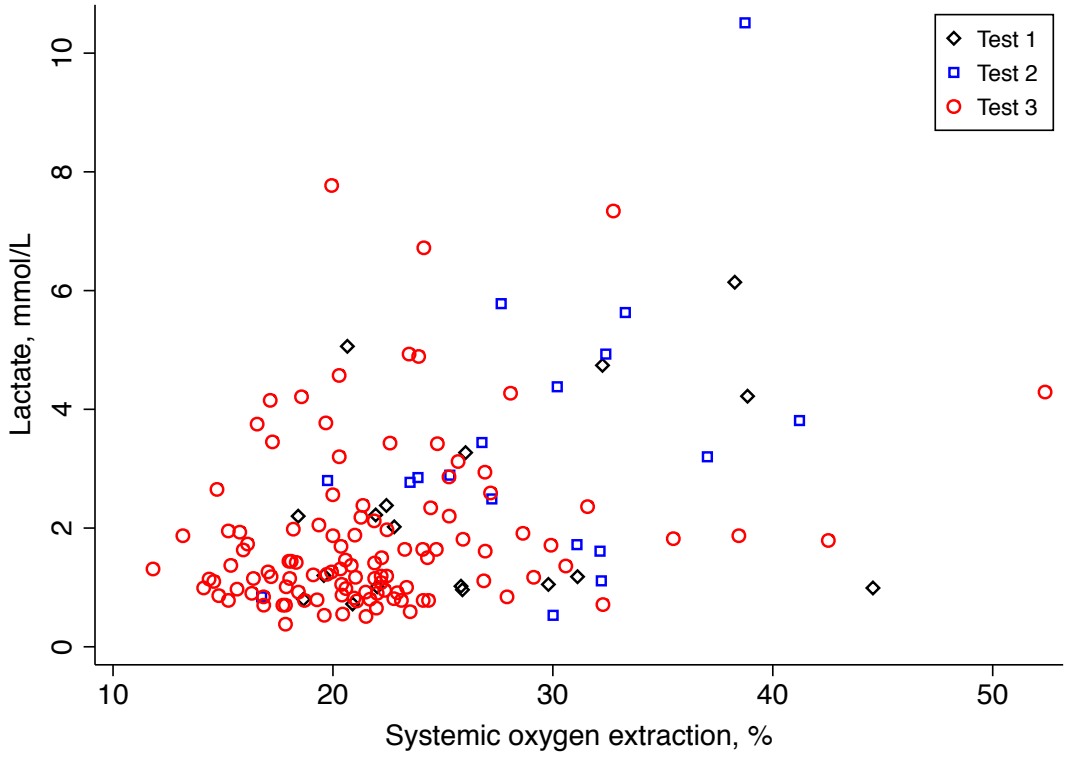

Figure 7.1: A dot plot of L-lactate concentration versus systemic oxygen extraction fraction (r = 0.37, p < 0.001) for steers at test 1 (black diamond), test 2 (blue square) and test 3 (red circle)

Analytes associated with mPAP

Systemic OEF, lactate and age were associated with mPAP when controlling for implant status (p = 0.20), altitude (p = 0.73), and clustering by pen (p = 0.95)(Table 7.2). Mean PAP increased by 1.5 mm Hg for every 10 % increment in sOEF and increased 4 mm Hg for every 100 days in the feedlot. Mean PAP decreased by 0.52 mm Hg for every 1 mmol/L increment in L-lactate.

Table 7.2: Change in mean pulmonary arterial pressure associated with a 1 unit change in the explanatory variable

Explanatory variable	Mean (95 % CI)	p-value
sOEF, %	0.15 (0.02, 0.29)	0.025
L-lactate, mmol/L	-0.52 (-1.03, -0.00)	0.048
Age, days	0.04 (0.02, 0.06)	< 0.001

Systemic OEF was significantly associated with sPAP when controlling for L-lactate ($p = 0.95$), age ($p < 0.001$), altitude ($p = 0.82$), implant status ($p = 0.40$) and clustering by pen ($p = 0.74$) (Figure 7.2). Systolic PAP increased by 3.0 mm Hg (95 % CI = 0.2, 5.8 mm Hg) for every 10 % increase in sOEF ($p = 0.038$). Systemic OEF was positively associated with dPAP when controlling for the same explanatory variables above but this was not statistically significant ($p = 0.13$). Although there was a positive association between sOEF and mPAP and between sOEF and sPAP there was considerable variation of individual observations about the least squares estimate (Figure 7.2).

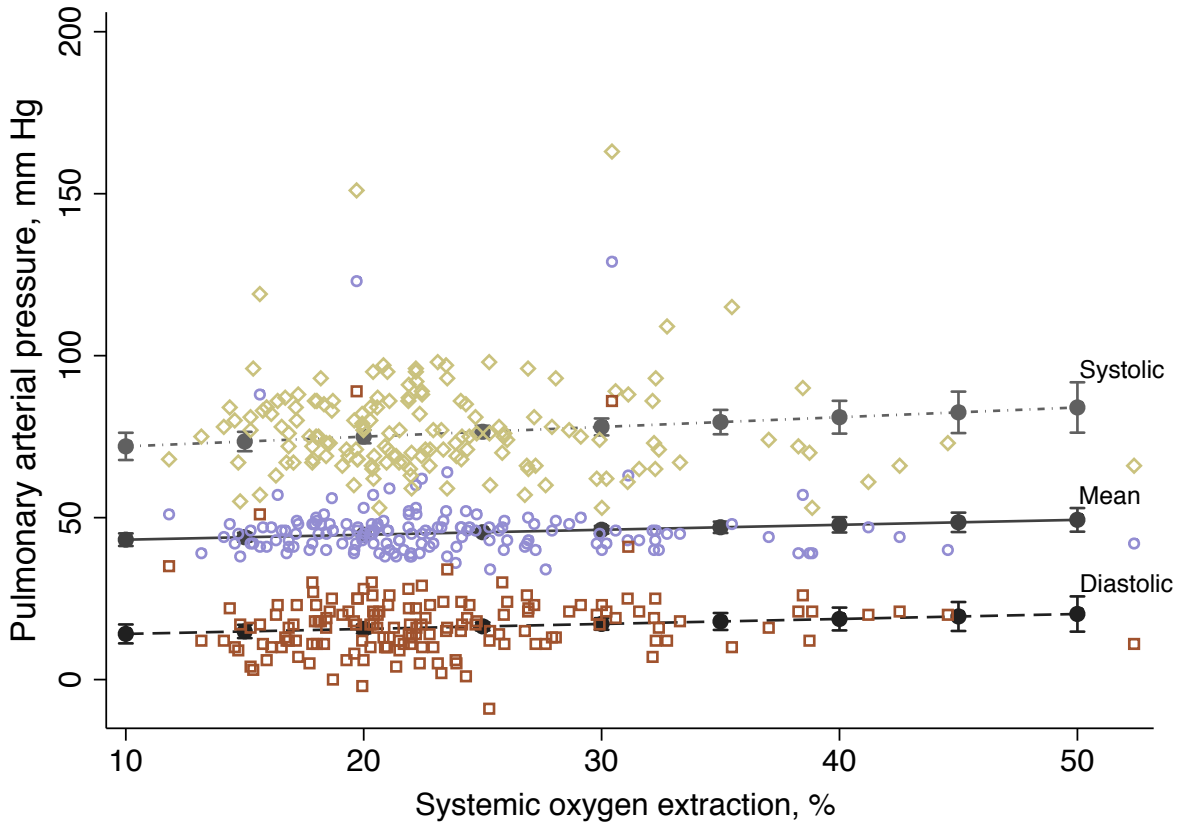

Figure 7.2: A graph of least squares mean and 95 % confidence interval of the mean for systolic, mean and diastolic pulmonary arterial pressure at systemic oxygen extraction fraction ranging from 10 to 50 %. Individual observations are shown for systolic (diamonds), mean (circles) and diastolic (squares) pulmonary arterial pressures

DISCUSSION

The results of this study showed that inadequate cardiac reserve was positively associated with mean pulmonary arterial pressure in feedlot cattle. A high proportion of the steers in this study, particularly during the early feeding period, showed evidence of low output failure, as indicated by a systemic oxygen extraction fraction (sOEF) greater than 30 %. Steers that had a high mPAP in the early feeding period tended to have a high mPAP in the late feeding period although, the correlation was not strong. Further studies

are necessary to determine how low-output failure, as indicated by increased sOEF, relates to increased mPAP and right-sided congestive heart failure.

One limitation of our study design is that causal inference cannot be determined. We cannot conclude that insufficient cardiac output relative to metabolic oxygen demand was a cause of increased mPAP. High mPAP may have caused a decrease in cardiac reserve (difference between maximum and resting cardiac output) by increasing right-ventricular afterload (forces opposing the ejection of blood out of the right-ventricle). Cattle with poor cardiac reserve at rest have a limited ability to adapt to stressors that require an increase in cardiac output. This may predispose cattle to heart failure. Poor cardiac reserve in broiler chickens selected for rapid growth has been associated with an increased risk of congestive heart failure (Olkowski et al., 2005). Further studies are necessary to determine how PAP, low-output heart failure and congestive heart failure are interconnected in cattle.

Interestingly, despite the high sOEF and L-lactate levels hematocrit did not increase. Failure to increase hematocrit in response to chronic hypoxia has been previously reported in cattle (Hays et al., 1978; Neary et al., 2013a). Increasing hematocrit is associated with increasing blood viscosity and therefore, increasing resistance to blood flow. Therefore, benefit of increasing the oxygen-carrying capacity of the blood must be balanced against the adverse effect of increased blood viscosity on resistance to blood flow.

L-lactate is a product of anaerobic metabolism that is used clinically as an indicator of ischemia and hypoxia. A study of 34 healthy, early-lactation dairy cows reported a median plasma concentration of 0.54 mmol/L (Figueiredo et al., 2006). The L-lactate values reported in our tissue were considerably higher. High sOEF was concurrent with high L-

lactate concentrations in the early feeding period. This suggests that the increased anaerobic metabolism in the early feeding period was due to insufficient cardiac output.

Alveolar ventilation, as indicated by arterial CO_2 tension, decreased from the early feeding period to the late feeding period. Hypoventilation has been suggested as a risk factor for pulmonary hypertension (Jensen et al., 1976). In our study, cattle did not show evidence of hypoventilation. However, a reduction in the degree of hyperventilation may have been sufficient to cause an increase in mPAP. Ventilation rate may have fallen in the late feeding-period because oxygenation of peripheral tissues, as indicated by reduced sOEF and L-lactate concentration, was improved relative to the early feeding period.

CHAPTER 8: HIGH GROWTH RATE AND HIGH FEED EFFICIENCY ARE POSITIVELY ASSOCIATED WITH MEAN PULMONARY ARTERIAL PRESSURE AND SYSTEMIC OXYGEN EXTRACTION IN CATTLE

INTRODUCTION

Rapid growth may be a risk factor for right-sided congestive heart failure (CHF) in cattle secondary to pulmonary hypertension. Mean pulmonary arterial pressure (mPAP) is reported to have a positive, unfavorable genetic correlation with birth weight and weaning weight (Shirley et al., 2008). It has also been suggested that rapid growth is a risk factor for congestive heart failure secondary to pulmonary hypertension in feedlot cattle (Jensen et al., 1976). However, to our knowledge the physiological association between rate of growth and mPAP has not been described.

Cattle have a small cardio-pulmonary system for their body mass and oxygen requirement (Veit and Farrell, 1978). Therefore, it is plausible that the risk of right-sided congestive heart failure in cattle may be increased in cattle with little cardiac reserve. Adequacy of cardiac output can be evaluated by measuring the systemic oxygen extraction fraction (sOEF); the proportion of oxygen within arterial blood that is utilized by the body. To our knowledge the association between rate of growth, mPAP and sOEF has not been evaluated in cattle.

The purpose of this study was to determine if the rate of body mass gain is associated with mPAP and sOEF in pre-weaned calves at high altitude (altitude 2,170 m) and in cattle during the feeding period (altitude 1,300 m). For the purpose of this study it

was assumed that high mPAP increases the risk of CHF. However, this is not necessarily true as CHF is a multifactorial disease. We hypothesized that average daily gain and feed efficiency would be positively associated with mPAP and sOEF.

MATERIALS AND METHODS

Study overview

A cohort of calves was followed from birth to slaughter. Mean pulmonary arterial pressure, systemic oxygen extraction and body mass were determined at two time points: first, when male calves were 6 months of age at 2,170 m (test 1) and second, when steers were 18.5 months of age at 1,300 m (test 2). Average daily gain was calculated between birth and weaning (test 1), between weaning and the late feeding period (test 2) and over a 70 day period when the cattle were approximately 15 months old. During the latter period feed efficiency was also evaluated by calculating residual feed intake. The study received approval from the Colorado State University Animal Care and Use Committee prior to the sampling or handling of any animals (Protocol ID 12-3513A).

Study sites

The study population consisted of calves from the Beef Improvement Center, Colorado State University (Saratoga, Wyoming). Due to herd management practices only male calves were studied. Approximately, 50 % of the steers studied were progeny of bulls with a mPAP < 40 mm Hg at 2,170 m above sea-level. The other 50 % of the steers were progeny of industry relevant bulls of an unknown mPAP. Heifers with low mPAP were

retained as herd replacements each year. Therefore, the maternal line has also been selected for 'low' mPAP.

A cohort of 60 calves was randomly selected for pulmonary arterial pressure (PAP) testing at 6 months of age (Table 8.1). Every second calf into the chute was sampled.

Table 8.1: The date, altitude, age and number of calves sampled according to test

Test	Altitude, m	Date	Number sampled			Mean age ± SD, days
			B[1]	S[2]	T[3]	
1	2,170	10/01/12	55	5[4]	60[4]	185 ± 19
2	1,300	10/8/13	0	119	119	554 ± 17

[1] B – The number of bull calves sampled
[2] S – The number of steer calves sampled
[3] T – The total number of calves sampled
[4] mPAP measured on an additional 93 steers for breeding management purposes.

The calves were born, raised and PAP tested at 2,170 m. Calves were weaned in mid-October. Some of the bull calves included in the cohort sampled at test 1 when 6 months of age were sold prior to test 2 (n = 30). Bull calves that were not sold were castrated and moved with the remaining steers to the Eastern Colorado Research Center (ECRC), Colorado State University (Akron, CO) located at an altitude of 1,300 m in mid-December (n = 123). On April 12th, 2013 half of the steers (n = 61) were transported to the Agricultural Research, Development and Education Center (ARDEC), Colorado State University (Fort Collins, CO) located at an altitude of 1,560 m for feed intake and efficiency assessment (Table 8.2). The other half of the steers (n = 62) was moved to ARDEC for feed intake and efficiency assessment in mid-May. These calves returned to ECRC in mid-August. All steers remained at the ECRC until completion of the feeding period.

Test 2 was performed at the ECRC, approximately 2 to 4 weeks prior to slaughter when the steers were approximately 19 months of age. One steer died from pneumonia between test 3 and 5. Another steer was sold due to ill-thrift and poor growth. Two steers escaped from the chute during sampling. Therefore, in total 119 steers were sampled.

Table 8.2: Rate of gain and residual feed intake in the early feeding period were determined at two time points

Group	n[1]	Altitude, m	Date at Feed Intake Unit Entry	Exit	Mean age at the midpoint of feed intake study ± SD, days
1	61	1,560	5/2/13	7/11/13	433 ± 16
2	62	1,560	6/13/13	8/22/13	469 ± 18

[1] The number of animals in a group

Calf health

The dams of calves studied were given a pre-breeding and pre-calving vaccination offering protection against *Bovine herpesvirus 1* (infectious bovine rhinotracheitis [IBR]), *Bovine viral diarrhea virus* (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. A record was kept of calf health and any treatments administered.

Pulmonary arterial pressure testing

A full description of the equipment, materials and facilities required for pulmonary arterial pressure testing is provided by Holt and Callan (2007). In brief, a large bore needle was inserted into the jugular vein. Flexible saline-filled catheter tubing was then fed through the needle, down through the right atrium, into the right ventricle, and then into

the pulmonary artery. A pressure transducer connected the catheter to an oscilloscope. The position of the catheter was determined from the pressure waveform on the oscilloscope. The jugular vein, right atrium, right ventricle and pulmonary artery have distinct pressure waveforms (Holt and Callan, 2007). Mean, systolic and diastolic pressures were recorded after the pulmonary pressure waveforms had stabilized.

Blood-gas analysis

Approximately, 2.5 ml of 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). Mixed venous blood was collected from the pulmonary artery via the catheter used for pulmonary arterial pressure measurement. 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 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 analyzer (VetScan i-STAT 1, Abaxis, Union City, CA, USA). A temperature 'correction' algorithm was used to adjust blood-gas tensions according to rectal temperature (CLSI, 2001).

Systemic Oxygen Extraction Fraction

The systemic oxygen extraction fraction (sOEF) is the systemic oxygen consumption (VO_2) expressed as a fraction of systemic oxygen delivery (DO_2). Normal systemic OEF is 0.2 to 0.3 (Dellinger, 2002; Leach and Treacher, 2002; McLellan and Walsh, 2004). This means that on average, approximately 4 times more oxygen is delivered to the microcirculation than is consumed. However, this varies by tissue type: at rest the mean OEF of cardiac and skeletal muscle have been estimated to be 68 % and 25 %, respectively (Binak et al., 1967). As demand for oxygen increases (VO_2) or delivery of oxygen decreases (DO_2) the OEF must increase in order to maintain aerobic metabolism. Cardiopulmonary insufficiency, a reduction in DO_2, results in sOEF to exceed 0.3, or 30 % (Olkowski et al., 2005; Rady et al., 1994). Systemic OEF is calculated using the following formula:

$$sOEF = \frac{((s_aHbO_2 \times Hb \times 1.39) + (0.003 \times p_aO_2)) - ((s_{mv}HbO_2 \times Hb \times 1.39) + (0.003 \times p_{mv}O_2))}{((s_aHbO_2 \times Hb \times 1.39) + (0.003 \times p_aO_2))}$$

Where s_aHbO_2 and s_aHbO_2 are arterial and mixed venous oxyhemoglobin saturation (%), respectively; Hb is hemoglobin concentration (g/L); and, p_aO_2 and $p_{mv}O_2$ are arterial and mixed venous oxygen tensions (mm Hg).

Average daily gain and residual feed intake

Average daily gain was calculated over 3 periods: from birth to test 1 (weaning), from test 1 to test 2, and for 70 days during the early feeding period (Table 8.2). Average daily gain was calculated as the difference in body mass divided by the number of days between the two time points (kg/d).

Residual feed intake was calculated as the difference between observed feed intake minus the expected feed intake given the animals level of performance. Individual feed intake was determined using an automated system (GrowSafe, GrowSafe Systems Ltd., Airdrie, AB, Canada). Least squares regression of expected intake was modeled using the following formula:

$$\text{Observed intake} = \mu + (\beta_w \times \text{mean metabolic body mass}) + (\beta_g \times \text{body mass gain}) + \text{residual error}$$

Where true intake is daily feed intake (kg, as fed), μ is a constant; mean metabolic body mass is (mean body mass of the animal for the intake test period)$^{0.75}$; and body mass gain is live body mass gain over the feed intake test period (kg/d). The residual feed intake is then the observed average daily feed intake minus the expected average daily feed intake.

Statistical analyses

Statistical analyses were performed using STATA version 12 (Stata Corporation, College Station, Texas, USA). Statistics are provided as mean ± SE unless otherwise indicated.

Differences in pulmonary arterial pressures between tests 1 and 2 were assessed using paired t-tests. Linear regression analyses were performed to evaluate the association between the explanatory variable, daily gain (kg per day), and the outcome variables, mean pulmonary arterial pressure and systemic oxygen extraction. The effects of other variables were controlled in the analyses. Between birth and test 1 these variables included: gender (bull or steer) and age at test 1 (days). Between tests 1 and 2 these variables included growth hormone implant status (implanted or non-implanted) and feedlot pen number (4

pens). Least squares linear prediction of mean pulmonary arterial pressure and systemic oxygen extraction fraction were performed while controlling for the variables listed above.

RESULTS

Change in pulmonary arterial pressures and oxygen extraction

On average, mPAP was higher during the late feeding period than at weaning. Mean PAP and sPAP were significantly higher in the late feeding period at 1,300 m than at weaning time at 2,170 m (Table 8.3). However, dPAP was significantly lower in the late feeding period than at weaning ($p = 0.02$). Mean sOEF values for both tests were within normal limits (< 30 %) but there was considerable variation about the mean values (Table 8.3). At test 1, 12 % of calves (7 of 59) had a sOEF > 30 %. At test 2, 8 % of calves (9 of 117) had a sOEF > 30 %. The interpretation of this indicates that approximately 10 % of animals at weaning and in the late feeding period had inadequate cardiac output to meet oxygen requirements. The daily gain from birth to test 1 was 0.98 ± 0.15 kg per day (mean ± SD) and 1.11 ± 0.13 kg per day from test 1 to test 2. The daily gain approximately 3 to 4 months prior to test 2 was 1.49 ± 0.32 kg per day (mean ± SD).

Table 8.3: Age, body mass, pulmonary arterial pressures and systemic oxygen extraction fraction according to test

Variable	Test 1 (Weaning)		Test 2 (Feedlot)		
	n	Mean ± SD	n	Mean ± SD	p-value
Age, days	60	185 ± 19	119	554 ± 17	
Body mass, Kg	60	218 ± 31	119	625 ± 62	
mPAP, mm Hg	60	42.3 ± 6.8	119	48.0 ± 12.0	< 0.001
sPAP, mm Hg	60	61.7 ± 9.1	119	80.4 ± 15.3	< 0.001
dPAP, mm Hg	60	26.4 ± 6.2	119	17.0 ± 12.4	0.016
sOEF, %	59	22.3 ± 8.3	117	21.9 ± 5.9	0.14

Daily gain from birth to test 1 and its association with mPAP and sOEF at test 1

Daily gain from birth to test 1 showed a positive and statistically significant association with sOEF (p = 0.04) (Figure 8.1).

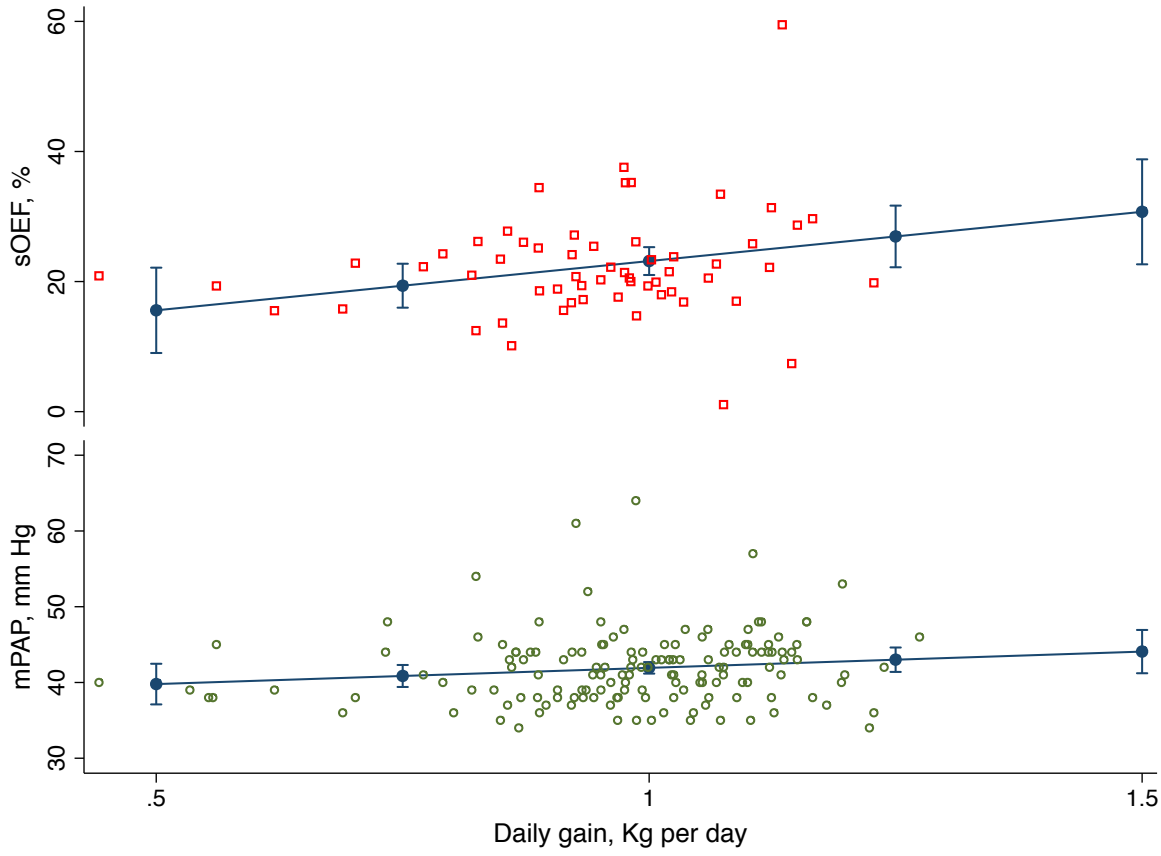

Figure 8.1: Least squares estimate and 95 % confidence intervals of systemic oxygen extraction fraction (sOEF) and mean pulmonary arterial pressure (mPAP) against daily gain from birth to test 1 while controlling for gender and age. Individual observations are shown as red squares (sOEF) and green circles (mPAP)

Variation in daily gain between birth and test 1 explained little of the variation in sOEF and was not associated with mPAP (Table 8.4).

Table 8.4: Change in mean pulmonary arterial pressure (mPAP) and systemic oxygen extraction fraction (sOEF) at test 1 associated with a 1 kg per day increase in daily gain between birth and test 1 while controlling for age and gender

Outcome	n	Mean (95 % CI)	Adjusted r^2, %	p-value
mPAP, mm Hg	60	4.3 (-1.1, 9.7)	6.3	0.12
sOEF, %	59	15.1 (0.8, 29.5)	11.3	0.04

Daily gain between tests 1 and 2 and its association with mPAP and sOEF at test 2

Both sOEF and mPAP showed a positive association with daily body mass gain between tests 1 and 2 (Figure 8.2).

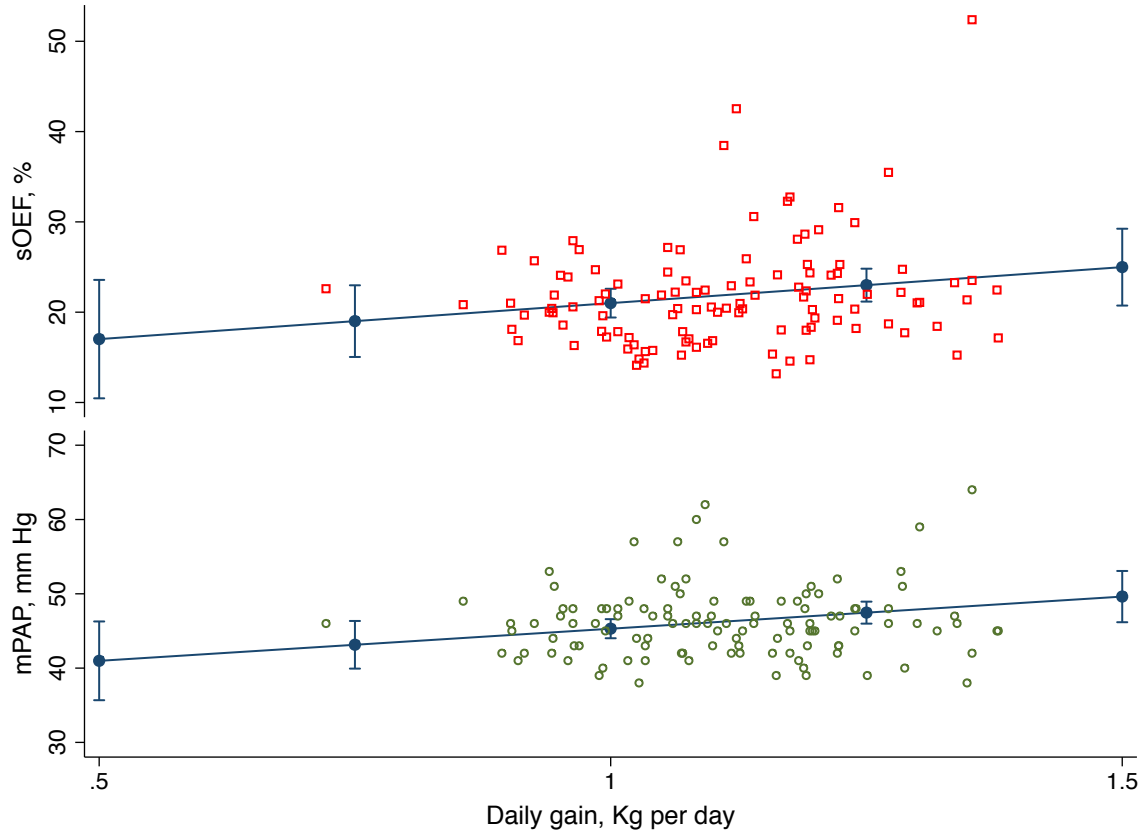

Figure 8.2: Least squares estimate and 95 % confidence intervals of systemic oxygen extraction fraction (sOEF) and mean pulmonary arterial pressure (mPAP) against daily gain from test 1 to test 2 while controlling for age, implant status, residual feed intake and clustering by pen. Individual observations are shown as red squares (sOEF) and green circles (mPAP)

Daily gain between tests 1 and 2 was associated with mPAP at test 2 and tended to be associated with sOEF at test 2 (Table 8.5). However, the variation in sOEF and mPAP explained by variation in growth rate was low (Table 8.5).

Table 8.5: Least squares estimated change in mean pulmonary arterial pressure (mPAP) and systemic oxygen extraction fraction (sOEF) at test 2 associated with a 1 kg per day increase in daily gain between tests 1 and 2 while controlling for age, growth hormone status, pen and treatment for disease

Outcome	n	Mean (95 % CI)	Adjusted r^2, %	p-value
mPAP, mm Hg	114	8.6 (0.0, 17.3)	3.5	0.05
sOEF, %	112	10.5 (-0.5, 21.5)	3.7	0.06

Daily gain and feed efficiency in the feedlot and its association with mPAP and sOEF at test 2

Increased growth rate and increased feed efficiency during the feeding period were positively associated with mPAP at test 2, which was approximately 3 months later (Table 8.6).

Table 8.6: Change in mean pulmonary arterial pressure (mPAP) and systemic oxygen extraction fraction (sOEF) at test 2 associated with a 1 kg per day increase in daily gain and a 1 unit increase in residual feed intake during the early feeding period while controlling for age, implant status, pen and treatment for disease

	Daily gain, Kg per day				Residual feed intake			
Outcome	n	Mean (95 % CI)	Adj. r^2, %	p value	n	Mean (95 % CI)	Adj. r^2, %	p value
mPAP, mm Hg	115	5.1 (1.2, 8.9)	8.3	0.01	115	-0.46 (-0.84, 0.08)	8.3	0.02
sOEF, %	113	2.4 (-2.5, 7.3)	2.3	0.33	113	-0.16 (-0.64, 0.32)	2.3	0.51

Growth rate and feed efficiency were not associated with sOEF at test 2 but appeared to show a relationship that was consistent with findings reported elsewhere in this study (Figures 8.3 and 8.4).

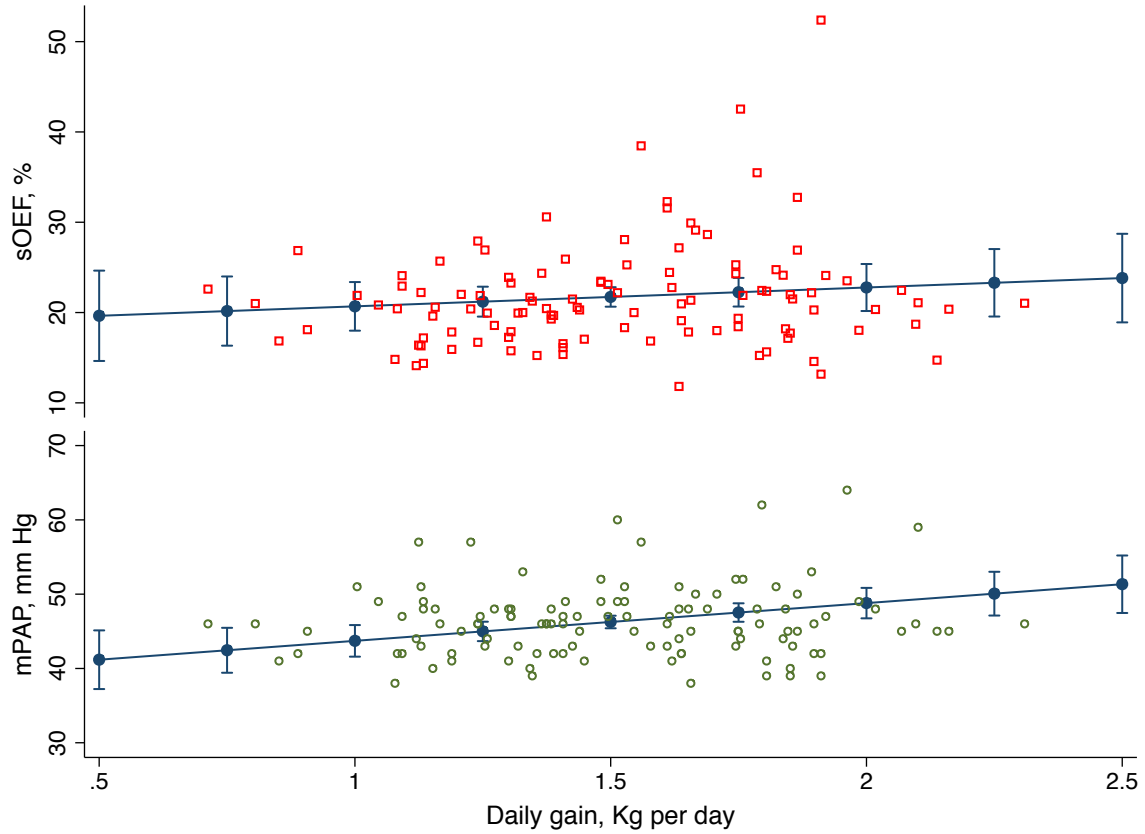

Figure 8.3: Least squares estimate and 95 % confidence interval of systemic oxygen extraction fraction (sOEF) and mean pulmonary arterial pressure (mPAP) in late-fed steers against daily gain during the early feeding period while controlling for age, implant status, residual feed intake and clustering by pen. Individual observations are shown as red squares (sOEF) and green circles (mPAP)

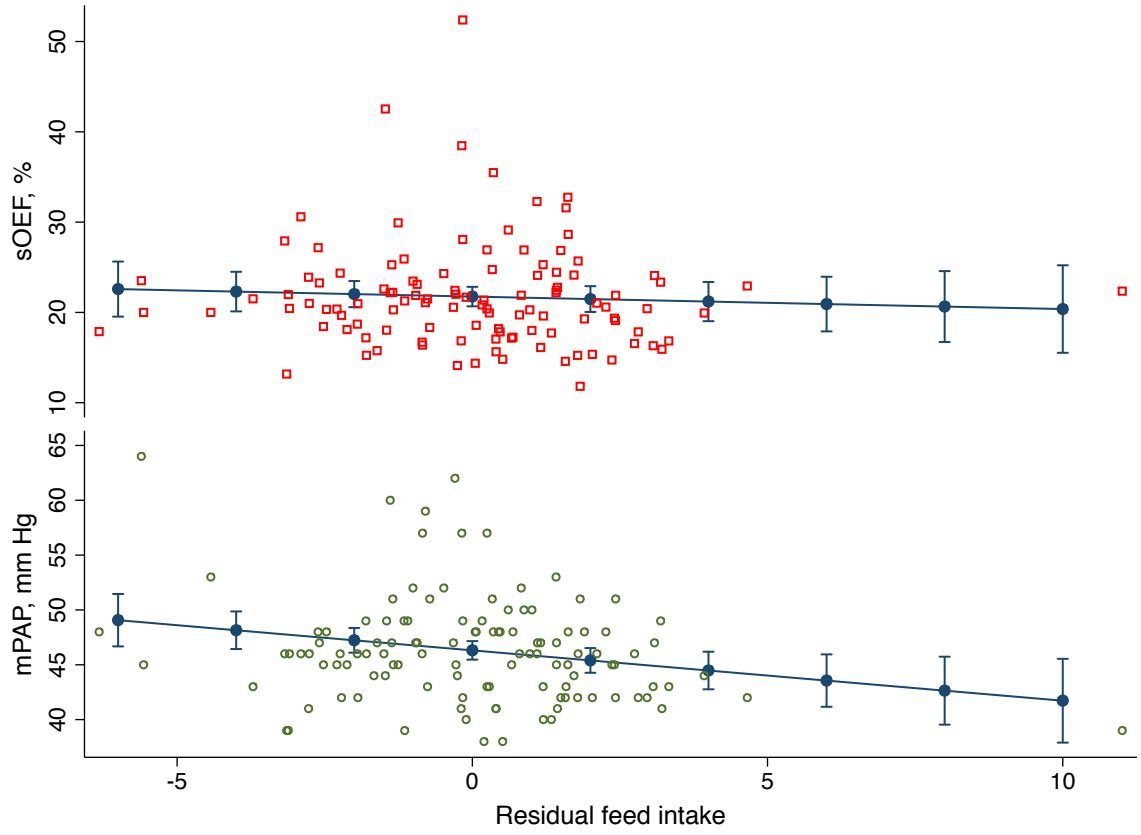

Figure 8.4: Least squares estimate and 95 % confidence interval of systemic oxygen extraction fraction (sOEF) and mean pulmonary arterial pressure (mPAP) in late-fed steers against residual feed intake during the early feeding period while controlling for age, implant status, residual feed intake and clustering by pen. Individual observations are shown as red squares (sOEF) and green circles (mPAP)

DISCUSSION

The results of this study showed that growth rate was positively associated with mean pulmonary arterial pressure (mPAP) and systemic oxygen extraction fraction (sOEF) in cattle. However, variation in growth rate explained little of the variation in mPAP or sOEF. Increased feed efficiency in the confined feeding period was positively associated with mPAP but not sOEF. However, variation in feed efficiency explained little of the variation in mPAP. These findings suggest that genetic selection for rate of growth and feed

efficiency may have the adverse effect of increasing mPAP. This, in turn, may predispose cattle to an increased risk of congestive heart failure (CHF).

A limitation of this study is that cattle from only one herd were studied. The results may be different for cattle of different breeds and under different management practices. However, the cattle studied were commercial cattle managed in a manner that is typical of many producers in the western United States.

Increased growth rate has been genetically correlated with increased mPAP in cattle (Levalley, 1978; Shirley et al., 2008). It has also been suggested that fast rate of growth is a risk factor for right-sided congestive heart failure (Jensen et al., 1976). Similarly, growth rate is correlated with mPAP in broiler chickens (Peacock et al., 1989). Fast-growing chickens (sOEF ≈ 0.4) and chickens with congestive heart failure (sOEF ≈ 0.56) had a significantly higher sOEF than slower-growing chickens (sOEF ≈ 0.16) (Olkowski et al., 2005). Inadequate cardiac output to meet the increased metabolic demand for oxygen associated with rapid growth is thought to predispose broiler chickens to congestive heart failure (Olkowski et al., 2005). Our findings indicate that a similar relationship between oxygen supply and demand and rate of growth exists for cattle.

Feed efficiency was also positively associated with mPAP but not sOEF. A weak but positive correlation between feed efficiency and susceptibility to congestive heart failure has been reported in broiler chickens (Pakdel et al., 2005). It is thought selection for feed efficiency in broiler chickens may have resulted in a functional hypothyroidism since thyroid function is an important regulator of metabolic rate (Decuypere et al., 2000). Hypothyroidism reported in chickens selected for fast rate of growth and increased feed efficiency is reported to contribute to congestive heart failure (Buys et al., 1999; Scheele et

al., 1992). Low thyroid hormone levels concurrent with selection for rapid growth and feed efficiency has been shown to impair lung development in broilers (Hassanzadeh et al., 2008). It is not clear why increased feed efficiency was associated with mPAP in feedlot cattle.

CHAPTER 9: A SLOW-RELEASE TRENBOLONE ACETATE AND 17-β-ESTRADIOL GROWTH IMPLANT REDUCED DIASTOLIC PULMONARY ARTERIAL PRESSURE IN FEEDLOT CATTLE

INTRODUCTION

Growth-promoting agents, such as steroidal implants and beta-adrenergic agonist, are used extensively in beef production. Over 90 % of feedlot cattle receive at least one steroidal implant for growth promotion from time of placement until marketing (NAHMS, 2013). Steroidal implants, such as trenbolone acetate and estradiol, are used to increase the efficiency and rate of growth (Mader, 1998).

We have shown that growth rate and feed efficiency are positively and unfavorably associated with mean pulmonary arterial pressure in cattle (Chapter 8). We have also shown that the risk of congestive heart failure in feedlot cattle increased from the year 2000 to the year 2012 (Chapter 3). The purpose of this study was to determine if growth promotion induced by a steroid implant increased mean pulmonary arterial pressure (mPAP) in feedlot cattle. We hypothesized that feedlot steers administered a growth hormone implant would develop a higher mPAP and systemic oxygen extraction fraction (sOEF) than nonimplanted control steers.

MATERIALS AND METHODS

Study overview

A cohort of steers (n = 121) was followed through the feeding period from approximately 13 months of age to 18.5 months of age. Baseline pulmonary arterial pressures and body masses were obtained from half of the steers on 5/2/13 at an altitude of 1,560 m and the remainder of the steers on 5/15/13 at an altitude of 1,300 m. Every second animal tested was implanted with a subcutaneous slow-release depot containing 40 mg estradiol and 200 mg trenbolone acetate. Approximately, 2 to 4 weeks prior to slaughter the steers were retested at an altitude of 1,300 m. In addition to pulmonary arterial pressures and body mass, mixed venous and arterial blood oxygen content was measured in order to calculate systemic oxygen extraction. The study received approval from the Colorado State University Animal Care and Use Committee prior to the sampling or handling of any animals (Protocol ID: 12-3513A).

Study site

The study population consisted of black-hided, Angus-based calves from the Beef Improvement Center, Colorado State University (Saratoga, Wyoming). The steers were born and raised at 2,170 m above sea-level. Approximately, half of the steers were sired by bulls with a mPAP < 40 mm Hg at 2,170 m. The remaining steers were born to cows bred by artificial insemination using semen from bulls of unknown mPAP. Heifers were retained as herd replacements each year. Therefore, the maternal line has also been selected for 'low' mPAP.

Calves were weaned in mid-October and all steer calves transported to the Eastern Colorado Research Center (ECRC), Colorado State University (Akron, CO) located at an altitude of 1,300 m in mid-December. All steers spent 70 days in a feed intake facility, after 21 days of adaptation, at the Agricultural Research, Development and Education Center (ARDEC), Colorado State University (Fort Collins, CO) located at an altitude of 1,560 m. The steers were divided into two groups. One group of steers was at the feed intake facility from May 2nd to July 11th (n= 61) and the other from June 13th to August 22nd (n = 62). Outside of these times all steers remained at the ECRC until completion of the feeding period. On May 2nd half of the steers were weighed and pulmonary arterial pressure (PAP) tested at the ARDEC (n= 61) (Test 1). The remaining calves were weighed and PAP tested 2 weeks later at the ECRC (n = 62) (Test 2). All steers were PAP tested at 19 months of age at the ECRC, approximately 2 to 4 weeks prior to slaughter.

Trenbolone acetate and estradiol implant

A growth-promoting hormone implant containing trenbolone acetate (200 mg) and estradiol (40 mg) was implanted in half of the calves (Revalor-XS®, Merck Animal Health, Summit, NJ). The implant consists of 10 pellets that disintegrate at differing rates providing a slow-release of hormone that is reported to increase the rate of weight gain and improve feed efficiency for up to 200 days in steers fed in confinement for slaughter.

Every second calf into the chute was implanted. The implant was inserted under the skin on the posterior aspect of the left ear below the midline approximately mid-way along the length of the ear. The ear was cleaned with a chlorhexidine solution prior to implantation.

Health

Calves were vaccinated against the respiratory pathogens *Bovine herpesvirus 1* (infectious bovine rhinotracheitis [IBR]), *Bovine viral diarrhea virus* (BVDV), *Bovine respiratory syncytial virus* (BRSV), and *Bovine parainfluenza virus 3* (BPIV-3) at 4-8 weeks of age and 2-4 weeks prior to weaning. A record was kept of calf health and any treatments administered.

Pulmonary arterial pressure testing

A full description of the equipment, materials and facilities required for pulmonary arterial pressure testing is provided by Holt and Callan (2007). In brief, a large bore needle was inserted into the jugular vein. Flexible saline-filled catheter tubing was 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 catheter to an oscilloscope. The position of the catheter was determined from the pressure waveform on the oscilloscope. The jugular vein, right atrium, right ventricle and pulmonary artery have distinct pressure waveforms (Holt and Callan, 2007). Mean, systolic and diastolic pressures were recorded after the pulmonary pressure waveforms had stabilized.

Blood-gas analysis

Approximately, 2.5 ml of 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). Mixed venous blood was collected from the pulmonary artery via the catheter used for

pulmonary arterial pressure measurement. 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 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 analyzer (VetScan i-STAT 1, Abaxis, Union City, CA, USA). A temperature 'correction' algorithm was used to adjust blood-gas tensions according to rectal temperature (CLSI, 2001).

Systemic Oxygen Extraction Fraction

The systemic oxygen extraction fraction (sOEF) is the systemic oxygen consumption (VO_2) expressed as a fraction of systemic oxygen delivery (DO_2). Normal systemic OEF is 0.2 to 0.3 (Dellinger, 2002; Leach and Treacher, 2002; McLellan and Walsh, 2004). This means that on average, approximately 4 times more oxygen is delivered to the microcirculation than is consumed. However, this varies by tissue type: at rest the mean OEF of cardiac and skeletal muscle have been estimated to be 68 % and 25 %, respectively (Binak et al., 1967). As demand for oxygen increases (VO_2) or delivery of oxygen decreases (DO_2) the OEF must increase in order to maintain aerobic metabolism. Cardiopulmonary insufficiency, a reduction in DO_2, results in sOEF to exceed 0.3, or 30 % (Olkowski et al., 2005; Rady et al., 1994). Systemic OEF is calculated using the following formula:

$$sOEF = \frac{((s_aHbO_2 \times Hb \times 1.39) + (0.003 \times p_aO_2)) - ((s_{mv}HbO_2 \times Hb \times 1.39) + (0.003 \times p_{mv}O_2))}{((s_aHbO_2 \times Hb \times 1.39) + (0.003 \times p_aO_2))}$$

Where s_aHbO_2 and s_aHbO_2 are arterial and mixed venous oxyhemoglobin saturation (%), respectively; Hb is hemoglobin concentration (g/L); and, p_aO_2 and $p_{mv}O_2$ are arterial and mixed venous oxygen tensions (mm Hg).

Statistical analyses

Statistical analyses were performed using STATA version 12 (Stata Corporation, College Station, Texas, USA). Statistics are provided as mean ± standard deviation (SD) unless otherwise indicated. Two sample t-tests were performed at tests 1 and 2 in order to determine if statistical differences in age, body mass and pulmonary arterial pressures existed between implanted and non-implanted steers at the start of the study period.

Linear regression analyses were performed in order to determine if implant status was associated with PAP or sOEF while controlling for age and clustering by feedlot pen. Least squares estimates of mPAP, systolic PAP (sPAP), diastolic PAP (dPAP) and sOEF were obtained while controlling for all other explanatory variable using the 'margins' command.

RESULTS

Baseline measures

Pulmonary arterial pressures and sOEF did not differ between steers that were subsequently implanted and steers that did not receive an implant ($p > 0.05$). Age of non-implanted group at test 2 was significantly higher than the implanted group ($p = 0.02$). Prior to implanting the non-implanted group tended to be heavier than the implanted group ($p = 0.07$) (Table 9.1).

Table 9.1: Age, body mass and pulmonary arterial pressures according to treatment group and test (mean ± SD)

Variable	Test 1 (Altitude 1,560 m)				Test 2 (Altitude 1,300 m)			
	n	Implanted	n	Control	n	Implanted	n	Control
Age, days	31	398 ± 18	29	398 ± 16	31	399 ± 20	30	409 ± 15
Body mass, kg	31	410 ± 32	29	418 ± 30	31	374 ± 39	30	391 ± 32
mPAP,[1] mm Hg	31	40.5 ± 4.4	29	42.1 ± 6.0	31	41.6 ± 3.8	30	42.8 ± 4.3
sPAP,[2] mm Hg	31	65.8 ± 8.4	29	67.4 ± 10.0	31	69.1 ± 8.0	30	68.9 ± 9.9
dPAP,[3] mm Hg	31	18.6 ± 5.7	29	20.7 ± 6.1	31	14.8 ± 5.9	30	16.4 ± 7.0

[1] Mean pulmonary arterial pressure
[2] Systolic pulmonary arterial pressure
[3] Diastolic pulmonary arterial pressure

Effect of estradiol and trenbolone acetate on PAP and sOEF

At the final test the mean age of control steers was 557 days (95 % CI = 553, 561) and the mean of implanted steers was 550 days (95 % CI = 546, 555) (p = 0 .04). The body mass of implanted steers was significantly greater than controls (Mean = 649 kg, 95 % CI = 634, 665 kg; Mean = 598 kg, 95 % CI = 585, 612 kg, p < 0.001). The daily gain of implanted steers was significantly greater than controls (Mean difference = 0.37 kg per day, 95 % CI= 0.29, 0.45, p < 0.001). Daily gain of control steers was 1.32 kg per day (95 % CI = 1.26, 1.38) and 1.68 kg per day for implanted steers (95 % CI = 1.63, 1.74). Implant status was not associated with feed efficiency (p = 0.80). Implant status was not associated with mPAP, sPAP or sOEF when controlling for age and clustering by pen (Table 9.2). However, implanted cattle had a significantly lower dPAP than controls (Table 9.2).

Table 9.2: Least squares estimates of mean pulmonary arterial pressures and oxygen extraction fraction when controlling for age and clustering by pen

Variable	Implanted (n = 61)	Control (n = 58)	p-value
mPAP,[1] mm Hg	45.9 (42.9, 48.9)	50.3 (47.2, 53.4)	0.20
sPAP, [2] mm Hg	79.1 (75.1, 82.9)	81.5 (77.5, 85.6)	> 0.5
dPAP, [3] mm Hg	14.2 (11.1, 17.4)	20.2 (16.9, 23.4)	0.04
sOEF, [4] %	22.2 (20.7, 23.7)	21.3 (19.7, 22.8)	> 0.5

[1] Mean pulmonary arterial pressure
[2] Systolic pulmonary arterial pressure
[3] Diastolic pulmonary arterial pressure
[4] Systemic oxygen extraction fraction

When controlling for age and clustering by pen the effect of the implant on PAP was greatest for dPAP (Figure 9.1).

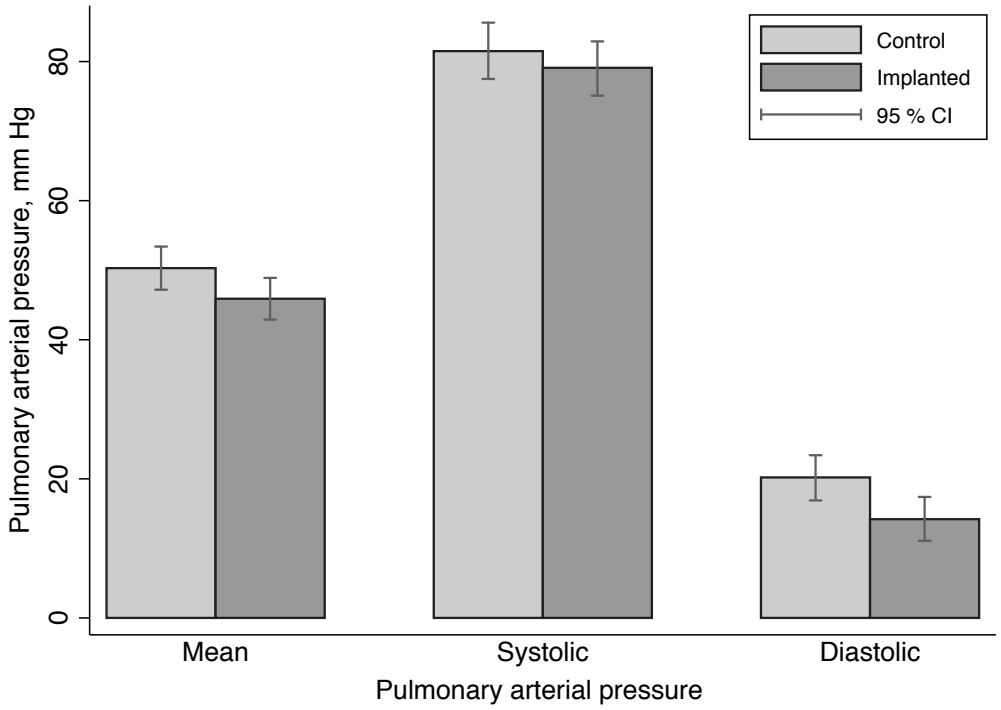

Figure 9.1: Least squares estimates of mean, systolic and diastolic pulmonary arterial pressures and 95 % confidence intervals according to growth hormone implant status while controlling for age and clustering by pen

DISCUSSION

The results of this study show that a growth-promoting implant containing trenbolone acetate and estradiol was not statistically associated with mean or systolic pulmonary arterial pressure when controlling for age and clustering by pen. However, diastolic pulmonary arterial pressure was significantly lower in implanted steers than non-implanted controls. Systemic oxygen extraction was not associated with implant status despite implanted steers having a significantly greater growth rate during the feeding period. These results indicate that hormonal growth implants containing trenbolone acetate and estradiol may have cardio-protective effects in feedlot steers.

A limitation of this study is that only steers from one herd were studied. However, we believe that the herd studied was characteristic of many cow-calf operations in the western U.S. High-altitude producers in the Rocky Mountain region select for low mPAP in order to reduce the incidence of CHF in their calf crop. Many producers also breed cows by artificial inseminate using semen from bulls maintained at low altitude and therefore, the suitability of their offspring for life at high altitude is unknown. Therefore, we believe that the study population was largely reflective of cattle born and raised in the Rocky Mountain region.

We had hypothesized that the increased growth rate associated with the implant would increase mPAP and systemic oxygen extraction fraction (sOEF) relative to non-implanted controls. However, we did not anticipate the magnitude of the growth-independent effects of the steroids on PAP. The beneficial cardio-vascular effects of 17-β-estradiol include: reduced oxidative damage, increased production of the vasodilatory

compound nitric oxide (LantinHermoso et al., 1997), reduced production of the vasoconstrictor endothelin-1 and inhibition of vascular smooth muscle proliferation (Mendelsohn and Karas, 1999).

There are also numerous studies reporting a beneficial action of estradiol for reversing or reducing the magnitude of pulmonary hypertension. Estradiol causes pulmonary vasodilation under normoxic conditions (English et al., 2001) and prevents pulmonary vasoconstriction under hypoxic conditions (Sylvester et al., 1985). Estradiol was found to improve pulmonary hemodynamics and vascular remodeling in perinatal lambs with pulmonary hypertension induced by partial ligation of the ductus arteriosus in utero (Parker et al., 2000). Estrogen therapy reversed right ventricular remodeling in both male and female rats with pulmonary hypertension (Nadadur et al., 2012), which may explain the significant reduction in dPAP associated with steroid supplementation in our study. However, the effects of estrogens appear paradoxical: in humans, females are more susceptible to pulmonary hypertension than males but the severity of the disease is less. Although there are numerous protective cardio-vascular effects associated with estrogens there are also numerous deleterious effects of estrogens and their metabolites reported (Austin et al., 2013; Tofovic, 2010).

Less is known about the effects of androgens, such as trenbolone, on pulmonary physiology. Testosterone is reported to have pulmonary vasodilatory properties (Jones et al., 2002; Rowell et al., 2009; Smith et al., 2008) and may be a more potent pulmonary vasodilator than estradiol (English et al., 2001). The endogenous steroid hormone dihydroepiandrosterone (DHEA) is metabolized to form a number of compounds including estradiol and testosterone. It has been shown that DHEA partially reverses the effect of

chronic-hypoxia on mPAP and pulmonary artery wall thickness in rats (Bonnet et al., 2003; Hampl et al., 2003; Oka et al., 2007).

In our study the potentially adverse consequences of a steroid-induced increased rate of growth on mPAP were likely offset by protective actions of the same steroids on the cardio-vascular system. Cattle, which are naturally susceptible to pulmonary hypertension, may serve as a suitable model for the study the effects of sex steroids on the development and progression of pulmonary hypertension. Unlike many other animal models of pulmonary hypertension neointimal lesions occur in bovine pulmonary hypertension (Neary et al., 2013b), which is a characteristic feature of severe pulmonary hypertension in humans.

CHAPTER 10: GENOME-WIDE ASSOCIATION STUDY OF PULMONARY ARTERIAL PRESSURE AND TRAITS ASSOCIATED WITH PULMONARY ARTERIAL PRESSURE IN BOVINE CALVES

INTRODUCTION

Pulmonary hypertension is a leading cause of right-sided heart failure in cattle (Blake, 1965; Will et al., 1975b). Historically, it was considered to be a disease of high altitude (Glover and Newsom, 1915; Hecht et al., 1962). The average herd incidence of congestive heart failure (CHF) has been estimated to be 2 % but as high as 10 % on ranches located at altitudes over 2,500 m (Glover and Newsom, 1915; Hecht et al., 1962) with calves less than 1-year old most at risk (Hecht et al., 1962). More recently, it has been reported to occur a altitudes around 1,600 m in feedlot cattle (Jensen et al., 1976) and Holstein heifers (Malherbe et al., 2012).

Measurement of mPAP is used to predict the likelihood of CHF. Cattle with a higher mPAP are considered to be at increased risk of CHF. However, the prognostic value of mPAP in cattle has not been determined. Mean PAP has been estimated to be moderately heritable (Shirley et al., 2008) and thought to be transmitted in an autosomal dominant manner (Anand et al., 1986; Weir et al., 1974), which means that susceptibility to hypoxia-induced pulmonary hypertension may lie within a small number of major genes. Cattle of any age with a mPAP > 49 mm Hg are considered to be at high risk of congestive heart failure and should not be used as herd sires due to the heritability of the trait (Holt and Callan, 2007).

We have found that the delivery of oxygen to peripheral tissues may be compromised in calves at high altitude (Neary et al., 2013a). Despite the chronic hypoxic exposure the hematocrit of calves did not increase (Neary et al., 2013a). We have also shown that poor cardiac reserve in pre-weaned calves at high altitude is positively associated with mean pulmonary arterial pressure (mPAP) and increased risk of mortality (Chapter 6).

The goal of this study was to identify chromosomal regions associated with pulmonary arterial pressure and traits associated with mPAP in calves. The first objective was to identify physiological traits associated with mean pulmonary arterial pressure. The second objective was to perform a high-density (800k) genome-wide association study (GWAS) of pulmonary arterial pressure and traits found to be associated with mPAP in calves at 4 and 6 months of age. Chromosomal regions associated with hematocrit were also evaluated in order to elucidate underlying mechanisms regulating erythrocyte production was performed on a cohort of calves at 4 and 6 months of age. We hypothesized that SNPs associated with mPAP would be shared with traits found to be physiologically associated with mPAP.

MATERIALS AND METHODS

Study overview

Physiological measurements, including pulmonary arterial pressure and blood-gas analyses of arterial and mixed venous blood, were collected from a cohort of calves at approximately 4 and 6 months of age. At 4 months of age 60 calves were tested. At 6

months of age the same 60 calves were tested plus an additional 5 calves of the same age that were found to have a high mean pulmonary arterial pressure. Whole-blood samples were collected from all calves for DNA. A GWAS was performed on pulmonary arterial pressure (mean, systolic and diastolic) and traits associated with mPAP. A GWAS was also performed on hematocrit in order to determine why hematocrit fails to increase in response to chronic hypoxic exposure. The study received approval from the Colorado State University Animal Care and Use Committee prior to the sampling or handling of any animals (Protocol ID: 13-4136A).

Study site and calf cohort

The study was conducted at the Colorado State University Beef Improvement Center (Saratoga, WY). The herd consisted predominantly of Black Angus cattle but < 10 % of the herd was Red Angus. Approximately 50 % of the calves studied were progeny of bulls with a mPAP < 40 mm Hg at 2,170 m above sea-level. The other 50 % of the calves were progeny of industry relevant bulls of an unknown mPAP. Heifers with low mPAP were retained as herd replacements each year. Therefore, the maternal line has also been selected for 'low' mPAP.

A cohort of approximately 60 male calves was randomly sampled at approximately 4 months of age (Table 10.1). Every other calf into the chute was sampled. The same 60 calves were studied at 6 months of age. All male calves within the herd were PAP tested at 6 months of age for herd management genetic selection purposes unrelated to the study. Five calves that were not tested at 4 months of age but had a notably high mPAP at 6months of age (53 to 64 mm Hg) were included in the dataset.

There were 17 sires of the 65 calves in this study: 3 bulls had 7 calves each, 4 bulls sired only 1 calf and the remaining 10 bulls sired between 2 and 6 calves each.

Table 10.1: **The number, gender and age of calves sampled according to the date of sampling**

Test	Number sampled			Date sampled	Altitude, m	Mean age ± SD, days
	Bull	Steer	Total			
1	55	5	60	07/31/12	2,166	124 ± 18
2	60	5	65*	10/01/12		186 ± 18

* Five additional calves with high mean pulmonary arterial pressures were included at test 2

The dams of calves studied were given a pre-breeding and pre-calving vaccination offering protection against *Bovine herpesvirus 1* (infectious bovine rhinotracheitis [IBR]), *Bovine viral diarrhea virus* (BVDV), *Bovine respiratory syncytial virus* (BRSV), and *Bovine parainfluenza virus 3* (BPIV-3). Calves were vaccinated against the same respiratory pathogens at 4 to 8 weeks of age and 2 to 4 weeks prior to weaning. This herd has tested negative for BVDV by ELISA.

Pulmonary arterial pressure measurement

A full description of the equipment, materials and facilities required for pulmonary arterial pressure testing was provided by Holt and Callan (2007) In brief, a 13 gauge needle was inserted into the jugular vein. Flexible saline-filled catheter tubing was then fed through the needle, through the right atrium, into the right ventricle, and then into the pulmonary artery. A pressure transducer connected the catheter to an oscilloscope. The position of the catheter was determined from the pressure waveform on the oscilloscope as

the jugular vein, right atrium, right ventricle and pulmonary artery have distinct pressure waveforms (Holt and Callan, 2007). Mean, systolic and diastolic pressures were recorded after the pulmonary pressure waveforms stabilized.

Blood-gas analysis

Approximately, 2.5 ml of 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 as the bovine coccygeal artery is a suitable source for blood-gas analysis (Collie, 1991; Nagy et al., 2002). Mixed venous blood was collected from the pulmonary artery via the catheter used for pulmonary arterial pressure measurement. 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 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 analyzer (VetScan i-STAT 1, Abaxis, Union City, CA, USA). A temperature 'correction' algorithm was used to adjust blood-gas tensions according to rectal temperature (CLSI, 2001).

Systemic Oxygen Extraction Fraction

The systemic oxygen extraction fraction (sOEF) represents the rate oxygen consumption by the peripheral tissues (VO_2) expressed as a fraction of the rate of oxygen delivered (DO_2). In healthy mammals at rest sOEF is approximately 0.2 (Cambier et al.,

2008; Dellinger, 2002; Leach and Treacher, 2002; McLellan and Walsh, 2004). As demand for oxygen increases (VO_2) or delivery of oxygen decreases (DO_2) the OEF must increase in order to maintain aerobic metabolism. Inadequate cardiac output (DO_2) to meet VO_2 results in sOEF to exceed 0.3, or 30 % (Olkowski et al., 2005; Rady et al., 1994). Systemic OEF is calculated using the following formula:

$$sOEF = \frac{((s_aHbO_2 \times Hb \times 1.39) + (0.003 \times p_aO_2)) - ((s_{mv}HbO_2 \times Hb \times 1.39) + (0.003 \times p_{mv}O_2))}{((s_aHbO_2 \times Hb \times 1.39) + (0.003 \times p_aO_2))}$$

Where s_aHbO_2 and s_aHbO_2 are arterial and mixed venous oxyhemoglobin saturation (%), respectively; Hb is hemoglobin concentration (g/L); and, p_aO_2 and $p_{mv}O_2$ are arterial and mixed venous oxygen tensions (mm Hg).

Genotyping

Blood was collected from the jugular vein during the pulmonary arterial pressure measurement process. DNA was genotyped on a SNP panel (BovineHD BeadChip, Illumina, San Diego, CA) by GeneSeek (Neogen, Lincoln, NE).

Statistical Analyses

Statistical analyses of pulmonary arterial pressures were performed using STATA version 12 (Stata Corporation, College Station, Texas, USA). Paired t-tests and pairwise correlation analyses were performed between ages 4 and 6 months of age for mPAP, dPAP and sPAP. Statistics are reported as mean ± standard deviation (SD) unless otherwise indicated.

Generalized estimating regression equations were used to evaluate physiological associations with mPAP as they account for repeated measures (Liang and Zeger, 1986; Zeger and Liang, 1986). An exchangeable correlation structure was used. Generalized estimating regression equations are robust to missing observations. The following explanatory variables were evaluated for association with the outcome variable mPAP: pH, pO_2 and pCO_2, L-lactate and sOEF. For the first 3 variables (pH, pO_2 and pCO_2), both arterial and mixed venous forms were evaluated. All variables were evaluated individually for association with mPAP when controlling for the covariate age and categorical variables ranch and gender. Variables achieving a statistical significance of < 0.20 were included in the full regression model. Backwards step-wise regression was then performed until all variables in the final model had a statistical significance ≤ 0.05 when controlling for the other explanatory variables herd, age and gender.

Genome-wide association was performed using SNP Variation Suite 7 (Golden Helix, Bozeman, MT). In this study, SNPs with a call rate < 0.95 (n = 17,934), a minor allele frequency < 0.05 (n = 197,595) or a LD $r^2 < 0.99$ (n = 32,0748) were removed from the analysis. Principle component analysis was performed to evaluate the sample substructure. Models were run using a Multi-Locus Mixed Model (Segura et al., 2012). All traits were transformed to a normal distribution prior to GWAS as necessary. Models were applied using both dominant and additive mechanisms of allele action. The age and gender of calf (bull or steer) and parity of dam were included in all models as potential sources of variation. Bonferroni correction was performed to provide a family-wise type I error of 0.05. The mapping and annotation of SNPs was performed using Ensembl (release 74, December 2013) and genome assembly UMD3.1. The top 10 SNPs for both additive and

dominant models of all traits are provided in the appendix. The closest single gene within 100,000 base pairs of each SNP was recorded. If two genes were within 5,000 base pairs from a SNP both were reported.

RESULTS AND DISCUSSION

Pulmonary arterial pressures

Mean PAP tended to be 2.5 mm Hg higher at 6 months of age than 4 months of age (95 % CI = -0.6, 5.6; p = 0.11) (Table 10.2). Systolic PAP was 4.6 mm Hg higher at 6 months of age than 4 months of age (95 % CI = 1.2, 8.1; p = 0.008). Diastolic PAP did not differ between age groups (p = 0.92).

Table 10.2: Pulmonary pressures (mean, systolic and diastolic) according to age (mean ± SD) and test of statistical difference between the cohort at 4 and 6 months of age

Variable	4 months old		6 months old			6 months old*	
	n	Pressure, mm Hg	n	Pressure, mm Hg	p-value	n	Pressure, mm Hg
mPAP	60	41.4 ± 7.9	60	43.9 ± 9.2	0.11	65	44.9 ± 9.5
sPAP	60	57.3 ± 9.6	60	62.0 ± 9.1	0.008	65	63.3 ± 10.3
dPAP	60	26.5 ± 7.0	60	26.6 ± 6.3	0.92	65	27.3 ± 6.5

* Five additional calves with high mean pulmonary arterial pressures were included in the study at 6 months of age.

Pairwise correlation of mPAP between 4 and 6 months of age was strong (r = 0.87; p < 0.001) (Figure 10.1) and moderate for dPAP (r = 0.40; p = 0.002). Pairwise correlation of sPAP between 4 and 6 months was weak (r = 0.23; p = 0.08).

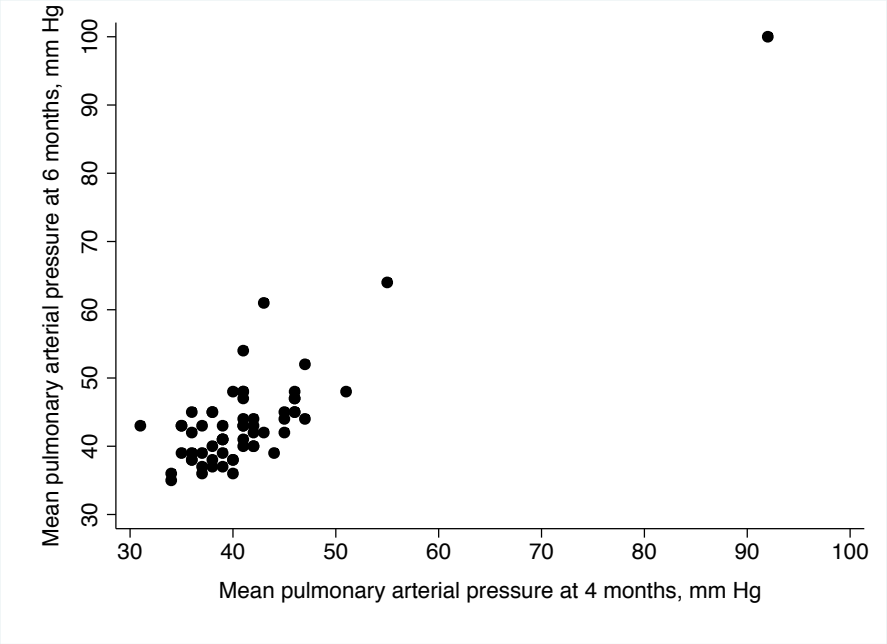

Figure 10.1: Dot plot of mean pulmonary arterial pressure at 4 months and 6 months of age, mm Hg

Oxygen extraction, lactate, mixed venous pCO_2 and hematocrit

Lactate and mixed venous CO_2 were significantly lower at 6 months of age than 4 months of age (Table 10.3). Oxygen extraction tended to be lower at 6 months relative 4 months of age. Hematocrit was significantly higher at 6 months than 4 months of age.

Table 10.3: Systemic oxygen extraction fraction (sOEF), lactate, mixed venous CO_2 tension (p_vCO_2) and hematocrit by test

Variable	4 months old		6 months old			6 months old*	
	n	Mean ± SD	n	Mean ± SD	p-value	n	Mean ± SD
sOEF, %	60	23.5 ± 5.0	57	21.7 ± 6.7	0.09	62	21.8 ± 7.0
L-lactate, mmol/L	59	1.8 ± 1.2	58	1.1 ± 0.8	< 0.001	63	1.1 ± 0.8
p_vCO_2, mm Hg	60	43.7 ± 3.9	58	40.3 ± 3.8	< 0.001	63	39.9 ± 4.2
Hematocrit, %	60	29.8 ± 3.7	60	30.9 ± 2.7	0.02	65	30.9 ± 2.7

* Five additional calves with high mean pulmonary arterial pressures were included at 6 months of age.

Pairwise correlation of sOEF (r = 0.05; p = 0.72) and L-lactate (r = 0.30; p =0.03) between ages 4 and 6 months was weak. Pairwise correlation between sOEF and L-lactate across all ages was also weak (r = 0.13; p = 0.15). High sOEF was not necessarily associated with a high L-lactate concentration (Figure 10.2). This suggests that inadequate oxygen delivery, as indicated by high sOEF, is not necessarily associated with anaerobic metabolism. The increased oxygen extraction may be sufficient to maintain aerobic respiration.

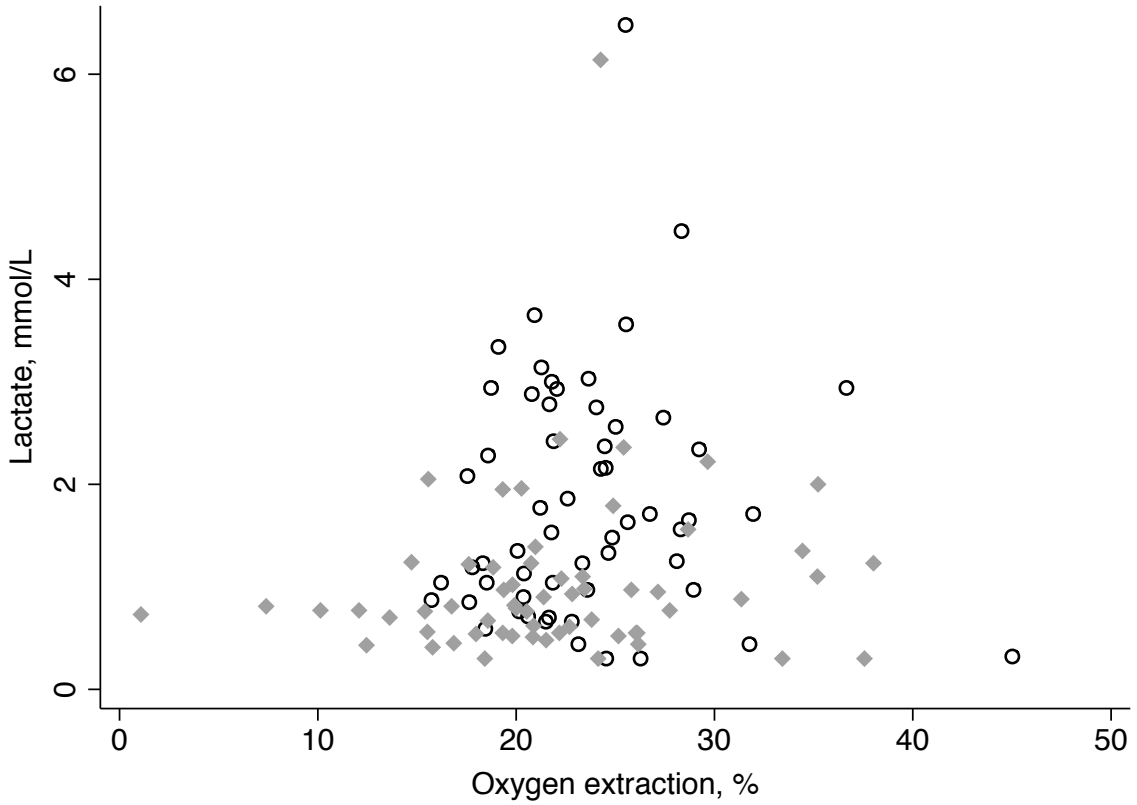

Figure 10.2: A scatter graph of L-lactate versus systemic oxygen extraction fraction at 4 months of age (circles) and at 6 months of age (diamonds). Pairwise correlation between systemic oxygen extraction and L-lactate across all ages was weak (r = 0.13; p =0.15)

Physiological associations with mean pulmonary arterial pressure

Systemic oxygen extraction fraction (p = 0.007), L-lactate (p = 0.05) and mixed venous pCO_2 (p = 0.05) were associated with mPAP when controlling for age and gender (Table 10.4).

Table 10.4: Change in mean pulmonary arterial pressure for a 1-unit change in systemic oxygen extraction fraction (sOEF), L-lactate, mixed venous CO_2 tension (p_vCO_2,) when controlling for age and gender

Variable	Mean	95 % CI	p-value
sOEF, %	-0.07	-0.28, 0.13	0.48
L-lactate, mmol/L	-4.60	-8.48, -0.72	0.01
sOEF x Lactate interaction	0.19	0.04, 0.35	0.02
$p_{mv}CO_2$, mm Hg	0.27	0.00, 0.54	0.05
Age, days	0.06	0.04, 0.08	< 0.001
Steer (relative to bull)	-5.8	-14.4, 2.8	0.18
Intercept	25.1	11.6, 38.6	< 0.001

There was an interaction between sOEF and L-lactate (p = 0.01). High L-lactate and high sOEF were associated with high mPAP (Figure 10.3).

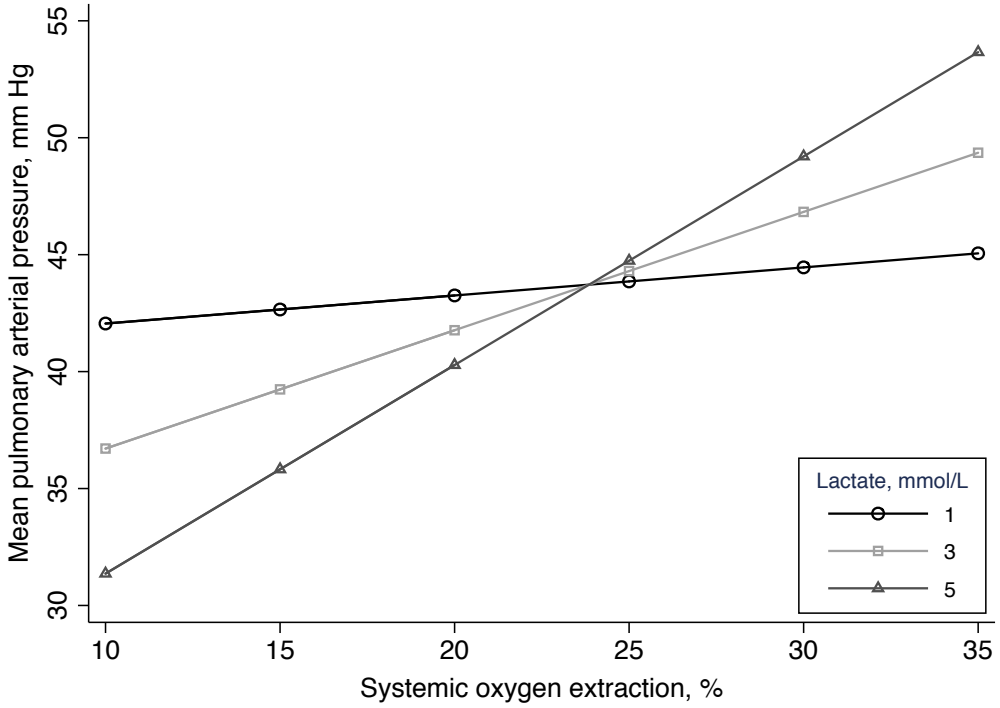

Figure 10.3: Least squares estimate of mean pulmonary arterial pressure (mPAP) against systemic oxygen extraction fractions at L-lactate concentrations of 1, 3 and 5 mmol/L while controlling for mixed venous CO_2, age and gender

Genotypic results

Principle component analysis revealed that the calves were genetically very similar (Figure 10.4).

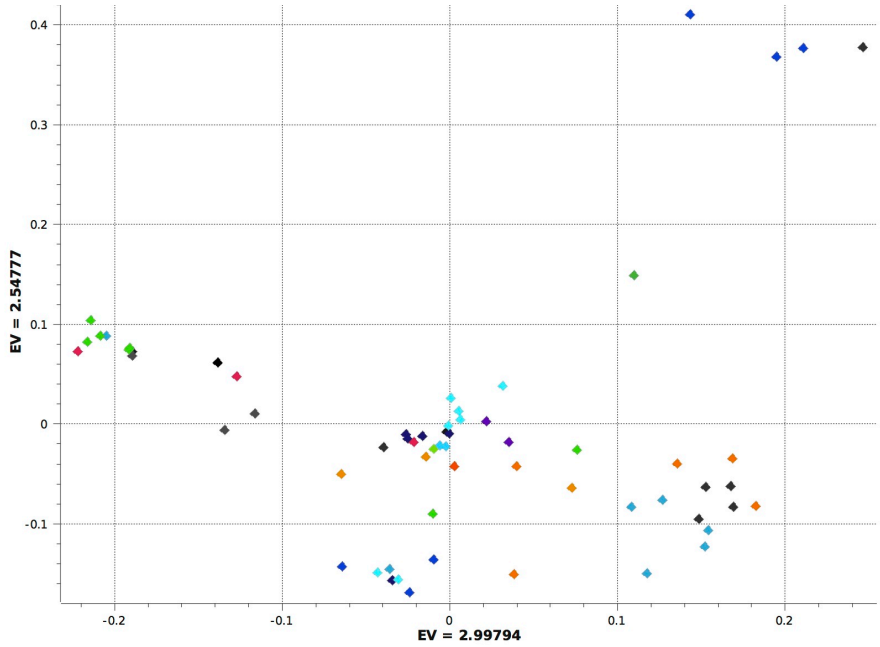

Figure 10.4: Principle component analysis showing high genetic similarity (Eigenvalue (EV) < 3.0) among calves (n = 65)

Mean pulmonary arterial pressure at 4 months of age

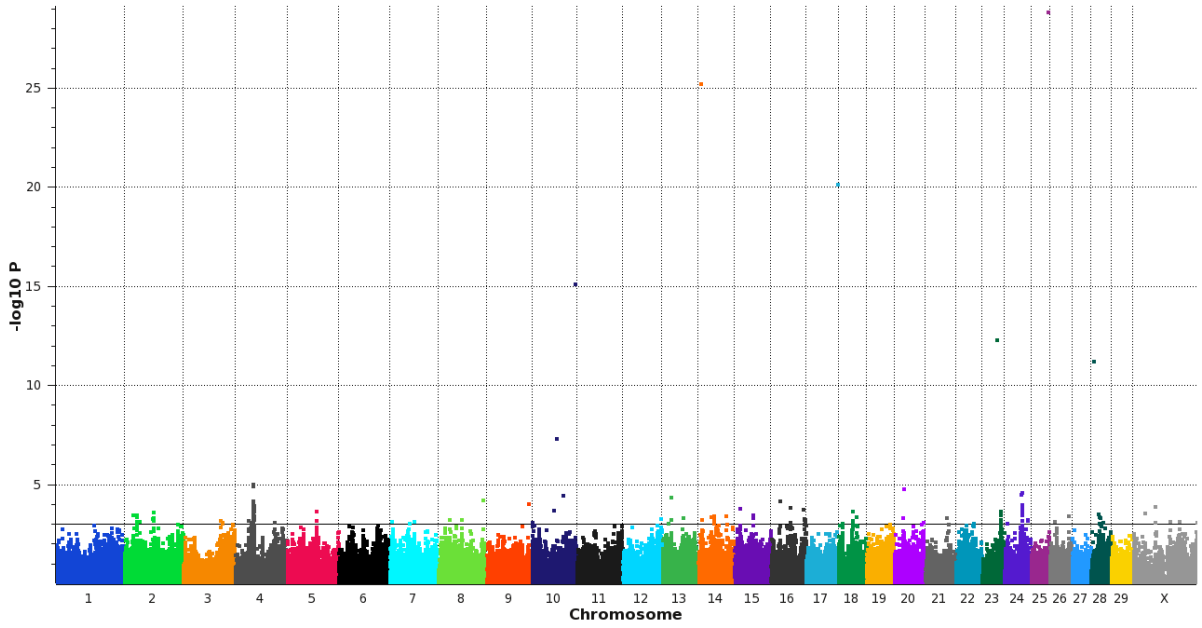

Figure 10.5: Manhattan plot showing genome-wide association p-values (-log 10) of single nucleotide polymorphisms (SNPs) associated with mean pulmonary arterial pressure at 4 months of age using an additive model of allele action (n = 60)

No SNPs were associated with mPAP using the dominant model of allele action. However, using an additive model of allele action, seven SNPs were associated with mPAP at 4 months of age (Figure 10.5). The first SNP (rs42080473) was located within an intergenic region approximately 30 kbp away from the nearest gene, neuronal pentraxin-2 (*NPTX2*) ($p < 0.001$). The frequency of the minor allele C was 0.08. Calves with alleles AC (n = 8) and CC (n = 1) had mPAPs that were 5.4 mm Hg (95 % CI = -1.4, 12.1) and 11.0 mm Hg (95 % CI = -4.8, 26.8) lower than calves with alleles AA (n = 55), respectively (Figure 6). In humans, aberrant methylation of *NPTX2* is predictive of pancreatic neoplasia (Yao et al., 2013) and is prognostic of a poor outcome in Ewing sarcoma (Alholle et al., 2013). These findings suggest that *NPTX2* may play a role in the modification of cellular plasticity. A change in cellular phenotype in response to hypoxia is reported in pulmonary hypertension (Shan et al., 2014; Stenmark et al., 2006). It is plausible that methylation of *NPTX2* may contribute to pulmonary vascular remodeling.

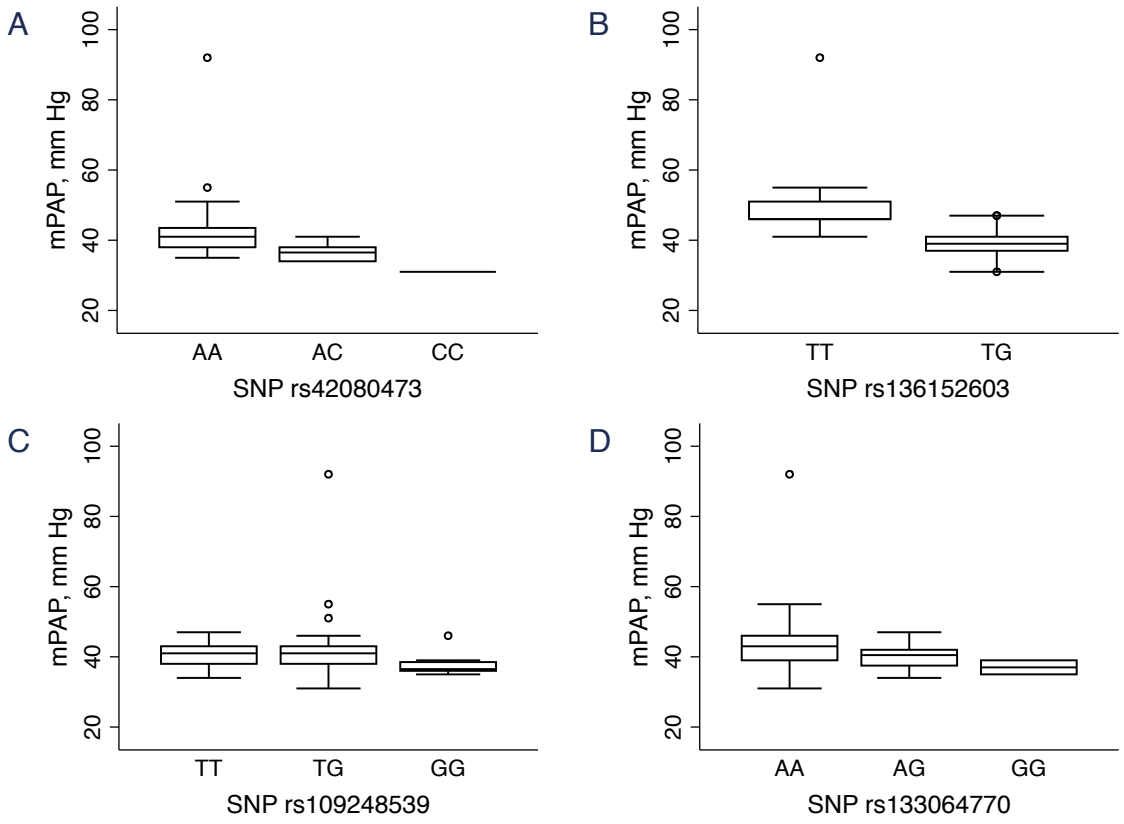

Figure 10.6: A box and whisker plot of mean pulmonary arterial pressure (mPAP) at 4 months of age against allelic variants of SNP A) rs42080473, B) rs136152603 C) rs109248539 and D) rs133064770. Hollow circles represent outliers (observations that are 1.5 times the interquartile range away from the median)

A second SNP (rs136152603) associated with mPAP at 4 months of age ($p < 0.001$) was located within an intron of gene *FAM135B* (family with sequence similarity, member B). Variation in this gene has been associated with susceptibility to extra-pulmonary tuberculosis (Oki et al., 2011). The frequency of the minor allele G was 0.08. Calves with alleles TT (n = 55) had an average mPAP that was 12.6 mm Hg higher (95 % CI = 7.9, 17.4) than calves with alleles TG (n = 9) ($p < 0.001$) (Figure 10.6).

The third SNP (rs109248539) associated with mPAP at 4 months of age ($p < 0.001$) was located within an intron of gene *SGLT1* (sodium-dependent glucose transporter-1)

(Figure 10.6). In humans, expression of *SGLT1* in the heart is high relative to most other tissues other than the small intestine (Zhou et al., 2003). Myocardial expression is reported to be up-regulated in disease states such as myocardial ischaemia (Banerjee et al., 2009). The frequency of the minor allele G was 0.43.

The fourth SNP (rs133064770) associated with mPAP at 4 months of age ($p < 0.001$) was located within an intergenic region; no genes were identified within 100 kbp of this SNP. It is possible that SNPs located within non-coding regions affected phenotypic expression of the various traits measured in this studied by regulating gene expression. For example, SNPs in microRNA genes influence cancer susceptibility in humans (Slaby et al., 2012). The frequency of minor allele G was 0.40. Calves with alleles AG (n = 38) and GG (n = 7) had mPAPs that were 5.2 mm Hg (95 % CI = 0.7, 9.7) and 8.5 mm Hg (95 % CI = 1.7, 15.3) lower than calves with alleles AA (n = 20), respectively (Figure 10.6).

The fifth SNP (rs137207558) associated with mPAP at 4 months of age ($p < 0.001$) was identified to be a 5' UTR variant of the gene with hormone activity *PRP3* (placental prolactin-related protein-3). The minor allele G had a frequency of 0.30. Calves with alleles AA (n = 12) and AG (n = 37) had mPAPs that were 12.5 mm Hg (95 % CI = 4.4, 20.5) and 12.4 mm Hg (95 % CI = 4.4, 20.5) lower than calves with alleles GG (n = 16), respectively (Figure 10.7). Prolactin and related molecules have recently been found to be produced by endothelial cells and may place a role in the regulation of angiogenesis (Corbacho et al., 2002; Ferrara et al., 1991). Misguided angiogenesis is thought to contribute to the pathology associated with pulmonary hypertension (Tuder and Voelkel, 2002).

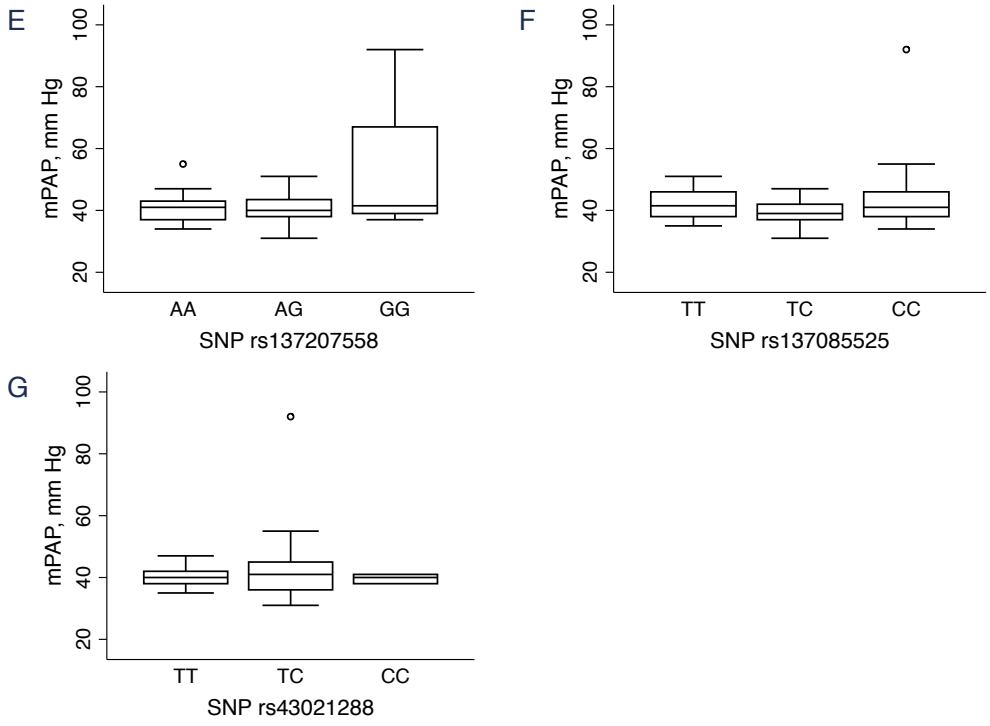

Figure 10.7: A box and whisker plot of mean pulmonary arterial pressure (mPAP) at 4 months of age against allelic variants of SNP E) rs137207558, F) rs137085525, and G) rs43021288. Hollow circles represent outliers (observations that are 1.5 times the interquartile range away from the median)

The sixth SNP (rs137085525) associated with mPAP at 4 months of age ($p < 0.001$) was located within an intron of gene *PCNXL2* (pecanex-like 2) a membrane constituent of unknown function. The frequency of minor allele T was 0.47. Calves with alleles TC (n = 37) and CC (n = 16) had mPAPs that were 2.3 mm Hg (95 % CI = -2.9, 7.6) lower and 2.6 mm Hg (95 % CI = -3.4, 8.6) higher than calves with alleles TT (n = 12), respectively (Figure 10.7).

The seventh SNP (rs43021288) associated with mPAP at 4 months of age ($p = 0.01$) was located within an intron of *UNC13C* (unc-13 homolog C), which is thought to be involved in intra-cellular signal transduction. The frequency of minor allele T was 0.40. Calves with alleles TC (n = 33) and CC (n = 3) had mPAPs that were 1.7 mm Hg (95 % CI = -

2.6, 6.0) higher and 0.9 mm Hg (95 % CI = 10.7, 8.9) lower than calves with alleles TT (n = 29), respectively (Figure 10.7).

Mean pulmonary arterial pressure at 6 months of age

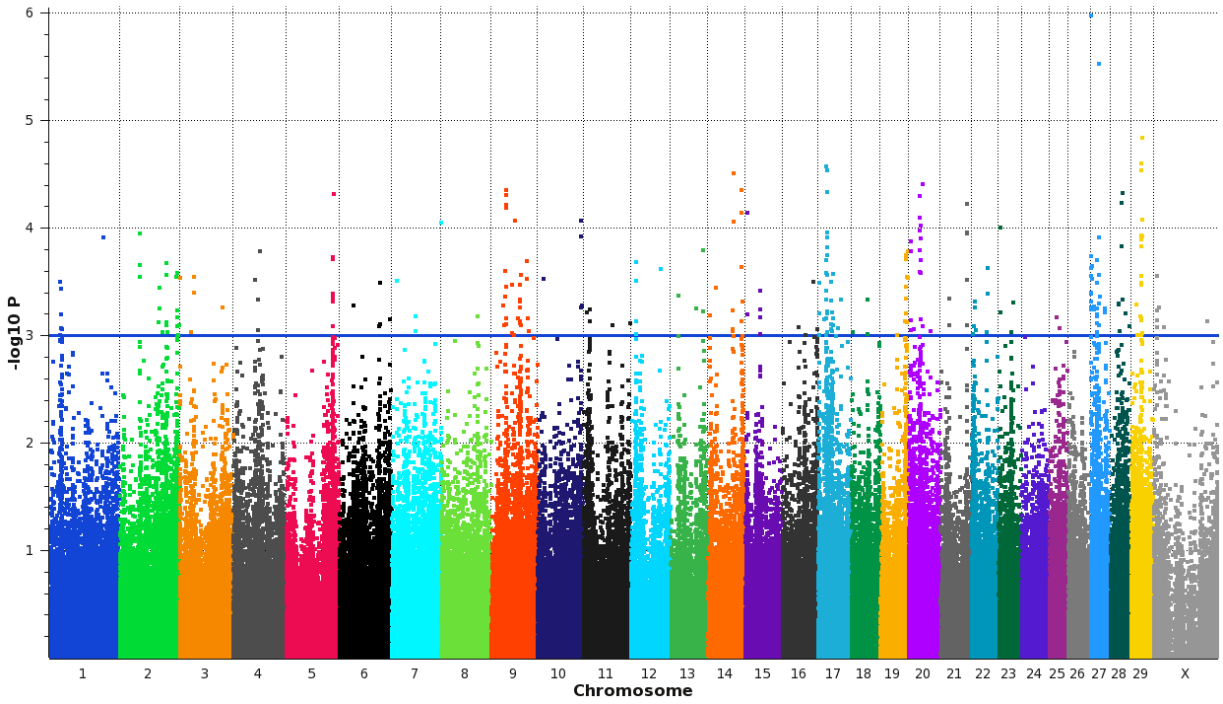

Figure 10.8: Manhattan plot showing genome-wide association p-values (-log 10) of single nucleotide polymorphisms (SNPs) associated with mean pulmonary arterial pressure at 6 months of age using an additive model of allele action (n = 65)

No SNPs were statistically associated with mean pulmonary arterial pressure at 6 months of age (Figure 10.8). The closest SNP to achieve statistical significance was rs137508599 (p = 0.25). At present there is no known gene within 100 kbp of this SNP. The minor allele C had a frequency of 0.46. Calves with alleles TC (n = 38) and CC (n = 11) had mPAPs that were 5.9 mm Hg (95 % CI = 0.8, 11.0) and 14.0 mm Hg (95 % CI = 7.3, 10.6) higher than calves with alleles TT (n = 16), respectively (Figure 10.9).

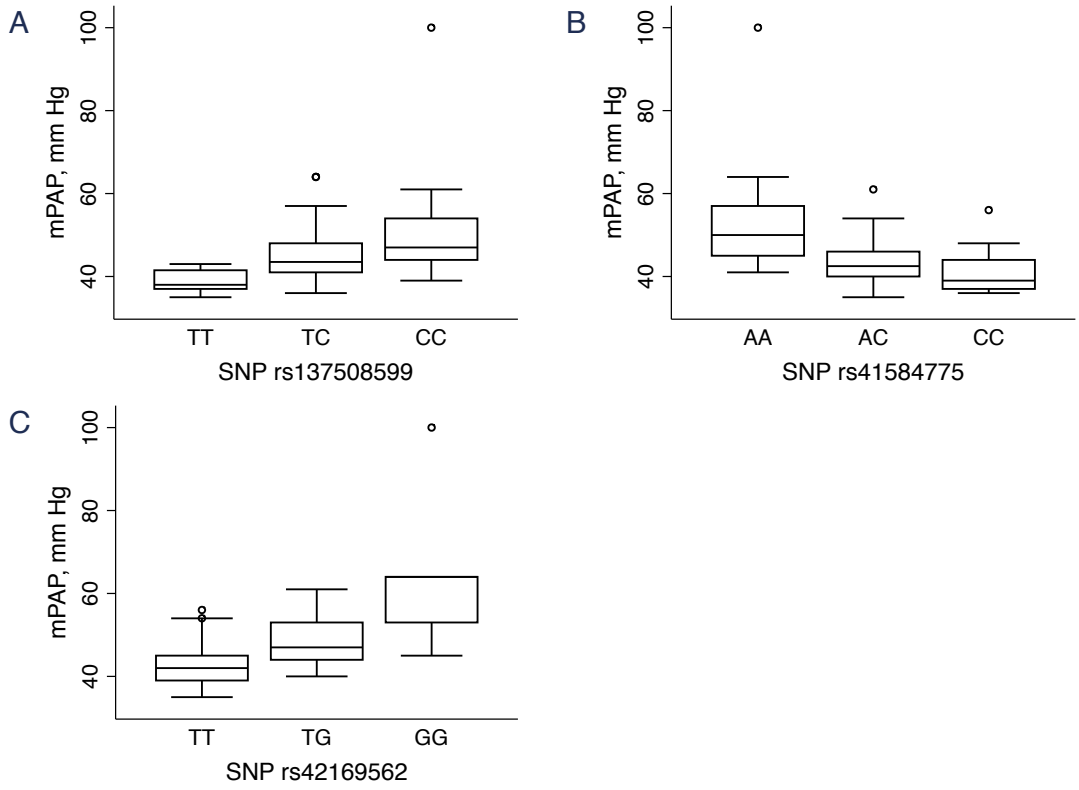

Figure 10.9: A box and whisker plot of mean pulmonary arterial pressure (mPAP) at 6 months of age against allelic variants of SNP A) rs137508599 B) rs41584775 and C) rs42169562. Hollow circles represent outliers (observations that are 1.5 times the interquartile range away from the median)

The SNP rs41584775 is located within an intron of gene *MTUS1* (microtubule associated tumor suppressor 1). Although not statistically associated with mPAP at 6 months of age (p = 0.72, Figure 10.9), the splice variants of *MTUS1* produce a family of ATIP (angiotensin type 2 interacting protein) proteins (Rodrigues-Ferreira and Nahmias, 2010), which could plausibly contribute to mPAP. It is thought that ATIPs are intimately involved in control of cell proliferation; down-regulation of ATIP has been reported in various cancers (Rodrigues-Ferreira and Nahmias, 2010). Studies of mice have found that ATIP expression is greatest in the heart (Zuern et al., 2012) and highly expressed in the developing cardiovascular system (Bundschu and Schuh, 2013). Interestingly, *MTUS1*

knockout (KO) mice had a significantly higher body weight than wild-type mice at 4 weeks and 10 months of age (Zuern et al., 2012). Heart hypertrophy and glomerulonephritis were identified in 28 % and 12 % of *MTUS1* KO mice, respectively. The heart to body weight ratio was significantly greater in *MTUS1* KO mice relative to wild-type (Zuern et al., 2012). A skin fibroblast proliferation assay of 3 wild-type and 3 KO mice found that cell proliferation rate was similar except for one of the KO mice lines, which had a fibroblast proliferation rate that was approximately double that of the other mice (Zuern et al., 2012). This suggests that the fibroblast proliferation may have been dependent on other factors. Proliferation of fibroblasts from the 3 KO mice was less sensitive to a reduction of growth factors present in the medium than fibroblasts from wild-type mice (Zuern et al., 2012). This supports previous findings that *MTUS1* has anti-proliferative effects (Nouet et al., 2004). Activation of the AT2 receptor is reported to oppose the pro-inflammatory effects of AT1 receptor activation through attenuating TNFα mediated nuclear factor-κB activation and expression of monocyte chemoattractant protein-1 in the vascular smooth muscle of fetal rats (Wu et al., 2004). In a mouse model of vascular injury overexpression of ATIP1 or its receptor, AT2 was protective against neointima formation, vascular proliferation, inflammation and oxidative stress (Fujita et al., 2009). These findings may have implications for pulmonary lesion development in association with pulmonary hypertension.

The third highest ranked SNP (rs42169562) associated with mPAP at 6 months of age was located within an intron of *NAV2* (neuron navigator 2) but a statistical association was not detected ($p > 0.99$, Figure 10.9). The gene *NAV2* encodes a member of the neuron navigator gene family, which is believed to be involved in cell migration and growth. NAV2 KO out showed a blunted baroreceptor reflex relative to wild-type mice; *NAV2* KO mice

showed a reduced ability to increase or decrease heart rate in response to the vasodilator sodium nitroprusside and the vasoconstrictor angiotensin II, respectively (McNeill et al., 2010). In healthy individuals, an increase in systemic vascular pressure and consequently distension of the carotid and aorta and baroreceptor activation initiates a slowing of the heart rate in order to bring systemic blood pressure back towards baseline. The *NAV2* gene has also been associated with carotid plaque formation in humans (Dong et al., 2012). Inappropriate baroreceptor reflex activity associated with *NAV2* and consequently vascular injury may be responsible for the development of such vascular lesions. Interestingly, *NAV3* (neuron navigator 3) was not associated with mPAP but was the closest gene to two SNPs associated with sPAP at 4 months of age: rs43424011 and rs42770702. It is thought that *NAV3* copy number changes may determine susceptibility to colorectal cancer (Carlsson et al., 2012). Therefore, although it is plausible that *NAV2* is linked to bovine pulmonary hypertension.

Systolic pulmonary arterial pressure

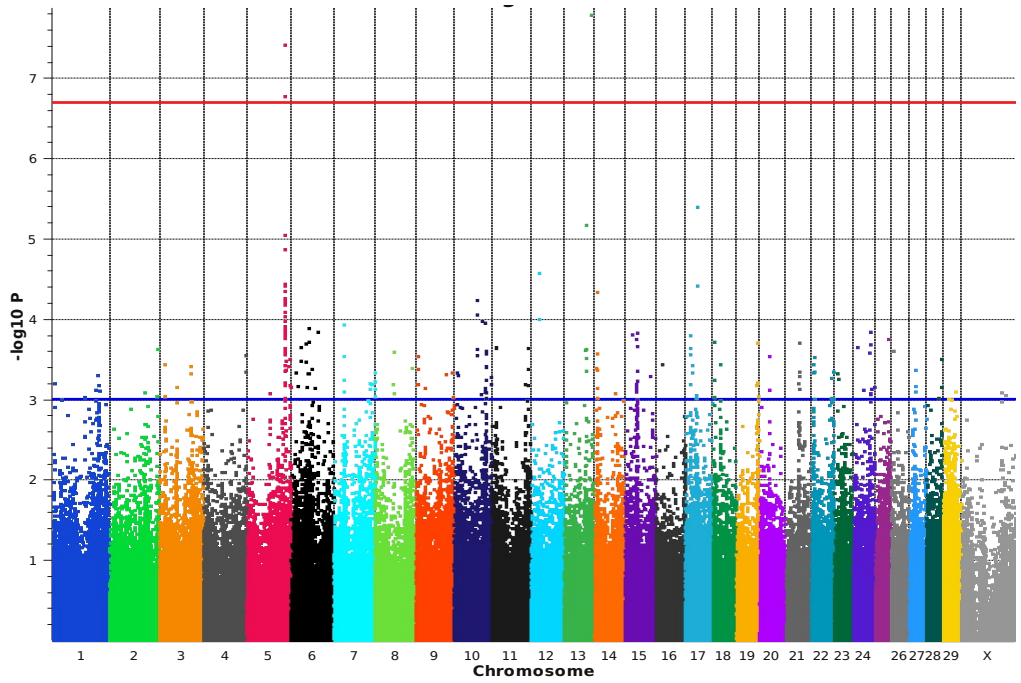

Figure 10.10: Manhattan plot showing genome-wide association p-values (-log 10) of single nucleotide polymorphisms (SNPs) associated with systolic pulmonary arterial pressure at 6 months of age using a dominant model of allele action (n = 65)

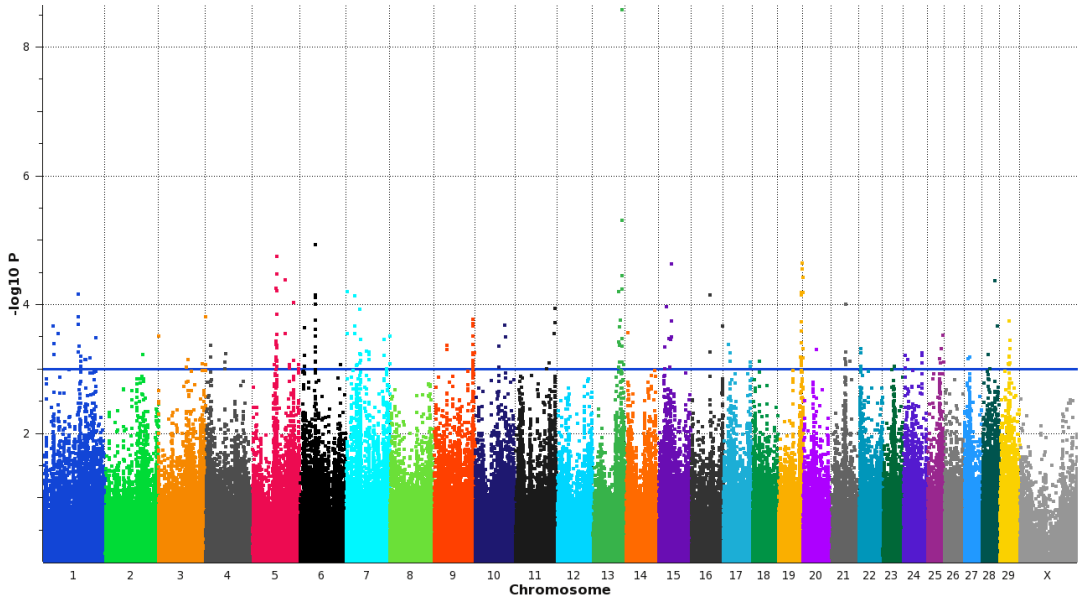

Figure 10.11: Manhattan plot showing genome-wide association p-values (-log 10) of single nucleotide polymorphisms (SNPs) associated with systolic pulmonary arterial pressure at 6 months of age using an additive model of allele action (n = 65)

Two SNPs were statistically associated with sPAP after Bonferroni correction. Both were associated with sPAP at 6 months of age (Figures 10.10 and 10.11). One SNP (rs135729312) was located with an intron of gene ENSBTAG00000045630 (p = 0.0006). This gene is reported to have serine-type endopeptidase inhibitor activity but its physiological role is unknown. Calves with alleles GT (n = 25) and TT (n = 5) had mPAPs that were 11.6 mm Hg (95 % CI = 7.8, 15.4) and 23.0 mm Hg (95 % CI = 16.1, 30.0) higher than calves with alleles GG (n = 35), respectively (Figure 10.12). The minor allele T had a frequency of 0.27.

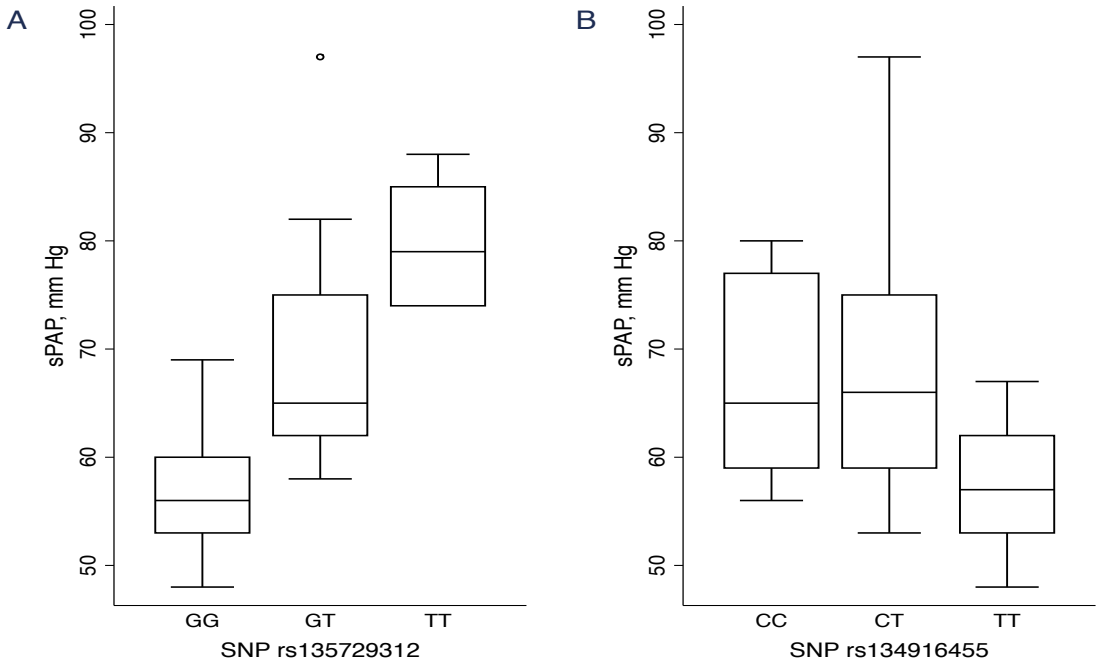

Figure 10.12: A box and whisker plot of systolic pulmonary arterial pressure (sPAP) at 6 months of age against allelic variants of SNP A) rs135729312 and B) rs134916455. Hollow circles represent outliers (observations that are 1.5 times the interquartile range away from the median)

Another SNP (rs134916455) associated with sPAP at 6 months of age was located within the intron of *vWF* (p = 0.009). The minor allele C had a frequency of 0.35. Calves

with alleles TT (n = 26) had a sPAP that was 10.1 mm Hg (95 % CI = 2.6, 17.7) lower than calves with CC alleles (n = 7) and 10.8 mm Hg (95 % CI = 6.4, 15.8) lower than calves with CT alleles (n = 32) (Figure 10.12). Von Willebrand Factor is a glycoprotein essential in the process of hemostasis. Abnormal vWF multimeric and oligomeric structure was reported in humans with pulmonary hypertension (Geggel et al., 1987; Lopes et al., 1993) and was prognostic of survival (Lopes et al., 1998). Von Willebrand Factor has also been identified as a genetic risk factor for hepatopulmonary syndrome in patients with liver disease (Roberts et al., 2010).

Two SNPs associated with mPAP at 4 months of age were located within genes also identified to be associated with sPAP at 4 months of age: rs136065769 located within an intron of *FAM135B* and rs42758526 located within an intron of *RXFP1* (relaxin/insulin-like family peptide receptor 1). The gene *RXFP1* is reported to be a hormone-binding G-protein involved in lung connective tissue development and extracellular matrix organization. The gene *RXFP1* encodes a receptor for the potent vasodilator relaxin, which is present on pulmonary vascular smooth muscles cells and throughout the systemic vasculature (Jelinic et al., 2014). Activation of *RXFP1* has anti-fibrotic properties that are protective against cardiovascular disease (Hossain et al., 2011; Samuel et al., 2006; Samuel et al., 2007).

Diastolic pulmonary arterial pressure

One SNP (rs43597502) was found to be marginally associated with dPAP at 4 months of age (p = 0.16). The closest gene (*ENSBTAG00000004890*) was 31,110 base pairs away; its function and physiological importance are unknown. CC calves (n = 52) had a

dPAP of 27.6 mm Hg (95 % CI = 25.8, 29.4). CT calves had a dPAP of 19.3 mm Hg (95 % CI = 14.4, 24.2). The one calf with alleles TT had a dPAP of 20 mm Hg (Figure 10.13).

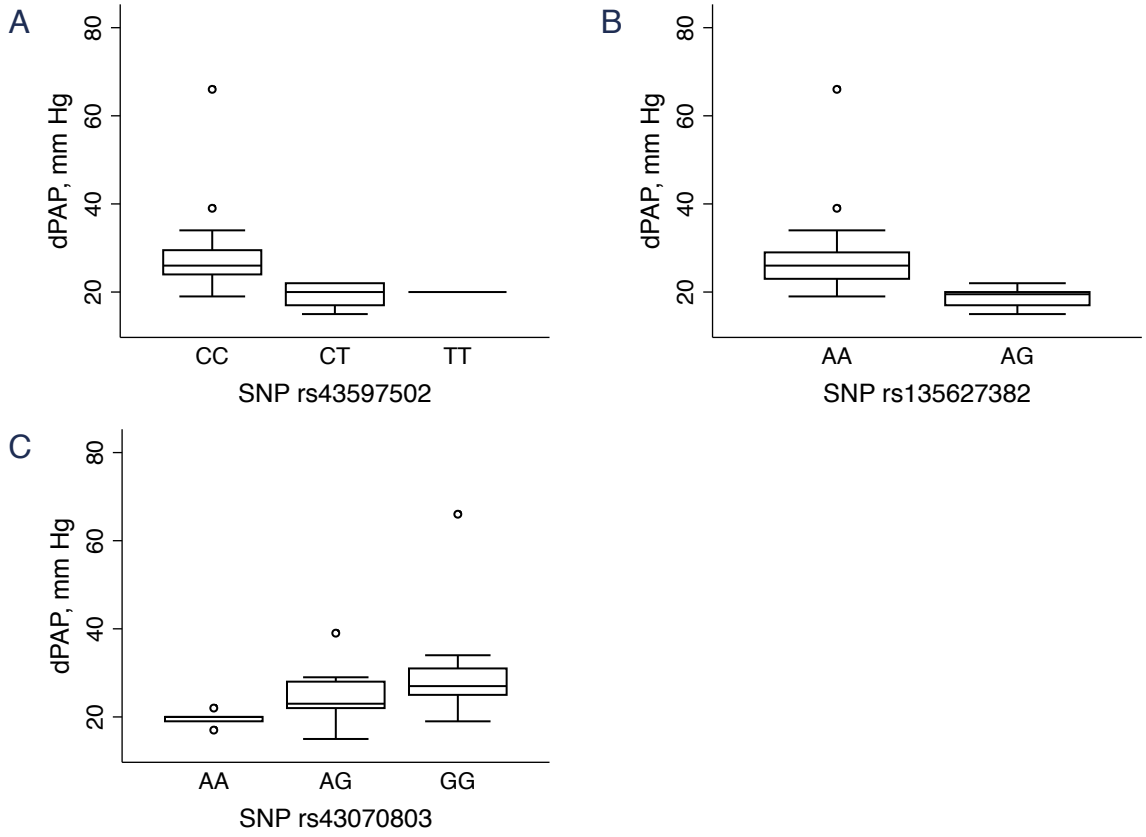

Figure 10.13: A box and whisker plot of diastolic pulmonary arterial pressure (dPAP) at 4 months of age against allelic variants of SNP A) rs43597502 B) rs135627382 and C) rs43070803. Hollow circles represent outliers (observations that are 1.5 times the interquartile range away from the median)

The second most statistically significant SNP (rs135627382) to be associated with dPAP at 4 months of age (p = 0.37, Figure 10.12) was located within an intron of gene *DCBLD1* (discoidin, CUB and LCCL domain containing-1). Little is currently known about this gene but it is believed to be an integral component of the cell membrane involved with cell adhesion. However, *EDIL3* (EGF-Like Repeats and Discoidin I-Like Domains 3), also

known as Del-1 (Developmental endothelial locus-1) is an important paralogue of *DCBLD1* that may regulate leukocyte recruitment and transendotheial migration (Chavakis et al., 2009). The immune system is thought to be extensively involved in the development of bovine pulmonary hypertension (Frid et al., 2006). Similarly, in humans (Pullamsetti et al., 2011) and broiler chickens (Wideman et al., 2013), the immune system was also reported to play a critical role in the etiology and progression of pulmonary hypertension.

SNP rs43070803 was located within an intron of gene *SLC35F1* (solute carrier family 35, member F1), which encodes for a nucleotide sugar transporter. Although not statistically associated with dPAP at 4 months of age ($p = 0.54$, Figure 12) there is plausible biological significance of this finding. In humans, *SLC35F1* is primarily expressed in the central nervous system but it is also expressed in the heart and peripheral tissues (Nishimura et al., 2009). It was reported that *SLC35F1* was associated with resting heart rate (Eijgelsheim et al., 2010) and left ventricular internal diastolic dimensions (Vasan et al., 2009). One SNP (rs11756438) located near *SLC35F1* has been found to be associated with QT interval, or the time to myocardial repolarization, in humans (Newton-Cheh et al., 2009). By altering cardiac function *SLC35F1* variants could influence cardiac workload and diastolic pulmonary arterial pressure.

Oxygen extraction ratio

No SNPs reached Bonferroni significance in association with systemic oxygen extraction fraction. One SNP (rs133993357) located within an intron of gene ALX1 had a Bonferroni statistical significance of 0.42 in association with oxygen extraction at 4 months of age. This gene has transcription factor activity and is involved in morphogenesis. Calves

with alleles AA, AB and BB had oxygen extraction ratios of 28.1 mm Hg (95 % CI = 26.1, 30.1; n = 20), 21.6 mm Hg (95 % CI = 19.9, 23.4; n = 27) and 21.3 mm Hg (95 % CI = 18.7, 23.9; n = 12), respectively (Figure 10.14).

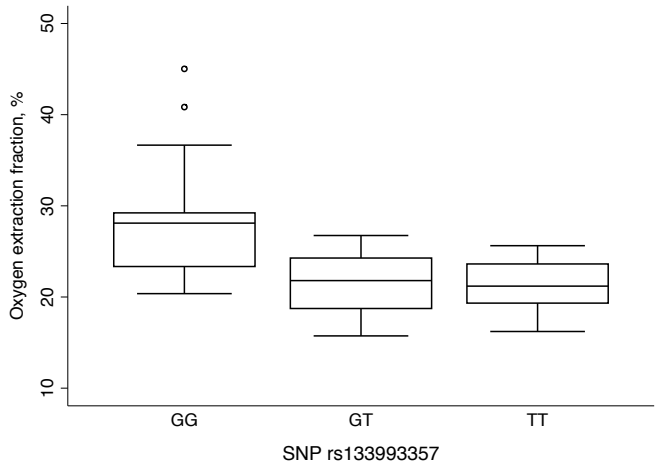

Figure 10.14: A box and whisker plot of systemic oxygen extraction fraction at 4 months of age against allelic variants of SNP rs133993357. Hollow circles represent outliers (observations that are 1.5 times the interquartile range away from the median)

L-lactate

One SNP (rs110578914) located within 9,000 base pairs of gene *FZD10* (frizzled family receptor-10) tended to be associated with L-lactate concentration at 4 months of age (p = 0.09). Calves with alleles AA, AG and GG had L-lactate concentrations 2.4 mmol/L (95 % CI = 1.7, 3.1; n = 8), 2.4 mmol/L (95 % CI = 2.0, 2.8; n = 24) and 1.1 mmol/L (95 % CI = 0.7, 1.5; n = 27), respectively (Figure 10.15). The gene *FZD10* encodes a G-protein involved in vascular development. There were no SNPs with a Bonferroni p-value < 0.50 at 6 months of age.

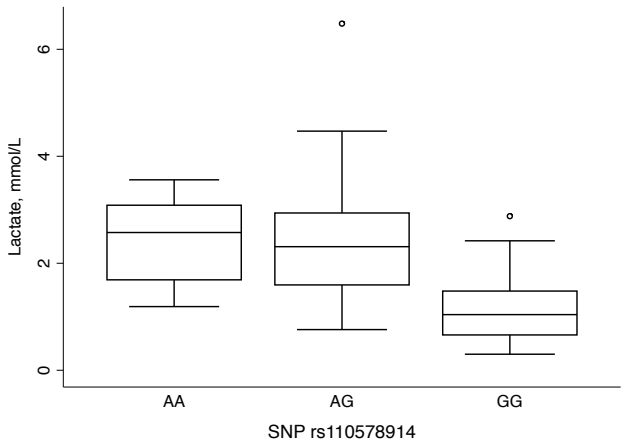

Figure 10.15: A box and whisker plot of L-lactate concentration at 4 months of age against allelic variants of SNP rs110578914. Hollow circles represent outliers (observations that are 1.5 times the interquartile range away from the median)

Although not statistically associated with L-lactate at 6 months of age SNPs rs110259300 (p = 0.13) and rs110636089 (p = 1.00) appeared to show a relationship (Figure 10.16). At present there are no genes located within 100 kbp either side of SNP rs110259300 or rs110636089, which are both located on chromosome 12.

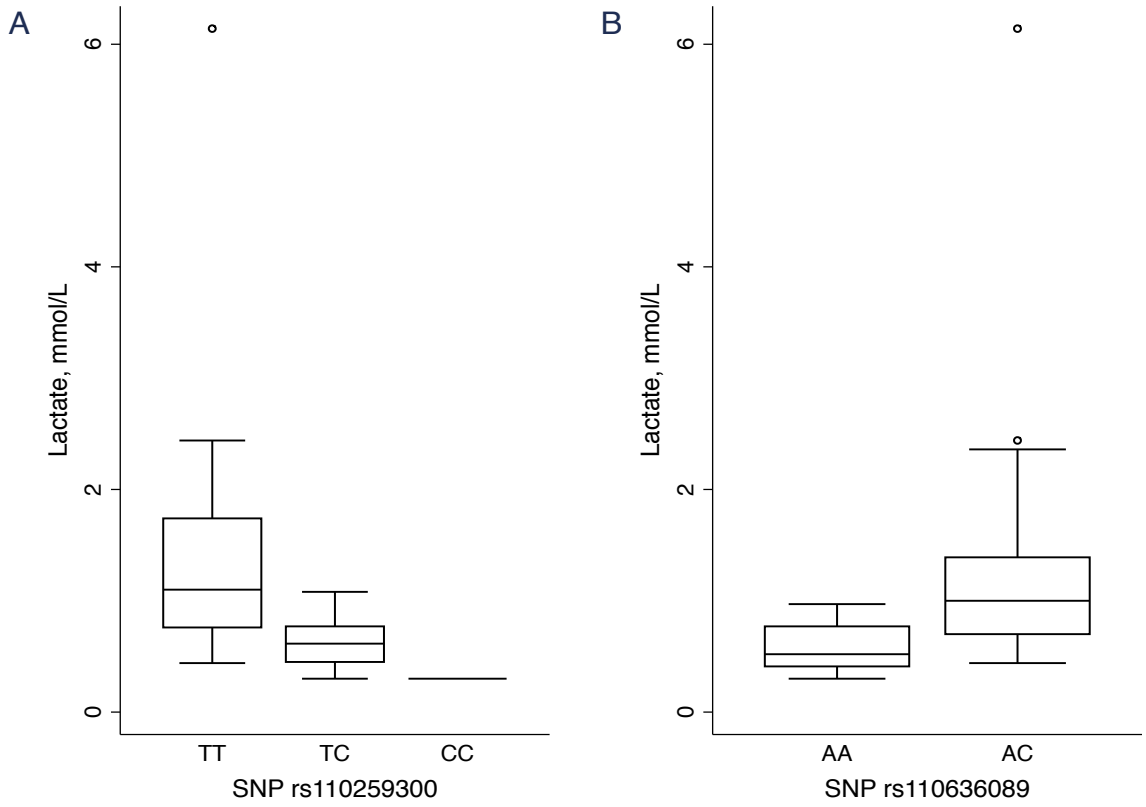

Figure 10.16: A box and whisker plot of L-lactate fraction at 6 months of age against allelic variants of SNP A) rs110259300 and B) rs110636089. Hollow circles represent outliers (observations that are 1.5 times the interquartile range away from the median)

Mixed venous CO_2

No SNPs were found to be statistically associated with mixed venous CO_2. The SNP (rs134318701) with greatest statistical association (p = 0.60) with mixed venous CO_2 at 6 months of age was located 53 kbp away from gene *GSTK1* (glutathione S-transferase kappa 1) (Figure 10.17). This gene has been found to affect the rate of respiration and metabolism of lipids in *C. elegans* (Petit et al., 2009). Therefore, it is plausible that polymorphisms in *GSTK1* may affect metabolic rate, and therefore CO_2 production, in other species such as cattle.

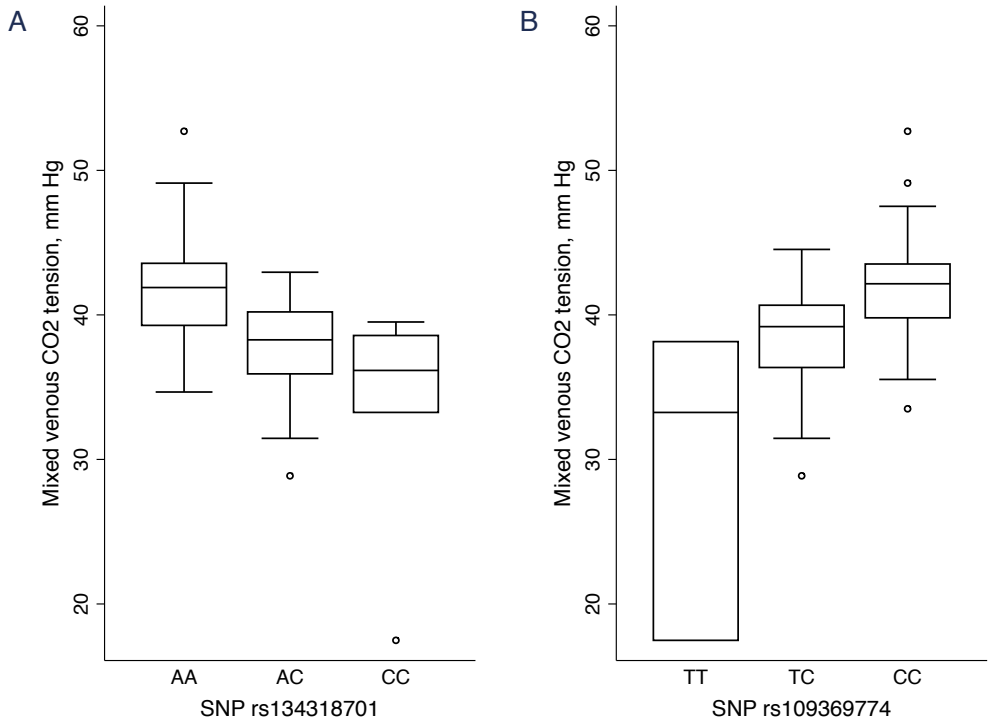

Figure 10.17: A box and whisker plot of mixed venous CO_2 at 6 months of age against allelic variants of SNP A) rs134318701 and B) rs109369774. Hollow circles represent outliers (observations that are 1.5 times the interquartile range away from the median)

The next SNP (rs109369774) to be associated with mixed venous CO_2 tension at 6 months of age was located within an intron of the gene *SIRT5* (sirtuin 5). This gene belongs to a family of NAD^+-dependent lysine deacylases localized to the mitochondria that play an important role in metabolic regulation (Wang et al., 2013).

Hematocrit

Two SNPs were found to have statistical association with hematocrit at $p < 0.30$; one at 4 months of age (rs41689970; $p = 0.17$) and the other at 6 months of age (rs136772915; $p = 0.21$). The SNP rs41689970 is located within an intron of gene *SLC24A3*

(sodium/potassium/Ca exchanger) also known as *NCKX3*. Calves with alleles GG, GT and TT at rs41689970 had hematocrits of 30.7 % (95 % CI = 29.7, 31.8; n = 43), 27.5 % (95 % CI = 25.8, 29.2; n = 16) and 25 % (n =1), respectively (Figure 10.18). Gene *NCKX3* is involved in transmembrane transport and is expressed in human pulmonary artery smooth muscle cells and regulates cytosolic Ca^{2+} concentration (Zhang et al., 2005) and vascular tone (Dong et al., 2006). Interestingly, *NCKX3* is highly expressed in the human endometrium and its level of expression increases in response to 17ß-estradiol (Yang et al., 2011). This implies that the gender hormone 17ß-estradiol may also affect vascular *NCKX3* expression and consequently vascular tone. Variation in this gene may affect hematocrit through its impact on platelet function and hemostasis. Expression of this gene was found to be downregulated in a human patient thrombocytopenia and impaired platelet aggregation (Sun et al., 2007).

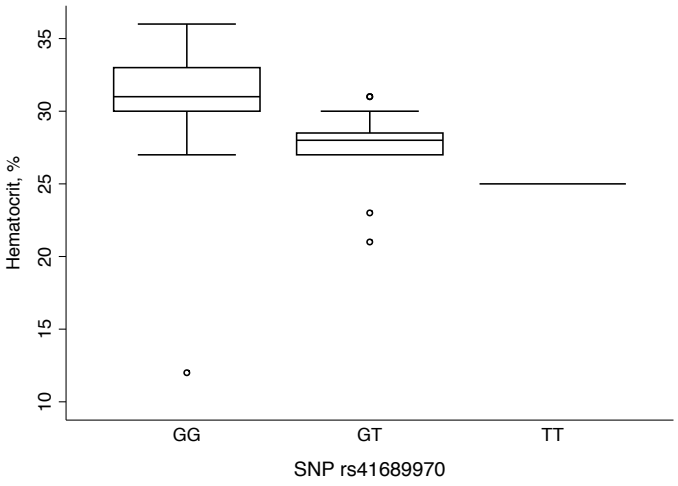

Figure 10.18: A box and whisker plot of hematocrit at 4 months of age against allelic variants of SNP rs41689970. Hollow circles represent outliers (observations that are 1.5 times the interquartile range away from the median)

The SNP rs136772915 is located 13,164 bases upstream of gene *ANXA8* (annexin A8) an anticoagulant protein with calcium binding properties. Calves with alleles AA, AG and GG had a hematocrit of 30.9 % (95 % CI = 29.4, 32.4; n = 11), 29.7 % (95 % CI = 28.9, 30.7; n = 26) and 32.0 % (95 % CI = 31.0, 33.1; n = 22), respectively (Figure 10.19).

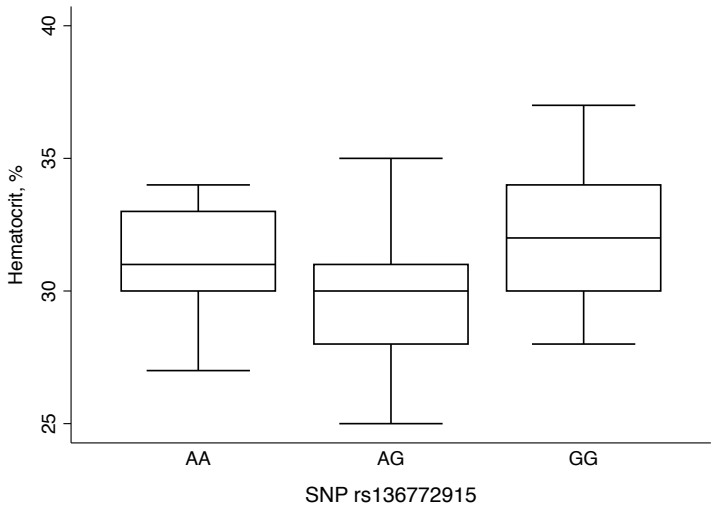

Figure 10.19: A box and whisker plot of hematocrit at 6 months of age against allelic variants of SNP rs136772915

Summary

Oxygen extraction, L-lactate and mixed venous CO_2 were found to be associated with mPAP in pre-weaned calves at 2,166 m above sea-level. This indicates that high metabolic oxygen demand relative to supply may be a risk factor for increased mPAP in pre-weaned calves at high altitude. However, we failed to reject the null hypothesis: a GWAS did not identify any SNPs concordant among the traits studied. This may be because the physiological traits found to be associated with mPAP explain little of the variation in mPAP. Seven SNPs were found to be associated with mPAP at 4 months of age. These SNPs were located within introns, or within 100 kbp, of genes that are reported to be involved in

cellular proliferation, susceptibility to myocardial and infectious disease and angiogenesis. No SNPs were significantly associated with mPAP at 6 months of age. However, the highest ranked SNPs have been previously associated with cellular proliferation, migration and growth. Two SNPs were significantly associated with sPAP at 6 months of age. One of these SNPs was located within an intron of von Willebrand factor, which indicates that hematostasis may play a role in determining susceptibility to pulmonary hypertension. No SNPs were found to be statistically associated with dPAP, oxygen extraction, L-lactate concentration or mixed venous CO_2 tension.

CHAPTER 11: DISCUSSION

Overview

The results of the investigations undertaken for this dissertation indicate that congestive heart failure (CHF) of cattle is not a disease that is restricted to high altitude. The incidence of the disease significantly increased in Canadian feedlots over a 12-year period. It is a particularly costly disease because mortality tends to occur in the late feeding period after substantial resources have been invested in cattle. Treatment for respiratory disease substantially increased the risk of CHF in feedlot cattle. This is a concern given the increasing incidence of respiratory disease reported in feedlot cattle (Loneragan et al., 2001).

High-performing cattle, that is cattle that have a high rate of gain and high feed efficiency, are significantly more likely to show cardiac insufficiency, as indicated by oxygen extraction, and have higher mean pulmonary arterial pressures (mPAP) than slower growing cattle and cattle with a lower feed efficiency. This suggests that increased oxygen demand associated with rapid growth is a risk factor for increased mPAP in cattle. Cattle that had the highest mPAP as calves at high altitude also tended to have the highest mPAP in the late feeding period at lower altitude. This suggests that pulmonary vascular lesions developed at high altitude may predispose those same cattle to increased mPAP during the confined feeding period. Alternatively, environmental or genetic risk factors for increased mPAP of calves at high altitude may be shared with those of feedlot cattle. Further epidemiological studies evaluating the risk of CHF among cattle sourced from various altitudes would help decipher between the possible risk factors.

A number of chromosomal regions were identified to be associated with mPAP in a small cohort of 60 calves. This suggests that a study of a larger sample size could identify many of the chromosomal regions that account for a large proportion of the genetic variation in mPAP. However, it should be remembered that mPAP and CHF are related but not synonymous. High mPAP may predispose cattle to CHF but it likely only explains a small amount of the variation in susceptibility to CHF. Therefore, a study evaluating variables associated with mPAP is not necessarily establishing risk factors for CHF. Further studies are necessary to determine the interplay among cardiac reserve, pulmonary function, pulmonary hemodynamics and vascular pressures, metabolic oxygen demand and risk of CHF.

In summary, the results of this dissertation indicate that CHF secondary to pulmonary hypertension in cattle should be considered to be a multifactorial disease rather than a disease associated with high altitude. High altitude accentuates the risk of CHF but it is not the only risk factor for CHF secondary to pulmonary hypertension. Selection for increased growth rate in beef cattle may predispose cattle to CHF by increasing mPAP. However, a causal association was not established. The physiological traits evaluated in this dissertation explain only a small amount of the variation in mPAP.

Diseases of livestock attributed to genetic selection for high production

Diseases of livestock resulting from genetic selection for increased productivity are becoming increasingly problematic (Rauw et al., 1998). Feed costs are typically the highest input cost associated with food animal production. Therefore, in order to maximize net economic return, the goal of any livestock operation is to maximize production and

minimize feed costs. Modern advances in genetic technology have substantially increased the accuracy and intensity of selection for production traits. Given that most production traits are moderately to highly heritable there has, and there will continue to be, marked improvements in animal productivity.

Up to 90 % of the increased rate of growth in broiler chickens has been attributed to genetics (Havenstein et al., 1994a). Selection for increased body mass in broiler chickens and turkeys has been associated with adverse health effects including: reduced immune performance (Miller et al., 1992) and increased susceptibility to infectious (Nestor et al., 1996) and non-infectious diseases such as congestive heart failure and lameness (Havenstein et al., 1994a; Peacock et al., 1990). Selection for rapid growth and leanness in pigs has been associated with a significantly increased risk of osteochondrosis in pigs (Busch and Wachmann, 2011).

Genetic selection may have reduced the ability of livestock species, such as cattle, pigs and poultry, to cope with environmental stressors. A shift in muscle fiber type from a low proportion to a high proportion of fast twitch glycolytic fibers that have a lower oxidative capacity, reduced capillary supply and larger fiber diameter appears to have occurred in pigs (Essen-Gustavsson and Lindholm, 1984), broiler chickens (Aberle and Stewart, 1983; Petracci and Cavani, 2012) and cattle (Karlstrom et al., 1994). It is not known if muscle fiber type or function contributes to increased mPAP in cattle. However, a study conducted in rats with pulmonary hypertension reported that skeletal muscle mitochondrial dysfunction preceded impairment of right-ventricular function (Enache et al., 2013).

Through continued genetic and technological developments, milk production of US dairy cattle is expected to increase by 200 kg per year from an average of 9,945 kg per cow per year in 2013 to 11,740 kg per cow per year in 2022 (USDA, 2013). The majority of cattle in US dairy herds survive for less than 5 lactations. The average lifetime parity is now less than 3 (Tsuruta et al., 2005). First-lactation heifers that partition greater energy into milk production are reported to suffer reduced longevity (Wathes et al., 2008).

Resource allocation theory predicts that selection for traits associated with high production could have adverse consequences for life history traits such as immune function (Rauw, 2012). Low thyroid hormone levels concurrent with selection for rapid growth and feed efficiency has been shown to impair lung development in broilers (Hassanzadeh et al., 2008). Further studies are needed to evaluate how selection for increased growth rate and feed efficiency in cattle impacts cardio-pulmonary development.

Anatomical considerations

Genetic selection for growth rate in chickens has been associated with small cardio-pulmonary system as a percentage of live body weight (Havenstein et al., 1994b). A similar response may have occurred among cattle in response to selection for increased productivity.

Per unit of oxygen consumed, the volume and gaseous exchange surface area of the bovine lung are substantially smaller than the mammalian average (Veit and Farrell, 1978). Subjective assessment of bovine pulmonary capillary density suggests that it is substantially less than other species (Epling, 1964). Combined, these 2 factors suggest that the pulmonary vascular capacity of the bovine may be reduced relative to other species.

The law of Laplace predicts medial hypertrophy of blood vessels to occur in response to high vascular pressure. Therefore, medial hypertrophy of small pulmonary arteries may be a product or a contributing factor in the development of bovine pulmonary hypertension as it is a cause. Broiler chickens have pulmonary arteries that are even more muscular than cattle (Sillau and Montalvo, 1982). Congestive heart failure of chickens was initially believed to be a disease of high altitude (Hall and Machicao, 1968) but subsequently became recognized as a disease associated with high production (Peacock et al., 1989). Therefore, it is plausible that species hyperresponsive to chronic hypoxic exposure, such as European cattle (*Bos taurus*), have muscular pulmonary arterioles (Tucker et al., 1975) because they have inadequate pulmonary arterial capacity to accommodate the large blood flow required to meet the oxygen demands of high production. It is also plausible that cattle have inadequate cardiac reserve to meet the increase in hydraulic resistance associated with medial hypertrophy of the pulmonary arterioles and increasing pulmonary vascular stiffness of the large elastic arteries (Lammers et al., 2008). Alternatively, cattle with low cardiac reserve may be predisposed to CHF because they have little ability to adapt to the increased right-ventricular afterload associated with an increased mPAP. From the studies undertaken for this dissertation it is not possible to determine that pathogenesis of CHF.

Cardiac mass may be substantially smaller in cattle today than it was 100 years ago. The average cardiac mass of heifers and steers (n = 224) raised at altitudes from 6,000 to 10,000 ft. and slaughtered in Denver in 1918 was 0.78 kg per 100 kg of carcass (Glover and Newsom, 1918). After excluding the atria, which are responsible for approximately $1/6^{th}$ of the weight of the heart (Jones, 1953) the total ventricular mass was approximately 0.65 kg per 100 Kg of carcass. The average total ventricular mass of a sample of 20 hearts recently

obtained from healthy cattle at slaughter from a feedlot at 1,440 m (4,725 ft.) was 0.51 kg per 100 kg of carcass (data not shown). If our results are reflective of the larger population, this suggests that cattle today have a smaller heart per unit of carcass than cattle almost one century ago. This supports our observations of high sOEF in calves at high altitude and feedlot steers at moderate altitude. Future studies should consider combining measurements of sOEF with metabolic oxygen requirements (VO_2) and measures of cardiac dimensions.

Other studies indicate that heart and lung weights of feedlot steers do not scale with growth rate or residual feed intake (Basarab et al., 2003; Mader et al., 2009). When corrected for body mass, Brown Swiss cattle have a significantly heavier heart, lung and spleen than Angus and Hereford breeds of cattle (Jenkins et al., 1986). This likely reflects the high altitude origins of the Brown Swiss breed of cattle.

Dietary involvement

The investigations undertaken focused on the epidemiological, physiological and genetic risk factors for CHF and increased mPAP. Nutrition was not evaluated but may also contribute to risk of bovine CHF. Maternal under-nutrition in early gestation may affect gene expression in the right ventricle of the fetus and be detrimental to health (Han et al., 2008).

Increasing water sulfate concentrations were found to be positively correlated with mPAP in feedlot cattle (Loneragan et al., 2005). Oxygen-dependent hydrogen sulfide metabolism may be responsible for hypoxia-induced pulmonary vasoconstriction in cattle (Olson et al., 2009).

There is now ample evidence that serotonin has a role in the development of pulmonary hypertension by affecting vascular remodeling and vasoconstriction (Delaney et al., 2013; Maclean and Dempsie, 2010). It is plausible that tryptophan, a by-product of nitrogen fertilizers and the biological precursor of serotonin (5-hydroxytryptamine), in cattle feedstuffs may increase susceptibility to pulmonary hypertension. Tryptophan, contained in lush forage, is converted to 3-methylindole by ruminal microorganisms, which when absorbed can cause acute bovine pulmonary edema and emphysema (Hammond et al., 1979). It remains to be determined if tryptophan increases the risk of pulmonary hypertension in cattle. However, in broiler chickens, a diet high in tryptophan has been shown to accelerate the development of pulmonary hypertension (Kluess et al., 2012).

Implications

The results of the investigations undertaken indicate that continued selection for high production in livestock species may increase the incidence of CHF in cattle. Genetic selection for increased animal productivity has already created many animal welfare concerns (Rauw et al., 1998). There may be other far-reaching consequences beyond animal welfare. Selection for productivity and the associated adverse consequences for immune function (Rauw, 2012) may mean reduced vaccine efficacy and a greater reliance on antimicrobials.

One such antibiotic licensed for use in pigs, beef cattle and poultry is the macrolide, tylosin. In beef cattle, tylosin is mixed in feed in order to reduce the incidence of liver abscesses associated with *Fusobacterium necrophorum* and *Arcanobacterium pyogenes*. Liver abscesses occur due to ruminal acidosis that results from feeding a diet high in

concentrate and deficient in roughage (Nagaraja and Lechtenberg, 2007), a typical finishing diet for feedlot cattle. One benefit of a diet low in roughage and high in concentrates is an improvement in feed efficiency (Stock et al., 1990).

Unfortunately, the majority of these adverse health effects are insidious. Many of these adverse health effects of selection for increased productivity can be attributed to 'black box physiology'; we do not know the physiological consequences of many selection practices.

The poultry and swine production industries have been able to counter the adverse consequences of genetic selection for enhanced productivity by tightly controlling the environmental conditions that animals are exposed in indoor-housing systems. For example, the risk of CHF in broiler chickens is minimized through the regulation of temperature in order to minimize pulmonary vasoconstriction in response to cold and light level in order to regulate feed intake and therefore rate of growth (Baghbanzadeh and Decuypere, 2008).

The cattle industry faces a greater challenge, as control of environmental conditions is less feasible. The most appropriate solution is likely to be the genetic selection of cattle that are most suited to their environment. For cattle, at high altitude this will likely mean more appropriate matching of selection for production traits that increase oxygen demand with concurrent selection for cardiopulmonary capacity.

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