DISSERTATION

CHARACTERIZATION OF PULMONARY HYPERTENSION STATUS AND UTILIZATION OF MULTI-OMICS ANALYSES TO DISCOVER VARIANTS THAT MAY INFORM SELECTION AGAINST HIGH MEAN PULMONARY ARTERIAL PRESSURE IN ANGUS CATTLE

Submitted by

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ABSTRACT

CHARACTERIZATION OF PULMONARY HYPERTENSION STATUS AND UTILIZATION OF MULTI-OMICS ANALYSES TO DISCOVER VARIANTS THAT MAY INFORM SELECTION AGAINST HIGH MEAN PULMONARY ARTERIAL PRESSURE IN ANGUS CATTLE

This multi-part research characterizes pulmonary hypertension (PH) from a physiologic and genetic point of view using the indicator trait mean pulmonary arterial pressure (PAP). Three aims were designed to address the research objective of investigating the genetics underlying PAP for the purposes of variant discovery.

The first aim sought to identify different PAP phenotypes based on altitude and diet during the stocker and finishing phases of production. This longitudinal study evaluated steers with a moderate PAP (41-48 mmHg) from yearling age until harvest, collecting PAP and blood gas parameters throughout the study and carcass data at the conclusion. Through this experimental approach, the role of different finishing systems was able to be evaluated and cattle with increased sensitivity to hypoxic conditions were identified. Results from this study indicated that regardless of finishing system, animals exhibited signs of respiratory alkalosis with renal compensation because of hypoxic conditions. The PAP data from this population corroborated that all steers were hypoxic. However, the only carcass quality differences observed were those between cattle that were grain finished compared to those that were grass finished, regardless of altitude.

Aim two was to perform next-generation whole genome sequencing for 30 Angus bulls and steers to compare those with high PAP (HPAP) to those with low PAP (LPAP) measures. All cattle sequenced originated from elevations ≥1,500m and were selected based on their pedigree information, as well as PAP observations. The sequence data from these 30 animals were then compared such that sequence from HPAP cattle was compared to that of LPAP cattle. There were 5,543 variants unique to HPAP cattle and 1,690 variants unique to LPAP cattle. Loci across all 30 chromosomes exhibited variation for PAP phenotype. Evaluation of these variants and validation will be necessary to sift through variants that are in linkage or may be less informative.

A multi-omics approach was used to perform variant discovery based on the PAP phenotype in aim three. Through combination of RNA sequence with DNA sequence, the list of variants relevant to PAP phenotype was filtered from millions of variants to hundreds of variants. Transcriptome data was compared for each of six tissues between HPAP and LPAP cattle. These variants were then compared to one another to determine which variants were detected in each PAP category across all six tissue types. Those variants were then compared to the DNA sequence from aim two to elucidate concordant variants for HPAP and LPAP cattle respectively. There were three variants that were unique to LPAP cattle and were concordant between DNA and RNA sequence. However, none of these variants were within 1,000bp of a gene recognized in the ARS-UCD1.2 bovine genome assembly and were therefore considered less informative. There were 523 variants unique to HPAP cattle. Within that population there was a subset that was either near or within a gene. There were six genes that were considered informative for further investigation. Three of those genes were uncharacterized genes on chromosome 16. The other three (U6, SIMC1, CDH23), while not well documented in cattle, had

functions in humans that would indicate their function could affect PAP phenotype expression. These genes and the variants within them could be useful for selection if validated in a larger population.

DEDICATION

This dissertation is dedicated to my family. First, to my mom, Karen Jennings (B.K.), and my dad, Phil Jennings, for your unwavering support as I have ventured far and wide for my education. I know it was not easy to let your little girl move away from home at the age of fifteen, but you have stood beside me with great strength and bravery through every peak and valley, even when I did not believe I possessed enough strength, bravery, or confidence myself. I may not have followed the "career blueprint" that I drew out for you at the age of four, and I may have been off-roading at many points throughout this adventure. However, you have stayed by my side through every bump, turn, jump, and free-fall. I love you both more than words can describe and will forever be indebted to you for the life you have both worked tirelessly to help me cultivate.

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CHAPTER 1

INTRODUCTION

High mountain disease (HMD) occurs in susceptible cattle grazing at elevations greater than 1,500 m (Pauling et al., 2018; Thomas et al., 2018). The condition results from pulmonary hypertension (PH) progressing into vascular remodeling that often culminates in congestive heart failure of susceptible cattle (Holt and Callan, 2007; Neary et al., 2015). Diagnosis of PH in cattle is conducted via a mean pulmonary arterial pressure (PAP) measurement, which is an indicator trait that assesses an animal's risk of developing HMD. It is well documented that PAP is moderately heritable and polygenic in nature, meaning that genetic improvement for PAP is possible (Schimmel, 1981; Schimmel, 1983; Enns et al., 1992; Shirley et al., 2008; Zeng, 2016; Crawford, 2016).

Studies to date have relied on SNP chip genotyping panels or gene-targeted approaches to detect variation within the genome related to PAP (Newman et al., 2011; Newman et al., 2015; Heaton et al., 2016; Zeng et al., 2016; Crawford et al., 2019; Heaton et al., 2020). However, these approaches can result in misrepresentation due to the difference in coverage of the genome (Hickey, 2013). With increased affordability of sequencing, variant detection approaches have been rapidly changing, including multi-omic bioinformatic strategies to combine different sequence types such as genomic (DNA) or transcriptomic (RNA) sequence to make inferences about biological systems as a whole (Heather and Chain, 2015; Muir et al., 2016; Cánovas et al., 2014; Nguyen et al., 2018). These next-generation approaches to variant discovery could enable for better understanding of PH from a molecular biology systems perspective through identification and validation of genes and variants related to PAP. Further, variants related to PAP may be utilized to enhance accuracies of genetic predictions through marker assisted

selection techniques, thus aiding ranchers of high altitude production systems (Glover and Newsom, 1915; Hecht et al., 1962; Northcutt, 2010; Garrick, 2011; Rolf et al., 2014; Neary et al., 2014; Zeng, 2016). Therefore, the objective of this research was to detect variants associated with PAP risk category using RNA and DNA sequence.

CHAPTER 2

A REVIEW OF PULMONARY HYPERTENSION AND ITS IMPACT ON THE BEEF INDUSTRY

Section 1: Introduction

Pulmonary hypertension (PH) has impacted cattle in the mountainous regions of the western United States. Since its discovery in 1913, the condition has been referred to as dropsy of high altitude, brisket disease, or high mountain disease (HMD), among other names. The pathophysiology of HMD is characterized as hypoxia-induced pulmonary hypertension, which can culminate in congestive heart failure of susceptible cattle. Despite years of research characterizing and working to reduce the incidence of this condition, HMD still impacts cattle today and results in death loss for ranchers across the mountainous western states (Holt and Callan, 2007). This chapter is an overview of the pathophysiology and genetics of HMD. In addition, this chapter provides insight into a lesser-known and understood manifestation of pulmonary hypertension known as feedlot heart disease and discusses improvements in genetic technologies that may aid in alleviating the impact of pulmonary hypertension on cattle production.

Section 2: Pulmonary Hypertension

Pulmonary hypertension (PH) is commonly recognized as high blood pressure. More specifically, PH affects the cardiopulmonary system and results in death if not properly managed (Mayo Clinic, 2017). While there are many causes for the development of PH, the disease is broadly characterized by cellular changes in the pulmonary system that result in stiffening of vessel walls and subsequent vascular remodeling. As these circulatory changes occur within the

vasculature of the lungs, greater strain is placed on the heart to maintain efficient blood flow. If vascular remodeling continues to progress in severity, the cardiac strain may become so severe that the patient succumbs to heart failure (HF). Some cases of PH are irreversible, while other incidents of PH may respond to treatment. The World Health Organization (WHO) has established five categories of PH based on etiology of the condition (Ryan et al., 2012; World Health Organization, 2013; Pulmonary Hypertension Association, 2019).

2.1 WHO Categories of PH

2.1.1 Category 1

The WHO has established that patients with Category 1 PH are experiencing pulmonary arterial hypertension (PAH). This condition is often diagnosed via elimination of Categories 2-5 of PH rather than a positive diagnosis of Category 1 PH. This type of PH includes idiopathic PH, familial PH, and PH associated with medical conditions (Ryan et al., 2012). Idiopathic derived PAH has no clear origin or cause of onset of the condition. Familial PAH is inherited (Pulmonary Hypertension Association, 2019). PAH may develop alongside other medical conditions such as liver cirrhosis, human immunodeficiency virus (HIV), and lupus. Further, PAH may develop due to past or present drug use or exposure to toxins (Mayo Clinic, 2017). Category 1 PH has a few treatments to alleviate the symptoms, but not cure, PAH (Pulmonary Hypertension Association, 2019).

2.1.2 Category 2

Category 2 PH derives from left-sided heart disease. This could develop from systolic or diastolic dysfunction of the left atrium and ventricle. Further, this condition can develop from valvular diseases that result in stenotic valves or leaky valves that impact the ability of the heart to pump blood without backflow. Ultimately, this type of PH is characterized as the inability to

maintain flow of oxygenated blood entering the left heart, resulting in increased pressure in the vasculature of the lungs. This form of PH is the most common in humans (Pulmonary Hypertension Association, 2019).

In addition to humans, manifestations of Category 2 PH have been observed in cattle. The condition, referred to as feedlot heart disease (FHD), is characterized as heart failure occurring in cattle in the latter stages of finishing (Jensen et al., 1976; Neary et al., 2015a; Neary et al., 2015b; Krafsur et al., 2019; Moxley et al., 2019). During the finishing phase of production, cattle are administered high energy diets to increase the rate of weight gain prior to harvest. Krafsur et al. (2019) concluded that cardiac and pulmonary tissues of late-fed cattle undergoing heart failure exhibited histopathologic features of remodeling similar to that of obesity-associated PH in humans. However, Neary et al. (2015b) described pathophysiology of FHD in steers as right-sided heart failure, which could indicate that FHD should be classified as Category 3 PH. Further research characterizing the pathophysiology FHD and comparing it to that of cattle experiencing hypoxia-induced PH through chronic high-altitude exposure is necessary to elucidate whether FHD should be classified as Category 2 PH.

2.1.3 Category 3

Chronic lung diseases, hypoxia, or a combination of both contribute to Category 3 PH (Mayo Clinic, 2017; Pulmonary Hypertension Association, 2019). The overall etiology of Category 3 PH results from lung restriction or narrowing of the airways that increase the difficulty of respiration. The cardiopulmonary system compensates for the decreased oxygen intake by reducing blood flow to regions of the lung that are not active in gas exchange. This increases pulmonary blood pressure (Pulmonary Hypertension Association, 2019).

Chronic lung diseases such as COPD or emphysema can result in Category 3 PH through restricting the ability of the lung to expand and contract during respiration. Sleep apnea and chronic exposure to high altitude also result in Category 3 PH (Mayo Clinic, 2017; Pulmonary Hypertension Association, 2019). Therefore, high mountain disease (HMD) in cattle is classified as Category 3 PH. High mountain disease is observed in cattle at elevations greater than 1,500m (Pauling et al., 2018). In extreme cases, HMD can result in vascular remodeling, heart failure, and death of susceptible cattle (Holt and Callan, 2007).

2.1.4 Category 4

Category 4 PH is caused by the development of pulmonary emboli (Mayo Clinic, 2017; Pulmonary Hypertension Association, 2019). The inability of the body to dissolve blood clots occludes pulmonary blood flow in the clotted regions of the lung. However, after a clot has resolved, scar tissue may remain that can occlude pulmonary blood flow permanently, resulting in strain on the right side of the heart due to vascular resistance in the lungs. Remedies exist for Category 4 PH, including surgical removal of clots and administration of drugs to resolve pulmonary clots (Pulmonary Hypertension Association, 2019).

2.1.5 Category 5

Category 5 PH encompasses incidences of PH not well understood (Mayo Clinic, 2017; Pulmonary Hypertension Association, 2019). More specifically, this category consists of cases of PH that are secondary to other diseases or conditions but have yet to be characterized well enough to be classified within WHO Categories 1-4 (Pulmonary Hypertension Association, 2019). Common conditions that are classified as Category 4 PH are blood disorders, metabolic disorders, cancer, and multi-system diseases (Mayo Clinic, 2017).

2.2 Physiology of vascular remodeling in response to chronic PH

The physiological response to chronic hypoxia resulting in PH is vascular remodeling. Small arteries (diameter < 500 μm) are the site of the cellular changes recognized in remodeling and can manifest as a decreased number of small arteries to transport blood, a loss of luminal space in arterial branches, or a combination of both. These changes result in an elevated PAP due to increased resistance in the branches narrowing off the pulmonary artery leading to the lungs (Rounds and Klinger, 2004; Shimoda and Laurie, 2013). A normal small artery that is part of the pulmonary vascular branches is comprised of numerous tissue substances including fibronectin, collagen, elastin, smooth muscle, and endothelial cells, which provide a rigid structure while allowing for adequate compliance to achieve blood flow to the lungs (Figure 2.1).

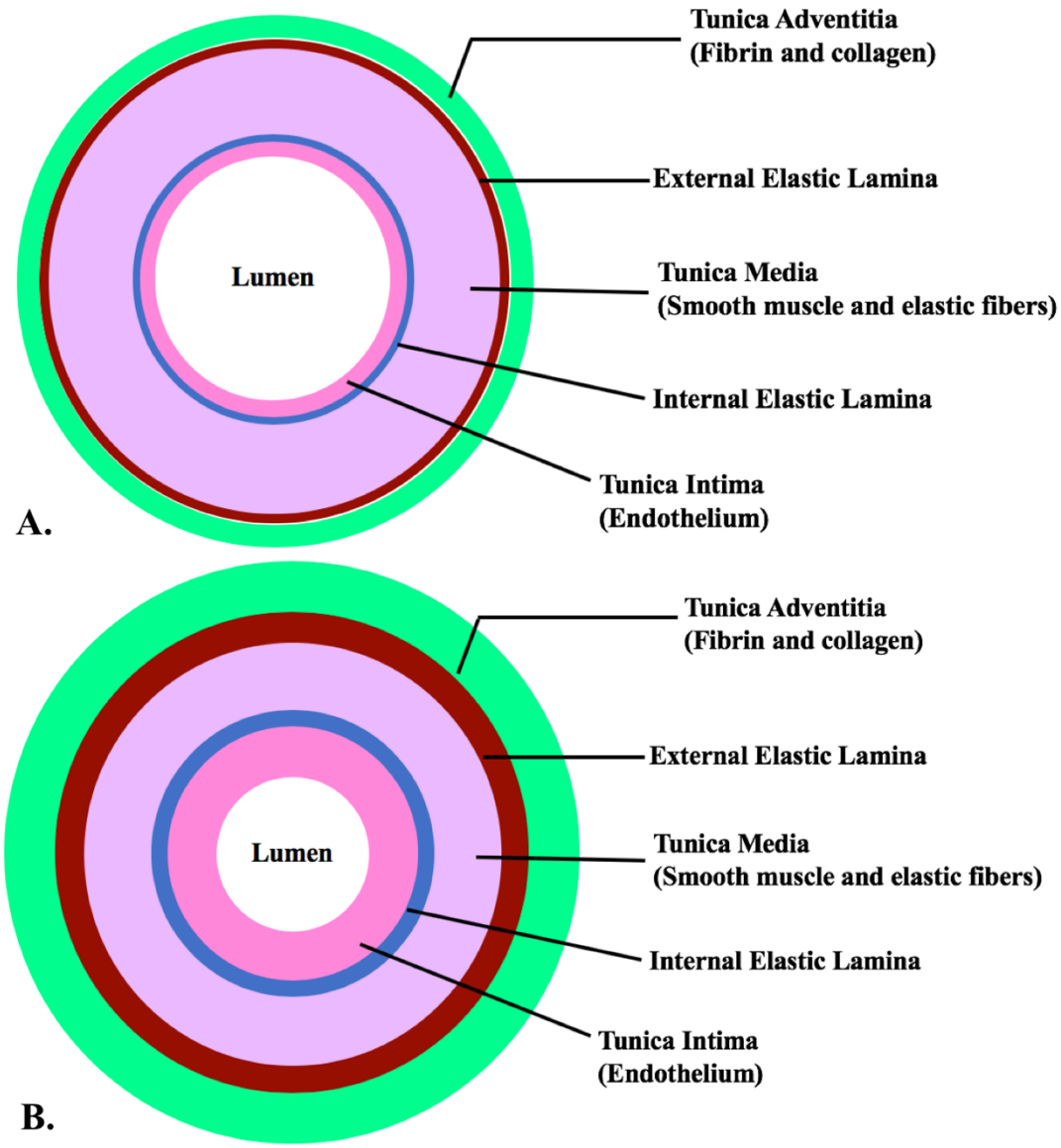

Figure 2.1 Comparison of the vascular structure of a normal and remodeled vessel. A.) Normal structure of a vascular branch off the pulmonary artery with the tissue composition of each major segment labeled. B.) Animals experiencing vascular remodeling from PH can exhibit a combination of thickening of the vessel layers, resulting in decreased luminal space. (Image created by the author).

Vascular remodeling of the pulmonary arterial circulation results in narrowing of the small vessels in the pulmonary branches. This reduction of luminal space derives from both hypertrophy and hyperplasia of cellular components in all layers of the vessel (Anderson et al., 1973; Chazova et al., 1995; Rounds and Klinger, 2004; Stenmark et al., 2009). Hypertrophy in

layers such as the tunica adventitia that contain cellular components such as fibrin and collagen can result in decreased compliance or increased rigidity of the outer portion of the vessel (Rounds and Klinger, 2004).

Stiffening of pulmonary vasculature due to remodeling leads to increased resistance, which puts increased strain on the right side of the heart as it works harder to eject blood through the pulmonary artery to the lungs. This increased pulmonary vascular resistance increases blood pressure and decreases overall blood flow due to reduced compliance of the vasculature. The heart compensates by increasing afterload, resulting in the heart working harder to eject blood. Over time, increased pumping efforts of the cardiac tissues will result in right ventricular hypertrophy, and loss of fluid in the extravascular spaces resulting from increased hydrostatic pressure (Louis and Fernandes, 2002). This loss of fluid is visible in cattle as edema in various regions of the body. Under prolonged duress, the right heart muscles will enlarge and, in extreme cases, will fail, resulting in death (Voelkel et al., 2006).

2.3 Factors influencing PH in cattle

2.3.1 Sex

Gender may impact the susceptibility or onset of PH through differences in cardiac performance and general pathology. Chu et al. (2005) observed that a survival advantage existed for females as compared to males when attempting to induce cardiac hypertrophy and heart failure. Further, induction of cardiac hypertrophy and subsequent progression to HF took longer in females than males (Chu et al., 2005). These results demonstrate that physiological changes associated with PH and subsequent HF are often more severe and have an earlier onset in males than females.

Quantitative genetic measures of cattle performance across genders have also been evaluated utilizing mean pulmonary arterial pressure (PAP) as an indicator of risk of developing high mountain disease (HMD) because of PH. In 2008, Shirley et al. evaluated fluctuations in PAP over time for males and females. Females exhibited an increased PAP of 0.022 $\pm$ 0.008 mmHg per day for each day of age. Conversely, males exhibited a decrease of 0.004 $\pm$ 0.01 mmHg per day for each day of age (Shirley et al., 2008). Cockrum et al. (2014) calculated different heritabilities for each of three gender categories (heifers, bulls, and steers) in yearling Angus cattle. The heritability measured for heifers was 0.21 $\pm$ 0.04, while the heritabilities for steers and bulls were 0.20 $\pm$ 0.15 and 0.38 $\pm$ 0.08 respectively (Cockrum et al., 2014). Zeng (2016) also estimated the heritability of PAP in yearling Angus heifers, bulls, and steers. The calculated heritabilities were 0.19 $\pm$ 0.03, 0.33 $\pm$ 0.06, and 0.37 $\pm$ 0.07 for heifers, steers, and bulls respectively (Zeng, 2016). These results suggest that sex could be a valuable source of variation when assessing PAP in cattle. Further, these differences could not only be attributed to gender itself but also to the fact that management strategies will differ between the three gender categories. Heifers, steers, and bulls have different breeding objectives or production endpoints to be met, which are achieved through different management strategies. For example, castration results in reduced testosterone levels, which increases fat deposition in steers (Owens et al., 1993). Conversely, heifers and bulls are maintained as breeding stock, and managed for their reproductive success. All three categories will have different nutrient requirements (National Research Council, 2016).

2.3.2 Age

The risk of developing HMD because of PH decreases with age. Majority of death loss due to progression of chronic PH. Cattle from birth to two years of age account for

approximately 75% of cattle succumbing to HMD (Pierson and Jensen, 1956; Blake, 1968). This was confirmed by literature released by a Utah Agriculture Experiment Station, who reported that of 397 cases of HMD in cattle, 269 confirmed cases were observed in cattle less than two years of age (Blake, 1968). Once cattle reach 2 to 5 years of age, incidence of HMD decreases from 75% to 3% (Blake, 1968). These studies did not report an explanation for such a drastic decrease in prevalence as age increases. However, this drastic decrease could be attributed to the fact that cattle either succumb to HMD or are culled for poor performance within the first two years of life.

The majority of data collected in cattle to infer PH status is recorded when cattle are weanling or yearling age. Measures recorded on cattle under one year of age may not be reliable as PAP measurements have greater variability in calves. As cattle age, the accuracy of their PAP measurement increases. Holt and Callan (2007) report that cattle that are at least 16 months of age will have more consistent and accurate PAP observations, which would be better to utilize in making management decisions if PAP is within a producer's breeding objective. However, some ranchers may wish to make culling decisions earlier than 12 to 16 months of age to reduce the amount of time, money, and resources being utilized on cattle that will not remain in the herd.

Studies that evaluate PAP over time are limited as most producers take a single measurement and utilize that value to determine whether the animal should remain in the herd. Neary et al. (2015a) evaluated a population of cattle over time and found that systolic PAP and pulmonary arterial pulse pressure increased uniformly with age. Zeng (2016) evaluated the correlation between weaning PAP and yearling PAP to determine if weaning PAP may be a good indicator of yearling PAP. The calculated correlation was 0.67 ± 0.18, coinciding with Holt and Callan (2007) who reported greater variability in PAP measurements recorded prior to 12 to 16

months of age. While the correlation reported by Zeng et al. (2015) depicts a moderate relationship between weaning and yearling PAP measurements, there remains a vast amount of variability between the two time points such that producers would be ill-advised to select based on weaning PAP. Enns et al. (1992) regressed PAP on age, concluding that for every day of age increase, PAP increased by 0.0387 mmHg. A similar study by Crawford (2015) regressed PAP on yearling age, reporting that for each day increased beyond weaning age, PAP increased by 0.03 mmHg. While these studies provide insight into the patterns of increased PAP over time, studies with repeated measures over the duration of an animal's life are limited.

2.3.3 Adaptation to hypoxic environments

Duration of exposure to environments with reduced atmospheric oxygen availability is one of many factors that can cause hypoxia, impact cardiopulmonary performance, and result in PH. However, not all cattle are equally impacted by changes in altitude. Cattle born at high elevations have reduced incidence of PH as compared to cattle relocated to high altitude later in life (Will and Alexander, 1970; Weir et al., 1974; Holt and Callan, 2007). Further, Tucker and Rhodes (2001) concluded that an acclimation period prior to assessment of PH results in the most accurate estimates. This acclimation period allows for cattle to overcome the acute hypoxia associated with increased altitude and instead evaluate the long-term implications of high altitude on an animal's health status (Tucker and Rhodes, 2001; Neary et al., 2015a).

The physiology underlying hypoxia-induced PH in cattle at high altitudes is predominantly characterized by alveolar hypoxia resulting in pulmonary vascular remodeling. Specifically, the reduced oxygen availability at the expense of an increased barometric pressure induces hypobaric hypoxia, a condition that also increases the risk of developing alveolar hypoxia (Neary et al., 2016a). In an effort to maintain alveolar sufficiency of oxygen ventilation

and perfusion, the pulmonary vasculature constricts, increasing pulmonary vascular resistance (Kuriyama and Wagner, 1981; Neary et al., 2016a). These changes are observed as an elevated PAP (Holt and Callan, 2007; Neary et al., 2016a).

Regarding cardiac response to vascular remodeling, Kuriyama and Wagner (1981) proposed that cattle with greater vascular tone or thicker arteries would have a more rapid and intense response to chronic hypoxia than their contemporaries with less vascular tone. This was confirmed by Tucker et al. (1975), who calculated a positive correlation between medial thickness of the vascular wall, the degree of PH, and right ventricular hypertrophy. Calves exhibited increased medial tone within the pulmonary vasculature in response to high altitude exposure (Naeye, 1965; Tucker et al., 1975). These findings by Naeye (1965) and Tucker et al. (1975) coincide with findings regarding the incidence of PH in calves as compared to cattle of yearling age or older.

Physiologic response to hypoxia induced by high altitude and therapies to lessen the impact of such responses from cattle have been evaluated. One of the most commonly recommended therapies in human medicine for acute hypoxia is the supplementation of oxygen to account for reduced oxygen availability (West et al., 2013). However, despite serving as a valuable therapy in humans, oxygen supplementation is not a viable therapy for calves suffering from PH (Neary et al., 2013). Neary et al. (2013) administered supplemental oxygen to calves unsuccessfully, finding the oxygen diffusion capacity to be low. This suggests that the issue results from a ventilation-perfusion mismatch. A 2016 study observed that calves responded to the physiological strain induced by high altitude by increasing the rate of alveolar ventilation. Increased rate of alveolar ventilation, more commonly called hyperventilation, can be detected

by not only measuring respiration rate, but also evaluating the partial pressure of carbon dioxide in arterial blood, which would decrease during hyperventilation (Gulick et al., 2016).

2.3.4 Health status and medical history

The medical history of a patient poses a great impact on their risk of developing PH. For example, humans with COPD, emphysema, liver disease, lupus, sleep apnea, blood disorders, or metabolic disorders have a greater risk of developing at least one form of PH (Mayo Clinic, 2017). Similarly, cattle with a history of respiratory disease or metabolic disease may be at a greater risk of developing PH (Holt and Callan, 2007; Neary et al., 2016a). Diagnosis of cattle with PH may be further complicated by the presence of a prior condition such as respiratory disease that can result in diagnosis of a recurring condition rather than development of PH in the animal (Neary et al., 2016a). This not only provides limited assistance in mitigating a condition with few therapeutic remedies but can also result in a gross underestimation of the number of cattle developing PH in the United States annually.

2.3.5 Genetics

The development of PH far exceeds environmental exposure that induces hypoxia. Research to date has demonstrated that genetics may infer tolerance to PH for some organisms, while negatively impacting the performance of others. This was demonstrated by Pauling (2017), who discovered that a re-ranking of cattle occurred based on mean pulmonary arterial pressure (PAP) measurements as cattle moved between high and low altitudes, suggestive of a genotype by environment interaction. The trait of interest when evaluating PH in cattle is PAP, which is an indicator trait that is utilized to make inferences about an animal's risk of developing high mountain disease (HMD) as a result of PH induced by high altitude (>1,500 m) exposure (Holt and Callan, 2007). Heritabilities for PAP have been estimated to be moderate (0.26 to 0.34) in

nature, which means that genetic improvement through selection is possible (Shirley et al., 2008; Crawford et al., 2016). However, due to a minute proportion of the population that succumbs to heart failure annually from progression of PH and HMD, it is difficult to cultivate selection tools such as expected progeny differences (EPDs). The establishment of EPDs for PAP is further complicated by the limited number of observations that are recorded in comparison to the number of animals within the record keeping system of a breed association.

2.4 Measures of cardiopulmonary performance

2.4.1 Pulmonary vascular Resistance

One of the principle assessments of cardiopulmonary health is pulmonary vascular resistance. Pulmonary vascular resistance (PVR) is measured by dividing the differential of mean pulmonary arterial pressure from mean pulmonary venous pressure and dividing it by the cardiac output (Equation 2.1). The units of measure for PVR are mmHg/L/min. Measuring PVR can elucidate vascular complications within the cardiopulmonary system compromising the overall health of the animal. An elevated PVR would be indicative of occlusion of blood flow. This occlusion is commonly classified as either reduction, obstruction, or restriction of flow (Griffin et al., 2008).

Equation 2.1. The calculation of pulmonary vascular resistance (PVR) is obtained by subtracting the mean pulmonary venous pressure, measured as the mean left atrial pressure (P_{LA}) from the mean pulmonary arterial pressure (P_{LA}). This differential is then divided by the cardiac output (Q).

$$PVR=\frac{\overline{P_{PA}}-\overline{P_{LA}}}{Q}$$

When measuring PVR in patients experiencing hypoxia, the most likely cause of an elevated PVR would be restriction. Restriction can be classified as either anatomic or vasoconstrictive in nature. These physiological responses are meant to maintain a normal

ventilation-perfusion ratio given reduced oxygen tension in the blood. The presentation of restriction will depend on whether the hypoxic state of the animal is considered to be acute or chronic in nature. Acute hypoxia will result in vasoconstriction. Vasoconstriction or constriction of the arteries within the pulmonary system serves as a temporary change to combat the hypoxic state of the animal. However, if an animal is in a long-term or chronic hypoxic state, they undergo anatomical changes that result in restriction of blood flow. This anatomical change is recognized as vascular remodeling, which is a change in the vascular tone and overall structure of the pulmonary vascular trunks to combat the chronic hypoxic state that an animal is facing.

2.4.2 Systemic oxygen extraction fraction

Systemic oxygen extraction fraction (sOEF) provides insight into tissue utilization of oxygen in the blood. More specifically, sOEF is the proportion of oxygen in arterial blood that is taken up by the peripheral tissues and organs of the body. Systemic oxygen extraction fraction is measured utilizing arterial oxyhemoglobin saturation, venous oxyhemoglobin saturation, hemoglobin concentration, partial pressure of oxygen in arterial blood, and partial pressure of oxygen in mixed venous blood (Equation 2.2).

Equation 2.2. The equation for systemic oxygen extraction fraction where s_aHbO_2 is arterial oxyhemoglobin saturation (%), $s_{mv}HbO_2$ is mixed venous oxyhemoglobin saturation (%), Hb is hemoglobin concentration (g/L), p_aO_2 is the arterial partial pressure of oxygen (mmHg), and $p_{mv}O_2$ is the mixed venous partial pressure of oxygen (mmHg).

$$sOEF = \frac{\left((s_aHbO_2 \times Hb \times 1.39) + (0.003 \times P_aO_2)\right) - ((s_{mv}HbO_2 \times Hb \times 1.39) + (0.003 \times P_{mv}O_2)}{\left((s_aHbO_2 \times Hb \times 1.39) + (0.003 \times P_aO_2)\right)}$$

A normal sOEF is approximately 0.2, meaning that only about 1/5 of the oxygen in arterial blood is being taken up throughout the systemic circulation to support peripheral tissues and basic physiological functions (Dellinger, 2002; Leach and Treacher, 2002; McLellan and Walsh, 2004). However, not all tissues have the same oxygen requirements. For example, cardiac

muscle has an oxygen extraction fraction (OEF) of about 68%, while skeletal muscle has an OEF of about 25% when at rest (Binak et al., 1967). The sOEF must fluctuate to accommodate an increased demand for oxygen by peripheral tissues as well as when delivery of oxygen decreases. This fluctuation is important as it serves to increase the systemic OEF as a means to maintain aerobic metabolic processes (Olkowski et al., 2005; Rady et al., 1994).

2.4.3 Pulmonary arterial pressure

An overview of PAP

Mean pulmonary arterial pressure (PAP) is a veterinary procedure consisting of threading a catheter containing a transducer through the jugular vein into the heart. The catheter is threaded through the right side of the heart into the pulmonary artery where a pressure measurement is taken. The calculation of PAP is a function of systolic pulmonary arterial pressure (sPAP) and diastolic pulmonary arterial pressure (dPAP). The calculation for PAP is not an arithmetic mean, meaning it is not a direct average of sPAP and dPAP (Equation 2.3). Modifications have been made to the original PAP equation in order to improve the accuracy of estimation (Equation 2.4) (Razminia et al., 2004).

Equation 2.3 The traditional PAP measurement used prior to modifications by Razminia et al. (2004).

$$PAP = \frac{1}{3}sPAP + \frac{2}{3}dPAP$$

Equation 2.4 The updated PAP calculation derived by Razminia et al. (2004) that includes heart rate (HR) in order to increase the accuracy of PAP estimation.

$$PAP = dPAP + \left[\frac{1}{3} + (HR \times 0.0012)\right] \times (sPAP - dPAP)$$

In livestock, PAP measurements are measured in high altitude production systems, where hypoxia-induced pulmonary hypertension (WHO category 3 PH) could be a concern for producers. Through measuring PAP, the degree to which cattle are experiencing PH can be

elucidated. Further, inferences can be made about an animal's risk of succumbing to heart failure (HF) due to the progression of PH (Table 2.1) (Holt and Callan, 2007).

Table 2.1 Interpretations of mean pulmonary arterial pressure (PAP) scores in cattle. (Holt and Callan, 2007).

PAP	Interpretation
30-35 mmHg	This score is considered excellent and highly reliable.
36-39 mmHg	This score is considered excellent for any animal over the age of 12 months. If the animal is less than 12 months of age, the score is still fairly reliable, but retesting before breeding is suggested.
<41 mmHg	Scores less than 41 mmHg are reliable measurements in all animals more than 12 months of age. It is recommended that yearling cattle have a PAP measurement less than 41 mmHg (depending on altitude of the test). The variation in scores 41 mmHg and above is inconsistent and difficult to predict in some cattle as they age. Any animal measuring 41 mmHg and greater should always be retested before use.
41-45 mmHg	This range is acceptable for older animals (ie, more than 16 months of age). Animals less than 16 months scoring in this range should be retested to predict the future PAP of the animal accurately.
45-48 mmHg	This range is acceptable only for older animals that have been in high elevations for an extended period of time. Animals with this score are more susceptible to environmental stresses leading to HMD and should be considered at some risk. Elevation of test site and where the animal lives must be evaluated closely for those in this PAP score range.
>49 mmHg	Animals that score in this range must always be considered high-risk candidates for developing HMD, not only for themselves but also their offspring. Many animals that have scored in this range have died of HMD. An option for these animals is to move them to a lower elevation for use there. It is also recommended that offspring of these animals never return to high altitude.

2.5 Clinical assessment of cardiopulmonary health

2.5.1 Clinical blood panels in cattle

Ahola et al. (2006) evaluated the efficacy of utilizing arterial blood parameter measurements in lieu of recording PAP measurements of cattle in an effort to find an alternative to the pricey and invasive procedure. This was evaluated by calculating correlations between arterial blood parameters (blood gases, blood electrolytes, and blood cellular components) and PAP. These parameters were measured utilizing three current technologies: hemogram, pulse oximetry, and portable clinical analyzer. The only parameters that were correlated with PAP were the hemogram measurements of packed cell volume, hemoglobin concentration, and red blood cell distribution width. None of the metrics recorded via pulse oximetry or portable clinical analyzer were correlated with PAP despite overlap between the three technologies, which all measured packed cell volume and hemoglobin (Ahola et al., 2006).

2.5.2 Clinical blood parameters and PH

The body has several physiologic responses to both acute and chronic hypoxia. These physiologic responses serve to maintain oxygen delivery to peripheral tissues in order to supply the mitochondria with oxygen to maintain functions at the cellular, tissue, and systemic levels. In addition to the changes within the cardiopulmonary tissues themselves, the blood chemistry of an animal will also alter in an effort to preserve oxygen levels in the blood (Cueva, 1967; Weir et al., 1974; Ahola et al., 2006; West et al., 2013).

Response of red blood cells and plasma volume to high altitude

Reduced atmospheric oxygen availability poses a challenge to animals as the body must compensate for respiratory insufficiencies. One of the best-known adaptations to this reduction in available oxygen is the increase of red blood cells (RBC) per unit of blood (Bert 1878; West et al., 2013). This increase in RBC results in an increased oxygen carrying capacity within the

blood due to a greater volume of hemoglobin to bind and carry oxygen throughout the body (Bert 1878; Viault, 1891; West, 1981; High altitude medicine and physiology 5th edition). However, despite the increase associated with hypoxia induced by high altitude, the benefits of an increased RBC count have been demonstrated to provide limited assistance to hypoxic humans in some instances (Winslow et al., 1985; Winslow and Monge, 1987; Calbet et al., 2002; West et al., 2013).

Plasma volume is also impacted by duration at high altitude. In the early stages of high-altitude exposure, plasma volume decreases. As duration at high altitude progressed, it was found that plasma volume increased but never reached the volume measured prior to altitude exposure (West et al., 2103). Sanchez et al. (1970) compared a high-altitude dwelling population to a sea-level dwelling population in Peru in order to estimate differences in blood volume as compared to plasma volume. After compensating for weight differences, Sanchez et al. (1970) concluded that people from high altitude had a reduced plasma volume and increased overall blood volume compared to the people at low altitude. This observed increase in blood volume in conjunction with a reduced plasma volume could be due to a compensatory increase in red blood cells. Individuals that experienced acute hypoxia due to high altitude exposure exhibited plasma volume measures greater than or equal to initial measures after 1-3 days back at sea-level (Robach et al., 2000).

Erythropoietin

Erythropoiesis is the process of forming red blood cells (West et al., 2013). In the absence of hemorrhage, anemia, bone marow disorders, iron deficiencies or other conditions that impact hemoglobin, erythropoiesis is regulated by levels of erythropoietin. Erythropoietin is a hormone produced by the liver and kidneys (Eckardt, 1996). Levels of erythropoietin increase in

the presence of tissue hypoxia with the common causes of elevated erythropoietin being blood loss and hypoxia. In the presence of tissue hypoxia, oxygen-sensing cells within the inner cortex and outer medulla of the kidney respond by increasing erythropoietin secretion, thus, inducing erythropoiesis (Eckardt, 1996; Semenza, 2009).

Hypoxic ventilatory response

Erythropoiesis in response to tissue hypoxia increases the red blood cells in the circulatory system. However, in cases of acute hypoxia, erythropoiesis does not occur at a rate conducive to alleviating the stress of hypoxia immediately. In addition to elevated erythropoietin to stimulate red blood cell formation, the carotid body mediates a hypoxic ventilatory response (Fisher and Langston, 1967). Unlike the liver and kidneys, the carotid body responds to the partial pressure of oxygen in the arterial blood. When the arterial pressure of oxygen decreases, the carotid body detects the change and induces increased ventilation as a means to increase oxygen intake (Eckardt et al., 1989). Hypoxic ventilator response occurs within seconds. However, full erythropoietin responses take multiple days (Eckardt et al., 1989; Semenza, 2009).

Maintenance of blood pH

Blood pH is important due to its relationship with oxygen affinity and, subsequently, oxygen transport (West et al., 2013). When a lowland acclimated individual relocates to high altitude, peripheral chemoreceptors induce hyperventilation, resulting in decreased arterial carbon dioxide in the circulation which increases blood pH. High altitude natives have arterial pH values of 7.4. Based on comparable arterial pH values between lowland and highland dwellers, it is speculated that highland dwellers have a fully compensated respiratory alkalosis (Winslow and Monge, 1987). Conversely, lowland dwellers have an increased pH when exposed

to high altitudes. However, when given time to acclimate to the increased elevation, the pH declines towards the normal value of 7.4 (Dill et al., 1937).

Oxygen dissociation curve

The oxygen dissociation curve (ODC) provides insight into how well oxygen has been bound to red blood cells and delivered to tissues. The ODC characterizes oxygen's affinity to bind to the hemoglobin within the red blood cells and is dependent upon pH, partial pressure of carbon dioxide, and 2,3-diphosphoglycerate in the blood. A shift to the left on the ODC is indicative of alkalosis (pH > 7.5) due to increased abundance of oxygen that is more tightly bound to hemoglobin. Conversely, a shift to the right on the ODC indicates acidosis (pH < 7.3). In the case of acidosis, partial pressure of carbon dioxide in the blood is elevated, resulting in a decreased affinity of oxygen to remain bound to hemoglobin. This decreased oxygen binding affinity results in easier release of oxygen to the tissues (T.N. Holt, personal communication).

In addition to blood pH and partial pressure of carbon dioxide, 2, 3-diphosphoglycerate concentrations also impact the ODC. Concentrations of 2, 3-diphosphoglycerate are dependent on red blood cell metabolism. When red blood cell metabolism increases, the concentration of 2, 3-diphosphoglycerate also increases as it is an end-product of this metabolic process. This increase is indicative of chronic hypoxia and results in an ODC shift to the right for easier oxygen release. Reduction of 2, 3-diphosphoglycerate means that red blood cells are more abundant in the blood, so the ODC will shift to the left and oxygen binding affinity to hemoglobin would increase (T.N. Holt, personal communication).

Blood gas tensions and electrolyte measures

Blood gas tensions are measured as a metric of blood acid-base chemistry. Measuring both venous and arterial blood gases provides insight into gas exchange processes and systemic

blood gas utilization. However, measurement of venous blood gases and references for venous blood gas metrics in humans, cattle, and other species are scarcely reported. Arterial blood gases are commonly reported when focusing on acid-base chemistry and provide insight into a number of physiological changes indicative of poor or declining health (West et al., 2013). While control measures for bovine diagnostics are limited, veterinarians that perform these tests on cattle have compiled their own control parameters of these blood panels that are not published (Table 2.2; Table 2.3).

Table 2.2 Normal ranges of blood gas measures impacting acid-base chemistry of arterial and venous blood samples in cattle.

Acid-base chemistry parameter	**Units**	**Normal bovine range (arterial)**	**Normal bovine range (venous)**	**Citations**
PO_2	mmHg	80-100 70-80[i]	35-40	(T.N. Holt, personal communication)
PCO_2	mmHg	35-45	35-44	(J. Kaneko et al., 2008; T.N. Holt, personal communication)
HCO_3	mEq/L	22-26	17-29 25-35	(J. Kaneko et al., 2008) (T.N. Holt, personal communication)
pH	--	7.35-7.45	7.31-7.53	(J. Kaneko et al., 2008; T.N. Holt, personal communication)
TCO_2	mEq/L	--	21-32	(J. Kaneko et al., 2008)

[i] Arterial PO_2 is 70-80 mmHg for cattle at $\geq$ 1,500 m elevation.

Table 2.3 Normal ranges for blood electrolytes and components of blood chemistry in arterial and venous blood samples of cattle.

Blood electrolytes and blood chemistry parameters	Units	Normal bovine range (arterial)	Normal bovine range (venous)	Citations
Sodium	mEq/L	--	136-147	(T.N. Holt, personal communication)
Potassium	mEq/L	--	4.0-5.0	(T.N. Holt, personal communication)
Chloride	mEq/L	--	96-107	(T.N. Holt, personal communication)
Calcium	mg/dL	--	7.6-10.2	(T.N. Holt, personal communication)
Phosphorus	mg/dL	--	4.0-8.6	(T.N. Holt, personal communication)
Magnesium	mg/dL	--	1.6-3.6	(T.N. Holt, personal communication)
Anion gap	mEq/L	--	14-26	(T.N. Holt, personal communication)

In cattle exposed to high altitudes, blood gas measurements are valuable because they can provide insight into an animal's cardiopulmonary health and hypoxic status prior to exhibiting physical symptoms indicative of HMD or FHD. For example, reduced arterial partial pressure of carbon dioxide accompanied by elevated serum bicarbonate and an elevated base excess is indicative of compensatory hyperventilation induced by hypoxia. Elevated serum lactate measures may be indicative of enhanced glycolysis, which may be induced by hypoxia. Evaluation of oxygen saturation in both arterial and venous blood is utilized to calculate the arterial-venous oxygen saturation of hemoglobin (A-V difference). An A-V difference of 30% or greater can also be indicative of hypoxia. All of these parameters would indicate that further evaluation of an animal's cardiopulmonary health may need to be evaluated, or if HMD or FHD is suspected, relocation to a lower altitude or feeding a different ration may be a viable option to alleviate signs of hypoxia (G.M. Krafsur, personal communication).

2.5.3 Sources of variation in clinical blood panels

Clinical assessment of blood samples, while informative in diagnosing patients and formulating a treatment plan, can be complicated by a number of factors that enhance the variation of the measures taken. Differences in laboratory as well as laboratory technician may increase variability of blood panel estimates. These can range from differences in standard laboratory handling procedures to variations in units of measurement, which can confound comparisons across laboratories if not detected. Blood panel results will also vary depending on age, sex, species, breed, and status within the respective livestock production setting. It is therefore of the utmost importance that such phenotypic data is available in order to determine which normal parameters are most feasible to utilize in making diagnoses.

Once samples are collected and analyzed, the interpretation of the results generated adds an additional level of complexity. Data should be compared to normal values generated from animals of similar age, sex, species, breed, and stage of production as the patient being evaluated. Further, the normal values utilized for diagnostic purposes should be validated either through the clinic or laboratory performing the blood panels, from reputable textbooks, or through peer reviewed journal publications. Lack of validation of the normal parameters being utilized to assess patient health, may result in discrepancies in the results as well as misdiagnoses in extreme cases.

2.5.4 Limitations of clinical blood panels in cattle

One of the primary challenges of utilization of modern technologies to measure blood gases and electrolytes is the lack of reference measures for comparative studies and diagnostic purposes. Research has demonstrated that metrics of cardiopulmonary health such as mean pulmonary arterial pressure (PAP) vary in cattle. More specifically, measurements may differ

based on breed, sex, age, production stage, etc. (Greatorex, 1957; Claxton and Ortiz, 1996). Therefore, a single reference value for each blood gas or blood electrolyte metric may present inaccurate conclusions and diagnoses when evaluating cattle. The Merck Veterinary Manual presents a single set of reference values for cattle, which is nondescript, not indicating age, breed, sex, or production stage (Fielder). While still a valuable resource for diagnostic purposes, as researchers work towards characterizing manifestations of PH in cattle, these gaps in knowledge are important to fill in order to make sure that the most accurate information possible is being implemented into future research efforts.

2.6 Signs and symptoms of PH in cattle

A number of complications exist in diagnosing PH in cattle. Symptoms commonly present in the latter stages of PH. Due to the late-stage diagnosis of PH, little can be done to save the animal (Holt and Callan, 2007). After signs have been recognized, cattle often die within 12 weeks, with the majority of cattle succumbing to HF within one month (Glover and Newsom, 1917; Pierson and Jensen, 1956). Crawford (2015) summarized ante-mortem and post-mortem signs and symptoms of PH in cattle (Table 2.4). However, further signs and symptoms are being evaluated to better detect incidences of PH. One of the new signs being used to evaluate pulmonary vascular remodeling and impending HF in cattle is post-mortem cardiac score.

Table 2.4 Ante-mortem and post-mortem signs and symptoms of HMD in cattle. (Crawford, 2015)

Ante-mortem	Post-mortem
Lethargy	Increased hepatic enzymes
Tachypnea (rapid breathing)	Enlarged, hard liver
Drooped ears	Enlarged, dilated heart
Rough hair coat	Lesions
Ataxia (lack of muscle control)	
Jugular vein distension	
Brisket edema	
Exophthalmia (protrusion of eyeballs)	
Ascites (fluid in abdomen)	
Generalized edema (intermandibular, ventral abdominal, limb)	
Decreased appetite	
Recumbent (lying down, inactive)	
Unable to rise	
Elevated heart and respiratory rates	
Muffled heart sounds	
Diarrhea	
Moist sporadic cough	
Gradual emaciation	
Inflammation	

2.6.1 Post-mortem cardiac scores

The diagnosis of HMD often does not occur until cattle have displayed visible symptoms or have succumbed to HF secondary to PH (Holt and Callan, 2007). Some cattle undergo vascular and cellular remodeling at a slower rate than those that have exhibited symptoms of HMD. These cattle may have survived through the finishing phase of production to be harvested and, as a result, are not classified as hypertensive or experiencing HMD. Therefore, a post-

mortem cardiac scoring system has been designed by Colorado State University and the United States Department of Agriculture Meat Animal Research Center (USDA-MARC) (T. N. Holt, personal communication). This system has been designed in order to not only further validate PAP as an indicator trait of susceptibility to PH and development of HMD, but to distinguish cattle that have never been PAP tested and may be undergoing vascular remodeling and other cardiopulmonary changes indicative of PH (T. N. Holt, personal communication).

The cardiac scoring system consists of looking at the heart and assigning a numeric score from 1 to 5, with 1 indicating a normal heart and 5 indicating a severely remodeled heart upon gross evaluation (Table 2.5) (Figure 2.2).

Table 2.5 Descriptions of the gross characteristics assessed to distinguish cardiac scores.

Cardiac Score	Description
1	<u>Normal Heart</u> • Normal conical shape • Normal left ventricle apex that is easily distinguished • Right ventricle is smaller than the left ventricle and ventricles are in normal proportions • Normal atrial anatomy such that the right atrium is smaller than the left atrium • No clinical evidence of infarction or aneurysm pending • No thinning of vessel walls • Normal pulmonary artery size
2	<u>Mild Change</u> • Normal conical shape • Blunting of left ventricle apex such that the apex is still distinguishable but losing some pointed shape • Right ventricle becoming larger than the left ventricle • Right ventricle is more pronounced • Right atrium beginning to enlarge to same sizer or slightly larger than the left atrium • No clinical evidence of infarction or aneurysm pending • Pulmonary artery beginning to exhibit mild enlargement • Upon palpation, cardiac muscle is stiff, suggestive of hypertrophy and loss of luminal space (may be bi-ventricular)
3	<u>Moderate Change</u> • Beginning to lose conical shape • Blunting of left ventricular apex such that apical shape is still visible but deviating and beginning to take the shape of a reversed or backwards letter "D" • Right ventricle is larger than the left ventricle • Right ventricle is more pronounced • Right atrium is larger than the left atrium • Can present with clinical evidence of infarction or aneurysm pending • Pulmonary artery enlarged • Upon palpation, cardiac muscle is stiff, suggestive of hypertrophy and loss of luminal space (may be bi-ventricular)
4	<u>Severe Changes</u> • Loss of conical shape • Loss of left ventricle apex • Right ventricle is larger than the left ventricle with the right ventricle becoming more pronounced and taking on a rounded shape • Reverse "D" shape of heart is apparent • Right atrium larger than the left atrium and is congested • Can present with clinical evidence of infarction or aneurysm pending • Pulmonary artery greatly enlarged • Upon palpation, cardiac muscle is becoming soft and without shape but still has some muscle tone
5	<u>Severe Changes and Flaccid Heart</u> • Severe loss of conical shape • Loss of left ventricular apex due to right ventricular rounding • Apparent reverse "D" shape of the heart • Right ventricle more pronounced and rounded • Right atrium larger than the left atrium and congested • Can present with clinical evidence of infarction or aneurysm pending • Pulmonary artery greatly enlarged • Upon palpation, cardiac muscle is soft, without shape, and without muscle tone (heart will lay flat)

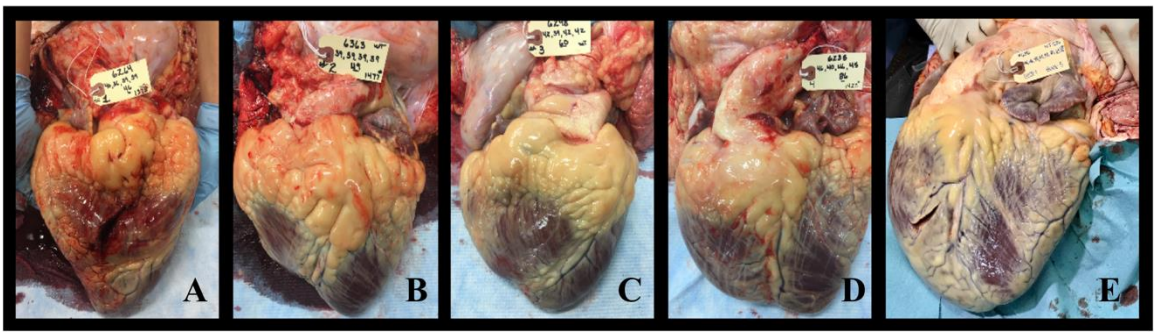

Figure 2.2 Images depicting each cardiac score. (A) This is cardiac score of 1. Notice the conical shape of the heart and the proportions of the chambers of the heart. This heart appears normal. (B) This is a cardiac score of 2. The heart is losing shape in the right ventricle (oriented on the reader's left side). However, the pulmonary artery appears normal and the overall conical shape of the heart is easily distinguished. (C) This is a cardiac score of 3. The right ventricle is beginning to bow outwards. The conical shape of the heart is becoming less prominent and more rounded. With the left ventricular blunting, the heart is beginning to take the shape of a backwards "D." The pulmonary artery appears enlarged. (D) This is a cardiac score of 4. The left ventricle apex has nearly disappeared, the right side of the heart is larger than the left side, and the pulmonary artery is enlarged. The overall reverse "D" shape of the heart is easily observed. (E) This is a cardiac score of 5. The overall conical shape of the heart has disappeared, the reverse "D" shape is still evident, the pulmonary artery is enlarged, and the heart has taken an overall rounded shape. Upon palpation of the cardiac muscle depicted, a loss of tone would be evident. Further, the heart would feel floppy and lay flat on a table (Figure designed by author).

2.7 PAP as an indicator trait for PH

2.7.1 Genetics of PAP

Mean pulmonary arterial pressure (PAP) measures PH and is also utilized to assess the risk of developing High Mountain Disease (HMD) resulting from the progression of PH (Holt and Callan, 2007). Through assessing PAP measurements, it has been concluded that genetic improvement to reduce incidence is possible due to the moderate heritability estimates that have been reported (Schimmel, 1981; Schimmel and Brinks, 1983; Enns et al., 1992; Shirley et al., 2008; Zeng, 2016; Crawford et al., 2016). Further, PAP is polygenic, meaning that multiple loci on multiple chromosomes affect the expression of the PAP phenotype (Cánovas et al., 2016). Studies have elucidated various genes that may affect PAP in cattle, humans, or mice (Table 2.6). However, this list is not exhaustive as most research has been conducted in mice or humans

rather than cattle. Research in cattle has used SNP chip genotype data which does not include every nucleotide within the bovine genome.

Table 2.6 A brief summary of genes related to high altitude exposure in humans, cattle, or other laboratory animals.

Gene Abbreviation	Gene Name	Function	Citations
EGLN1	EGL nine homolog 1	Post-translational formation of hydroxyproline in hypoxia-inducible factor (HIF) alpha proteins	Simonson et al., 2010; Buroker et al., 2012; Ge et al., 2012; Xiang et al., 2013; Zeng, 2016
EPAS1	Endothelial PAS domain protein 1	Induction of genes regulated by oxygen	Scortegagna, et al., 2003; Buroker et al., 2012; Xiang et al., 2013; Yang et al., 2013; Newman et al., 2015; Crawford et al., 2016; Zeng, 2016
PPAR-α	Peroxisome proliferator-activated receptor alpha	Expression of target genes involved in cell proliferation, cell differentiation and in immune and inflammation responses	Törüner et al., 2004; Simonson et al., 2010; Zeng, 2016; Heaton et al., 2016
PPAR-γ	Peroxisome proliferator-activated receptor gamma	Regulates adipocyte differentiation	Törüner et al., 2004; Oka et al., 2006; Simonson et al., 2010; Mahon et al., 2016; Zeng, 2016
ACE	Angiotensin-converting enzyme encoding	Regulates blood pressure, salt concentrations, and fluid concentrations as part of the renin-angiotensin system	Buroker et al., 2010; Srivastava et al., 2011; Luo et al., 2014; Zeng, 2016
ASIC2	Acid sensing ion channel subunit 2	Membrane ion channel; Activator of the calcineurin/NFAT signaling pathways	Grifoni et al., 2008; Lu et al., 2009; Abboud and Benson, 2015; Zhou et al., 2017; Crawford, 2019
EDN1	Endothelin 1	Vasoconstrictor	Schiffrin, 2005; Murphy and Eisner, 2006; Castro et al., 2007; Deacon et al., 2010; Calabro et al., 2012; Bkaily et al., 2015; Crawford, 2019
FBN1	Fibrillin 1	Extracellular matrix glycoprotein	Powell et al., 1997; Shen et al., 2011; Jeppesen et al., 2012; Chen et al., 2014; Crawford, 2019
KCNMA1	Potassium calcium-activated channel subfamily M alpha 1	Large conductance ion channel	Tomas et al., 2008; Barnes et al., 2016; D. Brown (results unpublished); Crawford, 2019
NOX4	NADPH oxidase 4	Catalytic subunit the NADPH oxidase complex; Acts as an oxygen sensor	Mittal et al., 2007; Li et al., 2008; Chen et al., 2012; Zhao et al., 2015; He et al., 2017; Crawford, 2019
P2RY6	Pyrimidinergic receptor P2Y6	G-protein coupled receptor; Mediates inflammatory responses	Hou et al., 1999; Nishida et al., 2008; Tovell et al., 2008; Nishimura et al., 2016; Sunggip et al., 2017; Crawford, 2019

PLA2G4A	Phospholipase A2 group IVA	Catalyzes the hydrolysis of membrane phospholipids to release arachidonic acid	Osanai et al., 1998; Handlogten et al., 2001; Magne et al., 2001; Ait-Mamar et al., 2005; Crawford, 2019
RCAN1	Regulator of calcineurin 1	Calcium/calmodulin-dependent phosphatase	Bush et al., 2004; van Rooij et al., 2004; Canaider et al., 2006; Grammer et al., 2006; Crawford, 2019
RGS4	Regulator G protein signaling 4	Regulator/inhibitor of G-protein signaling	Owen et al., 2001; Mittmann et al., 2002; Cho et al., 2003; Gu et al., 2010; Opel et al., 2015; Crawford, 2019
THBS4	Thrombospondin 4	Adhesive glycoproteins that mediate cell-to-cell and cell-to-matrix interactions	Stenina et al., 2005; Gabrielsen et al., 2007; Mustonen et al., 2012; Crawford, 2019
NFIA	Nuclear Factor I A		Heaton et al., 2020
ARRDC3	Arrestin domain containing 3		Oka, 2006; Zeng, 2016; Heaton et al., 2020
ROCK2	Rho associated coiled-coil containing protein kinase 2	serine/threonine kinase regulating cytokinesis, smooth muscle contraction, the formation of actin stress fibers and focal adhesions	Zeng, 2016

2.8 Physiological conditions in cattle resulting from PH

2.8.1 High Mountain Disease (HMD)

Introduction to HMD

Certain cattle respond especially negatively to the hypoxic conditions of high altitude. As a result, these cattle develop PH that further progresses into a condition recognized as High mountain disease. This condition is one of the leading causes of morbidity and mortality for cattle at elevations of 1,500 m or greater (Holt and Callan, 2007). Annual incidence of HMD in cattle native to high altitudes is 3% to 5% (Holt and Callan, 2007). However, for non-native cattle relocated to high altitude, incidence of HMD increases to 10% to 40% of cattle (Grover et al., 1963; Will et al., 1970).

Economics of HMD

The economic impact of HMD on high altitude beef production systems has not been extensively characterized, but there are studies that have attempted to quantify the annual losses resulting from this condition. Holt and Callan (2007) estimated the economic losses due to HMD

to be about $60 million annually. However, since 2007, the economic losses may have increased due to our ability to better detect and diagnose HMD in cattle. Beef producers managing cattle at high altitudes are acknowledging HMD and its resulting economic implications due to cattle death loss. As a result, producers are not only investing in PAP testing their cattle, but considering PAP when making selection decisions or purchasing cattle (Holt and Callan, 2007; Kessler, 2013). Based on data from Colorado State University Beef Improvement Center's annual bull sale, bull buyers place emphasis on PAP in addition to traits such as weaning weight, frame score, yearling weight, calving ease, and stayability when choosing a herd bull. More specifically, bull buyers were willing to pay premiums for bulls with PAP scores of 46 mmHg or less (Kessler, 2013). The premiums for a low PAP bull often range from $2,000 to $5,000 per bull, offsetting some of the financial losses accrued due to cattle that succumb to HMD (Holt and Callan, 2007).

PAP as an indicator of risk of HMD

All types of PH will result in an elevated PAP, regardless of whether the manifestation of PH is acute or chronic. Due to this relationship, PAP has been incorporated into cattle production and is utilized as an indicator trait for genetic selection at high altitudes. More specifically, PAP is utilized to measure PH and assess an animal's risk of developing HMD as a result (Holt and Callan, 2007). However, not all cattle that have an elevated PAP will develop HMD. It is important to recognize that PAP is a phenotype recorded in cattle in an effort to make selection decisions. A phenotype is dependent upon the genetics of the animal as well as the environment it is exposed to (Bourdon, 2000). Therefore, to fully understand PAP and its role in combatting HMD, the genetics underlying how the animal responds to the adversity of high altitude should be characterized.

Inheritance of HMD

Differences in incidence of HMD have been reported between native and non-native cattle (Grover et al., 1963; Will et al., 1970; Holt and Callan, 2007). These findings are indicative of an adaptation to high altitude or a genetic propensity for cattle to survive in a hypoxic environment with PH. Similar conclusions have been drawn from studies in humans as well, further supporting the premise that differences in adaptability across different demographics and populations could derive from genetic differences (Maclnnis et al., 2010). Genes that may impact response to HMD continue to be studied in both humans and animals.

2.8.2 Feedlot Heart Disease

Another manifestation of PH in cattle occurs in feedlot settings. This condition, known as feedlot heart disease (FHD), has been observed in cattle that have never been exposed to high altitudes (Jensen et al., 1976; Pringle et al., 1991; Malherbe et al., 2012). The symptoms of FHD resemble those of HMD, leading many to wonder if the cardiopulmonary changes as a result of PH are the same. Research the epidemiology and physiology of FHD is scarce. In 1976, HF resulting from FHD was reported to impact 2.85 out of every 10,000 cattle (Jensen et al., 1976). Further, Neary et al. (2016) reported an increase in incidence of FHD from year 2000 to 2012, when adjusting for period of feedlot placement, risk category, sex, age, feedlot, and death loss due to other conditions such as digestive disorders. In contrast to the incidence of FHD reported by Jensen et al. in 1976, Neary et al. (2016) reported that incidence of FHD in 2012 increased to 1.08 per 1,000 cattle. Upon further investigation, it was concluded that feedlot cattle treated for bovine respiratory disease (BRD) were three times more likely to succumb to HF in the feedlot. While death loss due to FHD is not as prevalent as digestive disorders or respiratory disease, the

increased prevalence of HF resulting from the condition is costly for the beef industry as half of the deaths reported occurred after 19 weeks in the feedlot (Neary et al., 2016).

Despite quantifying the increased incidence of FHD, management strategies to detect cattle experiencing the condition and administer treatment for the condition have yet to be characterized. It is speculated that, like HMD, PAP would be the best indicator of risk of developing FHD since both derive from PH in susceptible cattle. However, in a feedlot setting, PAP testing cattle is not only costly but labor-intensive due to the immense volume of cattle in feedlots (T.N. Holt, personal communication). Further research needs to be conducted to determine how similar HMD and FHD are to one another and how to efficiently identify cattle experiencing FHD. Similar to HMD, genetics may play a role in susceptible cattle, which could be a less-invasive tool to determine how cattle will perform during the late-stages of finishing.

Section 3: Sequencing Methodologies and Technologies

3.1 Early sequencing

Since Watson and Crick established the three-dimensional structure of DNA in 1953, the field of genetics has changed tremendously. However, many of the molecular breakthroughs that have resulted in the technologies and methodologies of next-generation sequencing, occurred decades after the structure of DNA had been published (Hutchison, 2007; Heather and Chain, 2015). Despite the discovery of DNA prior to the establishment of first-generation sequencing technologies, it was not the first hereditary component to be sequenced. Instead, scientists focused the earliest sequencing efforts on RNA of small bacteria or other microorganisms. The small genome size accompanied with the single-stranded nature of RNA provided a less complicated template to cultivate the earliest sequencing strategies, which were adapted in order to also sequence DNA.

Many strategies of sequencing were developed throughout the first-generation sequencing era, but none were as widely adapted as Sanger's chain-termination sequencing methodology, more commonly known as Sanger sequencing (Heather and Chain, 2015; Sanger et al., 1977a). Di-deoxy chain termination sequencing was an improvement from "plus and minus" sequencing as this methodology was quite inefficient and error-prone, and Maxam-Gilbert sequencing, another methodology at the time, was laborious compared to Sanger sequencing (Sanger and Coulson, 1975; Sanger et al. 1977a; Sanger et al., 1977b).

The basis of Sanger sequencing was to incorporate triphosphate analogs for each of the four nucleotide bases, which would each be placed in their own vesicle with a DNA template. The triphosphate analog corresponding to one of the four nucleotide bases terminates the DNA chain where that nucleotide appears in the DNA sequence. This is performed for all four nucleotides individually, and then the results are assessed by running the four samples in parallel via gel electrophoresis. After gel electrophoresis is complete, researchers would read the gel, assessing the distribution of bands across the four lanes to assemble the sequence (Atkinson et al., 1969; Sanger et al., 1977a).

Sanger sequencing continued to be modified in the years that followed to streamline the procedures. Changes made to the initial protocol included altering the method of radio-labeling specific nucleotides in order to combine the triphosphate analogs from all four bases into a single tube of DNA template, thus reducing the labor leading up to gel electrophoresis. In addition to modifying the laboratory procedure itself, further cultivation of Sanger sequencing ultimately resulted in development of the first automated sequencing technology, which enabled researchers to sequence more complex genomes than had been possible to date (Smith et al., 1985; Heather and Chain, 2015).

The earliest automated sequencing machines, still part of the first-generation wave of sequencing, generated less than one kilobase of sequence from a run. However, strategies of shotgun sequencing allowed for contiguous reads to be aligned to one another to create longer read fragments for downstream applications (Anderson, 1981; Heather and Chain, 2015). The earliest sequencing technologies were further streamlined through the development of polymerase chain reaction (PCR) and other wet-laboratory techniques that more efficiently allowed for preparation of a purified sample prepared for sequencing. Further technological advances made way to automated sequencers able to sequence multiple samples in parallel. The ability to run many samples simultaneously allowed for researchers to begin characterizing larger genomes through large-scale collaborations such as the Human Genome Project (Heather and Chain, 2015).

3.2 Next-generation sequencing

The first-generation of sequencing technologies inspired the development of new sequencing methodologies from companies such as 454 and Solexa (Heather and Chain, 2015). Sequencing companies were becoming more prevalent and cultivating their own sequencers. Competition amongst these companies resulted in rapid improvements in technology to reduce sequencing costs per sample. Further, some companies succumbed to the vast competition, liquidating their assets or being absorbed by one of the more successful companies. In the end, Illumina was most successful in meeting the demands of the scientific community throughout this time and continues to develop the most popular sequencing technologies available to date (Heather and Chain, 2015).

3.2.1 Sequencing by synthesis

The most popular next-generation sequencing methodology utilizes a technology known as sequencing by synthesis (SBS). Sequencing by synthesis accounts for approximately 90% of the next-generation sequence data generated and comes in three forms of sequencing chemistry: pyrosequencing, sequencing by reversible termination, and sequencing by detection of hydrogen ions (Illumina; Ambardar et al., 2016). The SBS chemistry is designed to perform sequencing projects in parallel. Illumina's newest sequencers utilize reversible termination sequencing, a technology that not only allows for greater throughput or sequencing capacity, but also reduces bias present in many prior methodologies through equal availability of reagents for all four nucleotides throughout the sequencing process (Ambardar et al., 2016). The overall SBS sequencing procedure outlined by Illumina consists of four major steps: sample or library preparation, cluster generation, sequencing, and data analysis.

Library preparation

The library preparation step can be performed utilizing a kit that contains all necessary reagents. The basic steps of a library preparation protocol consist of purification and fragmentation of the DNA template (M.D. Stenglein, J.S. Lee, and D.B. Sloan; personal communication) (Figure 2.3). Then one must add adapters specific to the Illumina instrument to be used, and through reduced cycle amplification, the sequencing binding site, sample index, and sequence regions complementary to the oligos on the Illumina flow cell for binding (Figure 2.4). This is performed for each library then they are pooled, diluted, and denatured before being loaded into a sequencing cartridge that contains all necessary reagents for the sequencing run. The cartridge and flow cell are then loaded into the sequencer.

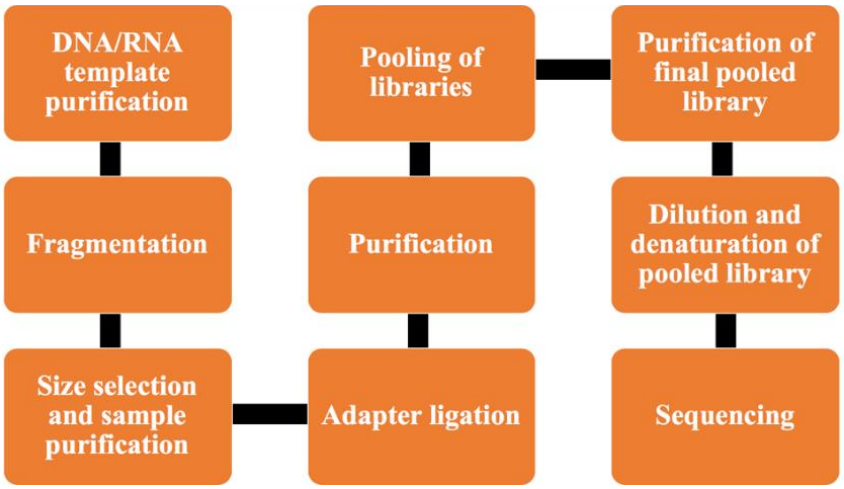

Figure 2.3 An overview of the library preparation and pre-sequencing steps for an Illumina sequencing run.

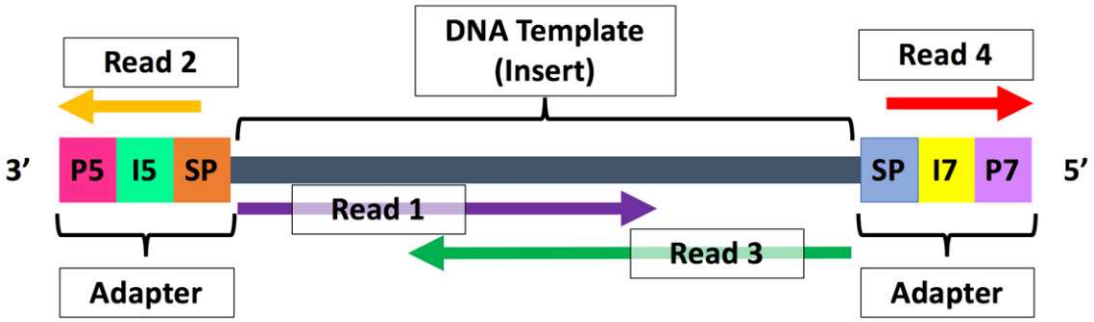

Figure 2.4 An example of a DNA fragment with ligated adapter components and illustration of how each read is generated for a paired-end sequencing run. The DNA template or DNA insert is the sequence region of interest. The DNA template is flanked on either side by ligated adapters

added via reduced cycle amplification. Within the adapter region is the sequencer binding site (P5 or P7) that is compatible with one of the two oligos within the lanes of the Illumina flow cell. Next to the binding site is the specific index that is unique to one of the animals being sequenced. I7 and I5 correspond to Illumina's index 1 and index 2 information, respectively. Samples can be dual indexed like is pictured above (I7 and I5 indexes) or single indexed (just I7 is present). Next to the index within the adapter region is the sequence primer binding region (SP). The sequencing primer binding region is where the polymerase binds to generate each of the reads. Each of the arrows denote a different read generated for each template strand through a paired-end sequencing run. Read 2 and read 4 allow for the index information in the adapter sequences to be read such that samples may be segregated from the pooled sequence data after the run is complete. Read 1 and read 3 are the paired-end reads of the DNA template, which are ideal for a complex sequencing project because the mid-read overlap allows for increased accuracy of called base pairs in the middle of the DNA template.

Cluster generation

The second phase of the Illumina sequencing pipeline is known as cluster generation. Cluster generation is the process in which each DNA fragment is isothermally amplified. During this process, one of the two varieties of oligos present within a lane of the glass flow cell will bind to the complement sequence that was added to the DNA fragment via reduced cycle amplification. A polymerase will then bind at the sequencing binding site to create a complement of the hybridized fragment. The sample is then denatured, and the original fragment that was bound to the flow cell is washed off. Bridge amplification then occurs using the newly formed read, which will bind to the complement oligo within the lane creating a bridge-like shape. The polymerase then copies the strand, creating a double-stranded bridge. The two reads are then denatured, becoming two unique reads that will be used as templates the next cycle of bridge amplification. This is repeated for millions of sequencing clusters for clonal amplification of all DNA fragments. Next, all reverse strands generated are washed off of the flow cell and an inhibitor is bound to the 3' end of each read to prevent unwanted priming of the remaining forward reads.

Sequencing

Upon completion of cluster generation, the actual sequencing process begins. All reverse reads are removed and the 3' ends of the forward reads are bound by an inhibitor to avoid unwanted priming. The sequencing process begins with the addition of the first primer corresponding to the complement of the nucleotide present on the strand starting with the sequencing binding site. This process consists of fluorescently tagged nucleotides being added to the growing chain one at a time based on the sequence of the template. At the end of each cycle, a fluorescent light is emitted and imaged to collect the sequence data. The number of cycles is used to determine the read length generated. During the sequencing process, hundreds of millions of clusters are sequenced in parallel.

After sequencing the first read, the read product is washed off of the flow cell so that a new read may be generated from the template. The index 1 read primer is hybridized to the same read template. This read is generated from the index 1 primer region to the oligo on the flow cell and then washed off. Currently, the 3' end of the read is de-protected to re-enable binding to the flow cell. The read will form a bridge again by binding the 3' end of the template to the complement oligo on the flow cell. At this point the index 2 read is generated and extends to the opposite oligo forming another double-stranded bridge. These strands are denatured to form individual linear strands. The original forward read is washed off the flow cell and the 3' end of the remaining reverse read is blocked to prevent unwanted primer binding. This entire process is then repeated for the reverse read. Sequencing of the forward and reverse strands of multiple DNA template strands is repeated hundreds of millions of times to generate multiple reads that correspond to all fragments in the prepared pooled library of DNA templates.

Data analysis

Upon completion of sequencing, reads can be demultiplexed using the unique index identifiers that were added in the library preparation steps. The demultiplexing process consists of taking pooled reads that were in the pooled library and sorting them into individual libraries again through the unique index information that was ligated to the fragments of each unique DNA library during the reduced cycle amplification step. Each sample can then be trimmed and assembled based on the overlap of reads to form contiguous reads (contigs) and scaffolds. These contigs and scaffolds can then be aligned to a reference genome or utilized to form a de novo assembly for further downstream analysis (Figure 2.5).

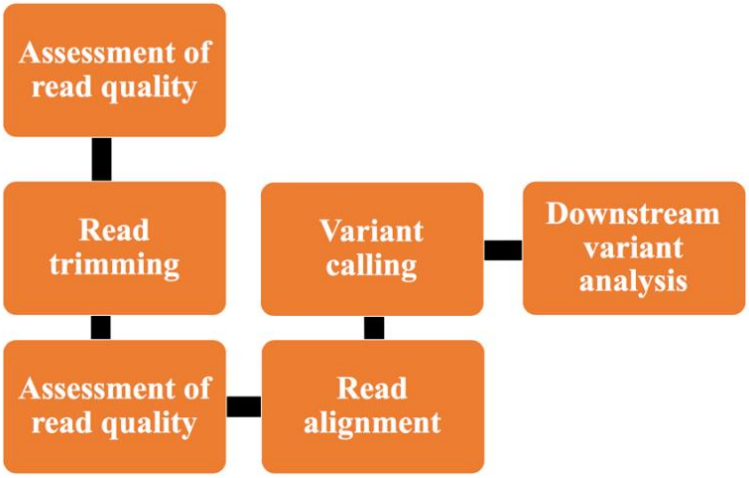

Figure 2.5 The basic steps of sequence data analysis.

3.2.3 SNP discovery

The process of determining where variants or polymorphisms exist within a genome is known as SNP discovery. During this process, sequence samples are compared to a reference genome in order to detect base pair differences (Nielsen et al., 2018). Due to the immense size of mammalian genomes such as the bovine genome, which is 2.7 gigabases in size, robust software and computer programs are utilized to compare sequences and call variants. Many of these SNP

discovery tools such as CLC Genomics Workbench (Qiagen Bioinformatics) or Genome Analysis Toolkit (GATK) (McKenna et al., 2010) allow for case-control comparisons in order to detect SNP between two separate populations in a study.

After SNP have been identified through comparisons of sequences, SNP must be investigated further in order to determine which genes contain SNP and what the functional consequences of each SNP may be. Tools such as the Ensembl Variant Effect Predictor (VEP) searches across the Ensembl database to determine functional consequences of a variant. The VEP indicates if a variant is an insertion, deletion, copy number variant, or structural variant. Further, the VEP will provide information about the genes and transcripts affected by a variant, the variant location, and the consequences of a variant on protein structure. The data provided about each variant provides insight into the physiological impact that a SNP may have on a gene and the overall function of an organism (Yates et al., 2020).

The final step of SNP discovery is validation of SNP. This is conducted by comparing genotypes at each discovered SNP to a trait of interest in a population of thousands of animals. The SNP that are concordant across a particular phenotype for the desired trait are considered to be valid and can proceed to be utilized for selection purposes. Conversely, SNP that are not concordant across animals with a particular phenotype would be eliminated from the pool of discovered SNP (Kumar et al., 2012).

Within livestock industries, SNP discovery is important for genomic selection. More specifically, SNP related to phenotypes of interest can be included on a SNP genotyping panel for genomic selection. Performing SNP discovery allows for relevant SNP to be incorporated into genetic evaluations and considered in breeding decisions.

3.2.4 Multi-omics analyses

Some traits within the beef industry are difficult to select for or against due to their polygenic nature (Moser et al., 2015). Further, genetic markers or polymorphisms associated with a complex trait often impact other traits, often resulting in pleiotropy. The overlap of loci that regulate multiple phenotypes can result in indirect selection for undesirable traits (Saatchi et al., 2014). It is difficult to parse loci related to multiple phenotypes in order to understand gene regulation and interactions for a single phenotype. However, through incorporation of multiple -omics technologies, a biological or physiological systems approach may be implemented to better understand genetic mechanisms underlying phenotypic expression for a given trait and create gene network models that depict how different genes and transcripts interact with one another to express a particular phenotype (Cánovas et al., 2014; Nguyen et al., 2018).

Multi-omics analyses benefit animal breeding in several ways. First, through combining -omics data, genes that may contain SNP valuable for genotyping may be easily identified and implemented in breeding programs. In addition, compilation of differential expression data and DNA sequence differences may be utilized to construct networks depicting how involved and interactive different loci are in phenotypic expression. In conjunction, pleiotropic effects may be closely evaluated in order to make genetic improvement for one trait while reducing the impact of selection against a separate economically relevant trait that shares an inverse relationship (Cánovas et al., 2014; Nguyen et al., 2018).

3.2.5 Genomics in livestock management

Commercial SNP genotyping has been widely adapted in the dairy and beef industries. Many breed associations are utilizing SNP panels to gather genomic information to enhance the accuracy of EPDs for genetic improvement. The resulting estimates of animal performance

incorporating SNP information is recognized as a genomic-enhanced EPD (GEPD). These measures are calculated by summing all marker effects across the genome that are associated with the phenotype of interest to form a direct genomic breeding value (DGV). These DGV are then combined with the phenotypic and pedigree information commonly utilized to calculate a standard EPD (Gray et al., 2012; Saatchi et al., 2012; Rolf et al., 2014).

Addition of genomic information into genetic evaluations enhances the amount of information included in calculations. This addition of genomic information results in increased accuracy of selection as it aids in measuring the "true" relationships between individuals. This resulting increase in accuracy will be more beneficial for traits that are expensive to measure or for young animals with no phenotypic observations yet (Garrick, 2011). In addition, selection intensity can be increased through incorporation of genomic information. Evaluation of livestock based on GEPDs will have an increased accuracy, meaning that producers can select fewer replacements with greater confidence (Weller, 2016). Increasing selection intensity also increases the rate of genetic change, resulting in faster genetic improvement (Bourdon, 2000). Alongside increasing selection intensity, incorporation of genomic information also reduces the generation interval, increasing the rate of genetic change (Weller, 2016; Bourdon, 2000). Overall, incorporation of genomic information into genetic evaluations increases accuracy and efficiency of selection.

There are multiple methods to calculate DGV for incorporation into genetic evaluations of livestock: (1) inclusion DGV estimations as a correlated trait; (2) inclusion of DGV as separate EPD measures; and (3) incorporating GEPD estimates into a selection index (Rolf et al., 2014). All of these methodologies are two-step methods. However, direct or single-step methods have also been developed for estimation of GEPD. Direct calculations of GEPD utilize a

genomic relationship matrix rather than calculating marker effects first. A genomic relationship matrix takes into consideration identity by descent, identity by state, and pedigree relationships. This genomic relationship matrix is included in a best linear unbiased prediction (BLUP) analysis to calculate GEPD (Misztal et al., 2009).

Section 4: 1000 Bulls Genome Project

The cost of next-generation sequencing has decreased substantially over the past decade. However, it is still thousands of dollars to sequence a population of cattle to a depth that would allow for detection of novel variants. Therefore, consortia are being established for a number of species. Through these consortia, researchers can submit sequence data from their research to obtain membership and gain access an expansive database of sequence data. One such consortium is the 1000 Bulls Genome Project, which consists of sequence data from modern dairy and beef cattle breeds (Hayes and Daetwyler, 2019).

The 1000 Bulls Genome Project aims to build a database of sequence variant genotypes from modern cattle breeds for genome-wide association studies (GWAS) and genomic prediction and enable the use of this data to identify mutations that may compromise cattle health, welfare, or productivity. Membership into this consortium is obtained through submission of a sequence data that satisfies the requirements indicated by the steering committee. Once membership has been obtained, members can download sequence data for utilization in future research endeavors. The data contained within this consortium has mutations or variants already identified, which can reduce the amount of work needed to perform GWAS or SNP discovery analyses (Hayes and Daetwyler, 2019).

Section 5: Conclusions

Pulmonary hypertension is increasing in prevalence within the beef industry, impacting not only high-altitude beef systems but also finishing cattle in the feedlots. With the recent cultivation of a PAP EPD, selection decisions can be made to combat PH in cattle. However, many challenges still exist as the beef industry work towards reducing incidence of PH. The first challenge to overcome is to determine if HMD and FHD are similar conditions that can be selected for in the same manner. In addition, the PAP EPD needs to continue to be enhanced in order to improve accuracy due the limited amount of PAP observations recorded annually. Through discovering SNP associated with PAP, a GEPD may be calculated that increases the accuracy of estimates of animal merit for genetic improvement across ranches at high altitudes.

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CHAPTER 3

SUMMARY OF STUDY ANIMALS AND EXPERIMENTAL DESIGN

3.1 Introduction

This chapter describes the steers utilized in studies described in chapters 4 through 6.

3.2 Animal selection

Animals utilized in chapters 4 to 6 of this dissertation were cared for according to the guidelines of the Colorado State University Animal Care and Use Committee (16-6397A & 19-8429A).

Steers from the 2016 calf crop at the Colorado State University Beef Improvement Center (BIC; John E. Rouse Angus) underwent initial pulmonary arterial pressure (PAP) testing at 10-12 months of age. Bulls and steers within the 2016 CSU BIC calf crop had an average PAP of 40.32 $\pm$ 1.22 mmHg. In order to be included in this study, steers had to have an initial PAP measurement within one standard deviation of the population mean (41.38 $\pm$ 0.46 mmHg; low risk of developing high mountain disease).

Forty steers were allocated to one of four treatment groups (n=10/group). The four groups were high altitude stockered and grain-finished (Grain_HA), high altitude stockered and grass-finished (Grass_HA), high altitude stockered and moderate altitude grain-finished (Ext_Mod_Stocker), moderate altitude stockered and grain-finished (Norm_Mod_Stocker). Stratification of the forty steers into finishing systems was performed based on their PAP, weight, and age such that the four groups were similar (Table 3.1).

Table 3.1 Mean PAP, age, and weight did not differ amongst the four treatment groups.

Finishing System	Mean PAP (mmHg)	Mean Age (Days)	Mean Weight (kg)
Grass_HA (n=10)	41.40 ± 0.96^{A}	313.00 ± 5.62^{A}	253.56 ± 5.67^{A}
Grain_HA (n=10)	41.20 ± 0.96^{A}	314.00 ± 5.62^{A}	270.90 ± 5.67^{A}
Ext_Mod_Stocker (n=10)	41.90 ± 0.96^{A}	309.00 ± 5.62^{A}	258.55 ± 5.67^{A}
Norm_Mod_Stocker (n=10)	41.00 ± 0.96^{A}	304.00 ± 5.62^{A}	262.18 ± 5.67^{A}

[A] Within each column, different superscripts represent statistically significant differences of the means between management groups for the specified trait ($P<0.05$).

3.3 Experimental design

The stockering phase of this study commenced in April of 2017. All groups that were stockered at high altitude (Grain_HA, Grass_HA, Ext_Mod_Stocker) were maintained at BIC at an elevation of 2,150 m. Groups that were stockered at moderate altitude (Norm_Mod_Stocker) were managed at the Eastern Colorado Research Center (ECRC) at an elevation of 1,420 m. Steers stockered at the BIC were grazed in pastures comprised of timothy and bromegrass, while steers stockered at the ECRC were grazed in pastures consisting of western wheat, sand bluestem, blue grama and prairie sandreed grasses.

In August 2017, steers were administered a finishing ration, which consisted of 13.35% crude protein and 1.47% net energy. Ext_Mod_Stocker steers were relocated from the BIC to ECRC in early August for finishing. Grain_HA and Grass_HA steers were finished at the BIC on the same ration as the Norm_Mod_Stocker and Ext_Mod_Stocker management groups. Finishing rations were administered from August 2017 until steers were harvested (Figure 3.1).

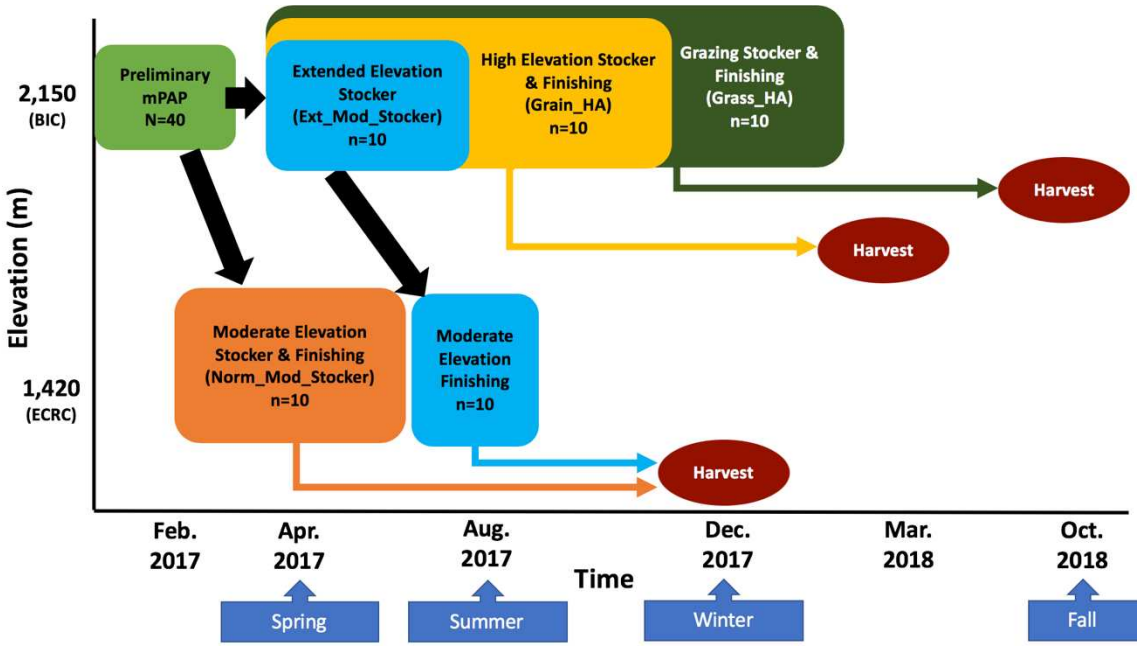

Figure 3.1 Summary of experimental design from allocation to treatment in February 2017 until harvest of each finishing system.

Pulmonary arterial pressure was recorded on each steer every six to eight weeks per the procedure described by Holt and Callan (2007) with the final measurement occurring within two weeks of harvest. Body weights were also recorded at these times. Once the average body weight for steers in a finishing system reached approximately 544 kg, steers were harvested within 30 days. Steers assigned to the Ext_Mod_Stocker and Norm_Mod_Stocker management groups were harvested in December of 2017. The Grain_HA steers were harvested in March 2018, and the Grass_HA steers were harvested in October 2018. After harvest, hot carcass weight (HCW), backfat thickness, yield grade (YG), ribeye area (REA), kidney, pelvis, and heart fat (KPH), marbling score (MARB), and quality grade (QG) were recorded.

3.4 Carcass quality grades

Quality grades were recorded on all steers after harvest according to the United States Department of Agriculture grading scale (Figure 3.2). The Norm_Mod_Stocker group had the highest quality grades of all finishing systems with 33.3% of the steers in that finishing system

graded prime-, 44.4%graded choice, and 22.2% graded choice-. Conversely, Grass_HA steers exhibited the lowest carcass quality grades with 20% graded choice-, 20% graded select+, 40% graded select-, and 20% graded standard+. All Ext_Mod_Stocker and Grain_HA steers were graded within the categories of choice and select.

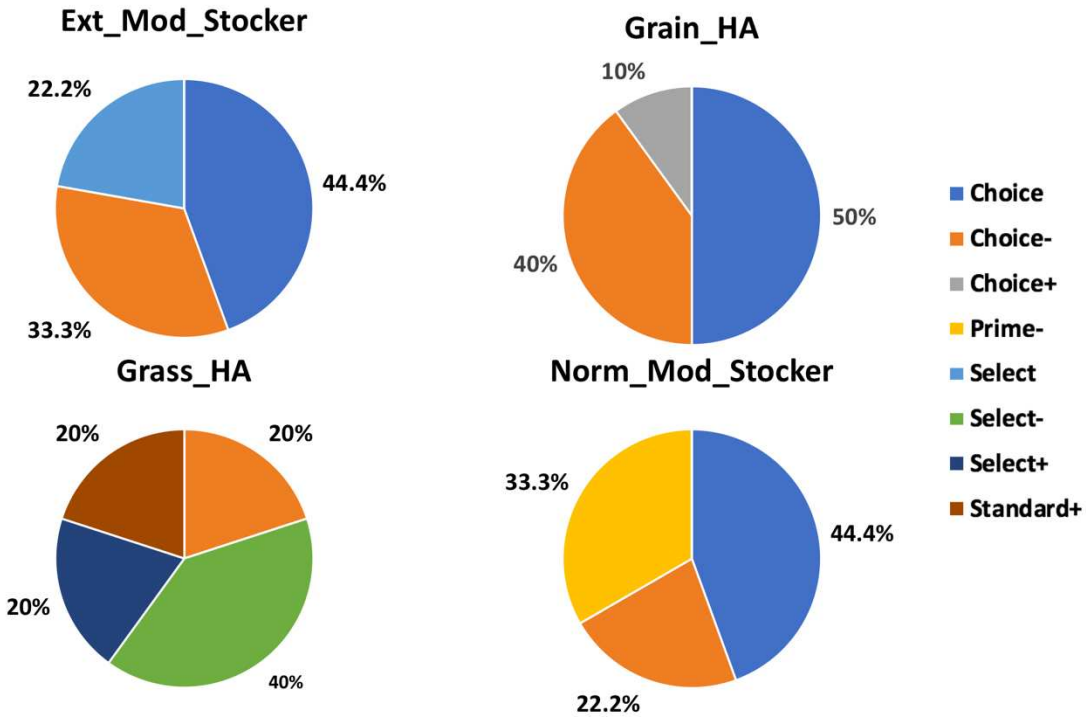

Figure 3.2 Comparison of quality grade across finishing systems.

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CHAPTER 4

DIFFERENTIATING THE ROLE OF ALTITUDE AND FINISHING STRATEGY ON PULMONARY ARTERIAL PRESSURE THROUGHOUT THE STOCKER AND FINISHING PHASES OF BEEF PRODUCTION

4.1 Introduction

High mountain disease (HMD) has challenged high altitude beef production systems for decades with reported herd death losses ranging from 2% to 25%. High mountain disease is characterized as remodeling of the cardiopulmonary system induced by pulmonary hypertension resulting from high altitude (>1,500 m) exposure (Jensen et al., 1976; Holt and Callan, 2007). This tissue remodeling results in increased cardiac strain and often culminates in right-sided heart failure (Jensen et al., 1976; Thomas et al., 2018). However, HMD is not the only pulmonary hypertension (PH) related disease that impacts the beef industry.

A similar condition known as feedlot heart disease (FHD) has been a concern for cattle from all altitudes since being discovered in the 1970's (Jensen et al., 1976). Research to date has suggested that while both HMD and FHD arise from vascular remodeling as a result of PH, they are separate conditions (Neary et al., 2015; Krafsur et al., 2017; Krafsur et al., 2019). Rapid weight gain that occurs in the latter stages of finishing cattle can result in late-term feedlot death (Neary et al., 2015; Neary et al., 2016; Krafsur et al., 2017; Krafsur et al., 2019).

The best indication of an animal's risk of HMD or FHD is a mean pulmonary arterial pressure measurement (PAP), which provides insight regarding the pulmonary health status (i.e. pulmonary hypertension) of an animal. A PAP of 49 mmHg or greater indicates that the animal is hypertensive and has a high risk of developing heart failure (Holt and Callan, 2007). Research to date has characterized HMD and FHD, but there is still much to learn about these conditions

(Neary et al., 2015; Thomas et al., 2018; Krafsur et al., 2019). Little is known about the impact of both altitude and finishing strategy on PAP simultaneously during stocker and finishing phases of cattle production. Therefore, the objective of this study was to evaluate the impact of finishing strategy and altitude on PAP throughout the stocker and finishing phases of beef production.

4.2 Materials and methods

Steers in the study population were selected, allocated, and managed according to the experimental design outlined in chapter three of this Dissertation.

Upon completion of data collection, automated model selection was performed using the dredge command from the multi-model inference (MuMIN) package in R (Bartoń, 2013; R core team, 2013). Effects considered in model selection predicting PAP were finishing strategy, period, and finishing strategy*period where period represents the time between the start of the study and the PAP measurement. This approach accounted for different sampling dates given steers were at multiple locations (Figure 4.1). Further, some finishing systems took longer to reach the average weight suitable for harvest, so coding the variable as Period allowed for comparisons of those pre-harvest time points. Period 1 corresponded to the initial PAP measure that was recorded prior to steers being assigned to a finishing system, and Period 5 corresponded to the final PAP measurement recorded within two weeks of harvest. A random effect of ID nested within finishing system was also fit for this model in order to account for variation across steers within the same finishing system. The best model for the analysis was selected based on the lowest corrected Akaike information criterion estimate (AICc). The final model included finishing system and Period as fixed effects.

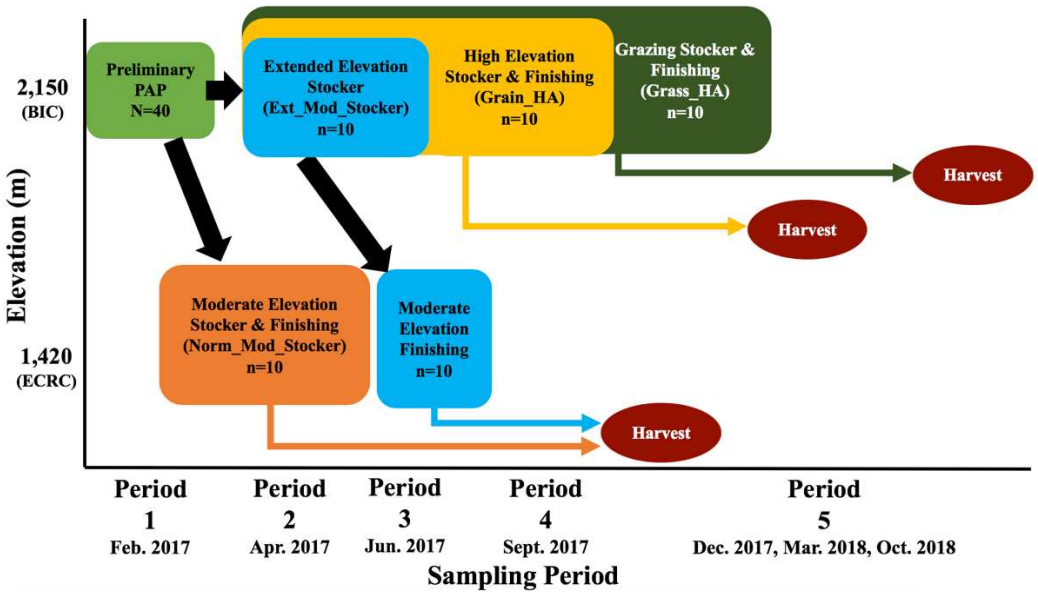

Figure 4.1 An outline of the experimental design that depicts how sampling time points were grouped into periods for comparisons across management groups.

The final model was fit using the linear mixed-effects (lmer) function from the linear mixed-effects using Eigen and S4 (lme4) package in R (Bates et al., 2015). Multiple covariance structures were evaluated with the model in order to determine the best covariance structure for the data. The first model was fit with a compound symmetry covariance structure, the second model was fit with unstructured covariances, and the third model was fit with an autoregressive covariance structure. The best covariance structure was determined based on which of the three models had the lowest AICc. Based on this criterion, covariances were fit as unstructured. Pairwise estimated marginal means (emmeans) analyses were performed to compare PAP across periods and to compare PAP across finishing strategies for each period (Searle et al., 1980). Statistical significance was accepted when probability greater than F was < 0.05.

4.3 Results

PAP did not differ between finishing systems at any Period within this study (Figure 4.2). Further, PAP did not differ across Periods 1 through 4 of the study. However, Period 5 PAP measurements differed from Periods 1 through 4 regardless of finishing strategy.

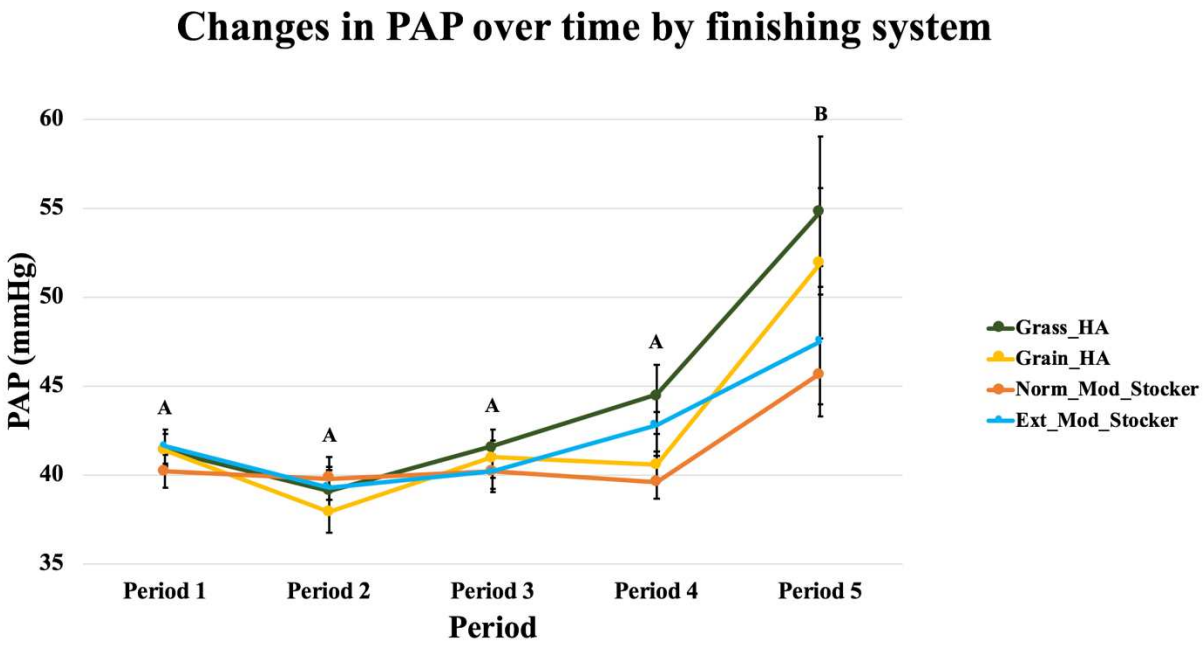

Figure 4.2 The fluctuations of PAP over time by finishing system, where A and B denote differences in the least square means between Periods.

4.4 Discussion

Mean pulmonary arterial pressures were similar among finishing systems at each period of this study. The Ext_Mod_Stocker steers that were moved from high altitude to moderate altitude mid-study for finishing still exhibited increased PAP as the study progressed. These results parallel the findings of Neary et al. (2015) that moved Angus steers from high altitude to moderate altitude for finishing, which exhibited an increased PAP over time regardless of altitude. Based on these results, neither finishing ration nor altitude appeared to be the primary cause of elevated PAP during the stocker and finishing phases of production. It appeared that both altitude and finishing ration contributed to the development of PH.

Over the duration of this study, PAP increased. However, PAP measurements recorded in periods 1 to 4 did not differ from one another and suggested these steers were at moderate risk of developing heart failure. Periods 1 to 3 occurred during the stockering phase of production in which all steers were consuming pasture or hay. Therefore, during periods 1 to 3 altitude was the only management challenge imposed on the steers. During this time, the groups at high altitude exhibited slight increases in PAP, but they were not different from the Norm_Mod_Stocker steers that were at moderate altitude throughout the study. These results are congruent with those of Neary et al. (2015). At younger stages of production, cattle did not exhibit steep fluctuations in PAP.

Period 4 was when PAP was recorded from steers consuming energy dense finishing ration. The HA_Grass steers were the only group that was not fed the finishing ration, yet they had PAP measurements similar to the steers on study during Period 4. It is hypothesized that, when Period 4 PAP measurements were recorded, steers had not yet gained enough weight to elevate PAP in contrast to those PAP measures recorded in Period 5. Neary et al. (2015) demonstrated that PAP increased regardless of altitude as steers progressed through the finishing phase. Krafsur et al. (2019) observed obesity-induced onset of PH progressing to heart failure, that paralleled the change from the stocker to finishing stages of production. However, the PAP measurements recorded in Period 4 did not agree with the findings of Neary et al. (2015) and Krafsur et al. (2019). This could be due to Period 4 measurements being recorded early enough in the finishing phase to not capture the increases of PAP at the expense of increased rate of gain. However, these increases in PAP during the finishing stage were observed in the Period 5 PAP measurements.

Mean pulmonary arterial pressure measurements recorded in Period 5 (pre-harvest) were greater than those recorded in Periods 1 to 4 regardless of finishing system. Based on Holt and Callan (2007), these steers were at high risk of developing heart failure. All steers had high PAP measurements pre-harvest regardless of finishing strategy or altitude. Ext_Mod_Stocker steers had a Period 5 PAP that was elevated compared to all other periods, which coincides with the conclusion of Neary et al. (2015) that alveolar hypoxia continued once steers were relocated to a moderate altitude for finishing. Therefore, altitude was not the only factor impacting PAP. This was also demonstrated through the changes from PAP in the Norm_Mod_Stocker group. Moving steers to moderate altitude and still observing elevated PAP measures ruled out hypobaric hypoxia as the only contributing factor of elevated PAP. Jensen et al. (1976) hypothesized that hypoventilation contributed to increased PAP. Hypoventilation may result from increased fat deposition or rumen size that compresses the lungs and results in rapid shallow breathing (Jensen et al., 1976, Krafsur et al., 2019).

In conclusion, steers exhibited elevated PAP over the finishing phase of production regardless of finishing strategy or altitude. These findings suggested that, while management and environment contributed to the development of PH, they were not the only cause of increased PAP and increased risk of developing heart failure.

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CHAPTER 5

CHARACTERIZING THE IMPACT OF ALTITUDE AND FINISHING SYSTEM ON MEAN PULMONARY ARTERIAL PRESSURE AND CARCASS CHARACTERISTICS IN ANGUS CATTLE

5.1 Introduction

High mountain disease (HMD) is a cardiopulmonary condition observed in cattle grazing at elevations greater than 1,500 m (Holt and Callan, 2007; Crawford et al., 2017; Pauling et al., 2018). The condition is caused by pulmonary hypertension (PH) resulting from chronic exposure to environmental hypoxia (Hecht et al., 1962). Cattle intolerant of hypoxic conditions often undergo pulmonary vascular remodeling, cardiopulmonary insufficiencies, right heart failure, and death. High mountain disease impacts three to five percent of cattle at high altitude on average, but some ranchers have reported death losses as great as twenty-five percent (Holt and Callan, 2007; Neary et al., 2013ab; Bruns et al., 2015).

Once cattle start showing clinical signs of PH, there are limited therapeutic remedies. The best indicator of risk of HMD is mean pulmonary arterial pressure (PAP) measured in cattle managed at high altitudes. Mean pulmonary arterial pressure is a veterinary procedure measured by threading a catheter containing a transducer through the jugular vein and right side of the heart in order to measure pressure in the pulmonary artery. This measurement indicates the animal's risk of developing HMD. Low-risk cattle have PAP measurements less than or equal to 41 mmHg, moderate-risk cattle will have a PAP ranging from 42 to 48 mmHg, and high-risk cattle have a PAP of 49 mmHg or greater (Holt and Callan, 2007). However, PAP is influenced by many factors such as age, breed, and altitude (Enns et al., 1992; Holt and Callan, 2007; Neary et al., 2015a, b; Crawford et al., 2017).

Symptoms similar to HMD have been observed in a portion of feedlot cattle never exposed to high altitude. The phenomenon of pulmonary hypertension occurring in feedlot cattle is described as feedlot heart disease (FHD). This disease can cause late-term feedlot death (Jensen et al., 1976; Neary et al., 2015ab). Common symptoms of HMD and FHD include, but are not limited to, lethargy, jugular vein distension, submandibular edema, and ascites (Holt and Callan, 2007; Krafsur et al., 2019). However, it is speculated that the conditions reflect distinct etiologies owing to the differing management practices that exist between ranches and feedlots. Krafsur et al. (2019) reported that the physiology of FHD is characterized as significant pathophysiologic remodeling of the left ventricle and pulmonary venous circulation accompanying right heart and pulmonary arterial remodeling. Whereas, HMD has been described as pulmonary hypertension and right ventricular dysfunction (Rhodes, 2005). Similarities and differences between HMD and FHD are still being investigated. Therefore, the objective of this study was to evaluate the influence of altitude and finishing ration on mean pulmonary arterial pressure and carcass characteristics.

5.2 Materials and methods

The steers utilized in this study were selected, managed, and allocated to treatments according to the procedures outlined in chapter 3. Steers were assigned to one of four groups: high altitude stockered and grain-finished (HA_Grain), high altitude stockered and grass-finished (HA_Grass), high altitude stockered and moderate altitude grain-finished (Ext_Mod_Stocker), moderate altitude stockered and grain-finished (Norm_Mod_Stocker).

The traits of interest for this study were pre-harvest PAP (PAP recorded within two weeks of harvest), hot carcass weight (HCW), backfat thickness (BF), kidney, pelvis, and heart fat (KPH), ribeye area (REA), yield grade (YG), marbling score (MARB), and quality grade

(QG). The carcass metrics were recorded by employees at the harvest facilities. Prior to analysis, MARB was converted from a categorical variable to a numerical marbling score utilizing the guidelines outlined by the Centralized Ultrasound Processing Laboratory (2007).

Upon completion of data collection, automated model selection was performed using the dredge command from the multi-model inference (MuMIN) package in R (R core team, 2013) with statistical significance being accepted when probability greater than F was <0.05. Random effects considered in model selection predicting pre-harvest PAP were initial PAP and age, and finishing system was included as a fixed effect. Random effects included for selecting the most appropriate model for HCW, backfat, KPH, REA, YG, and MARB were harvest age, initial PAP, pre-harvest PAP. Finishing system was included as a fixed effect for the model selection of the aforementioned carcass characteristics. The resulting models for dependent variables pre-harvest PAP, HCW, backfat, KPH, REA, MARB and YG (Table 5.1) were fitted. The estimated marginal means were utilized to compare means across finishing systems for pre- harvest PAP and carcass characteristics.

Table 5.1. Final model selected for each trait of interest utilizing the multi-model inference function in R, where an X signifies inclusion in the model for the dependent variable in that row.

Dependent Variable	Initial PAP (mmHg)	Age (days)	Finishing System	Harvest Age (days)	Pre-harvest PAP (mmHg)	Adjusted R^2
Pre-harvest PAP	X		X			0.012

HCW			X			0.670
Backfat			X	X		0.552
KPH	X		X			0.939
REA				X		0.188
YG			X	X	X	0.544
MARB			X	X		0.487

5.3 Results

Finishing system influenced pre-harvest PAP as HA_Grass steers had a PAP that was greater than Ext_Mod_Stocker steers (*P=0.006*) as well as Norm_Mod_Stocker steers (*P=0.024*). Steers in the HA_Grain finishing system were similar to the other three finishing systems when comparing pre-harvest PAP measures (Table 5.2).

Hot carcass weights (HCW) varied across finishing strategies (*P<0.05*). Specifically, steers stockered and grass finished at high altitude (HA_Grass) exhibited the lowest mean HCW compared to all other finishing systems (*P<0.001*) Steers of the Ext_Mod_Stocker treatment exhibited the second lowest HCW (*P<0.001*). Conversely, HA_Grain steers had the greatest HCW of the finishing systems (*P<0.001*) with Norm_Mod_Stocker steers exhibiting the second largest HCW (*P<0.001*) (Table 5.2).

Steers stockered and grain finished at high altitude (HA_Grain) exhibited an average carcass backfat thickness greater than Ext_Mod_Stocker (*P= 0.002*) and Norm_Mod_Stocker (*P=0.03*) steers. Backfat did not differ between HA_Grass steers and all other finishing systems. Furthermore, Ext_Mod_Stocker and Norm_Mod_Stocker steers did not exhibit differences in backfat (Table 5.2).

Yield grades differed such that HA_Grain steers exhibited a greater average yield grade than Ext_Mod_Stocker (*P=0.001*) and Norm_Mod_Stocker (*P=0.021*) steers, but not HA_Grass steers. Differences in yield grade were not significant when comparing HA_Grass, Ext_Mod_Stocker, and Norm_Mod_Stocker steers to one another (Table 5.2).

Steers in the HA_Grain finishing system had the greatest KPH ($P>0.001$). However, no differences in KPH were observed between the HA_Grass, Ext_Mod_Stocker, and Norm_Mod_Stocker finishing systems (Table 5.2).

Ribeye area differed across finishing systems such that Ext_Mod_Stocker steers had an average ribeye area that was larger than that of HA_Grain ($P<0.001$) and HA_Grass ($P<0.001$) steers, but not Norm_Mod_Stocker steers. The steers that were stockered and grass finished at high altitude (HA_Grass) had the smallest ribeye area ($P<0.001$; Table 5.2).

Table 5.2 Mean pre-harvest PAP measurement, hot carcass weight (HCW), backfat, yield grade (YG), ribeye area (REA), kidney, pelvic, and heart fat (KPH), and number of days on study for each finishing system.

Finishing System	Mean Pre-harvest PAP (mmHg)	Mean HCW (kg)	Mean Backfat (cm)	Mean YG	Mean REA (cm^2)	Mean KPH (%)	Mean MARB	Days on study
HA_Grass (n=10)	54.80 ± 1.54^{A}	322.50 ± 3.08^{A}	0.89 ± 0.23^{AB}	2.85 ± 0.24^{AB}	27.69 ± 0.41^{A}	1.26 ± 0.16^{A}	7.46 ± 1.84^{AB}	605
HA_Grain (n=10)	52.60 ± 1.80^{AB}	421.84 ± 3.58^{B}	1.42 ± 0.05^{A}	3.45 ± 0.04^{A}	29.97 ± 0.48^{B}	2.99 ± 0.03^{B}	6.27 ± 0.30^{AB}	407
Ext_Mod_Stocker (n=10)	46.60 ± 1.93^{B}	339.74 ± 3.83^{C}	0.76 ± 0.18^{B}	2.79 ± 0.18^{B}	32.77 ± 0.51^{C}	1.73 ± 0.12^{A}	4.01 ± 1.10^{A}	307
Norm_Mod_Stocker (n=10)	47.60 ± 1.95^{B}	382.38 ± 3.86^{D}	0.91 ± 0.18^{B}	2.94 ± 0.18^{B}	31.24 ± 0.51^{BC}	1.72 ± 0.13^{A}	5.41 ± 1.10^{B}	307

^{ABCD} Within each column, different superscripts represent differences between finishing system means for the specified trait ($P<0.05$)

Ext_Mod_Stocker carcasses had lower marbling scores than Norm_Mod_Stocker carcasses (*P<0.05)*, but not HA_Grass or HA_Grain carcasses (Table 3). However, Ext_Mod_Stocker carcasses had better quality grades than those of the Grass_HA group (Figure 1). Norm_Mod_Stocker carcasses had the best quality grades, with all carcasses being graded prime-, choice, or choice-. Conversely, Grass_HA carcasses had the poorest quality grades of all finishing systems. Collectively, the majority of carcasses, regardless of finishing ration, were graded within the range of choice+ to choice- (Figure 5.1).

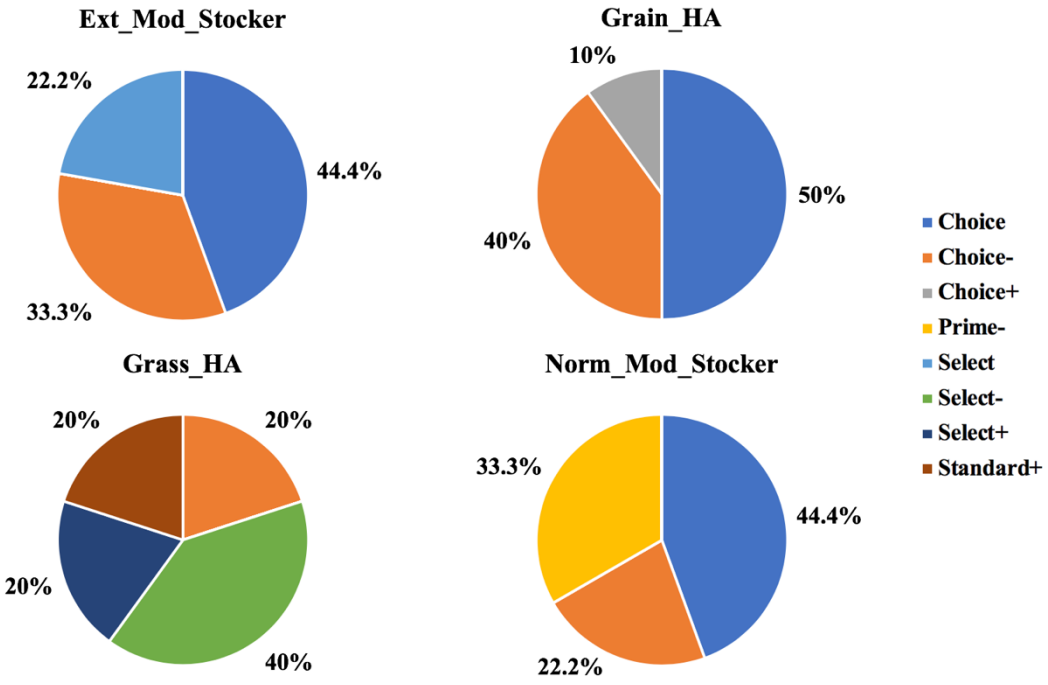

Figure 5.1 Comparison of quality grade across finishing systems.

5.4 Discussion

All finishing strategies within this study exhibited PAP measurements that would classify the steers as either moderate or high risk of developing pulmonary hypertension (Holt and Callan, 2007). However, the HA_Grass steers exhibited a higher average PAP than either Ext_Mod_Stocker or Norm_Mod_Stocker steers. HA_Grain steers were intermediate between

HA_Grass steers and the steers finished at moderate altitude when comparing pre-harvest PAP measures. The groups finished at moderate altitude had an average PAP consistent with a moderate risk of developing pulmonary hypertension, while the steers residing at high altitude for the duration of the study had an average PAP consistent with a high risk of developing pulmonary hypertension (Holt and Callan, 2007). Based on these results, it would appear that duration of exposure to altitude may pose a greater impact than finishing ration since the steers at high altitude took longer to reach finishing weights and were exposed to hypoxic conditions for the entire study.

It is important to note that within the HA_Grass finishing group, there was a single steer that had a pre-harvest PAP of 115 mmHg, which increased the average PAP of that group of steers. The HA_Grain finishing group had a steer with a pre-harvest PAP of 87 mmHg, which increased the average PAP of that group, but not to the same extent as the data point in the HA_Grass population. However, these data were included in the analysis due to their biological importance in addressing how steers respond to finishing strategies and altitude. Further, the percentage of the overall population that had "extreme" PAP measures was 2 out of 40 or 5% of the study population which is consistent with the percentage of cattle impacted by HMD in beef operations according to Holt and Callan (2007). Overall, it appears that duration of exposure to altitude may influence PAP more than finishing ration. Additional studies with larger numbers of cattle representing ranches across the western United States and Great Plains region may elucidate whether altitude is truly more impactful on PAP than finishing diet.

HCW differed across all finishing systems. HA_Grain steers had the heaviest carcasses, and HA_Grass steers had the lightest carcasses. These results agree with previous studies that concluded that when the amount of forage in the diet increases during the backgrounding and

finishing phases, HCW decreases (Cox et al., 2006; Pordomingo et al., 2012). Furthermore, Pordomingo et al. (2012) report that these changes are likely due to reduced average daily gain related to consumption of a predominantly forage-based diet. However, this was one of the first studies to evaluate carcass characteristics of Angus steers managed at moderate and high altitudes. Based on these results, it appears that finishing diet poses a greater impact on HCW than finishing altitude.

HA_Grain steers had greater backfat than steers finished at moderate elevation and HA_Grass steers. HA_Grass steers had similar backfat thicknesses to those finished at moderate elevation. Results for backfat thickness disagree with findings by Prodomingo et al., who concluded that as hay or forage content increased in the diet, backfat thickness decreased (2012). The elevated backfat thickness on HA_Grain carcasses may be attributed to scheduling conflicts with the abattoir that harvested the steers. If these steers were harvested two weeks sooner, we speculate that the backfat thickness recorded for HA_Grain steers would be comparable to that of the other finishing systems.

HA_Grain steers had greater YG than either group of steers grain finished at moderate elevation, but not HA_Grass steers. Contrary to the results from this study, Bennet et al. (1995), Camfield et al. (1999), and Cox et al. (2006) reported that pasture-fed steers exhibited lower YG than grain fed steers. A study by Garmyn et al. (2010) reported that grain finished heifers have greater YG than grass finished heifers. However, Bennett et al. (1995) reported no differences in yield grade between grass finished and grain finished steers. The finding that HA_Grass steers did not have lower YG than all grain finished groups on this study does not agree with results from previous studies and could indicate that high altitude impacts carcass quality. While little research has been conducted to evaluate the impact of altitude on carcass characteristics, a study

by Panjono et al. (2008) reported that altitude impacted meat color in Hanwoo cattle but saw no difference in YG between lowland and highland raised cattle.

HA_Grain steers had greater KPH than all other finishing systems. Cox et al. (2006) reported similar KPH between grass and grain finished steers. However, Garmyn et al. (2010) found that heifers finished on a concentrate grain-based diet had greater KPH than heifers finished on pasture. The fundamental difference between the studies by Cox et al. (2006) and Garmyn et al. (2010) was the gender within each study. This is relevant because research has established that there were differences in fat deposition between steers and heifers (Berg et al., 1979). Due to the two-week delay in harvesting HA_Grain steers, it is likely that KPH was increased during this time. Further studies should be conducted to validate whether HA_Grain steers would have increased KPH if harvested sooner. However, based on these results, it does not appear that altitude affected KPH.

HA_Grass steers had smaller REA than HA_Grain and Ext_Mod_Stocker, but not Norm_Mod_Stocker steers. These results agree with the report from Pordomingo et al. (2012), who suggested that grass finished cattle had smaller REA than steers finished on grain. However, Pordomingo et al. (2012) did not evaluate changes in altitude in addition to comparison of finishing strategies. Panjono et al. (2008) reported no difference in REA between lowland and highland finished cattle, but this study was conducted with Hanwoo cattle, whereas this dissertation only involved Angus cattle.

Ext_Mod_Stocker steers had lower MARB than Norm_Mod_Stocker steers, but not steers finished at high altitude. While HA_Grain steers produced the heaviest carcasses with the greatest amount of backfat, they did not differ from the other finishing systems when comparing MARB. Muir (1998) reported inconsistencies across multiple experiments evaluating HCW,

backfat, and MARB in multiple breeds of cattle. Results compiled suggested that the differences in marbling following grain finishing may be attributed to how cattle deposit fat and put on weight more than feed type. Therefore, factors such as breed, genetics, and how these measures were recorded could account for variability in MARB records. Further, Muir (1998) recommended using caution when comparing meat quality traits associated with fatness due inconsistent relationships between marbling and other fatness traits in experiments.

Results from this study were concordant with previous literature, which reported that quality grade was greater in grain finished beef than pasture raised beef (Bennet et al., 1995; Camfield et al., 1999; Cox et al., 2006). HA_Grass steers had the poorest quality grades of all finishing systems, while Norm_Mod_Stocker steers had the best quality grades. Ext_Mod_Stocker steers had poorer quality grades than HA_Grain steers, indicating that altitude did not impact quality grade. This coincides with findings from Panjono et al. (2008) which concluded that differences did not exist between lowland and highland finished Hanwoo cattle. However, little research has been conducted to evaluate the impact of altitude on quality grade in Angus cattle.

In summary, increased PAP was observed at ranches at both high and moderate altitudes, impacting PAP as well as carcass quality.

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CHAPTER 6

EXAMINATOIN OF ARTERIAL AND VENOUS BLOOD PARAMETERS ACROSS ALTITUDES AND FINISHING STRATEGIES THROUGHOUT THE STOCKER AND FINISHING PHASES OF PRODUCTION

6.1 Introduction

Cattle managed at altitudes greater than 1,500 m are exposed to reduced barometric pressures and reduced oxygen availability, resulting in pulmonary hypertension (PH). Select cattle that have been maintained at high altitude undergo pulmonary vascular remodeling as a result of PH and develop a condition known as High Mountain Disease (HMD). High Mountain Disease impacts 3% to 5% of cattle native to high altitude regions (Holt and Callan, 2007). However, incidence in cattle from altitudes less than 1,500 m that are relocated to high altitude production settings can be as great as 10% to 40% (Grover et al., 1963; Will et al., 1970). The best indicator of an animal's risk of developing HMD is measuring mean pulmonary arterial pressure (PAP) at 1 year of age, which has become a common practice for many ranchers with cattle at high altitudes (Holt and Callan, 2007).

A condition similar to HMD has been discovered in cattle in feedlots. These cattle were never exposed to high altitude production settings, indicating that PH may have a greater impact on the beef industry than previously estimated. This condition, known as Feedlot Heart Disease (FHD), while similar to HMD when evaluating symptoms, is a separate manifestation of PH (Jensen et al., 1976; Pringle et al., 1991; Malherbe et al., 2012; Neary et al., 2016; Krafsur et al., 2017; Krafsur et al., 2019). The rapid weight gain associated with finishing results in the development of PH in cattle that have been in feedlots for 19 weeks or more (Neary et al., 2016). This condition, like HMD, can result in congestive heart failure prior to harvest. The annual

death losses due to FHD are estimated to impact 1.08 out of 1,000 cattle as of 2012, following a trend of increased incidence since 1976 (Jensen et al., 1976; Neary et al., 2016). Despite quantifying the increased incidence of FHD, management strategies to detect cattle experiencing the condition and administer treatment for the condition have yet to be characterized. It is speculated that, like HMD, PAP would be a strong indicator of risk of developing FHD since both derive from PH in susceptible cattle. However, in a feedlot system, PAP testing cattle is not only costly but labor-intensive due to the immense concentration of cattle (T.N. Holt, personal communication).

In addition to PAP, blood parameters such as blood gases and electrolytes have been utilized in human medicine for diagnostic purposes (Singh et al., 2013). However, these parameters have not been tested widely in cattle exposed to hypoxic conditions that may result in development of HMD or FHD in susceptible cattle. A study in 2013 reported arterial blood gas data in calves up to six months of age as well as cows 24 to 27 months of age (Neary et al., 2013). Results from that study indicated that cattle were developing respiratory alkalosis. Despite these findings, PAP remains the best indicator of an animal's risk of developing either HMD or FHD. Additional research evaluating blood gas parameters and their utilization in distinguishing cattle at the greatest risk of developing HMD or FHD needs to be conducted in order to determine if these parameters can enhance the way veterinarians approach these two conditions.

In addition to understanding blood parameters to enhance risk assessment of cattle exposed to hypoxic conditions that may challenge their cardiopulmonary health, it is unknown whether altitude, rapid weight gain, or a combination of both pose a greater impact on susceptible cattle. Therefore, the objective of this study was to evaluate the impact of altitude

and finishing system on arterial and venous blood parameters throughout the stocker and finishing phases of beef production.

6.2 Materials and Methods

6.2.1 Experimental Design

Steers in the study population were selected, allocated, and managed according to the experimental design outlined in chapter III of this Dissertation. At each sampling period, arterial and venous blood samples were collected in a 2mL syringe. Arterial blood samples were collected from an auricular artery in the right ear. Venous blood samples were collected from the pulmonary artery. Arterial and venous blood gases and electrolytes were measured chute-side immediately using the Abaxis i-Stat 1 (Abaxis Inc., Union City, CA) blood gas analyzer for veterinary diagnostics. Arterial blood gases were measured using CG8+ cartridges, and venous blood gases were measured using CG4+ cartridges for the i-Stat 1 analyzer. These two cartridge varieties measured 21 blood chemistry, gases, and electrolytes (Table 6.1).

Table 6.1 Blood parameters measured from arterial and venous blood samples for each steer presented as an abbreviation and corresponding definition.

Arterial blood parameters (CG8+ cartridge)	**Venous blood parameters (CG4+ cartridge)**
P_aCO_2- arterial partial pressure of CO_2	P_vCO_2- venous partial pressure of CO_2
pH	pH
T_aCO_2- total CO_2 in arterial blood	T_vCO_2- total CO_2 in venous blood
s_aO_2- arterial oxyhemoglobin saturation	s_vO_2- venous oxyhemoglobin saturation
P_aO_2- arterial partial pressure of O_2	P_vO_2- venous partial pressure of O_2
HCO_3- blood bicarbonate	HCO_3- blood bicarbonate
BE- base excess	BE- base excess
Hct- hematocrit	Lac- blood L-lactate
Hgb- hemoglobin	
Na- sodium concentration	
iCa- ionized calcium concentration	
K- potassium concentration	
Glu- arterial blood glucose concentration	

In addition to the arterial blood gas parameters above, partial pressure of oxygen in the alveoli was calculated (Equation 6.1). Then the alveolar-arterial oxygen pressure gradient was calculated (Equation 6.2) in order to determine if a ventilation-perfusion mismatch reduced concentrations of oxygen in arterial blood (Neary et al., 2013; T.N. Holt, personal communication).

Equation 6.5 Partial pressure of oxygen in the alveoli (mmHg), where F_iO_2 is the fraction of inspired air (0.21), BP is barometric pressure (mmHg), pH_2O is the water vapor pressure at body temperature (52.4 mmHg at 39°C), P_aCO_2 (mmHg), and RQ is the respiratory quotient (0.9) (Tim Holt, personal communication).

$$P_AO_2 = F_iO_2 (BP - pH_2O) - (P_aCO_2/RQ)$$

Equation 6.6 Alveolar-arterial oxygen pressure gradient (mmHg), which is the differential between the partial pressure of oxygen in the alveoli (P_AO_2; mmHg) and the partial pressure of oxygen in the arterial blood (P_aO_2; mmHg)

$$\text{A-a } O_2 \text{ pressure gradient} = P_AO_2 - P_aO_2$$

6.2.2 Statistical Analysis

Arterial Samples

Model selection was performed using the dredge command multi-model inference (MuMIN) package in R statistical software (Bartoń, 2013; R core team, 2013). This function fit all possible models, including sampling period (Period), finishing system, and the interaction between finishing system and period as possible fixed effects. Period corresponded to the time that blood gas measurements were collected for each finishing system and resulted from the recoding of the number of days the steers were on study prior to harvest. This recoding from days on study to Period accounted for different dates of sampling for the same time point since steers were at multiple locations (Figure 6.1). Further, some finishing systems took longer to reach the average weight suitable for harvest, so coding the variable as Period allowed for

comparisons of those pre-harvest time points. Period 0 was the initial time point in which steers were selected for the study based on their PAP measurements, but no blood parameters were collected on this date. Period 1 corresponded to the first sampling recorded after steers were assigned to a finishing system, and Period 4 corresponded to the final sampling within two weeks of harvest. A random effect of ID nested within finishing system was also fit for this model to account for variation across steers within the same finishing system, but it was not detected as significant during model selection. The best model for the analysis was selected based on the lowest corrected Akaike information criterion estimate (AICc). The final model included finishing system and Period as fixed effects.

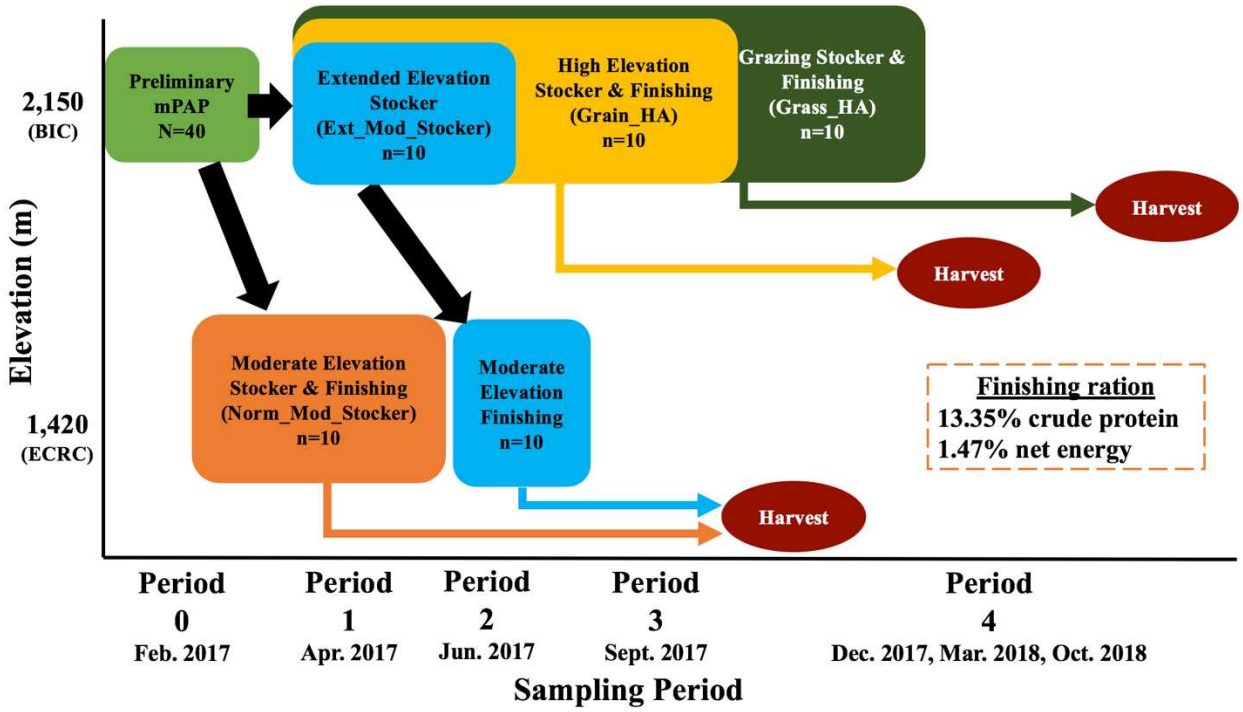

Figure 6.1 An overview of the experimental design including sampling dates. Note that Period 3 was the first sampling timepoint after the finishing phase had commenced.

Not all steers were sampled at every time point. Some steers had poor sample quality on the day of collection, which resulted in missing or unusable data. Further, one steer died between sampling periods 3 and 4. The number of steers sampled and included for analysis for each sampling period has been summarized in Table 6.2.

Table 6.2 A summary of the number of animals included in analysis for each finishing system by sampling period, where n_T represents the total number of steers prior to that sampling period, and n_e represents the number of steers excluded for a given sampling period for each finishing system. The "Reason" column for each period lists why a steer was excluded from the analysis for that time point.

	Period 1			**Period 2**			**Period 3**			**Period 4**		
Finishing System	**n_T**	**n_e**	**Reason**	**n_T**	**n_e**	**Reason**	**n_T**	**n_e**	**Reason**	**n_T**	**n_e**	**Reason**
Grass_HA	10	0	--	10	1	Poor sample quality	10	0	--	10	0	--
Grain_HA	10	0	--	10	0	--	10	0	--	10	0	--
Ext_Mod_Stocker	10	0	--	10	0	--	10	1	Poor sample quality	10	0	--
Norm_Mod_Stocker	10	0	--	10	0	--	10	0	--	10	1	Died*

* The steer death was unrelated to this study according to the results of a necropsy performed by a board-certified pathologist.

The final model for each blood parameter was fit using the linear mixed effects (lme) function from the linear mixed effects using Eigen and S4 (lme4) package in R (Bates et al., 2015). Multiple covariance structures were evaluated for each model in order to determine the best covariance structure for the data. The first model was fit with a compound symmetry covariance structure, the second model was fit with unstructured covariances, and the third model was fit with an autoregressive covariance structure. The best covariance structure was determined based on which of the three models had the lowest AICc. Based on this criterion,

covariances were fit as unstructured. Pairwise estimated marginal means (emmeans) analyses were performed to compare each blood parameter across Periods, between finishing systems, or across finishing system for each Period, depending on the variables included in the most suitable model for each dependent variable (Searle et al., 1980). Statistical significance was accepted when probability greater than F was < 0.05.

Venous Samples

The statistical methods for the venous blood parameters were the same as those used for the arterial blood gases. However, the periods for which data was analyzed differed. Venous blood parameter data was lost for periods 1 and 3 due to a software update of the iStat1 equipment. However, since periods 2 and 4 accounted for the end of the stocker and finishing phases respectively, the data was still informative in addressing the objectives of this research. Therefore, the same statistical methods as the arterial blood parameter analysis were followed for periods 2 and 4 of the venous data.

6.3 Results

6.3.1 Arterial Blood Parameters

Automated model selection revealed that the most relevant models to describe each blood parameter (Table 6.3). The interaction of finishing system and period was included in the model equations for Hb, Hct, HCO_3, tCO_2, pO_2, BE, Na, iCa, and Glu. Finishing system and period were both included in the model for sO_2, but not the interaction between the two fixed effects. Finishing system was the only effect included in the models for pH and pCO_2. However, period but not finishing system was informative when estimating K.

Table 6.3 The most appropriate models for each arterial blood parameter based on the lowest corrected AIC value.

Blood Parameter (y)	**Finishing System (x)**	**Period (x)**	**Finishing System*Period (x)**
pH	X		
Hb	X	X	X
Hct	X	X	X
HCO3	X	X	X
T_aCO_2	X	X	X
P_aCO_2	X		
P_aO_2	X	X	X
s_aO_2	X	X	
BE	X	X	X
Na	X	X	X
iCa	X	X	X
K		X	
Glu	X	X	X

The Ext_Mod_Stocker steers had a greater (P=0.0041) arterial pH than the Norm_Mod_Stocker steers, but not Grass_HA or Grain_HA steers (Table 6.4). Grass_HA and Grain_HA steers had arterial pH measurements that were intermediate to the Norm_Mod_Stocker and Ext_Mod_Stocker finishing systems, having similar pH measurements to both groups that were finished at moderate altitude.

Table 6.4 Arterial pH changes by finishing system regardless of sampling period expressed as mean ± standard error.

Finishing System	Arterial pH
Grass_HA	7.531 ± 0.009^{AB}
Grain_HA	7.541 ± 0.009^{AB}
Ext_Mod_Stocker	7.555 ± 0.010^{A}
Norm_Mod_Stocker	7.521 ± 0.009^{B}

Arterial hemoglobin measures fluctuated throughout the four sampling periods (Table 6.5). Norm_Mod_Stocker steers had a lower ($P \leq 0.0239$) arterial hemoglobin in period 1 than all other finishing systems at this time point. However, all steers, regardless of finishing strategy, had similar hemoglobin levels for sampling period 2. Ext_Mod_Stocker steers had lower ($P \leq 0.0185$) hemoglobin levels in period 3 than Grass_HA and Grain_HA steers but not Norm_Mod_Stocker steers, which were intermediate between the high-altitude finishing systems and the Ext_Mod_Stocker steers. Ext_Mod_Stocker steers had lower ($P \leq 0.0205$) arterial hemoglobin measures in period 4 than all other finishing systems. Norm_Mod_Stocker steers had arterial hemoglobin levels that were greater ($P=0.0205$) than the Ext_Mod_Stocker steers and similar to the Grass_HA steers in period 4. Grain_HA steers had greater ($P \leq 0.0063$) period 4 arterial Hb levels than Ext_Mod_Stocker steers and Norm_Mod_Stocker steers. Arterial Hb in Grass_HA steers for period 4 was similar to that of Norm_Mod_Stocker and Grain_HA steers, but greater ($P=0.0003$) than Ext_Mod_Stocker steers.

Table 6.5 Change in arterial hemoglobin over time by finishing system expressed as mean ± standard error.

	Arterial Hb (g/L)			
Finishing System	**Period 1**	**Period 2**	**Period 3**	**Period 4**
Grass_HA	9.210 ± 0.305^{A}	9.440 ± 0.322^{A}	9.530 ± 0.305^{A}	10.980 ± 0.305^{AB}
Grain_HA	9.970 ± 0.305^{A}	9.750 ± 0.305^{A}	9.750 ± 0.305^{A}	11.960 ± 0.305^{A}
Ext_Mod_Stocker	9.920 ± 0.305^{A}	9.620 ± 0.322^{A}	8.250 ± 0.305^{B}	9.190 ± 0.305^{C}
Norm_Mod_Stocker	7.970 ± 0.305^{B}	9.550 ± 0.305^{A}	8.800 ± 0.305^{AB}	10.490 ± 0.322^{B}

In period 1, Norm_Mod_Stocker steers had a lower ($P \leq 0.014$) Hct than all other finishing systems on study (Table 6.6). All finishing systems had similar Hct measurements in period 2. Grain_HA and Grass_HA steers had similar Hct measures in period 3 that were larger ($P \leq 0.022$) than those of Ext_Mod_Stocker steers, but not Norm_Mod_Stocker steers. Norm_Mod_Stocker steers had similar Hct measures to all other finishing systems in period 3. Ext_Mod_Stocker steers had the lowest ($P \leq 0.018$) period 4 Hct of all finishing systems. Norm_Mod_Stocker steers had a larger ($P=0.018$) Hct than Ext_Mod_Stocker steers, but a smaller ($P=0.007$) Hct than Grain_HA steers. Grass_HA steers had a period 4 Hct greater ($P=0.0003$) than the Ext_Mod_Stocker steers, but similar to the Grain_HA and Norm_Mod_Stocker steers.

Table 6.6 Change in arterial hematocrit over time by finishing system expressed as mean ± standard error.

	Arterial Hct (%)			
Finishing System	**Period 1**	**Period 2**	**Period 3**	**Period 4**
Grass_HA	27.400 ± 0.902^{A}	27.800 ± 0.951^{A}	28.000 ± 0.902^{A}	32.300 ± 0.902^{AB}
Grain_HA	29.300 ± 0.902^{A}	28.700 ± 0.902^{A}	28.700 ± 0.902^{A}	35.200 ± 0.902^{A}
Ext_Mod_Stocker	29.200 ± 0.902^{A}	28.300 ± 0.951^{A}	24.300 ± 0.902^{B}	27.000 ± 0.902^{C}
Norm_Mod_Stocker	23.500 ± 0.902^{B}	28.100 ± 0.902^{A}	25.900 ± 0.902^{AB}	30.900 ± 0.951^{B}

Arterial HCO_3 levels differed between finishing systems over time (Table 6.7). In periods 1and 2, Norm_Mod_Stocker steers had lower arterial HCO_3 levels than all other steers on study ($P \leq 0.0006$). Grass_HA, Grain_HA, and Ext_Mod_Stocker steers had similar arterial HCO_3 levels for periods 1 and 2. All steers had similar bicarbonate measures in periods 3 and 4.

Table 6.7 Change in arterial bicarbonate over time by finishing system expressed as mean ± standard error.

	Arterial HCO_3 (meq/L)			
Finishing System	**Period 1**	**Period 2**	**Period 3**	**Period 4**
Grass_HA	27.400 ± 0.949^{A}	32.300 ± 1.000^{A}	27.300 ± 0.949^{A}	28.800 ± 0.949^{A}
Grain_HA	28.900 ± 0.949^{A}	32.900 ± 0.949^{A}	28.100 ± 0.949^{A}	27.600 ± 0.949^{A}
Ext_Mod_Stocker	29.400 ± 0.949^{A}	33.100 ± 1.000^{A}	27.500 ± 0.949^{A}	25.700 ± 0.949^{A}
Norm_Mod_Stocker	23.500 ± 0.949^{B}	25.100 ± 0.949^{B}	26.400 ± 0.949^{A}	26.500 ± 1.000^{A}

Total arterial CO_2 differed between finishing systems over time (Table 6.8). Norm_Mod_Stocker steers had a reduced T_aCO_2 compared to all other finishing systems ($P \leq 0.0253$) in periods 1 and 2, which had similar T_aCO_2 measurements to one another. Grain_HA steers had a greater period 3 T_aCO_2 than Norm_Mod_Stocker steers ($P=0.038$) but not Grass_HA or Ext_Mod_Stocker finishing systems. Further, Grass_HA and Ext_Mod_Stocker

steers had T_aCO_2 levels similar to the Norm_Mod_Stocker steers in period 3. Grass_HA steers had a greater (P=0.046) T_aCO_2 than Ext_Mod_Stocker steers for period 4. Grain_HA and Norm_Mod_Stocker had T_aCO_2 levels similar to both Grass_HA and Ext_Mod_Stocker steers.

Table 6.8 Changes in total carbon dioxide in arterial blood by finishing system over time expressed as mean ± standard error.

	T_aCO_2 (mmHg)			
Finishing System	**Period 1**	**Period 2**	**Period 3**	**Period 4**
Grass_HA	28.400 ± 0.966^{A}	33.300 ± 1.018^{A}	29.200 ± 0.966^{AB}	30.000 ± 0.966^{A}
Grain_HA	29.000 ± 0.966^{A}	33.700 ± 0.966^{A}	31.000 ± 0.966^{A}	28.800 ± 0.966^{AB}
Ext_Mod_Stocker	30.400 ± 0.966^{A}	33.900 ± 1.018^{A}	28.500 ± 0.966^{AB}	26.400 ± 0.966^{B}
Norm_Mod_Stocker	24.500 ± 0.966^{B}	26.100 ± 0.966^{B}	27.300 ± 0.966^{B}	27.600 ± 1.018^{AB}

Arterial PCO_2 differed between finishing systems at high altitude and finishing systems at moderate altitude (Table 6.9). Grass_HA and Grain_HA steers had similar P_aCO_2 measurements. Ext_Mod_Stocker and Norm_Mod_Stocker also had similar P_aCO_2, regardless of sampling period. The high-altitude finishing groups had greater P_aCO_2 than those finished at moderate altitudes ($P \leq 0.015$).

Table 6.9 Changes in partial pressure of carbon dioxide of arterial blood by finishing system expressed as mean ± standard error.

Finishing System	**P_aCO_2 (mmHg)**
Grass_HA	34.700 ± 0.514^{A}
Grain_HA	34.600 ± 0.508^{A}
Ext_Mod_Stocker	32.500 ± 0.514^{B}
Norm_Mod_Stocker	30.900 ± 0.514^{B}

Grass_HA and Grain_HA steers had lower ($P \leq 0.0197$) P_aO_2 measurements in period 1 than Norm_Mod_Stocker steers (Table 6.10). Grain_HA steers had P_aO_2 measures in period 2 less ($P=0.0114$) than Norm_Mod_Stocker steers. Ext_Mod_Stocker steers had a P_aO_2 measurement that was intermediate between the aforementioned groups in periods 1 and 2. In period 3, Ext_Mod_Stocker steers had greater P_aO_2 than either Grass_HA or Grain_HA finishing groups ($P \leq 0.0059$). Norm_Mod_Stocker steers had greater P_aO_2 than Grass_HA steers in period 3 ($P=0.0047$), but not Grain_HA or Ext_Mod_Stocker steers. Grain_HA and Grass_HA steers had similar P_aO_2 in period 4 as did Ext_Mod_Stocker and Norm_Mod_Stocker steers. However, Ext_Mod_Stocker and Norm_Mod_Stocker steers had greater P_aO_2 in period 4 than Grass_HA and Grain_HA steers ($P \leq 0.0018$).

Table 6.10 Changes in arterial partial pressure of O_2 over time by finishing system expressed as mean $\pm$ standard error.

	P_aCO_2 (mmHg)			
Finishing System	**Period 1**	**Period 2**	**Period 3**	**Period 4**
Grass_HA	50.10 ± 3.05^{A}	56.90 ± 3.22^{AB}	50.10 ± 3.05^{A}	54.50 ± 3.05^{A}
Grain_HA	43.60 ± 3.05^{A}	53.50 ± 3.05^{A}	55.60 ± 3.05^{AB}	56.00 ± 3.05^{A}
Ext_Mod_Stocker	54.40 ± 3.05^{AB}	55.60 ± 3.22^{AB}	70.00 ± 3.05^{C}	71.90 ± 3.05^{B}
Norm_Mod_Stocker	62.80 ± 3.05^{B}	67.00 ± 3.05^{B}	64.80 ± 3.05^{BC}	78.10 ± 3.22^{B}

Arterial sO_2 differed between finishing systems (Table 6.11). Grass_HA and Grain_HA steers had similar s_aO_2 levels for periods 1 through 4. Ext_Mod_Stocker and Norm_Mod_Stocker steers had similar s_aO_2 levels for all four periods. However, Ext_Mod_Stocker and Norm_Mod_Stocker steers had greater s_aO_2 than Grass_HA and Grain_HA steers for periods 1 through 4 ($P \leq 0.004$).

Table 6.11 Changes in Arterial saturated oxygen over time by finishing system expressed as mean ± standard error.

	S_aO_2 (mmHg)			
Finishing System	**Period 1**	**Period 2**	**Period 3**	**Period 4**
Grass_HA	84.80 ± 1.39^{A}	90.50 ± 1.43^{A}	89.10 ± 1.39^{A}	90.70 ± 1.40^{A}
Grain_HA	84.60 ± 1.39^{A}	90.30 ± 1.40^{A}	88.90 ± 1.39^{A}	90.50 ± 1.39^{A}
Ext_Mod_Stocker	90.00 ± 1.39^{B}	95.70 ± 1.43^{B}	94.30 ± 1.39^{B}	95.90 ± 1.40^{B}
Norm_Mod_Stocker	90.50 ± 1.39^{B}	96.10 ± 1.40^{B}	94.70 ± 1.39^{B}	96.40 ± 1.42^{B}

Mean alveolar-arterial oxygen pressure gradient differed by period (Table 6.12). The period 4 oxygen pressure gradient measurements were less ($P \leq 0.0398$) than those of periods 1 and 2. The mean alveolar-arterial oxygen pressure gradient in period 3 was similar to the measurements from all other sampling periods.

Table 6.12 Mean alveolar-arterial oxygen pressure gradient by period expressed as mean ± standard error.

Period	**A-a O_2 Pressure Gradient (mmHg)**
1	24.10 ± 1.37^{A}
2	20.60 ± 1.41^{A}
3	19.80 ± 1.37^{AB}
4	15.30 ± 1.39^{B}

Arterial BE changed over time for each finishing system (Table 6.13). Norm_Mod_Stocker steers had a lower ($P<0.0001$) BE than Grass_HA, Grain_HA, and Ext_Mod_Stocker steers for periods 1 and 2. In periods 3 and 4, all finishing systems had similar BE measurements.

Table 6.13 Changes in arterial base excess over time for each finishing system expressed as mean ± standard error.

	Arterial BE (meq/L)			
Finishing System	**Period 1**	**Period 2**	**Period 3**	**Period 4**
Grass_HA	4.20 ± 1.09^{A}	10.44 ± 1.15^{A}	4.40 ± 1.09^{A}	6.10 ± 1.09^{A}
Grain_HA	6.20 ± 1.09^{A}	11.10 ± 1.09^{A}	6.10 ± 1.09^{A}	4.40 ± 1.09^{A}
Ext_Mod_Stocker	6.80 ± 1.09^{A}	11.44 ± 1.15^{A}	5.20 ± 1.09^{A}	3.10 ± 1.09^{A}
Norm_Mod_Stocker	0.30 ± 1.09^{B}	2.30 ± 1.09^{B}	3.80 ± 1.09^{A}	3.67 ± 1.15^{A}

Norm_Mod_Stocker steers had greater ($P \leq 0.0239$) arterial Na levels than all other finishing systems for periods 1 and 2 (Table 6.14). Norm_Mod_Stocker and Ext_Mod_Stocker steers had similar period 3 Na levels. Norm_Mod_Stocker, Grass_HA, and Grain_HA steers had similar period 3 Na levels. However, Ext_Mod_Stocker steers had greater ($P \leq 0.0172$) arterial Na than Grass_HA and Grain_HA steers. In period 4, Grass_HA steers had the largest ($P < 0.0001$) Na measure of all finishing systems. Grain_HA steers had a period 4 Na measure that was less ($P < 0.0001$) than the Grass_HA steers, but greater ($P \leq 0.0002$) than the Ext_Mod_Stocker and Norm_Mod_Stocker finishing systems.

Table 6.14 Change in arterial sodium over time by finishing system expressed as mean ± standard error.

	Arterial Na (mmol/L)			
Finishing System	**Period 1**	**Period 2**	**Period 3**	**Period 4**
Grass_HA	137 ± 0.615^{A}	138 ± 0.648^{A}	137 ± 0.615^{A}	152 ± 0.615^{A}
Grain_HA	138 ± 0.615^{A}	139 ± 0.615^{A}	138 ± 0.615^{A}	141 ± 0.615^{B}
Ext_Mod_Stocker	137 ± 0.615^{A}	138 ± 0.648^{A}	141 ± 0.615^{B}	136 ± 0.615^{C}
Norm_Mod_Stocker	143 ± 0.615^{B}	141 ± 0.615^{B}	139 ± 0.615^{AB}	137 ± 0.648^{C}

Arterial ionic calcium measures were similar among finishing systems for periods 1 through 3 (Table 6.15). In period 4, Ext_Mod_Stocker steers had an iCa level that was less than that of the Grain_HA steers (P=0.0083), but not Grass_HA or Norm_Mod_Stocker steers. Grain_HA steers had an iCa level in period 4 that was similar to that of the Grass_HA and Norm_Mod_Stocker finishing systems. Similarly, the Norm_Mod_Stocker and Grass_HA finishing systems had similar iCa measures in period 4.

Table 6.15 Changes in arterial ionic calcium over time by finishing system expressed as mean ± standard error.

	Arterial iCa (mmol/L)			
Finishing System	**Period 1**	**Period 2**	**Period 3**	**Period 4**
Grass_HA	1.081 ± 0.030^{A}	1.057 ± 0.031^{A}	1.086 ± 0.030^{A}	1.125 ± 0.030^{AB}
Grain_HA	1.107 ± 0.030^{A}	0.986 ± 0.030^{A}	1.024 ± 0.030^{A}	1.155 ± 0.030^{AC}
Ext_Mod_Stocker	1.125 ± 0.030^{A}	1.022 ± 0.031^{A}	1.030 ± 0.030^{A}	1.020 ± 0.030^{B}
Norm_Mod_Stocker	1.033 ± 0.030^{A}	1.032 ± 0.030^{A}	1.011 ± 0.030^{A}	1.114 ± 0.031^{ABC}

Arterial Glu differed between finishing systems over the four sampling periods (Table 6.16). Glucose levels in period 1 differed such that Norm_Mod_Stocker steers had greater (P=0.0194) Glu levels than Grass_HA steers, but not Grain_HA or Ext_Mod_Stocker steers, which were intermediate between the two groups. All finishing systems had similar Glu measures for period 2. Ext_Mod_Stocker steers had a greater ($P \leq 0.0451$) Glu level than Grass_HA and Norm_Mod_Stocker steers in period 3. Grain_HA steers were similar to all other finishing systems when comparing Glu in period 3. In period 4, Grain_HA, Ext_Mod_Stocker, and Norm_Mod_Stocker steers had similar Glu levels, which were larger (P<0.0001) than the Glu measure recorded for the Grass_HA finishing system.

Table 6.16 Changes in arterial glucose over time by finishing system expressed as mean ± standard error.

	Arterial Glu (mmol/L)			
Finishing System	**Period 1**	**Period 2**	**Period 3**	**Period 4**
Grass_HA	79.40 ± 2.44^{A}	75.90 ± 2.57^{A}	77.90 ± 2.44^{A}	70.90 ± 2.44^{A}
Grain_HA	85.10 ± 2.44^{AB}	74.50 ± 2.44^{A}	81.20 ± 2.44^{AB}	94.90 ± 2.44^{B}
Ext_Mod_Stocker	82.00 ± 2.44^{AB}	74.30 ± 2.57^{A}	90.00 ± 2.44^{B}	89.50 ± 2.44^{B}
Norm_Mod_Stocker	89.60 ± 2.44^{B}	80.80 ± 2.44^{A}	80.90 ± 2.44^{A}	91.10 ± 2.57^{B}

Arterial potassium levels were similar across finishing systems, but differed by period (Table 6.17). In periods 1, 3, and 4 steers had similar potassium levels. However, in period 2, steers had lower ($P \leq 0.0019$) potassium levels than in periods 1, 3, and 4.

Table 6.17 Changes in arterial potassium by period expressed as mean ± standard error.

Period	**Mean Arterial K**
1	3.530 ± 0.064^{A}
2	3.190 ± 0.066^{B}
3	3.620 ± 0.064^{A}
4	3.660 ± 0.065^{A}

6.3.2 Venous Blood Parameters

Automated model selection revealed the most relevant models to describe each venous blood parameter (Table 6.18). The interaction of finishing system and period was included in the model equations for pH, HCO_3, T_vCO_2, BE, and Lac. Period was informative in estimated P_vO_2, but not other effects that were tested in automated model selection. Further, P_vCO_2 and s_vO2 had no significant effects in the best model according to corrected AIC. Despite no significant effects, a model was fit including period as the only fixed effect in order to depict that there were no differences between periods.

Table 6.18 The most relevant models to predict each venous blood parameter based on corrected AIC values.

Blood Parameter (y)	Finishing System (x)	Period (x)	Finishing System*Period (x)
pH	X	X	X
HCO_3	X	X	X
T_VCO_2	X	X	X
P_VCO_2			
P_VO_2		X	
s_vO_2			
BE	X	X	X
Lac	X	X	X

Venous pH differed across sampling periods and finishing systems (Table 6.19). Norm_Mod_Stocker steers had a lower (P=0.0022) pH than all other steers in period 2. However, in period 4, all steers had similar pH measurements.

Table 6.19 Mean venous pH by finishing system and sampling period. All values have been expressed as the mean $\pm$ standard error.

	Mean Venous pH	
Finishing System	**Period 2**	**Period 4**
Grass_HA	7.545 ± 0.013^{A}	7.471 ± 0.013^{A}
Grain_HA	7.548 ± 0.013^{A}	7.487 ± 0.013^{A}
Ext_Mod_Stocker	7.528 ± 0.013^{A}	7.463 ± 0.013^{A}
Norm_Mod_Stocker	7.456 ± 0.014^{B}	7.475 ± 0.013^{A}

Bicarbonate levels in the venous blood differed between finishing systems for periods 2 and 4 (Table 6.20). Norm_Mod_Stocker steers had lower (P<0.0001) venous HCO_3 than all other steers in period 2. In period 4, Norm_Mod_Stocker and Ext_Mod_Stocker steers had similar HCO_3 concentrations that were lower (P< 0.03) than those of Grain_HA steers but not

Grass_HA steers. Grass_HA steers had HCO_3 concentrations that were comparable to all other finishing systems.

Table 6.20 Venous bicarbonate by finishing system over time expressed as mean ± standard error.

	Venous HCO_3 (meq/L)	
Finishing System	**Period 2**	**Period 4**
Grass_HA	14.10 ± 0.71^{A}	7.20 ± 0.71^{AB}
Grain_HA	13.10 ± 0.71^{A}	9.20 ± 0.71^{B}
Ext_Mod_Stocker	12.78 ± 0.74^{A}	5.67 ± 0.74^{A}
Norm_Mod_Stocker	4.33 ± 0.74^{B}	6.33 ± 0.74^{A}

Total venous CO_2 differed between finishing systems for each sampling period (Table 6.21). Levels of T_vCO_2 mirrored the results for venous HCO_3 such that Norm_Mod_Stocker steers had lower ($P<0.0001$) T_vCO_2 than all other steers in period 2. In period 4, Norm_Mod_Stocker and Ext_Mod_Stocker steers had T_vCO_2 levels similar to one another and to Grass_HA steers, but less ($P \leq 0.0311$) than Grain_HA steers. Further, Grass_HA steers and Grain_HA steers had T_vCO_2 measurements that were similar to one another.

Table 6.21 Total CO_2 in venous blood for each finishing system by period. Measures have been expressed as mean ± standard error.

	T_vCO_2 (mmHg)	
Finishing System	**Period 2**	**Period 4**
Grass_HA	37.9 ± 0.65^{A}	32.1 ± 0.65^{AB}
Grain_HA	36.8 ± 0.65^{A}	33.9 ± 0.65^{B}
Ext_Mod_Stocker	37.0 ± 0.69^{A}	30.7 ± 0.69^{A}
Norm_Mod_Stocker	29.6 ± 0.69^{B}	31.2 ± 0.69^{A}

Partial pressure of CO_2 in the venous blood did not differ between finishing systems or sampling periods (Table 6.22). All P_vCO_2 measures were similar to one another.

Table 6.22 Venous partial pressure of CO_2 over the two sampling periods expressed as mean $\pm$ standard error.

Sampling Period	**P_VCO_2 (mmHg)**
Period 2	41.7 ± 0.69^A
Period 4	42.0 ± 0.69^A

Partial pressures of O_2 in the venous blood differed between sampling periods but not finishing systems (Table 6.23). There was a greater (P=0.0387) mean P_vO_2 recorded for steers in period 4 than in period 2.

Table 6.23 Partial pressure of O_2 in the venous blood by sampling period expressed as mean $\pm$ standard error.

Sampling Period	**P_VO_2 (mmHg)**
Period 2	29.9 ± 0.51^A
Period 4	31.5 ± 0.51^B

Oxyhemoglobin saturation in the venous blood did not differ by finishing system or sampling period (Table 6.24). The s_vO_2 levels remained constant for periods 2 and 4.

Table 6.24 Oxyhemoglobin saturation in venous blood samples over the two sampling periods expressed as mean $\pm$ standard error.

Sampling Period	**s_vO2 (mmHg)**
Period 2	63.9 ± 1.39^A
Period 4	63.9 ± 1.39^A

Base excess measures in the venous blood differed between finishing systems for the two sampling periods (Table 6.25). In period 2, Norm_Mod_Stocker steers had a BE measure less ($P<0.0001$) than that of all other finishing systems. Ext_Mod_Stocker and Norm_Mod_Stocker steers had similar period 4 BE levels to each other as well as to steers in the Grass_HA finishing system. Grain_HA steers had a greater ($P\leq0.0332$) BE than Norm_Mod_Stocker and Ext_Mod_Stocker steers, but not Grass_HA steers.

Table 6.25 Venous base excess for each finishing system over time expressed as mean $\pm$ standard error.

	Venous Base Excess (meq/L)	
Finishing System	**Period 2**	**Period 4**
Grass_HA	14.10 ± 0.71^{A}	7.20 ± 0.71^{AB}
Grain_HA	13.10 ± 0.71^{A}	9.20 ± 0.71^{B}
Ext_Mod_Stocker	12.78 ± 0.74^{A}	5.67 ± 0.74^{A}
Norm_Mod_Stocker	4.33 ± 0.74^{B}	6.33 ± 0.74^{A}

L-lactate concentrations in venous blood samples differed between finishing systems by period (Table 6.26). All finishing systems had comparable lactate concentrations in period 2. However, in period 4, Grain_HA steers had a greater ($P=0.0371$) venous lactate concentration than Grass_HA steers but not Norm_Mod_Stocker or Ext_Mod_Stocker steers. Norm_Mod_Stocker and Ext_Mod_Stocker steers had similar period 4 Lac levels to one another and were similar to both the Grass_HA and Grain_HA finishing systems.

Table 6.26 Venous L-lactate concentrations for each finishing system over the two sampling periods expressed as mean $\pm$ standard error.

	Venous Lac

	(mmol/L)	
Finishing System	**Period 2**	**Period 4**
Grass_HA	1.02 ± 0.49^{A}	1.59 ± 0.49^{A}
Grain_HA	1.29 ± 0.49^{A}	3.49 ± 0.49^{B}
Ext_Mod_Stocker	2.44 ± 0.52^{A}	2.51 ± 0.52^{AB}
Norm_Mod_Stocker	2.67 ± 0.52^{A}	2.11 ± 0.52^{AB}

6.4 Discussion

6.4.1 Arterial Hematology

pH

Arterial pH was affected by finishing system, but not sampling period. A normal adult bovine arterial pH should be between 7.35 and 7.45. Cattle with arterial pH measures less than 7.35 are considered acidotic. Conversely, cattle with an arterial pH greater than 7.45 are considered alkalotic (Smith et al., 2014; T.N. Holt, personal communication). Based on these parameters, all finishing groups were experiencing alkalosis. In order to determine the likely cause of alkalosis, arterial levels of PCO_2 and HCO_3 should be evaluated in conjunction with the pH estimates (T.N. Holt, personal communication). Arterial PCO_2 levels for all finishing systems were less than 35 mmHg, indicative of respiratory alkalosis. Based on the fluctuation of HCO_3 levels throughout the four sampling periods, it appears that some steers might have been experiencing partially compensated respiratory alkalosis. This was evident by the slight decrease in HCO_3 over time in an effort to decrease pH in the presence of low PCO_2 levels (Prasse and Sexton, 1972; Patel et al., 2019; C.W. Miller, personal communication; T.N. Holt, personal communication). An alternative cause of alkalosis is metabolic alkalosis, but based on the low levels of PCO_2 measured in each finishing system, it was determined that respiratory alkalosis was the likely cause of alkalosis in these steers (T.N. Holt, personal communication). The arterial

pH estimates for steers on this study were similar to those by Neary et al. (2016), who reported an average pH of 7.47 $\pm$ 0.13 in cows 24 to 27 months of age at high altitude and concluded that cows were experiencing respiratory alkalosis.

Hemoglobin

Hemoglobin levels in arterial blood changed over time according to finishing system. However, despite the high-altitude exposure of the Grass_HA, Grain_HA, and Ext_Mod_Stocker finishing systems, hemoglobin levels were still within the normal range of 8-15 g/dL for cattle (Fielder, 2015a). In period 1, Norm_Mod_Stocker steers had a lower hemoglobin level than all other steers on study, and these steers were the only ones at moderate altitude at this time. The larger period 1 hemoglobin concentrations for the three finishing systems that were at the BIC were likely a physiologic response to increase arterial oxygen carrying capacity in the presence of reduced atmospheric oxygen at high altitude. However, this increase in hemoglobin also results in an increased binding affinity of oxygen to hemoglobin, which results in reduced oxygen uptake by systemic tissues (Storz, 2016; Patel et al., 2019; T.N. Holt, personal communication).

In period 2, there were no differences in hemoglobin concentrations across the four finishing systems. Further, all finishing systems had hemoglobin measures within normal range. Despite being at moderate elevation, the Norm_Mod_Stocker steers exhibited an increase in arterial hemoglobin from period 1 to period 2. This difference in hemoglobin concentration may be due to season. Warmer summer months results in heat stress for most cattle, and the most common response to heat stress in cattle is increased respiration rate or hyperventilation (Kadokawa et al., 2012; Das et al., 2016). This increase in arterial hemoglobin likely resulted from heat stress-induced hyperventilation.

Period 3 was the first period in which Ext_Mod_Stocker steers were relocated to ECRC for finishing along with the Norm_Mod_Stocker steers. The Ext_Mod_Stocker Steers had the lowest hemoglobin level. Norm_Mod_Stocker steers had a hemoglobin level intermediate between the Ext_Mod_Stocker and Grass_HA and Grain_HA steers, which remained at high altitude. Like in period 1, steers at high altitude had an elevated hemoglobin level compared to those at moderate altitude attributable to differences in atmospheric oxygen availability (Storz, 2016). However, all finishing systems had hemoglobin levels within the normal range.

Ext_Mod_Stocker steers had the lowest hemoglobin level of all finishing systems in period 4. Grain_HA steers had hemoglobin levels greater than both finishing systems at moderate altitude, but not Grass_HA steers which had a hemoglobin level comparable to Grain_HA and Norm_Mod_Stocker steers. The period 4 hemoglobin estimates for all finishing systems were within the normal range for cattle. However, the differences between Ext_Mod_Stocker and all other finishing systems indicates that more than altitude, the resulting barometric pressure, and atmospheric oxygen availability impacted hemoglobin levels in these cattle. If the altitude of management was the sole contributor to arterial hemoglobin concentrations, then Ext_Mod_Stocker and Norm_Mod_Stocker steers would have had similar average hemoglobin levels in period 4. These findings affirm that finishing diet and rapid weight gain also contribute to the hypoxic state of steers and can result in PH (Jensen et al., 1976; Pringle et al., 1991; Malherbe et al., 2012; Neary et al., 2016; Krafsur et al., 2017; Krafsur et al., 2019). The Norm_Mod_Stocker steers in this case exhibited signs of PH that may progress to FHD.

Hematocrit

Results for arterial hematocrit recapitulated the findings for arterial hemoglobin concentration. Since hemoglobin and hematocrit relate to one another, this was a logical finding. Hematocrit estimates the proportion of red blood cells to total blood volume, and hemoglobin is contained within red blood cells. Therefore, the measures of these parameters are dependent upon one another. Hematocrit and hemoglobin may vary based on hydration status which alters plasma volume (Billett, 1990). Further, in chronic hypoxic conditions, cattle undergo erythrocytosis in order to increase oxygen carrying capacity as was discussed with arterial hemoglobin levels (Storz, 2016). This increase in red blood cells and hemoglobin also increased hematocrit.

Base Excess

Base excess differed between finishing systems for each sampling period. In periods 1 and 2, Norm_Mod_Stocker steers had a lower BE than all other finishing systems. Further, the Norm_Mod_Stocker steers were the only finishing system in periods 1 and 2 that had BE values that were within the normal range of -2 to 2 (Taussig and Landau, 2008). No BE differences between finishing systems were observed for periods 3 and 4. All finishing systems had BE estimates that were above the normal range in periods 3 and 4. Overall, base excess indicates acid-base imbalances in blood that result from illness or changes in diet (Constable, 2002). Further, it can account for metabolic causes of fluctuations in blood pH and is an indicator of serum lactate levels (Chomsky-Higgins and Harken, 2017). Considering this information alongside pH observations indicative of alkalosis, these steers were impacted by either altitude, diet, or a combination of both, which resulted in acid-base fluctuations that elevated BE as well as pH, HCO_3, and PCO_2. While BE data supports the findings of respiratory alkalosis in this

research, it has not been reported in any similar studies comparing finishing altitude and diet of cattle during the stocker and finishing phases of production.

6.4.2 Arterial Blood Gases

Partial Pressure of Carbon Dioxide

Partial pressure of carbon dioxide differed between finishing systems but not sampling periods. Ext_Mod_Stocker and Norm_Mod_Stocker steers had PCO_2 concentrations that were similar to one another, but less than those of Grass_HA and Grain_HA steers, which also had similar concentrations to one another. The arterial PCO_2 concentrations observed in Grass_HA and Grain_HA steers were close to, but still slightly lower, than the normal range (Fielder et al., 2015b). The Ext_Mod_Stocker and Norm_Mod_Stocker finishing systems had PCO_2 concentrations that were further below the normal range than those steers finished at high altitude (Fielder et al., 2015b). Decreased PCO_2 is indicative of alkalosis, as was mentioned when evaluating arterial pH. As a result, altered blood bicarbonate levels have been known to change in an effort to lower pH back within the normal range for cattle (Prasse and Sexton, 1972; Patel et al., 2019; C.W. Miller, personal communication; T.N. Holt, personal communication). These results were concordant with findings by Neary et al. (2013), who evaluated arterial PCO_2 in calves up to six months of age and cows 24 to 27 months of age at high altitude and found that cattle were experiencing respiratory alkalosis.

Total Carbon Dioxide

Total carbon dioxide differed between finishing systems across sampling periods. In periods 1 and 2, Norm_Mod_Stocker steers had lower TCO_2 than all other finishing systems, which had similar measures to one another. Grain_HA steers had greater TCO_2 than Norm_Mod_Stocker steers, but not Grass_HA or Ext_Mod_Stocker steers, which were

intermediate and had TCO_2 measures that were similar to both finishing systems. Grass_HA steers had greater arterial TCO_2 than Ext_Mod_Steers, but not Norm_Mod_Stocker and Grain_HA steers, which had measures similar to both finishing systems. Despite these reported differences, all measurements were within normal ranges for bovine arterial blood (Fisher et al., 1980; Blood Gases, 2019). No prior research has been conducted to evaluate TCO_2 at varying altitudes with different finishing strategies. However, previous studies evaluated arterial bicarbonate, and total carbon dioxide has been established as an alternate way to estimate bicarbonate (Neary et al., 2013; Blood Gases, 2019).

Partial Pressure of Oxygen

Arterial PO_2 concentrations accepted as normal for cattle range from 80 to 100 mmHg (Fisher et al., 1980; Fields, 2015b). However, at altitudes greater than 1,500 m arterial PO_2 values have been observed to decline below the normal range to 70 to 80 mmHg (T.N. Holt, personal communication). All finishing systems were below the normal ranges for arterial PO_2 according to their altitudes at each sampling period. Neary et al. (2013) also reported low arterial PO_2 concentrations in calves and cows born and raised at high altitudes. Alveolar-arterial oxygen pressure gradient values were calculated in order to elucidate whether a ventilation and perfusion mismatch may be resulting in lower arterial PO_2 (Neary et al., 2013; T.N. Holt, personal communication; Hantzidiamantis and Amaro, 2019).

Oxyhemoglobin Saturation

Oxyhemoglobin saturation was below normal ranges (Nagy et al., 2003; Neary et al., 2013). This depressed percentage of oxyhemoglobin saturated can be attributed to the low arterial PO2 levels noted for all finishing systems across all sampling periods. The oxygen dissociation curve (ODC), a sigmoidal curve that describes the relationship between oxygen and

its affinity to bind to hemoglobin for delivery to tissues (C.W. Miller, personal communication; T.N. Holt, personal communication; Neary et al., 2013). As described by Neary et al. (2013), the sigmoidal shape of the ODC results in a steep decline in binding of oxygen to hemoglobin as arterial PO2 reaches 60 mmHg and below. Similar results have been reported in calves and cows born and managed at high altitude. As indicated by Neary et al. (2013), oxygen extraction fraction would be the best measure to elucidate issues with oxygen delivery.

Alveolar-Arterial Oxygen Pressure Gradient

Mean alveolar-arterial oxygen pressure gradient differed by sampling period but not by finishing system. Despite differences between periods, all steers had pressure gradient values greater than 10 mmHg. Elevated alveolar-arterial oxygen pressure gradient values reflect impaired oxygen transport from alveoli to the arterial blood, which is also recognized as a ventilation-perfusion mismatch (Lekeux et al., 1984; Nagy et al., 2003; Neary et al., 2013; T.N. Holt, personal communication). Neary et al. (2013) reported elevated mean alveolar-arterial oxygen pressure gradient in cows 24 to 27 months of age. Based on these data, impaired perfusion of oxygen across the alveolar membrane to the arterial blood has reduced arterial partial pressure of oxygen measurements.

6.4.3 Arterial Electrolytes and Chemistry

Bicarbonate

Bicarbonate differed across finishing systems and periods. In periods 1 and 2 Norm_Mod_Stocker steers had arterial bicarbonate concentrations that were less than all other finishing systems, which had similar concentrations to one another. However, in period 2, Grass_HA, Grain_HA, and Ext_Mod_Stocker finishing systems had elevated bicarbonate levels that reflected possible alkalosis (Smith et al., 2014; Fielder, 2015a). The results from period 2

indicated alkalosis, which when combined with arterial pH and base excess data supports the finding of respiratory alkalosis in steers. In periods 3 and 4, all steers had similar bicarbonate concentrations that were within normal range. These fluctuations in bicarbonate over time served to reduce pH when steers exhibited alkalotic acid-base parameters (Prasse and Sexton, 1972; Patel et al., 2019; C.W. Miller, personal communication; T.N. Holt, personal communication).

Sodium

Serum sodium levels in the arterial blood differed between finishing systems over the four sampling periods. In periods 1 and 2, Norm_Mod_Stocker steers had serum sodium levels greater than those of the Grass_HA, Grain_HA, and Ext_Mod_Stocker finishing systems. In period 3, Ext_Mod_Stocker steers had greater serum sodium concentrations than either Grass_HA or Grain_HA steers, but not those in the Norm_Mod_Stocker steers, which had a concentration similar to all three finishing systems. Grass_HA steers had a period 4 serum concentration greater than all other finishing systems. Grain_HA steers had a serum concentration for period 4 that was less than Grass_HA, but greater than the Ext_Mod_Stocker and Norm_Mod_Stocker finishing systems, which had similar serum sodium concentrations to one another. Despite the differences reported between finishing systems for each sampling period, all serum sodium levels were within normal range (Smith et al., 2014).

Fluctuations in serum sodium have been utilized to interpret hydration status of cattle. As an animal loses fluid, the sodium levels in the serum will increase due to an increased proportion of sodium ions to plasma volume (Smith et al., 2014). Sodium levels have also been recognized to be indicative of developing left heart failure in humans, which is an obesity-related model late-term finishing cattle have been compared to in literature (Lee and Packer, 1986; Gheorghiade et al., 2007; Forfia et al., 2008). Further, a study in 2008 linked hyponatremia to

human patients with pulmonary hypertension, concluding that decreases in sodium are related to right heart failure in patients with pulmonary hypertension (Forfia et al., 2008). However, no research has evaluated these findings in cattle experiencing pulmonary hypertension induced by either altitude or obesity, and based on the results from this study, there was no evidence of sodium levels that corroborated the results reported in humans.

Potassium

Arterial serum potassium differed by sampling period but not by finishing system. Period 2 serum potassium levels were less than those of all other periods. The decreased serum potassium in period 2 was at a level that may be indicative of moderate hypokalemia, resulting in reduced gut motility and increased recumbency in affected cattle (Constable, 2014). However, no increase in recumbent behavior was noted by ranch managers at either location. Period 2 was in the middle of the summer season, so steers may have been experiencing heat stress. Beede et al. (1983) reported that dairy cows in heat stress conditions exhibited reduced feed intake, thus, decreased potassium intake. Further, cows were losing additional potassium through sweating. Given that potassium levels returned to normal in period 3, after the summer season, steers may have been responding adversely to the summer heat, resulting in a slight decrease in serum potassium levels. Overall, serum potassium levels appeared normal.

Ionized Calcium

Ionized calcium did not differ between finishing systems for periods 1 through 3. In period 4, Grain_HA steers had greater ionized calcium levels than Ext_Mod_Stocker steers, but not Norm_Mod_Stocker or Grass_HA steers, which had measurements that were similar to both finishing systems as well as each other. All finishing systems were within the normal range for ionized calcium for their given age range (Lincoln and Lane, 1990; Agnes et al., 1993; Neary et

al., 2013). Overall, ionized calcium levels have remained consistent in the body despite increases in total calcium, which have been affected by increased protein-bound calcium or complexed calcium. In the presence of acid-base disturbances such as alkalosis, reductions in ionized calcium may be observed (Smith et al., 2014). However, despite observed alkalosis based on arterial pH, hemoglobin, hematocrit, and base excess, it does not appear that the alkalotic state of steers reduced ionized calcium levels. If the differences in acid-base chemistry were not large enough, ionized calcium may have been able remain within normal ranges despite an alkalotic state.

Glucose

Arterial glucose differed by finishing system over time. In period 1, Norm_Mod_Stocker steers had a greater glucose level than Grass_HA steers, but not Ext_Mod_Stocker or Grain_HA steers, which had glucose measurements intermediate between the two groups. However, in period 2, all steers had similar glucose measurements. During periods 1 and 2 all finishing systems were being stockered at their respective location and were, therefore, not being administered any sort of supplemental feed other than normal mineral supplementation. Instead, all 40 steers were on forage. These roughage-dense phases of feeding likely account for the similarities between finishing systems in period 2. This has been demonstrated through multiple studies that reported differences in plasma glucose levels between high-forage and low-forage diets fed to sheep or dairy cows such that high-forage diets reduced plasma glucose concentrations (Bickerstaffe et al., 1974; Evans and Buchanan-Smith, 1975; Evans et al., 1975). Further, as roughage was replaced with a concentrate or finishing ration, the rate of glucose metabolism increased, likely due to increased digestible energy in the diet (Ulyatt et al., 1974; Evans et al., 1975). However, the differences in period 1 do not reflect the reports comparable to

glucose levels on forage. The Grass_HA, Grain_HA, and Ext_Mod_Stocker steers were managed at the same high-altitude location during period 1 and were on the same forage. Norm_Mod_Stocker steers were at the moderate altitude location, where the pastures contain different forage types than the high-altitude location. Differences in forage types between the Norm_Mod_Stocker steers and the other 3 finishing systems, likely accounted for some of the differences noted between finishing systems in period 1.

Ext_Mod_Stocker steers had greater glucose levels than Grass_HA and Norm_Mod_Stocker steers, but not Grain_HA steers in period 3. Further, Grass_HA, Norm_Mod_Stocker, and Grain_HA steers had similar glucose levels at this sampling period. Period 3 was the first sampling period after commencement of the finishing phase of the study. All steers were fed a concentrate diet except for those in the Grass_HA finishing system. However, Ext_Mod_Stocker steers had greater plasma glucose than Norm_Mod_Stocker steers despite having the same diet, altitude, and management. This could indicate that some sort of compensatory change occurred as a result of relocation of the Ext_Mod_Stocker steers from high to moderate altitude as well as the change in climate from summer to fall. It has been well-documented in literature that various forms of stress, such as heat and altitude, can result in reduced grazing or feed consumption (Bianca, 1965; Pereira et a., 2007). Not only were Ext_Mod_Stocker steers relocated to a lower altitude between periods 2 and 3, but they also endured a season change from summer to fall, which reduced the temperature. As a result, Ext_Mod_Stocker steers may have exhibited increased intake compared to period 2. Further, these steers were transitioned from a forage-based diet to a finishing ration, which was more nutrient dense. These changes likely contributed to an elevated plasma glucose level in Ext_Mod_Stocker steers compared to Norm_Mod_Stocker steers in period 3.

All steers administered a finishing ration exhibited glucose levels that were similar to one another and greater than Grass_HA steers in period 4. Previous research reported that as cattle were placed on increased amounts of concentrates or finishing ration versus forage, plasma glucose levels also increased, likely due to increased digestible energy that promotes gluconeogenesis (Ulyatt et al., 1974; Evans et al., 1975). Further the differences in high forage and low or no forage diets has been recognized to result in differences in glucose levels such that greater forage in the diet resulted in reduced plasma glucose (Bickerstaffe et al., 1974; Evans and Buchanan-Smith, 1975; Evans et al., 1975). Overall, plasma glucose increased during finishing due to the nutrient dense ration that was administered to all steers except those in the Grass_HA finishing system.

6.4.4 Venous Hematology

pH

In period 2, Grass_HA and Grain_HA had venous pH levels slightly above the normal range (Smith et al., 2014). Norm_Mod_Stocker and Ext_Mod_Stocker steers had pH measures in period 2 that were within normal range. However, in period 4, all finishing systems had pH levels within normal range (Smith et al., 2014). No studies to date have reported venous blood gases in steers exposed to various altitudes during the stocker and finishing phases of production. However, based on the mean arterial pH parameters reported for each of the finishing systems, it would appear that blood pH has been maintained throughout the circulation.

The renal system has been noted as a vital organ system in the maintenance of blood pH through its excretion of H^+ ions, absorption of HCO_3, and creation of more HCO_3 in order to maintain blood pH around the range of 7.31-7.53 (Smith et al., 2014; Hamm et al., 2015). Therefore, urinalysis and other metrics of renal function may be useful in estimating the efficacy

of the renal system in its maintenance of blood pH. Further, such analysis could serve as an early indicator of renal failure in the event that an animal has begun to experience organ failure in the end stages of HMD or FHD.

Base Excess

Despite having a lower period 2 BE estimate than all other finishing systems, Norm_Mod_Stocker steers had venous BE levels above the normal range. Likewise, Ext_Mod_Stocker, Grain_HA, and Grass_HA steers had elevated period 2 BE levels. Elevated BE levels were also noted in all finishing systems for period 4. These BE levels have been noted to be indicative of alkalosis (Taussig and Landau, 2008). Overall, the venous blood pH levels did not agree with findings of alkalosis, but arterial results of blood gases and hematology were indicative of respiratory alkalosis. Therefore, there could have been a renal system reaction to the elevated arterial pH resulting in its mitigation in the venous blood. No research has been conducted to report BE in venous blood of cattle exposed to varying altitudes and diets during the stocker and finishing phases of beef production. Further research should be conducted to evaluate renal function and to gather further data of BE in cattle exposed to hypoxic conditions.

6.4.5 Venous Blood Gases

Partial Pressure of Carbon Dioxide

Partial pressure of CO_2 in the venous blood did not change between finishing systems or sampling periods. Further, the mean P_vCO_2 values that were reported for period 2 and period 4 were within the normal range for bovine venous blood (Smith et al., 2014). Based on the low P_aCO_2 and normal P_vCO_2 observations noted in this research, steers were experiencing compensated respiratory alkalosis. The primary means to alleviate alkalosis in affected cattle has been the renal system. In the presence of a P_aCO_2 below normal range, the renal system will

offset the acid-base imbalance by excreting HCO_3 in the urine and reabsorbing H^+ ions in the nephrons of the kidneys (Smith et al., 2014). Neary et al. (2013) has published the only study evaluating blood parameters in cattle exposed to high altitudes, and while findings of that study agreed with those of P_aCO_2 data in this research, they did not evaluate P_vCO_2 data. Despite this difference, it appears that results were concordant between this study and Neary et al. (2013), who also reported compensated respiratory alkalosis in calves up to six months of age and cows 24 to 27 months of age.

Total Carbon Dioxide

Total CO_2 in the venous blood differed between finishing systems for both periods that venous data was available. According to the normal ranges reported by Smith et al. (2014), the three finishing systems that remained at high altitude for the stocker phase of the study had T_VCO_2 levels that exceeded the acceptable range. However, Norm_Mod_Stocker steers had a normal T_vCO_2 in period 2. This could be attributed to increased stress during the summer months that resulted in hyperventilation of steers, thus, an increase in circulating CO_2 in the bloodstream that may manifest as either HCO_3 or CO_2. Further analysis investigating fluctuations of respiratory rates could elucidate if this was occurring. In period 4, all finishing systems had T_vCO_2 levels that were within the normal range (Smith et al., 2014). No studies to date have evaluated T_vCO_2 in cattle exposed to different finishing strategies and altitudes. However, it has been documented that TCO_2 measurements are utilized in lieu of measuring HCO_3 (Blood Gases, 2019). Neary et al. (2013) reported HCO_3 levels, but not for venous blood measures. Therefore, this is the first study that has reported T_vCO_2 for steers fed different finishing rations and managed at different altitudes.

Partial Pressure of Oxygen

Partial pressures of O_2 in the venous blood was below the normal range for steers in both sampling periods of this study (T.N. Holt, personal communication). This result was expected given the low P_aO_2 that was also recorded in this study. The A-a O_2 pressure gradient indicated that a perfusion issue existed such that O_2 was not crossing the alveoli to oxygenate the arterial blood. Given that the P_aO_2 measurements were below normal range, it was expected that P_vO_2 would also be less than the expected range for healthy adult cattle. Neary et al. (2013) reported differences in P_aO_2 that were also indicative of an alveolar perfusion insufficiency, but P_vO_2 was not evaluated.

Oxyhemoglobin Saturation

Oxyhemoglobin saturation in the venous blood was less than that of the arterial blood, which was expected since systemic tissues extract oxygen from the arterial blood for maintenance of physiologic function. Further, the s_vO_2 measures recorded were within the expected range (Smith et al., 2014). Therefore, oxygen extraction by the tissues was normal. Despite the amount of O_2 entering the arterial blood via alveolar perfusion being less than the normal range for cattle, the percentages of O_2 bound to hemoglobin in both venous and arterial blood samples were normal. Further, the s_vO_2 measures indicated that tissues were likely still receiving adequate oxygenation for maintenance. In order to validate these findings further, this study should be replicated in order to measure s_aO_2, s_vO_2, and cardiac output. Measuring cardiac output would elucidate any changes indicative of insufficient oxygen delivery to the tissues since one of the body's responses to low oxygen delivery to the tissues is to increase cardiac output in order to increase oxygen transport (C.W. Miller, personal communication; Smith et al., 2014).

6.4.6 Venous Electrolytes

Bicarbonate

Venous HCO_3 for periods 2 and 4 was below the normal range for all finishing systems (Smith et al., 2014). Contrary to the arterial blood for period 2, venous blood for the same time point indicates a reduction in HCO_3 such that it was well below the accepted range. This reduction was likely due to the observed alkalotic state of steers at this sampling period. As a physiological response in regulating blood acid-base balance, the renal system acts as a regulator of HCO_3 in the blood (Hamm et al., 2015). Therefore, it is likely that excess levels of HCO_3 were excreted to maintain an acceptable blood pH. Interestingly, the BE levels in the venous blood did not reflect a reduction in HCO_3, which should have lowered the pH and BE levels. No research has been conducted evaluating venous blood parameters in steers during the stocker and finishing phases at high altitudes. Follow-up research evaluating venous blood parameters and urinalysis would better reflect the changes that may be occurring via either excretion of HCO_3 in urine or reabsorption of HCO_3 by the nephrons within the renal system.

Lactate

In period 2, all steers had comparable Lac levels that were within the middle and upper levels of the normal reported range in cattle. However, in period 4, steers within the Grain_HA finishing system had a mean Lac measurement above the accepted range of 0.54 to 2.22 mmol/L (Figueiredo et al., 2006; Smith et al., 2014). Elevated Lac levels are indicative of poor perfusion of systemic tissues. These elevated Lac levels may be caused by dehydration, excessive muscle activity, increased anaerobic metabolism, or hypoperfusion, among other stressors (Broder and Weil, 1964; Weil and Afifi, 1970; Vincent et al., 1983; Bakker et al., 1991; Mizock and Falk, 1992; Bakker et al., 1996; Gernardin et al., 1996; Figueiredo et al., 2006). Reindl et al. (1998) speculated that pulmonary vascular tone may lead to pulmonary hypertension as well as alveolar

hypoperfusion in humans experiencing heart failure. Neary et al. (2013) reported elevated arterial L-lactate levels due to substantial anaerobic respiration of cows and calves. However, Neary et al. (2013) did not report venous lactate measurements. Based on previous research and results from this study, it is probable that elevated Lac levels observed in this study could be attributed to respiratory alkalosis or the onset of heart failure in susceptible cattle. However, histopathology results are not available yet.

6.5 Conclusions

Overall, arterial blood parameters were indicative of respiratory alkalosis in steers. Regardless of finishing system, steers exhibited signs of alkalosis. Further, most of the venous blood parameters were within normal ranges, which was indicative of compensation in the renal system in order to maintain acceptable acid-base blood chemistry. The findings of respiratory alkalosis were concordant with findings by Neary et al. (2013). However, this was the first study that evaluated the roles of altitude and finishing strategy on blood parameters during the stocker and finishing phases of beef production. In conclusion, altitude and diet both impacted blood parameters in steers that were stockered and finished at moderate or high altitudes. Future research should evaluate these parameters at low altitudes with each of the finishing diets in addition to the finishing systems investigated in this research.

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CHAPTER 7

VARIANTS ASSOCIATED WITH PULMONARY HYPERTENSION IN ANGUS CATTLE DETECTED WITH WHOLE GENOME SEQUENCE

7.1 Introduction

Pulmonary hypertension (PH) in cattle has been thoroughly characterized since its initial discovery (Glover and Newsom, 1915; Hecht et al., 1962; Krafsur et al., 2020). Further, substantial research has investigated mean pulmonary arterial pressures (PAP), which is still recognized by the beef industry as the best indicator of an animal's PH status and risk of developing high mountain disease (HMD; Will et al., 1962; Grover and Reeves, 1962; Grover et al., 1963; Holt and Callan, 2007; Pauling et al., 2018; Speidel et al., 2020). In addition to understanding the signs, symptoms, and pathology of the condition, studies have reported heritability estimates for PAP that have indicated the trait is moderately heritable (Schimmel, 1981; Schimmel, 1983; Enns et al., 1992; Shirley et al., 2008; Crawford et al., 2016; Pauling, 2018). Therefore, genetic improvement has been made possible through PAP expected progeny difference (EPD) estimates available through relatively limited resources for ranchers to consider in breeding and selection decisions (Enns et al., 2011; Pauling et al., 2017; Thomas et al., 2017; American Angus Association, 2019).

Development of EPD for traits such as PAP has been challenging since the accuracies of PAP EPD estimates are lower than those of more commonly measured traits due to the limited number of observations (Glover and Newsom, 1915; Hecht et al., 1962; Neary et al., 2014; Zeng, 2016). In cases such as these, genomic information has been useful to enhance accuracies for EPD estimates through genome-enhanced EPD (gEPD) or marker assisted selection (MAS) (Northcutt, 2010; Garrick, 2011; Rolf et al., 2014).

Research to date that has investigated potential variants and genes associated with PAP has revealed several regions within the genome that could be valuable for selection. However, these research efforts were limited with many efforts focusing on either SNP chip genotype data, transcriptome data, exome data, or searching specific regions of the bovine genome, which leaves regions of the bovine genome uninvestigated (Newman et al., 2011; Newman et al., 2015; Zeng, 2016; Crawford, 2019). Therefore, the objective of this study was to utilized whole genome sequence of steers and bulls of different PAP risk categories to discover candidate variants and genes that may be useful for selection to reduce incidence of HMD in cattle.

7.2 Materials and Methods

7.2.1 Selection of Angus cattle for sequencing

Thirty Angus bulls and steers were selected based on their PAP measurements and pedigree information. Through selection of cattle based on these criteria, the population to be sequenced contained both high and low PAP cattle and represented genetics from registered American Angus sires that are prevalent in many pedigrees and have been popular for artificial insemination. Sequencing candidates were from two ranches in Colorado. Five bulls were managed at Battle Creek Ranch LLC (Parshall, CO; 2,438 m altitude). Twenty-five bulls and steers were from the Colorado State University Beef Improvement Center (BIC; 2,150m). Cattle sourced from the BIC for whole-genome sequencing were a combination of herd bulls (n= 4), steers from a growth and development study (n=12), and steers from a comparative finishing system study across high and moderate altitudes (n=8) (Table 7.1).

Table 7.1 PAP summary statistics for each study population expressed as mean $\pm$ standard error

Data Source	Number of cattle	Ranch Location	Altitude (m)	Sex	PAP

2012 Angus RNA sequence study	12	Saratoga, WY	2,150	S	High: 64.00 ± 9.40 Low: 36.50 ± 1.43
2016 Angus finishing system study	8	Saratoga, WY Akron, CO	2,150 1,420	S	High: 73.25 ± 9.50 Low: 40.75 ± 1.49
Battle Creek Ranch	5	Parshall, CO	2,460	B	High: 51.00 ± 2.08 Low: 41.00 ± 1.00
CSU Beef Improvement Center	5	Saratoga, WY	2,150	B	High: 71.50 ± 18.5 Low: 38.33 ± 0.33

The twelve steers from the growth and development study were BIC steers born in 2012. These steers were a subset of the sample population in a study that compared high and low PAP steers administered a bull development ration (target body weight gain 1.5 kg/d) as a model for yearling bull performance. As part of this study, PAP measurements were recorded at three separate time points, so the subset of steers selected for sequencing were sorted into either low or high PAP categories for future comparisons based on their first on-ranch PAP measure. Further, these steers were considered of value for variant detection due to the RNA sequence data that was generated for each of the twelve steers for various cardiopulmonary tissues. Through combination of the transcriptomic data from this prior study (Crawford, 2019) and the genomic data generated in the current study, a multi-omics approach was possible. Thus, these steers were considered an informative subset within the whole-genome sequence population.

Eight of the BIC steers were a subset from a study that evaluated how diet and altitude impacted PAP postweaning. From each of four finishing strategies highlighted in Chapter 3, one high PAP and one low PAP steer from each group were chosen for whole-genome sequencing. Sequence information from this study was valuable because the PAP measurements were induced by physiologic response to altitude and diet, which provides additional phenotype data

to accompany the sequence data of the overall study population as described in Chapters 3 through 6.

7.2.2 Blood collection and storage

Blood samples were collected from 18 of the 30 bulls and steers via jugular venipuncture in a squeeze chute at the ranch where the animals were maintained. Samples were collected in 10 mL EDTA tubes and stored on ice for transport to the laboratory. Blood samples were centrifuged for 30 minutes at 2,500 rpm to fractionate the blood components. The white blood cell layer or "buffy coat" was evacuated for each sample and placed in a 1.5 mL microcentrifuge tube. The buffy coat was then suspended up to a volume of 1 mL in 1x phosphate buffered saline and stored at -20C until DNA was extracted for sequencing.

7.2.3 DNA extraction and quality assessment

Genomic DNA was extracted from blood (n=18) or liver caudate (n=12) from each animal using the Qiagen DNeasy Blood and Tissue Kit (Cat. No. 69504) according to the instructions provided for each sample type. Sample quality was determined utilizing a NanoDrop One (Thermofisher Scientific) to assess sample purity and a Qubit 4 Fluorometer using the broad range DNA assay (Cat. No. Q32850; Thermofisher Scientific) in order to assess sample concentrations (Table 7.2). Samples that did not have a satisfactory concentration according to the Rapid Genomics submission guidelines were cleaned and resuspended via an ethanol precipitation. In cases where an individual consistently had low DNA yields from extraction attempts, samples for that animal were combined and an ethanol precipitation was performed to increase concentration.

Table 7.2 A table of the absorbance ratios and concentrations measured by Nanodrop and Qubit machines that indicate acceptable DNA quality for submission for sequencing. The 260/230 ratio

measures the absorbance of nucleic acids (260 nm) compared to the absorbance of organic materials such as ethanol or phenol (230 nm). The 260/280 ratio measures the absorbance of nucleic acids (260 nm) compared to the absorbance of proteins (280 nm). Concentration is not noted for Nanodrop measurements because the Qubit has greater specificity in measuring concentration. However, the Qubit does not provide 260/230 or 260/280 ratio estimates.

Technology	**260/230 (nm)**	**260/280 (nm)**	**Concentration (ng/uL)**
Nanodrop	≥ 1.80	≥ 2.00	-
Qubit	-	-	≥ 30

Once samples were extracted and met sequencing standards, samples were assessed via an Agilent 2200 TapeStation machine with the Genomic DNA Screen Tape Analysis. The TapeStation assessed samples for DNA degradation and measured fragment sizes (Figure 7.1). This was the final quality assessment step prior to plating submission for DNA library preparation and next-generation sequencing and demonstrates that the samples were of acceptable quality and integrity for the proceeding steps.

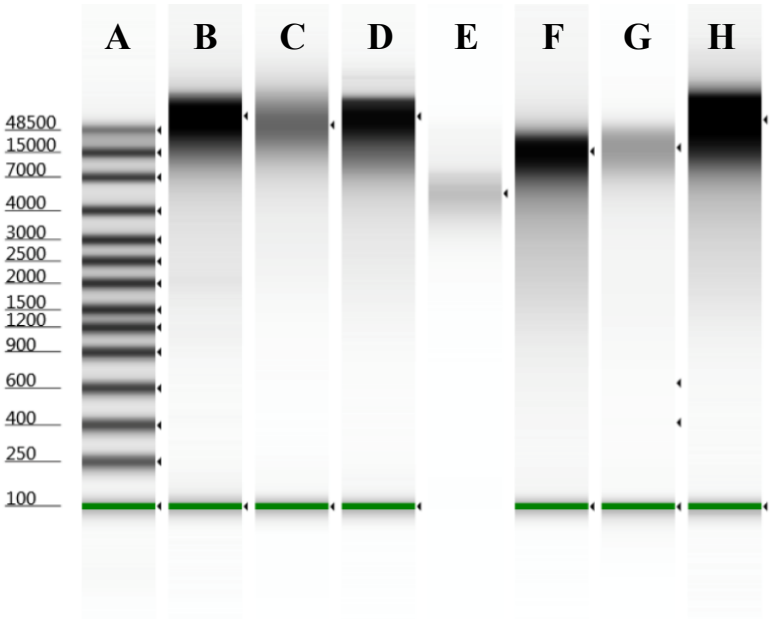

Figure 7.1 Tapestation images for a subset of animals to be submitted for sequencing. Lane A contains a DNA ladder, and lanes B through H contain genomic DNA from randomly selected samples that were submitted for sequencing. The large bands at the top of the gel image indicate

quantities of in-tact DNA in large enough fragments to submit for library preparation and subsequent sequencing. Note that an error occurred with the sample in lane E, which resulted in it appearing to have a low concentration with smaller fragments than other samples. This sample was tested a second time to verify sample integrity.

7.2.4 Dilution and plating of samples

Once all DNA samples were assessed for quantity, quality, and integrity, they were plated on a 96-well full-skirted plate. The well plate was then submitted to Rapid Genomics (Gainesville, FL) for library preparation and sequencing. Samples were diluted prior to plating with the intent to achieve a well volume of 40 ul each while maintaining a concentration above 30 ng/ul per well (Appendix A). The plating volumes and concentrations needed to be similar for the automated pipetting processes necessary for library preparation and subsequent sequencing.

7.2.5 Library preparation and sequencing

Extracted DNA from each bull or steer was submitted to Rapid Genomics for library preparation and subsequent sequencing. The library preparation was performed using a proprietary protocol to fragment the DNA into 150 base pair fragments then ligate adapters and barcodes compatible with an Illumina sequencing run. The samples were then pooled and sequenced using Illumina NovaSeq technology to generate 150 base paired-end reads with 30x median coverage.

7.2.6 Bioinformatic analysis

Samples were de-multiplexed by the bioinformatics team at Rapid Genomics (Gainesville, FL). The de-multiplexed reads were then processed and analyzed using CLC Genomics Workbench version 11 (Qiagen Bioinformatics). First, read quality was assessed using the "Create Sequencing QC Report" tool from the "NGS Core Tools" section of the software's toolbox. An adapter list was then created that contained all i5 and i7 adapter sequences that needed to be trimmed off of the reads. Reads were then trimmed using the "Trim Reads" tool within the "NGS Core Tools" section of the CLC toolbox using the default settings. A second QC report was then generated to verify that the trimming was effective. The trimmed reads were then aligned to the ARS-UCD1.2 (RefSeq ID: GCF_002263795.1) bovine reference genome assembly using the "Map Reads to Reference" tool within the "NGS Core Tools" section of the toolbox (Table 7.3).

Table 7.3 Settings used to map sequence reads to the reference genome in CLC Genomics Workbench (Qiagen Bioinformatics).

Parameter name	Parameter description	Parameter value
Match Score	Cost of a match between the reference and the sequence	1
Mismatch Score	Cost of a mismatch between the reference and the sequence	2
Insertion Cost	Cost of an insertion in the read resulting in a gap in the reference sequence	3
Deletion Cost	Cost of a gap in the read resulting in an insertion in the reference	3
Length Fraction	Minimum fraction of the length of a read that must match the reference	0.5
Similarity Fraction	Minimum fraction of the read that must be compatible with the reference	0.8

Known variants were called for the mapped sequences using the "Fixed Ploidy Variant Detection" tool within the "Resequencing Analysis" section of the CLC toolbox (Table 7.4). These files were then sorted into HPAP or LPAP groups such that any animal with a PAP $\geq$ 49 mmHg were classified as HPAP (n=14), and any animals with a PAP < 49 mmHg was classified as LPAP (n=16).

Table 7.4 Settings used for the "Fixed Ploidy Variant Detection" tool to call variants in CLC Genomics Workbench (Qiagen Bioinformatics) and the description for each parameter.

Parameter name	Parameter description	Parameter value
Ploidy	Ploidy of the species being evaluated (bovine)	2
Required Variant Probability	Minimum probability for which variants will be called	90%
Reference Masking	Ignore positions with coverage above a designated level	100,000 (default)
Read Filters	Ignore broken pairs	De-selected
Minimum Coverage	Minimum number of reads covering a region for a variant to be called	10
Minimum Count	Minimum number of reads to contain a variant for it to be called	2
Minimum Frequency	Minimum frequency for a variant to be called	5%
Base Quality Filter	Minimum quality necessary for a variant to be called	Selected and default values used
Relative Read Direction Filter	Compares observed read direction distributions compared to those expected for a region	Selected and default values used

7.2.7 Variant detection

Variants detected in both HPAP and LPAP cattle were compared using CLC Genomics Workbench. First, single tracks were generated for both HPAP and LPAP groups using the "Compare Variants Within Group" tool. The called variant tracks for each LPAP animal were compiled into a single track with the frequency percentage set to 1%, which would include any called variants present in a single animal. The same procedure was then performed for the HPAP group. In order to increase stringency of the comparisons between HPAP and LPAP cattle, the files were also compiled with the frequency percentage set to 100%, meaning that a variant had to be called in the individual files for each of the animals in that PAP category in order to be included in the single HPAP or LPAP track generated.

The compiled HPAP and LPAP variant tracks were then compared to one another using the "Compare Variants Between Groups" tool such that the 1% frequency HPAP and LPAP files were compared to one another, and the 100% frequency HPAP and LPAP files were compared to one another. Utilization of this function compares an input file to a designated file to call variant similarities or differences contained within the input file. For example, if the LPAP file was the input file that was compared to the HPAP file (LPAP vs HPAP), the tool would output a file of variants present in the LPAP file that were either identical or different from the HPAP file depending on whether the user instructed the tool to call for similarities or differences. Conversely, the inverse comparison was made such that the HPAP file was now the input file, which was compared to the LPAP file (HPAP vs LPAP). When evaluating variant similarities, the LPAP vs HPAP and HPAP vs LPAP results were identical. However, the variant differences were dependent on which file was the input file, meaning that the results from the LPAP vs HPAP comparisons were not the same as those of the HPAP vs LPAP comparisons.

Once the similarities and differences between HPAP and LPAP cattle were detected, the resulting files were annotated using the variant annotation that was compatible with the reference genome used. Further, functional consequences were evaluated to determine which variants may result in amino acid changes, loss of function, or other disruptions within the genome.

7.3 Results

The average number of reads generated exceeded 25 million per animal (Table 7.5). Of those generated reads, about 24.9 million per animal were successfully mapped to the ARS-UCD1.2 reference genome. Within the mapped reads for each animal, at least 45,599 known variants were called when compared to the reference genome.

Table 7.5 Statistics for the number of reads generated per animal, the number of mapped reads per animal, and the number of called variants per animal. All means have been expressed as mean ± standard error.

	Mean	Minimum	Maximum
Total Number of Reads	25,553,713.6 ± 7,049,402.1	3,713,082	149,616,664
Number of Mapped Reads	24,900,678.5 ± 6,862,141.7	3,649,144	145,757,570
Number of Called Variants	275,346.8 ± 99,472.4	45,599	2,530,785

The LPAP and HPAP categories had 3,421,234 and 1,512,287 variants respectively that were prevalent in at least 1% of samples. Comparison of these 1% filtered variant files for HPAP and LPAP categories revealed 977,861 common variants between the two populations (Figure 7.3). There were 514,050 variants unique to HPAP cattle and 2,214,162 variants unique to LPAP cattle.

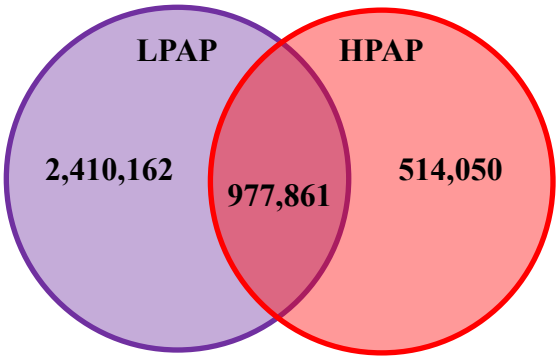

Figure 7.2 Comparison of variants between HPAP and LPAP cattle. Variants were compiled into HPAP and LPAP files for comparisons if they were present at a 1% frequency, meaning that the variant appeared in at least one animal within each of the two PAP categories.

Both LPAP and HPAP samples were also filtered at 100% frequency, meaning that a variant had to be called in all samples within a PAP category to be included in subsequent analysis steps. This resulted in 15,775 and 19,765 variants present in LPAP and HPAP groups respectively. Further, comparison of the 100% filtered variant files for HPAP and LPAP categories revealed fewer variants (Figure 7.4). There were 13,858 variants concordant between

HPAP and LPAP cattle. Only 2,750 of the 13,858 variants common between the two PAP categories were recorded in dbSNP. There were 1,690 variants unique to HPAP cattle and 5,543 variants found exclusively in LPAP cattle. Only 1,142 of the 5,543 variants unique to HPAP cattle were documented in dbSNP. Further, 263 of 1,690 variants unique to LPAP cattle were documented in dbSNP.

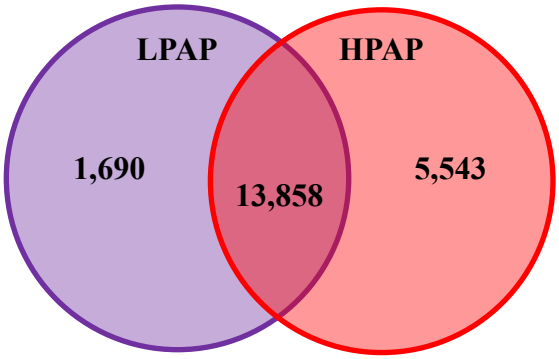

Figure 7.3 Comparison of variants between HPAP and LPAP cattle. Variants were compiled into HPAP and LPAP files for comparisons if they were present at a 100% frequency, meaning that the variant appeared in at least one animal within each of the two PAP categories.

Variants detected that were unique to HPAP cattle and were prevalent in 100% of the HPAP sequencing population spanned all 29 autosomes and the X chromosome (Figure 7.5). The chromosome with the fewest detected variants was chromosome 24. Conversely, autosomes 16 and 17 as well as the X chromosome had the most abundant number of variants detected in HPAP cattle.

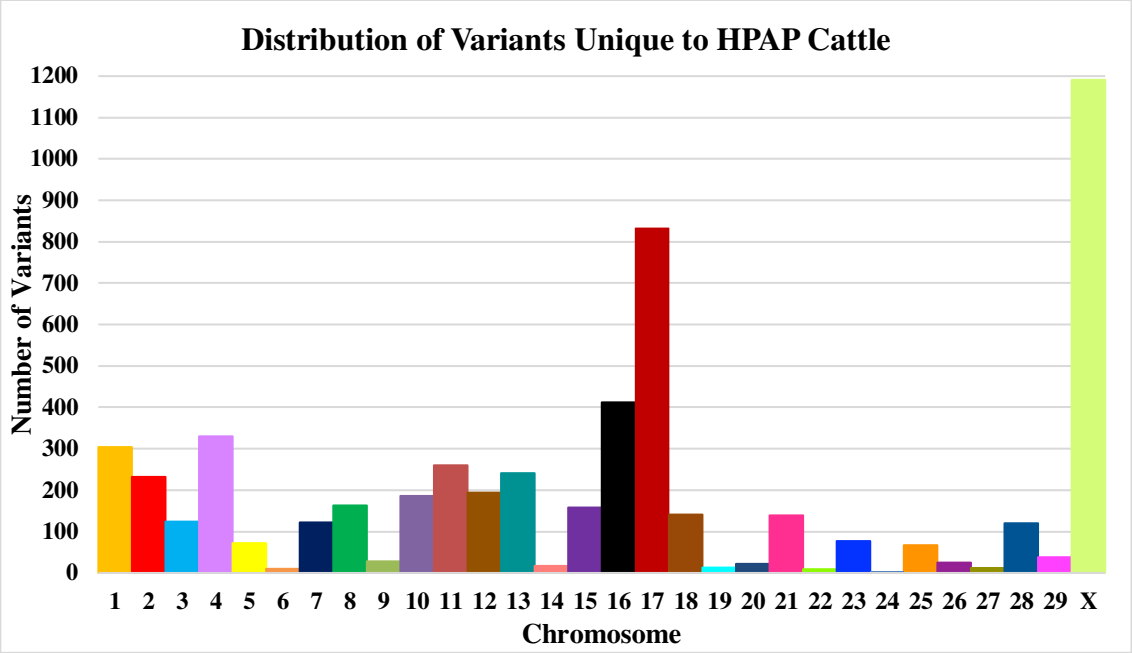

Figure 7.4 The distribution of variants detected at 100% prevalence and were unique to HPAP cattle by chromosome.

Variants that were prevalent in 100% of the LPAP cattle sequenced were also evaluated by chromosome (Figure 7.6). The distribution of detected variants spanned all 29 autosomes as well as the X chromosome. Autosome 24 had the fewest variants detected. Further, autosomes 7, 17, 21, and the X chromosome had the most abundant variants detected that were unique to LPAP cattle.

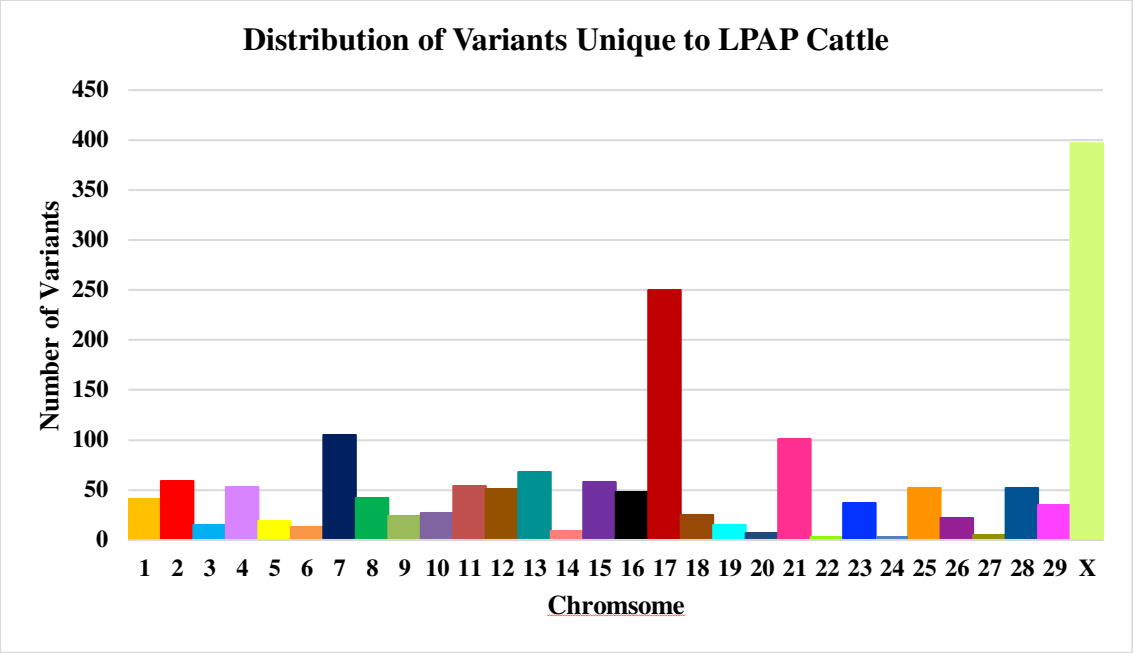

Figure 7.5 Variants that were prevalent in 100% of the LPAP cattle population and were unique to LPAP cattle distributed by chromosome on which they were located.

Variants that were prevalent in all cattle within each PAP category had concordant variants detected between HPAP and LPA cattle that spanned all 29 autosomes as well as the X chromosome (Figure 7.7). Autosomes 20 and 24 had the fewest detected variants. Conversely, autosomes 7 and 17 as well as the X chromosome contained the most variants concordant between HPAP and LPAP cattle.

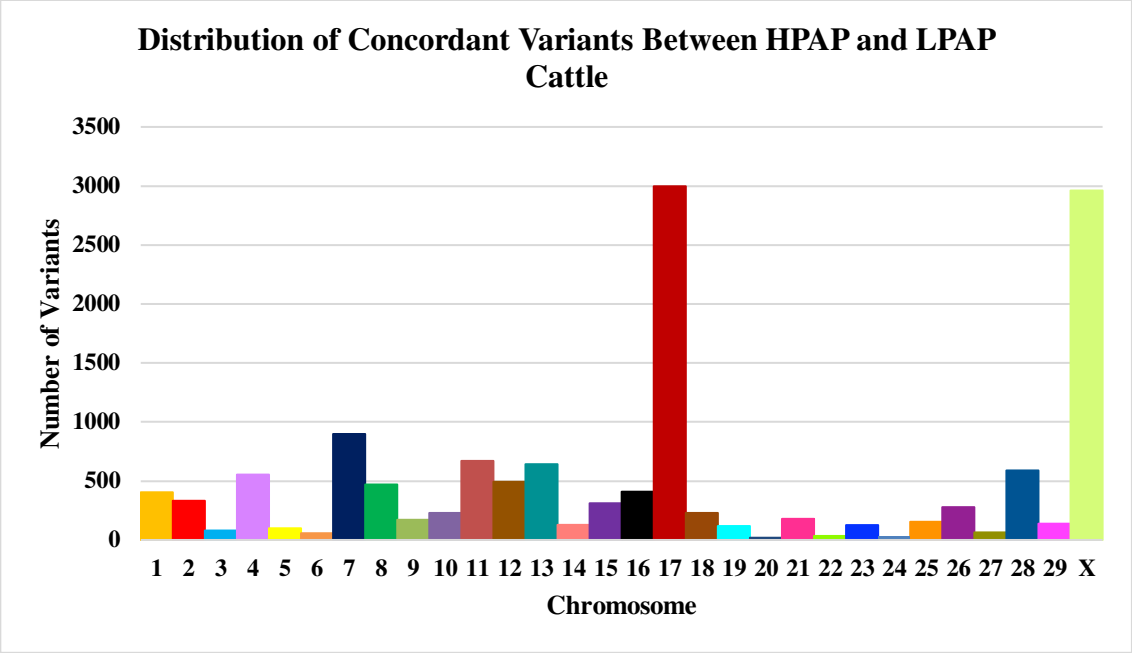

Figure 7.6 Variants common between both LPAP and HPAP cattle by chromosome. Note that only variants present in 100% of the LPAP and HPAP cattle respectively were compared to develop this subset of variants.

7.4 Discussion

The initial analysis in which a variant had to only be in one animal from either the LPAP or HPAP group to be included in the analysis revealed over 2 million variants warranting further investigation. Due to the immense number of variants detected with these parameters, a more stringent frequency was implemented to filter variants that may be less informative in understanding the PAP phenotype. The more stringent frequency of 100% eliminated additional variants, resulting in subsequent annotation and analysis of only those variants that appeared in all cattle within a PAP phenotype category.

The number of variants discovered was greater than that of Newman et al. (2011) who investigated 6,344 SNP from 8,011 variants detected from SNP chip genotype data. Further, the number of variants detected initially in this research was greater than those reported by Zeng (2016), who discovered and investigated 35,930 SNP from SNP chip genotype data. However,

the final list of variants for future analysis was less than those investigated by Zeng (2016). Similarly, Cockrum et al. (2019) investigated SNP associated with yearling PAP as well as growth traits in cattle using Illumina BovineSNP50 data via GWAS. That study yielded 37 variants of interest associated with PAP.

Most variants for HPAP cattle were located on autosomes 16 and 17 as well as the X chromosome. Further, most variants for LPAP cattle were located on autosomes 7, 17, and 21 as well as the X chromosome. These results reflected findings from previous research in which windows on autosome 7 and the X chromosome were associated with two category yearling PAP (Zeng, 2016). However, that study focused on SNP chip data in a genome-wide association study (GWAS) and compared 1 mb SNP windows to an older version of the bovine reference genome. This author also reported windows on autosomes 11 and 20 that accounted for more than 1% of the genetic variation of non-transformed two category yearling PAP (Zeng, 2016). Cockrum et al. (2019) detected eight variants on autosome 7 out of the 37 variants that were associated with PAP. Further, autosomes 16 and 21 each had one variant associated with PAP in the same study. However, the authors did not report any variants detected on autosome 17 or the X chromosome. While fewer variants were noted within autosomes 11 and 20 than other locations within the genome, the number of variants detected on these chromosomes were greater than those reported by Zeng (2016) or Cockrum et al. (2019).

Newman et al. (2011) reported SNP located on chromosomes 1, 3, 10, 24, and 29. While none of these chromosomes had the most variants detected of all chromosomes in this research, the number of variants that were detected on these chromosomes exceeded those detected by Newman et al. (2011). Like Zeng (2016), Newman et al. (2011) used an older reference genome assembly and did not utilize whole genome sequence data. Instead, they utilized low density SNP

genotyping data to detect SNP relevant to PAP, and then performed targeted RNA sequencing for those regions to evaluate fold-change differences. Overall, the approach in the current research was more exhaustive in detecting variants than studies that have evaluated markers associated with PAP to date through incorporation of 30x whole genome sequence data, and as a result, more variants were detected on each of the bovine chromosomes.

The regions of interest that differed between HPAP and LPAP cattle will be investigated further in the next chapter. Focus of variant detection would emphasize genes recognized for their association with PH in cattle from previous research (Table 7.6). Regions flanking these genes will also be investigated for variants that may be informative in estimating susceptibility of developing HMD of cattle in high altitude production systems. Additional genes may be detected based on concordance between transcriptome and genome sequence data. The ultimate objective of combining data from this chapter with transcriptome data was to find genes and variants for validation that may aid producers in distinguishing which cattle have greater risk of developing HMD.

Table 7.6 Genes from previous research that will be investigated using multi-omics data.

Gene	Gene Description	Chr.	Position	Citations
BMPR2	Bone morphogenetic protein receptor type 2	2	90,864,689-91,017,391	West et al., 2004
NFIA	Nuclear factor I A	3	84,197,144 - 84,620,790	Heaton et al., 2020
ARRDC3	Arrestin domain containing 3	7	90,839,580 - 90,853,625	Oka, 2006; Zeng, 2016; Heaton et al., 2020
ROCK2	Rho associated coiled-coil containing protein kinase 2	11	86,489,069 - 86,618,649	Zeng, 2016
EPAS1	Endothelial PAS domain protein 1	11	28,735,330 - 28,825,665	Gale et al., 2008; Newman et al., 2015; Zeng, 2016; Heaton et al., 2016; Crawford et al., 2016

In addition to genomic differences, variants that were similar between LPAP and HPAP were also detected in this study. While these variants may not be informative for diagnostic purposes or distinguishing between HPAP and LPAP cattle, they were collected for further investigation and inclusion as marker effects to estimate gEPD for PAP. These markers would enhance the accuracy of genetic predictions for PAP (Northcutt et al., 2010; Thomas et al., 2013; Garrick and Fernando, 2013; Zeng et al., 2016). Further, these markers would be useful as the beef industry works to provide selection tools for high altitude beef production systems. Only a limited number of resources have cultivated a PAP EPD for selection, with one of the most notable being the American Angus Association (American Angus Association, 2019). Through detection and validation of markers associated with the PAP phenotype, more selection tools can be made available for producers looking to reduce incidence of PH and subsequent HMD within their herds (Thomas et al., 2013; Zeng, 2016).

7.5 Conclusions

These results demonstrated a vast number of variants that may be informative for selection of cattle with reduced risk of developing HMD resulting from PH. Through stringently filtering the called variant files (vcf), those variants that were not prevalent in all cattle within a PAP category were eliminated prior to comparisons of HPAP and LPAP cattle. Variants were detected that differed between PAP categories, which, once validated, may be informative in distinguishing cattle less suitable for high altitude production settings. Further, variants that were similar between production systems may be useful to enhance accuracies of GEPD estimations for PAP. Despite the discovery of these markers, the genes and functional consequences still need to be evaluated.

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CHAPTER 8

DETECTION OF VARIANTS IN THE BOVINE GENOME ASSOCIATED WITH MEAN PULMONARY ARTERIAL PRESSURE AND HIGH MOUNTAIN DISEASE

8.1 Introduction

Increased affordability of sequencing technologies has resulted in thousands of animals being sequenced. In addition, sequencing efforts have been conducted with increased depth and coverage (Muir et al., 2016). For example, chapter 7 described 30 Angus cattle sequenced at 30x coverage. Through increased sequencing efforts as well as increased sequencing depth and/or coverage, novel variants may be discovered within a genome. Further, reduced costs associated with sequencing lends to the ability to deploy multi-omic and bioinformatic strategies to characterize genes, transcripts, and proteins within a species (Heather and Chain, 2015; Muir et al., 2016; Cánovas et al., 2014; Nguyen et al., 2018).

Within the beef industry, genomic information for selection has been contributed through SNP chip genotypes that cover the genome at varying densities. However, when assessing this information, regions of the bovine genome may be underserved or overlooked due to reduced coverage in a particular region (Hickey, 2013). These discrepancies complicate variant discovery efforts.

Variant discovery or detection can be important for development of genetic predictions for new traits, polygenic traits, or traits with few records. An example of a newer trait with few records is mean pulmonary arterial pressure (PAP), an indicator trait important for beef production systems that manage cattle at altitudes of 1,500 m or greater in order to select against high mountain disease (HMD). Due to the cost of measuring PAP and limited global regions concerned with PAP, limited records have been reported to breed associations for genetic

evaluations (Holt and Callan, 2007). Therefore, the estimates of expected progeny differences (EPD) for PAP have been accompanied by low accuracies of estimates. Accuracies of these EPDs can be increased by incorporation of genomic markers to create genome-enhanced EPDs (Glover and Newsom, 1915; Hecht et al., 1962; Northcutt, 2010; Garrick, 2011; Rolf et al., 2014; Neary et al., 2014; Zeng, 2016).

Research efforts to detect markers associated with PAP have used SNP genotype data or have focused on specific regions within the genome. However, the increased affordability of sequencing allows for more robust data to be generated to search the bovine genome for variants that may have been missed through previous variant detection efforts (Heather and Chain, 2015; Muir et al., 2016). Therefore, the objective of this study was to utilize RNA sequence to guide variant detection efforts within exons of bulls and steers with varying PAP risk categories. The research objective was addressed through four aims: (1) detection of variants in transcriptome data that vary between three category (high PAP sick (HPAPS); high PAP healthy (HPAPH); low PAP (LPAP)) and two category (high PAP (HPAP); low PAP (LPAP)) groups for each tissue; (2) determination of concordant variants across tissues; (3) investigation of regions within the common transcriptome variant list for variants, genes, or regions of increased interest pertaining to PAP phenotype; (4) evaluation of genes or regions of the genome associated with PAP that were detected using whole genome sequence data. Through isolation of regions of interest from genome sequence that accompanies the transcriptome data, variants may be detected for downstream validation.

8.2 Materials and methods

8.2.1 Selection of cattle, sample collection, and storage of samples

Thirty Angus bulls and steers were selected for sequencing based on their PAP measurements and pedigree information. These animals were from four different sources

described in Chapter 7. Template DNA was extracted from all thirty animals using either blood samples or liver caudate samples, which were processed and maintained as previously described (Chapter 7).

8.2.2 RNA sequence data

Twelve samples with varying PAP risk categories from a bull development study at the CSU Beef Improvement Center had RNA sequence that was generated from previous research efforts. Transcriptome sequences were available for right ventricle, pulmonary artery, left ventricle, aorta, lung, and *Longissimus dorsi* across each of the twelve steers. Steers had PAP measurements recorded at three separate time periods and were then selected for sequencing based on the three separate time points. Graphs of PAP over time for hypertensive and normotensive steers was summarized by Crawford in 2019 (Figure 8.1).

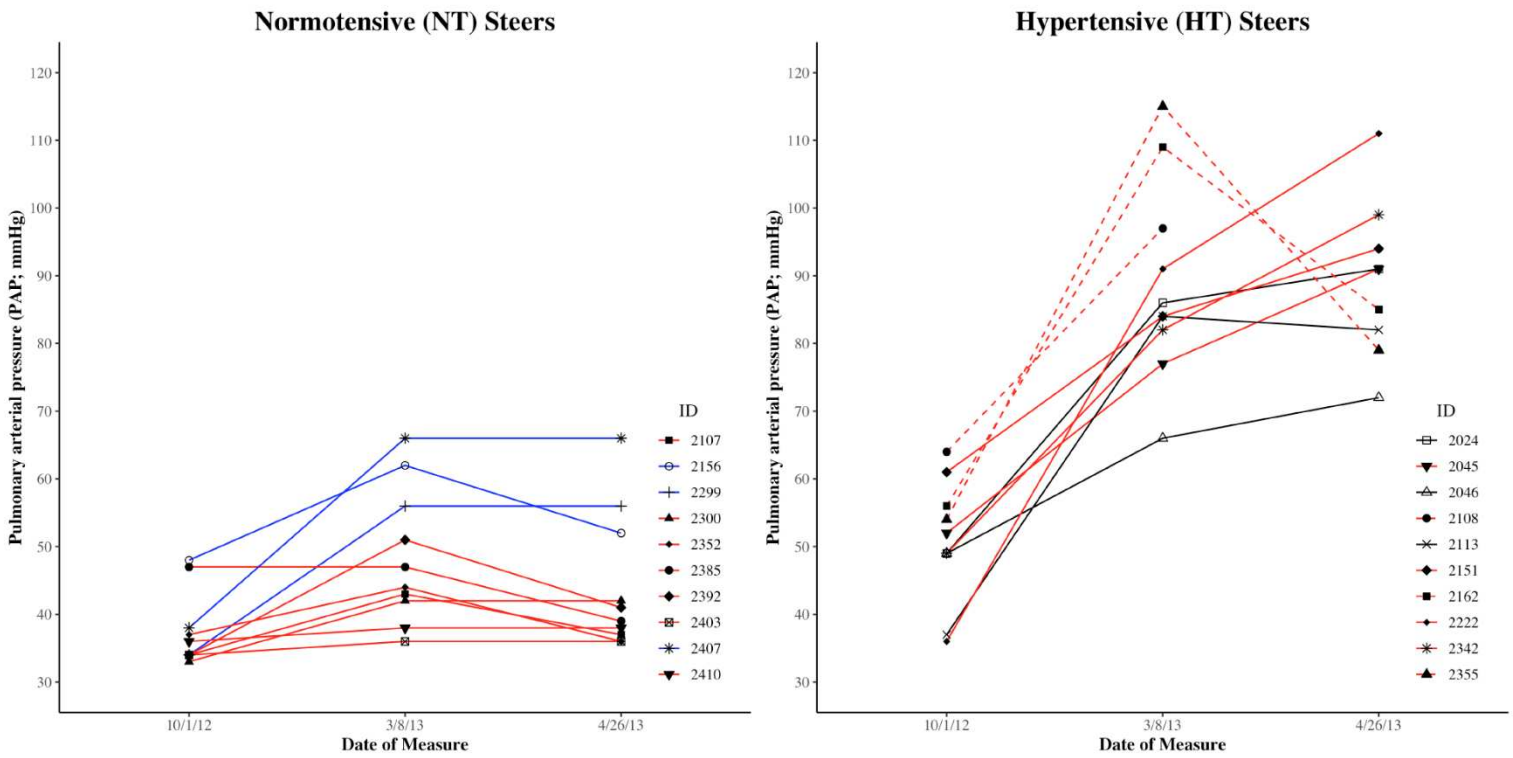

Figure 8.1 Image generated by Crawford (2019) that provides graphical representation of how PAP measures for the RNA sequence population changed over time prior to harvest.

The RNA sequences from these twelve steers also have DNA sequence to accompany them. When combining two data sources it is important to acknowledge factors that could impact the results of the study. These two sequence types (RNA and DNA) were sequenced in different years, at different sequencing facilities, utilizing different Illumina sequencer models. In addition, the sequencing coverage and depths likely differ from one another, which could result in differences in the variants called in the individual sequence types. The genomic data was sequenced to a median coverage of 30x, but the median coverage of the RNA sequence data in this study is unknown. Due to the increased affordability of sequencing since the RNA sequence data was generated in 2014, it was speculated that the coverage of the RNA sequence was less than that of the DNA sequence (Muir et al., 2016).

8.2.3 Bioinformatic analysis

Initial processing of the transcriptome data generated was performed by the research team at University of California- Davis that conducted the sequencing. At this time, all reads were demultiplexed, adapters were trimmed from the reads, and low-quality reads were either trimmed or separated from the data that was of acceptable quality. The trimmed data files were then processed and analyzed using CLC Genomics Workbench version 11 (Qiagen Bioinformatics). First, reads were aligned to the bovine reference genome, ARS-UCD1.2, using the RNA Seq Analysis tool.

8.2.4 Variant detection

Once reads were mapped to the ARS-UCD1.2 bovine reference genome, the files sorted by tissue (right ventricle, left ventricle, pulmonary artery, aorta, lung, and *longissimus dorsi*) as well as PAP risk categories (LPAP, HPAPH, and HPAPS; LPAP and HPAP). These variant tracks were then annotated with the annotation corresponding to ARS-UCD1.2. Variants within

the mapped files were called utilizing the Fixed Ploidy Variant Detection tool. Upon completion of variant calling, comparisons of variant tracks were performed according to a procedure described in Figure 8.2.

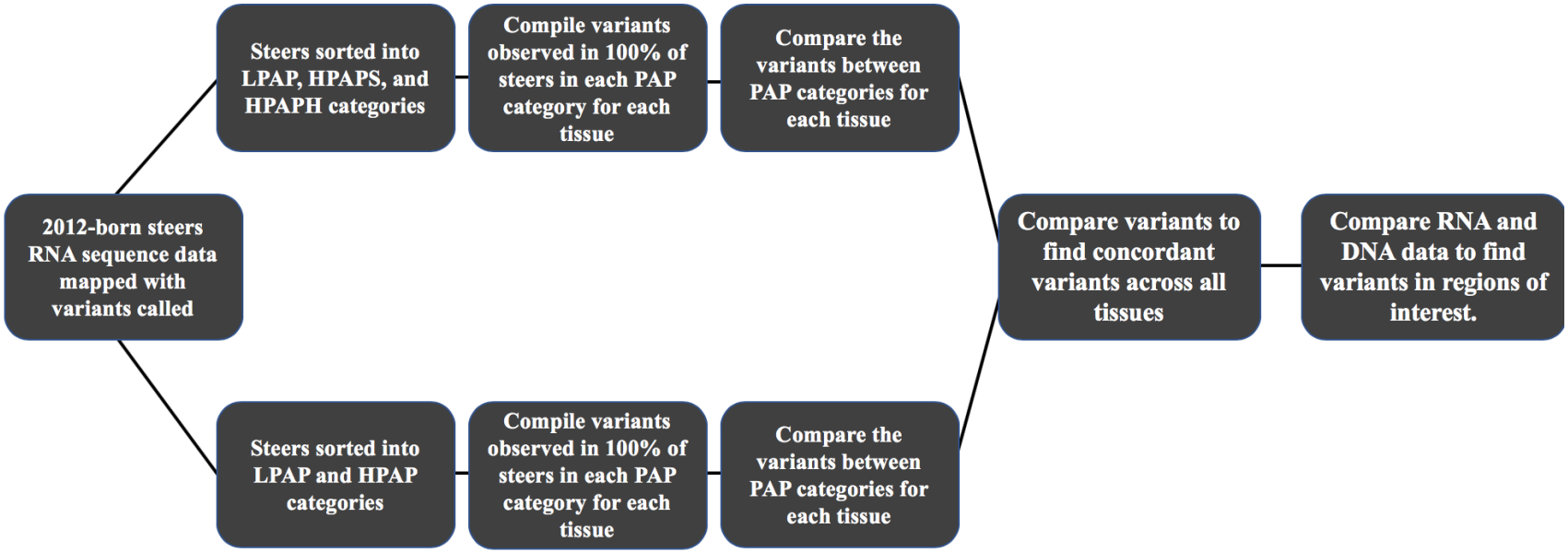

Figure 8.2 A summary of the workflow from variant calling through to comparisons of PAP categories and comparisons between RNA and DNA

8.2.5 Variant filtering

Transcriptome data for each tissue were analyzed separately by first filtering variants within each PAP category at a filtration rate of 100%. This filtration rate meant that a variant had to be called in all animals within the PAP category in order for it to be included in the next analytical step. Once this was performed for all PAP phenotypes within each of the six tissues, the variants were compared and contrasted to between PAP categories using the "Compare Sample Variants" tool to obtain variants that were the same and variants that were different between the different categories, keeping in mind that comparisons were order-dependent. These data were utilized to create Venn diagrams illustrating three category and two category PAP variants for each tissue (Microsoft Office, version 16.16.21).

Variants for each PAP category were then compared across all tissues in order to establish which variants were consistently called across all tissues for a particular PAP risk category. These variants were then compared to the genomic data that was described in chapter 7. Common variants between these two data types were then compared and contrasted to determine which variants may be most informative when evaluating the trait of PAP in cattle. These variants were then summarized by chromosome, evaluated for their proximity to a gene, and searched within Ensembl Genome Browser database ascertain known gene functions (Yates et al., 2020).

8.3 Results

8.3.1 Variants detected within *transcriptome data*

The number of variants detected in each tissue for three PAP category comparisons revealed different numbers of variants detected for each PAP category and tissue (Figure 8.3). Right ventricle samples revealed 4,889 variants unique to HPAPS cattle. Further, there were

9,289 and 2,159 variants unique to HPAPH and LPAP cattle respectively. Left ventricle analyses resulted in 10,449 variants detected only in HPAPS cattle, while 7,235 and 1,132 variants were only found in HPAPH and LPAP cattle respectively. The most variants detected for a single PAP category was detected in the pulmonary artery samples. There were 25,080 variants unique to HPAPS cattle. Pulmonary artery sequence analysis also revealed 4,382 variants exclusively in HPAPH cattle and 4,671 variants exclusive to LPAP cattle. For the aorta sequences, 13,603, 5,593, and 4,089 variants were unique to HPAPS, HPAPH, and LPAP cattle respectively. The *longissimus dorsi* results revealed 10,291 variants only in HPAPS cattle. There were also 5,925 and 2,519 variants unique to HPAPH and LPAP cattle respectively.

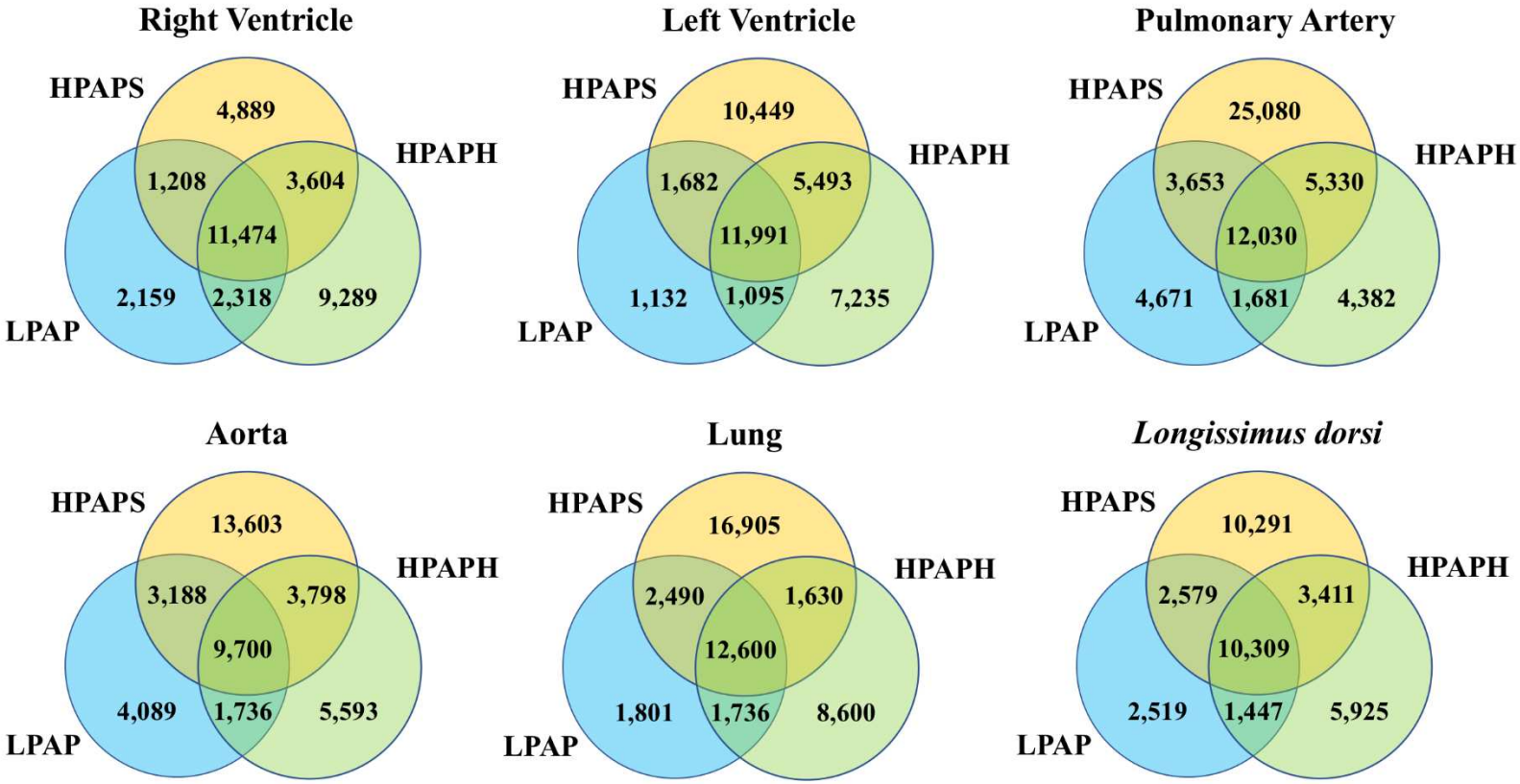

Figure 8.3 Three category PAP (high PAP healthy (HPAPH); high PAP sick (HPAPS); low PAP (LPAP)) comparisons by tissue, where each number represents the number of variants that were detected for each individual category or comparison of categories.

Like three category PAP, two category PAP was also compared for all of the tissues sequenced across the thirty animals (Figure 8.4). In right ventricle sequences, HPAP cattle had 3,555 variants that were different from LPAP cattle. Conversely, LPAP cattle had 5,674 unique variants detected in the right ventricle. Cattle within the HPAP category had 5,453 unique variants found in left ventricle sequence data, and cattle within the LPAP category had 3,870 variants not found in HPAP cattle. In pulmonary artery sequence data, LPAP cattle had nearly twice as many variants with 10,224 unique variants versus 5,277 in HPAP cattle. There were 3,739 detected exclusively in HPAP cattle from aorta sequence, which was less than the 9,123 variants unique to LPAP cattle in the same tissue. Both PAP categories were similar in the number of unique variants detected in lung tissue, with LPAP cattle having 5,876 variants and HPAP cattle having 5,432 variants. Cattle in the LPAP category had twice as many unique variants detected in *longissimus dorsi* tissue as HPAP cattle with 6,588 versus 3,307 respectively.

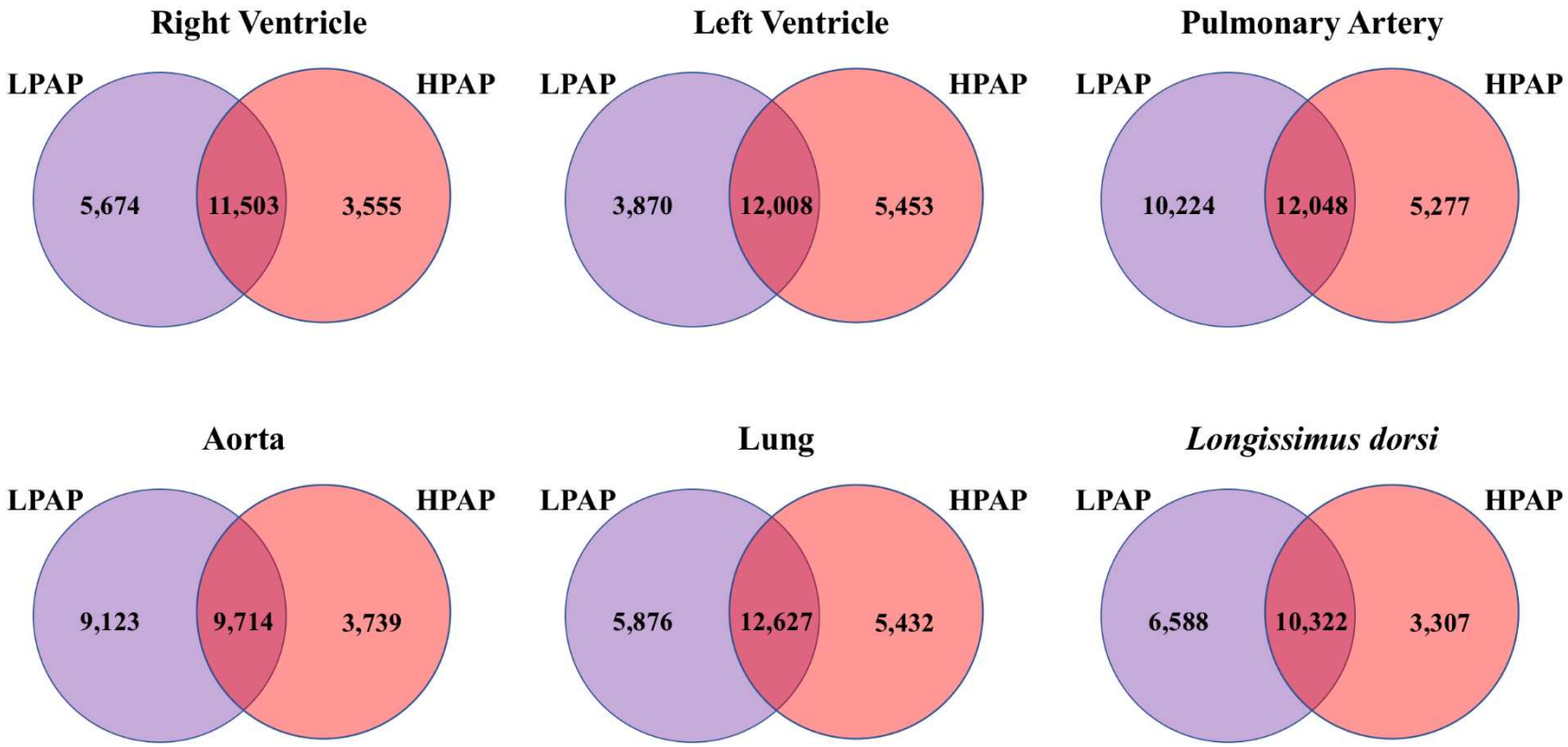

Figure 8.4 Comparisons of variants in HPAP (high PAP) and LPAP (low PAP) cattle across various tissues.

8.3.2 Concordant variants detected across all tissues

When comparing three category PAP variants detected in RNA across multiple tissues, 3,485 variants were detected that were unique to HPAPS cattle (Figure 8.5). Further, HPAPH cattle had 1,810 variants only detected in that PAP category. Comparatively, only 461 variants were identified exclusively in LPAP cattle. Common variants were noted between the three PAP categories such that 4,801 variants were common across all categories. There were 699 variants observed in both HPAPS and LPAP cattle. Cattle in the HPAPH and LPAP categories had 452 variants in common, and cattle in the HPAPS and HPAPH categories had 1,230 common variants.

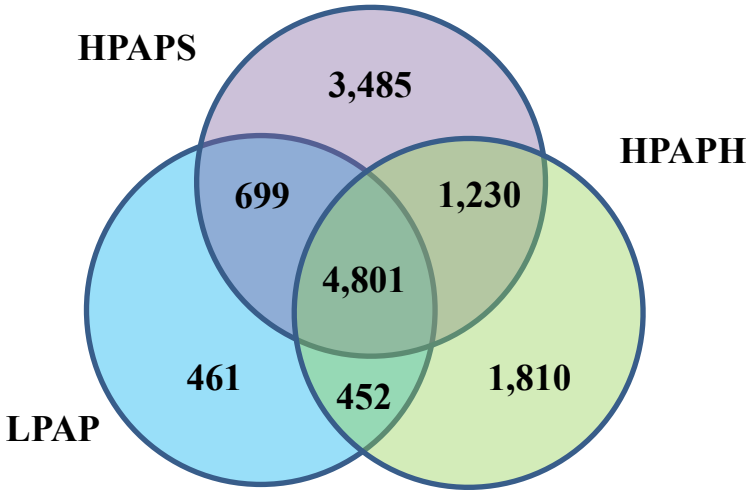

Figure 8.5 Comparison of detected variants in the RNA samples across all tissues for three category PAP.

Evaluation of variants detected in RNA across all tissues for two category PAP revealed 4,808 variants that were common between LPAP and HPAP cattle (Figure 8.6). Individually, there were 1,608 variants unique to LPAP cattle and 1,226 variants unique to HPAP.

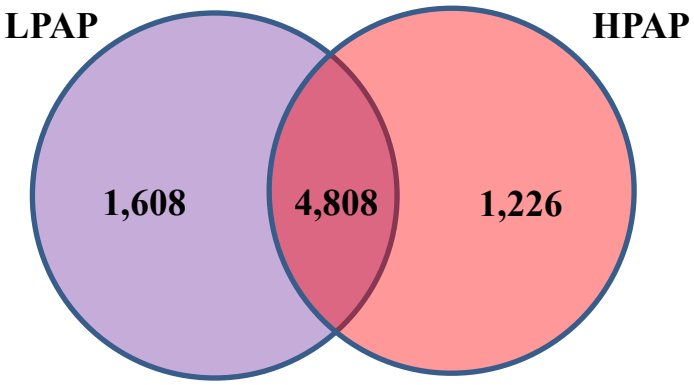

Figure 8.6 Comparison of detected variants in the RNA samples across all tissues for two category PAP.

8.3.3 Concordant variants between RNA and DNA

RNA and DNA were compared for LPAP cattle and HPAP cattle separately, revealing which variants were concordant between the two sequence data sources (Figure 8.7). There were 42 variants concordant between DNA and RNA sequence for LPAP cattle. For HPAP cattle, there were 562 concordant variants.

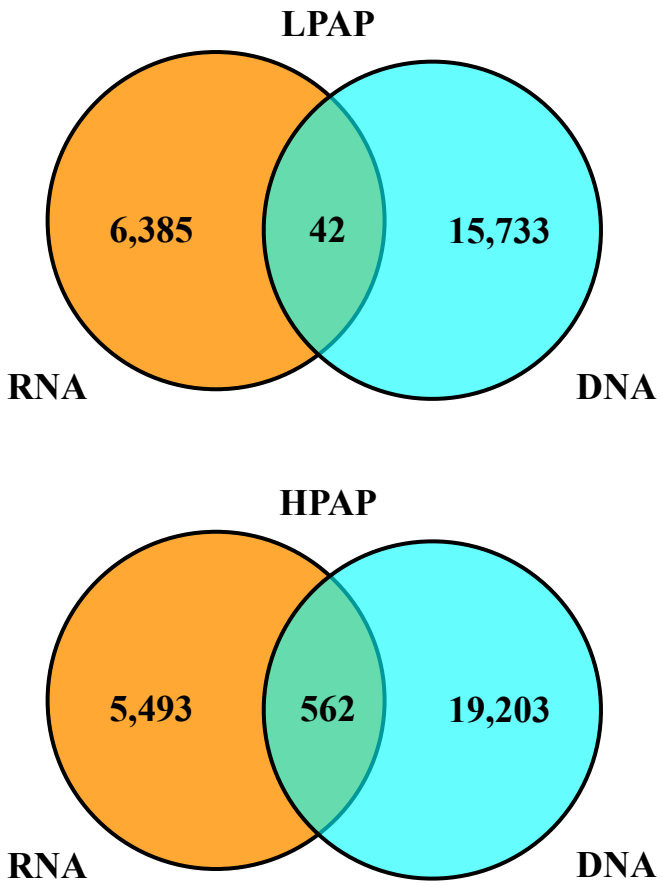

Figure 8.7 Comparisons of DNA and RNA variants for LPAP and HPAP cattle.

Variants concordant between DNA and RNA data sources for LPAP and HPAP cattle respectively were then compared to one another (Figure 8.8). This comparison yielded 39 common variants between HPAP and LPAP cattle. Conversely, there were 3 variants that were unique to LPAP cattle and 523 variants unique to HPAP cattle.

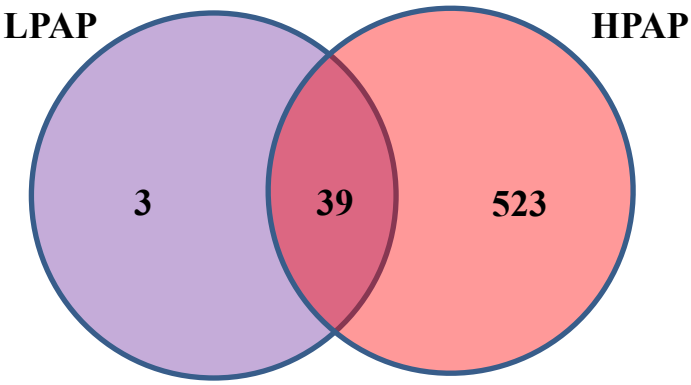

Figure 8.8 Comparison of concordant variants in RNA and DNA for each LPAP and HPAP.

Variants were then assessed by chromosome on which they were located for HPAP (Figure 8.9) and LPAP (Figure 8.10) groups respectively. In HPAP cattle, most of the variants were located on chromosome 16, and in LPAP cattle, two out of the three variants were located on the X chromosome.

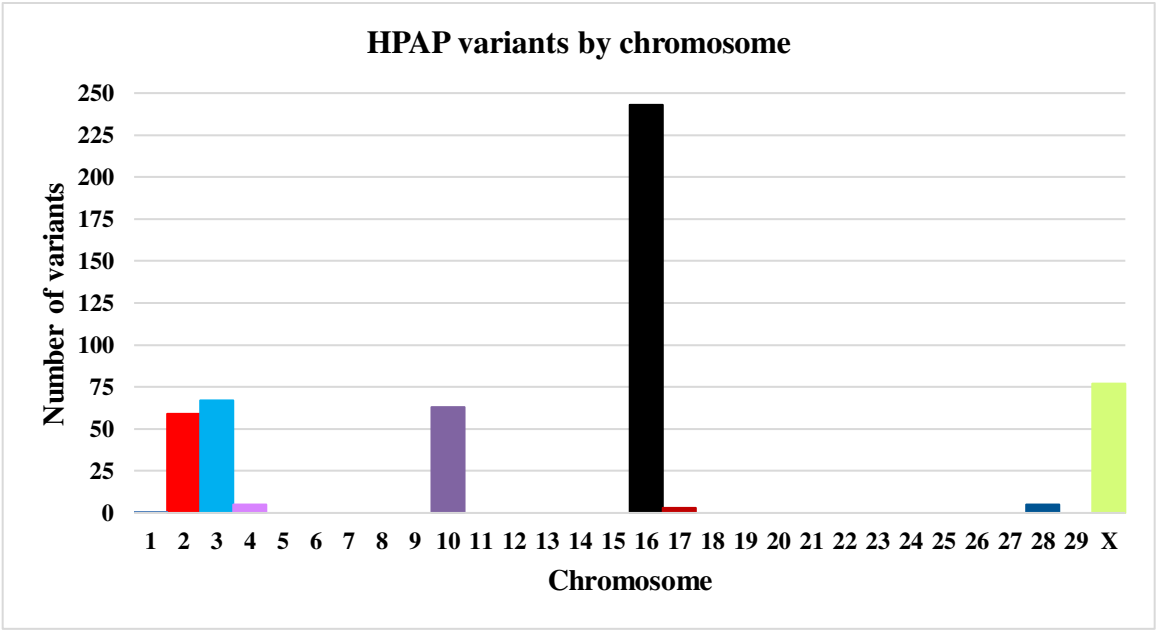

Figure 8.9 Variants that were concordant in RNA and DNA sequence and exclusive to HPAP cattle, organized by chromosome.

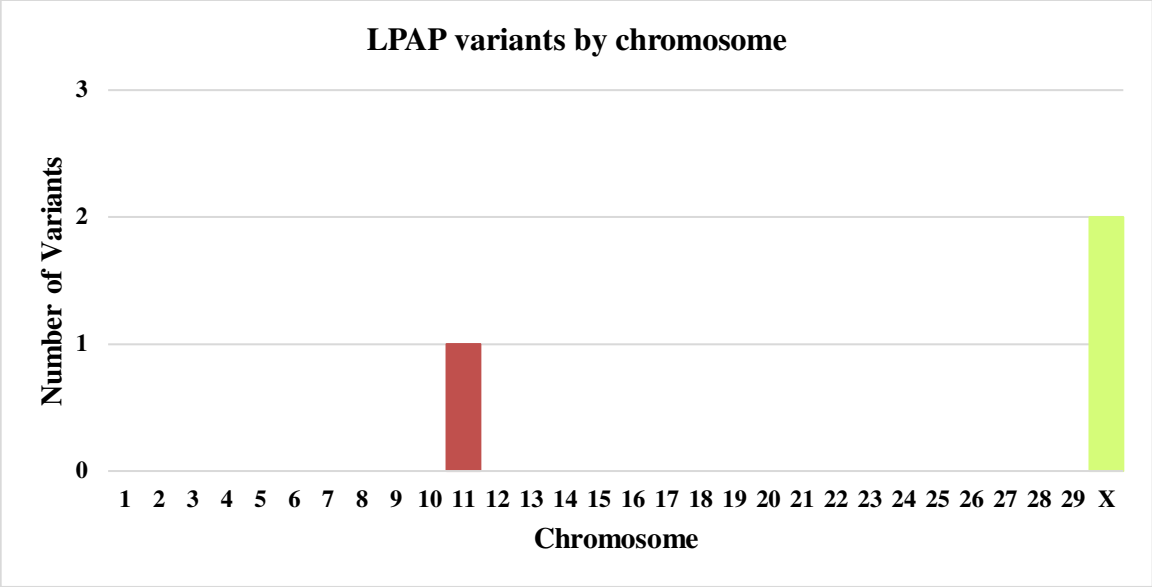

Figure 8.10 Variants that were detected in RNA and DNA and exclusive to LPAP cattle organized by chromosome.

8.3.4 Description of regions of interest

Of the variants detected in both DNA and RNA, those that were within 1,000 base pairs of a gene or RNA were considered worthy of further investigation. The three variants that were detected in both the DNA and RNA of LPAP cattle were not located within a gene or within 1,000 base pairs of a gene. However, in HPAP cattle, three variants on chromosome 2 were near a small nuclear RNA (snRNA) that may be of interest when evaluating the PAP phenotype (Table 8.2). Chromosome 10 contained 63 variants that were called within a single protein coding gene. There were 85 variants detected within 1,000 base pairs of one of three protein coding genes on chromosome 16. Further, within the 3 genes on chromosome 16, there were 21 observed variants. Five variants detected on chromosome 28 were within a single protein coding gene.

Table 8.1 *S*ummary of the number of variants that met the final filtration criteria based on proximity to a gene. Chromosome denotes the chromosome number, number of variants includes the number of variants that met the criteria on that chromosome, and proximity to gene lists whether variants were considered to be near a gene (less than 1,000bp away) or within the gene itself. Note that the numbers in parentheses for the "Proximity to gene" column denote the number of variants near or within a gene based on the total number of variants given in the corresponding "Number of variants" column.

Chromosome	Number of variants	Proximity to gene
2	3	Near
10	63	Within
16	106	Near (85) Within (21)
28	5	Within

The six genes that were near detected variants were researched in the Ensembl Genome Browser to determine gene names and locations (Table 8.3). This revealed three novel genes, two known protein coding genes, and a small nuclear RNA (snRNA) that accompanies a gene.

Table 8.2 Gene annotation information for the six genes that were near variants or contained variants with their Ensembl ID, gene name, gene description, location (chromosome : position in base pairs), and gene type

Ensembl ID	Gene Name	Description	Location	Gene Type
ENSBTAG00000052593	U6	U6 spliceosomal RNA	2: 2,097,072-2,097,178	snRNA
ENSBTAG00000034998	SIMC1	SUMO interactive motifs containing 1	10: 5,026,117-5,109,495	Protein coding
ENSBTAG00000049875	Novel gene	Unknown	16: 818,146-818,352	Protein coding
ENSBTAG00000048925	Novel gene	Unknown	16: 819,140-819,436	Protein coding
ENSBTAG00000050274	Novel gene	Unknown	16: 820,704-820,967	Protein coding
ENSBTAG00000021497	CDH23	Cadherin related 23	28: 27,570,723-27,963,222	Protein coding

8.4 Discussion

8.4.1 Tissue-specific transcriptome variants

Even with stringent filtering parameters, thousands of variants were detected for each tissue when comparing cattle based on PAP categories. The transcriptome data from these steers were previously evaluated in a targeted discovery approach in which a subset of genes related to calcium transport, availability, and utilization in cardiopulmonary tissues were evaluated with

PAP as the trait of interest (Crawford, 2019). The fundamental difference between this study and that of Crawford (2019) was the overall scope of the discovery effort. This study assessed the entire breadth of the transcriptome data to detect variants related to either two category or three category PAP by tissue.

8.4.2 Concordant variants across all tissues

Variants that were concordant in the transcriptomes of all steers within each PAP category were then compared across tissues. Through this approach the number of regions to investigate was further reduced. In addition, variants that were not present in all steers for all tissues were then eliminated from future steps. This approach allowed for detection of the variants highly likely related to PAP phenotype. A similar approach was applied to detect genetic variants associated with splicing, finding that variants of interest were present in many tissues (Xiang et al., 2017). Based on the conclusion from Xiang et al. (2017), this approach was implemented to detect variants that may be most likely to impact PAP phenotype. Further, this approach reduced the number of variants to be compared to the genomic data.

8.4.3 Concordant variants in RNA and DNA

Concordant variants in the RNA and DNA data were stringently evaluated due to the small number of animals in comparison to the immense amount of sequence data for each animal. Therefore, if a variant did not appear in all HPAP cattle or all LPAP cattle, it was excluded from downstream PAP category comparisons. This data sorting procedure greatly reduced the number of variants investigated. However, in addition to the stringency of the filtering process, variants may have been eliminated from downstream evaluation due to differences in sequencing coverage between the RNA and DNA sequence data or due to hemizygosity in the population.

The RNA and DNA data were generated at separate locations in different years using different Illumina sequencing platforms. In addition to these differences, the amount of data generated through each sequencing run differed. These differences in sequencing depth, coverage, and other parameters differed primarily because of increased affordability of sequencing between the two runs (Heather and Chain, 2015). Because the depth and coverage differed between the two data sets, variants that were called in the genomic data may have gone undetected in the transcriptome sequence (Sims et al., 2014). Therefore, more variants and genes may be present in all HPAP or LPAP cattle within this study population, but they were not detected in the RNA sequence and were therefore excluded from the final PAP category comparisons.

The resulting variants detected within the X chromosome in this study may be of interest for future research. However, prior to accepting any variants on this particular chromosome, the variants in question need to be evaluated in females with PAP records via a genotype to phenotype association study. Variants on the X chromosome that are not valuable in predicting PAP phenotype may have been detected through this study due to hemizygosity at the loci on the X chromosome. Because all cattle sequenced were male, hemizygosity was an important consideration during variant detection efforts due to a single X chromosome being present without possibility of being masked or altered in expression by the presence of a second X chromosome as would be observed in female cattle.

8.4.4 Variant descriptions

There were six genes that contained variants common to RNA and DNA sequence for all HPAP cattle within the study population. None of the genes that were found had been reported in previous gene and variant discovery efforts pertaining to PAP (Newman et al., 2011; Newman et

al., 2015; Heaton et al., 2016; Zeng, 2016; Crawford, 2019; Heaton et al., 2020). These genes and the variants within or flanking them should not be considered as superior to those discovered in previous literature, but should instead be considered an addition to the list of regions to include when working to cultivate selection tools for breeders using PAP as a trait in their breeding objective to combat HMD. It is well documented that PAP has a moderate heritability and is polygenic (Schimmel, 1981; Schimmel, 1983; Enns et al., 1992; Shirley et al., 2008; Cockrum et al., 2014; Zeng, 2016; Crawford, 2016). Therefore, inclusion of new genes and (or) variants would be in addition to those already identified.

The first gene observed in this study was located on chromosome 2. While the current bovine annotation recognizes the region as a snRNA, it's a highly conserved spliceosomal RNA that accompanies a single-copy gene. The U6 snRNA is part of a family of U RNAs that play various roles in the processing of genetic information and ultimately how a particular gene is expressed. The U6 snRNA often pairs with U4 snRNA to form a ribonucleoprotein complex that interacts with pre-mRNA to generate a spliceosome. Overall, this complex is responsible for splicing introns and joining exons for a particular region during transcription (Chen and Moore, 2015).

Within bovine genetics, research involving U6 snRNA has investigated the modification of this U RNA type for the purpose of RNA interference of short hairpin RNA as a means of gene-knockdown. The utilization of RNA interference allows for activation or inactivation of a gene of interest based on the ability of U6 snRNA to interact at a sequence-specific region of the genome (Lambeth et al., 2005).

Through the aforementioned research efforts describing the overall role and use of U6 snRNA, the potential for negative effects of variants in and around these regions was evident.

Within the context of PAP, if a variant were present in this particular region, it could negatively impact the ability of splicing in a particular region, which could result in mutations within surrounding genes. While the three variants that were indicated in this region were only flanking the U6 region, they were within 206 bases of the region and could interact or interfere with splicing. Further, the relationship between variants in this region and the HPAP phenotype in cattle is uncharacterized. Future research in this region should focus on exploration of this region of the genome to see if mutations in this region pose a specific consequence in translation and transcription and ultimately alter gene expression. If alterations in transcript abundance and overall gene expression exist in this region, variants in this region may be informative for selection of cattle that perform better at high elevations.

Sixty-three variants were found within a single gene on chromosome 10. The gene was annotated in the bovine reference genome as SUMO interactive motif containing 1 (SIMC1). Description of SIMC1 in cattle has been limited in literature. However, human research has described the gene as a regulator of autolysis for calpain-3 (CAPN3) (Ono et al., 2013). The gene has been identified as part of the calpain family of Ca^{2+}- regulated cysteine proteases and predominantly impacts skeletal muscle. Previous studies have reported that this gene has been related to intracellular processes such as cell proliferation, cell apoptosis, and muscle atrophy in humans (Roperto et al., 2010; Paco et al., 2012; Felicio et al., 2013; Wang et al., 2013; Liu et al., 2015). The Human Gene Expression Atlas reported expression of CAPN3 in epithelial cells of the bronchioles in the presence of carcinogenic compounds from tobacco smoke. In addition, other studies in humans reported deletions downstream of CAPN3 resulting Sotos syndrome, curvature of the spine, heart defects, and kidney defects (Ko, 2013; Dikow et al., 2013; Klaassens et al., 2014; Begum et al., 2016).

Both SIMC1 and CAPN3 have been located within the *Bos Taurus* genome (ARS_UCD1.2). However, while these genes were detected on chromosome 5 of the human genome, they are found on chromosome 10 of the bovine genome (Ensembl Release 101; Yates et al., 2020). Further, while no studies in cattle have evaluated SIMC1, there have been numerous studies describing the role of CAPN3. Most notably, variations in CAPN3 have been linked to tenderness in beef products through mechanisms of sarcomere remodeling and mitochondrial protein turnover (Cohen et al., 2006; Robinson et al., 2012; Barendse et al., 2008; Liu et al., 2015). While CAPN3 has been related to meat quality, it has not been evaluated within cardiopulmonary tissues or investigated within the context of PH in cattle. Given the expansive research of this gene in human literature, it is possible that CAPN3 may impact the cardiopulmonary tissue in the presence of variation within the SIMC1 gene. However, extensive research evaluating genomics, transcriptomics, and PAP phenotype within these genes would need to be performed in order to elucidate a relationship between PH and loci of interest within SIMC1 and CAPN3.

Three genes were found on chromosome 16. However, all three of these genes were classified as "novel" when they were investigated in Ensembl Genome Browser (Release 101; Yates et al., 2020). Each of these genes contained a single transcript but had no description or other classifying information. This lack of information may mean that the annotation did not include any information on these particular genes, or that ontological data has been submitted but not yet updated on any bioinformatic resource. Regardless, further research into these genes may be beneficial when working to characterize PAP phenotypes from a genetic perspective.

Cadherin related 23 (CDH23) has not been characterized in bovine research. However, it has been documented in humans as being related to cell adhesion and has been expressed in

tissues including brain, kidney, heart, lung, nose, eye, and ear. Further, research has indicated that, when upregulated, CDH23 may play a role in early metastasis of breast cancer cells (Apostolopoulou and Ligon, 2012; Takahashi et al., 2016). Further, ontology of this gene has revealed that in addition to cellular adhesion, CDH23 may also play a role in calcium transport (The UniProt Consortium, 2019). Within the context of PH, variants in this gene may reduce cardiopulmonary efficiency by interfering with calcium transport and resulting muscle contractility. Further, if variants in this gene resulted in cell to cell adhesions, a reduced efficiency in the heart or lungs may be observed, which may result in increased sensitivity to changes in altitude or other pulmonary diseases. This gene should be investigated in other cattle with known PAP categories in order to validate the role of CDH23 in cattle at high altitudes.

8.5 Conclusions

Through a multi-step filtration process, multi-omics data were utilized to detect variants associated with specific PAP phenotypes within coding regions of the bovine genome. This process revealed six genes for further exploratory efforts and variant validation. Of those, three were novel, not being characterized in the current genome annotation, which leaves the roles of those genes unknown. Two genes were protein coding genes that, while less characterized in cattle, had thorough descriptions in humans and mice that indicated they could play a role in what PAP phenotype an animal may develop. The last region was an snRNA that accompanies a gene, which, while less informative than other protein coding genes that were found, interacts with a gene in humans that may be relevant to cattle and how they express the PAP phenotype in hypoxic conditions.

These six variants, which have not been evaluated for their relationship to PAP in previous literature, should be investigated further in order to detect intergenic variants,

differential expression, and other information that may allow for better understanding of the genetics underlying PAP. Further, these variants along with variants from previous research efforts (Appendix B) should be validated on a larger population with known PAP phenotypes in order to determine which variants would be of value to genetic improvement programs if included on a SNP genotyping panel.

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APPENDIX A

WELL PLATE LAYOUT OF SAMPLES SUBMITTED FOR SEQUENCING

	1	2	3	4	5	6	7	8	9	10	11	12
A	Dark Knight 17815167 32.2 ng/ul 40ul ✧	Donnie 17048505 35 ng/ul 40ul ❊	103-3 1761654 35 ng/ul 40 ul ✧	186-6 18569631 35 ng/ul 40 ul ✧	400-1 17349449 32 ng/ul 40 ul ✧	519-2 17347109 35 ng/ul 40 ul ✧	1133 35 ng/ul 40 ul ❊	2107 35 ng/ul 40 ul ▲	2108 35 ng/ul 40 ul ▲	2151 35 ng/ul 40 ul ▲	2162 35 ng/ul 40 ul ▲	2222 35 ng/ul 40 ul ▲
B	2300 35 ng/ul 40 ul ▲	2342 35 ng/ul 40 ul ▲	2352 35 ng/ul 40 ul ▲	2355 35 ng/ul 40 ul ▲	2385 35 ng/ul 40 ul ▲	2403 35 ng/ul 40 ul ▲	2410 35 ng/ul 40 ul ▲	4019 35 ng/ul 40 ul ❊	5219 35 ng/ul 40 ul ❊	5227 35 ng/ul 40 ul ❊	6012 35 ng/ul 40 ul ■	6024 35 ng/ul 40 ul ■
C	6095 35 ng/ul 40 ul ■	6133 35 ng/ul 40 ul ■	6235 35 ng/ul 40 ul ■	6246 35 ng/ul 40 ul ■	6307 35 ng/ul 40 ul ■	6365 35 ng/ul 40 ul ■						
D												
E												

✧ Battle Creek Ranch bulls

❊ CSU Beef Improvement Center bulls

▲ CSU Beef Improvement Center 2012 born steers from RNA sequencing study

■ CSU Beef Improvement Center 2016 born steers from finishing system study

APPENDIX B

LIST OF GENES TO INVESTIGATE FURTHER

Ensembl ID	Gene name	Gene description	Chromosome	Position	Gene Type	Citations
ENSBTAG00000020035	*RCAN1*	Regulator of calcineurin 1	1	882,081-1,002,223	Protein coding	Bush et al., 2004; van Rooij et al., 2004; Canaider et al., 2006; Grammer et al., 2006; Crawford, 2019
ENSBTAG00000052593	*U6*	U6 spliceosomal RNA	2	2,097,072-2,097,178	snRNA	Lambeth et al., 2005; Chen and Moore, 2015; Jennings, 2020
ENSBTAG00000006420	*BMPR2*	Bone morphogenetic protein receptor type 2	2	90,864,689-91,017,391	Protein coding	West et al., 2004
ENSBTAG00000046277	*RGS4*	Regulator G protein signaling 4	3	6,290,852-6,298,224	Protein coding	Owen et al., 2001; Mittmann et al., 2002; Cho et al., 2003; Gu et al., 2010; Opel et al., 2015; Crawford, 2019
ENSBTAG00000000074	*NFIA*	Nuclear factor I A	3	84,197,144 - 84,620,790	Protein coding	Heaton et al., 2020
ENSBTAG00000008063	*PPARA*	Peroxisome proliferator-activated receptor alpha	5	116,438,987-116,507,065	Protein coding	Törüner et al., 2004; Simonson et al., 2010; Zeng, 2016; Heaton et al., 2016
ENSBTAG00000007116	*ARRDC3*	Arrestin domain containing 3	7	90,839,580 - 90,853,625	Protein coding	Oka, 2006; Zeng, 2016; Heaton et al., 2020
ENSBTAG00000034998	*SIMC1*	SUMO interactive motifs containing 1	10	5,026,117-5,109,495	Protein coding	Ono et al., 2013; Jennings, 2020
ENSBTAG00000012866	*THBS4*	Thrombospondin 4	10	11,005,591-11,060,139	Protein coding	Stenina et al., 2005; Gabrielsen et al., 2007; Mustonen et al., 2012; Crawford, 2019
ENSBTAG00000008868	*CAPN3*	Calpain 3	10	37,711,578-37,766,813	Protein coding	Cohen et al., 2006; Barendse et al., 2008; Roperto et al., 2010; Paco et al., 2012; Robinson et al., 2012; Felicio et al., 2013; Wang et al., 2013; Liu et al., 2015
ENSBTAG00000002278	*FBN1*	Fibrillin 1	10	61,653,913-61,919,176	Protein coding	Powell et al., 1997; Shen et al., 2011; Jeppesen et al., 2012; Chen et al., 2014; Crawford, 2019
ENSBTAG00000005847	*ROCK2*	Rho associated coiled-coil containing protein kinase 2	11	86,489,069 - 86,618,649	Protein coding	Zeng, 2016
ENSBTAG00000003711	*EPAS1*	Endothelial PAS domain protein 1	11	28,735,330 - 28,825,665	Protein coding	Gale et al., 2008; Newman et al., 2015; Zeng, 2016; Heaton et al., 2016; Crawford et al., 2016
ENSBTAG00000020399	*RNF139*	ring finger protein 139	14	15,593,322-15,607,732	Protein coding	Wang et al., 2017
ENSBTAG00000008432	*NUP98*	nucleoporin 98 and 96 precursor	15	51,264,707-51,350,656	Protein coding	Dogan et al., 2018
ENSBTAG00000038737	*P2RY6*	Pyrimidinergic receptor P2Y6	15	52,784,745-52,790,305	Protein coding	Hou et al., 1999; Nishida et al., 2008; Tovell et al., 2008; Nishimura et al., 2016; Sunggip et al., 2017; Crawford, 2019
ENSBTAG00000049875	Novel gene	Unknown	16	818,146-818,352	Protein coding	Jennings, 2020

Ensembl ID	Gene name	Gene description	Chromosome	Position	Gene Type	Citations
ENSBTAG00000048925	Novel gene	Unknown	16	819,140-819,436	Protein coding	Jennings, 2020
ENSBTAG00000050274	Novel gene	Unknown	16	820,704-820,967	Protein coding	Jennings, 2020
ENSBTAG00000013298	*PLA2G4A*	Phospholipase A2 group IVA	16	67,906,979-68,081,283	Protein coding	Osanai et al., 1998; Handlogten et al., 2001; Magne et al., 2001; Ait-Mamar et al., 2005; Crawford, 2019
ENSBTAG00000024950	*ACE*	Angiotensin-converting enzyme encoding	19	47,798,389-47,819,083	Protein coding	Buroker et al., 2010; Srivastava et al., 2011; Luo et al., 2014; Zeng, 2016
ENSBTAG00000025200	*ASIC2*	Acid sensing ion channel subunit 2	19	16,022,746-17,228,096	Protein coding	Grifoni et al., 2008; Lu et al., 2009; Abboud and Benson, 2015; Zhou et al., 2017; Crawford, 2019
ENSBTAG00000001823	*STC2*	stanniocalcin 2	20	4,999,725-5,011,125	Protein coding	Chang et al., 2008; Na et al., 2015
ENSBTAG00000001333	*PPARG*	Peroxisome proliferator-activated receptor gamma	22	56,709,248-56,835,386	Protein coding	Törüner et al., 2004; Simonson et al., 2010; Zeng, 2016; Heaton et al., 2016
ENSBTAG00000008096	*EDN1*	Endothelin 1	23	44,156,426-44,163,955	Protein coding	Schiffrin, 2005; Murphy and Eisner, 2006; Castro et al., 2007; Deacon et al., 2010; Calabro et al., 2012; Bkaily et al., 2015; Crawford, 2019
ENSBTAG00000053296	*EGLN1*	EGL nine homolog 1	28	4,087,440-4,150,379	Protein coding	Simonson et al., 2010; Buroker et al., 2012; Ge et al., 2012; Xiang et al., 2013; Zeng, 2016
ENSBTAG00000021497	*CDH23*	Cadherin related 23	28	27,570,723-27,963,222	Protein coding	Apostolopoulou and Ligon, 2012; Takahashi et al., 2016; The UniProt Consortium, 2019; Jennings, 2020
ENSBTAG00000013300	*KCNMA1*	Potassium calcium-activated channel subfamily M alpha 1	28	32,616,314-33,387,186	Protein coding	Tomas et al., 2008; Barnes et al., 2016; D. Brown (results unpublished); Crawford, 2019
ENSBTAG00000018540	*NOX4*	NADPH oxidase 4	29	6,120,515-6,303,004	Protein coding	Mittal et al., 2007; Li et al., 2008; Chen et al., 2012; Zhao et al., 2015; He et al., 2017; Crawford, 2019