Proc. Assoc. Advmt. Anim. Breed. Genet. 22:293-300

GENETICS OF BRISKET DISEASE IN BEEF CATTLE: A NOT SO HIGH ALTITUDE PROBLEM

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SUMMARY

Brisket disease, also termed high altitude disease (HAD), has been observed for a century in beef production systems at altitudes > 1,600 m. This disease is often diagnosed as cattle that died of right heart failure; however, there is limited data collection for this trait. Pulmonary arterial pressure (PAP) is an indicator trait that can be used to assess pulmonary hypertension. Cattle with PAP values ≤ 41 mmHg are considered as low risk of developing HAD, whereas cattle with values ranging from 42 to 49 mmHg are considered moderate and those with values ≥ 50 mmHg are considered high risk. This trait has moderate heritability and the phenotype and EPD are used in yearling bull and heifer selection programs. Multi-omics approaches involving SNP-chip genotypes and RNA-sequence gene expression levels indicate that yearling PAP is very polygenic and influenced by gene-pathways of inflammation, tissue remodeling, and metabolism; therefore, these results suggest that this trait could be improved with genomic selection. Recently, late feedlot death in fattening cattle at altitudes < 1,600 m has been observed with etiology of right and left heart failure. This finding adds complexity to understanding brisket disease and challenges the designing of breeding programs for healthy cattle.

INTRODUCTION

High altitude disease (HAD) has been a problem in beef cattle production systems within the Rocky Mountain region of the United States for approximately a century (Glover and Newsom 1917). This disease is commonly known as "Brisket Disease" due to the swelling of this anatomical region. The swelling is a result of the thoracic cavity and pericardium filling with fluid when an animal experiences the hypoxia-induced physiological cascade that leads to right heart failure. Economic losses associated with morbidity and mortality of cattle in response to this disease have been documented in several reports, despite the incidence being relatively low (< 5%) in native cattle. However, the incidence of this disease can expand to levels of approximately 40% in cattle not adapted to altitudes > 1,600 m (Will and Alexander 1970; Salman et al. 1991; Holt and Callan, 2007). High altitude disease is caused by hypoxia-induced pulmonary hypertension; however, collection of the true phenotype is often impossible in extensive pastures of mountain beef production systems; consequently, cattle are screened for disease susceptibility (i.e., risk) using an indicator trait, mean pulmonary arterial pressure (mPAP). This hemodynamic metric reflects the steady-state resistance to blood flow experienced by the right ventricle and a greater pressure, measured in mmHg, indicates greater pulmonary arterial resistance to flow (Holt and Callan 2007).

Mean PAP is a moderately heritable trait in growing Angus cattle (h^2 of 0.26 to 0.46; Crawford et al. 2016). Most cattle that are measured for this phenotype are yearlings (i.e., ~365 days of age) and British and Continental *Bos taurus* breeds although it should be noted that the neonatal calf is also very sensitive to hypoxia (for review: Stenmark et al. 2013). Since yearling mPAP is the most

common phenotype used to determine if cattle are tolerant of hypoxia, seedstock producers in high altitude production systems use this information in sire selection and heifer replacement programs. Expected progeny difference (EPD) for mPAP has been estimated for several breeding programs in the Western United States; however; breed association sponsored EPD for this trait are currently limited due to need for development of standardized data collection programs for this specific trait and due to the requirement that cattle be tested while physically residing at these elevations.

Genomic selection provides opportunity to improve accuracy of EPD and provide breeding values for traits that are difficult to measure, if ample data can be collected for training processes (Garrick and Fernando 2014). Therefore, it is very logical to suggest that genomic selection is a viable approach to ameliorate HAD. This review will describe the efforts of the research team at the Colorado State University Beef Improvement Center to combat HAD.

A more recent occurrence challenging our understanding of brisket disease in mountain production systems is the observation of the pulmonary hypertension and heart failure in fattening feedlot cattle (Neary et al. 2015ab). This is a growing concern to the feedlot segment of the beef industry that exist on what is known as the "Great-Plains" of North America, which are altitudes < 1,600 m (i.e., not so high altitude). This review will also describe what is becoming known as "late feedlot death" as to provide perspective as to the physiological and production challenges that hypoxia is causing beef production systems in the United States.

HIGH ALTITUDE DISEASE AND PULMONARY ARTERIAL PRESSURE

High altitude disease is recognized by the swelling of the brisket in live cattle; however, if the animal perishes, then postmortem evaluation of the organs in the thoracic cavity reveals malformation (i.e., enlargement) of the right ventricle of the heart and several anomalies of the pulmonary artery and lungs. As a consequence of restrictive-remodeling of the pulmonary artery, the liver will develop a unique nutmeg color. The World Health Organization classifies this form of pulmonary artery hypertension in people as Group 3. The phenotype of HAD in cattle can be confused with consequences of bronchiopneumonia or other respiratory infections that may have caused or exacerbated an animal's hypoxic state (Neary et al. 2013). There is no data collection process for the trait of HAD. This is primarily due to limited access to cattle in extensive and rugged pastures in mountainous beef production systems. Data collection from cattle in pastures with this topography has also led us to also study genetics of grazing distribution (Bailey et al. 2015).

Because of the challenges associated with collecting the phenotype of HAD, mPAP is used as an indicator trait to determine if cattle are tolerant of the hypoxic conditions related to increasing altitude. Mean PAP is estimated from the collection of systolic and diastolic PAP measured within the pulmonary artery. These measures are performed on cattle in a squeeze-chute (crush) by a licensed veterinarian that developed these specific skills. To effectively measure this phenotype, it is recommended that the cattle reside at an elevation greater than 1,500 m for at least 3 weeks. It should be noted that this phenotype is also influenced by sources of variation such as breed, gender, pregnancy, age, elevation, concurrent and (or) previous illness, environmental conditions, etc. (Holt and Callan 2007).

In the Rocky Mountain region of the United States, mPAP is annually measured on > 10,000 replacement bulls and heifers. Many beef producers use the raw phenotype and the following categories to cull high risk cattle. Specifically, cattle with PAP values ≤ 41 mmHg are considered low risk of developing HAD, whereas cattle with values ranging from 42 to 49 mmHg are considered moderate and those with values ≥ 50 mmHg are considered high risk (Holt and Callan, 2007). Colorado State University Department of Animal Sciences manages a research facility to study performance and adaptability of Angus cattle in a high elevation beef production system. This research facility is known as the Colorado State University Beef Improvement Center (i.e.,

Rouse Angus Ranch near Riverside Wyoming) This facility a seedstock and cow/calf operation that raises its own replacement females, has grazing lands that range from 2200 to 2800 m in elevation, and supports 430 mother cows and associated animals (e.g., bulls, replacement heifers, steers, etc.). This breeding program uses estrous synchronization and artificial insemination (AI) technologies to coordinate a progeny testing program involving the companies of Genex, American Breeder Services, and Select Sires. Each of these companies contributes bulls that they want to evaluate for tolerance to high altitude. In the past 15 years, > 300 Angus AI sires have been mated in this program. There are several groups of Angus cattle from the Rouse Angus breeding program described in Table 1. The distribution of the mPAP data from this herd appears to have an out of proportion and non-Gaussian distribution of elevated pressures (Figure 1). Therefore, approximately 50.8% of the cattle would be categorized as low risk, 38.1% as moderate risk, and 11.1% as high risk for developing HAD.

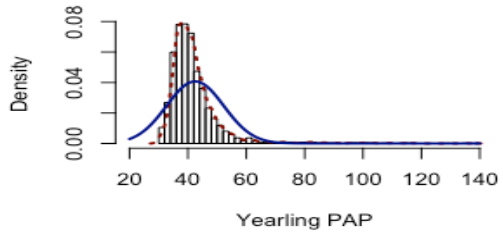

Figure 1. Histogram of mPAP (mmHg, x-axis) in yearling Angus bulls, heifers, and steers (n = 5,659) studied at the Colorado Beef Improvement Center (i.e., Rouse Angus Ranch; elevation 2,200 m; Zeng, 2016). The y-axis is the percentage (density) of the number of animals at each level of the mPAP

In addition to the mPAP data from Rouse Angus cattle, Table 1 also presents results from additional groups of cattle and species. Note that grazing crossbred cows sampled at sea-level have PAP values approximately 9 mmHg higher than healthy humans and other large ruminants thought to be adapted to high altitude (i.e., American Bison and Yak)Also, the two groups of yearling bulls and fattening steers, which would typically be gaining 1.5 kg/day of body weight, have substantially higher PAP values than other types of animals. These data provide evidence to suggest that cattle have higher PAP than most animals and these values increase when the animals are fed high-gain diets. It should also be noted that the fattening Angus steers in the first row have PAP values within the range as people suffering from hypertension described in the last row of the data; therefore, providing validation evidence that mPAP is effective indicator trait for HADThe health consequences of mPAP in these fattening steers will be described further in the section titled “Late Feedlot Death”.

HERITABILITY AND EXPECTED PROGENY DIFFERENCE

Mean PAP was reported to be a trait of moderate ($h^2 = 0.34$) to high ($h^2 = 0.46$) heritability using data from a registered Angus herd in Carbondale Colorado (elevation 1,981 m; Enns et al. 1992; Shirley et al. 2008). Using data from the Rouse Angus herd, which are purebred cattle, but not registered, a moderate ($h^2 = 0.26$) heritability was estimated using records from 1993 to 2014 (n = 5,776; bulls and heifers; Crawford et al. 2016). Zeng (2016) reported similar results using bull, heifer, and steer data, which also included the 2015 calf crop from this breeding program. A genetic correlation of 0.67 was observed between yearling (365 days of age) and weaning (205 days of age) in mPAP in the Dissertation of Zeng (2016). Slight to moderate, and positive genetic correlations (≤ 0.22) were observed between growth traits and yearling mPAP in these two studies; however, stronger relationships (≤ 0.50) were reported by Shirley et al. (2008), but the PAP values in that study were collected at weaning. Nonetheless, the positive relationships between growth traits and PAP values could be unfavorable for overall animal health with aggressive selection for growth.

Colorado State University's Center for Genetic Evaluation of Livestock has calculated EPD for PAP for several cattle breeding programs located in the western mountainous states of the United States. For the two Angus breeding programs described in the previous paragraph, genetic trend was slight. This is most likely a result of the unique distribution of the numeric trait of PAP (Figure 1), the continuous introduction of new sires through the progeny test program, and (or) a selection program that typically only culls medium and high risk animals. In general, 90% of the animals will have an EPD ranging -4 to +4, with the remaining 10% increasing up to +19 mmHg. The underlying physiology of this trait and its interaction with altitude suggest that there is likely a limit in genetic reduction of PAP; therefore, Zeng (2016) conducted research evaluating genetic correlations among PAP and growth traits, heritability, and EPD using the categorical veterinary risk descriptions of PAP (i.e., low, moderate, and high)These genetic correlations and heritability estimates were calculated using a threshold model that assumed a continuous underlying normal distribution of liabilities. The overall results of this effort were very similar to those obtained using the raw phenotypes. The EPD from the threshold models were converted to a probability scale. These types of EPD are often difficult for the beef producers to interpret; therefore, the decision was made to continue to calculate the EPD using the raw data as the breeders and bull buyers in this region of the world are very familiar with the phenotype of PAP, which is expressed in the units of mmHg.

MULTIOMICS STUDY OF PULMONARY ARTERIAL PRESSURE

Pulmonary arterial pressure data is currently being used for EPD calculations of traits indicative of tolerance to high altitude in cattle. There are no data collection systems for incidence of high altitude disease as collection of "death" data is difficult in these extensive environments where predators and topography may mean mortalities are never observed. To date, 3,000 cattle from the Rouse Angus herd have been high density genotyped. Sixty-six Angus animals have been genotyped with the BovineSNPHD (~777,000 SNP) and the rest with BovineSNP50 (54,0001 SNP). Genome-wide association studies (GWAS) have been performed to identify quantitative trait loci (QTL). Figure 2 presents results from Angus weanling steers (i.e., 6 months of age) described in the studies of Neary et al. (2014). Figure 3 presents the results from yearling Angus cattle described in the studies of Zeng (2016). Cumulatively, these results indicate that PAP is a very polygenic trait. These findings parallel the results from various gene expression studies that extracted RNA from heart, lung, and blood peripheral blood mononuclear cells in cattle exposed to hypoxic conditions and compared to samples from calves in normoxic conditions. Fibroblasts harvested from the pulmonary artery were an important cell type in these studies as their unique-phenotype is indicative of hypoxia-indued arterial remodeling. In these results, multi-gene pathways of inflammation, tissue remodeling, and metabolism were prominent. The latter is a very intriguing result and suggests that hypoxic cells have altered-glycolytic metabolism (i.e., Warburg effect; Stenmark et al. 2013; Newman et al. 2015; Li et al. 2016).

Since PAP EPD uses data collected from yearling cattle, an RNA-Seq study was conducted to obtain gene expression data from steers being grown as contemporaries to the bulls at the Rouse Angus ranch. This study involved identifying High- and Low-PAP individuals as to obtain RNA from muscle (i.e., right and left heart ventricle and longissimus dorsi) and the pulmonary system tissues (pulmonary artery, aorta, and lung). This approach allowed study of transcriptome-gene expression and also provided sequence for SNP. The initial results of this effort suggested that more than 1,000 genes were differentially expressed between high and low PAP groups in the right ventricle (Canovas et al. 2016). Splice variant analyses revealed several hundred differentially expressed genes in RNA from the right ventricle, aorta and pulmonary artery. Pathway and transcription factor (i.e., gene regulatory analyses) also revealed numerous genes involved in inflammation and several other indicators of heart failure. Examples of such gene-pathways were

IL-8/IL-10 signaling, leukocyte extravasation and factors promoting cardiogenesis, coagulation, thrombin and cardiac hypertrophy signaling. There were some responses among the high and low groups in hypoxia inducible factors (HIF); however, this is a very large and complex gene family requiring additional study.

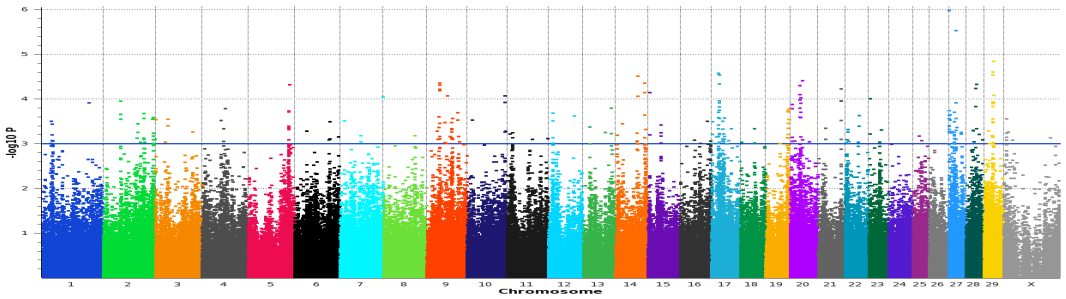

Figure 2. Manhattan plot of mean pulmonary arterial pressure (mPAP; y-axis) in weanling Angus calves (n = 66) at 2200 m. Genotypes were from BovineSNPHD (770,000 SNP) and were used in a single SNP analysis (Neary, 2014)

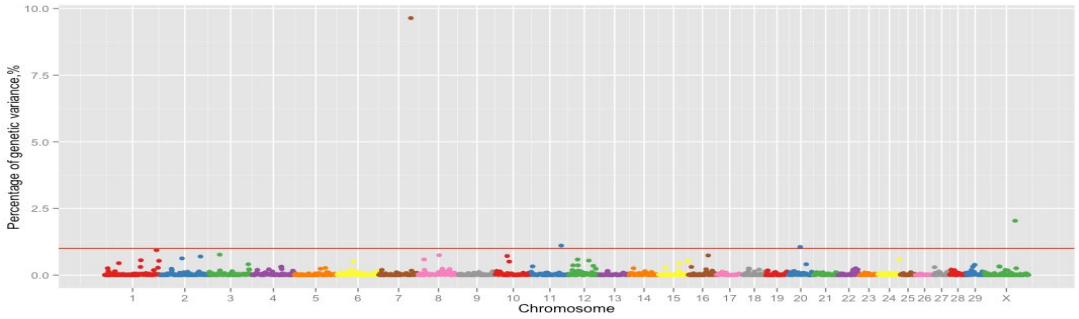

Figure 3. Manhattan plot of the proportion of genetic variance explained by 1 Mb SNP windows for deregressed estimated breeding value of mean pulmonary arterial pressure (mPAP; y-axis) in yearling Angus cattle (n = 2,582) at 2,200 m. Genotypes were from BovineSNP50 (54,001 SNP) and marker effects and associated variances were generated with Bayes B (Zeng, 2016)

LATE FEEDLOT DEATH

Between the years of 2000 and 2012, the incidence of death due to right heart failure doubled in an epidemiologic study of North American feedlot cattle (Neary et al. 2015a). This study involved 1.56 million cattle at 15 feedlots at elevations < 1,300 m. The death rate from congestive heart failure in this study was ~10 steers per 10,000 steers on feed. This is an interesting observation, as also during these span of years, carcasses of finished steers reached their highest weight since the United States Department of Agriculture started reporting these data in 1944. This organization reported average live and carcass weights of steers on January 17, 2017 as 661 and 425 kg, respectively.

Pulmonary artery remodeling and subsequent right heart failure has been thought to be a disease related to hypoxia at altitudes > 1,500 m, so most of the animals affected by this condition were calves within cow/calf operations in mountainous beef production systems; however,

veterinary diagnostic laboratory reporting programs have observed an increase in feedlot cattle post-mortem submissions. Many of these cattle came from feedlots of < 1,500 m. The first row of Table 1 presents PAP data collected from fattening feedlot steers. The PAP values are extremely high relative to several other animal groups and somewhat analogous to values in people that are suffering from various cardiopulmonary conditions involving pulmonary hypertension (Moraes et al. 2000; Bossone et al., 2013). Krafsur et al. (2017) are studying feedlot steers that perished from heart failure in the late feeding period. Remodeling of the pulmonary artery and the right heart ventricle was observed in these cattle as would be expected. However, an interesting observation in these tissues was that these steers also contain evidence of left ventricle heart malformations as exhibited in Figure 4. The World Health Organization would categorize this type of pulmonary hypertension as Group 2, which is often associated with obesity. Feedlot steers with United States quality grades of choice and prime are approximately 30% body fat, which is comparable to obese people with body mass indexes ≥ 30 kg/m^2. Late feedlot death is a growing concern and data collection systems are needed to understand if these traits are related to the measures commonly collected on yearling cattle for risk of HAD.

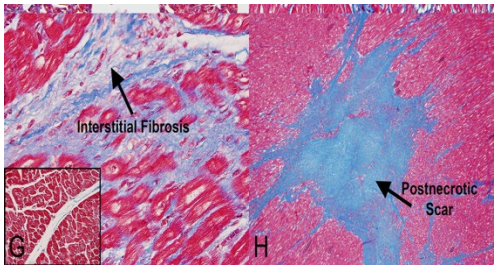

Figure 4. Histopathological images from the left ventricle of the heart of a fattening *Bos taurus* steer from a low elevation (550 m) feedlot. G. Interstitial fibrosis of left ventricle. High magnification (400x); arrow: fibrocollagenous matrix separating and entrapping left ventricular cardiomyocytes. Inset: normal myocardium. **H.** Heart with postnecrotic scar (100x). Arrow: fibro-collagenous matrix replacing cardiomyocytes in the left ventricle

CONCLUSIONS

High altitude disease is a problem in mountainous beef production systems; however, there are no data collections systems for this phenotype, so an indicator trait, mPAP, is used in selection. This indicator trait from measures of yearling Angus cattle has unique a non-Gaussian distribution, but appears suitable for EPD calculation. Mean PAP is very polygenic and results of multi-omics studies suggest genomic selection approaches could provide opportunity to improve breeding value estimations. Evidences suggest that all age classes of cattle (i.e., neonate, weanling, yearling, and finishing steers) are responsive to hypoxia and additional research is needed to help understand genetic relationships among these age groups and the influences of their growth rates on HAD.

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Table 1. Review of mean pulmonary arterial pressure (mPAP) values in cattle and closely related ruminant species and humans

Animals	Altitude (m)	mPAP (mmHg)	mPAP Range (mmHg)	References
Angus fattening steers; n = 30	2,200/1,400	54.1 ± 2.7	42-143	Krafsur et al. 2017 Neary et al. 2015
Yearling Angus bulls (gain-test); n = 1,397	2,200	45.8 ± 0.3	29-139	Zeng 2016
Yearling bulls of several *Bos taurus* breeds (gain test); n = 2,426	2,200	45.1 ± 0.8	29-145	Crawford et al. 2017
Mature Angus cows; n = 44	2,200-2,800	42.8 ± 0.8	31-55	Bailey et al. 2016
Yearling Angus heifers (grazing); n = 3,489	2,200	41.4 ± 0.2	22-135	Zeng 2016
Yearling Angus steers as grazing stockers; n = 773	2,200	41.1 ± 0.2	27-138	Zeng 2016
Angus-crossed cows (*Bos taurus* and *Bos indicus*), n = 49	0	34 ± 0.5	28-41	Holt, personal communication
Mature American Bison; n = 6	2,200	29.8 ± 0.8	28-34	Holt, personal communication
Mature Himalayan Yak; n = 6	3,700	20.2 ± 1.4	18-21	Anand et al. 1986
Healthy Human (meta-analysis)	≤ 400	25	15-35	Bossone et al. 2013
Human hypertension (meta-analysis)	≤ 150	>35	15-70	Moraes et al. 2000

Research in this report supported with funds from NIH-5-PO1 HL014985-40A1 (Stenmark) and the John E. Rouse Endowments of Colorado State University (Thomas and Enns).