DISSERTATION

# ANGUS CATTLE AT HIGH ALTITUDE: PULMONARY ARTERIAL PRESSURE, ESTIMATED BREEDING VALUE AND GENOME-WIDE ASSOCIATION STUDY

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ABSTRACT

ANGUS CATTLE AT HIGH ALTITUDE: PULMONARY ARTERIAL PRESSURE, ESTIMATED BREEDING VALUE AND GENOME-WIDE ASSOCIATION STUDY

In high altitude states such as Colorado, New Mexico, Utah and Wyoming, hypoxia-induced bovine pulmonary hypertension (PH) commonly referred to as "brisket disease" or "high altitude disease (HAD)", has been observed within the cattle industry. This disease is a major cause of morbidity for beef cattle ranches and feedyards above 1500 m and contributed to an estimated $60 million (based on cattle price of $800/head) loss each year in beef herds at high altitude of the United States. This disease in humans and animals is a response to hypoxia, which results in pulmonary vasoconstriction, vascular remodeling, elevated pulmonary arterial pressure (PAP), pulmonary hypertension, right heart ventricular hypertrophy, and finally death from congestive heart failure. Due to the close physiological relationship between HAD and PAP, this measurement has been used as an indicator trait for studying HAD. The objectives of this study were to explore the phenotypic and genetic characteristics of various yearling PAP phenotypes, develop multivariate models for genetic evaluation of yearling PAP phenotypes, conduct genome-wide association studies (GWAS) on yearling PAP phenotypes and performance traits and evaluate the genomic relationships among traits.

Yearling PAP measurements (42.45 ± 0.56 mmHg) and performance phenotypes were collected from Angus cattle born from 1993 to 2015 at John E. Rouse Colorado State University Beef Improvement Center (CSU-BIC, 2,170 m in elevation). Beside the non-transformed yearling PAP measurements (RAW), power-transformed PAP measurements (PT), an ordinal

three-category phenotype (CAT3), an ordinal two-category phenotype (CAT2) were investigated in this study. The PT ($10000 \times PAP^{-2}$) was determined via Box-Cox analysis, the CAT3 observations were defined as low risk (PAP < 41mmHg), moderate risk (41mmHg ≤ PAP ≤ 49mmHg) and high risk (PAP > 49mmHg) for HAD. The CAT2 observations were constructed by combining low and moderate risk categories of CAT3. Performance traits included birth weight (BWT; 36.21 ± 0.50 kg), weaning weight (WW; 213.88 ± 0.34 kg), post-weaning gain (PWG; 127.29 ± 0.88 kg) and yearling weight (YW; 344.85 ± 1.10 kg). Genotype data on 2,765 Angus cattle born from 1997 to 2015 in the CSU-BIC were merged and used in this study. Most individuals were genotyped using various formats of Illumina Bovine SNP50 Beadchip version 2 assays (54,609) over three year-groups (i.e. 2013, 2014 and 2015) and in two labs (i.e. Zoetis and GeneSeek), and with a subset (n=65) steers genotyped in 2013 using Illumina BovineHD BeadChip (777,962 SNP) through GeneSeek (Lincoln, NE).

The fixed effects in the models for yearling PAP phenotypes included sex, age of dam, PAP measurement date and age at PAP measurement as a covariate. For performance traits, fixed effects included sex, age of dam, age of measurements (covariate) and contemporary groups. Significance of fixed effects was tested using log-likelihood ratio test from generalized linear models and maximum likelihood method. Univariate linear and threshold models were applied to estimate heritability for yearling PAP phenotypes (i.e. RAW, PT, CAT3 and CAT2), and bivariate and multivariate linear and threshold models were used to obtain genetic correlations between yearling PAP phenotypes and performance traits, and between yearling PAP in different sex categories.

Deregressed EBV (DEBV) and associated reliability of various yearling PAP phenotypes and performance traits were developed from their EBV and accuracy from multivariate animal

models and used as dependent variables in GWAS. The linear models for GWAS were executed using Bayes B and Bayes C methods. The percentage of genetic variance of specific trait explained by a genomic window (~ 1 Mb) was applied to identify significant QTL regions. The SNP effects on each trait from GWAS were obtained to construct associated weight matrix (AWM) and calculate SNP effect based genetic correlation between yearling PAP phenotypes and performance traits.

The estimated heritabilities were 0.24, 0.24, 0.25, and 0.32 for RAW, PT, CAT3 and CAT2, respectively. Sire EBV accuracies from univariate models of RAW, PT, CAT3 and CAT2 ranged from 0.03 to 0.67, 0.03 to 0.68, 0.01 to 0.65 and 0.01 to 0.58 with means of 0.31, 0.31, 0.27 and 0.21, respectively (pooled $sd = 0.13$). The absolute genetic correlations between them were above 0.91, and the rank correlations between EBV from RAW and PT, CAT3 or CAT2 were 0.92, 0.84 and 0.77, respectively. The RAW, CAT3 and CAT2 had a downward sloping genetic trend, and the PT (the inverse transformation of PAP) has an upward sloping genetic trend that consistent with the other PAP phenotypes' genetic trend. The estimated heritability of yearling PAP phenotypes of bulls were significantly different ($P < 0.05$) from that of heifers. Genetic correlations between yearling PAP phenotypes in bulls and heifers were 0.82, 0.79, 0.96 and 0.87, and EBV rank correlations were 0.94, 0.93, 0.99 and 0.96 for RAW, PT, CAT3 and CAT2, respectively. Results suggested violation of assumptions in linear modeling had limited influence on genetic evaluation results, and losses in EBV accuracy and some re-ranking of sires was observed in ordinal categorical phenotypes compared to continuous PAP scores. The non-transformed yearling PAP measurements were preferred in genetic evaluation of PAP measurements because of its similar genetic heritability with PT, higher accuracy than categorical phenotypes and easier interpretation. Ordinal categorical phenotypes can be

alternative dependent variables in studying PAP, however, they would cause some re-ranking of sires related to non-transformed PAP measruments. The PAP phenotypes in different sexes were identified genetically un-identical ($P < 0.05$). However, it is not necessary to treat yearling PAP as separate traits by sex in genetic evaluation because the EBV from all PAP and PAP of different sexes were highly correlated and would yield similar rank of animals.

The estimated genetic correlations between various yearling PAP phenotypes and BWT, WW, PWG, YW and MILK ranged from 0.22 to 0.27, 0.16 to 0.22, 0.03 to 0.16, 0.11 to 0.20, and 0.07 to 0.16 respectively. The average EBV accuracy of yearling PAP phenotypes was improved by 0.011 (*sd = 0.0026;* 7.20%) and 0.0018 (*sd=0.0021,* 1.15%) in multivariate (including PAP, BWT, WW, PWG) and bivariate models (including PAP and YW), respectively. This multivariate model was preferred in estimating EBV for yearling PAP phenotypes, since it would increase the accuracy of the EBV and the number of animals used in GWAS.

We identified 4, 12 and 9 windows (1-Mb) associated with RAW, CAT3 and CAT2, respectively. The majority of these lead-SNP (with the highest model frequency in identified window) resulted in significant ($P < 0.05$) additive effects, and only one lead-SNP had significant dominant effect. This demonstrated the polygenetic characteristics of yearling PAP phenotypes, additive effects of most of yearling PAP phenotypes associated SNP and dominant effects of limited number of yearling PAP phenotypes associated SNP. Five concordant windows located on chromosome 7, 11, 12, 15 and 20 were identified across the PAP phenotypes when considering top 2% windows of each phenotype. Gene enrichment and ontology analysis suggested these windows were related to ion binding and transportation, inflammation, innate immunity and cell proliferation mechanisms. This gave evidences of the identified QTL's association with hypoxia-induced elevated PAP in cattle.

Twenty-two windows were identified to be associated with performance traits (i.e. BWT, WW, PWG, YW, MILK), which illustrated the polygenetic characteristics of these traits. Seven of them located on chromosome 7, 14, 20 and X were pleiotropic across these performance traits. Gene enrichment and ontology of these windows clustered gene functions in categories such as adipose tissue development and innate immunity.

Only two windows, located on chromosome 7 at 93 Mb and Chromosome 20 at 4 Mb, were recognized as pleiotropic between yearling PAP phenotypes and performance traits. Low to moderate SNP-based genetic correlation were identified between yearling PAP phenotypes and performance traits, and the SNP-based genetic correlation explained 61 % variation of pedigree-based genetic correlation. Results suggested SNP effects could be used to estimate genetic correlation between traits. Genes in two pleiotropic windows and AWM have roles in intracellular transportation, cellular metabolism, inflammatory, hypoxia response and cell proliferation, which demonstrated the effects of SNP in AWM on PAP phenotypes. Our findings will improve the understandings of biological process involving health and growth in Angus cattle managed at high altitude, and also help genetic improvement in these cattle against PH and HAD.

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# CHAPTER 1

# INTRODUCTION

## 1.1 Background

There is economic relevance to high altitude disease (HAD), with an incidence of 3% to 5% typically in native cattle managed at high altitude (Holt and Callen, 2007), and about 10% to 40% of cattle when they were moved from low altitude to high altitude (Grover et al., 1963, Will et al., 1970). Therefore this disease would lead to economic loss in high altitude states such as Colorado, New Mexico, Utah and Wyoming in the United States.

High altitude disease in humans and animals is a consequence of response to hypoxia in high altitude. In response to alveolar hypoxia, the pulmonary artery constricts resulting in hypertension, right heart ventricular hypertrophy, vascular remodeling, and death from congestive heart failure (Holt and Callan, 2007). Therefore, pulmonary hypertension is one of the important characteristics for HAD, and it is typically diagnosed using measurement of pulmonary arterial pressure (PAP). The measurement of PAP has been used as indicator trait for selection against HAD, and reported to be moderately heritable in cattle (with heritability ranging from 0.25 to 0.46; Enns et al., 1992; Shirley et al., 2008; Crawford et al., 2016). Previous results were based on the PAP measures without studying the data structure and potential transformation needs, or the potential genetic differences among PAP measures at different environments (e.g. age and sex). However, violation of model assumptions may influence the analyses and results (Maas and Hox, 2004; Nimon, 2012;), and different genetic structure of PAP may be associated with different environmental situations (Holt and Callen, 2007; Zeng et al., 2015).

Based on physiological studies of HAD and PAP, animal frame, weights, and obesity could be somehow related to the PAP measurements and susceptibility of HAD (Koda et al., 2007; Neary 2013). Therefore, the PAP measurements and susceptibility of HAD may be genetically related to performance traits in cattle (e.g. birth weight (BWT), weaning weight (WW), yearling weight, etc.). Crawford et al. (2016) reported low but non-zero (0.19 to 0.24) genetic correlations between PAP and pre-weaning growth traits (i.e. BWT and WW), and moderate genetic correlations between them were also previously observed (Shirley et al., 2008). This suggested the potential improvement in genetic prediction of PAP with using correlated performance traits in multi-variate models.

With the advance in molecular genetic techniques and statistical methods, genome-wide association study (GWAS) has been successfully executed to detect associations between single-nucleotide polymorphisms (SNP) and traits. Moderate heritability of PAP measurements provided us the potential to identify its QTL. Identification of QTL could help studying hypoxia-induced pulmonary hypertension in both human and animals and improve selection of cattle against HAD. Also, the concordances observed in GWAS of PAP and performance traits can help us explore genes influencing both PAP and performance traits. However, there are few published GWAS of PAP, except for unpublished works and proceeding papers from Animal Breeding and Genetics group in Colorado State University based on non-transformed and log10-transformed PAP phenotypes.

The true genetic merit observed among unrelated animal in the absence of selection is an ideal training population data for genomic selection (Garrick et al., 2009). However, true breeding values are not available in practice, and the estimate of true breeding value (EBV) of an individual is usually calculated using the data of related animals. The EBV estimated from this

procedure is a shrinkage estimator of true breeding value and could introduce family relatedness into the GWAS. Family relatedness can reduce the power and increase the false positive rate of QTL identification in GWAS. Therefore, deregressed estimated breeding value (DEBV), with removing the parent average and un-shrinking estimates appeared to be the most appropriate response variable for GWAS of PAP.

## 1.2 Objectives

The goal of this dissertation effort is to study data structure, estimate EBV and conduct DEBV based GWAS of PAP using data from a herd of Angus cattle at CSU Beef Improvement Center (CSU-BIC; One Bar Eleven, Rouse Ranch in WY) located at elevation of 2,170 m. This herd has bred Angus cattle for high altitude adaptability for more than 50 years through phenotypic and genetic selection (from year of 2002) on PAP. The breeding program also cooperates with AI companies by progeny testing the PAP genetic merit of bulls via breeding cows of this herd with semen from outside AI sires. The goal of this doctoral study is achieved through the following objectives:

1. **Study the phenotypic and genetic characteristics of yearling PAP measurements.** This part investigated distribution of yearling PAP phenotype, tested the fixed effects and estimated the genetic parameters of yearling PAP in alternative phenotypic forms, and inspected the sex's influence on genetic parameters of yearling PAP phenotypes. The information helped explore the potential violation of modeling assumptions and its influence on genetic analysis, develop models for yearling PAP phenotypes and determine the phenotypic form of yearling PAP to be used in genetic evaluation.

2. **Investigate the genetic relationship between yearling PAP measurements and performance traits.** Genetic correlations were estimated between performance traits (i.e. BWT, WW, PWG and YW) and the various yearling PAP phenotypes (i.e. continuous and categorical scale). The genetic correlation between performance traits and yearling PAP phenotypes in different sex categories (i.e. bull, heifer and steer) were also examined. These analyses provided information needed to construct the multivariate models to be used in genetic evaluation of yearling PAP phenotypes and obtained the EBV from the most appropriate model.
3. **Conduct GWAS on various yearling PAP phenotypes using deregressed EBV.** This part identified genomic windows (1-Mb QTL) for each yearling PAP phenotype. The DEBV of various yearling PAP phenotypes were developed from the EBV and used as dependent variable in GWAS. The concordant genomic windows across alternative yearling PAP phenotypes were also studied. Candidate genes (located in identified genomic window regions) associated with yearling PAP phenotypes were also identified and studied.
4. **Conduct GWAS of performance traits using deregressed EBV.** Estimated breeding values of performance traits (i.e. BWT, WW, PWG, YW) from multivariate models, and developed DEBV for GWAS from them. The DEBV were used to conduct GWAS and locate the genomic window (1-Mb QTL) associated with each of the performance trait and identify the concordant QTL windows across performance traits.
5. **Study the SNP-based genetic relationship between yearling PAP phenotypes and performance traits.** This section identified the concordant genomic regions and genes between yearling PAP phenotypes and performance traits, and obtained the SNP effects

of yearling PAP phenotypes and performance traits from GWAS. An association weight matrix (Reverter and Fortes, 2013) was constructed based on estimated SNP effects to calculate the SNP-based genetic correlations between yearling PAP phenotypes and performance traits. These SNP-based correlations were compared with the estimated genetic correlations from traditional pedigree-based quantitative genetic methods to assess the relationship between the two types of genetic data.

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# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

In high altitude states such as Colorado, Wyoming, New Mexico, and Utah, hypoxia-induced bovine pulmonary hypertension (PH) commonly referred to as "brisket disease" or "high altitude disease (HAD)", has been observed within the cattle industry (Holt and Callen, 2007). Glover and Newsome (1915) first studied this disease in cattle as to advise Colorado and New Mexico stockman to protect their herds. Because HAD is highly economically relevant, it has been studied by agriculture scientists for a century. These studies coved multiple aspects of the disease, including physiology, pathology, genetics and now "omics" areas (i.e. Genomics, transcriptomics, proteomics and metabolomics). Due to the close physiological relationship between HAD and pulmonary arterial pressure (PAP), this measurement has been used as an indicator trait for studying HAD. This section reviewed the factors, methods and previous reports discussing HAD and PAP. The contents of these reviews included economic aspect of HAD, physiology of HAD, the relationship between PAP and HAD, factors influencing HAD or PAP, genetics and "omics" information of HAD and PAP.

## 2.2 Economics

There is a economic relevance to HAD, since it is a major cause of calf morbidity for beef cattle ranches and feedyards above 1500 m (Hecht et al., 1962; Jensen et al., 1976). Williams et al. (2012) reported HAD (or Bovine PH) attributed to an estimated $60 million loss each year in beef herds at high altitudes of the United States. High altitude disease has been attributed to a

producer losing 20% of his 600 calves between summer turnout and weaning, which equates to $78,864 of lost potential income based on a market price of $1.24/lb. live weight (November 7th 2011) and the herd average weaning weight in 2009 (529.8± 72.4lbs; Neary, 2013). Cattle native to high altitude may be more resistant to HAD than cattle that originally lived at low altitude due to natural and artificial selection (Will et al., 1975). These long-term selections would produce cattle that are adapted to high altitude environment and resistant to HAD (Qiu et al., 2012). The general incidence of HAD was 3% to 5% typically in native cattle (Holt and Callen, 2007), whereas 10% to 40% of non-native cattle developed HAD when they were moved from low altitude to high altitude (Grover et al., 1963, Will et al., 1970). It should be note that reports on HAD are limited due to the fact that beef production system in mountain terrain are extensive cow/calf operation.

## 2.3 Physiology of HAD

Alexander and Jenson (1959, 1963) reported that, hypoxia in regions of high elevation is the major cause of HAD. As altitude increased, although the proportion of oxygen in the air is the same at all altitudes, the barometric pressure falls (Peacock, 1998). The reduction in barometric pressure causes a corresponding drop in partial pressure of oxygen, which results in less oxygen reaching the lungs and pulmonary artery, sequentially leading to the hypoxia in the lungs (Peacock, 1998).

Due to the hypoxia, some areas of the lung are poorly oxygenated. This leads to vasoconstriction in the pulmonary distal vessel as to distribute the blood away from poorly oxygenated areas within the lung to the areas that better oxygenated (Neary, 2014). The closure of some pulmonary capillaries causes the increased pressure in pulmonary capillaries and the

primary pulmonary artery. If an animal stays in a hypoxic condition for longer than 3 weeks, a remodeling process begins in the pulmonary artery. Specifically, The smooth muscle growth in the pulmonary arteriole wall leads to vascular hypertrophy and thickening of the medial layers of the pulmonary arterioles (medial hypertrophy, Stenmark et al., 2006). Vascular remodeling leading to loss of peripheral pulmonary arteries contributes to increased pulmonary vascular resistance. In early stages of remodeling, a combination of events (e.g. pulmonary vasoconstriction, pulmonary remodeling and unchanged cardiac output) causes the increase in PAP and significant PH, which makes it harder for the right ventricle to pump the same amount of blood through the lung in the later stage (Holt and Callen, 2007; Neary, 2014). This leads to changes in cardiac function: right ventricular hypertrophy, followed by right ventricle dilation, and finally right congestive heart failure. In addition, Holt and Callen (2007) described the increased vascular hydrostatic pressure (intravascular hypertension) causes ventral edema in the brisket region and the loss of fluid into the extra vascular space. Therefore, HAD is usually characterized by the presence of ventral edema in the brisket region (i.e. Brisket Disease).

In order to adapt to PH, a complex physiological process happens in right ventricle (Vonk-Noordegraaf et al., 2013). Generally, PH increases right ventricle wall stress, which leads to the ischemia, mitochondrial remodeling, neurohormonal and immunological activation, and sequentially causes myocardial remodeling. Hypertrophy and matrix remodeling from the myocardial remodeling can increase right ventricle contractility to adapt to the increased PAP. There are two patterns of ventricular remodeling on the basis of morphometric and molecular characteristics: adaptive and maladaptive remodeling. Adaptive remodeling preserves systolic and diastolic function, whereas maladaptive remodeling is associated with unhealthy systolic and diastolic function. This maladaptive remodeling finally leads to the arrhythmias, right ventricular

dilatation and failure. The right heart failure is the eventual clinical characteristic of HAD, and ultimately may lead to the death of the animal.

High altitude disease in human is classified into four categories although they are all associated with elevated PAP and PH: acute mountain disease, high-altitude pulmonary edema, high-altitude cerebral edema or chronic mountain sickness (Jin et al., 2009; Luo et al., 2014). These classifications depend on the duration, stage and level of severity of the high altitude disease. In addition, an acclimatization processes is also involves in the development of HAD (Vonk-Noordegraaf et al., 2013; Luo et al., 2014).

Several physiology responses can be initiated in the human or animal during high altitude acclimatization, and the acclimatization is a comprehensive effect of various organ systems. This process includes: elevated ventilation leading to a rise in arterial oxygen saturation; pulmonary vasoconstriction, a mild diuresis and contraction of plasma volume leading to more oxygen per unit of blood; elevated blood flow and cardiac output; and greater hemoglobin mass and red blood cells (Chawla and Saxena, 2014; Julian et al., 2009). In addition, molecular responses regulate many physiological processes to defend hypoxia. For instance, the up-regulation of hypoxia inducible factor 1 (HIF-1) expression regulates the genes involved in glucose uptake, glycolysis, metabolism, pH balance, angiogenesis, erythropoiesis to help human and animal adapt hypoxia environment (Chawla and Saxena, 2014).

Although physiology mechanisms for acute mountain sickness, high-altitude pulmonary edema and high-altitude cerebral edema remain elusive, they generally occur between the initial hypoxia exposure and the onset of acclimatization (optimal acclimatization takes days to weeks, or perhaps months) and associated with elevated PAP and PH (Imray et al., 2010). The acute mountain sickness, high-altitude pulmonary edema and high-altitude cerebral edema have similar

the same pathophysiology and considered to represent different points along a single spectrum of a same disease, and high-altitude pulmonary edema and high-altitude cerebral edema are considered more serious form of acute mountain sickness (Bärtsch and Bailey, 2014). These diseases were only caused by hypoxia, and it was associated with the general mechanism described above. The HAPE contributes to the increased pressure and damages of pulmonary capillary and lung alveolar (Maggiorini et al., 2001). Under hypoxia, in order to satisfy the oxygen requirement of brain, the cerebral blood flow elevates to bring more oxygen to the brain, which may increase blood-brain barrier permeability or cerebral vascular permeability (Bärtsch and Bailey, 2014). This can be a mechanism observed in high-altitude cerebral edema.

The chronic mountain sickness is a disease that develops after spending an extended time (years) living at high altitude (> 3,000 meter), and it is an important high-altitude disease in mountain regions (León-Velarde et al., 2010). The chronic mountain sickness of humans and animal results from the loss of capacity to adapting hypoxia (developed thicken pulmonary arteries), which was related to age, disease (e.g. lung diseases and obesity), unhealthy behavior (e.g. smoking) and contamination (Penaloza and Arias-Stella, 2007; Jin et al., 2009). All these factors can incorporate the hypoxia on high altitude to cause alveolar hypoventilation (Jin et al., 2009). Beside PH, this disease was also characterized by excessive erythrocytosis with the typical symptoms of polycythemia, hypoxemia, breathlessness, palpitations, sleep disturbances, cyanosis, venous dilatation, headaches, tinnitus, and dizziness (Jiang et al., 2014). The level of erythrocytosis is used to define the CMS (Jiang et al., 2014; Villafuerte et al., 2014). The mechanism of CMS is different from AMS, HAPE or HACE, which may explain the large difference of the HAD case rate in high altitude native cattle and cattle that relocated from low elevation regions to high elevation regions.

## 2.4 Relationship between PAP and HAD

Increased PAP is a direct outcome of all types of PH and HAD. As a physiological indicator of PH, PAP measurements have been used to assist selection of cattle to reduce the incidence of HAD in recent decades in high altitude regions. Holt and Callen (2007) provided guidelines to help breeders make selection decisions using PAP phenotypes to reduce risk of HAD in high altitude beef production systems (Table 2.1). Producer reports collected in veterinary health studies suggest that, in some cases, low PAP cows should have significantly reduced the incidence of HAD within their calf crop (Neary, 2013). However, elevated PAP dose not necessary lead to the consequences of HAD (e.g. death and brisket edema). Holt (Personal communication, 2015) described that a bull of normal healthy at high altitude region (>2,300m in elevation), although his measured PAP measurement was larger than 100 mmHg. Neary (2013) also described that reports from some producers showed that the selection on low PAP has minimum influence on reducing the mortality of pre-weaned beef calves. The actual genetic relationship between PAP and HAD has not been described because of the difficulty to obtain data on the incidence of HAD. Therefore, additional studies should be conducted to understand that genetic selection on low PAP would reduce the chance of cattle for developing HAD. Genomic studies could be effective methodology to genetically tie up HAD and PAP via identifying common genes and pathways associated with both HAD and elevated PAP.

Table 2.1. Evaluation of pulmonary arterial scores[1]

| 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. |
| 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. |

[1]These figures are based on cattle tested at or above 1800 m (6000 ft) and12 months of age or greater. If the animal does not meet these criteria then adjustments must be made (Holt and Callen, 2007).

## 2.5 Measurement of PAP

### 2.5.1 Technical procedure

Pulmonary arterial pressure is a measure of blood pressure found in the primary pulmonary artery. The procedure used to measure PAP in cattle has been used for more than 30 years. This measurement can only be taken by one licensed veterinarian in one herd in order for selection to be more effective, because PAP measures can be influenced by any unprofessional action in the process. With the right equipment and facilities, a veterinarian can take PAP score for a large number of animals daily (about 200 to 300 cattle), which makes PAP a measurable and affordable trait for selection (about $20/head). Pulmonary arterial pressure is measured through a right heart catheterization procedure, which requires jugular venipuncture, catheter insertion and passing flexible catheter tubing through a large bore needle inserted into the jugular vein (Holt and Callen, 2007). The catheter is passed via the jugular vein, through the right atrium, into the right ventricle, and then into the pulmonary artery. Once the catheter is inside the pulmonary artery, the systolic, diastolic and mean blood pressures are recorded from a heart monitor, which is attached to the catheter via a transducer (Ahola et al., 2007; Holt and Callen, 2007).

### 2.5.2 Calculation of mean PAP

Although the mean PAP can be directly recorded from cardio graphic monitor, it is not directly measured. There are two phase of the arterial pressure: systole and diastole (Fucuta and Little, 2008, Homoud, 2008, PysiologyWeb, 2011). During the systole, blood is ejected from right ventricle to the pulmonary artery, and during the diastole, the heart relaxes, and blood flows from the pulmonary artery into pulmonary circulation. In the systolic phase, the blood pressure

rise during right ventricular ejection after opening of the pulmonary valve, which is followed by a general decrease in pressure while blood is being ejected from the right ventricle until closure of the pulmonary valve. The closure of the pulmonary valve causes a short increase in pressure, which is called dicrotic notch. In the diastolic phase and after the dicrotic notch, PAP decreases as the heart relaxes. The maximum and minimum pressures during cycle of ventricular contraction and relaxation are defined as the systolic and diastolic PAP, which can be directly measured and used to calculate mean PAP (Homoud, 2008, PysiologyWeb, 2011, Chemla et al., 2004). However, the mean PAP is not the simple average of systolic and diastolic PAP. The formula used to calculate the mean PAP is (Homoud, 2008, PysiologyWeb, 2011):

$$mean\ PAP = diastolic\ PAP + (\frac{1}{3} pulse\ PAP)$$
$$pulse\ PAP = systolic\ PAP - diastolic\ PAP$$

This mathematic formula was constructed based on the duration of systolic and diastolic phases of ventricles. In reality, the ventricles spend one-third (1/3) of their time in systole, and two-thirds (2/3) in diastole. Chemla (2004) reported another formula to approximate the mean PAP using the statistical relationship between mean PAP and systolic PAP. As the mean PAP was highly correlated with systolic PAP, a regression model is suitable for calculate mean PAP form systolic PAP:

$$mean\ PAP = 0.61\ systolic\ PAP + 2mmHg$$

The author suggested that mean PAP could be accurately predicted from systolic PAP. The mean PAP used in the regression analysis was defined as the area under the pressure curve divided by the pulse interval, which can also be considered an appropriate measurement.

The PAP measurements (mean PAP, systolic PAP, diastolic PAP) are associated with many other hemodynamic parameters including cardiac output (CO), pulmonary vascular resistance

(PVR), and mean pulmonary artery wedge pressure (PAWP; or Pulmonary Artery Occlusion Pressure; Chemla, 2002; Klabunde, 2010). Blood pressure is the force of blood against the walls of the arteries as the heart pumps blood throughout the body. Assuming a constant diameter for the pulmonary arteries, the high CO brings more volume of blood and higher force against the arterial wall, which causes higher pressure (Mayet and Hughes, 2003). Less elastic and thicker arterial wall provides more react force to the blood volume and cause higher pressure (Mayet and Hughes, 2003). Like a water pipe, the resistance on one end of an artery can also cause increased pressure inside the artery (Chemla, 2002). Mean PAWP serves as the resistant force in forming mean PAP. Thus, another formula to estimate mean PAP is expressed as (Klabunde, 2010, Chemla, 2002):

$$mean\ PAP = (CO \times PVR) + mean\ PAWP$$

The increase in CO, PVR and PAWP will increase mean PAP. The CO measured the amount of blood the heart pumps through the systemic and pulmonary circulation in a minute (Wingfield and Raffe, 2002). Sufficient CO is needed to sustain blood pressure and supply oxygen to the whole body, and it is influenced by heart rate and stroke volume (Wingfield and Raffe, 2002). The PAWP is an indicator for the left atrial pressure, and its increase causes more resistant for blood flow in pulmonary arteries resulting in higher PAP (Luchsinger et al., 1962). The PVR is the resistance that the blood flow must overcome to go through the pulmonary circulation. Vasoconstriction and vascular remodeling (e.g. cell proliferation) both influence PVR and therefore blood pressure (Elzouki et al., 2012). Generally, the blood flowing through the pulmonary circulation is essentially the same as the blood flowing through the systemic circulation, but the blood pressure in systemic circulation is about 10 times higher than that in pulmonary circulation because the systemic vascular resistance is 10 to 15 times higher than

pulmonary vascular resistance (Klabunde, 2010). The PVR can be estimated using the modification of equation (3) (Griffin et al., 2008; Homoud, 2008).

$$PVR = \frac{mean\ PAP - mean\ PAWP}{CO}$$

2.6 Environmental factors influencing PAP measurements

The PAP measurements can be influenced by many factors including age, elevation, breed, gender, pregnancy statue, temperature, production level, feed and other diseases (Holt and Callen 2007; Jin et al., 2011; Neary, 2014). In order to understand and use PAP measurements, these factors should always be considered.

2.6.1 Elevation

Humbert (2010) reported that important changes in oxygen saturation and PAP occurred with mild and moderate increases of elevation above 3000 m, and elevation degree was inversely related to the level of oxygen saturation and directly related to PAP. Also, PAP measurements were reported to increase 1 to 2 mmHg per 305 m (1000 ft) rise in elevation (Holt and Callen, 2007). In addition, the risk of CHF appeared to increase with rising elevation (Neary, 2014). Penaloza (2012) reported that people (across different ages) residing high altitude had higher PAP than people at sea level (Figure 2.1). Thus, when using PAP in cattle, it is important for ranchers to know the associated elevation of the PAP score.

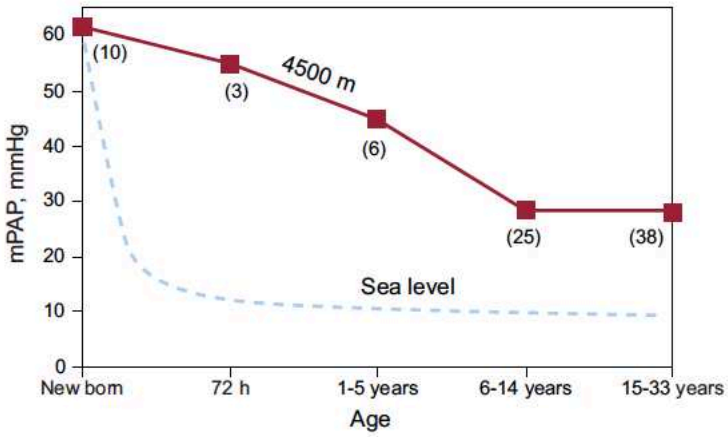


Figure 2.1 Relationship between mean pulmonary arterial pressure and age in natives with normal health who live at a high altitude, at 4540 m (solid line), compared to the data reported for sea level residents (dashed line; the numbers in parenthesis indicate the number of cases). The mean pulmonary arterial pressure decreases rapidly at sea level; in contrast, in high-altitude natives, the grade of pulmonary hypertension decreases slowly and can persist into adulthood (Penaloza, 2012).

In human, the hypoxia-induced pulmonary response would not be seen until individuals reached an elevation of 1524 m (5000 ft; Aghababian, 2010; Netzer et al., 2013). Venugopalan (2014) indicated that it is difficult to demonstrate significant clinical changes due to hypoxia in people at elevations lower than 3000 m. In order to exclude PH from other illnesses, PAP measurements performed above 1524 m should be used to identify animals that are sensitive to the hypoxic condition. Therefore, PAP measurement would be more accurate and reliable, when the data are collected at higher elevation. Thus, the reliable PAP for studying HAD should be recorded from animals that stayed at regions higher than 1524 m in elevation for at lease three weeks (Holt and Callen, 2007).

### 2.6.2 Age

Holt et al. (2007) implied that PAP measurements were likely to change greatly from younger ages to older ages. Will et al. (1975) reported that PAP rose with increasing age, and

Neary (2014) demonstrated that, even at moderate altitude, PAP significantly increased with age among pre-weaned calves. Rhodes (2005) also showed a potential non-linear relationship between incidence of hypoxia-induced PH and age as the majority of hypoxia-induced PH of cattle occurs between birth and 2 years of age. Similarly in humans, it was reported that systolic PAP increased with age regardless of altitude, which would suggest pulmonary vascular remodeling with increasing age (Lam et al., 2009). Badesch et al. (2009) reported that although the mean PAP of rest persons was not different among different age groups, the mean PAP was significant higher in older persons during slight and submaximal exercise. However, Penaloza (2012) reported higher PAP in younger man (Figure 2.1). Therefore, age should always be considered when we use individuals' PAP measurements.

### 2.6.3 Gender

Different high PAP incidences were observed in male and female cattle (Holt and Callen), and heifer calves were reported having significantly lower mean PAP than bull calves (Neary, 2014). In this discussion (Neary, 2014), it was illustrated that broiler (male) chickens had significant greater muscular hypertrophy of the pulmonary arteries than hen-chicks when they were raised at an altitude of 3000 m until 4 weeks old. Jin (2010) presented that differences existed between males and females in their response to hypoxia, and CMS and AMS appears to be more frequent in men than women. In addition, gender differences were reported in breath patterns of humans at high altitude that was related to HAD, and males were more sensitive to central sleep apneas than females (Lombardi, 2012).

It is not clearly why these gender differences exits. Holt and Callen suggested that no physiological bases were identified for different PAP between males and females, yet

management factors (e.g. feed, genetic background and husbandry) can often lead to these differences (Holt and Callen, 2007). Jin (2010) attributed the gender differences in chronic mountain sickness and acute mountain sickness incidences between men and women to the female hormones that exert a positive effect on ventilation, oxygen utilization, and oxygen metabolism. Neary (2014) also suggested that the female gender hormone estrogen could have cardio-protective action that may be related to gender differences in pulmonary hypertension. The estrogen-hormone in this example was a growth promoting implant.

### 2.6.4 Production level

In cattle, chickens and humans, excess weight gain may be risk factors for PH and right-side congestive heart failure (Neary 2014; Jesen, 2009; Peacock et al., 1989). Rapid growth and high efficiency cattle were reported to have higher mean PAP and be more susceptible to bovine PH and brisket disease (Neary, 2013). This phenomena was not only seen at high altitude, but also be reported in feedlot non-high-attitude region (e.g. Texas at elevation of 518 m; Neary, 2014). Shirley (2008) reported a positive but unfavorable genetic relationship between weaning PAP and pre-weaning growth traits (i.e. birth weight and weaning weight). Holt (2013) presented that fast growth and muscling may affect pulmonary function and give rise to PH. In humans, body mass was positively related to systolic PAP, which may attribute to the increased cardiac output and decreased left ventricular function in obese persons at high altitude (McQuillan et al., 2001). Researchers indicated that animals at high production levels require more oxygen than animals at low production levels, which puts more pressure on the cardio-pulmonary system of high production animals and increase theirs susceptibility to PH, especially at high altitude (Veit and Farrell, 1978; Neary, 2013).

Smith (2011) also implied that fore-stomach engagement and recumbency can cause intra-abdominal pressure, hypoventilation and alveolar hypoxia. Ge et al. (2005) reported that genes might contribute to the susceptibility of obese individuals to acute mountain sickness. The obesity hormone, Leptin, was associated with acute mountain sickness and chronic mountain sickness, because it is reported over expressed in individuals in hypoxia condition and associated with higher levels of *HIF-1α* (Koda et al., 2007). The Leptin were also reported to function on many traits in beef including feed intake and fact content (Houseknecht et al., 1998), which tied up the relationship between production level and HAD.

2.6.5 Other factors

Besides the factors stated above, many other factors could influence PAP measurements including temperature, feed, and concurrent illness. Holt and Callen (2007) reported that cold environment would increase PAP measurements and cause PH in cattle. Kashimura (1993) illustrated that cold exposure would increase the incidence of high altitude sickness in rats, such as high altitude pulmonary edema. Ingestion of toxin contained in feeds (e.g. swainsonine in locoweed) and ionophores feed would increase the incidence and severity of HAD in calves at high altitude (Holt and Callen, 2007). Besides HAD, other infectious and noninfectious bovine respiratory diseases and lung disease can also lead to alveolar hypoxia, increased PAP and cause some similar clinical signs with HAD. In human, the lung disease, heart disease and blood clots also can also cause PH.

## 2.7. Inheritance of PAP and HAD

### 2.7.1 Inheritance of HAD

Holt and Callen (2007) reported that there are variations in incidence and susceptibility between individual animals, breeds and other species of cattle. Humans native to three high altitude geographic regions have been reported to be better adapted to a high altitude environment than other nationalities (e.g. Han). These people were from the Qinghai-Tibetan Plateau, the Andean Altiplano, and the Semien Plateau of Ethiopia (Bigham et al., 2013), and these geographic regions are at altitude of 4,500 m, 3,750 m and 2,000 m, respectively. In human and animals, some individuals or families appeared susceptible to HAD while others appeared resistant (Maclnnis et al., 2011). This indicated that the genetic make up might play a role in different adaptability between different populations, animal breeds, families or individuals.

Various "omics" studies of high altitude acclimatization/adaptation, acute mountain sickness, high altitude pulmonary edema, high altitude cerebral edema and chronic mountain sickness revealed many genes differentially expressed with different health condition people. Two genes identified to be strongly associated with the evolutionary adaption to high altitude were egl nine homolog 1 (EGLN1) and endothelial PAS domain protein 1 (EPAS1). In the study of Simonson et al. (2010), they reported that EGLN1 and Peroxisome proliferator-activated receptor alpha (PPAR-α) were associated with the hypoxia response factor (HIF), and the some variants in this gene were identified in high altitude adapted individuals, which can be used to study the high altitude adaption pathway in humans. The EPAS1 gene, also known as hypoxia-inducible factor-2alpha (HIF-2α) encoding gene, the expression of which is involved in the body's adaptation to hypoxia and high altitude. Several mutations in this gene were identified in Tibetan population adapted to living at high altitude, and associated the high altitude acclimation, HAPE and CMS

(Scortegagna et al., 2003; Buroker et al., 2012; Ge et al., 2012; Xiang et al., 2013; Yang et al., 2013).

Besides these two genes, many other genes have been identified associated with HAD. The mu-type opioid receptor 1-encoding gene (OPRM1) was determined to be important in cardiopulmonary adaptation to high-altitude environments, and it encodes mu opioid receptors (e.g. MOR) that were implicated in deceasing respiration (Jin et al., 2012). Higher expression of peroxisome proliferator-activated receptor gamma (PPAR-γ) was identified in Tibetan populations, so it was recognized to be associated with the high altitude adaptation (Simonson et al., 2010). The increased PPAR-γ level was associated with leptin level in diet, which induced obese rats and might imply the relationship between production level and HAD (Törüner et al., 2004). Also, it is reported that the Angiotensin-converting enzyme encoding gene (ACE) is related to acute mountain sickness, high altitude pulmonary edema and chronic mountain sickness (Buroker et al., 2010; Luo et al., 2012ab). The gene encodes angiotensin-converting enzyme, and its activation results in narrowed blood vessels, increased blood pressure through converting angiotensin I to angiotensin II and cleaving bradykinin. Angiotensin II is the protein causing blood vessels to constrict, and the coding genes of itself (AGT) and one of its receptor (AGTR1) were also identified relating to high altitude pulmonary edema and acute mountain sickness, respectively (Buroker et al., 2010; Luo et al., 2012; Srivastava et al., 2012). The ACE, AGT, and AGTR1 are parts of renin-angiotension system that regulates blood pressure, and the balance of fluids and salt in the body through cleaving proteins.

2.7.2 Inheritance of PAP

As a widely used indicator trait for HAD, the heritability and repeatability of PAP have been estimated in many studies to reveal the genetics aspect of HAD. Heritability is the proportion of phenotypic variation that is explained by additive genetic variation. Table 1 summarizes the heritability of PAP reported in historical literatures. Pulmonary arterial pressure (PAP) has been shown to be moderately to highly heritable and repeatable in cattle (Schimmel, 1981; Enns et al., 1992; Shirley et al., 2008; Crawford et al., 2016). The heritability and repeatability of PAP were first estimated in a dissertation work of Schimmel (1981). The PAP values in this study were collected from weaning calves and mature cow of Hereford, Angus and Red Angus that were raised at the San Juan Basin Research Center, Hesperus, Colorado (elevation at 2,316m). He reported heritabilities of PAP as 0.77 ± 0.21, 0.60 ± 0.24, 0.40 ± 0.13 and 0.13 ± 0.23 for bull, heifer, calves and cows. Enns (1992) reported a heritability estimate of 0.46 ± 0.16 of PAP measured at both weaning and yearling, which were from Angus cattle from western Colorado. The heritability of PAP at 0.34 ± 0.05 and 0.25 ± 0.03 reported by Shirley et al. (2008) and Crawford et al. (2016) using records from Angus cattle in Colorado.

Table 2.2. Estimated heritability and repeatability for pulmonary arterial pressure (PAP) in previous literatures

| Author | Heritability | Repeatability | Age of cattle |
|---|---|---|---|
| Schimmel (1981) | 0.13 to 0.23 | 0.25 to 0.26 | Mature Cow |
| Schimmel (1983) | 0.40 | - | Weaning |
| Enns et al. (1992) | 0.46 | - | 166d-662d |
| Shirley et al. (2008) | 0.34 | - | Weaning |
| Crawford et al. (2016) | 0.25 to 0.26 | - | Yearling |

In addition, estimated heritability for PAP of different sexes were reported differently. Cockrum et al (2014) showed the estimated heritability of PAP measured in yearling Angus cattle was 0.21 ± 0.04, 0.37 ± 0.08, 0.19 ± 0.14 and 0.23 ± 0.03 for bulls, heifers, steers and compiled data. The reports from Cockrum (2014) were similar to results from Schimmel (1981), in that the heritability of PAP of bulls was higher than that of heifers. The genetic correlation between yearling PAP of bulls and heifers (0.67 ± 0.15) was not high. These estimates suggested that PAP measurements of heifers and bulls were potentially different traits, and implied a genetic difference between bulls and heifers in response to high altitude environment. However, we must consider these results as potentially confound with growth management. All of the estimates were based on the data from John E. Rouse Ranch of Colorado State University Beef Improvement Center (CSU-BIC) in the study of Cockrum (2014). In this production system, bulls were developed within a grain-supplemented performance test, whereas heifers were grazed. Therefore, there may be a genetic by environmental interaction among two source of information: sex and diet environment.

The heritability of PAP measured at different ages tends to be different. Zeng et al. (2015) reported heritability of weaning and yearling PAP as 0.56 and 0.30 using the same dataset used in this presented study, respectively. Schimmel (1981) showed difference between heritabilities of calves and cows. Although the estimated heritability varied in studies, all of them showed a moderate to high heritability for PAP measurements (0.23 to 0.77). The estimated repeatability were limited, the reason for which may be that the PAP score is usually measured one time in cattle's life (i.e. yearling), as it's a difficult to measure trait requiring veterinary expertise. However, we can expect a moderate repeatability of PAP based on the report Schimmel (1981) who showed PAP repeatability of cows ranging from 0.16 to 0.25.

Genomic and transcriptomic studies were also conducted on PAP measurements from Angus cattle, whose results demonstrated potential inheritance influence of PAP measurements. Newman et al. (2011) provided the first molecular interrogation on Bovine PH based on case-control (designed using mean PAP score) GWAS and gene expression study (using peripheral blood mononuclear cells) of Angus cattle. The authors identified 15 up and down expressed genes associated with cattle group with high-altitude pulmonary hypertension (whose PAP measurements were range from 72 mmHg and 116 mmHg), and 10 disease processes were reported to be associated with high-altitude pulmonary hypertension. These disease processes include respiratory disease, inflammatory response, connective tissue disorders, skeletal and muscular disorders, immunological disease, genetic disorder, hematological disease, cardiovascular disease and metabolic disease. In addition, Newman et al. (2015) reported that two cis variants in EPAS1 were highly associated with HAPH in Angus cattle at high altitude (5,200-7,850 ft) through studying two cattle groups: HAPH group (whose PAP scores were larger than 50 mmHg) and unaffected group (whose PAP scores were smaller than 38mmHg).

## 2.8 Genetic selections and PAP

Because of genetic influence on HAD, selection can be used to reduce the incidence of HAD in cattle at high elevation. Expected progeny difference (EPD) is used as a tool to aid selection of potential breeding stock for a specific trait within a breed. The EPD provides estimates of the breeding value of an animal as a parent, and it is expressed as the expect difference between the mean performance of the specific individual's progeny and the mean performance of all progeny in a population. Specifically, differences in EPD between two individuals of the same breed

predict differences in performance between their future offspring when each is mated to animals of the same average genetic merit.

A genetic evaluation requires estimation of genetic parameters, and the reliability of these parameters are influenced by the heritability, the size and the quality of data (Falconer 1989). Koots (1996) illustrated that variation of estimated genetic parameters become constant after the sample size for analysis reaches 500, and the lower the heritability of a trait, the larger sample size are needed to obtain reliable estimates. Generally, the heritability for health traits are low (< 0.2), so over one thousand animal' records are needed to achieve acceptable statistical power for the heritability estimated for healthy traits (Klein, 1974). However, consistent recording system of cattle health traits are limited in the United States, which impedes the implementation of genetic evaluation of health traits in cattle industry (Gaddis, 2014). These facts can also apply to HAD. Fortunately, given the physiology relation with between PAP and HAD, the PAP-EPD can be estimated and used in selection against HAD. The individuals with lower EPD are expected to produce offspring with lower PAP who will be more tolerant to the high altitude and have less risk of HAD (Holt and Callen, 2007).

The EPD for PAP were first estimated with data from Angus cattle at Tybar Ranch, Carbondale, CO and used for selection of resistance to HAD in 1992 (Enns, 2011). Since then, the EPD for PAP has been used in Angus cattle breeding in Colorado. Also, the PAP EPD has been used in the selection program in John E. Rouse Ranch of Colorado State University known as the Beef Improvement Center (CSU-BIC) since 2002. Figure 2 presents the genetic trend of PAP EPD from both Tybar Ranch and CSU-BIC. The downward (favorable) genetic trend has been observed in both of the breeding programs.

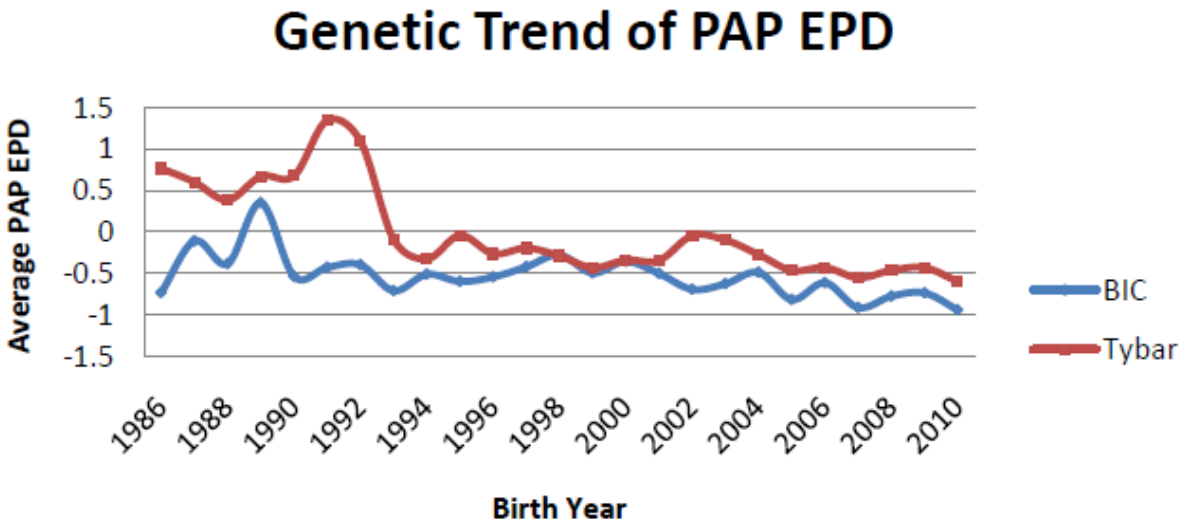


Figure 2.2. Genetic trend for pulmonary artery pressure (PAP) in Angus cattle at the Tybar Ranch (Tybar) and the CSU John E. Rouse Beef Improvement Center (BIC) since selection with EPD began in 1992 in Tybar) and 2002 in BIC (Enns et al., 2011).

## 2.9 Model for genetic evaluation

The condition of the data and the fitness of the model determine the quality of a genetic evaluation (Werf, 2002). Mixed models using the BLUP methodology, which developed by Henderson (1949), have been widely applied in genetic evaluation of livestock animals (Mrode, 2014). This methodology allows estimating fixed effects and breeding values (random effects) simultaneously and potentially delivering the most accurate and unbiased predictions (Werf, 2002). The livestock industries have applied several types of mixed models in genetic evaluation, including animal model, sire model, reduced animal model, random regression model, maternal trait model, single trait model, multivariate model, threshold model, etc. as explained by Mrode (2014).

### 2.9.1 Multivariate models

The single trait model uses information from one trait, while the multivariate model uses information from more than one traits and the genetic and environmental correlation among traits. The multivariate model allows evaluating animals for two or more traits simultaneously. Multivariate models have several advantages. First, the use of multivariate model would increase the accuracy of genetic evaluation of a trait. The larger the absolute difference between the genetic and residual correlations between the traits, the greater the gain in accuracy of evaluations (Schaeffer, 1984; Thompson and Meyer, 1986). The traits with lower heritabilities tend to gain more in accuracy from use of multivariate models, although all traits can benefit to some degree from multivariate analysis. The increases in accuracy from these multivariate models come from additional information from correlated traits and better connection in the data due to residual covariance between traits (Thompson and Meyer, 1986). Second, multivariate models allow the prediction of breeding value of correlated traits. Third, the multivariate analysis can account for the culling selection bias for the traits that are measured after sequential rounds of selection. For this kind of traits, only the better individuals are measured. For instance, yearling weights are only measured on individuals who passed culling based on weaning weights.

There are also a couple of disadvantages associated with multivariate model. First, multivariate analysis requires high computing, time, memory and disk storage. Second, multivariate analysis relies on accurate estimates of genetic and residual correlations among traits. However, the improvement of computational technology and data recording system diminished the influences of the challenges associated with multivariate analysis.

2.9.2 Threshold models

Wright (1934a,b) first introduced the threshold model to the discipline of animal quantitative genetics. Threshold models are preferred to analyze categorical traits in genetic evaluation (e.g. calving ease, heifer pregnancy, stayability and healthy traits), although they are more complex and higher computing cost than linear models (Gianola and Hammond, 2012). Linear models can also be applied to categorical traits, but they have limitations on this situation. The widely used best linear unbiased prediction (BLUP) linear models assumed the normally distributed additive genetic effects and residual effects, and the homogenous variances (Henderson, 1975). These assumptions were violated and some of the properties of BLUP do not hold when a linear model applied to a categorical trait (Fernando et al., 1983). Categorical traits are not normally distributed, and Gianola (1982) reported that the variance of categorical records was heterogeneous when there are fixed effects. This occurs because the variance of categorical traits is a function of its expectation, and the expectation is related to the fixed effects. A threshold model assumes an underlying normally distributed liability, and can overcome the limitations. A threshold model scales categorical responses to conform to intervals of normal distribution, and applies a linear model to the scale data (i.e. liability; Kendall and Stuart, 1961; Snell, 1964; Gianola and Norton, 1981, Gianola and Foulley, 1983).

Meijering and Gianola (1985) demonstrated that threshold models performed better than linear models in estimating breeding values for categorical traits with fixed effects. Ramirez-Valverde et al. (2001) reported that the best option to predict the genetic effect of calving difficulty might be threshold-linear animal model with calving ease and birth weight. However, similar performance of threshold and linear models, and better performance of linear models than threshold models were both appeared in literatures (Weller et al., 1988; Renand et al., 1990;

Matos et al., 1997; Hagger and Hofer, 1989). Meijering and Gianola (1985) also implied that threshold and linear models performed similarly when there are no fixed effects.

One disadvantage associated with a threshold model is its computing cost. Misztal (1989) indicated that the computing cost for a threshold model is three to five folds higher than a linear model. Another limitation of a threshold model comes from the extreme category problem, and the analysis would not converge in this situation. This problem comes from the no-data variation within a fixed effect level and the extreme small category size. Modification of data can be used to deal with this problem.

### 2.9.3 Other types of models

All animals were evaluated in an animal model, while only sires' effects were estimated in the sire model. In a reduced animal model, the equations for estimating breeding values are only built for the individuals having offspring records, and the breeding value of progenies in the dataset are calculated by back-solving from the predicted breeding values of parents (Quaas and Pollak, 1980). Both of the breeding values for individuals and the maternal genetic effect (the additive genetic ability of the dam to provide a suitable environment, e.g. milk) are evaluated simultaneously in a maternal trait model (Quaas and Pollak, 1980). The random regression models are dealing with longitudinal data such as milk production and cattle weights, which can account for the curve of lactation and growth.

### 2.9.4 Model development

No matter where data is derived (i.e. simulated data or field data) and what is the type of the data (i.e. experimental data or observed data), we need to develop statistical models to analyze them and mining meaning of them. Jakeman et al. (2006) present ten iterative steps in develop models (Figure 3). Generally, these steps can be summarized in four steps: purpose specification, exploratory data analysis, model selection and model validation.

The purpose of a study or an analysis is always important in model development. Based on the purpose of a study, Shmueli (2010) summarized the purpose of model development into three general categories: explanatory modeling, descriptive modeling and predictive modeling. The explanatory modeling is used to test hypotheses and causal relationships; descriptive modeling summarizes and mines the relationship between variables; predictive modeling focus on producing accurate predictions from data. The study and modeling purpose guide and influence the study design, data collection, dataset building, modeling method selection (Jakeman et al., 2006; Shmueli, 2010). Well definition of purpose would prevent missing important information, and wasting time, labor and money for unnecessary information. For instance, in animal breeding and genetics, the collection of performances data should match the specific breeding objective.

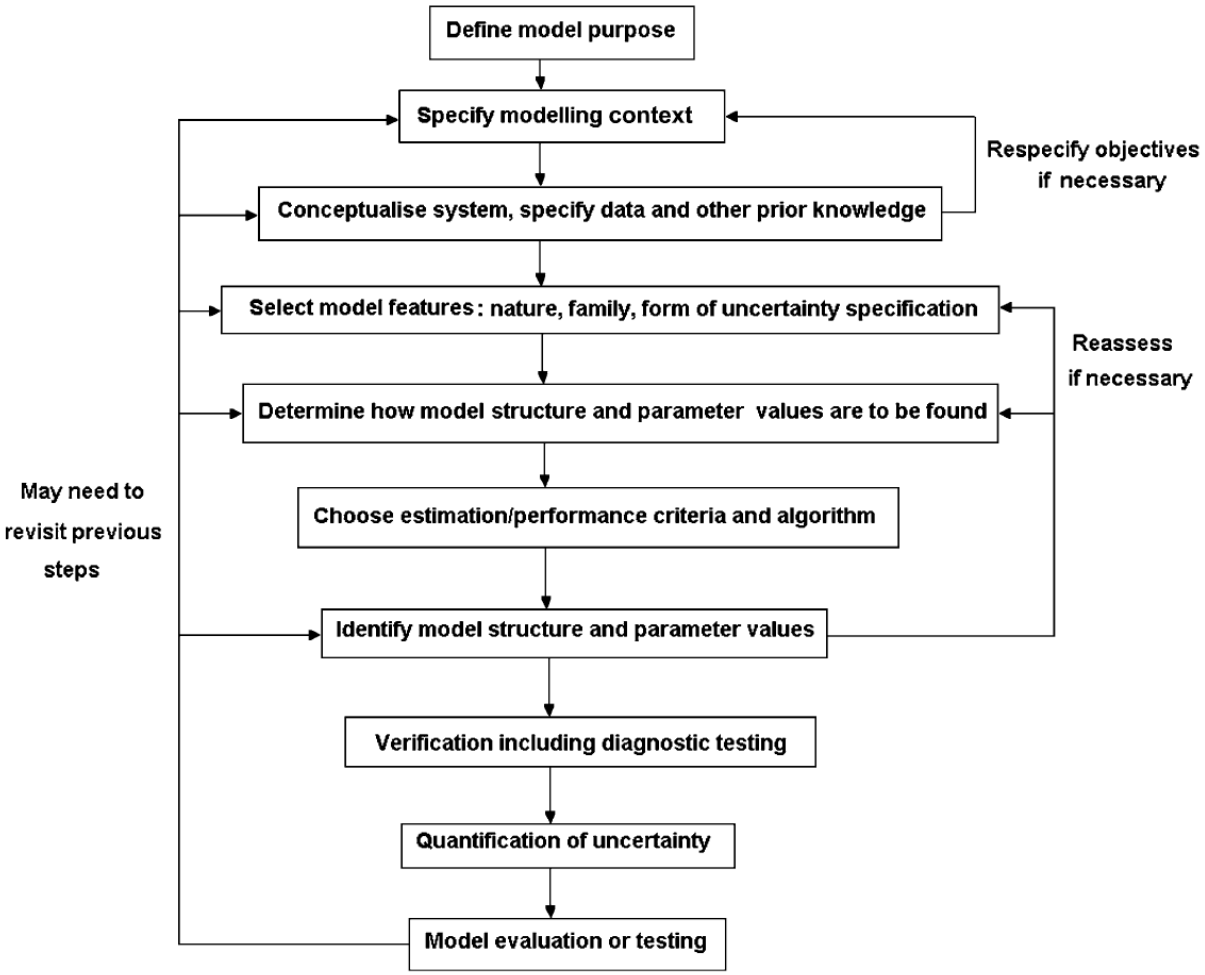


Figure 2.3. General iterative steps in model building, but these steps are not always clearly separable. These steps can be used by both modeler and end-user (Jakeman et al. 2006).

Exploratory data analysis is the initial step for all kinds of modeling (Shmueli, 2010). Exploratory data analysis has effective been used to examine the structure of data, check assumptions, detect mistake in the data and identify outliers, preliminarily assess the relationship between variable and helps select a preliminary model (Seltman, 2012). The exploratory data analysis summarizes data numerically (e.g. first and second moments of data, and correlation between variables) and graphically (e.g. Scatter plot, histogram and box-plot). Different statistical methods have different assumptions on residual distribution and variance structure, so

analyses of data variance and distribution are important components to assure the efficient modeling. Given that normally distributed residuals and homogeneous variance are the assumptions for widely used linear regression, the homoscedasticity and normality of data are usually tested in the first step of modeling (Seltman, 2012; Shmueli, 2010). Many numerical and graphical methods can determine if a variable is normally distributed or needs transformation. These methods include skew test (e.g. Kolmorogov-Smirnov or Shapiro-Wilk's W test), histogram and Q-Q plot.

If assumptions in a statistical model are violated, more complex model (e.g. threshold model) or data transformation may be needed. Many transformations were traditionally used in scientific data analyses like logarithmic transformations, square root transformation and invers transformation. All these transformations are members of power transformation (Box Cox transformation) (Box and Cox, 1964). The form of Box-Cox transformation were illustrated as follows:

$$y^{\lambda} = \begin{cases} \dfrac{y^{\lambda}-1}{\lambda} & \text{if } \lambda \neq 0 \\ \ln(y) & \text{if } \lambda = 0 \end{cases}$$

The Box-Cox analysis estimates the transformation coefficient λ, which indicates the best transformation to make a random variable nearly normally distributed. For example, λ = 1 means no transformation needed, λ = -1 means reciprocal transformation and λ = 0 indicates log transformation. The Box-Cox method has been implemented in SAS, R, SPASS and many other statistical softwares. Although the data transformation may benefit an analysis, it should be used with care because it may bring issues in interpreting results and alter the fundamental nature of the data (Osborne, 2002).

Model selection is usually recognized as variable selection, but it also includes the selection of modeling methods. The selection of the modeling methods is based on the purpose of study and modeling and the exploratory data analysis. For example, multi-trait model should be used to study the relationship between traits, threshold model was preferred to analyze categorical traits (Meijering and Gianola, 1985; Ramirez-Valverde et al. 2001), and generalized linear model are suitable to deal with data that has classic distribution other than normal distribution (Wolfinger et al., 1993). The traditional statistical algorithms (e.g. least square and maximum likelihood) would be preferred in explanatory modeling to study the relationship between variables, while some new algorithms (e.g. machine leaning and data mining methods) appeared preferable in predictive modeling because they can capture more complex associations between variables.

Besides selecting variables to match the modeling objective, there are many criteria that can be used to statistically test and select the variables. Commonly used criteria includes F test (or Wald F test), log likelihood ratio test, Akaike information criterion, Bayesian information criterion and Mallows's Cp (Kutner, et al., 2004). However, all of them are nested model (using the same explanatory variables) selection criteria, and they are not suitable to test select model from non-nested models (e.g. having different fixed effects, different variance structures or different form of variables; Smith, 2015). In addition, the likelihood value is not comparable between models with different fixed effects when using REML, because the likelihood value depends on the fixed effects in this situation (Zuur et al., 2009). Therefore, we cannot apply them to test fixed effects when the likelihood values are from REML analysis. However, we can test random effects using the likelihood value from REML because they are comparable with changing random effects in REML. The likelihood value (from maximum likelihood) should be used to test fixed effects (Oehlert, 2012). In the non-nested model, researchers suggested that the

Cox test (Cox, 1961) could be used to test non-nested model hypothesis in some situations (e.g. different fixed effects). When using a predictive model, one of the most important criteria we considered is predictive performance, so some model determination criteria could be another option to compare non-nested models, e.g. adjusted $R^2$ and prediction accuracy (Wilson et al., 2012; Kizilkaya et al., 2014).

The prediction accuracy is one of the most important tools to validate a predictive model. Estimating genetic parameters and breeding values are important aspects of quantitative genetics. Therefore, comparison of the resulting estimations (e.g. genetic parameters, EBV and accuracy) from different models can be used to select or validate a model (Wilson et al., 2012; Kizilkaya et al., 2014). Prediction accuracy can be used to determine if a transformation or a more complex model is necessary for a dataset. Tanner and Wells (2001) and Maas and Hox (2004) reported that the violation of normality in mixed model analysis would not influence the estimation of breeding value and parameters, but it would introduce bias to statistical tests. Therefore, the violation of assumption would not be a problem in estimate breeding value and variance components, but a robust method should be preferred as a diagnostic tool.

### 2.9.5 Models for genetic evaluation of PAP

Because of the influences of environmental factors, the fixed effects involved in the model for PAP included age of PAP measurements, sex, age of dam and management contemporary group (e.g. herd, year of birth, season of birth, etc.; Enns et al., 1993; Shirley et al., 2008). Univaraite animal models were previously used to develop PAP EPD, but a multivariate model was preferred in genetic evaluation. Although PAP can be measured on large number of cattle by a veterinary, PAP data size was still relatively small (i.e. much smaller than growth traits).

Therefore, genetic evaluation on PAP could benefit from multivariate models with growth traits. Generally, growth traits are moderate to high heritable and have the largest data size in beef cattle industry, which can be potentially used as correlated traits in models to improve the accuracy of PAP EPD. The degree of the benefit that the PAP can obtain from a multi-trait model depends on the heritability of correlated traits, the genetic and residual correlation between PAP and correlated traits, and the sample size of correlated traits.

As stated above, the BLUP method that was widely used in quantitative genetics assumed the normally distributed response variable conditionally on fixed effects. However, the distribution of PAP measurements violates the assumption with a heavy right side tail (Cockrum et al., 2014). This violation might limit the properties of BLUP, and influence some outcomes from BLUP. In this situation, some researchers use the raw PAP measurements in analyses and ignore the potential influences (Shirley et al., 2008; and Shimmel, 1981), but some researcher transformed the PAP score in analyses (e.g. log transformation) to reduce the potential problems (Cockrum et al., 2014). When use PAP as an indicator for susceptibility of HAD, we worry about the extreme large PAP values that indicate the high risk for HAD. Therefore, some researchers used the raw PAP scores to divided animals into different categories (i.e. resistance and susceptibility to HAD), and then conducted studies and analyses using the categorical data (Newman et al, 2012; 2015). Each of the studoes led to valuable results, but no evidence demonstrated which way to utilize PAP scores was preferred in genetic evaluation.

An important problem associated with the categorical "transformation" is how to determine the PAP thresholds to classify cattle. In order to assist breeders make breeding decisions against HAD, Holt and Callan (2007) presented a guideline to classify cattle based on PAP measurements (Table 1). It indicated that: cattle having PAP less than 41 mmHg should be

considered low risk to HAD and good breeding stock at high altitude region; cattle with PAP larger than 49 mmHg are recognized having pulmonary hypertension and high risk to HAD, and cattle having PAP between 41 mmHg and 49 mmHg are considered moderate risk to HAD, and they should be used with caution. This guideline is based on the test of cattle that were 12 months of age or older and lived at about 1829 m in elevation. It can be an accurate reference to deal with cattle that are 9 to 16 month old and raised at regions from 1524 m to 2195 m in elevation (Holt, 2016; personal communication).

Because elevation and age are two important factors influencing PAP measurements (Neary, 2014), some limitations are associated with this guideline when data is collected outside the age or elevation range. Holt and Callan (2007) suggested that PAP measurements from cattle younger than 9 month of age are only reliable in identifying cattle that are developing pulmonary hypertension (PAP > 49 mmHg) and high-risk for HAD, so the lower threshold (PAP <41 mmHg) from this guideline is less accurate in predicting low-risk cattle. For the elevation factor, PAP measurements would increase 1 to 2 mmHg per 305 m rise in elevation (Holt and Callan, 2007), so the lower threshold can be increased to 44 mmHg to determine low-risk cattle for HAD when the cattle resident above 2195 m. In addition, since the hypoxia-induced pulmonary response would not be seen until individuals reached on elevation of 1524 m (Holt and Callan, 2007; Aghababian, 2010; Netzer et al., 2013), it is not possible to determine the low-risk cattle for HAD at low elevation (< 1500 m) using PAP measurements. However, the higher threshold in the guideline (PAP > 49 mmHg) is always reliable to identify high-risk cattle (at any age or elevation), and we can even relax it to a lower level when cattle (older than 9 month) live at regions lower than 1500 m. In summary, when PAP is measured in cattle younger than 9 mouth of age, we can only use PAP measurements to identify high-risk cattle for HAD (PAP >

49mmHg). Several altitude levels can be used to advise procedures (Table 2). Therefore, 49 mmHg PAP score can be a general recommendation to identify high-risk cattle to HAD at any elevation, and 41 mmHg can generally serve as a conservative criterion to determined low-risk cattle to HAD at high elevation (> 1524 m). One advantage that may come from categorical variable is that the phenotype is less sensitive to changing environmental factors (e.g. ages and elevation; Table 2.2), and it allow capturing much more complicated relationship (Pasta, 2009).

Table 2.3. Guidelines for using pulmonary arterial pressure to evaluate cattle's (older than 9 month) susceptibility for high altitude disease (Holt , 2016; *personal communication*)

| Elevation | Low risk | Moderate risk | High risk |
|---|---|---|---|
| < 1524 m | Not applicable | Not applicable | > 45 mmHg |
| 1524 - 2195 m | < 41 mmHg | 41 to 49 mmHg | > 49 mmHg |
| 2195 - 2743 m | < 45 mmHg | 45 to 49 mmHg | > 49 mmHg |
| > 2743 m | | Not available for study | |

2.10 Genetic relationship between PAP and growth traits

In order to develop multivariate animal model for PAP measurements, we need consider genetic correlation between PAP and growth traits. Schemmel (1981) reported genetic correlations between weaning PAP (from Hereford, Angus and Red Angus) and BWT, WW as -0.43, 0.19, respectively. Shirley et al. (2008) reported the genetic correlation between PAP weaning PAP and BWT, maternal BWT, WW and maternal WW as 0.49, 0.01, 0.51 and -0.05, respectively. Absolute genetic correlations between original PAP measurements and performance traits were ranged from 0.10 to 0.24 in Crawford et al. (2016), which were remarkably lower than the estimated from Shirley et al. (2008). The reported relationship between PAP and growth traits was mainly positive, which is in accordance with positive phenotypic relationship between them (Neary, 2014). Also, these results indicated a low to

moderate genetic relationship between PAP measurements and growth traits, which implied that it might be beneficial to conduct genetic evaluation on PAP using multivariate model.

## 2.11 Genomic wide association study

Genomics is a discipline in genetics using genotyping, DNA sequencing and bioinformatics technologies to study the functional units of genome, and reveal the association between genome segments and phenotype. The goal of genomic analyses is to detect phenotype-associated genes using data from microarray and genotyping technology. Because of these advanced molecular genetics techniques, high-density marker maps and tools are available, and large number of animals can be genotyped with a reasonable investment. This fact allows genome wide association study (GWAS), which utilizes high-density single-nucleotide polymorphisms (SNP) platforms. The GWAS is an approach to revel common genetic variants in different individuals to assess if any variant is associated with a trait. Actually, GWAS has been widely used in identifying significant SNP, biological pathways and networks underlying complex traits in human disease and associated treatment methods. It highly improved the understanding of different kind of cancer, diabetes, tuberculosis and high altitude disease (Vasseur and Quintana-Murci, 2013). In the beef industry, GWAS helped developing genomic-enhanced estimate breeding value (GE-EBV) for higher accuracy genomic selection (Northcutt, 2010). Therefore it is beneficial to conduct GWAS on PAP and use GEBV or marker assisted selection to conduct selection of cattle at both low and high altitude for resistant to HAD. However, there are few published GWAS studies on PAP or HAD on cattle, except for the work from Newman et al. (2011) and works from Colorado State University.

### 2.11.1 Response variable in GWAS

To guarantee the accuracy of GEBV prediction, the ideal data for training would be true genetic merit data observed on unrelated animals in the absence of selection (Garrick et al., 2009). However, the true breeding values are not available in practice. Alternative sources of information were used in GWAS and genomic prediction, including single or repeated measures of individual phenotypic performance, information on progeny, estimated breeding value (EBV) from genetic evaluations, or a pooled mixture of more than one of these information sources (Garrick et al., 2009).

Although phenotypes and EBV were commonly used for training, they have limitations that would increase the false positive rate in GWAS (Ekine et al., 2014). In the livestock industry, the data normally come from individuals who are related to each other, which would introduce family relatedness into the association study. The EBV is the estimated measure of the true additive genetic merit, and it is estimated from its own performance and the performance of related individuals, thus familial information influences the EBV. Because the goal of GWAS is to reveal the major gene and polygenic effects (Mendelian sampling term), involving familial information in GWAS can affect both power and false positive rate of the study (Ekine et al., 2014). The familial information can dilute the effect of some important SNP, while lead to the false positive relationship between some genomic regions and traits. In addition, the variance of the EBV is lower than the variance of true breeding value, and EBV is a shrinkage estimator of true breeding value (shrink towards the mean; Garrick et al., 2009). The degree of shrinkage is different on varied accuracy of individuals, which leads to the heterogeneous variance across individuals.

In order to avoid these problems associated with EBV, Garrick (2009) developed a "deregressed" EBV that adjusts for the heterogeneous variance across individuals and parent average effects (remove effects from other relatives). Because of these properties, this DEBV was widely used as dependent variable in GWAS and to estimate SNP/marker effects. Therefore, a deregressed estimated breeding value (DEBV) may be the best response variable used in future GWAS on PAP.

### 2.11.2 Methods used in GWAS

Even though published GWAS of PAP or HAD on cattle are forthcoming, the statistical methods used in GWAS for different traits are generally the same. In order to improve the accuracy of GWAS, many statistical methods have been applied during the past 20 years. Actually, these methods are different kinds of model selection methods. The most widely used methods include ridge regression BLUP (RR-BLUP), BayesA, BayesB, BayeCπ, Bayesian LASSO, GBLUP, machine learning etc. Hayes and Goddard (2010) concluded that the highest accuracies of GWAS were achieved when the prior distribution of SNP effects matches the true distribution. The method assuming many SNP effects of zero and a small proportion of SNPs with moderate to large effects yield higher accuracy GEBV.

The RR-BLUP, BayesA and BayesB were first introduced, compared and discussed in the paper of Meuwissen et al. (2001). The BLUP method assumed a normal distribution of SNP effects with null mean and covariance matrix $I\sigma_\alpha^2$, which suggested a very large number of QTL with small effects. In RR-BLUP, $\sigma_\alpha^2$ is identical for all loci and assumed known. The best linear prediction of SNP effects can be obtained through solving the corresponding Henderson's mixed model equations. BayesA assumed a distribution of SNP effects, which is based on a large

number of QTL with small effects and a small proportion with moderate to large effects. In BayesA, the variance of each SNP effect was assumed unequal and under an inverted chi-square distribution with scale parameter S and v degree of freedom, whereas it is assumed that the error variance was under an inverted chi-square with scale parameters 2. BayesB is a method assuming mixture distribution of zero effects and t distribution of effects for SNP, which suggest a large number of genome regions with zero effect (as a proportion of π) and a small proportion (1- π) of QTL with moderate effects. The variance distribution assumption for QTL loci and error term are the same as BayesA. Bayes A can be referred as a specific case of Bayes B, with π equals 0.

Habier et al. (2011) developed BayesC and BayesCπ methodologies. Both assumed that there is π proportion of loci have 0 effect and (1-π) proportion of loci have moderate to large effect with common variance across these loci. The π is a fixed value in BayesC while in BayesCπ, π is sampled from a beta distribution based on data. The error variance is assumed under an inverted chi-square distribution with scale parameter 2 as other Bayes methods.

Table 2.4 summarizes different Bayesian methods used in animal breeding and genetics. In most analyses, Bayes B performed better than Bayes C or Bayes Cπ in predict genomic effects (given appropriate value of π and scale parameters for SNP effect; Garrick and Fernando, 2013), since the prior assumptions of SNP effects in Bayes B match the true distribution better. However, the appropriate values of π and scale parameters are usually not available. In these cases, the Bayes Cπ can be used to estimate π, total genetic variance and scale parameters, and then these estimates can be applied in Bayes B to develop better prediction of SNP effects (Garrick and Fernando, 2013).

Table 2.4. Summary of different Bayesian methods

| Method | Bayes A | Bayes B | Bayes C | Bayes Cπ |
|---|---|---|---|---|
| Reference | Meuwissen et al. (2001) | Meuwissen et al. (2001) | Habier et al. (2011) | Habier et al. (2011) |
| Prior distribution[1] | $t-distribution$ | $\begin{cases} 0 \\ t-distribution \end{cases}$ | $\begin{cases} 0 \\ t-distribution \end{cases}$ | $\begin{cases} 0 \\ t-distribution \end{cases}$ |
| Implication | A large number of SNP of small effect, a small proportion with moderate to large effect | π proportion of SNP with zero effect, (1-π) proportion with moderate to large effect | π proportion of SNP with zero effect, (1-π) proportion with moderate to large effect | π proportion of SNP with zero effect, (1-π) proportion with moderate to large effect |
| π | NO | YES | YES | YES |
| Sample π | NO | NO | NO | YES |
| Constant variance | NO | NO | YES | YES |
| Sampler | Gibbs sampling | Metropolis-Hastings | Gibbs sampling | Gibbs sampling |

[1]Prior marginal distribution of SNP effects

Another method is Bayesian Lasso introduced by Yi and Xu (2008), which also assumed a very large proportion of SNP effects close to zero and small proportion with a moderate to large effect. In this method, the SNP effect is assumed a normal distribution and the variance of QTL is under an exponential distribution.

The GBLUP is based on the restricted maximum likelihood (REML) concept. The SNP effects and variance can be estimated from a mixed model described by Henderson (1976) based on REML with treating the SNP as random effects and including a genomic relationship matrix. Using this methods, fixed effects can be estimated too. This GWAS method can be accomplished using many software packages including SVS (Golden Helix, Inc., Bozeman, MT), R, SAS (SAS Institute, Cary NC), ASReml (Gilmour et al., 2009), etc. In R, some GWAS packages written by other researchers can be used directly.

In addition to the previous methods, Long et al. (2007) developed a machine learning method. This method can be used to classify suspect and healthy animals with high accuracy and identify disease related SNPs. Specifically, a case-control experiment is designed, then machine learning method was used to select SNPs. Besides the naïve Bayes used in Long's study, the machine learning method has many algorithms including support vector machine, decision tree, artificial neural machine, etc.

In recent years, a method named as multiple locus mixed model (MLMM) were used in GWAS studies. It is a method using a simple stepwise mixed-model regression with forward inclusion and backward elimination of genotypic markers as fixed effect covariates with a genomic relationship matrix (Segura, 2012). The variance components are re-estimated between each forward and backward step. Currently, the MLMM is available in the SVS (Golden Helix, Inc., Bozeman, MT).

2.11.3 Bayesian Inference

Thomas Bayes first developed Bayes' theorem, and it is a theorem of probability. The real expression of Bayes theorem used in most scientific disciplines is described as Bayesian inference. Bayesian inference is statistical inference in which evidence or observations are used to update or to newly infer the probability that a hypothesis may be true. It can be expressed as the following formula (Box and Tiao):

$$f(\theta \mid y) = \frac{f(y \mid \theta) f(\theta)}{f(y)} \propto f(y \mid \theta) f(\theta) \qquad (1)$$

where, $\theta$ denote the unknown parameter we want to estimate; y is the observed data. This formula indicates that the conditional probability density function (posterior distribution) of unknown given observed data ($f(\theta \mid y)$) equals the likelihood of unknown with fixed observed data or the conditional probability density function of data if the assumed value for unknown is true ($f(y \mid \theta)$) multiplied by prior probability density function of unknown ($f(\theta)$) divided by the marginal distribution of ($f(y)$). Because $f(y)$ is only independent of unknown parameters (only related to the observed data), the posterior probability density function for unknown is proportional to the product of likelihood (given observed data) and prior distribution of unknown parameters. Bayesian methods can be generalized in three steps: deciding prior distribution of unknown parameters, deriving posterior distribution, and estimating unknown parameters. In Bayesian analyses, the unknowns are estimated through making inferences from their posterior distribution (derived from equation 1).

The Bayesian thought process is different from the traditional Frequentists thought process. These differences can be divided into three categories: the expression of uncertainty, the methods and used information, and the statistical concept. The Frequentist way of inference is based on how a large number of estimates would be distributed around the true value if a large number of

samples were taken or an infinite number of repetitions of the experiment were performed, whereas Bayesians examine the probability distribution of the true value, given the data (Blosco, 2001). In other words, from the view point of Frenqutists, the true value of parameters are constant and fixed and the sample is variable, while from the view point of Bayesianist, the parameter is a random variable and the data are fixed. In the Frequentist school, we treat effects as fixed and random, but all unknowns are considered as random effects in Bayesian School. The expression of uncertainty is based on maximum likelihood or least square in Frequentist school, while the Bayesian scientists derive the uncertainty of unknowns from posterior distribution. The Bayes methods can also apply previous knowledge in estimating unknowns. In addition, there is no "bias" term in Bayesian School because Bayesianist studies the distribution of true values, and the "confidence interval" is called "credibility interval". However, according to the illustration of Blasco (2001), most of the methods (e.g. BLUP, REML and maximum likelihood), belonging to the traditional Frequentist school, can be described in Bayesian School.

The Bayesian methods have many advantages over Frequentist approaches. Bayesian combines prior information with data in estimating unknowns, which could provide higher accuracy for estimates. The inferences from small and large samples are similar in Bayesian methods, because asymptotic approximation in maximum likelihood is not necessary in Bayesian inference. In addition, Bayesian inference can avoid problems with model identification (number of unknowns is larger than the sample size) in methods of Frequentist school (e.g. OLS) by manipulating prior distributions. This is main reason for the wide usage of Bayesian methods in GWAS study, because the number of markers available for analysis greatly exceeds the number of observations (genotyped animals) in most GWAS. However, there are also some limitations associated with Bayesian analyses. Given a small data sample size, we could generate misleading

results if the chosen prior distribution is improper. Bayesian analyses are not suitable in multivariate analyses, because it is impossible to fix previous beliefs in the multivariate cases. Furthermore, the posterior of unknowns are usually not the well-known distributions (e.g. normal, passion, gamma, etc.), so we cannot directly make inference from the posterior distribution for some computational reasons. This fact hindered the adoption of Bayesian methods in statistical analyses, although the Bayesian theory has been introduce earlier than many of the theories of frequentists school. The development of Markov chain Monte Carlo (MCMC) solved this challenge, which made the Bayesian methods practical.

Table 5 presented a timeline of Bayesian inference applied in animal breeding. Even though the theorem was introduced several centuries age, it became popular since 1960s, and Gianola and Fernando first used it in animal breeding for a threshold model paper in 1986. Bayes methodologies have been widely used in GWAS in animal breeding since the study of Meuwissen et al. (2001).

Table 2.5. History of Bayesian Inference in animal breeding and genetics

| Event | Time | Personage |
|---|---|---|
| Bayes' Theorem | 1700s | Bayes and Price (1973) |
| Bayesian school | 1774-1812 | Laplace (1812) |
| Bayesian in animal breeding | 1986 | Gianola et al. (1986) |
| Bayesian in GWAS | 2001 | Meuwissen et al. (2001) |

### 2.11.4 Markov chain Monte Carlo

The Markov chain Monte Carlo (MCMC) can be explained separately as Markov chain and Monte Carlo, and Gelf and and Smith (1990) first provide its application in Bayesian statistics. A Markov chain is a random process with the following properties: conditional on its present value, the future is independent of the past, and stationary distribution of the random process (Grimmett and Stirzaker, 2001). Monte Carlo is a statistical approximated estimation of an integral value

using evaluations of an integrand at a set of points drawn randomly from a distribution with support over the range of integration (Murphy, 2012). The MCMC was placed in the top 10 most important algorithms of the 20th century (Murphy, 2012). It is associated with many characteristics: easier to implement, suitable for boarder range of models, and able to sample from high-dimensional distributions. This algorithm allows drawing samples from unknowns' posterior distribution and indirectly making inferences of them. Two widely used MCMC methods are Gibbs sampling and Metropolis-Hasting (MH), which have been applied in GWAS of livestock. Many software have implemented Bayesian and MCMC methods, including GenSel software (Fernando and Garrick, 2008), SVS (Golden Helix, Inc., Bozeman, MT), BLUPF90 family (Misztal et al, 2014) and MCMCglmm package in R (Hadfield, 2010).

#### 2.11.4.1 Gibbs Sampling algorithm

The Gibbs sampling allows sampling joint distribution for parameters through drawing samples from full conditional distribution for each parameter. It is an efficient and simple algorithm, and a special case of MH (Murphy, 2012). The process of Gibbs sampling was stated as follows:

1. Specify initial values: $(x_1^0, x_2^0, x_3^0 \cdots, x_n^0)$
2. Reapeat sampling:

$x_1^1$ from $p(x_1 \mid x_2^0, x_3^0 \cdots, x_n^0)$

$x_2^1$ from $p(x_2 \mid x_1^1, x_3^0 \cdots, x_n^0)$

$x_n^1$ from $p(x_n \mid x_1^1, x_2^1 \cdots, x_{n-1}^1)$

$x_j^t$ from $p(x_j \mid x_1^t, \cdots, x_{j-1}^t, x_{j+1}^{t-1}, \cdots, x_n^{t-1})$

3. Study the posterior inference: $(x_1^t, x_2^t, x_3^t \cdots, x_n^t)$

which indicates that this algorithm sample each variables in turn conditionally on the value of other variables in the joint distribution. Through the repeated sampling, the algorithm achieves the required number of samples for each variable, and these samples are Markov Chain with a

stationary distribution. The estimates of unknowns can be obtained through summarizing these resulting samples. This algorithm has been implemented in software (e.g. GenSel) to execute Bayes A, Bayes C and Bayes Cπ. Known full conditional distribution functions are required for Gibbs Sampling, which makes this algorithm not applicable for the cases without known full conditional distributions for variables. Because Gibbs sampling only updates one variable at a time, it can be quite slow if the variables are highly correlated (Murphy, 2012).

2.11.4.2 Metropolis-Hasting algorithm

Metropolis-Hasting is another widely used MCMC algorithm, and it is more general than Gibbs sampling. In some situations, Gibbs Sampling is not applicable, therefore Metropolis-Hasting can be a better option. The processes of the Metropolis-Hasting are as follows:

1. Specify initial values $x^0$
2. Drawn sample y from a proposal distribution: $q(x^* | x^{t-1})$
3. Compute acceptance probability:

$$r = \frac{p(x^*)q(x^{t-1} | x^*)}{p(x^{t-1})q(x^* | x^{t-1})}$$

4. Accept $x^*$ as new sample $x^t$ with probability $\min(r,1)$. If $x^*$ is not accepted, $x^t = x^{t-1}$.
5. Repeat Setp 2 to Step 4 to get T draws of samples from joint distribution of unknowns.

The basic idea in Metropolis-Hasting is that we define the proposal distribution $q(x^* | x^{t-1})$ as the probability of the movement from current state $x^{t-1}$ to a new state $x^t$ in each step. The proposal distribution is chosen by modelers, which makes it is a flexible method. The normally used proposal distributions are symmetric Gaussian distribution conditionally on the current sample and independent distribution of x*, which lead to random walk Metropolis algorithm and independent sampler, respectively. The proposal distribution is also used to compute the acceptance probability that is used to decide whether to accept a new sample. In this process, a value U is drawn from Uniform(0,1), then if U<=r, we accept x* as $x^t$, otherwise use $x^{t-1}$ as $x^t$. As

a result, candidate draws with higher probability than current samples are always accepted, because r is larger than 1 in this situation. An efficient Metropolis-Hasting requires monitoring acceptance rate at a reasonable level (not too high or too low), which limits its usefulness for automatic modeling process. In addition, the performance is sensitive to the chosen proposal distribution when using independent sampler. If the proposal distribution is far different from the posterior distribution, the independent sampler would be extremely inefficient.

## 2.12 Post GWAS process

The significant phenotype-associated genomic regions and their associated effects are estimated from a GWAS. Many actions can be executed based on these results, including development of genomic-enhance EBV and analyses of pathways and networks.

### 2.12.1 Genomic selection

The direct genomic breeding values (DGV) can be calculated by summing up the effect of markers (SNP) across the whole genome from GWAS (Saatchi et al, 2012). The DGV can be directly used in selection tools, but the selection accuracy is dependent on the percentage of genetic variation that the DGV can explain. In most cases, the estimated DGV can only explain a small portion of genetic variation of complex traits, so it is not an accurate selection tool by itself. In the beef cattle industry, DGV were used as additional information to phenotypic and pedigree information to derive genomic-enhanced EBV (GEBV) and conduct genomic selection (Gary et al., 2012; Saatchi et al, 2012; Rolf et al, 2014).

Many advantages are associated with genomic selection. First, increasing the accuracy of selection is the main advantage of this process in the beef industry (Rolf et al., 2014). This

achievement was accomplished by genomic technique through providing additional genomic information to genetic evaluation, and helps reveals the "true" relationship between individuals (relationship based on pedigree and genomic information). This information helps increase the accuracy of the estimated breeding value, and sequentially improves the selection response. Traits that are hard or expensive to be measured may benefit the most from the genomic technology, including: feed efficiency traits, carcass traits and longevity traits (Garrick, 2011). Also, the young animals with no measurements and few offspring and relatives would gain great benefit from it, because the genomic selection can use genomic information at early age (Garrick, 2011).

Second, genomic selection helps increase selection intensity (Weller, 2016). The rise in selection intensity would speed the selection response. The increased selection accuracy of genomic selection allows breeder to select less individuals with high confidence (decrease the percentage of selected animals). In addition, this technique allows cross-herd selection that increases the base size of the animal population, and sequentially increases the selection intensity.

Third, genomic selection would help decrease the generation interval (Weller, 2016). The generation interval influences the speed of genetic gain, and large generation interval slows down the genetic improvement (Bourdon, 1997). The genomic technology allows selecting animals at earlier age (before the measures of phenotypes and abandoning progeny testing), because the genomic information can be available shortly after birth of an animal. This would significantly shorten the generation interval in selection, and improve traits expressed late in an animal's life, including carcass, longevity and reproductive traits (Garrick, 2011).

Fourth, genomic selection reduces breeding costs (Bassi et al., 2016), since genomic selection would cut down the number of progeny needed to develop as seedstock. It can make up

the loss in accuracy from the reduction in progeny test. In addition, it will reduce the cost in measuring phenotype, because it allows selection of animals without phenotypes for traits that are expensive to be measured (e.g. feed efficiency traits).

In beef industry, three methods were developed to incorporate the estimated DGV in to transitional genetic evaluation to develop GEBV (Rolf et al, 2014): (1) the DGV was treated as correlated trait; (2) the DGV was used as external EPDs; (3) the GEBV was used in a selection index. The National Cattle Evaluation adapted the first method to develop the genome-enhanced EPD for breeders (Kachman, 2008). In the method, the DGV is implemented in the multi-trait model as another correlated traits in genetic evaluation. The accuracy of EPD of traits in interest increases as the genetic correlation between DGV and the trait in interest rising. The American Simmental Association adapted the second method in its evaluation. In this method, the DGV influences the accuracy of EPD differently for each animal due to the relationship between the animal with the DGV and the training population. The index method develops genomic-enhanced EBV through combine direct genomic value and parent average and pedigree indexes using an index equation. American Angus Association adapts this method in their EPD reports.

Besides these two-step methods, some "direct" methods, using genomic derived relationship matrix, can estimate GEBV from BLUP directly without obtaining marker effects first. These methods include GBLUP, TABLUP and single-step methods, which construct genomic relationship matrix based on IBD, IBS and the combination of IBD and pedigree-based relationship matrix, respectively (Misztal et al., 2009).

Many advantages are associated with these "indirect" methods (using DGV): (1) more computational feasible, and it can be used for large data set analysis; (2) quick and easy to predict GEPD for young genotype animal (like traditionally add data to a evaluation); (3) these

methods allow the using of MBV that is constructed from portion of genotyped markers. The disadvantages of these methods are: (1) deregression is needed to solve the problem of double counting relationship from records and individuals; (2) they are not feasible for multi-trait analysis; (3) the genomic prediction models are needed to be re-trained on a regular base, because the accuracy of genomic predictions decays with the increases in generation between training and test population. Among these methods of the indirect category, the selection index method is simple, and does not need modification in traditional genetic evaluation. The external EPD method allows varying impacts on accuracies of genomic enhanced EPD depending on the relationship between the genotyped animal and the training population.

### 2.12.2 Genes and pathway analysis

In order to investigate the physiologic function of genomic regions detected as significant in GWAS, bioinformatics related analysis should be done. These analyses may include genes identification, pathway analysis, cluster analysis, transcription factor analysis, etc. Given the significant regions, we can identify related genes by searching published assembly and annotation of the bovine genome (e.g UMD3.1, BTAU4.0). This can be done using public website tools, such as Ensembl, NCBI, BMC, etc. Also, the cattle assembly sequence of significant windows can be aligned against other species' genome such as human, pig and mouse to determine if any homologous genes were present in the putative region. This method can be used to filter less significant SNP. Then, all the selected genes from assemble results can be searched on several data bases to obtain pathway information, such as MetaCyc (Caspi et al., 1008) or KEGG (Kanehisa and Goto, 2000) through tools like DAVID (Dennis et al., 2003), Ingenuity (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity), g:Profiler (Reimand et

al., 2016), etc. Cluster analyses integrate pathway analysis to provide more complete picture of a gene network. Cluster software (e.g. MCODE plug-in of Cytoscape) can construct cluster and tree graphic, and explore the connectivity between genes to find highly dense clusters as well as the node and hubs (Fortes et al., 2010).

### 2.12.3 Network analysis

By knowing QTL using SNP, we can conduct network analysis on single trait or multi-trait associated genes. Cluster analyses integrate the pathway analysis to provide more complete picture of gene network. Network analysis can be executed using the procedurals described by Reverter and Fortes (2013) and Fortes et al. (2011). These network studies generated gene regulatory network and epistatic network by association weight matrix (AWM). The partial correlation and information theory (Reverter and Chan, 2008) and regulatory impact factor (Reverter et al., 2010) can be integrated with associated weight matrix to infer gene co-association and regulatory networks and identify transcription factor. We can generate a co-association network and identify highly connected regulators using the GWAS results across a variety of phenotypes with this method.

### 2.12.4 Additional "omics" studies

Although GWAS can identify SNP explaining high common variance of a specific trait, they explain small portion of total variance and far less of the rare variance, which may be causally linked to phenotype. Also, GWAS cannot directly identify the variants that are causally linked to the phenotype (Marian and Belmont, 2011). However, GWAS can provide target regions (QTL) for fine mapping on transcriptomics analysis using next generation sequence, which would detect

new causal genomic variation and associated mutations (markers). It can also incorporate other "omic" studies to reveal the systems biology of a trait or disease. Genomics and transcriptomics (using genotyping and RNA or DNA sequencing) identifies all potential QTL, associated markers and corresponding genes through association study; proteomics and metabolomics can provide the functional information to these genes, which is defined as functional genomics. Lots of GWAS have been done in this area in livestock, the identified genomic makers can only explain relatively small portion of the genetic variation, which yeild low accuracy of prediction based on these markers. In order to build an effective genomic prediction system, more genomic markers accounting for high genetic variation should be identified for economic relevant traits (including susceptibility to HAD). Advances in functional genomics make the possibility to develop trait (or disease)-specific genotype assay platforms with less but more important makers (they explain relatively high genetic variation) than traditional general chips (e.g. BovineSNP50 or BovineHD). These specific chips would result in high prediction accuracy, but low cost and data storage or computation requirement (Rolf et al., 2014). This will largely improve the genetic improvement in livestock industry and reduce the breeding cost. Therefore, genomic study of PAP in cattle would provide information for functional genomics of HAD and help conduct accurate genomic selection against HAD.

## 2.13 Conclusions

Selection for resistance to HAD is important for beef cattle, because HAD influences calf mortality at high altitudes (above 1500m). Pulmonary arterial pressure is an indicator trait for selection of tolerance to high altitude, especially since it is physiologically related to HAD and moderately heritable. Genetic selection for low PAP for beef production system at high altitudes

could potentially improve profitability by reducing morbidity and mortality. However, more genetic evidence is needed to ensure that selection for low PAP could reduce the incidence of PH and HAD. The GWAS of PAP can be used to identify the most significant SNP or inference to genes potentially related to PH and HAD, and estimate GEBV to serve as a selection tool. Thus, genomic information can help the selection of cattle for resistance to HAD at earlier ages. Besides the benefit of traditional genetic selection on PAP, GWAS of PAP will also help reveal the genomic architecture of PH by studying genes, and increase the selection efficiency for less susceptibility to HAD. However, case/control data of HAD are needed to help expose information of the complex high altitude disease. Thus, it is important and beneficial to collaborate with breeders of cattle in mountains regions of the country to collect the PH and HAD in the future.

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# CHAPTER 3

# PHENOTYPIC AND GENETIC CHARACTERISTICS OF PULMONARY ARTERIAL PRESSURE IN ANGUS CATTLE MANAGED AT HIGH ALTITUDE

## 3.1. Introduction

Exploratory data analysis and descriptive statistics are the first steps in analyses and modeling of complex traits. This chapter addresses the phenotypic and genetic characteristics of pulmonary arterial pressure (PAP) in Angus cattle managed at high altitude. The information herein helped determine the phenotypic form of yearling PAP in genetic evaluation in the subsequent studies. This part examined the data structure, checked modeling assumptions, and determined the influences of fixed effects on PAP. In addition, genetic parameters, EBV and accuracies were estimated for four yearling PAP phenotypes (non-transformed (RAW), power-transformed (PT), three-category (CAT3) and two category (CAT2) phenotypes). Also, the results from different forms of PAP phenotypes were compared to decide which form was most appropriate in genetic evaluation.

## 3.2. Materials and methods

### 3.2.1 Data

The PAP measurements were collected from Angus cattle from 1993 to 2015 at the John E. Rouse Colorado State University Beef Improvement Center. The headquarter of CSU-BIC is located at 2,170 m in elevation, and the growth traits and PAP were measured at this elevation, but the actual elevation environment ranged from 2,170 m to 2,740 m. In this herd, heifers and steers were developed post-weaning by grazing and alfalfa hay supplementation with an

expected average daily gain of 0.5 kg/d, while bulls were fed a high concentrate diet in a gain test for approximately 120 days with an expected average daily gain of 1.5 kg/d. Measurements were taken on spring born calves by the same licensed veterinarian when the cattle were about 365 days of age. The PAP was measured in millimeters of mercury (mmHg) for every animal using the procedures outlined by Holt and Callen (2007). Only the yearling PAP measurements (n = 5,659; age ranged from 260 d to 450 d) were used in this study, and the PAP measurements that were less than 30 mmHg (n = 18) were considered biologically impossible at high elevation and excluded from analyses. Cattle having usable yearling PAP scores were progeny of 299 sires and 1,600 dams. The average yearling weight of animals with PAP measurement was 344.85 ± 81.90 kg with an average weaning hip height of 109.17 ± 21.11 cm. The pedigree file used in genetic evaluation contained 11,715 Angus cattle. Fixed effects involved in yearling PAP analyses included sex, date of PAP measurements, age of dam and age of PAP measurements as a covariate. Table 3.1 presents the descriptive statistics of yearling PAP and age of measurement in various sex categories.

Table 3.1. Descriptive statistics of yearling pulmonary arterial pressure measurements (PAP) and age in each sex category of Angus cattle managed at high altitude (elevation at 2,170 m)

| Item | n | Mean | Minimum | Maximum | SD |
|---|---|---|---|---|---|
| PAP, mmHg | 5659 | 42.45 | 22.00 | 139.00 | 9.88 |
| Age, days | 5659 | 348.32 | 261.00 | 450.00 | 29.65 |
| PAP of heifers, mmHg | 3489 | 41.36 | 22.00 | 135.00 | 8.57 |
| Age of heifers, days | 3489 | 351.16 | 261.00 | 420.00 | 25.81 |
| PAP of bulls, mmHg | 1397 | 45.76 | 29.00 | 139.00 | 11.58 |
| Age of bulls, days | 1397 | 352.47 | 261.00 | 414.00 | 21.72 |
| PAP of steers, mmHg | 773 | 41.11 | 27.00 | 138.00 | 10.25 |
| Age of steers, days | 773 | 327.98 | 261.00 | 450.00 | 45.79 |

### 3.2.2 Distribution of yearling PAP measurements

Normality of yearling PAP measurements were tested using the Shapiro-Wilk normality test and the density plots, Q-Q plots of PAP phenotypes, and residuals after adjusting for potential fixed effects (Ghasemi and Zahediasl, 2012). In order to deal with the non-normality and heterogenous variance associated with non-transformed PAP measurements, alterative phenotypes of yearling PAP were tested and compared to determine the appropriate analysis. These phenotypes included PT, and ordinal categorical phenotypes (i.e. CAT2 and CAT3). A Box-Cox analysis was used to determine the power transformation (Box and Cox, 1964). The categorical phenotypes were constructed in accordance with the guidelines of PAP measurement described by Holt and Callen (2007). The categories of CAT3 were defined as low risk (PAP < 41 mmHg), moderate risk (41 mmHg ≤ PAP ≤ 49 mmHg) and high risk (PAP > 49 mmHg) for high altitude disease (HAD). The CAT2 categories (PAP ≤ 49 mmHg or PAP > 49 mmHg) were constructed by combining low and moderate risk categories in CAT3 to indicate the with/without risk of pulmonary hypertension (PH) in cattle ( ≥ 25 mmHg is used to define PH in humans (Humbert et al., 2013)). Additional genetic analyses were conducted on all the alternative phenotypic forms and raw yearling PAP measurements.

### 3.2.3 Testing fixed effects

The potential fixed effects for analyses of yearling PAP included sex, age of dam, PAP measurement date and age of pap measurements as covariate. The fixed effects were examined for each phenotypic form (RAW, PT, CAT2 and CAT3) through log-likelihood ratio test (LR) using linear and threshold models. The phenotypic relationship between yearling PAP phenotypes and 365-day-adjusted yearling weight were also analyzed using linear and threshold

model with also accounting for fixed effects. The estimats of fixed effects and likelihood values of models were obtained from regression analyses using the Maximum likelihood method in R (R core Team, 2013; using packages "stat" and "ordinal"). Linear models were used to analyze RAW and PT, while threshold models were spplied to CAT3 and CAT2. These likelihood values were used to calculate the log-likelihood test (LR) for each fixed effect, which were expressed as:

$$LR = -2(LogL_r - LogL_F) \quad \text{(Equation 3.1)}$$

$LogL_r$ denoted the log-likelihood of model with less number of parameters, and $LogL_f$ stood for log-likelihood of the model with more parameters. In addition, Nagelkerke $R^2$ values (Nagelkerke, 1991) were calculated to illustrate the performance of each model for the varied yearling PAP phenotypes as follows:

$$\text{Nagelkerke's } R^2 = \frac{1-\exp(-\frac{2}{n}(LogL_\beta - LogL_0))}{1-\exp(\frac{2}{n}LogL_0)} \quad \text{(Equation 3.2)}$$

where $n$ was the sample size, $LogL_\beta$ was the log likelihood of the test model, and $LogL_0$ denoted the log-likelihood of the null model (only the mean was included as explanatory variable). Nagelkerke's $R^2$ is a pseudo coefficient of determination, which is preferred in threshold models (Nagelkerke, 1991).

### 3.2.4 Genetic evaluations

Heritability, EBV and accuracies were estimated for the different phenotypic forms using univariate linear and threshold mixed animal models. Bivariate and multivariate linear and threshold animal models were used to estimate the genetic correlations between different PAP phenotypic forms or PAP phenotype of different sex categories, respectively. Linear animal

models were used to analyze models associated with phenotypes in continuous scale (RAW and PT), while threshold animal models were used to study models associated with categorical phenotypes (CAT2 and CAT3). Equation 3.3 and Equation 3.5 present the form of these models.

$$\begin{vmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{vmatrix} = \begin{vmatrix} \mathbf{X}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_2 \end{vmatrix} \begin{vmatrix} \beta_1 \\ \beta_2 \end{vmatrix} + \begin{vmatrix} \mathbf{Z}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_2 \end{vmatrix} \begin{vmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \end{vmatrix} + \begin{vmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{vmatrix} \quad \text{(Equation 3.3)}$$

$$\text{var} \begin{vmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \\ \mathbf{e}_1 \\ \mathbf{e}_2 \end{vmatrix} = \begin{vmatrix} \mathbf{A}\sigma^2_{a_1} & \mathbf{A}\sigma_{a_{12}} & \mathbf{0} & \mathbf{0} \\ \mathbf{A}\sigma_{a_{12}} & \mathbf{A}\sigma^2_{a_2} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma^2_1 & \mathbf{I}\sigma_{12} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_{12} & \mathbf{I}\sigma^2_2 \end{vmatrix}$$

Specifically, $\mathbf{y}_1$ and $\mathbf{y}_2$ were vectors of observations of each pair of RAW, CAT3 and CAT2 when this represented regular linear models. $\beta_1$ and $\beta_2$ denoted the vectors of fixed effects on the two traits. $\mathbf{X}_1$ and $\mathbf{X}_2$ were incidence matrices relating observations in $\mathbf{y}_1$ and $\mathbf{y}_2$ to effects $\beta_1$ and $\beta_2$. $\mathbf{u}_1$ and $\mathbf{u}_2$ were vectors of random effects on $\mathbf{y}_1$ and $\mathbf{y}_2$, and $\mathbf{Z}_1$ and $\mathbf{Z}_2$ were incidence matrices relating these effects to observations. The $\mathbf{e}_1$ and $\mathbf{e}_2$ denoted the random residual effects on observation $\mathbf{y}_1$ and $\mathbf{y}_2$. The **A** was Wright's numerator relationship matrix, $\sigma^2_{a_1}$ and $\sigma^2_{a_2}$ were genetic variances of trait 1 and trait 2, $\sigma^2_1$ and $\sigma^2_2$ were the residual variance of these two traits, and $\sigma_{a_{12}}$ and $\sigma_{12}$ were genetic and residual covariance between $\mathbf{y}_1$ and $\mathbf{y}_2$. This equation also represented the threshold model for ordered categorical traits. In this situation, the $\mathbf{y}_1$ and $\mathbf{y}_2$ denoted the liability (*l*) of categorical observations on a standard normal scale (*l~N(0,1)*), and the relationship between the liability and the standard normal distribution curve was (Mrode, 2014):

$$\phi(l_i) = \exp(-0.5l_i^2) / \sqrt{2\pi} \quad \text{Equation (3.4)}$$

the $\phi(l_i)$ was the height of the normal distribution at $l_i$. The following equation represent the model for analyzing genetic relationship between PAP phenotypes in different sex categories.

$$\begin{vmatrix} \mathbf{y}_h \\ \mathbf{y}_b \\ \mathbf{y}_s \end{vmatrix} = \begin{vmatrix} \mathbf{X}_h & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_b & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{X}_s \end{vmatrix} \begin{vmatrix} \beta_h \\ \beta_b \\ \beta_s \end{vmatrix} + \begin{vmatrix} \mathbf{Z}_h & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_b & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{Z}_s \end{vmatrix} \begin{vmatrix} \mathbf{u}_h \\ \mathbf{u}_b \\ \mathbf{u}_s \end{vmatrix} + \begin{vmatrix} \mathbf{e}_h \\ \mathbf{e}_b \\ \mathbf{e}_s \end{vmatrix} \quad \text{(Equation 3.5)}$$

$$\text{var} \begin{vmatrix} \mathbf{u}_h \\ \mathbf{u}_b \\ \mathbf{u}_s \\ \mathbf{e}_h \\ \mathbf{e}_h \\ \mathbf{e}_h \end{vmatrix} = \begin{vmatrix} \mathbf{A}\sigma^2_{a_h} & \mathbf{A}\sigma_{a_{hb}} & \mathbf{A}\sigma_{a_{hs}} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{A}\sigma_{a_{hb}} & \mathbf{A}\sigma^2_{a_b} & \mathbf{A}\sigma_{a_{bs}} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{A}\sigma_{a_{hs}} & \mathbf{A}\sigma_{a_{bs}} & \mathbf{A}\sigma^2_{a_s} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma^2_h & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma^2_b & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma^2_s \end{vmatrix}$$

where $\mathbf{y}_h$, $\mathbf{y}_b$ and $\mathbf{y}_s$ were vectors of heifer, bull and steer observations of each PAP phenotype when this represented regular linear models. $\beta_h$, $\beta_b$ and $\beta_s$ denoted the vectors of fixed effects on heifer, bull and steer observations. The fixed effects used in these models included age of dam, date and age (covariate) of PAP measurements. $\mathbf{X}_h$, $\mathbf{X}_b$ and $\mathbf{X}_s$ were incidence matrices relating observations to fixed effects. The $\mathbf{u}_h$, $\mathbf{u}_b$ and $\mathbf{u}_s$ were vectors of random effects on heifer, bull and steer observations, and $\mathbf{Z}_h$, $\mathbf{Z}_b$ and $\mathbf{Z}_s$ were incidence matrices relating these effects to heifer, bull and steer observations. The $\mathbf{e}_h$, $\mathbf{e}_b$ and $\mathbf{e}_s$ denoted the random residual effects on these observations. The $\sigma^2_{a_h}$, $\sigma^2_{a_b}$ and $\sigma^2_{a_s}$ were genetic variances of heifer, bull and steer PAP phenotypes, $\sigma^2_h$, $\sigma^2_b$ and $\sigma^2_s$ were the residual variances of observations in different sex categories. The $\sigma_{a_{hb}}$, $\sigma_{a_{hs}}$ and $\sigma_{a_{bs}}$ were genetic covariance between heifer, bull and steer observations. Unlike equation 3.3, the residual covariance between heifer, bull and steer PAP phenotypes were fixed as 0 in analyses, because there was no individual that appear in more than one sex category.

The presented model for estimating variance components, EBV and accuracy were analyzed with Gibbs sampling in software: renumf90, thrgibbs1f90 and postgibbs1f90 (Tsuruta and Misztal, 2006; Aguilar et al., 2014). A total of 250,000 iterations were run with the first 50,000 discarded as burn-in, thinning every 10 samples, which resulted in sample size 20,000 for each estimated parameter and EBV. The simple two-sample *z* test were used to test the significance of differences between estimates of heritabilities, and the *z* score was calculated as (Åkesson et al., 2008):

$$z = \frac{h_i^2 - h_j^2}{\sqrt{se_i^2 + se_j^2}} \text{ (Equation 3.6)}$$

where $h_i^2$ and $h_j^2$ were two heritability estimates, and $se_i$ and $se_j$ were corresponding standard error of the estimates. The *z* scores corresponding two-tail p-values were obtained from the standard identity normal distribution to assess the significance level. In order to assess the differences between genetic evaluation of different PAP phenotypic forms, Pearson and rank correlations between EBV from different phenotypic forms were estimated, and the Beef Improvement Federation (BIF) accuracies from different phenotypic forms were compared. The EBV and predicted error variance (PEV) were direct output of software, and the BIF accuracies (acc) were calculated as:

$$acc = 1 - \sqrt{\frac{PEV}{G \times (1+F)}} \text{ (Equation 3.7)}$$

where the *G* denoted the genetic variance of each PAP phenotypic forms, and *F* is the inbreeding coefficient. The genetic trends were also calculated and plotted for the comparison of genetic evaluations of yearling PAP phenotypes.

To evaluate the differences between genetic evaluations of heifer, bull and steer yearling PAP, Pearson and Rank correlations between EBV from PAP measurements of different sexes

were calculated. A likelihood ratio test was used to assess the significance of fitting yearling PAP of different sexes as separate traits. The likelihood values used to conduct the likelihood ratio test were obatained from ASReml 3.0 (Gilmour et al., 2009). Because the likelihood values from mixed models using REML with different fixed effects were not comparable (not nested model; Pinheiro and Bates, 2000), the likelihood values from univariate model (including all PAP scores and sex as a fixed effect) and multivariate model (treating PAP scores as different traits by sex without sex as a fixed effect) cannot be used to conduct log likelihood ratio test. A similar multivariate model was parameterized to approximate the univariate model of PAP scores through the procedure described in Shirley et al. (2008). The converged variance parameters from the univariate animal model were applied and fixed in an alternative multivariate model fitting PAP as different traits by sex. In this alternative model, the genetic correlations between sexes were fixed as 0.99, because these genetic correlations could not be parameterized to unity to avoid a singular genetic variance-covariance matrix. The likelihood ratio test was obtained using likelihood of the two multivariate models treating PAP as same or different traits by sex in ASReml, and the associated degree of freedom was the difference in number of estimated parameters between the two models. Because ASReml can only analyze multivariate linear models, the tests involving CAT3 and CAT2 phenotypes (should be tested in multivariate threshold models) were approximated by treating them as linear traits.

## 3.3. Results and discussion

### 3.3.1 Distribution of yearling PAP measurements

The Shapiro-Wilk normality test on the residual of PAP while adjusting for fixed effects (i.e. sex, age of PAP, age of dam and measurement date) resulted in $W = 0.687$ ($P < 0.0001$). Figure

3.1 presentes the distribution of yearling PAP measurements and residuals, the Q-Q plot and box plot of PAP marginal residuals. This information suggested that the residuals significantly violated the assumption of normality, and a long and thin tail existed on the right side of the distributions. The normally used linear statistical methods (i.e. LMM) for estimating variance components or predicting breeding values assume that the error/random effect is normally distributed and homoscedastic (Mrode, 2014). Non-normal and heteroskedastic data can potentially introduce bias into estimation and statistical tests (Osborne, 2005). The long right side tail caused non-normality of yearling PAP measurements, but we chose not to exclude these "extreme" measurements from the data to solve the non-normality problem because these are valuable phenotypes to understand the relationship of PAP and risk of HAD. In addition, the long right side tail is a general characteristic of the PAP distribution, this could also be associated with other cardiac diseases (e.g. congestive heart defects) that leads to the elevated PAP. Transformation of the original data was an option to accommodate non-normality and heterogeneity of variance (Box and Cox, 1964; Osborne 2005). Many other scientific studies ave applied different transformations to solve non-normality problem (Ali and Shook, 1980; Nusser et al., 1996). Box-Cox analysis was used to decide the most appropriate power transformation, which suggested the power for transforming original yearling PAP measurements was -2 (Figure 3.2). Specifically, the power transformation was recommended as $(PAP)^{-2}$. Because of the small scale of the transformed data ($10^4$), an additional linear transformation was made by multiplying $10^4$ to the transformed data for easier reporting. Therefore, the final transformation was $10^4*(PAP)^{-2}$. This transformation reduced the non-normality issue as suggested by the distribution, Q-Q plot and box plot of residuals of yearling PAP (Figure 3.2). The Box-Cox data transformation is a simple transformation method to effectively resolve the non-normal and

heteroscedastic problem, which were applied in many scientific studies involving livestock (Besbes et al., 1993; Becerril et al., 1994; Peltier et al, 1998).

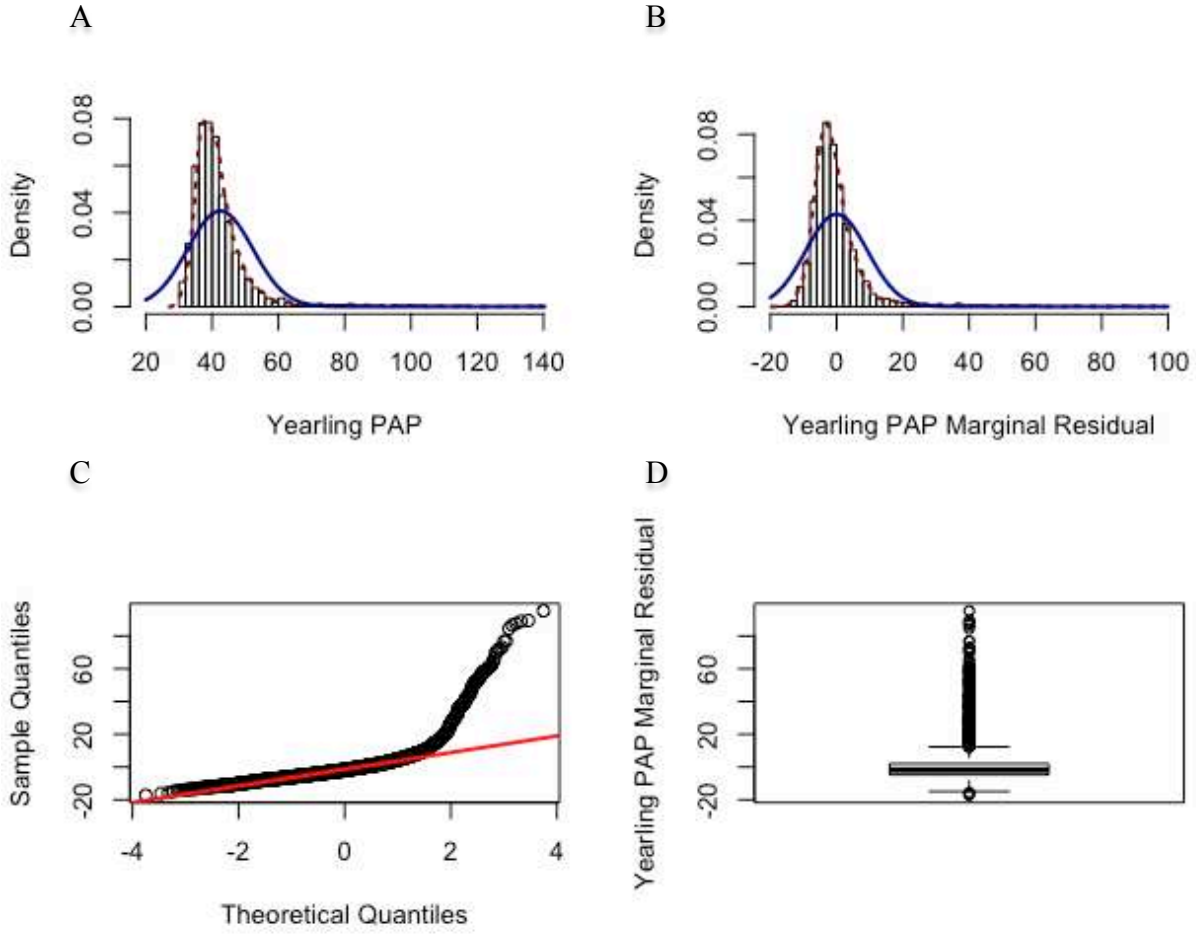


Figure 3.1. Histogram of (A) original measurements and (B) associated marginal residuals, and (C) Q-Q plot and (D) boxplot of residuals of yearling pulmonary arterial pressure (PAP)

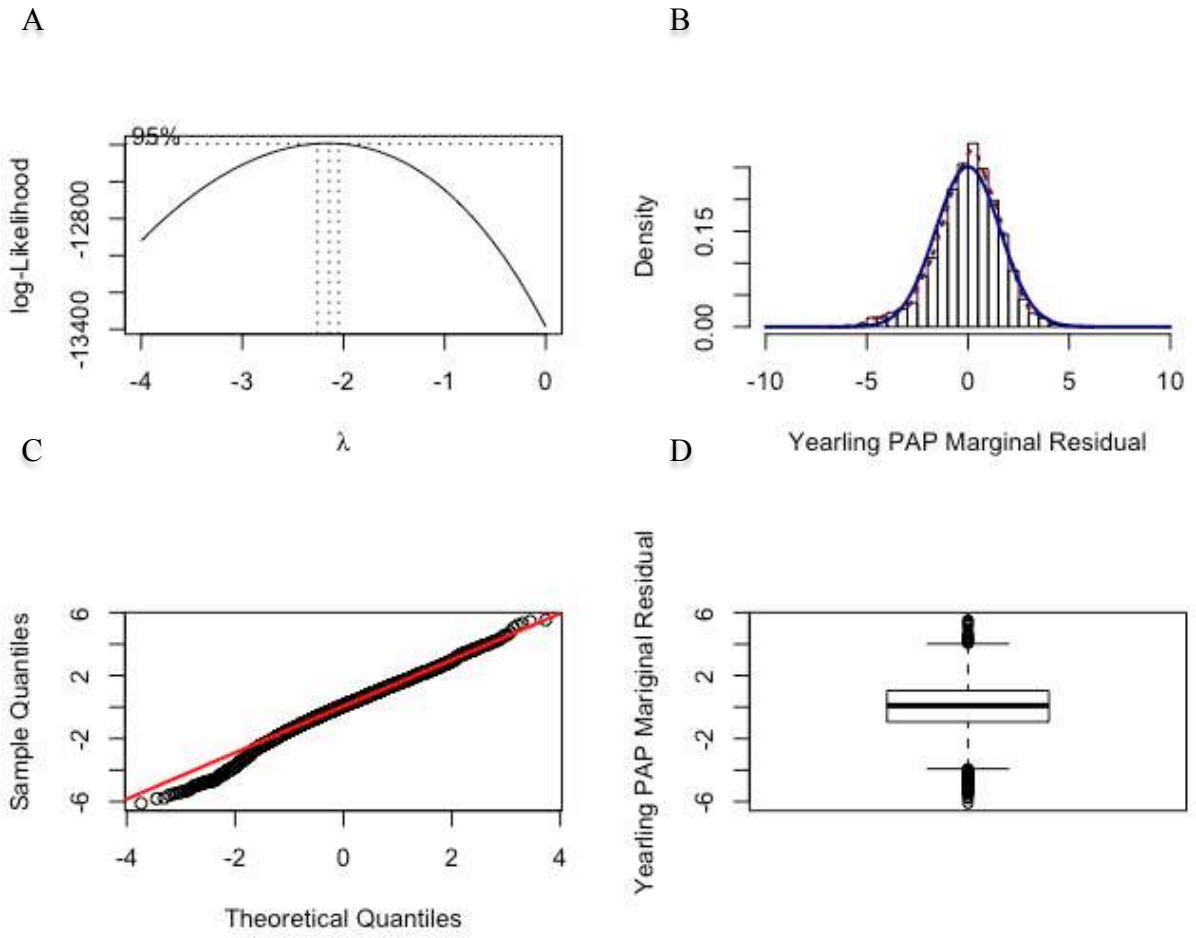


Figure 3.2. (A) Box-Cox analysis result, (B) histogram, (C) Q-Q plot and (D) boxplot of residuals of the power-transformed (λ = -2) yearling pulmonary arterial pressure (PAP) measurements

According to Holt and Callen (2007) and Holt (2016, personal communication), PAP > 49 mmHg is a general threshold to distinguish high-risk cattle for HAD at high elevation (elevation > 1,524 m), and a measure of PAP < 41 mmHg can be used to determine low-risk cattle to HAD when the elevation ranges from 1,524 m to 2,195 m. In addition, a preliminary study that scaned the heritability of categorical phenotypes based on different trunctation points implicated that using of truncation points at 41 mmHg and 49 mmHg also resulted in relatively higher heritability, and they were the average of the trauncation values of top 10 heritable three-category PAP phenotypes (Apeendix 3.1).

Therefore, the categories of CAT3 were defined as low risk (PAP < 41 mmHg), moderate risk (41 mmHg ≤ PAP ≤ 49 mmHg) and high risk (PAP > 49 mmHg) for HAD. The CAT2 categories were constructed by combining low and moderate risk categories of CAT3 to represent the with (PAP ≤ 49 mmHg) or without (PAP > 49 mmHg) presenting PH. Figure 3.3 presents the frequency of categorical phenotypes. The proportions of the second category for CAT2 were the same with proportion of the third category for CAT3. The proportions of the first category of CAT2 were obtained by summing the first and second category in CAT3, which were 88.89%, 91.93%, 80.59% and 91.72% for all, heifer, bull and steer PAP, respectively. The category frequencies varied among sexes (i.e. bull, heifer and steer). Figure 3.3 and the Chi-Squared tests of independence different sexes on CAT3 suggested that different sexes were associated with different percentage of animals in each yearling PAP category ($P < 0.05$). Larger proportion of bulls was in second and third categories, but higher percentage of heifers and steers were clustered first category, as well as proportion of animals in each category is similar between heifers and steers. For original PAP measurements, the heifer PAP measurements were similar with steer PAP measurements based on the Student t test on the mean ($P = 0.76$), and they were lower than the bull PAP measurements ($P < 0.05$; Table 3.1). This suggested a potential influence for management and genetic differences of PAP measurements recorded in bulls and heifers or steers, since the bulls were fed in a gain test (with average daily gain of 1.5 kg/d) but heifer and steers were grazed (average daily gain of 0.5 kg/d) in post-weaning period, which is a typical beef production system in U.S. mountain regions.

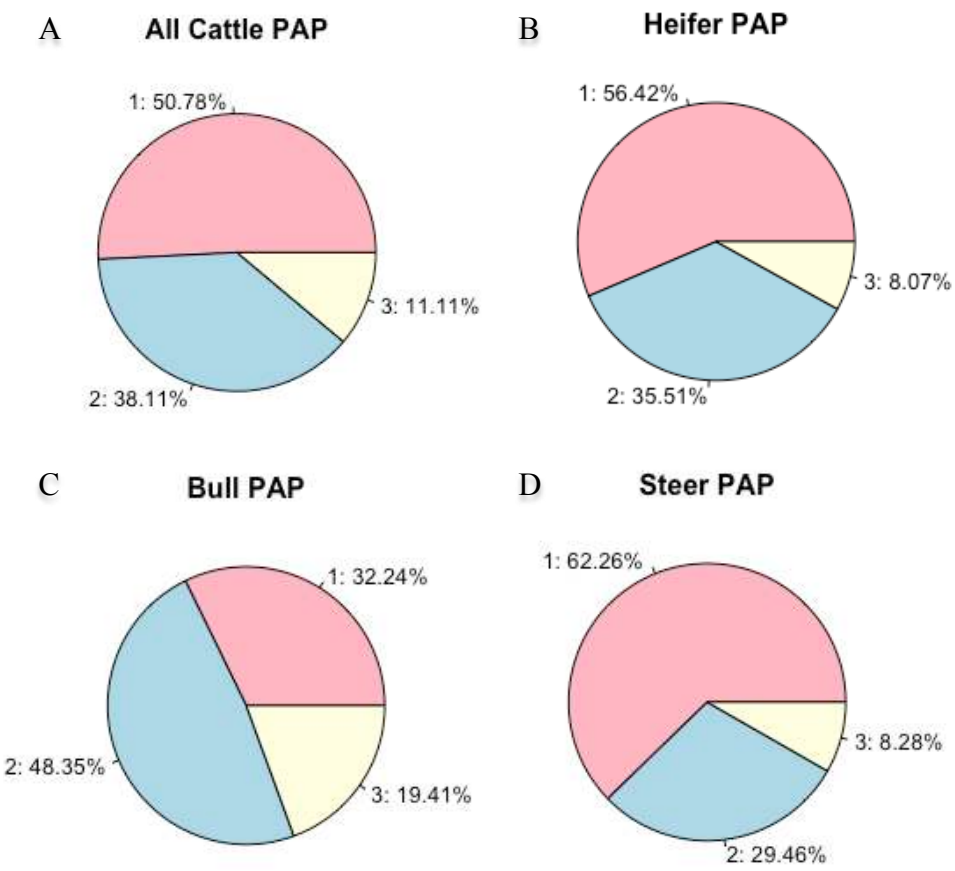


Figure 3.3. Proportion of calves in each of the three phenotypic categories based on all, heifer, bull and steer pulmonary arterial pressure (PAP) measurements. 1: (PAP < 41mmHg) ; 2: (41mmHg <= PAP <= 49 mmHg); 3: (PAP > 49 mmHg).

### 3.3.2 Fixed effects

Table 3.2 presents the log likelihood ratio test results for potential fixed effects in models to analyze PAP phenotypes. All the potential fixed effects (i.e. sex, age of dam, PAP measurement date and age of pap measurements as a covariate) were significantly associated with described yearling PAP phenotypes, so these were included in all analyses of yearling PAP. These fixed effects for PAP phenotypes were similar to those reported in Enns et al. (1992), Shirley et al. (2008) and Crawford et al. (2016). It appeared the non-normality of yearling PAP did not influence the statistical significance of fixed effects. Nagelkerke $R^2$ values were calculated to illustrate the performance of each model. Nagelkerke $R^2$ values were estimated as 0.10, 0.16,

0.15 and 0.14 for models of RAW, PT, CAT2 and CAT3. These results suggested that, with the same fixed effects, the model for PT, CAT3 and CAT2 performed better than raw PAP scores because the model terms explained slightly more variation in the data. In all phenotypes, bulls resulted in significant higher yearling PAP than heifers, and the mature cows (i.e. > 5 years of old) gave birth to individuals with higher yearling PAP than those from first calving heifers (Appendix 3.2 to Appendix 3.5).

Table 3.2. Results of log-likelihood ratio tests for fixed effects of each form of yearling pulmonary arterial pressure (PAP) phenotypes in Angus cattle managed at high altitude (elevation at 2,170 m)

| Effect[1] | $LogL_f$[2] | $LogL_r$[2] | $-2(LogL_r-LogL_f)$[2] | df | P-Value |
|---|---|---|---|---|---|
| Raw | | | | | |
| PDATE | -20456.74 | -20643.14 | 372.80 | 43 | <0.01 |
| AOD | -20456.74 | -20466.68 | 19.88 | 4 | <0.01 |
| SEX | -20456.74 | -20508.14 | 102.80 | 2 | <0.01 |
| AOP | -20456.74 | -20475.47 | 37.45 | 1 | <0.01 |
| PT | | | | | |
| PDATE | -10522.04 | -10796.81 | 549.53 | 43 | <0.01 |
| AOD | -10522.04 | -10530.84 | 17.59 | 4 | <0.01 |
| SEX | -10522.04 | -10617.88 | 191.69 | 2 | <0.01 |
| AOP | -10522.04 | -10548.39 | 52.69 | 1 | <0.01 |
| CAT3 | | | | | |
| PDATE | -4997.69 | -5203.55 | 411.71 | 43 | <0.01 |
| AOD | -4997.69 | -5008.63 | 21.88 | 4 | <0.01 |
| SEX | -4997.69 | -5057.55 | 119.72 | 2 | <0.01 |
| AOP | -4997.69 | -5025.01 | 54.64 | 1 | <0.01 |
| CAT2 | | | | | |
| PDATE | -1760.05 | -1892.54 | 264.98 | 43 | <0.01 |
| AOD | -1760.05 | -1768.78 | 17.46 | 4 | <0.01 |
| SEX | -1760.05 | -1784.63 | 49.17 | 2 | <0.01 |
| AOP | -1760.05 | -1772.85 | 25.61 | 1 | <0.01 |

[1]RAW: non-transformed PAP; PT: power-transformed PAP; CAT3: three-category phenotype; CAT2: two-category phenotype; PDATE: PAP measurement date; AOD: age of dam; AOP: age of cattle when taking PAP measurements.

[2] $LogL_f$: log likelihood value of the complete model with including all the fixed effects; $LogL_r$: log likelihood value of the reduced model with including all the fixed effects except the one being tested (indicated by the raw name of the table).

The regression coefficients and statistical test of 365-day-adjusted YW on yearling PAP phenotypes after adjusting for other fixed effects (i.e. sex, age of dam, date and age of PAP measruments) are presented in Table 3.3. The regression coefficients of adjusted YW were significantly different from zero when regressed on each yearling PAP phenotypes based on our sample size (n = 4,981), and these positive estimates suggested that the increase in adjusted YW would result in a rise in yearling PAP measurements and the probability to be higher risk for HAD. However, these estimates were relatively small, for example, the regression coefficient for adjusted YW on non-transformed PAP was 0.01, which indicated that 100 kg difference in YW would result in only 1 mmHg difference in PAP measurement under the same environmental situation. Therefore, although the adjusted YW differed extremely about 300 kg (661 lb), only a 3 mmHg range in yearling PAP measurement was expected. Also, very small amount (< 0.01) of improvement in $R^2$ were obtained from including adjusted YW as covariate in models for yearling PAP phenotypes. This could support the weak genetic correlation (0.13) between yearling weight and PAP in Crawford et al. (2016). Weight measurements were influenced by many factors (e.g. age and management), and these environmental factors were already included the model for yearling PAP phenotypes. In addition, the potential relationship between animal weights and yearling PAP phenotypes may be attributed to the pleiotropic gene effects, and we want to study the genetic relationship between them, so we do not want ignore them from our genetic studies. Mortimer et al. (2014) reported the variance partition between effects would be altered and the gentic correlation between traits would be overcorrected when using other traits as covariate in gentic evaluation of another trait. Therefore, the weights were not included as covariate in the model for yearling PAP phenotypes, and their relationship were assessed in multivariate models.

Table 3.3. Results of regression coefficients, statistical tests and $R^2$ of 365-day-adjusted yearling weights on yearling pulmonary arterial pressure phenotypes of Angus cattle managed at high altitude (elevation at 2,170 m)[1]

| Phenotype | Coefficients | LR | P-value | $\Delta R^2$ |
|---|---|---|---|---|
| RAW | 10.00e-03 | 7.17 | 0.01 | 1.28e-03 |
| PT | -4.06e-03 | 30.52 | <0.01 | 5.18e-03 |
| CAT3 | 2.80e-03 | 25.43 | <0.01 | 5.22e-03 |
| CAT2 | 2.10e-03 | 6.62 | 0.01 | 2.47e-03 |

[1]LR: Log likelihood ration test for 365-day-adjusted yearling weights; $\Delta R^2$: the change of $R^2$ between models with or without including 365-day-adjusted yearling weights as covariate

Tables 3.4 to 3.7 present the results of log-likelihood ratio tests for fixed effects when modeling RAW, PT, CAT3 and CAT2 phenotypes in sex categories. The date of PAP measurement was significant in models for all sex categories of each of the phenotype forms. The date information included year information, which accounted for the yearly environmental conditions. Age of PAP was significantly ($P < 0.05$) associated with yearling PAP phenotypes of bulls and heifers, while it was not significantly ($P > 0.05$) related to steer yearling PAP phenotypes in analyses. Generally, the PAP went up with the increases in age (Appendix 3.2 to Appendix 3.5).

Varied levels of significance were observed in age of dam when modeling bull and heifer yearling PAP in different phenotypic forms ($P = 0.01$ *to* $0.24$). The age of dam was significant at least at the 0.05 level in the model for bulls of all PAP phenotyeps. Also, age of dam was significant in the model for heifers of RAW and PT but not significant in models for heifers of CAT3 and CAT2. This may contribute to the losses of some phenotypic variation in constructing category phenotypes from continuous scal phenotypes. The significant test on age of dam mainly resulted from that PAP associated with heifer dam was significantly lower than the PAP associated with mature dam (i.e. age of dam as 5 years old). In addition, the non-significant age of dam effects in categorical phenotypes showed the similar pattern with thoses are significant in continuous phenotypes (Appendex 3.2 to Apeendix 3.5).

Table 3.4. Results of log-likelihood ratio tests for fixed effects of non-transformed yearling pulmonary arterial pressure (PAP) of Angus cattle managed at high altitude in each sex category (elevation at 2,170 m)

| Effect[1] | $LogL_f$[2] | $LogL_r$[2] | $-2(LogL_r-LogL_f)$[2] | df | P-Value |
|---|---|---|---|---|---|
| Bull | | | | | |
| PDATE | -5303.69 | -5380.21 | 153.04 | 25 | <0.01 |
| AOD | -5303.69 | -5308.97 | 10.56 | 4 | 0.03 |
| AOP | -5303.69 | -5308.57 | 9.78 | 1 | <0.01 |
| Heifer | | | | | |
| PDATE | -12128.35 | -12252.25 | 247.80 | 31 | <0.01 |
| AOD | -12128.35 | -12134.31 | 11.93 | 4 | 0.02 |
| AOP | -12128.35 | -12146.47 | 36.25 | 1 | <0.01 |
| Steer | | | | | |
| PDATE | -2771.34 | -2882.01 | 221.24 | 19 | <0.01 |
| AOD | -2771.34 | -2772.53 | 2.26 | 4 | 0.69 |
| AOP | -2771.34 | -2772.91 | 3.04 | 1 | 0.08 |

[1]PDATE: PAP measurement date; AOD: age of dam; AOP: age of cattle when taking PAP measurements.
[2] $LogL_f$: log likelihood value of the complete model that included all the fixed effects; $LogL_r$: log likelihood value of the reduced model that include all the fixed effects except the one being tested (indicated by the row name of the table).

Table 3.5. Results of log-likelihood ratio tests for fixed effects of power-transformation yearling pulmonary arterial pressure (PAP) of Angus cattle managed at high altitude in each sex category (elevation at 2,170 m)

| Effect[1] | $LogL_f$[2] | $LogL_r$[2] | $-2(LogL_r-LogL_f)$[2] | df | P-Value |
|---|---|---|---|---|---|
| Bull | | | | | |
| PDATE | -2507.56 | -2613.67 | 212.23 | 27 | <0.01 |
| AOD | -2507.56 | -2515.34 | 15.57 | 4 | <0.01 |
| AOP | -2507.56 | -2515.45 | 15.78 | 1 | <0.01 |
| Heifer | | | | | |
| PDATE | -6145.94 | -6272.41 | 252.94 | 32 | <0.01 |
| AOD | -6145.94 | -6150.90 | 11.79 | 4 | 0.04 |
| AOP | -6145.94 | -6495.60 | 44.91 | 1 | <0.01 |
| Steer | | | | | |
| PDATE | -1441.64 | -1558.36 | 233.44 | 19 | <0.01 |
| AOD | -1441.64 | -1442.20 | 1.12 | 4 | 0.89 |
| AOP | -1441.64 | -1442.42 | 1.57 | 1 | 0.21 |

[1]PDATE: PAP measurement date; AOD: age of dam; AOP: age of cattle when taking PAP measurements.
[2] $LogL_f$: log likelihood value of the complete model that included all the fixed effects; $LogL_r$: log likelihood value of the reduced model that included all the fixed effects except the one being tested (indicated by the row name of the table).

Table 3.6. Results of log-likelihood ratio tests for fixed effects of three-category yearling pulmonary arterial pressure (PAP) of Angus cattle managed at high altitude in each sex category (elevation at 2,170 m)

| Effect[1] | $LogL_f$[2] | $LogL_r$[2] | $-2(LogL_r-LogL_f)$[2] | df | P-Value |
|---|---|---|---|---|---|
| Bull | | | | | |
| PDATE | -1342.02 | -1435.08 | 186.13 | 27 | <0.01 |
| AOD | -1342.02 | -1352.10 | 20.16 | 4 | <0.01 |
| AOP | -1342.02 | -1349.57 | 15.58 | 1 | <0.01 |
| Heifer | | | | | |
| PDATE | -2993.28 | -3090.93 | 195.31 | 32 | <0.01 |
| AOD | -2993.28 | -2997.78 | 9.00 | 4 | 0.06 |
| AOP | -2993.28 | -3015.06 | 43.56 | 1 | <0.01 |
| Steer | | | | | |
| PDATE | -604.42 | -664.52 | 120.22 | 19 | <0.01 |
| AOD | -604.42 | -605.38 | 1.91 | 4 | 0.75 |
| AOP | -604.42 | -605.15 | 1.45 | 1 | 0.23 |

[1]PDATE: PAP measurement date; AOD: age of dam; AOP: age of cattle when taking PAP measurements.
[2] $LogL_f$: log likelihood value of the complete model that included all the fixed effects; $LogL_r$: log likelihood value of the reduced model that included all the fixed effects except the one being tested (indicated by the row name of the table).

Table 3.7. Results of log-likelihood ratio tests for fixed effects of two-category yearling pulmonary arterial pressure (PAP) of Angus cattle managed at high altitude in each sex category (elevation at 2,170 m)

| Effect[1] | $LogL_f$[2] | $LogL_r$[2] | $-2(LogL_r-LogL_f)$[2] | df | P-Value |
|---|---|---|---|---|---|
| Bull | | | | | |
| PDATE | -611.23 | -683.26 | 144.06 | 27 | <0.01 |
| AOD | -611.23 | -620.45 | 18.43 | 4 | <0.01 |
| AOP | -611.23 | -618.61 | 14.77 | 1 | <0.01 |
| Heifer | | | | | |
| PDATE | -922.48 | -941.55 | 104.88 | 32 | <0.01 |
| AOD | -922.48 | -925.05 | 5.14 | 4 | 0.27 |
| AOP | -922.48 | -931.70 | 18.43 | 1 | <0.01 |
| Steer | | | | | |
| PDATE | -182.38 | -224.96 | 85.16 | 19 | <0.01 |
| AOD | -182.38 | -183.74 | 2.71 | 4 | 0.61 |
| AOP | -182.38 | -182.39 | 0.03 | 1 | 0.87 |

[1]PDATE: PAP measurement date; AOD: age of dam; AOP: age of cattle when taking PAP measurements.
[2] $LogL_f$: log likelihood value of the complete model that included all the fixed effects; $LogL_r$: log likelihood value of the reduced model that included all the fixed effects except the one being tested (indicated by the row name of the table).

The observed slight differences between age of dam significance between RAW and PT suggested that the violation of normality could slightly alter the statistical test (i.e. log likelihood ratio test) on significance of fixed effects, which was implicated in previous reports (Nimon, 2012). Categrocial phenotypes were analyzed using threshold models without assuming the normal distriubution of data, so they did not violate the models assumptions. Also, the fixed effects appeared to influence yearling PAP measurements of different sex categories at different degrees. This result agreed with the report of Callen and Holt (2007) that different high PAP incidences were observed between male and female cattle. Because all fixed effects were statistical significant in some models used in the study, and in order to keep consistent across all models for yearling PAP measurements, all of these potential fixed effects were included in each model for yearling PAP phenotypes in the following studies.

### 3.3.3 Genetic evaluation

#### 3.3.3.1 Genetic parameters

Table 3.8 presents the heritability, genetic variance, and genetic and residual correlation of RAW, PT, CAT3 and CAT2 phenotypes. Although the estimated heritabilities were not statistically different across various yearling PAP phenotypes (i.e. RAW, PT, CAT3 and CAT2), the heritability estimates appeared slightly higher for ordinal categorical yearling PAP phenotypes than raw PAP measurements. The estimated heritabilities of RAW, PT and CAT3 were smaller than the heritability of PAP reported previous literatures (i.e. 0.30 and 0.46), and the heritability of CAT2 was similar with these reports (Enns et al., 1992; Shirley et al., 2008). Enns et al. (1992) used small number of PAP records and a univariate model to obatain the heritability as 0.46, and Shirley et al. (2008) used a multivariate model with BW and WW to get

a lower heritability as 0.30. Our estimates from univariate animal model on non-transformed PAP were consistent with the heritability estimate in the study of Crawford et al. (2016) who used the data from the same herd but analyzed them using multivariate model and REML based on method. The differences observed in the heritability of the current study and previous studies may be attributed to the different PAP measurements in these studies, since Enns et al. (1997) and Shirley et al. (2008) included weaning PAP (measured before 260 days of age) in their study and the current study analyzed yearling PAP. Holt and Callen (2007) implicated that weaning PAP scores of cattle could not be used to correctly infer the yearling PAP, and Zeng et al. (2015) reported the genetic correlation between weaning and yearling PAP measruments as 0.67 (0.18), and higher weaning PAP heritability than yearling PAP heritability, which suggested weaning and yearling PAP was not genetically identical.

No heritability estimate has been reported on the incidence of PH or the susceptibility of HAD in cattle, but Williams et al. (2012) assumed the missing yearling weight at high altitude were predominantly due to brisket disease and obtained the heritability of 0.36 for the cattle survivability to yearling weight at high altitude. This heritability estimated was similar to our heritability report on CAT2. However, the assumption in Williams et al. (2012) may introduce some biases to the data, since many other factors (e.g. other health issues and selection decisions) could infleucne the survivability to yearling age at high altitude, and some sick aniamals may not be identified and moved to low altitude.

Table 3.8. Heritability, genetic correlation (above diagonal) and residual correlation (below diagonal) among non-transformed, power-transformed and categorical yearling PAP of Angus cattle managed at high altitude (elevation at 2,170 m)

| Phenotype[1] | Raw | PT | CAT3 | CAT2 |
|---|---|---|---|---|
| Raw | 0.24 (0.03)[a] | -0.95 (0.02) | 1.00 (0.00)[2] | 0.92 (0.05) |
| PT | -0.82 (0.01) | 0.24 (0.03)[a] | -0.99 (0.00) | -0.91 (0.06) |
| CAT3 | 1.00 (0.00) | -1.00 (0.00) | 0.25 (0.03)[a] | 1.00 (0.00) |
| CAT2 | 1.00 (0.00) | -0.99 (0.01) | 1.00 (0.00) | 0.32 (0.05)[a] |

[1]PT: powered transformed yearling PAP, 10000*(PAP)$^2$; CAT_3: three-category phenotype, 1: PAP < 41 mmHg, 2: 41 mmHg ≤ PAP ≤ 49 mmHg, 3: PAP > 49 mmHg; CAT_2: two-category phenotype, 1: PAP ≤ 49 mmHg, 2: PAP > 49 mmHg

[2] The value in the parentheses indicated standard error, and the 0.00 indicate any value less than 0.005.

[a] Within the diagonal, the heritability estimates without a common superscript differ (P < 0.05).

Results herein suggested that a larger portion of phenotypic variance was explained by genetic components in analyzing CAT3 and CAT2 (higher heritability than continuous phenotypes). The better fits of the fixed effects in models for CAT3 and CAT2 would decrease the portion of residual variance and result in higher heritability than RAW. However, Kizilkaya et al. (2014) reported that the threshold model for analyzing categorical phenotypes would over-estimate heritability, which may also contribute to the higher heritability obtained from CAT3 and CAT2 using threshold models. Heritabilities obtained from PT and RAW were the same, and no improvement was gained in the heritability estimated from PT than RAW. This implied that the violation of normality of the original PAP data had little effects on genetic parameter estimates. High absolute genetic correlations (> 0.91) were estimated between RAW, PT, CAT3 and CAT2 (Table 3.8). The correlation coefficients associated with PT were negative, because PT was inversely transformed from RAW. Genetic correlation informs us how much common genetic influence exists across two traits. Therefore, these high genetic correlations suggested that the various phenotypic forms of yearling PAP were genetically similar, and the categorical phenotypes can be alternative response variables in study of the genetic characteristics of PAP measruments, PH and HAD.

3.3.3.2 EBV

Table 3.9 presents the correlations between EBV of all animals in the pedigree from RAW, PT, CAT3 and CAT2 using a univariate animal model. The correlations between sire EBV of these phenotypes have the same pattern as shown in Table 3.9. The Pearson's correlations ranged from 0.74 to 0.91, and the resulting Rank correlations ranged from 0.69 to 0.92. The Pearson and Rank correlations were high (0.89 and 0.92) between RAW and PT. This suggested that the violation of normality had limited influence on yearling PAP EBV and the ranking of the Angus cattle. Considering these similar heritability estimates, high correlations and ease of interpretation, RAW was the preferred dependent variable in estimating breeding value of to select against HAD.

The resulting EBV rank correlations between RAW and categorical phenotypes were 0.84 and 0.77, although they are high correlations, these correlations suggest some re-rankings of animals based on EBV among RAW and categorical phenotypes. Both of the lowest Pearson and Rank correlations were between CAT2 and PT, and suggested use of two-category phenotype would lead to the most re-ranking compared to EBV from PT or RAW. The EBV from CAT3 yielded to higher correlations with EBV from RAW and PT (with correlations larger than 0.8). These results implied that the fewer the categories involved in categorical phenotypes, the more re-rankings of animals would be. In summary, these categorical PAP phenotypes can be alternative response variable to study genetic characteristics of cattle's PAP at high altitude and develop EBV for selecting for low PAP and against HAD based on their moderate heritability, high genetic correlations with non-transformed PAP measruments, but some re-ranking of animals would be identified compared to non-transformed PAP measurements.

Table 3.9. Results of Pearson (above diagonal) and Rank (below diagonal) correlations among EBV of raw, continuous transformed and categorical transformed pulmonary arterial pressure (PAP) records of Angus cattle managed at high altitude (elevation at 2,170 m)

| Phenotype[1] | Raw | PT | CAT3 | CAT2 |
|---|---|---|---|---|
| Raw | 1 | -0.89 | 0.81 | 0.78 |
| PT | -0.92 | 1 | -0.91 | -0.74 |
| CAT3 | 0.84 | -0.91 | 1 | 0.81 |
| CAT2 | 0.77 | -0.69 | 0.76 | 1 |

[1]PT: powered transformed yearling PAP, 10000*$(PAP)^2$; Category_3: three-category phenotype, 1: PAP < 41 mmHg, 2: 41 mmHg ≤ PAP ≤ 49 mmHg, 3: PAP > 49 mmHg; Category_2: two-category phenotype, 1: PAP ≤ 49 mmHg, 2: PAP > 49 mmHg

Table 3.10 showed the average EBV accuracies of all animals in the pedigree file from univariate animal models of RAW, PT, CAT3 and CAT2, and also included average accuracies of sires that have offspring with PAP records. The average number of offspring with PAP records for a sire was 19 (associated with accuracy around 0.39 for non-transformed PAP), and the average accuracy for these sires was ranged from 0.21 to 0.31 in the four PAP associated phenotypes. As expected, average EBV accuracies from PT were similar with those from RAW based on the similar heritability obtained from them ($P > 0.05$). Although higher heritabilities were obtained from categorical phenotypes using threshold models, the resulting EBV accuracies are higher for RAW and PT than CAT3 and CAT2 ($P < 0.05$). The difference between sire's accuracies of CAT3 and phenotypes on a continuous scale was about 0.04, and the difference between accuracies of CAT2 and continuous phenotypes was about 0.1. Therefore, approximately 3 and 8 additional offspring would be needed to compensate for the losses in accuracies of CAT3 and CAT2 compared to continuous phenotypes based on the accuracy equation for offspring data from Bourdon (1997) and a heritability at 0.24. These results suggested losses in EBV accuracy from ordered categorical phenotypes compared to continuous yearling PAP scores, and these losses increased as the number of category decreased, which could imply that some information was lost when constructing categorical phenotypes based on

non-transformed PAP scores. Although the losses in accuracies were not reported in previous genetic evaluation studies, Kärkkäinen et al. (2013) and Kizilkaya et al. (2014) reported that categorical phenotypes yielded lower genomic prediction accuracy than continuous phenotypes, and illustrated that the accuracies increased with increases in ordered categories. In constructing the categorical phenotypes, it assumed that animals in the same risk categories for PH had the same levels of exposure to genetic risk factors. However, some animals considered "unaffected" could be higher risk than others in this group, and some animal considered "affected" could be in a more severe hypertensive state than other "affected" animals. This could contribute to the losses in information from categorical phenotypes relative to continuous scale phenotypes (Kizilkaya et al., 2014).

Table 3.10. Summary of accuracy from univariate model for each form of yearling pulmonary arterial pressure (PAP) phenotypes of Angus cattle managed at high altitude (elevation at 2,170 m)

| Form[1] | Average | Min | Max | SD | Sire_average |
|---|---|---|---|---|---|
| Raw | 0.17[a] | 0 | 0.68 | 0.09 | 0.31[a] |
| PT | 0.17[a] | 0 | 0.68 | 0.09 | 0.31[a] |
| CAT3 | 0.15[b] | 0 | 0.65 | 0.08 | 0.27[b] |
| CAT2 | 0.11[c] | 0 | 0.60 | 0.06 | 0.21[c] |

[1]PT: powered transformed yearling PAP, 10000*(PAP)$^2$; Category_3: three-category yearling PAP, 1: PAP < 41 mmHg, 2: 41 mmHg <= PAP <= 49 mmHg, 3: PAP > 49 mmHg; Category_2: two-category yearling PAP, 1: PAP <= 49 mmHg, 2: PAP > 49 mmHg

[abc] Within the column, the mean of accuracy without a common superscript differ (P < 0.05).

### 3.3.3 Genetic trend

Figure 3.4 illustrates the genetic trends (on genetic standard deviation scale) and the associated regression lines from RAW, PT, CAT3 and CAT2. The genetic trends of continuous traits indicated the average genetic changes of yearling PAP across years. The genetic trends of categorical phenotypes reflected the genetic changes of liability under a normal distribution.

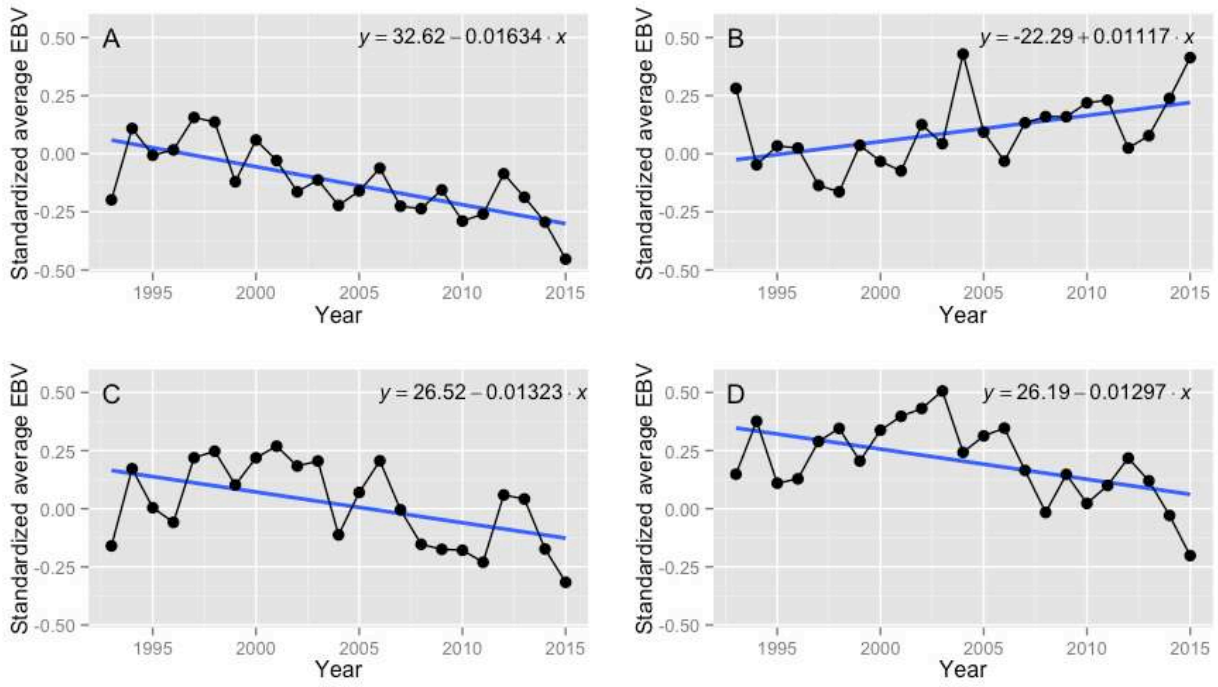


Figure 3.4. Genetic trends and associated regression lines and equations across year from 1993 to 2015 from raw yearling pulmonary arterial pressure (PAP) (A), power-transformed yearling PAP (B), and three-category (C) and two-category (D) phenotypes.

All forms of phenotypes showed the same overall direction of selection response – decreased PAP measurements over years (i.e. negative regression coefficients for these genetic trends). The resulting genetic trend for PT was positive, since PT is the inverse transformation of PAP. Enns et al. (2011) also reported a similar downwards PAP genetic trend based on information from Tybar ranch in Colorado. The resulting genetic trends could be attributed to the CSU-BIC Angus herd selecting against HAD using PAP for decades. These results suggest that genetic improvements were and can continue to be made on PAP for cattle managed at high altitude through selection on PAP measurements. The resulting genetic improvement rate was approximated by the regression coefficient of the genetic trend, which suggested a slight (1.1% to 1.6% of genetic standard deviation; Figure 3.4) genetic improvement rate per year for these PAP phenotypes. Considering the non-transformed PAP phenotype, the PAP was genetically

improved by only -0.075 mmHg per year based on the genetic variance of RAW as 21.96 $mmHg^2$. Although the overall genetic trend was decreasing, large fluctuations were identified across years. This may be due to external sires (without genetic information on PAP) were introduced in this herd.

Beside the genetic trend on all animals, we also constructed the genetic trends of non-transformed PAP phenotypes of animals (whose dams were all CSU bred) from three different sire groups, the registered external sires, the CSU-BIC sires (whose sire and dam were both CSU-BIC bred), and the partial CSU-BIC sires (one of whose parents were outside bred; Figure 3.5). The genetic trends of these different sire groups had the same direction with overall genetic trend (i.e. decreasing PAP measurements). The genetic improvement in cattle sired by external sires may be attributed to the genetic improvement of their CSU-BIC dams. It may also come from the genetic improvement of PAP of external herds because increasing number of PAP tests were done in seedstock herds at high altitude for selecting against HAD in recent years, and there is demand for cattle are adapted to high altitude. The average PAP EBV of cattle from external sires was larger than those from CSU-BIC sires ($P<0.05$, Figure 3.5), which suggested that the CSU-BIC cattle tend to have lower yearling PAP and less susceptibility to HAD than those external registered cattle under the same environmental conditions.

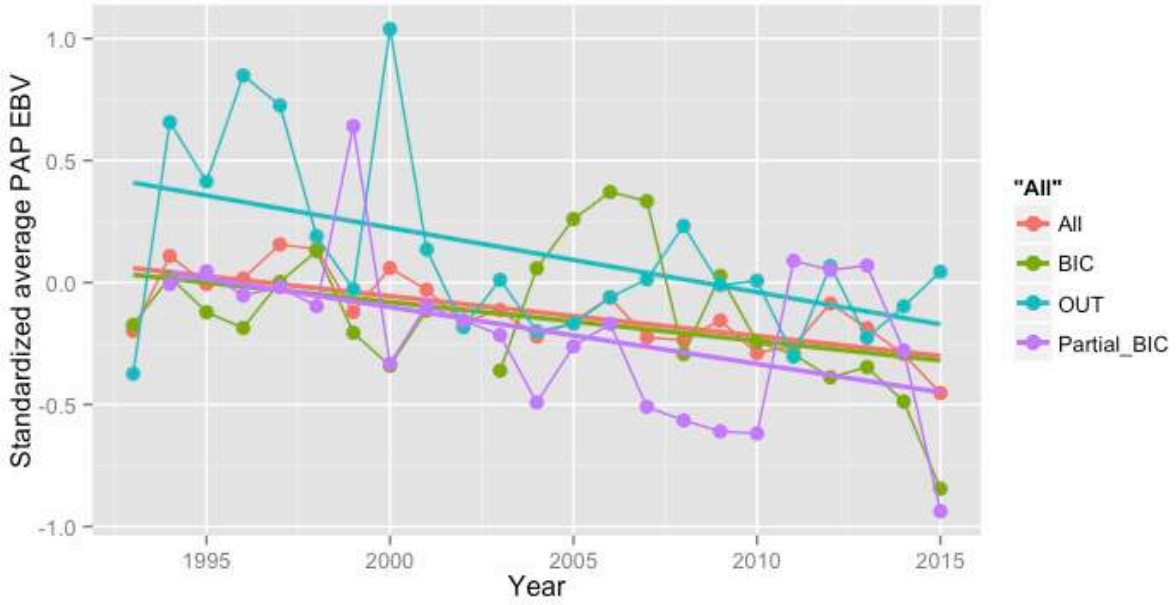


Figure 3.5 Genetic trends and associated regression lines across year from 1993 to 2015 of animals from all sires (All), the registered external sires (OUT), the CSU-BIC sires (BIC), and the partial CSU-BIC sires (Partial_BIC) on original yearling pulmonary arterial pressure measurements in Angus cattle managed at high altitude (elevation at 2,170 m)

3.3.3.4 Genetic relationship between PAP in different sex categories

Tables 3.11 to 3.14 present the genetic correlations between sex categories of RAW, PT, CAT3 and CAT2. Table 3.15 summarizes the log-likelihood ratio tests on sex-differences for different yearling PAP phenotypes. The log-likelihood ratio tests revealed difference ($P < 0.05$) among bulls, heifers and steers, which suggested that PAP phenotypes in different sex categories were not genetically identical for all forms of PAP phenotypes. Non-identical genetic correlations and varied heritability estimates, genetic and residual variances across heifer, bull and steer PAP in different phenotypic forms may contribute to these results. The cross-sex genetic correlations were examined on a large number of traits of different species, Poissant et al. (2009) summarized these correlations for 310 traits from 42 animal species and reported that the average cross sex correlations were about 0.80 ± 0.03, 0.77 ± 0.09, 0.73 ± 0.05 and 0.62 ± 0.07

for morphological, behavioral, developmental and physiological traits, respectively. The genetic correlation estimates between heifers and bulls in current study were close to these average values.

The genetic correlations between RAW in varied sex categories was similar to those associated with PT ($P>0.05$), and the heritability estimate of bulls, heifers and steers were similar between RAW and PT ($P>0.05$). These results demonstrated the limited influence of the violation of normality. It was reported that violation of normality should not cause major problem in regression analysis when sample size was large, while it could introduce different statistic results when sample size was small (Ghasemi and Zahediasl, 2012; Statistics Solutons, 2013). In RAW and PT, the genetic correlations between heifer and steer were high ( > 0.95), but the genetic correlation between bull and heifer or steer were lower (< 0.82). Also, the average PAP score of bulls were higher than the average of heifers and steers ($P < 0.05$), and the average PAP scores of heifers were similar with that of steers ($P > 0.05$; Table 3.1).

Holt and Callen (2007) implied no physiologic basis for a different in PAP measurements between male and females cattle. This presented information suggested that, other than sex effects, the different management environments might contribute to the genetic difference between the yearling PAP measurements of sex categories, since the post-weaning managements were different between bulls and heifers or steers, but similar between heifers and steers. In this Angus herd, before yearling, the bulls were fed and managed in a gain test (with average daily gain at about 1.5kg/day), and the steers were castrated and grazed with heifers (with average daily gain at about 0.5 kg/day). Neary et al. (2015) suggested that cattle were more susceptible to develop pulmonary hypertension when they were managed for high levels of gain. However, Crawford et al. (2016) reported weak genetic relationship between PAP measurements and post-

weaning growth traits (i.e. PWG and YW). The results supported there may be a genetic by environmental effect from production management type on yearling PAP measurements.

In categorical phenotypes, the genetic correlation between bulls and heifers were higher than those with continuous scale phenotypes, but bulls still had higher heritability than heifers. The genetic correlations between steers and bulls or heifers were all moderate in category phenotypes and lower than those of continuous phenotypes. This illustrated the categorical PAP was not genetically identical across heifers, bulls and steers, and both the sex effect and management of gain would contribute to these genetic difference between sexes. Shirley et al. (2008) reported the genetic correlation between male and female weaning PAP measurements as 0.64. Darling and Holt (1999) suggested that an abnormal Y chromosome could be associated with susceptibility of male cattle to HAD. Also, Jin (2010) reported that the incidence of chronic high altitude disease and acute high altitude disease were more frequent in human males. This information collectively suggest possible different genetic basis between males and females for susceptibility of HAD and the PAP.

Table 3.11. Heritability, genetic variance and genetic correlation (above diagonal) between non-transformed PAP measurements of heifer, bull and steer Angus cattle managed at high altitude (elevation at 2,170 m)

| Sex | Heifer | Bull | Steer |
|---|---|---|---|
| Heifer | 0.19 (0.03) | 0.82 (0.10) | 0.99 (0.01) |
| Bull | 0 | 0.37 (0.07) | 0.81 (0.09) |
| Steer | 0 | 0 | 0.33 (0.06) |

Table 3.12. Heritability, genetic variance and genetic correlation (above diagonal) between power-transformed pulmonary arterial pressure measurements of heifer, bull and steer Angus cattle managed at high altitude (elevation at 2,170 m)

| Sex | Heifer | Bull | Steer |
|---|---|---|---|
| Heifer | 0.23 (0.06) | 0.78 (0.08) | 0.95 (0.04) |
| Bull | 0 | 0.41 (0.06) | 0.67 (0.17) |
| Steer | 0 | 0 | 0.25 (0.06) |

Table 3.13. Heritability, genetic variance and genetic correlation (above diagonal) between three-category pulmonary arterial pressure phenotype of heifer, bull and steer Angus cattle managed at high altitude (elevation at 2,170 m)

| Sex | Heifer | Bull | Steer |
|---|---|---|---|
| Heifer | 0.21 (0.03) | 0.96 (0.06) | 0.46 (0.18) |
| Bull | 0 | 0.43 (0.07) | 0.58 (0.16) |
| Steer | 0 | 0 | 0.45 (0.12) |

Table 3.14. Heritability, genetic variance and genetic correlation (above diagonal) between two-category pulmonary arterial pressure phenotype of heifer, bull and steer Angus cattle managed at high altitude (elevation at 2,170 m)

| Sex | Heifer | Bull | Steer |
|---|---|---|---|
| Heifer | 0.30 (0.06) | 0.87 (0.12) | 0.32 (0.33) |
| Bull | 0 | 0.49 (0.07) | 0.63 (0.19) |
| Steer | 0 | 0 | 0.54 (0.11) |

Table 3.15 Log likelihood ratio test for sex effects of four yearling pulmonary arterial pressure phenotypes of Angus cattle managed at high altitude (elevation at 2,170 m)

| Effect | $LogL_f$ | $LogL_r$ | $-2(LogL_r-LogL_f)$ | df | P-Value |
|---|---|---|---|---|---|
| RAW | -4960.61 | -5060.56 | 199.90 | 7 | <0.01 |
| PT | -5319.08 | -5331.15 | 24.14 | 7 | <0.01 |
| CAT3 | -352.94 | -362.90 | 19.92 | 7 | <0.01 |
| CAT2 | 3793.07 | 3670.87 | 244.40 | 7 | <0.01 |

[1]PT: powered transformed yearling PAP, $10000*(PAP)^2$; CAT3: three-category phenotype, 1: PAP < 41 mmHg, 2: 41 mmHg ≤ PAP ≤ 49 mmHg, 3: PAP > 49 mmHg; CAT2: two-category phenotype, 1: PAP ≤ 49 mmHg, 2: PAP > 49 mmHg

Table 3.16 to 3.19 present the Pearson and Rank correlations between EBV predicted from all cattle, bull, heifer and steer yearling PAP phenotypes (i.e. RAW, PT, CAT3 and CAT2). The yearling PAP EBV correlations among sexes were consistent between RAW and PT. They were all high with the lowest observed value as 0.89. The lowest correlations associated with RAW

and PT were identified between PAP EBV from bulls and steers, which corresponded to estimated genetic correlations and also suggested that management of gain has an influence on PAP measurements. The EBV correlations between all animals and steers were larger than 0.76 in categorical phenotypes, and some re-ranking was identified between the two sets of EBV. The smaller sample size of steers compared to heifers and bulls and the estimated moderate genetic correlation in categorical phenotypes would lead to these EBV correlations. In addition, high Pearson and Rank correlations (>0.9) were identified between EBV from all cattle and heifer or bull yearling PAP data in each PAP phenotypic form, which suggested similar rank of animals based on all, heifer and bull PAP EBV. These results suggested that designating yearling PAP among sexes as separate traits was not necessary when calculating EBV because of these high correlations between EBV from all animals' PAP phenotypes and PAP measurements in different sexes. In addition, it should be noted that since the EBV was shrunk toward the mean and the parents average, especially for individuals with less amount of information, and different individuals had missing phenotypes in bulls, steers and heifers data compared to data for all cattle, the EBV from all, bull, heifer, steer data were shrunk differently, which may introduce a few bias in the correlations between EBV.

Van Vleck and Cundif (1998) identified high cross-sex correlations (>0.85) of cattle for WW and YW, and reported that these genetic correlations were large enough to consider male and female weight as the same traits in genetic selection. Moderately to highly positive cross-sex correlations were identify for weaning gain and post-weaning gain in the study of Stålhammar and Philipsson (1997), and they suggested that sex-specific parameters should be used in an evaluation system of post-weaning gain. However, treating phenotypes from different sexes as separate traits would result in more than one set of EBV in an evaluation system, which would

introduce more complexity in selection systems and subsequently how to use or incorporate these EBV. It should also be noted that moderate to high cross-sex correlations between bulls and heifers were identified for real time ultrasound traits (e.g. subcutaneous fat thickness, longissimus muscle area and intramuscular fat percentage); thus bull and heifer ultrasound traits could be considered as separated traits in models for genetic evaluation of carcass traits (all measurements were considered as the same trait) because of the high genetic correlations between ultrasound and carcass traits (Crews and Kemp, 2001; Crews et al, 2003; Reverter et al., 2000). However, there is no end-use trait when applying PAP phenotypes among sexes as separate traits. Besides the high cross-sex EBV correlations, treating PAP of different sexes as the same trait would result in simpler evaluation model and easier interpretation and application of EBV.

Table 3.16. Pearson (above diagonal) and rank (below diagonal) correlations between estimated breeding value from non-transformed pulmonary arterial pressure measurements of all animals, heifer, bull and steer Angus cattle managed at high altitude (elevation at 2,170 m)

| Sex | All | Heifer | Bull | Steer |
|---|---|---|---|---|
| All | 1 | 0.95 | 0.93 | 0.95 |
| Heifer | 0.95 | 1 | 0.96 | 1.00 |
| Bull | 0.93 | 0.94 | 1 | 0.95 |
| Steer | 0.95 | 1.00 | 0.93 | 1 |

Table 3.17. Pearson (above diagonal) and rank (below diagonal) correlations between estimated breeding value from power-transformed pulmonary arterial pressure measurements of all animals, heifer, bull and steer Angus cattle managed at high altitude (elevation at 2,170 m)

| Sex | All | Heifer | Bull | Steer |
|---|---|---|---|---|
| All | 1 | 0.96 | 0.90 | 0.96 |
| Heifer | 0.95 | 1 | 0 .92 | 0.99 |
| Bull | 0.90 | 0.92 | 1 | 0.89 |
| Steer | 0.95 | 0.99 | 0.88 | 1 |

Table 3.18. Pearson (above diagonal) and rank (below diagonal) correlations between estimated breeding value from three-category pulmonary arterial pressure phenotype of all animals, heifer, bull and steer Angus cattle managed at high altitude (elevation at 2,170 m)[1]

| Sex | All | Heifer | Bull | Steer |
|---|---|---|---|---|
| All | 1 | 0.95 | 0.95 | 0.80 |
| Heifer | 0.95 | 1 | 0.99 | 0.72 |
| Bull | 0.95 | 0.99 | 1 | 0.78 |
| Steer | 0.77 | 0.65 | 0.72 | 1 |

[1]three-categorical phenotype: 1: PAP < 41 mmHg, 2: 41 mmHg ≤ PAP ≤ 49 mmHg, 3: PAP > 49 mmHg

Table 3.19. Pearson (above diagonal) and rank (below diagonal) correlations between estimated breeding value from two-category pulmonary arterial pressure phenotype of all animals, heifer, bull and steer Angus cattle managed at high altitude (elevation at 2,170 m)[1]

| Sex | All | Heifer | Bull | Steer |
|---|---|---|---|---|
| All | 1 | 0.93 | 0.93 | 0.78 |
| Heifer | 0.92 | 1 | 0.97 | 0.72 |
| Bull | 0.92 | 0.96 | 1 | 0.85 |
| Steer | 0.76 | 0.72 | 0.84 | 1 |

[1]two-categorical phenotype: 1: PAP ≤ 49 mmHg, 2: PAP > 49 mmHg

## 3.4 Conclusions

Although the non-transformed yearling PAP measurements were not normally distributed, the violation of normality had limited influence on the significance of fixed effects tests and genetic evaluation of yearling PAP measurements. Sex, age of dam, measurement date of PAP and age of PAP (covariate) were important explanatory variables of PAP phenotypes and the overall $R^2$ of these variables on RAW, PT, CAT3 and CAT2 were 0.10, 0.16, 0.15 and 0.14, respectively. Losses of EBV accuracy were identified for ordinal categorical phenotypes compared with continuous phenotypes. Ordered categorical phenotypes can be alternative dependent variables in studying characteristics of PAP and selecting against elevated PAP, however, they would cause some re-ranking of sires related to non-transformed PAP scores. In genetic evaluation, the non-transformed yearling PAP measurements were preferred based on the

similar heritability, higher accuracy and same scale of the trait interpretation that ease breeder understanding. The PAP measurements of different sexes were identified genetically un-identical. However, it is not necessary to treat yearling PAP as separate traits by sex in genetic evaluation because the EBV from all PAP and PAP of different sexes would yield similar ranking of animals.

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# CHAPTER 4

# GENETIC RELATIONSHIP BETWEEN PULMONARY ARTERIAL PRESSURE PHENOTYPES AND PERFORMANCE TRAITS OF ANGUS CATTLE MANAGED AT HIGH ALTITUDE

## 4.1 Introduction

This chapter addresses the relationship between yearling PAP phenotypes and commonly measured performance traits. Excess weight gains in human and animals (i.e. cattle and chicken) could increase PAP and the risk for PH, HAD and right-side congestive heart failure (Peacock et al., 1989; Jin et al., 2009; Neary, 2014), which suggests that performance traits could be genetically related to PAP measurements, PH and susceptibility to HAD. Shirley (2008) reported a moderate-positive but unfavorable genetic relationship between weaning PAP measurements and pre-weaning growth traits (i.e. birth weight and weaning weight) in Angus cattle. Crawford et al. (2016) reported a low genetic correlation between PAP measurements and performance traits using data similar to this study, but this study included additional phenotypes from steers of this herd and phenotypes from the year of 2015.

In order to assess the influence of the violation of normality on genetic correlations and examine the genetic correlation between PH and susceptibility to HAD, genetic correlations were also studied for alternative phenotypes (power-transformation, three-category and two-category phenotypes). These genetic correlations and the EBV accuracies between alternative multivariate models helped develop the multivariate model to estimate EBV of PAP measurements (un-transformed PAP measurements) and susceptibility to HAD (categorical phenotypes) in Angus cattle. Although the resulting EBV were highly correlated between PAP phenotypes from

different sex categories (i.e., bull, heifer and steer; Chapter 3), this study found the genetic dissimilarity between them (i.e., different heritability and moderate to high genetic correlations; Chapter 3). Therefore, it is reasonable to explore the genetic correlations between performance traits and yearling PAP by sex categories to help understand more about the relationship between yearling PAP phenotypes and performance traits under various management-environmental situations.

## 4.2 Materials and methods

### 4.2.1 Data

Yearling PAP records used in this section were the same with those used in Chapter 3. Besides the non-transformed yearling PAP measurements (RAW), the alternative phenotypes included power-transformed (PT) yearling PAP measurements, three-category (CAT3) and two-category (CAT2) phenotypes that were defined in Chapter 3. The performance data used in this section were collected from 1993 to 2015 from the Angus herd that was described in Chapter 3. The studied performance traits included birth weight (BWT), weaning weight (WW), post-weaning gain (PWG) and yearling weight (YW). Three standard deviations around the mean and the phenotypic distribution of each phenotyope were used to decide the ranges of the phenotypes to be included in analyses. Table 4.1 presents the descriptive statistic of these traits and their associated ages. Also, the pedigree information used in Chapter 3 was also applied in this section.

Table 4.1. Summary statistics of performance traits and associated ages in Angus cattle managed at high altitude (elevation at 2,170 m)

| Traits[1] | n | Mean | Min | Max | SD |
|---|---|---|---|---|---|
| BWT (kg) | 9024 | 36.21 | 18.14 | 54.43 | 5.05 |
| WW (kg) | 8328 | 213.88 | 71.67 | 338.834 | 31.43 |
| WAGE (days) | 8328 | 184.30 | 106.00 | 256.00 | 22.56 |
| PWG (kg) | 5529 | 127.29 | 0.91 | 311.62 | 65.59 |
| YW (kg) | 5569 | 344.85 | 180.53 | 584.23 | 81.90 |
| YAGE (days) | 5569 | 354.30 | 261.00 | 528.00 | 38.65 |

[1]BWT: birth weight; WW: weaning weight; PWG: post-weaning gain; YW: yearling weight; WAGE: weaning age; YAGE: yearling age

4.2.2 Fixed effects

The fixed effects for all yearling PAP phenotypes included sex, age of dam, PAP measurement date and age (covariate). When studying yearling PAP separately by sexes, the fixed effect "sex" was not included in these models. The potential fixed effects for performance traits included sex, age of dam, age of measurements (except BWT; covariate) and contemporary group (CG). Sex categories for BWT were male and female, and those for other performance traits involved bull, heifer and steer. The birth CG for BWT only included year of birth; the weaning CG for WW was defined as birth CG and weaning date; and the yearling CG for PWG and YW included weaning CG and yearling date. These fixed effects were evaluated using log-likelihood ratio tests (LR; Equation 3.1) for each trait by comparing the likelihood value of the full model (including all potential effects) and reduced model (including all effects except the one in test; Equation 3.1). The estimats of fixed effects and likelihood values of models were obtained from regression analyses using the Maximum likelihood method in R (R core Team, 2013; using packages "stat" and "ordinal"). Linear models were used to analyze RAW and PT, while threshold models were spplied to CAT3 and CAT2.

4.2.3 Genetic parameters

Genetic correlations between yearling PAP phenotypes and direct BWT, direct WW, maternal WW (MILK), and PWG were estimated from multivariate linear (used for RAW and PT) and threshold (used for CAT3 and CAT2) maternal models, and bivariate linear (used for RAW and PT) and threshold (used for CAT3 and CAT2) animal models were used to estimate genetic correlation between yearling PAP phenotypes and YW. The differences in genetic evaluation of the various yearling PAP phenotypes were evaluated by analyzing the differences between estimated genetic correlations (with performance traits) across RAW, PT, CAT3 and CAT2. The multivariate models for estimating genetic correlations between yearling PAP phenotypes and performance traits were expressed as

$$\begin{vmatrix} \mathbf{y}_{pap} \\ \mathbf{y}_{bw} \\ \mathbf{y}_{ww} \\ \mathbf{y}_{pwg} \end{vmatrix} = \begin{vmatrix} \mathbf{X}_{pap} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_{bw} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{X}_{ww} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{X}_{pwg} \end{vmatrix} \begin{vmatrix} \beta_{pap} \\ \beta_{bw} \\ \beta_{ww} \\ \beta_{pwg} \end{vmatrix} + \begin{vmatrix} \mathbf{Z}_{pap} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_{bw} & \mathbf{Z}_{M_{bw}} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{Z}_{ww} & \mathbf{Z}_{M_{ww}} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{Z}_{pwg} \end{vmatrix} \begin{vmatrix} \mathbf{u}_{pap} \\ \mathbf{u}_{bw} \\ \mathbf{u}_{M_{bw}} \\ \mathbf{u}_{ww} \\ \mathbf{u}_{M_{ww}} \\ \mathbf{u}_{pwg} \end{vmatrix} + \begin{vmatrix} \mathbf{e}_{pap} \\ \mathbf{e}_{bw} \\ \mathbf{e}_{ww} \\ \mathbf{e}_{pwg} \end{vmatrix} \quad \text{(Equation 4.1)}$$

$$\text{var} \begin{vmatrix} \mathbf{u}_{pap} \\ \mathbf{u}_{bw} \\ \mathbf{u}_{M_{bw}} \\ \mathbf{u}_{ww} \\ \mathbf{u}_{M_{ww}} \\ \mathbf{u}_{pwg} \\ \mathbf{e}_{pap} \\ \mathbf{e}_{bw} \\ \mathbf{e}_{ww} \\ \mathbf{e}_{pwg} \end{vmatrix} = \begin{vmatrix} \mathbf{A}\sigma^2_{a_{pap}} & \mathbf{A}\sigma_{a_{pap\cdot bw}} & \mathbf{0} & \mathbf{A}\sigma_{a_{pap\cdot ww}} & \mathbf{A}\sigma_{a_{pap}\cdot m_{ww}} & \mathbf{A}\sigma_{a_{pap\cdot pwg}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{A}\sigma_{a_{pap\cdot bw}} & \mathbf{A}\sigma^2_{a_{bw}} & \mathbf{0} & \mathbf{A}\sigma_{a_{bw\cdot ww}} & \mathbf{0} & \mathbf{A}\sigma_{a_{bw\cdot pwg}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{A}\sigma^2_{m_{bw}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{A}\sigma_{a_{pap\cdot ww}} & \mathbf{A}\sigma_{a_{bw\cdot ww}} & \mathbf{0} & \mathbf{A}\sigma^2_{a_{ww}} & \mathbf{0} & \mathbf{A}\sigma_{a_{ww\cdot pwg}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{A}\sigma_{a_{pap}\cdot m_{ww}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{A}\sigma^2_{m_{ww}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{A}\sigma_{a_{pap\cdot pwg}} & \mathbf{A}\sigma_{a_{bw\cdot pwg}} & \mathbf{0} & \mathbf{A}\sigma_{a_{ww\cdot pwg}} & \mathbf{0} & \mathbf{A}\sigma^2_{a_{pwg}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma^2_{pap} & \mathbf{I}\sigma_{pap\cdot bw} & \mathbf{I}\sigma_{pap\cdot ww} & \mathbf{I}\sigma_{pap\cdot pwg} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_{pap\cdot bw} & \mathbf{I}\sigma^2_{bw} & \mathbf{I}\sigma_{bw\cdot ww} & \mathbf{I}\sigma_{bw\cdot pwg} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_{pap\cdot ww} & \mathbf{I}\sigma_{bw\cdot ww} & \mathbf{I}\sigma^2_{ww} & \mathbf{I}\sigma_{ww\cdot pwg} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_{pap\cdot pwg} & \mathbf{I}\sigma_{bw\cdot pwg} & \mathbf{I}\sigma_{ww\cdot pwg} & \mathbf{I}\sigma^2_{pwg} \end{vmatrix}$$

where $\mathbf{y}_{pap}$ was each phenotypic form of PAP measurements (i.e. RAW, PT, CAT3 and CAT2), and $\beta_{pap}$ were the vectors of fixed effects of PAP phenotypes. The $\mathbf{X}_{pap}$ were incidence matrices relating PAP observations to fixed effects. The $\mathbf{u}_{pap}$ were vectors of direct random effects on PAP observations, and $\mathbf{e}_{pap}$ was the random residual effect for PAP. The $\sigma^2_{a_{pap}}$ and $\sigma^2_{pap}$ were genetic and residual variance for PAP phenotypes. The $\sigma_{a_{pap\cdot bw}}$, $\sigma_{a_{pap\cdot ww}}$, $\sigma_{a_{pap}\cdot m_{ww}}$, and $\sigma_{a_{pap\cdot pwg}}$ represented the genetic covariance between PAP phenotypes and performance traits, and $\sigma_{pap\cdot bw}$, $\sigma_{pap\cdot ww}$, and $\sigma_{pap\cdot pwg}$ were residual covariance between PAP phenotypes and performance traits. The $\mathbf{y}_{bw}$, $\mathbf{y}_{ww}$, and $\mathbf{y}_{pwg}$ denoted the observations of BWT, WW and PWG, and $\beta_{bw}$, $\beta_{ww}$, and $\beta_{pwg}$ were the vectors of fixed effects on these traits. The $\mathbf{X}_{bw}$, $\mathbf{X}_{ww}$, and $\mathbf{X}_{pwg}$ were incidence matrices relating performance observations to their fixed effects. The $\mathbf{u}_{bw}$, $\mathbf{u}_{ww}$, and $\mathbf{u}_{pwg}$ were vectors of direct random effects on BWT, WW and PWG observations, and $\mathbf{u}_{M_{bw}}$ and $\mathbf{u}_{M_{ww}}$ were maternal random effects for BWT and WW. The $\mathbf{Z}_{bw}$, $\mathbf{Z}_{ww}$, $\mathbf{Z}_{pwg}$ and were incidence matrices relating direct random effects on these observations, and $\mathbf{Z}_{M_{bw}}$ and $\mathbf{Z}_{M_{ww}}$ denoted the incidence matrices relating maternal random effects on phenotype observations. The $\mathbf{e}_{bw}$, $\mathbf{e}_{ww}$, and $\mathbf{e}_{pwg}$ denoted the random residual effects on these observations. The $\sigma^2_{a_{bw}}$, $\sigma^2_{a_{ww}}$, $\sigma^2_{a_{pwg}}$, $\sigma^2_{m_{bw}}$, and $\sigma^2_{m_{ww}}$ were genetic variances of direct effects of BWT, WW, and PWG and maternal effects of BWT and WW, respectively. The $\sigma^2_{bw}$, $\sigma^2_{ww}$, and $\sigma^2_{pwg}$ were the residual variances of phenotype observations. The, $\sigma_{a_{bw\cdot ww}}$, $\sigma_{a_{bw\cdot pwg}}$ and $\sigma_{a_{ww\cdot pwg}}$ were direct genetic covariance between BWT, WW and PWG. The direct-maternal covariance involved genetic and environmental components, and the direct-maternal environmental covariance would inflate the estimates of direct-maternal genetic

covariance (Meyer, 1997). In addition, direct-maternal genetic covariance could influence the heritability of traits. In this, the genetic correlations between direct effects for performance traits (BWT, WW, PWG and YW) and maternal effects for BW and WW were fixed as 0. In order to explore the genetic correlation between PAP phenotypes and maternal effects weaning weight (Milk), $\sigma_{a_{pap} \cdot m_{ww}}$ was not fixed as 0 in these models. The genetic covariance between yearling PAP phenotype and maternal BWT were fixed as 0, since this parameter did not converage well. The bivariate models for estimating genetic correlations between yearling weight and PAP phenotypes were expressed as:

$$\begin{vmatrix} \mathbf{y}_{pap} \\ \mathbf{y}_{yw} \end{vmatrix} = \begin{vmatrix} \mathbf{X}_{pap} & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_{yw} \end{vmatrix} \begin{vmatrix} \beta_{pap} \\ \beta_{yw} \end{vmatrix} + \begin{vmatrix} \mathbf{Z}_{pap} & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_{yw} \end{vmatrix} \begin{vmatrix} \mathbf{u}_{pap} \\ \mathbf{u}_{yw} \end{vmatrix} + \begin{vmatrix} \mathbf{e}_{pap} \\ \mathbf{e}_{yw} \end{vmatrix} \quad \text{(Equation 4.2)}$$

$$\text{var} \begin{vmatrix} \mathbf{u}_{pap} \\ \mathbf{u}_{yw} \\ \mathbf{e}_{pap} \\ \mathbf{e}_{yw} \end{vmatrix} = \begin{vmatrix} \mathbf{A}\sigma^2_{a_{pap}} & \mathbf{A}\sigma_{a_{pap \cdot yw}} & \mathbf{0} & \mathbf{0} \\ \mathbf{A}\sigma_{a_{pap \cdot yw}} & \mathbf{A}\sigma^2_{a_{yw}} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma^2_{pap} & \mathbf{I}\sigma_{pap \cdot yw} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_{pap \cdot yw} & \mathbf{I}\sigma^2_{yw} \end{vmatrix}$$

where $\mathbf{y}_{pap}$, $\mathbf{X}_{pap}$, $\mathbf{Z}_{pap}$, $\beta_{pap}$, $\mathbf{u}_{pap}$, $\mathbf{e}_{pap}$, $\sigma^2_{a_{pap}}$, $\mathbf{A}$ and $\mathbf{I}$ were defined the same as Equation 4.2. The $\mathbf{y}_{yw}$ denoted the observations of YW, and the $\beta_{yw}$, $\mathbf{u}_{yw}$ and $\mathbf{e}_{yw}$ were the fixed, random additive genetic and residual effects for YW, respectively. The $\mathbf{X}_{yw}$ and $\mathbf{Z}_{yw}$ were the incidence matrices relating the YW observations to their fixed and random effects, respectively. The $\sigma^2_{a_{yw}}$ and $\sigma^2_{yw}$ were the additive genetic and residual variances of YW, and the $\sigma_{a_{pap \cdot yw}}$ and $\sigma_{pap \cdot yw}$ denoted the genetic and residual covariance between yearling PAP phenotypes and YW. These multivariate and bivariate models were also used to obtain genetic correlations between performance traits and PAP phenotypes separated by sex by replacing the PAP phenotypes with heifer, bull and steer PAP phenotypes. Because of the issue of parameters' computational converagence, genetic

correlation between maternal WW and PAP phenotypes were also controlled to be zero in these sex-separate multivariate analyses to correctly assess the genetic correlations between PAP phenotypes and direct effect of growth performance traits..

4.2.4 EBV and accuracy

In order to be consistent across all models in estimating breeding value and associated accuracies for varied PAP phenotypes, genetic parameters of growth traits used in multivariate or bivariate models were from performance-traits only models. The genetic parameters that were associated with BWT, WW and PWG were estimated from multivariate maternal mixed models. The fixed effects for growth traits in this model were the same with those used in the multivariate or bivariate models for PAP (Equation 4.1 and Equation 4.2). The maternal effect was included in the model for BWT and WW (maternal WW effects were used to study the genetic parameters for milk), and the permenant maternal effect was included for WW. Because of the part-whole relationship between WW, PWG and YW, the genetic parameters for YW were estimated separately. The heritability of YW and genetic correlations between YW and other growth traits were estimated from univaraite or bivariate animal models, respectively. The multivariate model for performance traits was expressed in the following Equation 4.3, and the model terms in this equation were defined in Equation 4.2.

$$\begin{vmatrix} \mathbf{y}_{bw} \\ \mathbf{y}_{ww} \\ \mathbf{y}_{pwg} \end{vmatrix} = \begin{vmatrix} \mathbf{X}_{bw} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_{ww} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{X}_{pwg} \end{vmatrix} \begin{vmatrix} \beta_{bw} \\ \beta_{ww} \\ \beta_{pwg} \end{vmatrix} + \begin{vmatrix} \mathbf{Z}_{bw} & \mathbf{Z}_{M_{bw}} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{Z}_{ww} & \mathbf{Z}_{M_{ww}} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{Z}_{pwg} \end{vmatrix} \begin{vmatrix} \mathbf{u}_{bw} \\ \mathbf{u}_{M_{bw}} \\ \mathbf{u}_{ww} \\ \mathbf{u}_{M_{ww}} \\ \mathbf{u}_{pwg} \end{vmatrix} + \begin{vmatrix} \mathbf{e}_{bw} \\ \mathbf{e}_{ww} \\ \mathbf{e}_{pwg} \end{vmatrix} \quad \text{(Equation 4.3)}$$

$$
\begin{vmatrix} \mathbf{u}_{bw} \\ \mathbf{u}_{M_{bw}} \\ \mathbf{u}_{ww} \\ \mathbf{u}_{M_{ww}} \\ \mathbf{u}_{pwg} \\ \mathbf{e}_{bw} \\ \mathbf{e}_{ww} \\ \mathbf{e}_{pwg} \end{vmatrix} = \begin{vmatrix} \mathbf{A}\sigma^2_{a_{bw}} & \mathbf{0} & \mathbf{A}\sigma_{a_{bw\text{-}ww}} & \mathbf{0} & \mathbf{A}\sigma_{a_{bw\text{-}pwg}} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{A}\sigma^2_{m_{bw}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{A}\sigma_{a_{bw\text{-}ww}} & \mathbf{0} & \mathbf{A}\sigma^2_{a_{ww}} & \mathbf{0} & \mathbf{A}\sigma_{a_{ww\text{-}pwg}} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{A}\sigma^2_{m_{ww}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{A}\sigma_{a_{bw\text{-}pwg}} & \mathbf{0} & \mathbf{A}\sigma_{a_{ww\text{-}pwg}} & \mathbf{0} & \mathbf{A}\sigma^2_{a_{pwg}} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma^2_{bw} & \mathbf{I}\sigma_{bw\text{-}ww} & \mathbf{I}\sigma_{bw\text{-}pwg} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_{bw\text{-}ww} & \mathbf{I}\sigma^2_{ww} & \mathbf{I}\sigma_{ww\text{-}pwg} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_{bw\text{-}pwg} & \mathbf{I}\sigma_{ww\text{-}pwg} & \mathbf{I}\sigma^2_{pwg} \end{vmatrix}
$$

Multivariate and bivariate models were excuted to obatained the EBV and associated accuracy for each PAP phenotype: Model 1 (including PAP, BWT, WW, PWG) and Model 2 (only including PAP and YW). The software outputted the EBV and associated standard error, and the EBV associated BIF accuracies were calculated from equation 3.6 discussed in Chapter 3. The accuracies of PAP EBV from univariate models (from Chapter 3), multivariate and bivariate models were compared to quantify the improvement in genetic evaluation on PAP from multivariate or bivariate analysis. The EBV and accuracies of RAW, PT, CAT3 and CAT2 were also compared to assess the difference in multivariate genetic evaluation of PAP in different phenotypic forms. The models used for estimating genetic parameters, breeding value and corresponding accuracies were analyzed using Gibbs sampling algorithm in software packages: renumf90, thrgibbs1f90 and postgibbs1f90 (Tsuruta and Misztal, 2006; Aguilar et al., 2014). A total of 250,000 iterations were run with the first 50,000 discarded as burn-in, thinning every 10 samples, which resulted in sample size 20,000 for each estimate.

## 4.3 Results and Discussion

### 4.3.1 Fixed effects

The fixed effects were tested in Chapter 3 (Table 3.2). The studied fixed effects were all associated ($P < 0.05$) with BWT, WW, PWG and YW based on the log-likelihood ratio tests (Table 4.2). Males had higher weights than females for all the performance traits, and the bulls had higher average weights than heifers and steers ($P < 0.05$). These results were consistent with the physiology of bulls and the management strategies in the herd (Soffe, 2011). The mature dam (from 5 years old to 10 years old) yielded significant higher BWT, WW and YW for calves than other age of dam categories, while calves from them were associated with alightly lower PWG than other dam category (except heifer dam). Claves from heifer dams yielded ligher weights in all measurement stages. This coincided with reports that the mature dam should produce larger calves (BIF, 2010). The lower PWG for mature cows may because their calves had higher weaning weights; therefore, their calves experienced less compensatory growth when the pre-weaning environment provide by the dam was sufficient (Young et al., 1978; Elzo et al., 1987). All these effects were included in the models for performance traits in following studies.

Table 4.2. Results of log-likelihood ratio tests of fixed effects for performance traits in Angus cattle at high altitude (elevation range from 2,170 m to 2,740 m)

| Effect[1] | $LogL_f$[2] | $LogL_r$[2] | $-2(LogL_r-LogL_f)$[2] | df | P-Value |
|---|---|---|---|---|---|
| BWT | | | | | |
| YOB | -33413.56 | -33790.56 | 753.99 | 22 | <0.01 |
| AOD | -33413.56 | -33964.58 | 1102.04 | 4 | <0.01 |
| SEX | -33413.56 | -33726.26 | 625.40 | 1 | <0.01 |
| WW | | | | | |
| WCG | -44177.44 | -44908.84 | 1462.80 | 25 | <0.01 |
| AOD | -44177.44 | -44712.46 | 1070.03 | 4 | <0.01 |
| SEX | -44177.44 | -44929.49 | 1504.104 | 2 | <0.01 |
| wage | -44177.44 | -45670.53 | 2986.17 | 1 | <0.01 |
| PWG | | | | | |
| YCG | -28248.60 | -30961.51 | 5425.81 | 56 | <0.01 |
| AOD | -28248.60 | -28271.26 | 45.31 | 4 | <0.01 |
| SEX | -28248.60 | -28277.61 | 58.02 | 2 | <0.01 |
| yage | -28248.60 | -28337.76 | 178.31 | 1 | <0.01 |
| YW | | | | | |
| YCG | -30755.03 | -32199.29 | 2888.52 | 57 | <0.01 |
| AOD | -30755.03 | -31009.52 | 508.97 | 4 | <0.01 |
| SEX | -30755.03 | -30788.69 | 67.32 | 2 | <0.01 |
| yage | -30755.03 | -31508.26 | 1506.44 | 1 | <0.01 |

[1]BWT: birth weight; WW: weaning weight; PWG: post-weaning gain; YW: yearling weight; YOB: year of birth; AOD: age of dam; WCG: weaning contemporary group; wage: weaning age; YCG: yearling contemporary group; yage: yearling age.
[2] $LogL_f$: log likelihood value of the whole model; $LogL_r$: log likelihood value of the reduced model with excluding one of the fixed effects for each traits

4.3.2 Genetic correlations between yearling PAP and performance traits

The genetic correlations between performance traits and yearling PAP in different phenotypic forms are presented in Table 4.3. Low to moderate genetic correlations were identified between these performance traits and yearling PAP phenotypes. These estimated correlations were similar between RAW and PT, which suggested that the violation of normality had limited influence on the estimated genetic correlations between yearling PAP measurements and performance traits. The genetic correlations involving CAT3 and CAT2 were slightly larger than those from RAW and PT, but the difference were not statistically significant ($P > 0.05$). These similar genetic correlations also supported that the ordered categorical phenotypes can be

alterative phenotypes to study characteristic of PAP in cattle. The estimated genetic correlations between RAW and growth traits were similar with the reports of Crawford et al. (2016) who reported genetic correlation ranged from -0.10 to 0.23, except that we identified positive genetic correlation between RAW and PWG although it is weak. This difference may be due to the exclusion of steer data and the addition of one more year of data (2015).

Table 4.3 Genetic correlations between yearling pulmonary arterial pressure phenotypes (PAP) and performance traits of Angus cattle managed at high altitude (elevation at 2,170 m)

| Phenotypes[1] | Model | BWT | WW | MILK | PWG | YW |
|---|---|---|---|---|---|---|
| RAW | Linear | 0.22 (0.09) | 0.16 (0.13) | 0.10 (0.15) | 0.03 (0.11) | 0.11 (0.09) |
| PT | Linear | -0.22 (0.08) | -0.21 (0.12) | -0.10 (0.12) | -0.08 (0.10) | -0.19 (0.08) |
| CAT2 | Threshold | 0.26 (0.10) | 0.22 (0.16) | 0.07 (0.21) | 0.16 (0.13) | 0.13 (0.11) |
| CAT3 | Threshold | 0.27 (0.08) | 0.19 (0.13) | 0.16 (0.14) | 0.13 (0.11) | 0.20 (0.09) |

[1]RAW: non-transformed yearling PAP measurements; PT: power-transformed yearling PAP measurements; CAT3: three-category yearling PAP phenotypes, 1: PAP < 41 mmHg, 2: 41 mmHg ≤ PAP ≤ 49 mmHg, 3: PAP > 49 mmHg; CAT2: two-category yearling PAP phenotypes, 1: PAP ≤ 49 mmHg, 2: PAP > 49 mmHg; BWT: birth weight; WW: weaning weight; MILK: maternal weaning weight; PWG: post-weaning gain; YW: yearling weight

The estimated genetic correlation between BWT and yearling PAP phenotypes ranged from 0.22 to 0.27, which were lower than the estimate of genetic correlation between PAP and BWT (0.49) from the study of Shirley et al. (2008) who analyzed data from another Angus seedstock operation at high altitude (elevation, 1981 m) in Colorado. This study also reported a genetic correlation of 0.51 between direct WW and yearling PAP, which was larger than the estimates herein between yearling PAP phenotypes and WW. In addition, when compared to the same study, the genetic correlation estimates of 0.07 to 0.16 between milk (maternal WW) and yearling PAP tended to be higher, but the differences were not significantly different from their estimates (-0.05). Although the genetic correlations between maternal BWT and PAP phenotypes

were not estimated in the full model, they were assessed from bivariate models with PAP phenotype and BWT, which were resulting in genetic correlation as 0.13, 0.16, 0.15 and 0.14 for RAW, PT, CAT3 and CAT2, respectively. Although the estimates tend to be higher than Shirley et al. (2008; 0.01), they were not statistically different ($P>0.05$).

The differences between the two studies may be because that PAP used in studies were measured at different age. My presented study used the PAP measured at yearling (aged from 260 d to 450 d with a average of 365 d), but they used PAP from younger animals (aged from 171 d to 343 d with average of 277 d; Shirley et al., 2008). Neary et al. (2015) reported that PAP measurements increased from younger ages to older ages, and Zeng et al. (2015) suggested that PAP measured at weaning and yearling was not genetically identical and age might influence the expression of genes associated with PAP. Schimmel (1981) studied Hereford, Angus and Red Angus cattle managed at high altitude (elevation at 2,316 m) in Colorado and reported the genetic correlations between PAP measurements and BWT or WW as -0.43 and 0.09, which were different from our estimates, and Shirley et al. (2008), especially for BWT (different direction). Schimmel (1981) obtained genetic correlations using sire models on a small sample size (n = 667) of historical weaning PAP measurements. The small sample size, type of PAP measurements and the statistical models may all help explain the differences in estimates observed across these studies.

Genetic correlations in this presented study yield non-zero, positive and unfavorable (except BWT) relationships between growth traits and yearling PAP phenotypes, which suggested that the genetic improvement in performance traits tended to be associated with increased PAP in Angus cattle. However, there would be limited influences on yearling PAP phenotypes based on these low to moderate genetic correlations. It was reported in chicken and humans that excess

weight gain may be risk factors for pulmonary hypertension and right-side congestive heart failure (Poirier et al., 2009; Friedman and Andrus,2012; Scheele et al., 1997). When studing the steers from suckling (4 mo; at elevation of 2,170 m) to finishing (18 mo; at elevation of 1,560 m and 1,300 m), rapid growth cattle were associated with higher mean PAP and likely to be more susceptible to PH and HAD in steers (Neary et al., 2015).

Most of the genetic correlations between yearling PAP and pre-weaning performance traits (i.e. BWT and WW) were slightly higher than the correlations between yearling PAP phenotypes and post-weaning performance traits (i.e. PWG and YW) in this study. The rapid growth before weaning tends to have a stronger relationship to increased PAP than the post-weaning period. Furthermore, the non-zero and nearly moderate genetic correlation between yearling PAP phenotypes and performance traits could provide benefits for improving the accuracy of the genetic evaluation of yearling PAP using multivariate models.

Table 4.4 to 4.7 presents the genetic correlations between performance traits and RAW, PT, CAT3 and CAT2 for each sex category. The estimated genetic correlations were low to moderate. Some differences in estimated genetic orrelations were observed across various yearling PAP phenotypes, especially for steer phenotypes. The genetic correlations between heifer or bull yearling PAP phenotypes and performance traits in RAW and PT were similar, but the genetic correlations between WW and steer yearling PAP phenotypes tend to be different between RAW and PT. In our analyses, the sample size of PAP for heifers (n = 3,456) and bulls (n = 1,392) was much larger than it for steers (n = 761), which may supported that the small samples size may be more sensitive to the violation of normality because the PT was close to normal (Ghasemi and Zahediasl, 2012; Statistics Solutions, 2013).

Table 4.4. Genetic correlations between growth traits and non-transformed pulmonary arterial pressure (PAP) phenotypes in different sex categories (heifers, bulls, steers) of Angus cattle managed at high altitude (elevation at 2,170 m)

| Traits[1] | Heifer PAP | Bull PAP | Steer PAP |
|---|---|---|---|
| BWT | 0.29 (0.10) | 0.25 (0.13) | 0.11 (0.24) |
| WW | 0.20 (0.13) | 0.29 (0.15) | 0.28 (0.28) |
| YW | 0.15 (0.11) | 0.08 (0.12) | 0.03 (0.22) |
| PWG | 0.04 (0.13) | 0.11 (0.13) | 0.08 (0.32) |

[1]BWT: birth weight; WW: direct weaning weight; PWG: post-weaning gain; YW: yearling weight

Table 4.5. Genetic correlations between growth traits and power-transformed pulmonary arterial pressure (PAP) phenotype of different sex categories (heifers, bulls, steers) of Angus cattle managed on high altitude (elevation at 2,170 m)[1]

| Traits[1] | Heifer PAP | Bull PAP | Steer PAP |
|---|---|---|---|
| BWT | -0.28 (0.10) | -0.31 (0.11) | 0.03 (0.19) |
| WW | -0.24 (0.12) | -0.28 (0.12) | -0.13 (0.26) |
| YW | -0.26 (0.09) | -0.08 (0.11) | 0.03 (0.20) |
| PWG | -0.10 (0.11) | -0.08 (0.11) | -0.14 (0.24) |

[1]power-transformed yearling PAP phenotype: $10000*PAP^{-2}$
[2]BWT: birth weight; WW: direct weaning weight; PWG: post-weaning gain; YW: yearling weight

Table 4.6. Genetic correlation between growth traits and three-category pulmonary arterial pressure (PAP) phenotype of different sex categories (heifers, bulls, steers) of Angus cattle managed on high altitude (elevation at 2,170 m)[1]

| Traits[2] | Heifer PAP | Bull PAP | Steer PAP |
|---|---|---|---|
| BWT | 0.33 (0.10) | 0.30 (0.12) | -0.01 (0.21) |
| WW | 0.27 (0.13) | 0.29 (0.13) | 0.01 (0.22) |
| YW | 0.26 (0.10) | 0.03 (0.13) | 0.06 (0.19) |
| PWG | 0.16 (0.12) | 0.02 (0.13) | 0.17 (0.23) |

[1]three-category yearling PAP phenotype: 1: PAP < 41 mmHg, 2: 41 mmHg ≤ PAP ≤ 49 mmHg, 3: PAP > 49 mmHg
[2]BWT: birth weight; WW: direct weaning weight; PWG: post-weaning gain; YW: yearling weight

Table 4.7. Genetic correlations between growth traits and two-category pulmonary arterial pressure (PAP) phenotype of different sex categories (heifers, bulls, steers) of Angus cattle managed on high altitude (elevation at 2,170 m)[1]

| Traits[2] | Heifer PAP | Bull PAP | Steer PAP |
|---|---|---|---|
| BWT | 0.38 (0.13) | 0.19 (0.13) | -0.08 (0.26) |
| WW | 0.27 (0.17) | 0.22 (0.15) | 0.10 (0.25) |
| YW | 0.06 (0.04) | -0.05 (0.14) | 0.24 (0.28) |
| PWG | 0.17 (0.16) | -0.09 (0.13) | 0.12 (0.15) |

[1]two-category yearling PAP phenotype: 1: PAP ≤ 49 mmHg, 2: PAP > 49 mmHg
[2]BWT: birth weight; WW: direct weaning weight; PWG: post-weaning gain; YW: yearling weight

Genetic correlations between PAP phenotypes and performance traits differed across different sex categories. Genetic correlations between performance traits and heifer or bull PAP phenotypes were similar with those from the whole PAP dataset (Table 4.4) and the estimates from Crawford et al. (2016), which reported moderate relationships for BWT and WW, but weak relationships for PWG. Nearly moderate positive genetic correlations were obtained between WW and heifer or bull PAP phenotypes, but the genetic correlations were still smaller than the estimate (0.51) of Shirley et al. (2008) using weaning PAP. Generally, low absolute genetic correlations were identified between steer yearling PAP phenotypes and BWT and WW. In addition, close to zero genetic correlations were identified between YW and male yearling PAP phenotypes, which tended to be lower than those obtained between YW and female yearling PAP phenotypes. The low genetic correlations between PWG and CAT3 or CAT2 at high altitude in different sexes were lower ($P < 0.05$) than the estimates (i.e., 0.62) from Williams et al. (2012).

It was reported that sex hormones, such as estrogen and testosterone, have an influence on pulmonary vasculature, which could lead to the different incidence of PH between males and females (Jones et al., 2002; Lahm et al., 2007; Pugh and Hemnes, 2010). The hormone could interact with genes that influenced the different heritability between males and female PH (West et al., 2004 and 2008). In addition, differences in susceptibility to HAD between male and females in other species or humans were observed (Wu et al., 1998; Zubieta-Castillo et al., 1998; Kayser et al., 1991; Baspyat et al., 2000). The information may be associated with different estimated genetic correlations between yearling PAP phenotypes and performance traits across various sex categories. In general, the PAP phenotypes in each sex category appeared to be weakly to moderately relate to growth traits. Schimmel (1981) reported a negative moderate to high genetic correlations between bull PAP scores and BWT (-0.22) or WW (-0.75), which were

different from the estimates presented by this study. These differences may have been a result of small sample size, multiple breeds (Hereford, Angus and Red Angus), younger age of PAP measurements, and (or) the different statistical approach used in the study for Schimmel (1981).

### 4.3.3 EBV and Accuracy

The genetic parameters of performance traits, used in multivariate models to develop EBV and accuracy of yearling PAP phenotypes, are presented in Table 4.8. High heritabilities were identified for BWT and YW, and low heritabilities were estimated for WW and PWG. The genetic correlations between performance traits were from moderate to high. These estimates presented in this study were within the ranges of heritability and genetic correlations of performance traits (i.e. BWT, WW, PWG and YW) in previously reported studies (Crews and Kemp, 1999; MacNeil et al., 2011; American Angus Association, 2016). Based on the estimated parameters for yearling PAP phenotypes and performance traits, the genetic evaluation of yearling PAP phenotypes could take advantage of multivariate models involving performance traits. The reasons were: 1. difference between genetic and residual correlations between yearling PAP phenotypes and performance traits (Table 4.3); 2. higher heritabilities for performance traits than yearling PAP phenotypes (Table 4.8; Mrode, 2014).

Table 4.8. Heritability, genetic variance, genetic correlation (above diagonal) and residual correlation (below diagonal) between performance traits of Angus cattle managed on high altitude (elevation at 2,170 m)

| Traits[1] | BWT | WW | MILK | YW | PWG |
|---|---|---|---|---|---|
| BWT | 0.42 (0.04) | 0.69 (0.05) | 0 | 0.57 (0.05) | 0.49 (0.07) |
| WW | 0.26 (0.03) | 0.21 (0.03) | 0 | 0.97 (0.01) | 0.62 (0.08) |
| MILK | - | - | 0.14 (0.02) | 0 | 0 |
| YW | 0.25 (0.03) | 0.72 (0.01) | 0 | 0.41 (0.03) | 0.75 (0.04) |
| PWG | 0.05 (0.03) | 0.01 (0.03) | 0 | 0.73 (0.01) | 0.22 (0.03) |

[1]BWT: birth weight; WW: direct weaning weight; MILK: maternal weaning weight; PWG: post-weaning gain; YW: yearling weight

Tables 4.9 to 4.11 summarizes the EBV accuracy of all individuals in the pedigree file and sires of offspring with PAP records of RAW, PT, CAT3 and CAT2 from univariate and two multivariate (Model 1: PAP, BWT, WW and Model 2: PAP and YW) models, respectively. Multivariate Model 1 and 2 both resulted in higher accuracy than univariate model as expected, and the Model 1 were associated the highest yearling PAP accuracy. The average difference between accuracies from univariate model and Model 1 for RAW, PT, CAT3 and CAT2 were 0.01 to 0.02. This suggested that approximately 1 to 2 additional offspring with yearling PAP phenotypes were needed in univaraite model to achieve the same accuracy obtained from multivariate model based on the accuracy equation for offspring phenotypes from Bourdon (1997) and the heritability of yearling PAP phenotypes at 0.24 (Table 3.8). When using a genetic correlation of approximately 0.25 between a target trait and an indicator trait in a simulation study, Calus et al. (2011) reported that the multivariate model could increase accuracy by 0.01 for target trait. There was almost no accuracy improvement from Model 2 compared to univariate model. It was reported that the absolute difference between the genetic and residual correlations between the traits determined the gain in accuracy of evaluations (Schaeffer, 1984; Thompson and Meyer, 1986). The estimated residual correlations between yearling PAP and performance traits were all near zero, so the higher genetic correlations resulted in larger accuracy improvement (i.e. continuous versus categorical yearling PAP phenotypes and Model 1 versus Model 2).

Results of this chapter suggested that the multivariate model would improve the EBV accuracy of each PAP phenotypic forms. Guo et al. (2014) suggested that the EBV from multivariate models could be used in GWAS and resulted in more reliable results than the EBV from single trait model. Jia et al. (2012) also reported that taking advantage of the genetic

relationship with higher heritable traits would increase the genomic prediction accuracy of lower-heritable traits and the traits with missing phenotypes. Therefore, Model 1 was preferred to generate EBV of PAP phenotypes for the following GWAS study.

The presented average accuracies of all aniamls in the pedigree were relative low (approximate 0.2), but a large portion of the pedigree did not related to any PAP records. However, the largest BIF accuracy was 0.69, and the average EBV accuracy of sires having offspring with PAP records was approximately 0.32 with the average number of offspring at 19 ± 1. The moderate sire accuracies implied that we could make genetic improvement against HAD based on these estimated EBV. Although higher heritability and genetic correlations between performance traits and yearling PAP phenotypes were used in models to develop EBV for categorical yearling PAP phenotypes, lower EBV accuracies were observed when compared to continuous scale yearling PAP phenotypes. These losses in the genetic prediction accuracy in categorical phenotypes compared to continuous phenotypes using the same genetic parameters were also observed in other studies (Kärkkäinen et al., 2013; Kizilkaya et al., 2014).

Table 4.9. Comparison of EBV accuracies from univariate and two multivariate models for non-transformed pulmonary arterial pressure (PAP) phenotype of Angus cattle managed at high altitude (elevation at 2,170 m)

| Model[1] | Mean | Min | Max | SD | Sire_average[2] |
|---|---|---|---|---|---|
| Univariate | 0.176 | 0 | 0.684 | 0.091 | 0.314 |
| Model 1 | 0.184 | 0 | 0.688 | 0.093 | 0.323 |
| Model 2 | 0.176 | 0 | 0.685 | 0.091 | 0.314 |
| Difference | Mean=0.008; 4.55% higher accuracy gained in Model 1 than univariate model | | | | |

[1]Model 1: yearling PAP, birth weight weaning weight and post-weaning gain; Model 2: yearling PAP and yearling weight

[2]Sire_average: average of accuracies of sires who have offspring with PAP scores

Table 4.10. Comparison of EBV accuracies from univariate and two multivariate models for power transformed pulmonary arterial pressure (PAP) phenotype of Angus cattle managed at high altitude (elevation at 2,170 m)

| Model[1] | Mean | Min | Max | SD | Sire_average[2] |
|---|---|---|---|---|---|
| Univariate | 0.174 | 0 | 0.681 | 0.090 | 0.311 |
| Model 1 | 0.184 | 0 | 0.690 | 0.09 | 0.321 |
| Model 2 | 0.174 | 0 | 0.680 | 0.090 | 0.312 |
| Difference | Mean=0.010; 5.747% higher accuracy gained in Model 1 than univariate model | | | | |

[1]Model 1: yearling PAP, birth weight weaning weight and post-weaning gain; Model 2: yearling PAP and yearling weight

[2]Sire_average: average of accuracies of sires who have offspring with PAP scores

Table 4.11. Comparison of EBV accuracies from univariate and two multivariate models for three-category pulmonary arterial pressure (PAP) phenotype of Angus cattle managed at high altitude (elevation at 2,170 m)

| Model[1] | Mean | Min | Max | SD | Sire_average[2] |
|---|---|---|---|---|---|
| Univariate | 0.149 | 0 | 0.652 | 0.078 | 0.271 |
| Model 1 | 0.162 | 0 | 0.658 | 0.081 | 0.285 |
| Model 2 | 0.153 | 0 | 0.658 | 0.079 | 0.275 |
| Difference | Mean=0.013; 8.72% higher accuracy gained in Model 1 than univariate model | | | | |

[1]three-category yearling PAP phenotype: 1: PAP < 41 mmHg, 2: 41 mmHg ≤ PAP ≤ 49 mmHg, 3: PAP > 49 mmHg

[2]Model 1: yearling PAP, birth weight weaning weight and post-weaning gain; Model 2: yearling PAP and yearling weight

[3]Sire_average: average of accuracies of sires who have offspring with PAP scores

Table 4.12. Comparison of EBV accuracies from univariate and two multivariate models for two-category pulmonary arterial pressure (PAP) phenotype of Angus cattle managed at high altitude (elevation at 2,170 m)

| Model[1] | Mean | Min | Max | SD | Sire_average[2] |
|---|---|---|---|---|---|
| Univariate | 0.112 | 0 | 0.596 | 0.062 | 0.207 |
| Model 1 | 0.127 | 0 | 0.607 | 0.066 | 0.226 |
| Model 2 | 0.115 | 0 | 0.596 | 0.063 | 0.211 |
| Difference | Mean=0.015; 13.39% higher accuracy gained in Model 1 than univariate model | | | | |

[1]two-category yearling PAP phenotype: 1: PAP ≤ 49 mmHg, 2: PAP > 49 mmHg

[2]Model 1: yearling PAP, birth weight weaning weight and post-weaning gain; Model 2: yearling PAP and yearling weight

[3]Sire_average: average of accuracies of sires who have offspring with PAP scores

## 4.4 Conclusions

Low to moderate genetic correlations were identified between yearling PAP phenotypes and performance traits in Angus cattle managed at high altitude. Similar genetic correlations were identified across non-transformed and power transformed yearling PAP measurements. The violation of normality appeared to have limited influence on estimated genetic correlation between PAP measurements and performance traits. These estimated genetic correlations between performance traits and yearling PAP phenotypes in different sex categories also ranged from low to moderate, but these performance traits related differently to yearling PAP phenotypes among bulls, heifers, and steers. Since the similar ranking of animals based on EBV from models with/without treating PAP in different sex categories as separate traits and the ease in applying EBV in selection, PAP in different sex category were not treat as sepertate traits in genetic evaluation. In both analyses of yearling PAP phenotypes with and without separating them by sexes, the pre-weaning performance traits appeared to have stronger relationship with yearling PAP phenotypes than post-weaning performance traits because of larger genetic correlations between pre-weaning performance traits and yearling PAP phenotypes. The multivariate models appeared to improve the genetic evaluation of non-transformed PAP measurements and susceptibility of HAD (i.e. CAT3 and CAT2). The multivariate models including yearling PAP phenotype, BWT, WW and PWG were preferred to estimate breeding values of RAW, PT, CAT3 and CAT2.

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# CHAPTER 5

# GENOMIC WIDE ASSOCIATION STUDY OF YEARLING PULMONARY ARTERIAL PRESSURE PHENOTYPES IN ANGUS CATTLE AT HIGH ALTITUDE REGION

## 5.1 Introduction

With the advances in molecular biological techniques, the genome of animals can be genotyped for a large number of animals with relatively limited cost (Boichard et al., 2016). This allows the frequent use of genotype information for genome wide association study (GWAS) of complex traits (e.g. disease and fertility traits). Based on the studies in previous sections, the yearling PAP phenotypes are moderately heritable, which implies that it is possible to find sequence variants associated with PAP phenotypes. This chapter reports GWAS of yearling PAP phenotypes using EBV and genotype data from the BovineSNP50 BeadChip. The objective of this study was to detect QTL that were associated with yearling PAP phenotypes using GWAS. The resulting QTL were also investigated in public databases to identify candidate genes. This study also enhanced understanding of adaptation and susceptibility to HAD, and provided biomarkers for selecting against elevated PAP and HAD in cattle.

## 5.2 Materials and Mathods

### 5.2.1 Deregressed EBV

These GWAS were conducted on each of the three yearling PAP phenotypes, including un-transformed PAP (RAW), ordinal three-category (CAT3; 1: PAP < 41 mmHg, 2: 41 mmHg ≤ PAP ≤ 49 mmHg, 3: PAP > 49 mmHg) and two-category phenotypes (CAT2; 1: PAP ≤ 49 mmHg, 2: PAP > 49 mmHg). Deregressed EBV (DEBV) were used as response variables in

these GWAS. The procedure developed by Garrick et al. (2009) was used to calculate DEBV free of parent average from the raw EBV and reliabilities ($r^2$; Equation 5.1) of genotyped animals and their parents, and this method also accommodated the heterogeneous variances due to differences in reliability among animals. These EBV, associated prediction error variance (PEV), genetic variance and heritability were obtained from multivariate linear or threshold animal models involving each PAP phenotype (i.e. RAW, CAT3 and CAT2), birth weight (BWT), weaning weight (WW) and post weaning gain (PWG) through software renumf90, thrgibbs1f90 and postgibbs1f90. The models used to develop PAP EBV were expressed as Equation 4.1. To calculate the DEBV, the reliability for each RAW EBV were calculated first as:

$$r^2 = 1 - \frac{PEV}{G \times (1+F)} \quad \text{(Equation 5.1)}$$

where $r^2$ denoted the reliability of raw EBV and G was the genetic variance used for estimating EBV and accuracies. The genetic variance used for RAW, CAT3 and CAT2 were 22.69 $mmHg^2$, 0.16, and 0.51, respectively. The DEBV that were free of parents' average and associated DEBV reliabilities ($r_i^{2*}$) were calculated as:

$$DEBV = \frac{\gamma^*}{\mathbf{Z}_i'\mathbf{Z}_i} \quad \text{(Equation 5.2)}$$

$$r_i^{2*} = 1 - \lambda / (\mathbf{Z}_i'\mathbf{Z}_i + \lambda) \quad \text{(Equation 5.3)}$$

where $\gamma_i^*$ was information equivalent to a right-hand-side element pertaining to the individual *i* and expressed as:

$$\gamma_i^* = -2\lambda \hat{g}_{PA} + (\mathbf{Z}_i'\mathbf{Z}_i + 2\lambda)\hat{g}_i \quad \text{(Equation 5.4)}$$

and the $\lambda$ was an assumed known parameter that was defined as: $\lambda = (1-h^2)/h^2$, where $h^2$ was the heritability corresponding to each phenotypes. These were 0.25, 0.26 and 0.34 for RAW,

CAT3 and CAT2, respectively. The $\hat{g}_i$ denoted the EBV of this individual *i,* and $\hat{g}_{PA}$ was the parent average EBV that was calculated from EBV of parent ($\hat{g}_{sire}$ and $\hat{g}_{dam}$) of animal *i* as $\hat{g}_{PA} = \frac{\hat{g}_{sire} + \hat{g}_{dam}}{2}$. The $\mathbf{Z}_i'\mathbf{Z}_i$ reflected the unknown information of individual *i*. The $\mathbf{Z}_i'\mathbf{Z}_i$ was expressed as:

$$\mathbf{Z}_i'\mathbf{Z}_i = \delta\mathbf{Z}_{PA}'\mathbf{Z}_{PA} + 2\lambda(2\delta - 1) \quad \text{(Equation 5.5)}$$

where $\delta = (0.5 - r_{PA}^2)/(1 - r_i^2)$ . Here $r_i^2$ denoted the reliability of individual *i*, and the $r_{PA}^2$ was the reliability of parent average that was calculated as: $r_{PA}^2 = \frac{r_{sire}^2 + r_{dam}^2}{4}$. The $r_i^2$ , $r_{sire}^2$ and $r_{dam}^2$ were calculated using Equation 5.1. The $\mathbf{Z}_{PA}'\mathbf{Z}_{PA}$ reflected the unknown information content of the parent average of individual *i* that was expressed as:

$$\mathbf{Z}_{PA}'\mathbf{Z}_{PA} = \lambda(0.5\alpha - 4) + 0.5\lambda\sqrt{(\alpha^2 + \frac{16}{\delta})} \quad \text{(Equation 5.6).}$$

In this equation, the α was defined as: $\alpha = \frac{1}{(0.5 - r_{PA}^2)}$ . Because the $r_i^2$ varies among different individuals, the variance of DEBV was heterogeneous. In order to address this problem, a vector of scaled weights was needed in the analysis of DEBV to weight the DEBV information. The scaled weight ($w_i$) of each individual's DEBV of each phenotype was calculated as:

$$w_i = \frac{1 - h^2}{[c + (1 - r_i^{2*})/r_i^{2*}]h^2} \quad \text{(Equation 5.7)}$$

where the *c* was defined as the genetic variance of a trait that was not explained by the genotype markers. The value of the c used in this presented study was assumed as 0.4. Animals with low accuracy (<0.05) DEBV for each trait were removed from GWAS study to keep the quality of analyses.

5.2.2 Genotype data

In total, 2,700 Angus cattle from the Colorado State University Beef Improvement Center were genotyped using various formats of Illumina Bovine SNP50K Beadchip version 2 assays (54,609 SNP; Matukumalli et al., 2009). These cattle were born from 1997 to 2015 (Appendix 5.1), and genotyping was completed in three year-groups (i.e. 2013, 2014 and 2015) and in two labs (i.e. Zoetis and GeneSeek). In addition, 65 Angus steers in this herd were genotyped in 2013 using Illumina BovineHD BeadChip (777,962 SNP) through the lab work of GeneSeek. Therefore, 2,765 genotyped animals were originally considered in this study.

In each group of genotyped animals, only samples with call rate larger than 0.95 were used in GWAS. Data from these genotyped animals were merged together and used in GWAS, which resulted in 2,582 samples (Appendix 5.1). Due to the fact that some SNP markers differed across the various genotyping platforms, there were 43,918 SNP markers in common across all marker results. Marker quality control were performed on autosome markers of these remaining SNP, and genotype at particular loci were filtered according to the following criteria: average call rate lower than 0.85 across; minor allele frequency of each loci smaller than 0.01; and Hardy-Weinberg equilibrium test results of P-value less than $1\times10^{-4}$. The call rate and minor allele frequency criteria were also used to filter genotype on X chromosome. Then SNP pruning was executed on the remaining marker to remove some SNP that were in very high linkage disequilibrium ($r^2 > 0.99$) with other SNP markers. These quality control analyses were performed using the SNP & Variation Suite v8.x (Golden Helix, Inc., Bozeman, MT, www.goldenhelix.com). Only autosomal and X chromosome markers that passed these quality control were kept in analyses (Kizikaya et al., 2014; Saatchi et al, 2014; Snelling et al., 2014). Overall, 35,930 SNP makers were used in each GWAS. Genotypes in analyses were coded as -10

(AA), 0 (AB) and 10 (BB) based on the suggestion of Fernanco and Garrick (2008). Missing genotypes were replaced with the average value (ranged from -10 to 10) for each SNP marker.

5.2.3 Statistical method

The 35,930 SNP makers were fitted in a model simultaneously to study their association with yearling PAP phenotypes. For each phenotype, a mixed model was used to estimate maker effects, which was expressed as:

$$\mathbf{y} = \mathbf{X}\beta + \sum_{m=1}^{M} \mathbf{Z}\mathbf{a} + \mathbf{e} \quad \text{(Equation 5.8)}$$

where $\mathbf{y}$ was the vector of DEBV for each PAP phenotype; $\beta$ was a vector of fixed effects that only contained the population mean in this study, because DEBV was developed from EBV that has already adjusted out the the fixed effects in genetic evaluation. The *a* was the vector of random marker substitution effects. The $\mathbf{X}$ was the incidence matrix relating DEBV to the population mean, respectively, and $\mathbf{Z}$ was the value (i.e. -10, 0, and 10) of covariate for marker m of individuals. As usual, *e* denoted the random residual effect. The prediction models for DEBV of RAW, CAT3 and CAT2 were analyzed using Bayes methods. Bayes C (Habier et al., 2011) was used to obtain genetic and residual variance to construct priors of genetic and residual scale parameters for Bayes B (Meuwissen et al., 2001), because Bayes C is less sensitive to prior assumption than Bayes B (Garrick and Fernando, 2013). Then Bayes B was used to estimate the marker effects and associated variances. When using Bayes B, the marker effects were assumed to follow a mixture distribution: $a = 0$ with probability $\pi$ and $a \sim N(0, \sigma^2_{a_m})$ with probability $1-\pi$. The $\pi$ was assumed as 0.995, which indicated that most of the presented markers (99.5%) have no effect on studied traits, and a small portion of them (0.5%) have effects. The $\sigma^2_{a_m}$

denoted the variance of maker substitute effect at locus *m*, and the marker effect variance was assumed varied across different locus.

In Bayes analyses, $\sigma^2_{a_m}$ was also assumed unknown and following a scaled inverse chi-square distribution with a degree of freedom $\upsilon_a = 4$ and a scale parameters $S_a = \frac{\sigma^2_g(\upsilon_a - 2)}{(1-\pi)\sum_{m=1}^{M} p_m(1-p_m)\upsilon_a}$, where $p_m$ was the minor allele frequency at locus *m* and the $\sigma^2_g$ was the marker explained additive genetic variance of the studied trait. Fixed effects were assumed following flat distributions. The residual distribution was assumed as $e \sim N(0,\sigma^2_e)$ with degree of freedom of 10. The assumptions of Bayes C were the same with Bayes B except the assumption on marker effects variances. In Bayes C, marker variance was assumed the same across all genomic loci, but variances of different markers was assumed varied in Bayes B. The Markov chain Monte Carlo methods (i.e. Gibbs Sampling and Metropolis–Hastings) were used to perform Bayes B and Bayes C on DEBV to estimate marker effects, maker variances, and genetic and residual variances of studied traits.

The effects of consecutive 1-Mb windows based on the position along the whole genome (n = 2,648) were also studied. The genetic variance explained by markers in a window was defined as $\sigma^2_{g_w} = \frac{\sum_i^n (g_{w_i} - \bar{g}_w)}{n}$, where the *w* was the 2,648 windows across the bovine genome, the $g_{w_i}$ was sum of the effect of each marker in a window for animal *i*, and the $\bar{g}_w$ denoted the average window *w* effect across all samples, the *n* denotes the number of samples in a GWAS.

The GeneSel software (Fernando and Garrick, 2008) that implemented the MCMC methods was used to conduct these GWAS. These analyses were executed using 41,000 iterations for each run with the first 1000 samples as burn-in. As a result, 40,000 samples were used to provide

posterior distribution of each estimable parameter. Marker effects, additive genetic variance, residual variance and proportion of genetic variance explained by a genomic window were direct output from the software. A genomic window was recognized to be associated with a trait if the proportion of the genetic variance accounted by this window was larger than 1%. When jointly studying the three PAP phenotypes, the top 2% (53) windows were considered of each phenotype were compared, and the SNP effects correlation between these three yearling PAP phenotypes were estimated.

Lead-SNP of an identified yearling PAP phenotypes associated window was recognized as the SNP having the largest model frequency in the window region. The model frequency was defined as the proportion of fitted models including that maker. Then, the lead-SNP were analyzed separately using ordinal least square models to evaluate the significance, least square mean of genotypes, additive and dominance effect. In this model, the genotypes (i.e. AA, AB and BB) of a single lead-SNP were treated as fixed effects and regressed on DEBV of yearling PAP phenotypes, and the least square mean genotypes were obtained from the model. The least squares mean of genotypes were used to calculate the additive and dominance effect of the lead-SNP (Weng et al., 2016),

$$\text{addtive effect} = \frac{BB - AA}{2} \quad \text{(Equation 5.9)}$$

and

$$\text{dominance effect} = AB - \frac{AA + BB}{2} \quad \text{(Equation 5.10)}$$

We used the software R to execute the analysis, estimate and compare least square mean, and estimate and test additive and dominance effect of lead-SNP.

5.2.4 Identification of candidate gene and associated pathways

The identified windows from PAP phenotypes were aligned to the *Bos taurus* genome (UMD 3.1) to identify the genes within these windows using Ensembl (Kinsella et al., 2011; http://www.ensembl.org/). The NCBI dbSNP and gene databases (Sherry et al., 2001; Coordinators et al., 2013; http:// www.ncbi.nlm.nih.gov/SNP; http:// www.ncbi.nlm.nih.gov/gene) were also used to provide additional information for these genes. The genes' associated molecular functions, cell components and pathways were obtained from DAVID (The Database for Annotation, Visualization and Integrated Discovery) 6.7 database (Ashburner et al., 2000). Gene ontology enrichment analysis on genes locating within concordant windows across phenotypes was conducted using g:Profiler (Reimond et al., 2016 ;http://biit.cs.ut.ee/gprofiler/).

5.3 Results and discussion

5.3.1 DEBV

The number of cattle analyzed for RAW, CAT3 and CAT2 phenotypes were 2,243, 2,259 and 2,148 respectively, as there were 339, 323 and 434 animals with DEBV reliability less than 0.05. Results showed that lower accuracy of EBV from CAT2 used in DEBV calculation might lead to more individuals with extremely low DEBV accuracies. The standard deviations of DEBV (i.e. 8.9, 0.9 and 1.6 for RAW, CAT3 and CAT2, respectively) were larger than those of EBV (i.e. 2.5, 0.2 and 0.3 for RAW, CAT3 and CAT2, respectively), which supported that DEBV expanded the distribution of the raw estimates. The average DEBV accuracy of genotyped animals of non-transformed PAP was larger than those of categorical phenotype, which is consistent with the characteristics of higher EBV accuracies in RAW than categorical PAP phenotypes. The GWAS using DEBV were also considered the weighted linear model

analyses, and the calculated weights for DEBV were applied to the matrices to adjust the heterogeneous variances of DEBV across different individuals having varied reliability (Garrick et al., 2009). The weights reflected the emphasis of information (e.g. DEBV) in the analyses, so the weights are consistent with DEBV accuracies. Larger weights correspond to the DEBV is having more value in the analyses.

Ekine et al. (2014) reported that original phenotypes or EBV in GWAS may increase rate of false positive and decrease power of GWAS, since they could introduce family relatedness into the analysis. Therefore, the DEBV in this study removed parent averages and accommodated the heterogeneous variances of EBV, was considered a better approach for GWAS than phenotypes and EBV (Garrick et al., 2009; Ostersen et al., 2011). A study on purebred pigs demonstrated that DEBV yielded more accurate direct genomic values than raw EBV (Ostersen et al., 2011). Therefore, the DEBV have been used in several GWAS of cattle and swine (Do et al., 2013; Saachi et al., 2013; Boddhireddy et al., 2014; Sevillano et al., 2015).

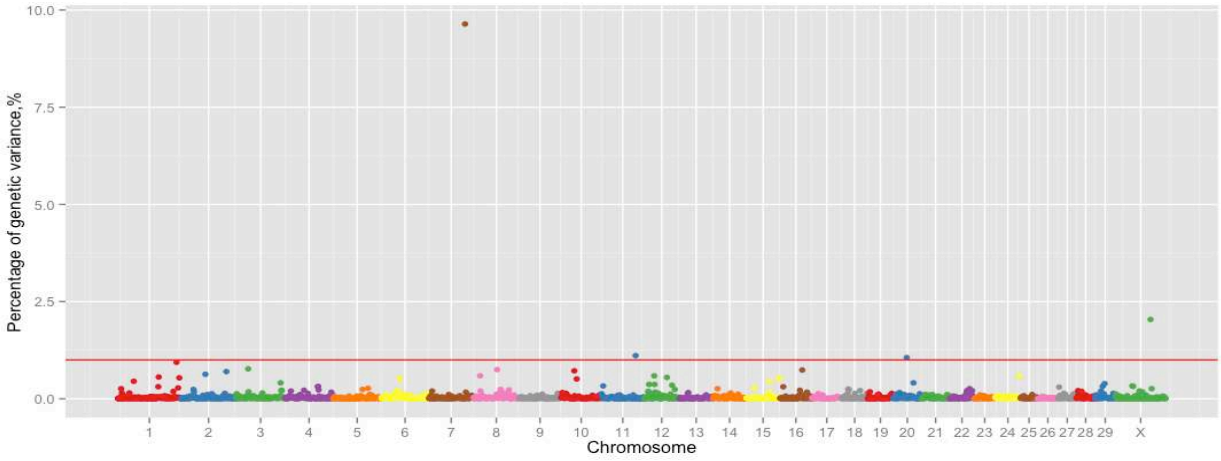
### 5.3.2 GWAS of PAP phenotypes

Nineteen genomic windows (QTL, defined as 1-Mb windows of the genome explaining ≥ 1% of additive genetic variance) were identified across the three phenotypes. There were 4, 12 and 9 windows associated with RAW, CAT3 and CAT2, respectively. Figure 5.1 includes Manhattan plots of each yearling PAP phenotype and shows the proportion of genetic variance explained by each of the 2,648 1-Mb SNP windows with respect to their genomic positions. Note the genome-windows had varying degrees of association with PAP continuous and categorical scale PAP phenotypes, and small number of regions had large effect on these phenotypes, which was consistent with our previous assumptions. Because the GWAS results were based on the

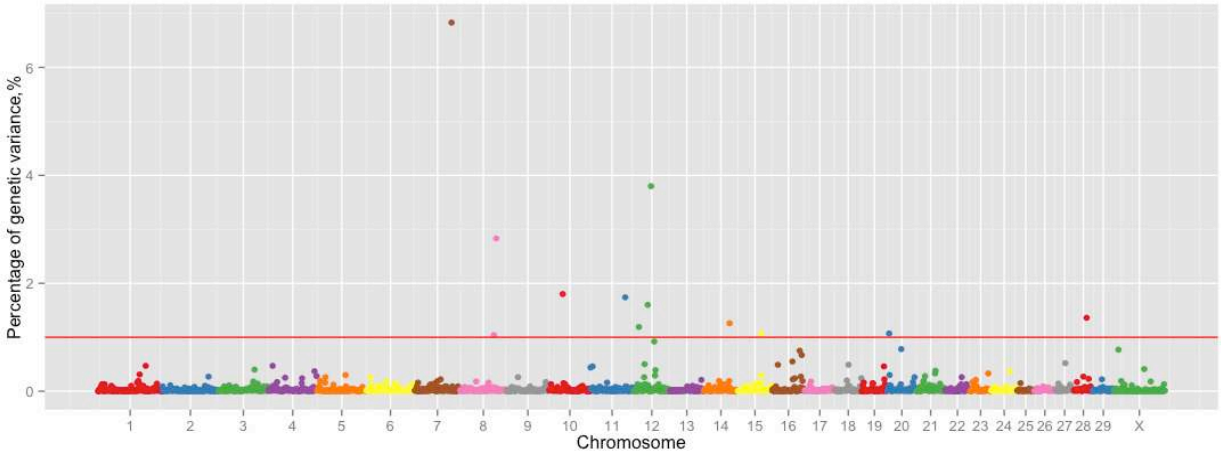
DEBV (developed from the EBV), different genetic parameters used in the model to estimate breeding value would slightly vary the GWAS results, for example, the percentage value of genetic varation explained by a 1-Mb window. These reported results were based on EBV from multivariate models with fixing zero correlations between maternal and direct effects for growth traits. Without the restriction on genetic correlations, the resulting genetic parameters may be varied even though the difference would be small. Some minor changes in estimates for genomic window and SNP effects could be observed because of different genetic parameters, but its influence on identification of top genomic windows for each phenotypes would be limited.

Neary (2014) presented GWAS on PAP measured before or at weaning age of cattle (i.e. 6 mo of age), yet these identified SNP were different from the presened results. These differences among results may be explained by several reasons, inclduding the age of PAP measurements (i.e. weaning versus yearling PAP scores), the dependent variable (raw observations versus DEBV), the sample sizes of GWAS (60 versus 2300), the number of SNP (BovineHD versus BovineSNP50) and the statistical methods (REML using SNP versus Bayes using windows). Based on the samples size, the current study was a much stronger approach to identify QTL for PAP. The GWAS of Cockrum et al. (2014) reported PAP measurements (using $\log_{10}$ transformed PAP data) associated loci on chromosome 7 and 28 that were also identified in this study. In human GWAS, many loci and genes have been reported to be associated with pulmonary arterial hypertension, high altitude adaptation and disease, and various blood pressures phenotypes (International Consortium for Blood Pressure Genome-Wide Association Studies, 2011; Ji et al., 2012; Mishra et al., 2012; Germain et al., 2013). The results of this study in cattle and reports in other species all demonstrated the polygenic effects of PAP measurement indicative of susceptibility to PH and HAD.

A.




B.




C.

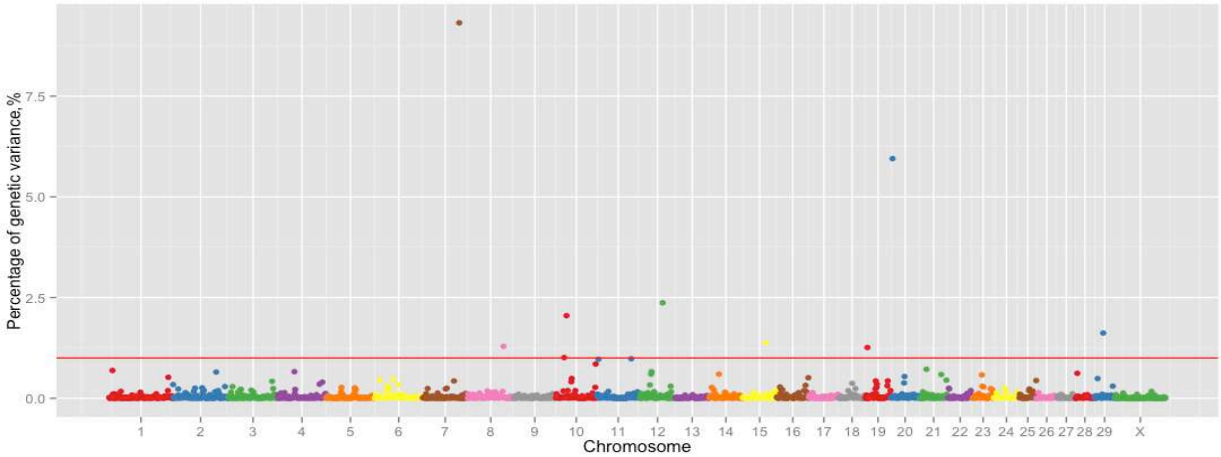



Figure 5.1 Manhattan plots of proportion of genetic variance explained by 1-Mb windows from genomic wide association study of deregressed EBV from A) non-transformed PAP; B) three-category; C) two-category yearling pulmonary arterial phenotypes of Angus cattle managed at high altitude (elevation at 2,170 m) with the line reprenting 1% of genentic variation.

The SNP used in this study (n = 35,930) explained 17.9%, 28.3% and 62.3% of the total variance of DEBV from RAW, CAT3 and CAT2, respectively. Golan et al. (2014) also illustrated that the phenotypic variation explained by genotype (common variants) was positively related with heritability. Among these genetic variances (variation explained by markers), the significant windows (windows that explained >1% genetic variation) explained 13.9%, 25.6% and 26.3% of total genetic variation, and the top 52 (2%) windows explained 34.2%, 42.0% and 47.2% of total genetic variation explained by all markers RAW, CAT3 and CAT2, respectively. A GWAS logically initial genomic analyses on common variants (with MAF > 5%; Visscher et al., 2012), but the common variants could not explain all genetic variation for complex traits. There are many other factors that could also contribute to the genetic variation. The unexplained portion of genetic variance in a GWAS may be from gene-by-gene interactions, gene-by-environment interactions, genomic structural variations, epigenetics and rare variants (Eichler et al., 2010). In addition, De los Campos et al. (2015) suggested that limited number of genotype markers could hardly cover all QTL for a complex trait in a 3 gigabase genome such as cattle. However, this presented GWAS provides the best knowledge obtainable at the present time.

Some concordant windows were identified across the three PAP phenotypes although the actual portions of genetic variance explained by these windows were slightly different (Figure 4.1). A genome window on Chromosome 7 appeared to be the most important genomic region associated with all these PAP phenotypes. The GWAS results were more similar between RAW and CAT3 than RAW and CAT2, which coincided with genetic correlation between RAW and CAT3 and CAT2 (Table 3.7).

Table 5.1 Genomic windows explaining > 1% genetic variation of non-transformed pulmonary arterial pressure (PAP) measurements of yearling Angus cattle from Beef Improvement Center of Colorado State University (elevation at 2,170 m)[1]

| BTA_Mb | Start (bp) | End (bp) | Number of SNP | % Variance Explained | Lead-SNP | Model Frequency | % Variance Explained by Lead-SNP |
|---|---|---|---|---|---|---|---|
| 7_93 | 93007435 | 93886136 | 6 | 9.64 | rs109819349 | 0.51 | 2.44 |
| X_110 | 110003891 | 110719987 | 9 | 2.04 | rs41618346 | 0.28 | 0.18 |
| 11_86 | 86048363 | 86965492 | 15 | 1.11 | rs110993632 | 0.16 | 0.09 |
| 20_34 | 34051201 | 34981347 | 18 | 1.06 | rs109633897 | 0.30 | 0.24 |

[1]Bovine chromosome and n$^{th}$ 1 Mb window on the same chromosome based on the UMD3.1 assembly; Start: start position of the window; End: end position of the window; Num. SNP: number of SNP in the window; % Variance Explained: proportion of the genetic variance explained by the window; Lead-SNP: SNP with the highest model frequency; Model Frequency: the proportion of fitted models including that maker; % Variance Explained by Lead-SNP: proportion of the variance explained by the lead-SNP

5.3.2.1 Non-transformed PAP measurements

Four windows on chromosomes 7, 11, 20 and X were identified to be associated with RAW. Table 5.1 described the details of these windows and their lead-SNP. In the window on chromosome 7, besides the lead-SNP presented in Table 5.1, another SNP (*rs41625563*) also had high model frequency (49%) in the window, which suggested that *rs109819349* and *rs41625563* could be of physically linked with each other, and the QTL could be distributing its effect over the two markers. Therefore, *rs41625563* can also be considered an important SNP this RAW associated genomic window. The rs109819349 is within a gene (*LOC507513*), which was reported as a protein-coding gene and described as *G-protein coupled receptor 98*. The *rs41625563* is inter-genic between LOC507513 (20 Kb downstream) and *LOC104968987* (1.5 Kb upstream). The *LOC104968987* is a non-coding RNA gene. Therefore, these uncharacterized genes may have influences on PAP measurements. However, additional investigations on function of these genes are needed.

Besides the lead-SNP related genes, four other uncharacterized genes were identified and one annotated gene (*ARRDC3*) was obtained under this QTL window on chromosome 7. The *ARRDC3* (*arrestin domain containing 3*) is a protein-coding gene and was recognized associated with the plasma membrane, endosomes, lysosomes during endocytosis, and cell proliferation (Oka et al., 2006). It was also reported to be a HIF-2α regulate gene and influence the endothelial sprouting during prolonged hypoxic culturing (Nauta et al., 2016). The *ARRDC3* was identified by many studies to be associated with breast cancer in human (Draheim et al., 2010; Wang et al., 2015). Considering the metabolic theory of PH, Stenmark et al. (2015) and Li et al. (2016) described the relationship between PH and a metabolic adaptation often observed in cancerous cells (i.e. “Warburg effect”). This adaptation has been described in various other studies of

cancer and PH cells (Pavlides et al., 2009; Paulin and Michelakis, 2014). These metabolic changes may contribute to the tissue remodeling and inflammation observed in PH (Stenmark et al., 2015). Also, Patwari et al. (2009) suggested that Arrdc3 protein might play role in metabolism regulation, which corroborated that cellular metabolic changes occur with increase in PAP and PH.

A window on chromosome 11 was another important region associated with RAW. The lead-SNP (*rs110993632*) in this window does not reside near (< 2,500 bp) of any annotated genes. Besides the lead-SNP, *rs109850195* also had a relatively high model frequency (19%), which was located in an un-annotated gene region. However, this lead-SNP is about 7.4 Kb downstream away from *KCNF1* (*potassium channel*, *voltage gated modifier subfamily F, member 1*). This gene encodes subfamily F, which is a member of the voltage-gated potassium channels, and these channels function in many aspects in human including neurotransmitter release, heart rate, and smooth muscle contraction (Grant, 2009). This gene's role in the smooth muscle contraction and (or) the cardiac system may relate this gene to the PAP phenotypes in Angus cattle.

Nine other genes were also identified in the region of window 11_86 (Appendix 5.2). The protein encoded by *ROCK2* (*Rho associated coiled-coil containing protein kinase 2*) is a serine/threonine kinase regulating cytokinesis, smooth muscle contraction, the formation of actin stress fibers and focal adhesions and the activation of the c-fos serum response element (Coordinators, 2013). Shimizu et al. (2013) reported that *ROCK2* in vascular smooth muscle cells contributed to the pathogenesis of hypoxia-induced pulmonary arterial hypertension. Do et al. (2009) illustrated that the expression of *ROCK2* was significantly increased in patients with idiopathic pulmonary arterial hypertension. In addition, studies reported that it was related to

other aspects of pulmonary and cardiac systems including cardiovascular disease, idiopathic pulmonary fibrosis with oxidative stress and non-small cell lung cancer (Vigil et al., 2012; Liu et al., 2013; Shimizu et al., 2014). These studies support our finding that *ROCK2* could potentially be associated with PH and HAD in Angus cattle at high altitude regions. The protein encoded by *PDIA6* can catalyze formation, reduction, and isomerization of disulfide bonds in proteins and are thought to play a role in folding of disulfide-bonded proteins. This gene was overexpressed in the lung adenocarcinoma patients. The *NTSR2* encodes neurotensin receptor 2, and Leyton et al. (2002) indicated that functional neurotensin receptors might play roles in lung cancer cells. Gene *E2F6* encodes transcription factors that can control cell cycle, which was reported to have function on controlling hypoxia-induced apoptosis and hematopoietic progenitor cells apoptosis during proliferation.

On chromosome 11 of the *Bos taurus* genome, *EPAS1* (*HIF-2α*) located at the position of 28.57 to 28.67 Mb, and some previous studies tested its association with PAP and HAD in both human and cattle. Newman et al. (2015) reported two cis variants in *EPAS1* associated with PAP of Angus cattle at high altitude, and the mutations in this gene were also reported to be related to the high altitude adaptation of humans (Buroker et al., 2012; Xiang et al., 2013; Yang et al., 2013). The window containing this gene was not associated with any PAP phenotypes in the presented study, and it only explained about 0.02% to 0.04% of the genetic variation of these PAP phenotypes. Also, a variant in *EPAS1* was not expressed differently between low PAP and high PAP groups in a study on Angus cattle managed at high altitude (Crawford et al., 2016). This gene was normally identified to be associated with HAD or high altitude adaptation through comparing the low and high altitude residents. However, all the individuals in our study were

exposed to high altitude since birth, so this may lead to the non-significant result on *EPAS1* in our analysis.

A QTL on the X chromosome was associated with PAP measurements. The lead-SNP of this window was rs41618346 and was not close to any annotated genes. However, another SNP (*rs110159935*) with similar model frequency with the lead-SNP was located within *TSPAN7* (*tetraspanin 7*). The encoded protein of this gene is a member of the transmembrane 4 superfamily proteins (cell-surface proteins), which has a role in cell development, activation, growth and motility. It was reported to be associated with X-linked mental retardation and neuropsychiatric diseases such as Huntington's chorea, fragile X syndrome and myotonic dystrophy (Raymond, 2006; Bassani et al., 2012). In this window, there were 4 other annotated genes (i.e. *SRPX*, *RPGR*, *OTC* and *SYTL5*; Appendix 4.1). Shimakage et al. (2009) suggested that *SRPX* is down regulated when developing small-cell lung carcinoma. The other three genes were reported possibly related to retinitis pigmentosa, hyperammonemia and Rab27A-dependent membrane trafficking in specific tissues (Kuroda et al., 2002, Beltran et al., 2014; Mohamed et al., 2015). However, they have not been reported associated with maladaptation of the pulmonary and cardiac system.

Another important window for RAW was identified on chromosome 20 at 34.05 to 34.98 Mb, but there are no annotated genes in this window. Compared to human and mouse, the bovine genome is poorly annotated (Weikard at al., 2013), with better annotation in the future, there may be some genes in this region to help study the association with PAP and HAD. This GWAS study was based on UMD3.1 sequence assemble map, which has substantial differences from and a higher coverage than BTAU4.6. However, a new cattle optimal map (BtoM1.0) was

developed, and will assist improvements to existing sequence builds (i.e. UMD3.1 and BTAU4.6) and genomic study in bovine community in future (Zhou et al., 2015).

5.3.2.2 Three-category phenotypes for PAP

We observed 12 windows on chromosomes 7, 8, 10, 11, 12, 14, 15, 20 and 28 that were associated with DEBV from CAT3. The most important window associated with CAT3 was also the window on chromosome 7, and the lead-SNP was also *rs41625563*. Another SNP *rs109819349* also had relatively high model frequency (42%) in the window. Another concordant genomic windows between RAW and CAT3 was 11_86, but the lead-SNP were different between RAW and CAT3. Table 5.2 summarizes the QTL windows associated with CAT3.

There were three windows identified in chromosome 12. Beside the lead-SNP of window 12_12, the *rs110675288* also had relatively high model frequency (27.3%). This SNP was 1,284 bp upstream of *AKAP11* (A-kinase anchoring protein 11), and the silencing of this gene retarded cell migration in human cancer cell (Logue et al., 2011). Four other genes were in this window (Appendix 5.2), among them, the expression of the *DGKH (diacylglycerol kinase eta)* was associated with failing human hearts (Bilim et al., 2011).

Table 5.2 Genomic windows explaining > 1% genetic variation of three-category pulmonary arterial pressure (PAP) measurements of yearling Angus cattle from Beef Improvement Center of Colorado State University (elevation at 2,170 m)[1]

| BTA_Mb | Start (bp) | End (bp) | Number of SNP | % Variance Explained | Lead-SNP | Model Frequency | % Variance Explained by Lead-SNP |
|---|---|---|---|---|---|---|---|
| 7_93 | 93007435 | 93886136 | 6 | 6.83 | rs41625563 | 0.49 | 1.42 |
| 12_42 | 42046435 | 42709211 | 8 | 3.80 | rs110660529 | 0.60 | 1.50 |
| 8_89 | 89007062 | 89960384 | 20 | 2.83 | rs43567728 | 0.80 | 1.30 |
| 10_36 | 36065111 | 36978160 | 14 | 1.80 | rs108977212 | 0.60 | 0.79 |
| 11_86 | 86048363 | 86965492 | 15 | 1.74 | rs109850195 | 0.42 | 0.44 |
| 12_34 | 34013716 | 34960812 | 15 | 1.60 | rs11021769 | 0.33 | 0.30 |
| 28_31 | 31053570 | 31960726 | 8 | 1.36 | rs109614495 | 0.56 | 0.81 |
| 14_64 | 64005605 | 64968182 | 14 | 1.26 | rs109608699 | 0.55 | 0.79 |
| 12_12 | 12041734 | 12805107 | 13 | 1.19 | rs43706907 | 0.30 | 0.19 |
| 15_59 | 59020999 | 59937374 | 15 | 1.07 | rs42596067 | 0.60 | 0.07 |
| 20_4 | 4145679 | 4962725 | 22 | 1.07 | rs109724258 | 0.40 | 0.41 |
| 8_83 | 83023897 | 83888935 | 15 | 1.04 | rs41570498 | 0.36 | 0.31 |

[1]Bovine chromosome and n$^{th}$ 1 Mb window on the same chromosome based on the UMD3.1 assembly; Start: start position of the window; End: end position of the window; Num. SNP: number of SNP in the window; % Variance Explained: proportion of the genetic variance explained by the window; Lead-SNP: SNP with the highest model frequency; Model Frequency: the proportion of fitted models including that maker; % Variance Explained by Lead-SNP: proportion of the variance explained by the lead-SNP.

The lead-SNP (*rs110217699*) in the window 12_34 was intragenic of gene *SPATA13 (spermatogenesis associated 13)*, which appears to have role in cell migration in humans (Kawasaki et al, 2007). Beside this gene, seven genes were located in this window (Appendix 5.2). Among them, Srivastava et al. (2005) suggested that the *MTMR6* could regulate $Ca^{2+}$-activated $K^+$ channel and T cells, and be associated with pathological cell proliferation, such as cancer and atherosclerosis. The $Ca^{2+}$-activated $K^+$ channel was also reported to be associated with innate immunity (Ahluwalia et al., 2010). The product of *C1QTNF9* in serum played an important role in metabolic phenotypes, arterial stiffness, inflammation and coronary atherosclerosis in human (Hwang et al., 2013; Jung et al., 2014; Wang et al., 2015). The flow of $Ca^{2+}$ and $K^+$, the metabolic changes and the innate immunity are involved in PH and HAD (Stenmark et al., 2015 and 2016), which could demonstrate this window's association with PAP phenotype of cattle managed at high altitude.

The SNP *rs41593489* was recognized as the lead-SNP in the window 12_42. Another SNP (*rs110660529*) also resulted in relative high model frequency (35%). Therefore, they were both considered important markers in this window that were potentially associated with PAP phenotype of cattle managed at high altitude. Unfortunately, neither of them resident near (< 2,500 bp) any annotated genes based on the *Bos taurus* gene map; as there were no genes annotated within this window. Thus, future studies using more advanced techniques or better sequence and annotation were needed to validate the potential functions of this locus.

Two other CAT3 associated windows were located on chromosome 8. No genes were located near (< 2,500 bp) the lead SNP of window 8_83 (*rs41570498*) and 8_89 (*rs43567728*). One gene in the window 8_89 was *PDCD1LG2* (*programmed cell death 1 ligand 2*), whose expression may down-regulate autoimmune and allergic reactions in human. Kim et al. (2015)

suggested that *PDCD1LG2* and *CD274* were frequently expressed in human pulmonary squamous cell carcinoma. The *CD274* gene was also located in this window 8_89, which plays important role in human immune response. Besides the discussed genes, six other genes were also identified in this window. These genes have diverse biological function and were not previously reported to be associated with the cardiac or pulmonary system (Appendix 5.2).

The CAT3 genomic window approach also identified a QTL window on chromosome 28, and its lead-SNP (*rs109614495*) was located in an open reading frame but ditant from characterized genes. The result suggested that this open reading frame on the bovine genome may be associated with the mechanisms of HAD; however, the bovine genome is not currently annotated as well as the human genome so no functional genes were annotated to this region. Five genes were located in this window region (Appendix 5.2). Among them, Katagiri et al. (2011) showed that *DUSP13* may inhibit the stress-activated MAPKs, and it may play a role in regulating cell proliferation and differentiation (Nakamura et al., 1999). The MAPK signaling and cell proliferation were involved in the mechanism of PH and HAD (Stenmark et al., 2006 and 2013), which could support this genes association with HAD. Other genes functioned on many cellular processes including controlling ceramide homeostasis (*SAMD8*) and realizing metabolite diffusion across the mitochondrial outer membrane (*VDAC2*).

Five annotated genes were located in the identified window from 64 Mb to 65 Mb on chromosome 14 (Appendix 5.2), but the lead SNP (*rs109608699*) of this window was not located within 2,500 bp of any annotated genes. Among them, the interaction between UBR5 (*ubiquitin protein ligase E3 component n-recognin 5*) in this window and *MRE11-RAD50-NBS1* complex was regulated by *PPARγ* to control the DNA damage response, which is an impaired signaling pathway in pulmonary arterial hypertension.

In the region of genomic window 15_59, the SNP *rs42596067* had a relative high model frequency and was considered the lead-SNP, but there were no genes located nearby (< 2,500 bp). However, three genes were observed in this window regions: *BDNF*, *KIF18A* and *METTL15*. The closest gene to the lead SNP was *BDNF* (*brain-derived neurotrophic factor*), which encodes a member of the nerve growth factor family of proteins related to many diseases (e.g. Huntington's, Alzheimer's, Parkinson's), and plays roles in regulating stress. Baker-Herman et al. (2004) suggested that *BDNF* was necessary and sufficient for spinal respiratory plasticity and long-term facilitation. In addition, *BDNF* and their receptors (TrkB) were reported to be associated with pulmonary hypertension through influencing the intracellular $Ca^{2+}$ and NO generation and pulmonary arterial smooth musle cell proliferation (Meuchel et al., 2011; Kwapiszewska et al., 2012; Prakash et al., 2006; Aravamudan et al., 2012). Hartman et al. (2015) also illustrated that a potential mechanism by which hypoxia can promote changes in pulmonary arterial structure and function in humans was the enhanced expression and signaling of the *BDNF-TrkB* system in pulmonary arterial smooth muscle cells. This information coincides with the presented finding that *BDNF* is a potential gene influencing PAP, PH and finally HAD in Angus cattle. The other two genes were not reported to be associated with PAP or HAD, and the *KIF18A* (*kinesin family member 18A*) could play a role in controlling mitotic chromosome positioning as it regulates kinetochore microtubule dynamics, but little functions are known for *METTL15* (*methyltransferase like 15*).

Another window was detected on chromosome 20. The lead-SNP (*rs43350564*) of this window was located 269 bp downstream from *ERGIC1* (*endoplasmic reticulum-golgi intermediate compartment 1*). The *ERGIC1* encodes a cycling membrane protein that interacts with other proteins in the family to enhance each other's turn over. This gene has been associated

with prostate cancer in humans (Vainio et al., 2012), but no previous evidence suggests it is related to PH, HAD or blood pressure.

In addition, 7 other genes located in within the region. Among them, *DUSP1* could regulate MAPK (mitogen-activated protein kinases) and function on inflammation and cell proliferation in humans and animals (Kim et al., 2011; Shah et al., 2014). Jin et al. (2010) suggested that a deficiency of *DUSP1* (*MKP-1*) may be related to the progression of hypoxic PH in mice, and Jin et al. (2014) reported that up-regulation of *DUSP1* (*MKP-1*) in pulmonary arterial smooth muscle cell would decrease their cell proliferation under hypoxia that may prevent PH. The *CREBRF* encodes regulatory factor for *CREB3* (*cAMP responsive element binding protein 3*), and the *CREB3* was reported to promote atherosclerosis and vascular smooth muscle cell migration. The *NKX2-5* (*NK2 homeobox 5*) functions in heart formation and development in humans and was reported to be related to many congenital cardiac defects (Schott et al., 1998; Goldmuntz et al., 2001, Peng et al., 2010). Another gene, *STC2* (*Stanniocalcin-2*) involved in the regulation of calcium and phosphate transport in the kidney was also reported to play multiple roles in human growth and several cancers (Madsen et al., 1998; Jansen et al., 2015; Hou et al., 2015). Na et al. (2015) showed that *STC2* could enhance lung cancer metastasis and progression and be a potential biomarker for lung cancer. The *SH3PXD2B* was located at the edge of this window. The abnormality in this gene-associated protein could cause cardiac anomalies in animals (Iqbal et al., 2010). This may predispose humans and animals' risk of cardiac related diseases including HAD. The other genes within this window had little relationship with PAP, PH or HAD based on previous reports.

5.3.2.3 Two-category phenotype for PAP

Compared to the results from three-category phenotype, fewer genomic windows (n = 9) were identified that explained more than 1% genetic variation of CAT2 (Table 5.3). Window 7_93 was the most important window in RAW and CAT3, and this genomic window also explained the highest portion of genetic variation of CAT2. In addition, the lead-SNP (*rs109819349* and *rs41625563*) of this window were the same across RAW and CAT3, although the model frequency varied. This result implicated that the *G-protein coupled receptor 98* (LOC507513) gene was associated with the PAP measurements and susceptibility to HAD. Window 8_89, 15_59 and 20_4 also explained > 1% genetic variation of both CAT3 and CAT2, and the lead-SNP of 8_89 and 20_4 were the same between the two phenotypes, but the lead-SNP of window 15_59 for CAT2 was *rs108980174* that is different from CAT3 (*rs42596067*). The other top windows were unique for CAT2.

The lead-SNP (*rs41589721*) of this window at 21 to 22 Mb on chromosome 10 was intragenic to *RBM23* (*RNA binding motif protein 23*). The protein encoded by *RBM23* increases transcription of steroid-responsive transcriptional reporters in a hormone-dependent manner (Dowhan et al., 2005). Besides the lead-SNP, another SNP (*rs41647560*) loci yielded relative high model frequency (21.1%). The *rs41647560* also located in *RBM23* and located near (~ 1,300 bp downstream) *LRP10*, but little function was reported to *LRP10*.

Table 5.3 Genomic windows explaining > 1% genetic variation of two-category pulmonary arterial pressure measurements of yearling Angus cattle from Beef Improvement Center of Colorado State University (elevation at 2,170 m)[1]

| BTA_Mb | Start (bp) | End (bp) | Number of SNP | % Variance Explained | Lead-SNP | Model Frequency | % Variance Explained by Lead-SNP |
|---|---|---|---|---|---|---|---|
| 7_93 | 93007435 | 93886136 | 6 | 9.32 | rs41625563 | 0.99 | 9.32 |
| 20_4 | 4145679 | 4962725 | 22 | 5.59 | rs43350564 | 1.00 | 5.59 |
| 12_57 | 57020102 | 57900635 | 15 | 2.37 | rs108987669 | 0.82 | 1.75 |
| 10_29 | 29028329 | 29865159 | 15 | 2.05 | rs43417234 | 0.73 | 1.38 |
| 29_22 | 22019432 | 22930524 | 18 | 1.62 | rs41626199 | 0.58 | 0.70 |
| 15_59 | 59020999 | 59937374 | 15 | 1.37 | rs108980174 | 0.38 | 0.30 |
| 8_89 | 89007062 | 89960384 | 20 | 1.29 | rs43567728 | 0.62 | 0.78 |
| 19_5 | 5057128 | 5934293 | 12 | 1.26 | rs41605392 | 0.62 | 0.75 |
| 10_21 | 21008360 | 21986516 | 15 | 1.01 | rs41589721 | 0.28 | 0.14 |

[1]Bovine chromosome and n$^{th}$ 1 Mb window on the same chromosome based on the UMD3.1 assembly; Start: start position of the window; End: end position of the window; Num. SNP: number of SNP in the window; % Variance Explained: proportion of the genetic variance explained by the window; Lead-SNP: SNP with the highest model frequency; Model Frequency: the proportion of fitted models including that maker; % Variance Explained by Lead-SNP: proportion of the variance explained by the lead-SNP.

Beside these two genes, there were 30 other genes located in this window (Appendix 5.2). The *MYH6* and *MYH7* encode the alpha and beta heavy chain subunits of cardiac myosin, which were associated with familial hypertrophic cardiomyopathy. The *IL-25* encoded a cytokine protein that may be a pro-inflammatory cytokine favoring the Th2-type immune response, and *IL-25* was a key mediator of RV-induced exacerbations of pulmonary inflammation and developed responses to viral respiratory diseases (Beale et al., 2014; Valizadeh et al., 2015). Hams et al. (2014) also reported that *IL-25* is involved in an innate immunity mechanism for generating pulmonary fibrosis. The *IL-25* may have a role in inducting and maintaining eosinophilic-inflammation in the airways by acting on lung fibroblasts (Severine et al., 2006).

The overexpressed *BCL2L2* was reported to promote the growth of a non-small cell lung cancer cell (Kawasaki et al., 2007). Lim et al. (2014) demonstrated that the activation of *PRMT1* could mediate hypoxia- and ischemia-induced apoptosis in human lung epithelial cells and in the lung of miniature pigs. Xuan et al. (2013) identified two novel heterozygous missense mutations in *HOMEZ* gene exon-2 in isolated ventricular septal defect patients in the Chinese population, which were directly linked with the etiology of isolated Cardio Ventricular Septal Defect that can also cause elevated PAP and PH. Also, the non-coding region of *HOMEZ* still had the possibility of harboring recessive mutations leading to congenital heart disease in the Indian population. The mechanism of PH and HAD involve inflammation, immunity and cell proliferation in pulmonary and cardiac system (Stenmark et al., 2013 and 2015), so the functions of these genes could explain their association with HAD.

Another window (10_29) was also identified on chromosome 10, and three genes were located in this region (Appendix 5.2). The lead-SNP *(rs43417234)* is intragenic to *FMN1,* which was reported to be involved in cell polarity, cytokinesis, cell migration and transcriptional

activity (Hu et al., 2014). The *RYR3* (ryanodine receptor 3) in this window is one member of ryanodine receptors, which are the major $Ca^{2+}$ release channels and control cardiac and skeletal muscle contraction in human and animals (Santulli et al., 2015). The expression of ryanodine receptors could influence the intracellular $Ca^{2+}$ level in pulmonary arterial smooth muscle cells was important for hypoxic $Ca^{2+}$ and contractile response in PH (Wang and Zheng, 2010).

The lead-SNP of window 19_5 was located intragenic to *TOM1L1,* and the amplification of this gene could enhance the metastatic progression of *ERBB2*-positive breast cancers. Among the four other genes in this window (Appendix 5.2), the MMD was associated with differentiation from monocytes to macrophages, and its interaction with miR-140-5p could affect the non-small cell lung cancer in human (Li and He 2014). Another CAT2 unique window was on chromosome 29, and the *SLC17A6* contained the lead-SNP of this window. This window contains three additional genes (Appendix 5.2), ANO5 was one of them and is a putative calcium-activated chloride channel, whose mutation may enhance dilated cardiomyopathy (Wahbi et al., 2013). The increased calcium-activated chloride channel activity was reported to be associated with PH induced by various factors in a mice model (Wang et al., 2015). Another CAT2 associated QTL was identified on chromosome 12 at 57 Mb, but no genes were annotated in this region.

#### 5.3.2.4 Additive and dominant effects

This section presents the significance, least square means (LSM), additive and dominant effects resulted from analyzing genotypes of each lead-SNP on EBV of yearling PAP phenotypes separately in Table 5.4. The majority of these lead-SNP had significant effects ($P < 0.05$) on PAP phenotypes except three SNP (*rs41618346*, rs42596067, *rs41605392*). In windows of these three lead-SNP, there were other SNP having relatively high model frequency, which could share

the QTL variation of the yearling PAP phenotypes and reduce the effect of the lead-SNP. For example, the *rs41618346* had model frequency of 28% but *rs110159935* in the same window also had a model frequency of 27%, so the summing of the variation of the SNP in the window elevated this region to the top window on a Manhattan plot.

The LSM of three genotypes (i.e. AA, AB, BB) were different from each other ($P < 0.05$) in studying most of the lead-SNP, which demonstrated these SNP's association with yearling PAP phenotypes. Based on the LSM, the favorable and unfavorable genotype can be identified. For example, the individual having AA on *rs109819349* could have a unfavorable (0.48) performance on yearling PAP phenotypes, while individual with BB could show favorable (-1.31) performance. In addition, the majority of these lead-SNP resulted in significant ($P < 0.05$) additive effects but non-significant dominant effects except *rs41589721* whose dominant effect test was significant. This demonstrated the polygenetic characteristics of yearling PAP phenotypes, additive effects of most of yearling PAP associated SNP and dominant effects of limited number of yearling PAP associated SNP.

Historically, studies suggested a single autosomal dominant genetic effect for PAP and susceptibility to PH or HAD based on breeding experiments in cattle (Weir et al., 1974; Anand et al., 1986). Neary et al. (2014) described a gene (*MYH15*) linking to lower PAP in a dominant manner. However, compared to our study, these reports involved limited sample size and genes, or in studies that are preceding the development of the bovine genome and its tools, which would lead these older studies to misinterpret the overall inheritance pattern of PAP and susceptibility to PH and HAD.

Table 5.4 Significance, estimate least square means (s.e.) of each genotype, and estimated additive and dominant effects of lead-SNP (identified in QTL windows) on EBV of yearling pulmonary arterial pressure (PAP) phenotypes in Angus cattle managed at high altitude (elevation at 2,170 m)

| Phenotype[1] | Lead-SNP | P-Value[2] | Genotype[3] | | | Effects[4] | |
|---|---|---|---|---|---|---|---|
| | | | AA | AB | BB | Additive | Dominance |
| RAW | rs109850195 | <0.001 | 0.19(0.18)$^{a}$ | -0.34(0.080)$^{a}$ | -0.73(0.070)$^{b}$ | -0.35*** | 0.0097 |
| RAW | rs110993632 | <0.001 | -0.061(0.13)$^{a}$ | -0.51(0.073)$^{b}$ | 1.11 (0.084)$^{c}$ | -0.52*** | 0.071 |
| RAW | rs109633897 | 0.002 | -0.32(0.12)$^{a}$ | -0.64 (0.075)$^{ab}$ | -0.83(0.087)$^{b}$ | -0.26*** | -0.067 |
| RAW | rs110159935 | 0.116 | -0.68(0.056)$^{a}$ | -0.65(0.14)$^{a}$ | -0.13(0.26)$^{a}$ | 0.28* | -0.24 |
| RAW | rs41618346 | 0.073 | -0.096(0.25)$^{a}$ | -0.63(0.14)$^{a}$ | -0.68(0.056)$^{a}$ | -0.29* | -0.24 |
| RAW | rs41625563 | <0.001 | 0.36(0.11)$^{a}$ | -0.66(0.070)$^{b}$ | -1.28 (0.091)$^{c}$ | -0.82*** | -0.20 |
| RAW | rs109819349 | <0.001 | 0.48(0.12)$^{a}$ | -0.60(0.070)$^{b}$ | -1.31 (0.086)$^{c}$ | -0.89*** | -0.18 |
| CAT3 | rs108977212 | <0.001 | 0.034(0.033)$^{ab}$ | 0.023(0.0090)$^{a}$ | -0.029(0.0050)$^{b}$ | -0.031 | 0.021 |
| CAT3 | rs109850195 | <0.001 | 0.044(0.015)$^{a}$ | -0.00078(0.0069)$^{b}$ | -0.039(0.0061)$^{c}$ | -0.041*** | -0.00035 |
| CAT3 | rs109767777 | <0.001 | 0.017(0.0081)$^{a}$ | -0.014(0.0059)$^{b}$ | -0.067(0.0088)$^{c}$ | -0.042*** | 0.011 |
| CAT3 | rs110675288 | <0.001 | 0.0078(0.0083)$^{a}$ | -0.016(0.0061)$^{a}$ | -0.048(0.0095)$^{b}$ | -0.028*** | 0.0040 |
| CAT3 | rs110217699 | 0.046 | -0.0026(0.0073)$^{a}$ | -0.021(0.0061)$^{a}$ | -0.033 (0.012)$^{a}$ | -0.015* | -0.0031 |
| CAT3 | rs110660529 | <0.001 | -0.052(0.0070)$^{a}$ | -0.0058(0.0061)$^{b}$ | 0.055(0.012)$^{c}$ | 0.054*** | -0.0073 |
| CAT3 | rs41593489 | <0.001 | -0.051(0.0073)$^{a}$ | 0.0053(0.0062)$^{b}$ | 0.058(0.012)$^{c}$ | 0.054** | -0.0085 |
| CAT3 | rs109608699 | <0.001 | -0.069(0.016)$^{a}$ | -0.019(0.0069)$^{b}$ | -0.0058(0.0061)$^{c}$ | 0.031*** | 0.018 |
| CAT3 | rs42596067 | 0.313 | -0.027(0.0094)$^{a}$ | -0.016(0.0063)$^{a}$ | -0.0086(0.0080)$^{a}$ | 0.0094 | 0.0023 |
| CAT3 | rs43350564 | <0.001 | -0.036(0.0073)$^{a}$ | -0.012(0.0063)$^{b}$ | 0.015(0.011)$^{b}$ | 0.025*** | -0.0019 |
| CAT3 | rs41625563 | <0.001 | 0.061(0.0098)$^{a}$ | -0.014(0.0060)$^{b}$ | -0.069(0.0078)$^{c}$ | -0.065*** | -0.010 |
| CAT3 | rs109819349 | <0.001 | 0.080(0.010)$^{a}$ | -0.0087(0.0060)$^{b}$ | -0.074(0.0071)$^{c}$ | -0.078*** | -0.011 |
| CAT3 | rs41570498 | <0.001 | 0.0063(0.0059)$^{a}$ | -0.037(0.0070)$^{b}$ | -0.066(0.016)$^{b}$ | -0.036*** | -0.0075 |
| CAT3 | rs43567728 | <0.001 | 0.052(0.023)$^{a}$ | 0.0020(0.0076)$^{a}$ | -0.029(0.0054)$^{b}$ | -0.040*** | -0.0094 |

Table 5.4. Continue

| Phenotype[1] | Lead-SNP | P-Value[2] | Genotype[3] | | | Effects[4] | |
|---|---|---|---|---|---|---|---|
| | | | AA | AB | BB | Additive | Dominance |
| CAT2 | rs41589721 | <0.001 | 0.037(0.022)[ab] | 0.046(0.010)[a] | -0.013(0.10)[b] | -0.025* | 0.034* |
| CAT2 | rs43417234 | <0.001 | 0.073(0.021)[a] | 0.028(0.010)[a] | -0.0087(0.10)[b] | -0.041*** | -0.0040 |
| CAT2 | rs108987669 | <0.001 | 0.077(0.011)[a] | -0.0037(0.011)[b] | -0.090(0.019)[c] | -0.078*** | 0.0020 |
| CAT2 | rs108980174 | <0.001 | 0.076(0.011)[a] | 0.0010(0.010)[b] | -0.079(0.019)[c] | -0.077*** | 0.0025 |
| CAT2 | rs41633546 | <0.001 | -0.079(0.018)[a] | 0.0034(0.010)[b] | 0.077(0.011)[c] | 0.078*** | 0.0048 |
| CAT2 | rs41605392 | <0.469 | -0.024(0.035)[a] | 0.021(0.012)[a] | 0.019(0.0089)[c] | 0.021 | 0.024 |
| CAT2 | rs43350564 | <0.001 | -0.020(0.012)[a] | 0.022(0.010)[b] | 0.085(0.017)[c] | 0.052*** | -0.010 |
| CAT2 | rs41626199 | <0.001 | 0.044(0.010)[a] | -0.0098(0.011)[b] | 0.0017(0.024)[ab] | -0.021 | -0.032 |
| CAT2 | rs41625563 | <0.001 | 0.15(0.015)[a] | 0.020(0.0095)[b] | -0.075(0.013)[c] | -0.11*** | -0.019 |
| CAT2 | rs43567728 | <0.001 | 0.13(0.038)[a] | 0.049(0.012)[a] | -0.0031(0.0087)[b] | -0.067*** | -0.014 |

[1]RAW: non-transformed yearling PAP measurements; CAT3: three-category yearling PAP phenotypes; CAT2: two-category yearling PAP phenotypes.
[2]The significance of single SNP analysis on EBV.
[3]Different letter within SNP denote significant at *P-value < 0.05*.
[4]***Significant at *P-value < 0.001*; **Significant at *P-value < 0.01*; *Significant at *P-value < 0.05*.

5.3.3 Effect of different phenotypic forms on GWAS

Table 5.5 presents correlations between the SNP effects estimated from the three yearling PAP phenotypes. These SNP effect correlations were lower than the estimate genetic correlations and EBV correlations between these phenotypes using pedigree-based quantitative genetic methodologies; however, these SNP correlations reflected a similar pattern to genetic and EBV correlations. Note the relationship between RAW and CAT3 appeared closer than that between RAW and CAT2 (Table 3.7 and Table 3.8). These results showed genetic differences between different yearling PAP phenotypes, and confirmed that genetics may play roles in causing different levels of resistance or susceptibility for PH or HAD in Angus cattle.

Table 5.5. Pearson (above diagonal) and Rank (below diagonal) correlations between genome-wide SNP effect of three yearling pulmonary arterial pressure (PAP) phenotypes of Angus cattle at high altitude regions

| Phenotypes[1] | RAW | CAT3 | CAT2 |
|---|---|---|---|
| RAW | 1 | 0.48 | 0.42 |
| CAT3 | 0.73 | 1 | 0.37 |
| CAT2 | 0.67 | 0.67 | 1 |

[1]RAW: non-transformed yearling PAP measurements; CAT_3: three-category phenotype, 1: PAP < 41 mmHg, 2: 41 mmHg <= PAP <= 49 mmHg, 3: PAP > 49 mmHg; CAT_2: two-category phenotype, 1: PAP <= 49 mmHg, 2: PAP > 49 mmHg

The only window that explained greater than 1% genetic variance in RAW, CAT3 and CAT2 was window 7_93 on chromosome 7, and the window 7_93 was the most important window for all the studied phenotypes. As discussed, the lead-SNP (*rs109819349 and rs41625563*) in this window were associated with two un-annotated genes on the bovine genome. Nonetheless, these two genes have a potential influence on PAP measurements and susceptibility of HAD in Angus cattle. Although the *LOC507513* was not annotated in the *Bos taurus* genome, it was predicted as a potential *G-protein coupled receptor 98* (*GPCR98*). When aligning the

sequence of this gene to genome sequences of other species using BLAST of NCBI (Johnson et al., 2008), this gene sequence was homologous with the *G-protein coupled receptor 98* gene in many other species. The G-protein coupled receptors are the largest superfamily of related proteins and the most diverse group of membrane receptors in eukaryotes, which assist cells to respond to their environment (O'Connor et al., 2010; OMIM 602851). This group of proteins has important roles in modern medicine, as they were one of the most targeted sources for modern medicinal drugs. This gene in human genome was named as *ADGRV1* (*adhesion G protein-coupled receptor V1*). It is calcium-binding G-protein coupled receptor expressed in the central nervous system. Several reported studies suggested that *ADGRV1* was associated with Usher syndrome type 2 (a hearing and vision disease), and mutations in this gene can be used in genetic diagnosis of this disease (Scheel et al., 2002, Besnard et al., 2012; Moteki et al., 2015). In addition, it was also reported that a mutation in *ADGRV1* had a role in regulating bone mineral density in human and mouse (Urano et al., 2012) and its low expression risk factor of epileptic seizures in patients with low-grade glioma (Wang et al., 2015). Results of the current study suggested that *GPCR98*'s association with elevated PAP, PH and HAD susceptibility, and which may be attribute to its role in calcium-binding and regulating cellular response to hypoxia of high altitude because increasing of intracellular calcium level in pulmonary arterial smooth muscle cells would contribute to the development of hypoxic PH (Wang et al., 2006; Whitman et al., 2008).

When considering the most important genomic windows across the three phenotypes (i.e. top 2%), 5 windows were in concordance across phenotypes (Table 5.6). Four of these windows explained >1% genetic variation of RAW, CAT3 or CAT2, but window 12_25 consistently explained similar and moderate genetic variation of phenotypes across RAW, CAT3 and CAT2,

it were also considered important genomic regions associated with PAP measurements and susceptibility risk to HAD.

Table 5.6. Common top 2% (n=52) windows across non-transformed (RAW), three-category (CAT3) and two-category (CAT2) pulmonary arterial pressure phenotypes of Angus cattle managed at high altitude (elevation at 2,170 m)[1]

| BTA_Mb | Start (bp) | End (bp) | Number of SNP | %Variance Explained | | |
|---|---|---|---|---|---|---|
| | | | | RAW | CAT3 | CAT2 |
| 7_93 | 93007435 | 93886136 | 6 | 9.64 | 6.83 | 6.78 |
| 11_86 | 86048363 | 86965492 | 15 | 1.11 | 1.74 | 0.93 |
| 12_25 | 26358215 | 26967177 | 5 | 0.59 | 0.26 | 0.44 |
| 15_59 | 59020999 | 59937374 | 15 | 0.44 | 1.07 | 1.43 |
| 20_34 | 4145679 | 4962725 | 22 | 1.06 | 0.78 | 0.52 |

[1]Bovine chromosome and $n^{th}$ 1 Mb window on the same chromosome based on the UMD3.1 assembly; Start: start position (base pair) of the window; End: end position (base pair) of the window; Number of SNP: number of SNP in the window; %Variance Explained: proportion of the genetic variance explained by the window.

Four genes were located in this window (Appendix 5.2). The *SPG20* encoded protein regulated endosomal trafficking and mitochondria function, and this gene could inhibit BMP signalling that is important in pulmonary hypertension by promoting BMP receptor degradation (Tsang et al., 2009). Other genes in this window had roles in cancer and cancer development.

Within the concordant windows, 19 genes were observed, and Table 5.7 summarizes their gene ontology information (i.e. biological processes and KEGG pathways) from gene enrichment analysis of these genes based on their biological functions and related pathways. Results suggested that these genes have roles in metabolic and neural regulation. The significant KEGG pathway was cAMP signaling pathway. Serezani et al. (2008) reported that cAMP could regulate metabolism and gene regulation, and influence innate immune function of human and animals via controlling phagocyte function. Innate immune response and vascular inflammation were reported to be involved in the development of PH and HAD (Stenmark et al., 2006 and 2013).

Sitbon and Morrell (2012) reported that the cAMP signaling pathway was involved in pathogenesis of pulmonary arterial hypertension.

Table 5.7 Significant gene ontology (GO) terms from gene enrichment analysis on genes within identified concordant 1-Mb windows with genome-wide association studies across yearling PAP phenotypes of Angus cattle managed at high altitude (elevation at 2,170)

| GO[1] | ID | P-value[2] | Name | Gene list |
|---|---|---|---|---|
| BP | GO:0051234 | 1.49E-02 | Adipose tissue development | *ARRDC3, SPG20* |
| BP | GO:0098660 | 7.00E-03 | Collateral sprouting | *SPG20, BDNF* |
| ke | KEGG:05231 | 3.27E-02 | cAMP signaling pathway | *ROCK2, BDNF* |

[1]BP: Biological process; ke: KEGG pathway
[2] Benjamini-Honchberg FDR corrected P-Value
[3]Associated chromosome and n$^{th}$ 1-Mb window based on UMD3.1 assembly

Besides the enrichment analysis, the gene ontology information (i.e. molecular function, cellular component and associated pathways) of single gene was also investigated (Table 5.8). The molecular function of the most important gene identified in this study (*GPR98/ADGRV1*) includes ion binding (i.e. calcium and metal ion), cytoskeletal protein binding, myosin binding and cation binding. Wilkins et al. (2015) suggested that the mechanism of the acute and chronic hypoxic pulmonary vasoconstriction involves hypoxia-induced release of vasoactive mediators (i.e. endothelin 1, prostacyclin and nitric oxide), changes in intracellular $Ca^{2+}$ and changes in pulmonary arterial small muscle cell myofilament sensitivity to $Ca^{2+}$ (arising from inhibition of myosin light chain phosphatase; via RhoA/Rho kinase or protein kinase C, or decreased nitric oxide signaling; Figure 4.2 and Figure 4.3). Remillard et al. (2005) and Yuan (2005) also reported the important roles of $Ca^{2+}$ and $K^{+}$ on pulmonary vasoconstriction and vascular remodeling in chronic hypoxia-induced PH via their functions on cellular volume, gene transcription, apoptosis and cell cycle progression. Therefore, this gene may influence $Ca^{2+}$ and other ion transportation process and subsequentially may have roles in PH and HAD in Angus cattle. Newman et al. (2011) also identified a gene (i.e. *EMR1*) having molecular function on G protein-coupled receptor and up-regulated in cattle with hypoxia-induced PH.

Among the gene related pathways, there were several pathways were previously reported to be associated with high altitude adaptation, PH and HAD in humans and cattle, including MAPK signaling pathway, Wnt signaling pathway and TGF-β signaling pathway (De et al., 2013; Newman et al. (2011). The MAPK signaling pathway is involved in cell proliferation, differentiation and migration in mammals (Sun et al., 2015), and was reported to be involved in pulmonary vascular remodeling from PH in both human and cattle (Wilson et al., 2015 and Archer et al., 2010). The Wnt signaling pathway is another important pathway included three different parts: the canonical pathway, the planar cell polarity pathway and the Wnt/$Ca^{2+}$ pathway. This pathway was related to the MAPK signaling pathway and has a role in cell-fate specification, progenitor-cell proliferation and the control of asymmetric cell division (Kenehesa, et al., 2008). Also, Königshoff and Eickelberg (2010) summarized and reported that Wnt signaling pathway was involved in lung development and lung diseases including lung homeostasis, lung cancer, lung fibrosis and pulmonary arterial hypertension. The TGF-β signaling pathway, involving TGF-β, activins and bone morphogenetic proteins (BMPs), is related to both MAPK signaling pathway and Wnt signaling pathway, and also have roles in regulating cell proliferation, apoptosis, differentiation and migration (Massagué, 2012). A mutation in the *BMPR2* was found in humans and thought to contribute to the abnormal growth in pulmonary artery smooth muscle cells (Morrell, 2006). It was also unregulated in the peripheral pulmonary vasculature during hypoxia-induced PH (Anderson et al., 2010).

Cell proliferation pathways therefore could related these genes involved in these pathways to PH and HAD, because chronic hypoxia vascular remodeling (i.e. cell proliferation) is an important characteristic of PH and was demonstrated by histological changes observed in necropsy examination in animal model with PH (e.g. cattle and rats; Hislop et al., 1976; Neary et

al., 2015). The increased muscularization of distal vessels with extension of muscle into previously un-muscularized arterioles was reported in rats and human residing in hypoxic environment (Arias-Stella and Saldana.,1974; Hislop et al., 1976). Alexander (1965) and Neary et al. (2015) reported the narrowing of the pulmonary arteries and veins in response to hypoxia. The histological changes in pulmonary vascular tree including expanded adventitia with micro-vascular proliferation were also observed in dairy cattle having PH (Malherbe et al., 2012). In addition, Stenmark et al. (2006) described marked adventitial thickening in hypoxic neonatal calves.

Besides the proliferation of the smooth muscle cell and fibroblasts in pulmonary distal vascular, inflammation and metabolic reprogramming (i.e. Warburg effect) of fibroblast and vascular remolding was also identified in individuals with PH (Li et al., 2011 and 2016). Burke et al. (2009) and Stenmark et al. (2015) implicated that the change in cell metabolism and infiltration of inflammatory cell, dendritic cells, and T cells were evidence of the pulmonary vascular remodeling. Newman et al. (2011) reported that the inflammatory response and immunological disease could be associated with hypoxia-induced PH in cattle using gene enrichment analysis, and also identified several hypoxia-induced PH associated genes that have roles in immune and inflammatory response (e.g. *TCRB* and *PTX3*). In this present study, the *ROCK2* involved in pathways for chemokine signaling pathway and vascular smooth muscle contraction were observed. The vascular smooth muscle contraction pathway could influence the pulmonary vasoconstriction in PH, and the chemokine signaling pathway is involved in inflammatory immune response system (Wong and Fish, 2003), which supported this gene's association with PAP in cattle.

Table 5.8 Summary of genes located in concordant genomic windows from genome-wide association study of yearling PAP phenotypes of Angus cattle at high altitude (elevation at 2,170 m)[1]

| BTA_Mb[2] | Gene | CC[3] | MF[3] | Pathway[3] |
|---|---|---|---|---|
| 7_93 | *ADGRV1* | Stereocilium, plasma membrane and cell surface | Calcium ion and protein binding | - |
| | *ARRDC3* | Endosome and plasma membrane | Protein binding | - |
| 11_86 | *LPIN1* | Nucleus | Phosphatidate phosphatase activity | Metabolic pathways, Glycerolipid metabolism and Glycerophospholipid metabolism |
| | *NTSR2* | Membrane and plasma membrane | G-protein coupled (neurotensin) receptor activity | Neuroactive ligandreceptor interaction |
| | *E2F6* | Nucleus and transcription factor complex | DNA and protein binding, and transcription factor and corepressor activity | - |
| | *ROCK2* | Cytoskeleton, plasma membrane, centrosome And cytosol | protein serine/threonine kinase activity, meta ion binding, ATP binding | Chemokine signaling pathway, Vascular smooth muscle contraction, Wnt signaling pathway, TGFbeta signaling pathway, Axon guidance, Focal adhesion, Leukocyte transendothelial migration, Regulation of actin cytoskeleton, Pathogenic Escherichia coli infection |
| | *PQLC3* | Endoplasmic reticulum, integral component of membrane | - | - |
| | *ATP6V1C2* | Proton-transporting V-type ATPase, V1 domain | Hydrolase activity, protein dimerization activity | ATPase, V1 complex, subunit C |

Table 5.8 continue

| BTA_Mb | Gene | CC | MF | Pathway |
|---|---|---|---|---|
| 11_86 | *PDIA6* | Endoplasmic reticulum, endoplasmic reticulum-Golgi intermediate compartment, and melanosome, | Protein disulfide isomerase activity, intra-molecular oxidoreductase activity, | Protein processing in endoplasmic reticulum |
| | *NOL10* | Nucleolus | Poly(A) RNA binding | - |
| | *HIST1H4A* | Nuclear chromosome, nucleus | DNA, histone, poly(A) RNA and protein binding, histone demethylase activity | - |
| | *KCNF1* | Voltage-gated potassium channel complex and membrane | Potassium channel activity | - |
| | *GREB1* | Integral component of membrane and extracellular exosome | - | - |
| 12_25 | *CCNA1* | - | - | Cell cycle, Progesterone-mediated oocyte maturation |
| | *SPG20* | - | - | - |
| | *NBEA* | Plasma membrane, cytosol | Protein kinase binding, phospholipid binding | - |
| 15_59 | *BDNF* | Extracellular region, cytoplasmic membrane bounded vesicle, cytoplasm | Growth factor activity and neurotrophin TRKB receptor binding | MAPK signaling pathway, Neurotrophin signaling pathway, Huntington's disease |
| | *KIF18A* | Ruffle, cytoskeleton, microtubule and caveola, | Microtubule motor activity, and actin, protein ATP, microtubule and ubiquitin binding | - |
| | *METTL15* | - | protein binding, methyltransferase activity | - |

[1]Three yearling PAP phenotypes included non-transformed, three-category (1: PAP < 41 mmHg; 2: 41 mmHg ≤ PAP ≤ 49 mmHg; 3: PAP > 49 mmHg) and two category PAP (1: PAP ≤ 49 mmHg; 2: PAP > 49 mmHg))
[2] Bovine chromosome and $n^{th}$ 1 Mb window on the same chromosome based on the UMD3.1 assemble.
[3]CC: cellular component; MF: molecular function; Pathway: Kyoto Encyclopedia of Genes and Genomes pathways.

## 5.4 Conclusions

Four, twelve and nine 1-Mb genomic windows were identified through GWAS to be associated with RAW, CAT3 and CAT2, respectively, and demonstrated the polygenetic basis for PAP phenotypes in Angus cattle. Five 1-Mb windows located on chromosome 7, 11, 12, 15, 20 were concordant across various PAP phenotypes. Genes within these identified windows were also recognized to be potentially associated with PAP phenotypes. These genes are related to ion binding and transportation, inflammation, innate immunity and cell proliferation mechanisms, which provided evidences of their potential functions on chronic hypoxia induced elevated PAP in Angus cattle. The identified QTL regions can be used to help understanding of bovine PH and HAD, identifying cattle's susceptibility to elevated PAP, and selecting against PH and HAD with genomic selection. The studied yearling PAP phenotypes were moderate heritable polygenetic traits and influenced by a lager number of loci with small effects and only a small number of loci with large effects. Combining these data in multi-omics studies (i.e. RNA-seq studies) on the same phenotypes can be helpful to verify our current findings.

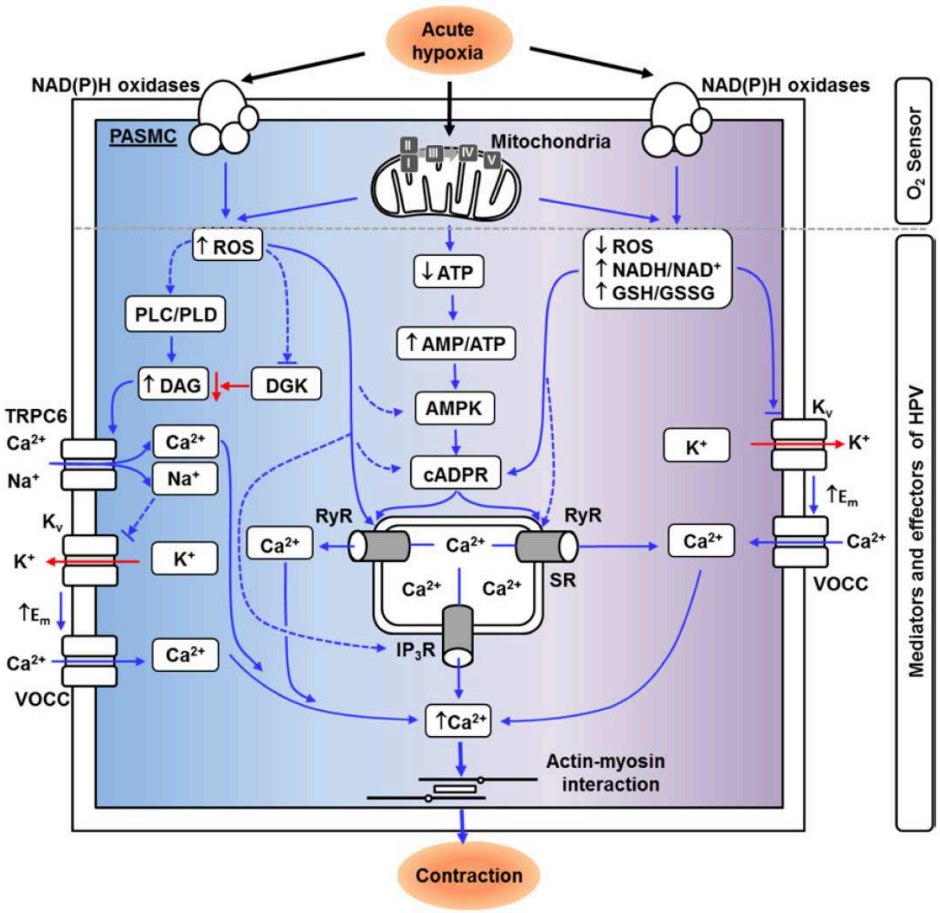



Figure 5.2 Signaling mechanisms underlying acute hypoxic pulmonary vasoconstriction (HPV). Pathways activated by hypoxia are depicted in blue; those inhibited by hypoxia are depicted in red. Both mitochondria and nicotinamide adenine dinucleotide (phosphate) oxidases have been suggested as oxygen sensors. A reduction in the cytosolic redox state could inhibit voltage-dependent potassium channels, subsequent membrane depolarization of PASMCs, opening of l-type calcium channels and $Ca^{2+}$ influx.20 By contrast, increased cytosolic ROS levels can result in $Ca^{2+}$ release from the SR, possibly through the oxidation of cysteine residues in RyRs and the opening of IP3-gated calcium stores.19 Increased ROS could also provoke an influx of extracellular $Ca^{2+}$ or $Na^{+}$ through transient receptor potential channels (TRPC6).21 In this scenario, the increase of acute hypoxia-induced ROS triggers an accumulation of DAG, resulting from the activation of phospholipase C or phospholipase D or inhibition of DAG-degrading DAG kinases. Another proposal assumes that acute hypoxia leads to inhibition of the respiratory chain and a subtle decrease in ATP production, which does not affect energy state, but rather acts as a mediator and alters the cellular AMP/ATP ratio. An increase in the AMP/ATP ratio activates AMPK, followed by an increase in cADPR that triggers the release of $[Ca^{2+}]i$ through RyR of SR.9 The level of ROS could be relevant through ROS-dependent alteration of function of AMPK and cADPR. (Wilkins et al., 2015)

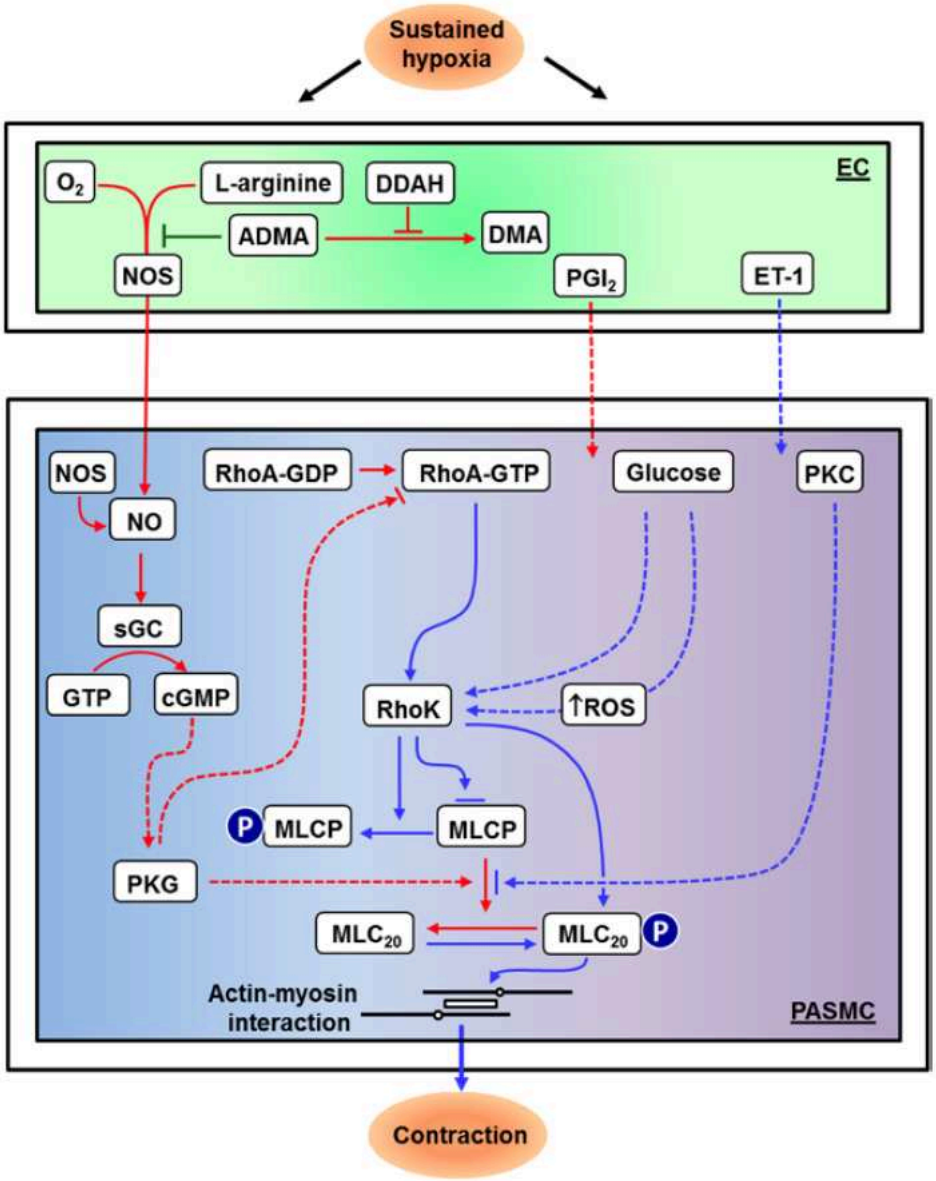



Figure 5.3 Signaling mechanisms underlying sustained hypoxic pulmonary vasoconstriction (HPV). The endothelium releases a variety of vasoactive mediators, such as endothelin 1, prostacyclin, and nitric oxide (NO),9,16 and their production is perturbed by hypoxia. In addition to changes in intracellular Ca2+ levels, changes in PASMC myofilament sensitivity to Ca2+, arising from inhibition of MLCP via RhoA/Rho kinase or protein kinase C (PKC), or a decreased activation of MLCP by decreased NO signaling,9 also contributes to sustained HVP. ADMA indicates asymmetrical dimethylarginine; cGMP, cyclic guanosine monophosphate; DDAH, dimethylarginine dimethylaminohydrolase; DMA, dimethylamine; ET-1, endothelin 1; EC, endothelial cell; MLC20, regulatory myosin light chain; MLCP, myosin light chain phosphatase; NO, nitric oxide; NOS, nitric oxide synthase; O2, oxygen; PASMC, pulmonary arterial smooth muscle cell; PGI2, prostacyclin; Rho, Ras homolog gene family; ROS, reactive oxygen species; and sGC, soluble guanylyl cyclase. Phosphorylated proteins are indicated by a white "P" in a blue circle. (Wilkins et al., 2015)

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# CHAPTER 6

# GENOME-WIDE ASSOCIATION STUDY OF GROWTH PERFORMANCE TRAITS OF ANGUS CATTLE MANAGED AT HIGH ALTITUDE

## 6.1 Introduction

Growth performance traits in cattle are moderately to highly heritable, and it is feasible to identify QTL associated with them using genomic wide association study (GWAS; Saatchi et al., 2014). This section reports GWAS of birth weight, weaning weight, maternal weaning weight, post-weaning gain and yearling weight using deregressed EBV and genotype data from the BovineSNP50 BeadChip. The objective of this study was to determine the chromosomal regions associated with growth performance traits. Chapter 4 suggested a low to moderate genetic correlation between performance traits and yearling pulmonary arterial pressure (PAP) phenotypes. Therefore, another objective of this chapter was to compare resulgts of the growth performance GWAS and PAP phenotypes to help understand the genetic relationships between them.

## 6.2 Materials and Methods

Genome-wide association studies were conducted on performance traits of Angus cattle managed at high altitude (elevation at 2,170 m). These traits included birth weight (BWT), weaning weight (WW), maternal weaning weight (MILK), post-weaning gain (PWG) and yearling weight (YW). Deregressed EBV (DEBV) of these growth traits were used as dependent variables in GWAS. Estimated breeding value of BWT, WW, MILK and PWG were developed from the multivariate models described in Equation 4.3. The EBV of YW was obtained by

summing up the EBV of WW and PWG, and the accuracy of PWG was used to approximate the accuracy for YW. The DEBV and associated scale wights for growth traits were calculated using procedures described by Garrick et al. (2009), and was presented in Chapter 5. Heritability of BWT, WW, MILK, PWG and YW used in developing DEBV were 0.41, 0.21, 0.23 and 0.41, respectively. Animals with low DEBV reliability (< 0.05) were removed from these GWAS for quality of analyses.

Genotype data of 2,582 samples were used in this study, and 35,930 SNP were used in these GWAS (for details on quality control, see material and methods in Chapter 5). These SNP were simultaneously included in GWAS models for these growth traits (Equation 5.7). Similar with GWAS of PAP phenotypes, growth performance traits DEBV were analyzed using Bayes B (Meuwissen et al., 2001) and Bayes C (Habier et al., 2011) methods with $\pi$ equaling 0.995. The Bayes C was used to obtain genetic and residual variance to construct priors of genetic and residual scale parameters for Bayes B, the reason for this was that Bayes C is less sensitive to prior assumptions than Bayes B (Garrick and Fernando, 2013). Also, GeneSel software (Fernando and Garrick, 2008) with Marlov chain Monte Carlo (MCMC) method was used to conduct these GWAS analyses. These analyses were executed using 41,000 iterations for each run with the first 1000 samples as burn-in. As a result, 40,000 samples were used to provide posterior distribution of each estimable parameter. The QTL regions of each growth trait were identified through the evaluation of 1-Mb windows (explained larger than 1% of genetic variation) across the whole genome (n = 2,648), which were explained in Chapter 5. Pleiotropic window regions were recognized as those identified for at least two of these studied performance traits.

Associated genes were identified via aligning identified genomic windows on *Bos taurus* genome (UMD 3.1) in Ensembl database (Kinsella et al., 2011; http://uswest.ensembl.org/index.html). Additional information of identified windows and genes were also obtained from NCBI dbSNP and gene database (Sherry et al., 2001; Coordinators et al., 2013; http://www.ncbi.nlm.nih.gov/). Gene enrichment analysis on genes located within identified pleiotropic genomic windows was conducted via the web tool g:Profiler (Reimond et al., 2016; http://biit.cs.ut.ee/gprofiler/). The biological process and KEGG pathways with Benjamini–Honchberg false discovery rate (FDR) corrected *P* value at 0.05 were reported.

## 6.3 Results and Discussion

### 6.3.1 DEBV

The number of animals with usable DEBV for BWT, WW, MILK, PWG and YW were 2,553, 2,553, 548, 2,551 and 2,551, respectively, since there were 29, 29, 2,034, 31 and 31 cattle that had DEBV reliability less than 0.05 for these traits, respectively. There were only 510 dams with offspring having weaning weights, and the parent average accuracy were removed in DEBV, which may contribute to the small number of samples used in GWAS for maternal WW. Applying appropriate non-zero direct by maternal genetic correlations in develop maternal WW EBV may improve this issue. Table 6.1 summaries the resulting EBV, DEBV, DEBV-associated reliability and scale weights for each performance trait.

Table 6.1. Summary of EBV and deregressed EBV of performance traits of genotyped Angus cattle managed at high altitude region (elevation at 2,170 m)

| Item[1] | n | Mean | Min | Max | SD |
|---|---|---|---|---|---|
| BWT | | | | | |
| EBV | 2553 | -1.89 | -17.79 | 14.91 | 4.35 |
| DEBV | 2553 | -1.62 | -39.42 | 44.91 | 10.08 |
| ACC | 2553 | 0.44 | 0.09 | 0.90 | 0.05 |
| WEIGHTS | 2553 | 0.83 | 0.13 | 2.73 | 0.22 |
| WW | | | | | |
| EBV | 2553 | -3.89 | -61.16 | 43.30 | 14.09 |
| DEBV | 2553 | -2.59 | -214.05 | 222.57 | 44.71 |
| ACC | 2553 | 0.33 | 0.09 | 0.83 | 0.05 |
| WEIGHTS | 2553 | 1.49 | 0.35 | 6.01 | 0.34 |
| PWG | | | | | |
| EBV | 2551 | -0.31 | -61.03 | 66.02 | 10.78 |
| DEBV | 2551 | 0.93 | -283.01 | 288.47 | 46.79 |
| ACC | 2551 | 0.27 | 0.07 | 0.76 | 0.10 |
| WEIGHTS | 2551 | 1.20 | 0.27 | 5.08 | 0.52 |
| YW | | | | | |
| EBV | 2551 | -4.21 | -108.46 | 106.09 | 23.44 |
| DEBV | 2551 | -0.69 | -847.30 | 718.43 | 120.60 |
| ACC | 2551 | 0.27 | 0.07 | 0.76 | 0.10 |
| WEIGHTS | 2551 | 0.48 | 0.11 | 2.06 | 0.21 |
| Milk | | | | | |
| EBV | 548 | 7.48 | -27.45 | 33.66 | 10.49 |
| DEBV | 548 | 9.00 | -169.46 | 108.80 | 33.88 |
| ACC | 548 | 0.29 | 0.05 | 0.53 | 0.12 |
| WEIGHTS | 548 | 2.51 | 0.36 | 5.43 | 1.19 |

[1]BWT: birth weight; WW: weaning weight; PWG: post-weaning gain; YW: yearling weight; Milk: maternal weaning weight; DEBV: deregressed EBV; ACC: accuracy associated with DEBV; weights: weight factor used in analysis with DEBV

The DEBV weights were related to the accuracy and heritability (Equation 5.7), so the weights were unique to individuals and traits. A weight of a trait indicated the emphasis of a DEBV in the analysis of the trait, and the higher the weight in an evaluation was interpreted to mean the DEBV having more value in the analysis (Garrick et al., 2009). The SD of DEBV were larger than those of EBV, which suggested the DEBV un-shrunk the EBV (Ostersen et al., 2011).

The calculation of the DEBV involving individual's EBV, accuracy, and heritability, and parent's EBV and accuracy, so the degree of expansion was also unique across traits and individuals (see details in material and method for developing DEBV in Chapter 5). Because EBV were shrunk towards the mean (i.e. 0; Garrick et al., 2009), the deviating degree from the mean should be smaller for values close to mean than those on tails of distributions when calculating DEBV. Therefore, the differences between EBV and DEBV means were relatively small compared to those between minimum and maximum values.

### 6.3.2 GWAS of performance traits

The GWAS results of BWT, WW, MILK, PWG and YW are presented in Manhattan plots that showed the proportion of genetic variance of each performance trait explained by each of the 2,648 1-Mb SNP windows spanning the bovine genome (Figure 6.1 to Figure 6.4). Peak QTL regions were identified on chromosome 7, 14 and 20. These peaks were similar across four of the performance traits, except MILK, although the portion of genetic variation explained by these regions were slightly different between traits. Based on the graphs, the MILK had limited concordance with other performance traits, which may attribute to the small sample size used for MILK GWAS. These results were from multivariate model (Equation 4.3) that restricted the growth traits maternal and direct genetic correalation to zero to develop DEBV. Without the restrictions, there could be some changed in estimates for genomic window and SNP effects, but limited influence on identification of top genomic windows would be limited.

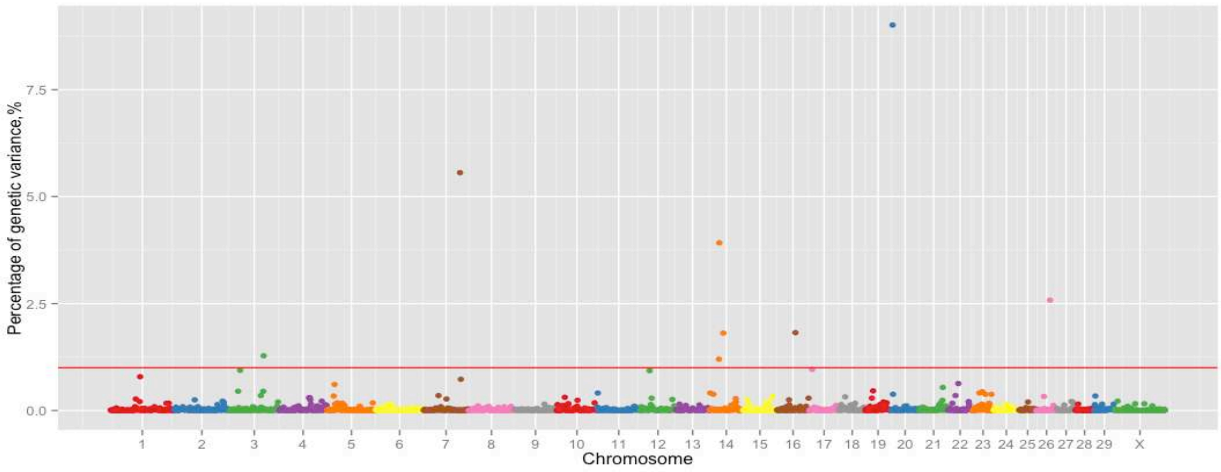


Figure 6.1 Manhattan plot of proportion of genetic variance explained by 1-Mb windows from genome-wide association study of deregressed EBV of birth weight in Angus cattle managed at high altitude (elevation at 2,170 m) with the line representing the 1% of the genetic variation.

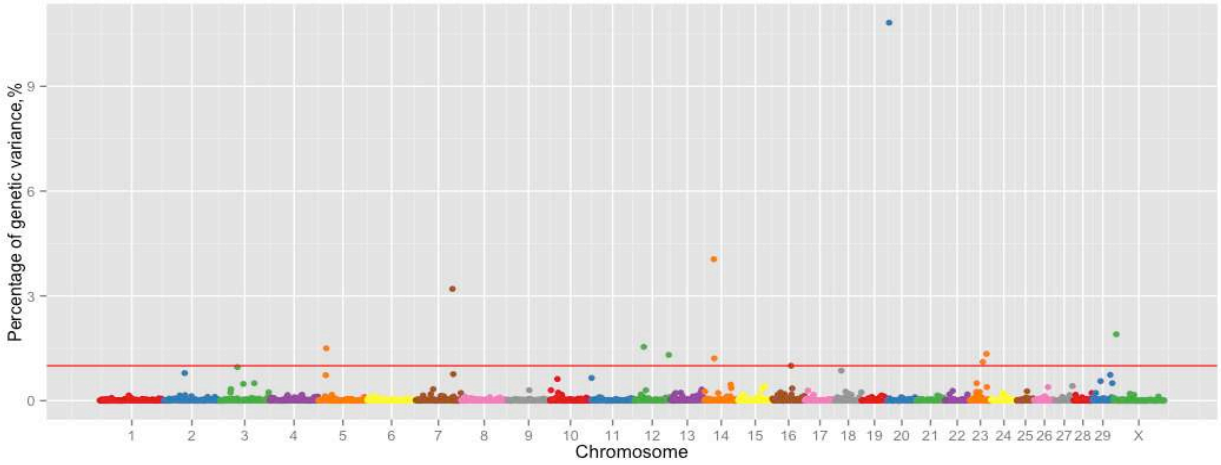


Figure 6.2 Manhattan plot of proportion of genetic variance explained by 1-Mb windows from genome-wide association study of deregressed EBV of weaning weight in Angus cattle managed at high altitude (elevation at 2,170 m) with the line representing the 1% of the genetic variation.

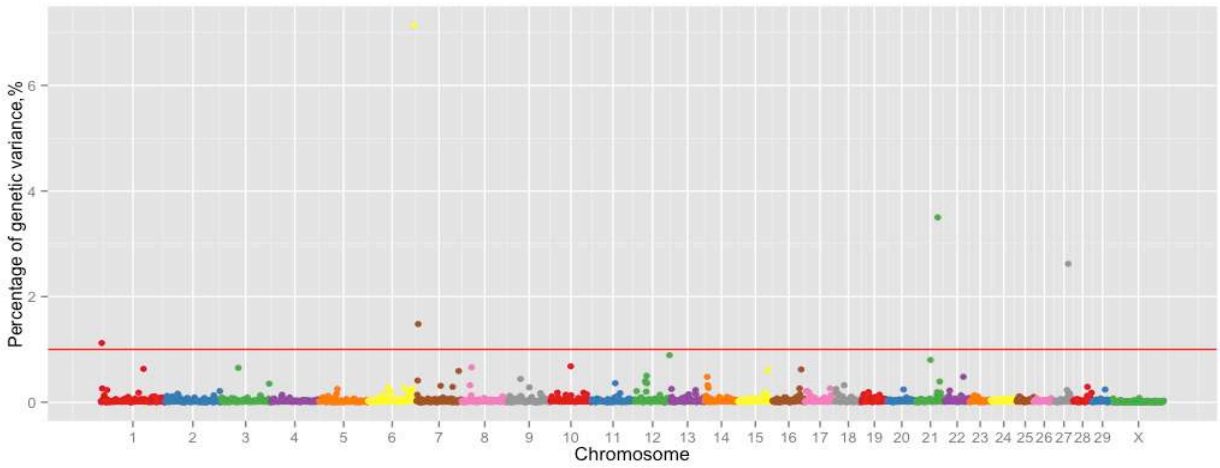


Figure 6.3 Manhattan plot of proportion of genetic variance explained by 1-Mb windows from genome-wide association study of deregressed EBV of maternal weaning weight (MILK) in Angus cattle managed at high altitude (elevation at 2,170 m) with the line representing the 1% of the genetic variation.

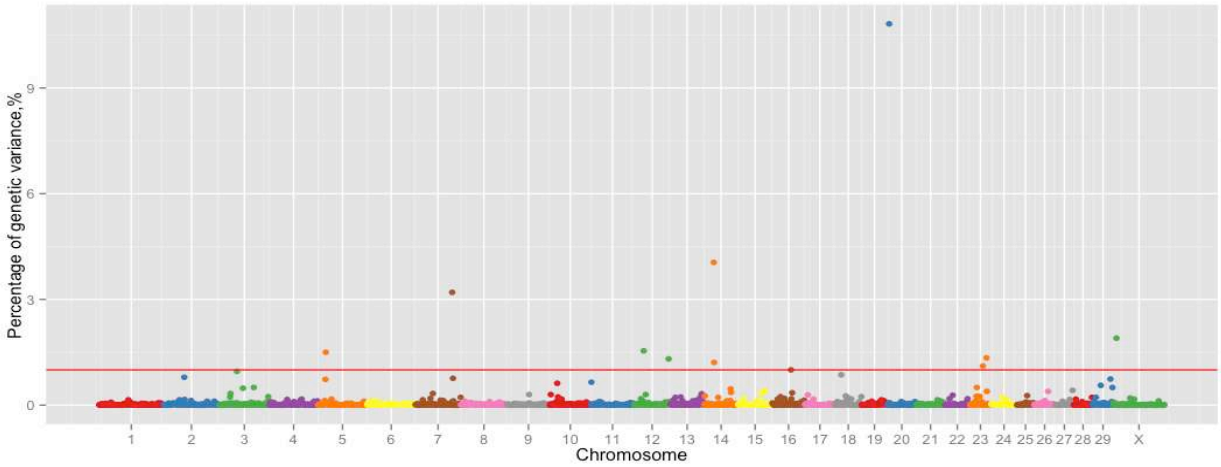


Figure 6.4 Manhattan plot of proportion of genetic variance explained by 1-Mb windows from genome-wide association study of deregressed EBV of post-weaning gain in Angus cattle managed at high altitude (elevation at 2,170 m) with the line representing the 1% of the genetic variation.

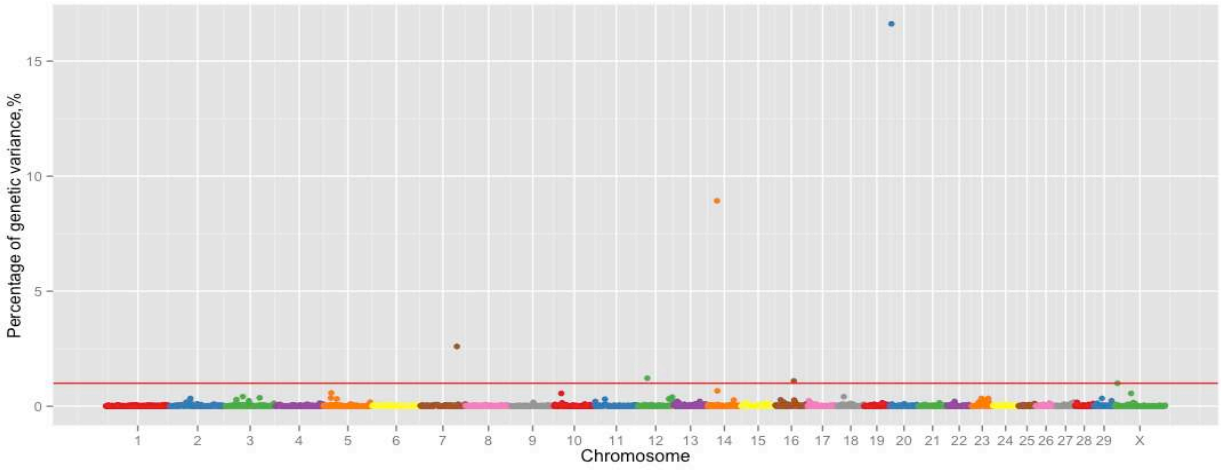


Figure 6.5 Manhattan plot of proportion of genetic variance explained by 1-Mb windows from genome-wide association study of deregressed EBV of yearling weight in Angus cattle managed at high altitude (elevation at 2,170 m) with the line representing the 1% of the genetic variation.

Twenty-two QTL windows (defined as 1-Mb genomic windows explaining > 1% of additive genetic variance) were identified for the five performance traits (i.e. BWT, WW, MILK, PWG and YW), and they were distributed across 11 chromosomes. Generally, QTL on chromosome 7, 14, and 20 had the largest impact on development of performance traits (Figure 6.1 – Figure 6.6), and these windows were also associated with the largest number of traits. These four windows, a window on chromosome 12, a window on chromosome 16 and another window on the X chromosome were considered pleiotropic QTL windows, which were associated with more than one performance trait (Table 6.2). Fifteen of the windows were trait-specific QTL regions (Figure 6.1 to Figure 6.5). These pleiotropic 1-Mb QTL were located on chromosome 7 at 93 Mb, chromosome 12 at 23 Mb chromosome 14 at 24 and 25 Mb, chromosome 16 at 47 Mb, chromosome 20 at 4 Mb and chromosome X at 7 Mb.

They were similar to the reports of Saatchi et al. (2014) of 10 beef cattle breeds and Weng et al. (2016) of Brangus cattle, but our data did not result in the QTL region on chromosome 6 at 38 Mb, which was recognized important QTL region for cattle performance (Snelling et al., 2010;

Saatchi et al., 2014). Kneeland et al. (2004) identified a haplotype QTL at approximately 26 Mb on chromosome 14 to be associated with BWT and pre-weaning average daily gain. When scanning potential QTL with influencing growth traits in commercial Angus cattle, McClure et al. (2010) also reported growth-trait associated regions on BTA 7, 14 and 20. Another study of crossbred beef cattle involving Angus, Simmental, Hereford, etc. and their performance traits, identified genomic regions on BTA 7, 14 and 20 were also identified (Snelling et al., 2010). Mao et al. (2016) reported loci on chromosome 7 (at 93 Mb) and 14 (at 25 Mb) that were associated with growth traits in dairy cattle, and these regions were similar with the regions described in the current study involving Angus cattle managed at high altitude.

Table 6.2 Pleiotropic QTL 1-Mb windows associated with performance traits of Angus cattle managed at high altitude (elevation at 2,170 m)

| BTA_Mb[1] | Start[1] | End[1] | Number of SNP[1] | Associated traits[2] |
|---|---|---|---|---|
| 7_93 | 93007435 | 93886136 | 6 | BWT, WW, YW |
| 14_24 | 24057354 | 24643266 | 10 | BWT, WW, PWG, YW |
| 14_25 | 25107556 | 25982072 | 16 | BWT, WW |
| 16_47 | 47026456 | 47942535 | 14 | BWT, WW, PWG, YW |
| 20_4 | 4145679 | 4962725 | 22 | BWT, WW, PWG, YW |
| X_7 | 7042383 | 7909757 | 10 | WW, YW |

[1]BTA_Mb: Bovine chromosome and $n^{th}$ 1 Mb window on the same chromosome based on the UMD3.1 assembly; Start: start position of the window; End: end position of the window; Number of SNP: number of SNP in the window.
[2]BWT: birth weight; WW: weaning weight; MILK: maternal weaning weight; PWG: post-weaning gain; YW: yearling weight

There were 8, 11, 5, 5, and 7 genomic windows were identified to be associated with BWT, WW, MILK, PWG and YW in this study of Angus cattle managed at high altitude. The numbers of identified QTL for these performance traits in this study were larger than the results of Angus cattle presented by Saatchi et al. (2014). Many factors can contribute to this difference in power of QTL window detection. Hong and Park (2012) reported that increasing sample size would result in enhanced statistical power in identifying QTL in GWAS. Besides sample size, the

genetic variation level of studied animals (Saatchi et al., 2014), the patterns of linkage disequilibrium and the frequencies of causative variants (Spencer et al., 2009; Hong and Park, 2012) could also increase power of QTL detection.

The genomic windows explained 36.4%, 17.3%, 20.2%, 14.8% and 22.7% of the variance of DEBV and the windows explaining >1% genetic variation explained 27.2%, 29.0%, 15.9%, 28.5% and 31.5% of the total genetic variation of BWT, WW, MILK, PWG and YW, respectively. The proportions of genetic variance explained by these genomic windows of growth performance traits were consistent with the heritability estimates of these traits. Specifically, higher heritability estimates of performance traits corresponded to higher proportions of the genetic variance explained by genotypes of these traits. Golan et al. (2014) reported that the phenotypic variation explained by genotype (common variants) could be positively related to heritability.

### 6.3.3 Trait-specific QTL windows

There were 3 genomic regions that were unique for birth weight (Table 6.3). One BWT specific QTL window was located on chromosome 3 spanning on 85 to 86 Mb locus. The window 3_85 had a lead-SNP located in an uncharacterized gene. There were two other annotated genes in this region (Appendix 6.1), but no publication supported their potential effects on cattle birth weight at the present time.

Table 6.3 Genomic windows explaining > 1% genetic variation in birth weight of Angus cattle managed at high altitude (elevation at 2,170 m)[1]

| BTA_Mb | Start (bp) | End (bp) | Number of SNP | % Variance Explained | Lead-SNP | Model Frequency | % Variance Explained by Lead-SNP |
|---|---|---|---|---|---|---|---|
| 20_4 | 4145679 | 4962725 | 22 | 9.01 | rs43350564 | 1.00 | 8.68 |
| 7_93 | 93007435 | 93886136 | 6 | 5.56 | rs41625563 | 0.86 | 4.69 |
| 14_25 | 25107556 | 25982072 | 16 | 3.92 | rs29021334 | 0.90 | 3.32 |
| 26_34 | 34020541 | 34955110 | 15 | 2.58 | rs41567908 | 0.84 | 1.85 |
| 16_47 | 47026456 | 47942535 | 14 | 1.82 | rs110974545 | 0.57 | 0.73 |
| 14_35 | 35052708 | 35962028 | 14 | 1.81 | rs41628883 | 0.77 | 1.27 |
| 3_85 | 85043515 | 85901731 | 16 | 1.28 | rs43271697 | 0.49 | 0.43 |
| 14_24 | 24057354 | 24643266 | 10 | 1.20 | rs42646708 | 0.18 | 0.07 |

[1]Bovine chromosome and n$^{th}$ 1 Mb window on the same chromosome based on the UMD3.1 assembly; Start: start position of the window; End: end position of the window; Num. SNP: number of SNP in the window; % Variance Explained: proportion of the genetic variance explained by the window; Lead-SNP: SNP with the highest model frequency; Model Frequency: the proportion of fitted models including that maker; % Variance Explained by Lead-SNP: proportion of the variance explained by the lead-SNP.

Beside the uncharacterized gene that contained the lead-SNP of window 14_35, this window contained four other genes (Appendix 6.1). One of these genes, *SULF1* had elevated levels of expression in fully differentiated osteoblasts and osteoclasts (Zaman et al., 2016) and played a key role in cartilage development and joint formation in a quail study (Zhao et al., 2006). Holst et al. (2007) also suggested that *SULF1* has a role in embryonic and neonatal development, and it may influence neonatal survival, the skeletal defects and the birth weight of mice through controlling the pattern of heoaran sulfate proteoglycans that interact with several key growth factors.

Another window associated with BWT was located at 34 Mb on chromosome 26. This region was also reported by Saatchi et al. (2014) to influence BWT in several beef cattle breeds (i.e. Angus, Gelbvieh, and Simmental). Nine genes (Appendix 6.1) existed in this window, and none of them were close (< 2,500 bp) to the lead-SNP of this window. The *ADRB1* is a member of β-adrenoceptors, whose functions were well documented in heart rate and heart failure in humans (Kang et al., 2015; Yogev et al, 2016). One Gly allele polymorphism on *ADRB1* was identified to confer lower risk for hypertension in eastern Asian population (Wang et al., 2013). Additional studies also suggested the functions of β-adrenoceptors on skeletal muscle growth, development and hypertrophy in mammals (Ryall et al, 2010), and reported that β-adrenoceptors might also respond to the rapid fetal and neonatal growth (Auman et al., 2002 and Lagercrantz and Slotkin,1986). This information could support the finding of the current study and the relationship between *ADRB1* and BWT of cattle managed at high altitude.

Table 6.4 Genomic windows explaining > 1% genetic variation of weaning weight of yearling Angus cattle managed at high altitude (elevation at 2,170 m)[1]

| BTA_Mb | Start (bp) | End (bp) | Number of SNP | % Variance Explained | Lead-SNP | Model Frequency | % Variance Explained by Lead-SNP |
|---|---|---|---|---|---|---|---|
| 20_4 | 4145679 | 4962725 | 22 | 10.82 | rs43350564 | 1.00 | 10.70 |
| 14_24 | 24057354 | 24643266 | 10 | 4.05 | rs41724332 | 0.34 | 0.58 |
| 7_93 | 93007435 | 93886136 | 6 | 3.20 | rs41625563 | 0.64 | 1.67 |
| X_7 | 7042383 | 7909757 | 10 | 1.90 | rs110977907 | 0.69 | 0.85 |
| 12_23 | 23046778 | 23991213 | 16 | 1.54 | rs41610326 | 0.56 | 0.79 |
| 5_19 | 19128124 | 19983794 | 8 | 1.50 | rs110476952 | 0.57 | 0.79 |
| 23_41 | 41103432 | 41992435 | 20 | 1.34 | rs41589765 | 0.46 | 0.49 |
| 12_88 | 88018471 | 88979628 | 19 | 1.31 | rs42359835 | 0.52 | 0.59 |
| 14_25 | 25107556 | 25982072 | 16 | 1.21 | rs29021334 | 0.29 | 0.30 |
| 23_32 | 32110882 | 32998188 | 14 | 1.11 | rs29011699 | 0.40 | 0.35 |
| 16_47 | 47026456 | 47942535 | 14 | 1.00 | rs110974545 | 0.26 | 0.14 |

[1]Bovine chromosome and n$^{th}$ 1 Mb window on the same chromosome based on the UMD3.1 assembly; Start: start position of the window; End: end position of the window; Num. SNP: number of SNP in the window; % Variance Explained: proportion of the genetic variance explained by the window; Lead-SNP: SNP with the highest model frequency; Model Frequency: the proportion of fitted models including that maker; % Variance Explained by Lead-SNP: proportion of the variance explained by the lead-SNP.

Four trait-specific QTL windows were associated with WW besides the three pleiotropic QTL regions (Table 6.4). Window 5_19 contained three genes, but none of them were within 2,500 bp of the lead-SNP (rs110476952) of this window (Appendix 6.1). These genes have roles in cellular proliferation (*DUSP6*; Wang et al., 2010), ciliogenesis (*POC1B*; Pearson et al., 2009), blood pressure and risk to hypertension in humans (*ATP2B1*; Tabara et al., 2010). Cell proliferation maybe involved in vascular remodeling mechanisms described in bovine PH and HAD (Stenmark et al., 2006 and 2013). However, their association with growth traits and PH in cattle needs further research and validation.

The window 12_88 were novel regions identified on chromosome 12 associated with WW in cattle. In this window, no genes contained the lead-SNP (*rs42359835*) of this window. Among the three genes in this window (Appendix 6.1), the *IRS2* could be a potential link to type 2 diabetes (Brady, 2004), and a mutation of *COL4A1* was related to muscular defects in humans (Kuo et al., 2012).

The other two windows were located on chromosome 23 at 32Mb and 41 Mb. The QTL at 31.7 Mb was previously reported to be associated with average daily gain in a *Bos taurus* commercial line of *Bos taurus* (Kneeland et al., 2004). Lead-SNP of window 23_32 was intragenic to *ALDH5A1*, which were related to body weigh and growth in human and pig (Xiong et al., 2015). Seven additional genes were in this QTL window for WW direct (Appendix 6.1). The *LRRC16A* was potentially related to growth traits in cattle and pigs (Puig-Oliveras et al., 2014). Only two genes were located in window 23_41, and the lead-SNP was far away (> 2,5000 bp) from these genes. Neither of them was reported to be associated with performance traits.

The GWAS identified five windows for maternal weaning weight (MILK), and they were all trait-specific QTL (Table 6.5). The identified window on chromosome 1 was at 2 Mb, and its

lead-SNP (*rs110875985*) was not near any genes. This window was also identified to be associated with maternal WW of Charolais, Hereford and Simmental in Saatchi et al. (2014). Among the seven MILK associated genes in this widow (Appendix 6.1), *MRAP* encodes a member of melanocortin receptor-interacting protein, whose polymorphisms could be associated with milk production traits (Fontanesi et al., 2011). The lead-SNP of window 7_4 was located intragenic to *ELL*, and 20 additional genes were in this window (Appendix 6.1). The *MEF2B* could affect growth traits Ujumqin sheep (Zhang et al., 2016). The *GDF15* in this window were reported to be associated with obesity, diabetes and cardiovascular diseases in human and animals (Adela and Banerjee, 2015).

Another MILK associated window was on chromosome 27. This window contained one fibroblast growth factor receptor (FGFR1), whose associated pathway has a promotion role in proliferation and differentiation of the epithelial tissue in the gastrointestinal tract in calves and may influence digestion. The *TACC1* was reported to be associated with gastric carcinoma. The *ADAM9* and *ADAM32* are members of a disintegrin and metalloproteinase domain family, which was involved in muscle development, inflammation and cancers (Seals and Courtneidge, 2003). There is any one (*FSCB*) gene in window 21_54, and no gene in window 6_114. Li et al. (2007) reported that the protein encoded by FSCB was calcium-binding protein. Previous reported MILK associated QTL windows, mainly located on chromosome 6 and 14 (Khatkar et al., 2004), and were not concordant with those presented in this study. This GWAS of MILK was based on genotypes of only 548 animals, such small sample size may influence the power and accuracy of QTL identification.

Table 6.5 Genomic windows explaining > 1% genetic variation of maternal weaning weight (MILK) of Angus cattle managed at high altitude region (elevation at 2,170 m)[1]

| BTA_Mb | Start (bp) | End (bp) | Number of SNP | % Variance Explained | Lead-SNP | Model Frequency | % Variance Explained by Lead-SNP |
|---|---|---|---|---|---|---|---|
| 6_114 | 114019660 | 114938340 | 21 | 7.14 | rs41573388 | 0.36 | 1.01 |
| 21_54 | 54056137 | 54923774 | 9 | 3.50 | rs110176118 | 0.51 | 1.79 |
| 27_33 | 33035739 | 33947904 | 14 | 2.62 | rs110131802 | 0.28 | 0.43 |
| 7_4 | 4058780 | 4953801 | 15 | 1.48 | rs43500370 | 0.22 | 0.21 |
| 1_2 | 2049400 | 2977063 | 15 | 1.12 | rs110875985 | 0.19 | 0.14 |

[1]Bovine chromosome and n$^{th}$ 1 Mb window on the same chromosome based on the UMD3.1 assembly; Start: start position of the window; End: end position of the window; Num. SNP: number of SNP in the window; % Variance Explained: proportion of the genetic variance explained by the window; Lead-SNP: SNP with the highest model frequency; Model Frequency: the proportion of fitted models including that maker; % Variance Explained by Lead-SNP: proportion of the variance explained by the lead-SNP.

Table 6.6 Genomic windows explaining > 1% genetic variation of post-weaning gain of Angus cattle managed at high altitude region (elevation at 2,170 m)[1]

| BTA_Mb | Start (bp) | End (bp) | Number of SNP | % Variance Explained | Lead-SNP | Model Frequency | % Variance Explained by Lead-SNP |
|---|---|---|---|---|---|---|---|
| 20_4 | 4145679 | 4962725 | 22 | 14.74 | rs43350564 | 1.00 | 14.36 |
| 14_24 | 24057354 | 24643266 | 10 | 8.25 | rs41724332 | 0.67 | 3.71 |
| 16_47 | 47026456 | 47942535 | 14 | 3.06 | rs110974545 | 0.54 | 1.14 |
| 4_13 | 13061952 | 13968183 | 14 | 1.30 | rs109388623 | 0.43 | 0.53 |
| X_49 | 49506722 | 49939818 | 2 | 1.13 | rs41594577 | 0.35 | 0.25 |

[1]Bovine chromosome and n$^{th}$ 1 Mb window on the same chromosome based on the UMD3.1 assembly; Start: start position of the window; End: end position of the window; Num. SNP: number of SNP in the window; % Variance Explained: proportion of the genetic variance explained by the window; Lead-SNP: SNP with the highest model frequency; Model Frequency: the proportion of fitted models including that maker; % Variance Explained by Lead-SNP: proportion of the variance explained by the lead-SNP.

Table 6.7 Genomic windows explaining > 1% genetic variation of yearling weaning of Angus cattle managed at high altitude region (elevation at 2,170 m)[1]

| BTA_Mb | Start (bp) | End (bp) | Number of SNP | % Variance Explained | Lead-SNP | Model Frequency | % Variance Explained by Lead-SNP |
|---|---|---|---|---|---|---|---|
| 20_4 | 4145679 | 4962725 | 22 | 16.62 | rs43350564 | 1.00 | 16.04 |
| 14_24 | 24057354 | 24643266 | 10 | 8.93 | rs41724332 | 0.44 | 1.83 |
| 7_93 | 93007435 | 93886136 | 6 | 1.49 | rs41625563 | 0.27 | 0.28 |
| 12_23 | 23046778 | 23991213 | 16 | 1.22 | rs41610326 | 0.29 | 0.26 |
| 16_47 | 47026456 | 47942535 | 14 | 1.10 | rs110974545 | 0.19 | 0.11 |
| X_7 | 7042383 | 7909757 | 10 | 1.00 | rs110977907 | 0.27 | 0.16 |

[1]Bovine chromosome and $n^{th}$ 1 Mb window on the same chromosome based on the UMD3.1 assembly; Start: start position of the window; End: end position of the window; Num. SNP: number of SNP in the window; % Variance Explained: proportion of the genetic variance explained by the window; Lead-SNP: SNP with the highest model frequency; Model Frequency: the proportion of fitted models including that maker; % Variance Explained by Lead-SNP: proportion of the variance explained by the lead-SNP.

Five windows were identified to be associated with PWG DEBV, and two of them were unique to PWG (Table 6.6). There are no annotated genes in window X_41. The lead-SNP of window 4_13 was intragenic to *SLC25A13*, and the lead-SNP of the other windows did not reside within 2,500 bp of any genes. The *SLC25A13* appears to have a role in development of citrin deficiency, which influences the urea cycle and the malate-aspartate shuttle in humans (Avdjieva-Tzavella et al., 2014; Song et al., 2013; Zhang et al., 2014). However, there are no reports describing genes' role in post-weaning growth of cattle.

For YW DEBV, six genomic windows were identified explaining larger than 1% of genetic variation, and all of them were identified for other growth traits (Table 6.1). Table 6.7 summarizes the information of these windows for YW. These results correspond to the high estimated genetic correlation between YW and WW or PWG and the fact that the YW EBV was constructed by summing EBV of WW and YW. It should also be noted that there were more unique windows for YW that explained < 1% of the genetic variation. The details of genes in theses windows were discussed in the pleiotropic window section.

### 6.3.4 Pleiotropic QTL windows

Window 7_93 was identified as a pleiotropic QTL influencing BWT, WW and PWG (Table 6.2). This region explained 5.6%, 2.4% and 1.5% of genetic variation of BWT, WW, and YW, respectively. Chromosome 7 contains many cattle growth performance QTL including this window region (e.g. BWT, WW, YW and mature weight; Decker et al., 2012; Saatchi et al., 2014; Weng et al., 2016). Besides growth traits, they also reported this genomic window's association with several carcass traits (e.g. marbling score, rib eye area, carcass weight and fat thickness). The detection of the lead-SNP (*rs41625563*) of this region was consistent across

these traits. This lead-SNP was located *G protein-coupled receptor 98* (*GPR98*). The role of *GPR98* in cattle is yet to be described; however, this gene is known to influence cell growth and proliferation (Gutkind, 1998). The only characterized gene, *ARRDC3* (*arrestin domain containing 3*), in this window has been shown to affect obesity in humans and mice through regulating β-adrenergic signaling (Patwari et al., 2011). Intake of β-adrenergic agonists (a growth-promoting agent) in cattle increases muscle and decrease fat accretion in cattle (Mersmann, 1998; Johnson et al., 2014). Neary (2014) studied the role of growth-promoting agents in PAP of fat cattle in feedlot and found that it significantly ($P < 0.05$) influenced the diastolic PAP but didn't significantly influence the mean PAP ($P > 0.5$). In addition, the current study identified this window to be associated with yearling PAP and susceptibility to HAD, which suggested this region's pleiotropic genetic effect on both performance traits on yearling PAP phenotypes.

Window 12_23 was pleiotropic between WW and YW, which explained 1.5% and 1.2% of genetic variance of them, respectively. McClure et al. (2010) identified a BWT-associated QTL contained the window 12_23. The lead-SNP (*rs41610326*) of window 12_23 is not located within 2,500 bp of any annotated gene, and the five genes in this window were not reported to be associated with growth performance.

Another two pleiotropic window was observed on chromosome 14 at 24 and 25 Mb. Window 14_24 explained 1.2%, 4.1%, 8.3% and 8.9% of genetic variation in BWT, WW, PWG and YW, respectively; and window 12_25 explained 3.9% and 1.2% of genetic variation in BWT and WW, respectively (Table 6.2). These findings supported reports of Saatchi et al. (2014), who illustrated a big performance traits associated window region, ranging from 23 Mb to 26 Mb on chromosome 14. Specifically, the window 14_24 was associated with BWT, WW and YW in

Gelbvieh and Simmental cattle. Weng et al. (2016) also described a close region at 26 Mb being associated with WW in Brangus cattle. In addition, Chromosome 14 was reported to be important in milk production and other performance traits in cattle (Hu et al., 2013). Two lead-SNP were identified for window 12_24 across studied performance EBV. The *rs42646708* was the lead-SNP for BW, while the *rs41724332* was the lead-SNP for WW, PWG and YW. The model frequency for *rs42646708* in BWT was relatively low compared to other lead-SNP, because several other SNPs in this window had similar model frequency, suggesting the QTL distributed the effect across the whole window. The *rs42646708* SNP is intragenic to *XKR4* (*X-linked Kx blood group related 4*). Utsunomiya et al. (2013) reported a SNP within intron 2 of the *XKR4* gene that was associated with BWT of Nellore cattle and suggested *XKR4* could be a candidate gene for performance and carcass traits. This gene was also reported to be associated with rump fat thickness in *Bos taurus*, *Bos indicus* and composite cattle (Bolormaa et al., 2011; Porto Neto et al., 2012). The *XKR4* was also reported to be associated with average daily feed intake and average daily gain (Lindholm-Perry et al., 2012). The *rs41724332* SNP does not reside within any gene regions, but it is located approximately 30,000 bp upstream from *XKR4*.

Five other characterized genes resided in this 1-Mb window region. The *LYPLA1* encodes ghrelin deacylation enzyme, which regulates appetite for signals of the stomach (Shanado et al., 2004). In addition, they were reported to have pleiotropic genetic effects on growth (e.g BWT), and feed intake traits (e.g. average daily intake) in various breeds of cattle (Karim et al., 2011; Lindholm-Perry et al., 2012; Utsunomiya et al., 2013). Gene *RPS20* could also affect calving ease via regulating fetal growth traits (Pausch et al., 2011). Gudbjartsson et al. (2008) described this gene's association with human height. Therefore, this genomic region could be considered a concordant QTL influencing stature, growth and feed intake traits of beef cattle.

The lead-SNP (*rs29021334*) of window 14_25 was not located near (< 2,500 bp) any gene, but five genes were located in this window. Among them, the *PENK (proenkephalin),* involved in the endogenous opioid systems, could play role in maternal adaptation to pregnancy and in supporting embryo growth in mice (Zhu and Pintar, 1998). The variants in this gene were widely reported to be associated with fertility traits (e.g first service conception, heifer pregnancy and puberty) in populations of Brangus, Brahman and tropical composite beef cattle (Hawken et al., 2012; Peters et al., 2013; Cánovas et al., 2014). In a study of Nelore and Nelore-Angus cattle, *PENK* was suggested to be related to birth weight (Utsunomiya et al., 2013; Riley et al., 2014), which could support results of the current study. In addition, Grissom et al. (2013) suggested that obesity during pregnancy in mice would lead to offspring obesity, via altered the expression level of *PENK*.

Mutations in *IMPAD1* that encodes Golgi-resident 3'-phosphoadenosine-5'-phosphate phosphatase could cause chondrodysplasia abnormal joint development and skeletal elements (Vissers et al., 2011). The *PLAG1* and *CHCHD* in this region were linked to each other in previous studies of cattle, and the *PLAG1-CHCHD* inter-genic region was identified in previous studies to be associated with bovine stature, body weight gain and carcass traits in dairy and beef cattle (Karim et al., 2011; Nishimura et al., 2012; Hoshiba et al., 2013). Littlejohn et al. (2011) suggested *PLAG1* could play a role in fetal development, neonatal body weight gain and growth in *Bos taurus* cattle.

Window 16_47 explained 1.8%, 1.0%, 3.1% and 1.1% of genetic variation of BWT, WW, PWG and YW, respectively. This is a novel QTL was reported to be associated with growth traits in cattle, but Alexander et al. (2007) showed a QTL for average daily gain near 46.7 Mb on this chromosome. The same lead-SNP (*rs110974545*) was found across these traits, and was

intragenic to an un-annotated gene. Among the 16 annotated genes in this window (Appendix 6.1), *PLEKHG5* could regulate *ROCK* activity and influence the cell migration in cancer (Dachsel et al., 2013), and HES3 is expressed in adult pancreatic islet that could regulate cell growth and insulin release in human (Masikur et al., 2014). However, their roles in growth performance in human and animal are limited.

The genomic window on chromosome 20 at 4 Mb also had pleiotropic effects on BWT, WW, PWG and YW (Table 6.2), which explained 9.0%, 10.8%, 14.7% and 16.6% of genetic variation of these traits, respectively. The 20_4 window was previously reported to be associated with BWT, WW, YW and mature weight in Angus, Hereford, Red Angus and Simmental cattle, and explained relatively large percent of the (> 5%) genetic variation of these traits (Saatchi et al., 2014; Weng et al., 2016). Saatchi et al. (2014) also reported its association with carcass weight, fat thickness and yield grade. The lead-SNP (*rs43350564*) of this window was the same for these associated traits, and Saatchi et al. (2014) also reported *rs43350564* SNP as the lead-SNP for several growth traits across several beef cattle breeds. The B allele effects of this SNP were in the same direction (i.e. positive), and the estimated model frequencies were above 0.99 in different performance traits. This suggested *rs43350564* was very likely in linkage disequilibrium with a causal mutation for performance traits of Angus cattle.

The lead-SNP at chromosome 20_4 Mb resides approximately 269 bp downstream from the gene *ERGIC1* (*endoplasmic reticulum-Golgi intermediate compartment protein 1*). In humans, *ERGIC1* influences membrane selective transport of cargo between the endoplasmic reticulum and the golgi apparatus, which regulates early secretory pathways (Breuza et al., 2004). This gene was found to be potentially associated with prostate cancer (Vainio et al., 2012). Limited information has been written about *ERGIC1* in cattle, and no evidence was reported to support

*ERGIC1*'s role in growth traits of cattle. Nine other genes were located in this window (Appendix 6.1). Khadir et al. (2015) showed that the up-regulation of *DUSP1* was strongly linked to human adiposity. The *DUSP1* (*dual specificity phosphatase 1*) was also related to TGF-β1 and MAPK, which influenced inflammation and cell proliferation in human and animals (Kim et al., 2011; Shah et al., 2014). These mechanisms were reported to be involved in vascular remodeling in cattle having PH and HAD (Stenmark et al., 2006 and 2013).

In mouse models, *STC2* appears to have a role as a growth inhibitor, which negatively influences postnatal growth (Chang et al., 2008). It could inhibit pregnancy-associated plasma protein-A mediated IGF receptor signaling in vitro, and reduce intramembranous and endochondral bone development and skeletal muscle growth (Gagliardi et al., 2005; Jepsen et al., 2015). Another gene on the edge of this window was *SH3PXD2B*, and it was also reported to be essential in postnatal growth and development (Mao et al., 2009). Iqbal et al. (2010) demonstrated an important role for *SH3PXD2B* in development of bone, heart, and eye of animals. Besides some genes' roles in inflammation and cell proliferation, this window was also identified as an important QTL for yearling PAP phenotypes (Chapter 5), which suggested this region's association with both performance traits and PAP or susceptibility to HAD.

In addition to the autosome chromosome pleiotropic genomic window, there was a window identified on the X chromosome being associated with WW and YW (Table 6.2). This window X_7 explained 1.9% and 1.0% of genetic variation of WW and YW, respectively. No previous literature reported this region's association with cattle growth. However, Saatchi et al. (2014) identified a window at 145 Mb on chromosome X being associated with WW in Brangus, and reported the window X_7 having potential relationship with rib eye area in Gelbvieh cattle. The lead-SNP (*rs110977907*) of this window was concordant between WW and YW. There were five

genes located in this region that influence cell growth and death, but none of them were reported to have effects on growth in animals.

### 6.3.5 Gene enrichment analysis

Forty-five genes were located in these pleiotropic window regions, and they were used in the gene ontology analysis. All the candidate genes were clustered based on their involved biological function and pathways. Table 6.8 summarizes the significant gene enrichment for biological processs and KEGG pathways. Two genes were involved in adipose tissue development. The process of adipose tissue development was also reported by the study of Saatchi et al. (2014). Two genes were also enriched in a KEGG pathway - NF-kappa B signaling pathway. This pathway involves the nuclear factor-kappa B transcription factors, which regulates genes in innate immunity, inflammation and cell survival in human and cattle (KEGG PATHWAY: 04064). Because the infinitesimal-type models were used in this study to identified traits-associated window and only considered the genes to be within the most important QTL regions (explain >1% genetic variation), small numbers of genes were used in this gene enrichment analysis and identified in a biological processes. None of these significant processes contained all the candidate genes, and this may attribute to that we simply considered all the genes in a 1-Mb QTL window as candidate genes to be used in this analysis, and these biological processes are very polygenetic involving large number of genes.

Table 6.8 Significant gene ontology (GO) terms from gene enrichment analysis on genes in identified in pleiotropic genomic windows

| GO[1] | ID | P-value[2] | Name | Gene list | BTA_Mb[3] |
|---|---|---|---|---|---|
| BP | GO:0060612 | 5.00E-02 | Adipose tissue development | ARRDC3, SH3PXD2B | 7_93, 20_4 |
| ke | KEGG:04064 | 1.47E-02 | NF-kappa B signaling pathway | BIRC8 LYN | 14_24, X_7 |

[1]BP: Biological process; ke: KEGG pathway
[2] Benjamini-Honchberg FDR corrected P-Value
[3]Associated chromosome and $n^{th}$ 1-Mb window based on UMD3.1 assembly

## 6.4 Conclusions

We identified 22 QTL windows associated with performance traits of Angus cattle managed at high altitude using genotype data from the Bovine SNP50 Beadchip. Besides the previously reported QTL regions, several novel performance-related genomic window were identified. This supported the polygenetic characteristics of growth performance traits. Further studies need to validate these novel QTL regions. Seven of these windows were pleiotropic across performance traits, which are located on chromosome 7, 12, 14, 16, 20 and X. The function of the genes in these pleiotropic windows had roles in adipose tissue accretion and innate immunity, which could influence cattle growth, performance and health. Our findings will improve the understandings of biological process involving growth and health of Angus cattle managed at high altitude.

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# CHAPTER 7

# GENOMIC RELATIONSHIP BETWEEN YEARLING PULMONARY ARTERIAL PRESSURE PHENOTYPES AND GROWTH PERFORMANCE TRAITS OF ANGUS CATTLE AT HIGH ALTITUDE

## 7.1 Introduction

Pulmonary arterial pressure (PAP) and susceptibility to high altitude disease (HAD) could be associated with body mass and growth performance levels of humans and animals (Jin et al., 2009; Neary 2014), and low to moderate genetic correlations were estimated between performance traits (birth weight and weaning weight) and yearling PAP phenotypes in Chapter 4. Pleiotropic QTL for growth traits were described in Chapter 6, and these QTL were likely influencing yearling PAP measurements. This chapter describes the study of concordant QTL and genes that were associated with both performance traits and PAP phenotypes and the genomic relationship (SNP effects relationships) between these traits via an associated weight matrix (AWM).

## 7.2 Material and methods

### 7.2.1 QTL

Concordant QTL regions and genes were identified with genomic-wide association study (GWAS) of non-transformed PAP scores (RAW), three-category PAP phenotype (CAT3) and two-category PAP phenotype (CAT2), birth weight (BWT), weaning weight (WW), maternal weaning weight (MILK), post-weaning gain (PWG) and yearling weight (YW; Chapters 5 and 6). The 1-Mb windows considered in this section were those explained greater than 1% of

genetic variation of each trait. Pleiotropic QTL windows across yearling PAP phenotypes and growth performance traits were described as those associated with at least one yearling PAP phenotype (i.e. RAW, CAT3 and CAT2) and at least one growth performance trait DEBV (i.e. BWT, WW, PWG, MILK and YW). Gene enrichment analysis of genes located within these pleiotropic QTL regions was performed with g:Profiler (Reimand et al., 2016 ;http://biit.cs.ut.ee/gprofiler/). The biological process and KEGG pathways with Benjamini–Honchberg FDR corrected *P* value at 0.05 were reported.

### 7.2.2 Genomic correlations

Effects of 35,930 loci were used to calculate genomic correlation among genomic correlations among growth performance traits and yearling PAP phenotypes. The SNP effects were developed from Bayes B method (Meuwissen et al., 2001) using DEBV of each phenotype. Bayes C (Habier et al., 2011) was used to estimate the prior of the genetic and residual scale parameters used in Bayes B. The details of the Bayes B and Bayes C methods were discussed in Chapter 5. Two sets of correlations were calculated to assess the genomic correlations: 1. Correlations between effects of all SNP used in the GWAS; 2. Construction of an AWM. Specifically, the SNP that explained larger than the average genetic variation of SNP were considered in AWM. The resulting SNP correlations from all SNP and AWM, which could assess the genetic correlations, were compared to the estimated genetic correlations between EBV among PAP phenotypes and performance traits. In order to visualize the SNP effect correlations and estimate pedigree-based genetic correlations, the SNP effects correlations were regressed on the estimate genetic correlations.

The concept of an AWM was developed based on results of single trait GWAS and the procedures of Reverter and Fortes (2013). The traits considered in construction of this AWM included RAW, CAT3, CAT2, BWT, WW, MILK, PWG and YW, and the RAW was used as the key trait in AWM. In this study, the AWM was constructed with the following steps:

1. Select the SNP explaining larger than average genetic variation of the key phenotype (RAW).

2. Calculate the average number of associated non-key phenotypes ($\mu_p$) for each selected SNP in Step 1. The SNP explained larger than average genetic variation of each of non-key phenotype were considered.

3. For each non-key phenotype, consider the SNP explained larger than average genetic variation, list the number of their associated non-key phenotypes, and select the SNP influencing more than $\mu_p$ of non-key phenotypes. Then merge these selected SNP with those in Step 1.

4. Calculate the SNP-to-Gene distance for each potential SNP in the AWM. The gene map information was based on Bovine UMD 3.1, which was obtained from Ensembl (http://uswest.ensembl.org/index.html). The BovineSNP50 manifest based on the Bovine UMD3.1 was used in this calculation.

5. Remove the unmapped SNP and the SNP mapped distant (2.5 Kb < SNP-to-Gene-distance < 1.5 Mb) from any characterized gene. In this step, the selected SNP in Step 1 and Step 3 that mapped close to (SNP-to-Gene-distance $\leq$ 2.5 Kb) and very distant (SNP-to-Gene-distance $\geq$ 1.5 Mb) from any genes were only kept in the AWM.

6. Prune SNPs (located close to genes) that correspond to the same gene to maintain the one SNP to one gene relationship. In these examples, if a gene was represented by more than one

SNP, the SNP that represented the most phenotypes and explained the highest average percentage of genetic variation across phenotypes was used in the AWM.

7. Standardize and assign the SNP effect of each phenotype to the selected SNP and corresponding gene in Step 6. In order to directly compare the SNP effects across phenotypes, the SNP effects used in the AMW were standardized for each phenotype by dividing each SNP effect for each phenotype by the standard deviation of all SNP effects from the GWAS of a phenotype.

8. To complete the AWM, the corresponding gene name was used as the row name if the SNP was located closed to gene (SNP-to-Gene-distance < 2.5 Kb); the SNP identification was directly used as row name if it was located far (SNP-to-Gene-distance > 1.5 Mb) away from genes. Therefore, the gene or SNP identification was the row name for the AWM, the phenotypes represented the columns, and the cells in the matrix contained the standardized effects of SNP for phenotypes.

## 7.3 Results and discussion

### 7.3.1 Pleiotropic genomic windows of PAP and growth performance traits

Table 7.1 summarizes the genomic windows that could have pleiotropic effects on PAP phenotypes and growth traits. Two windows were considered having pleiotropic effect on PAP phenotypes and performance traits, and both of them were identified as pleiotropic QTL of performance traits, which suggested the role of performance associated QTL and genes in PAP and susceptibility of HAD in Angus cattle managed at high altitude. It is possible that these QTL could contribute to the estimated genetic correlation between performance traits and yearling

PAP phenotypes. However, there was just one window (7_93) that was identified to be concordant between RAW and performance traits and explained an average of 3.61 ± 2.14 % of genetic variances of these phenotypes (Table 7.1). This could support the low to moderate genetic correlations observed with pedigree-based quantitative analyses between RAW PAP phenotypes growth and growth performance traits (Table 4.3, Crawford et al., 2016).

Table 7.1 Pleiotropic QTL windows associated with yearling pulmonary arterial pressure (PAP) and performance traits of Angus cattle managed at high altitude (elevation at 2,170 m)

| BTA_Mb[1] | Associated traits[2] |
|---|---|
| 7_93 | RAW (9.64)[3], CAT3 (6.83), CAT2 (9.32), BWT (5.56), WW (3.20), PWG (0.38), YW (1.49), MILK (0.06) |
| 20_4 | RAW (0.03), CAT3 (1.07), CAT2 (5.59), BWT (9.01), WW (10.82), PWG (14.74), YW (16.62), MILK (0.02) |

[1]BTA_Mb: Bovine chromosome and n[th] 1 Mb window on the same chromosome based on the UMD3.1 assembly

[2]RAW: non-transformed yearling PAP; CAT3: three-category yearling PAP; CAT2: two-category yearling PAP; BWT: birth weight; WW: weaning weight; MILK: maternal weaning weight; PWG: post-weaning gain; YW: yearling weight

[3]Trait (percentage): percentage (%) of genetic variation of certain trait explained by certain genomic window

The window 7_93 explained the largest portion of genetic variation of all yearling PAP phenotypes (i.e. RAW: 9.6%, CAT3: 6.8% and CAT2: 9.3%), and also contributed to the relatively high genetic variation of BWT (5.6%), WW (3.2%) and YW (1.4%). Besides its important role in cattle growth reported in the presented study and previous studies (Decker et al., 2012; Saatchi et al., 2014; Weng et al., 2016), we also revealed that the genomic region on chromosome 7 at 93 Mb was the most important QTL for PAP and susceptibility of HAD (Chapter 5). Genes in this window region (*GPR98* and *ARRDC3*) have roles in cell proliferation, ion and adrenergic receptor binding (Gutkind, 1998; Patwari et al., 2011), which may also

support this window's diversified roles in PAP phenotypes and performance of cattle at managed at high altitude.

Window 20_4 explained the largest portion of genetic variation of BWT, WW, PWG and YW, and it was also identified to explain a relatively large portion of genetic variance of CAT3 and CAT2. The lead-SNPs of these windows were consistent across yearling PAP phenotypes and cattle performance traits. Ten genes were located in this window, and these genes have roles in heart formation, pulmonary arterial smooth muscle cell development, cell proliferation and growth in humans and animals (Mao et al., 2009; Peng et al., 2010; Kim et al., 2011;), which supported the observed pleiotropic effects of this window on cattle yearling PAP phenotypes and growth. Only one of the pleiotropic windows was associated with RAW PAP, while both of them were identified in CAT2 and CAT3. This was coincident with the estimated genetic correlation between yearling PAP phenotypes and growth traits as categorical yearling PAP phenotypes yielded higher genetic correlation than RAW (Chapter 4).

Although there were only two pleiotropic windows (containing 11 genes), the gene enrichment analysis provided general function information for the genes. These genes were clustered in adipose tissue development and response to unfolded protein biological processes. The adipose tissue development was reported in the GWAS of performance traits (Chapter 6, Saatchi et al., 2014). The development of adipose tissue could have negative effects on body metabolism and increase incidence of various illnesses (Berry et al., 2013). Some adipose tissue-derived products (e.g. adiponectin and resistin) were reported to be associated with PH and other pulmonary vascular disease development (Mu et al., 2006; Musaad and Haynes, 2007). Hypoxia can stimulate unfolded protein response in pulmonary artery smooth muscle cell and inflammatory processes to vascular remodeling in PH (Yeager et al., 2012ab). Li et al. (2016)

reported metabolic changes in pulmonary vascular cells under PH, so the reported biological processes may contribute to the metabolic changes and subsequently be related to PAP measurements and PH in humans and cattle. The identified pathways included collecting duct acid secretion and SNARE interactions in vesicular transport, which have roles in acid excretion and biological product transportation. The SNARE complexes were involved in the intracellular protein trafficking, whose dysfunction were observed in pulmonary endothelial cells and pulmonary arterial smooth muscle cells (Sehgal and Lee, 2011). The gene ontology information supported the identification of pleiotropic QTL on yearling PAP phenotypes and performance traits.

Table 7.2 Significant gene ontology (GO) terms from gene enrichment analysis of genes identified in pleiotropic windows across PAP phenotypes and performance traits in Angus cattle managed at high altitude (elevation at 2,170 m)

| GO[1] | ID | P-value[2] | Name | Gene list |
|---|---|---|---|---|
| BP | GO:0060612 | 2.39E-03 | Adipose tissue development | *ARRDC3, SH3PXD2B* |
| BP | GO:0006986 | 1.60E-02 | Response to unfolded protein | *STC2, CREBRF* |
| keg | KEGG:04010 | 3.79E-02 | Collecting duct acid secretion | *ATP6V0E1* |
| keg | KEGG:04130 | 5.00E-02 | SNARE interactions in vesicular transport | *BNP1* |

[1]BP: Biological process; keg: KEGG pathway
[2] Benjamini-Honchberg FDR corrected P-Value

### 7.3.2 Genomic correlation

The number of selected SNP of the studied traits was ranged from 195 to 678, which was consistent with our assumption in GWAS that only a small portion of the SNP (n = 35,930) had large effects on the studied traits. The summary of the selected SNP in AWM is presented in Appendix 7.1. After filtering based on SNP-to-gene distance and pruning the SNP, 673 genes were associated with these SNP. The AWM contained SNP/genes on rows and the eight traits on

columns (Appendix 7.2). Column-wise correlations denoted the genetic correlation based on SNP effects (Table 7.3).

The pair-wise correlations of each SNP effect were calculated for all SNP and SNP in AWM, which were presented in Table 7.3. Generally, most of the correlations from all SNP were the same with those from SNP in the AWM. These resulting SNP effect correlations between performance traits and PAP growth phenotypes were low to moderate, which supported the pedigree based genetic correlation. Also, SNP-based genetic correlations between performance traits and RAW were lower than those between performance traits and CAT3 or CAT2, which was on the same pattern of pedigree-based genetic correlations (Table 4.4).

Table 7.3 Pearson correlations between SNP effects of yearling pulmonary arterial pressure phenotypes (PAP) and performance traits in association weight matrix (above diagonal) and all studied SNP (below diagonal)

| Traits[1] | RAW | CAT3 | CAT2 | BWT | WW | PWG | YW | MILK |
|---|---|---|---|---|---|---|---|---|
| RAW | 1.00 | 0.48 | 0.44 | 0.18 | 0.15 | 0.02 | 0.07 | 0.01 |
| CAT3 | 0.48 | 1.00 | 0.39 | 0.17 | 0.18 | 0.10 | 0.14 | 0.01 |
| CAT2 | 0.41 | 0.37 | 1.00 | 0.52 | 0.50 | 0.33 | 0.44 | 0.00 |
| BWT | 0.18 | 0.17 | 0.48 | 1.00 | 0.66 | 0.42 | 0.54 | 0.01 |
| WW | 0.15 | 0.18 | 0.46 | 0.66 | 1.00 | 0.75 | 0.88 | 0.03 |
| PWG | 0.03 | 0.10 | 0.30 | 0.42 | 0.75 | 1.00 | 0.94 | 0.01 |
| YW | 0.07 | 0.14 | 0.40 | 0.54 | 0.88 | 0.94 | 1.00 | 0.01 |
| MILK | 0.01 | 0.01 | 0.01 | 0.01 | 0.03 | 0.01 | 0.01 | 1.00 |

[1]RAW: non-transformed PAP score; CAT3: three-category phenotype; CAT2: two-category phenotype; BWT: birth weight; WW: weaning weight; PWG: post-weaning gain; YW: yearling weight; MILK: maternal weaning weight.

Figure 7.1 presents plots of the SNP effect correlations against traditional estimated pedigree-based genetic correlations and the associated regression line. A point in the graph represented the genetic correlation between a pair of phenotypes from pedigree and SNP based analyses. The SNP-based correlations from all SNP and AWM-SNP showed moderate agreement

on estimate genetic correlations, and they explained the same variation of the estimated genetic correlations ($R^2$ = 0.61 and 0.61 for AWM-SNP and all SNP, respectively). In addition, the correlation between genotype and pedigree-based quantitative genetic correlations was 0.78. Theoretically, we can obtain SNP effect correlations that were the same with the estimated genetic correlations if all QTL were identified, but it may require at least 200,000 SNPs in GWAS (De Roos, 2008) and we only used 35,930 SNP within 1 Mb windows in this analysis. Our results suggested that the SNP effects from GWAS could be used to estimate genetic correlations between traits.

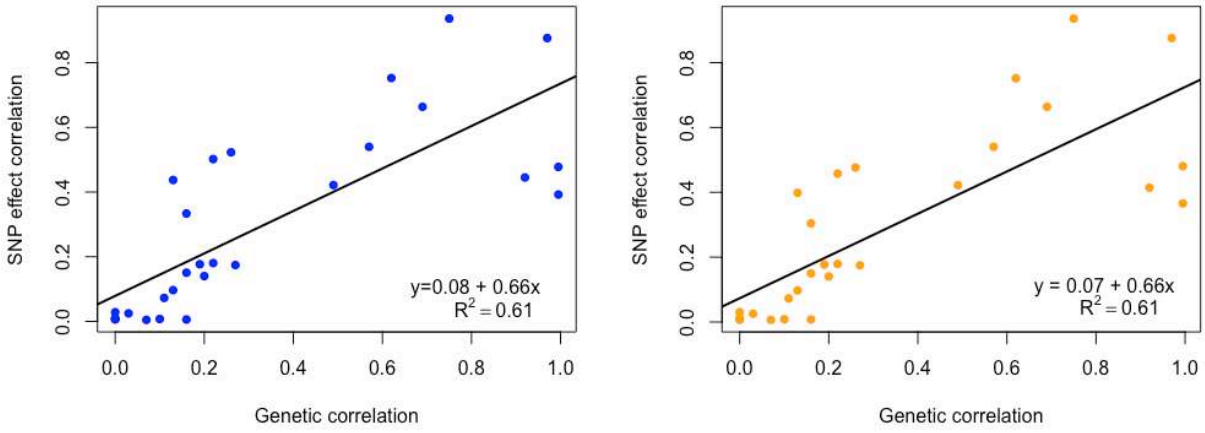


Figure 7.1 Comparison of traditional estimated genetic correlations and SNP-based correlations across eight phenotypes. Genetic correlations estimated from traditional quantitative analyses were compared with AWM-SNP-based correlations (A; n = 2,105), and all-SNP-based correlations (B, n = 35,930) in orange.

The SNP effect correlations between maternal WW and yearling PAP phenotypes were nearly 0. Although these correlations from pedigree based analysis is also not statistically significant from 0, they were slightly larger than the SNP effect based correltions. Low DEBV accuracy animals were removed from GWAS, which led to only 548 samples for MILK and may contribute to these zero correlations. In addition, the genetic correlations between maternal and

direct effects of growth traits were fixed as zero, and this restriction could influence the EBV and DEBV (used for GWAS) for maternal WW in some degree and may also subsequently influence the SNP effects. The correlations were all positive, which suggested the QTL or genes improving Angus cattle performance may also increase the PAP. However, the influence of performance associated QTL on PAP measurements is limited because of the low genomic correlations between RAW and performance traits.

The SNP effect correlations across yearling PAP phenotypes (RAW, CAT3 and CAT2) were moderate and much lower than the estimated genetic correlation (about 0.99), which suggested that they were correlated traits but not the same with each other. This difference may be attributed to the relatively small number of SNP used in GWAS, and increasing the number of SNP may improve the similarity between them. The SNP effect correlations between growth performance traits also supported the moderate to high estimated genetic correlations between them (Chapter 4).

The summary of biological process and pathways from gene enrichment analysis on the genes in AWM were presented in Appendix 7.2. These genes were clustered in several biological processes, including ion transportation, growth, development, metabolic process, etc. Among them, 13 and 12 of these genes were clustered in a role for regulating MAPK and Wnt signaling pathway, which were previously reported in association with PH. The identified KEGG pathways were Ras signaling pathways, insulin secretion, cAMP signaling pathway, the HIF-1 signaling pathway etc. The intra- and extra- cellular flow of $Ca^{2+}$ and $K^{+}$ were reported had important role in pulmonary vasoconstriction and vascular remodeling in chronic hypoxia-induced PH via their role in cellular volume, gene transcription, apoptosis and cell cycle progression (Remillard and Yuan 2005; Stenmark el al., 2006). Insulin secretion pathway is

associated with glucose metabolism in human and animal and is critical to maintain fuel homeostasis (Jitrapakdee et al., 2010). HIF-1 signaling pathway responded to hypoxia stress of organisms that not only come from reduced oxygen availability but also inflammation, energy deprivation or intensive proliferation, and it has important roles in development of PH in human and cattle (Zagórska and Dulak, 2004; Stenmark etal., 2015). The Ras signaling pathway could regulate cell proliferation, differentiation and migration regulate (Karnoub and Weinberg, 2008), which was reported to have an important role in performance development and vascular remolding in pulmonary hypertension in both human and cattle (Archer et al., 2010; Saatchi et al., 2014; De et al., 2013; Wilson et al., 2015; Stenmark 2006 and 2013).

## 7.4 Conclusions

This study identified two genomic windows on chromosome 7 and 20 with pleiotropic effects for yearling PAP phenotypes and performance traits in Angus cattle managed at high altitude. Genes in these windows were involved in adipose tissue development, response to unfolded protein, collecting duct acid secretion and SNARE interactions in vesicular transport. These biological processes and pathways have roles in intracellular transportation and cell metabolic changes, and that could be related to tissue remodeling and cell proliferation, which could support the pleiotropic genetic effects of these identified QTL regions. In addition, low to moderate genomic (SNP effects based) correlations were identified between PAP phenotypes and performance traits. In addition, the genes involved in AWM may influence mechanisms of PH and HAD via their roles in cellular metabolism, immunity, hypoxia responses and cell proliferation.

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APPENDIX 3.1 HERITABILITY ASSOCIATED WITH DIFFERENT THRESHOLD POINTS TO CONSTRUCT THREE-CATEGORY PULMONARY ARTERIAL PRESSURE PHENOTYPE

| Low | High | Genetic variance | Residual variance | Heritability | s.e. |
|---|---|---|---|---|---|
| 41 | 47 | 0.23 | 0.69 | 0.25 | 0.03 |
| 40 | 47 | 0.16 | 0.50 | 0.25 | 0.03 |
| 41 | 49 | 0.15 | 0.46 | 0.25 | 0.03 |
| 43 | 49 | 0.33 | 1.00 | 0.25 | 0.04 |
| 41 | 48 | 0.19 | 0.57 | 0.25 | 0.03 |
| 40 | 49 | 0.11 | 0.35 | 0.24 | 0.03 |
| 40 | 48 | 0.14 | 0.42 | 0.24 | 0.03 |
| 43 | 48 | 0.44 | 1.37 | 0.24 | 0.04 |
| 43 | 51 | 0.24 | 0.74 | 0.24 | 0.04 |
| 43 | 50 | 0.28 | 0.88 | 0.24 | 0.04 |
| 43 | 47 | 0.60 | 1.89 | 0.24 | 0.04 |
| 42 | 47 | 0.33 | 1.06 | 0.24 | 0.04 |
| 41 | 50 | 0.13 | 0.43 | 0.24 | 0.03 |
| 42 | 49 | 0.20 | 0.65 | 0.24 | 0.04 |
| 41 | 51 | 0.12 | 0.38 | 0.24 | 0.03 |
| 43 | 52 | 0.20 | 0.65 | 0.24 | 0.04 |
| 42 | 48 | 0.26 | 0.84 | 0.23 | 0.04 |
| 42 | 51 | 0.16 | 0.51 | 0.23 | 0.04 |
| 43 | 53 | 0.18 | 0.59 | 0.23 | 0.04 |
| 40 | 51 | 0.09 | 0.30 | 0.23 | 0.03 |
| 40 | 50 | 0.10 | 0.33 | 0.23 | 0.03 |
| 42 | 50 | 0.18 | 0.59 | 0.23 | 0.04 |
| 43 | 54 | 0.16 | 0.53 | 0.23 | 0.04 |
| 43 | 61 | 0.10 | 0.35 | 0.23 | 0.04 |
| 43 | 59 | 0.11 | 0.39 | 0.23 | 0.04 |
| 43 | 56 | 0.13 | 0.45 | 0.23 | 0.04 |
| 43 | 57 | 0.12 | 0.42 | 0.23 | 0.04 |
| 43 | 58 | 0.12 | 0.40 | 0.23 | 0.04 |
| 43 | 55 | 0.14 | 0.49 | 0.23 | 0.04 |
| 41 | 52 | 0.10 | 0.35 | 0.23 | 0.03 |
| 43 | 60 | 0.11 | 0.38 | 0.22 | 0.04 |
| 42 | 53 | 0.12 | 0.42 | 0.22 | 0.04 |
| 41 | 57 | 0.07 | 0.25 | 0.22 | 0.03 |

| | | | | | |
|---|---|---|---|---|---|
| 41 | 53 | 0.09 | 0.32 | 0.22 | 0.03 |
| 42 | 52 | 0.13 | 0.46 | 0.22 | 0.04 |
| 43 | 69 | 0.08 | 0.28 | 0.22 | 0.04 |
| 43 | 70 | 0.08 | 0.28 | 0.22 | 0.04 |
| 43 | 64 | 0.09 | 0.31 | 0.22 | 0.04 |
| 43 | 63 | 0.09 | 0.32 | 0.22 | 0.04 |
| 43 | 71 | 0.08 | 0.27 | 0.22 | 0.04 |
| 43 | 62 | 0.09 | 0.33 | 0.22 | 0.04 |
| 41 | 54 | 0.09 | 0.30 | 0.22 | 0.03 |
| 41 | 56 | 0.08 | 0.26 | 0.22 | 0.03 |
| 41 | 61 | 0.06 | 0.22 | 0.22 | 0.03 |
| 42 | 54 | 0.11 | 0.39 | 0.22 | 0.04 |
| 43 | 72 | 0.08 | 0.26 | 0.22 | 0.04 |
| 43 | 65 | 0.09 | 0.31 | 0.22 | 0.04 |
| 41 | 55 | 0.08 | 0.28 | 0.22 | 0.03 |
| 40 | 53 | 0.07 | 0.26 | 0.22 | 0.03 |
| 43 | 66 | 0.08 | 0.30 | 0.22 | 0.04 |
| 40 | 52 | 0.08 | 0.28 | 0.22 | 0.03 |
| 41 | 59 | 0.07 | 0.23 | 0.22 | 0.03 |
| 41 | 58 | 0.07 | 0.24 | 0.22 | 0.03 |
| 42 | 56 | 0.09 | 0.34 | 0.22 | 0.04 |
| 43 | 68 | 0.08 | 0.28 | 0.22 | 0.04 |
| 43 | 74 | 0.07 | 0.25 | 0.22 | 0.04 |
| 43 | 73 | 0.07 | 0.26 | 0.22 | 0.04 |
| 41 | 60 | 0.06 | 0.23 | 0.22 | 0.03 |
| 43 | 67 | 0.08 | 0.29 | 0.22 | 0.04 |
| 40 | 54 | 0.07 | 0.24 | 0.22 | 0.03 |
| 42 | 57 | 0.09 | 0.32 | 0.22 | 0.04 |
| 42 | 55 | 0.10 | 0.36 | 0.22 | 0.04 |
| 41 | 62 | 0.06 | 0.21 | 0.22 | 0.03 |
| 41 | 69 | 0.05 | 0.18 | 0.22 | 0.03 |
| 41 | 70 | 0.05 | 0.18 | 0.22 | 0.03 |
| 41 | 71 | 0.05 | 0.18 | 0.22 | 0.03 |
| 41 | 72 | 0.05 | 0.17 | 0.22 | 0.03 |
| 42 | 61 | 0.08 | 0.27 | 0.22 | 0.04 |
| 42 | 58 | 0.08 | 0.30 | 0.22 | 0.04 |
| 43 | 75 | 0.07 | 0.25 | 0.22 | 0.04 |
| 41 | 64 | 0.05 | 0.20 | 0.22 | 0.03 |
| 41 | 74 | 0.05 | 0.17 | 0.22 | 0.03 |
| 41 | 63 | 0.06 | 0.20 | 0.22 | 0.03 |
| 42 | 59 | 0.08 | 0.30 | 0.21 | 0.04 |

| | | | | | |
|---|---|---|---|---|---|
| 43 | 78 | 0.07 | 0.24 | 0.21 | 0.04 |
| 43 | 77 | 0.07 | 0.24 | 0.21 | 0.04 |
| 43 | 76 | 0.07 | 0.24 | 0.21 | 0.04 |
| 41 | 66 | 0.05 | 0.19 | 0.21 | 0.03 |
| 40 | 55 | 0.06 | 0.23 | 0.21 | 0.03 |
| 41 | 78 | 0.04 | 0.16 | 0.21 | 0.03 |
| 41 | 67 | 0.05 | 0.19 | 0.21 | 0.03 |
| 40 | 56 | 0.06 | 0.22 | 0.21 | 0.03 |
| 41 | 73 | 0.05 | 0.17 | 0.21 | 0.03 |
| 40 | 57 | 0.06 | 0.21 | 0.21 | 0.03 |
| 41 | 68 | 0.05 | 0.18 | 0.21 | 0.03 |
| 41 | 77 | 0.04 | 0.16 | 0.21 | 0.03 |
| 41 | 65 | 0.05 | 0.20 | 0.21 | 0.03 |
| 41 | 76 | 0.04 | 0.16 | 0.21 | 0.03 |
| 42 | 69 | 0.06 | 0.22 | 0.21 | 0.04 |
| 42 | 70 | 0.06 | 0.22 | 0.21 | 0.04 |
| 42 | 71 | 0.06 | 0.22 | 0.21 | 0.04 |
| 41 | 75 | 0.04 | 0.16 | 0.21 | 0.03 |
| 42 | 74 | 0.05 | 0.20 | 0.21 | 0.04 |
| 42 | 60 | 0.08 | 0.29 | 0.21 | 0.04 |
| 42 | 72 | 0.06 | 0.21 | 0.21 | 0.04 |
| 40 | 59 | 0.05 | 0.19 | 0.21 | 0.03 |
| 43 | 84 | 0.06 | 0.21 | 0.21 | 0.04 |
| 43 | 79 | 0.06 | 0.23 | 0.21 | 0.04 |
| 41 | 84 | 0.04 | 0.14 | 0.21 | 0.03 |
| 42 | 62 | 0.07 | 0.26 | 0.21 | 0.04 |
| 42 | 73 | 0.06 | 0.21 | 0.21 | 0.04 |
| 43 | 85 | 0.05 | 0.20 | 0.21 | 0.04 |
| 40 | 58 | 0.05 | 0.20 | 0.21 | 0.03 |
| 42 | 64 | 0.07 | 0.24 | 0.21 | 0.04 |
| 41 | 85 | 0.04 | 0.14 | 0.21 | 0.03 |
| 40 | 61 | 0.05 | 0.18 | 0.21 | 0.03 |
| 42 | 68 | 0.06 | 0.23 | 0.21 | 0.04 |
| 42 | 63 | 0.07 | 0.25 | 0.21 | 0.04 |
| 42 | 65 | 0.06 | 0.24 | 0.21 | 0.04 |
| 40 | 74 | 0.04 | 0.14 | 0.21 | 0.03 |
| 42 | 66 | 0.06 | 0.24 | 0.21 | 0.04 |
| 40 | 78 | 0.04 | 0.13 | 0.21 | 0.03 |
| 42 | 78 | 0.05 | 0.19 | 0.21 | 0.04 |
| 43 | 80 | 0.06 | 0.23 | 0.21 | 0.04 |
| 41 | 83 | 0.04 | 0.14 | 0.21 | 0.03 |

| | | | | | |
|---|---|---|---|---|---|
| 41 | 79 | 0.04 | 0.16 | 0.21 | 0.03 |
| 40 | 60 | 0.05 | 0.19 | 0.21 | 0.03 |
| 42 | 67 | 0.06 | 0.23 | 0.21 | 0.04 |
| 43 | 81 | 0.06 | 0.22 | 0.21 | 0.04 |
| 42 | 77 | 0.05 | 0.19 | 0.21 | 0.04 |
| 42 | 76 | 0.05 | 0.19 | 0.21 | 0.04 |
| 42 | 75 | 0.05 | 0.20 | 0.21 | 0.04 |
| 40 | 77 | 0.04 | 0.14 | 0.21 | 0.03 |
| 43 | 83 | 0.06 | 0.21 | 0.21 | 0.04 |
| 40 | 72 | 0.04 | 0.15 | 0.21 | 0.03 |
| 40 | 76 | 0.04 | 0.14 | 0.21 | 0.03 |
| 40 | 71 | 0.04 | 0.15 | 0.21 | 0.03 |
| 40 | 75 | 0.04 | 0.14 | 0.21 | 0.03 |
| 40 | 69 | 0.04 | 0.15 | 0.21 | 0.03 |
| 40 | 70 | 0.04 | 0.15 | 0.21 | 0.03 |
| 41 | 80 | 0.04 | 0.15 | 0.21 | 0.03 |
| 41 | 81 | 0.04 | 0.15 | 0.21 | 0.03 |
| 40 | 84 | 0.03 | 0.12 | 0.21 | 0.03 |
| 40 | 83 | 0.03 | 0.12 | 0.21 | 0.03 |
| 40 | 73 | 0.04 | 0.14 | 0.21 | 0.03 |
| 42 | 84 | 0.04 | 0.17 | 0.21 | 0.04 |
| 40 | 63 | 0.04 | 0.17 | 0.21 | 0.03 |
| 40 | 62 | 0.05 | 0.17 | 0.20 | 0.03 |
| 42 | 79 | 0.05 | 0.19 | 0.20 | 0.04 |
| 40 | 85 | 0.03 | 0.12 | 0.20 | 0.03 |
| 41 | 82 | 0.04 | 0.15 | 0.20 | 0.03 |
| 43 | 82 | 0.06 | 0.22 | 0.20 | 0.04 |
| 40 | 64 | 0.04 | 0.17 | 0.20 | 0.03 |
| 42 | 85 | 0.04 | 0.17 | 0.20 | 0.04 |
| 40 | 79 | 0.03 | 0.13 | 0.20 | 0.03 |
| 42 | 83 | 0.04 | 0.17 | 0.20 | 0.04 |
| 40 | 66 | 0.04 | 0.16 | 0.20 | 0.03 |
| 42 | 80 | 0.05 | 0.18 | 0.20 | 0.04 |
| 42 | 81 | 0.05 | 0.18 | 0.20 | 0.04 |
| 40 | 67 | 0.04 | 0.16 | 0.20 | 0.03 |
| 40 | 68 | 0.04 | 0.16 | 0.20 | 0.03 |
| 40 | 80 | 0.03 | 0.13 | 0.20 | 0.03 |
| 40 | 65 | 0.04 | 0.17 | 0.20 | 0.03 |
| 40 | 81 | 0.03 | 0.13 | 0.20 | 0.03 |
| 40 | 82 | 0.03 | 0.13 | 0.20 | 0.03 |
| 42 | 82 | 0.04 | 0.18 | 0.20 | 0.04 |

APPENDIX 3.2 REGRESSION ESTIMATES OF SEX, AGE OF DAM AND AGE OF MEASUREMENT ON NON-TRANSFORMED ALL, BULL, HEIFER AND STEER PULMONARY ARTERIAL PRESSURE PHENOTYPE

| Effect level[1] | Number of records[2] | Estimate | s.e | P-value |
|---|---|---|---|---|
| All | | | | |
| Intercept | 5609 | 20.20 | 3.30 | <0.001 |
| aop | 5609 | 0.05 | 0.01 | <0.001 |
| Heifer | 3456 | . | . | . |
| Bull | 1392 | 4.24 | 0.42 | <0.001 |
| Steer | 761 | 1.47 | 0.59 | 0.014 |
| 2 | 963 | . | . | . |
| 3 | 780 | 1.40 | 0.52 | 0.007 |
| 4 | 703 | 0.97 | 0.53 | 0.069 |
| 5-10 | 2648 | 1.85 | 0.44 | <0.001 |
| ≥11 | 515 | 1.74 | 0.60 | 0.003 |
| Bull | | | | |
| Intercept | 1392 | 12.91 | 9.72 | 0.185 |
| aop | 1392 | 0.08 | 0.03 | 0.002 |
| 2 | 251 | . | . | . |
| 3 | 167 | 3.00 | 1.34 | 0.025 |
| 4 | 181 | 2.64 | 1.31 | 0.045 |
| 5-10 | 714 | 3.43 | 1.10 | 0.002 |
| ≥11 | 79 | 1.94 | 1.63 | 0.233 |
| Heifer | | | | |
| Intercept | 3456 | 20.50 | 3.44 | <0.001 |
| aop | 3456 | 0.05 | 0.01 | <0.001 |
| 2 | 622 | . | . | . |
| 3 | 518 | 1.01 | 0.56 | 0.073 |
| 4 | 434 | 0.80 | 0.58 | 0.172 |
| 5-10 | 1564 | 1.52 | 0.48 | 0.002 |
| ≥11 | 318 | 1.62 | 0.66 | 0.014 |
| Steer | | | | |
| Intercept | 761 | 33.01 | 11.37 | 0.004 |
| aop | 761 | 0.04 | 0.03 | 0.087 |
| 2 | 90 | . | . | . |
| 3 | 95 | 1.53 | 1.52 | 0.314 |

| | | | | |
|---|---|---|---|---|
| 4 | 88 | 0.60 | 1.62 | 0.711 |
| 5-10 | 370 | 1.68 | 1.38 | 0.224 |
| ≥11 | 118 | 1.56 | 1.55 | 0.314 |

[1]Age of dam levels contains 2, 3, 4, 5-10, ≥11 year of age
[2]Number of pulmonary arterial pressure records associated with each sex and age of dam level

APPENDIX 3.3 REGRESSION ESTIMATES OF SEX, AGE OF DAM AND AGE OF MEASUREMENT ON POWER-TRANSFORMED ALL, BULL, HEIFER AND STEER PULMONARY ARTERIAL PRESSURE PHENOTYPE

| Effect level[1] | Number of records[2] | Estimate | s.e | P-value |
|---|---|---|---|---|
| All | | | | |
| Intercept | 5609 | 10.40 | 0.56 | 0.000 |
| aop | 5609 | -0.01 | 0.00 | 0.000 |
| Heifer | 3456 | . | . | . |
| Bull | 1392 | -0.96 | 0.07 | 0.000 |
| Steer | 761 | 0.01 | 0.10 | 0.887 |
| 2 | 963 | . | . | . |
| 3 | 780 | -0.15 | 0.09 | 0.089 |
| 4 | 703 | -0.19 | 0.09 | 0.038 |
| 5-10 | 2648 | -0.29 | 0.08 | 0.000 |
| ≥11 | 515 | -0.23 | 0.10 | 0.025 |
| Bull | | | | |
| Intercept | 1392 | 12.91 | 9.72 | 0.185 |
| aop | 1392 | 0.08 | 0.03 | 0.002 |
| 2 | 251 | . | . | . |
| 3 | 167 | 3.00 | 1.34 | 0.025 |
| 4 | 181 | 2.64 | 1.31 | 0.045 |
| 5-10 | 714 | 3.43 | 1.10 | 0.002 |
| ≥11 | 79 | 1.94 | 1.63 | 0.233 |
| Heifer | | | | |
| Intercept | 3456 | 20.50 | 3.44 | <0.001 |
| aop | 3456 | 0.05 | 0.01 | <0.001 |
| 2 | 622 | . | . | . |
| 3 | 518 | 1.01 | 0.56 | 0.073 |
| 4 | 434 | 0.80 | 0.58 | 0.172 |
| 5-10 | 1564 | 1.52 | 0.48 | 0.002 |
| ≥11 | 318 | 1.62 | 0.66 | 0.014 |
| Steer | | | | |
| Intercept | 761 | 33.01 | 11.37 | 0.004 |
| aop | 761 | 0.04 | 0.03 | 0.087 |
| 2 | 90 | . | . | . |
| 3 | 95 | 1.53 | 1.52 | 0.314 |

| | | | | |
|---|---|---|---|---|
| 4 | 88 | 0.60 | 1.62 | 0.711 |
| 5-10 | 370 | 1.68 | 1.38 | 0.224 |
| ≥11 | 118 | 1.56 | 1.55 | 0.314 |

[1]Age of dam levels contains 2, 3, 4, 5-10, ≥11 year of age
[2]Number of pulmonary arterial pressure records associated with each sex and age of dam level

APPENDIX 3.4 REGRESSION ESTIMATES OF SEX, AGE OF DAM AND AGE OF MEASUREMENT ON ALL, BULL, HEIFER AND STEER THREE-CATEGORY PULMONARY ARTERIAL PRESSURE PHENOTYPE

| Effect level[1] | Number of records[2] | Estimate | s.e | P-value |
|---|---|---|---|---|
| All | | | | |
| aop | . | 0.01 | 0.00 | 0.000 |
| Heifer | 3456 | . | . | . |
| Bull | 1392 | 0.56 | 0.05 | 0.000 |
| Steer | 761 | 0.07 | 0.08 | 0.332 |
| 2 | 963 | . | . | . |
| 3 | 780 | 0.07 | 0.06 | 0.192 |
| 4 | 703 | 0.15 | 0.06 | 0.008 |
| 5-10 | 2648 | 0.22 | 0.04 | 0.000 |
| ≥11 | 515 | 0.14 | 0.06 | 0.028 |
| Bull | | | | |
| aop | . | 0.01 | 0.00 | 0.000 |
| 2 | 251 | . | . | . |
| 3 | 167 | 0.31 | 0.11 | 0.006 |
| 4 | 181 | 0.45 | 0.11 | 0.000 |
| 5-10 | 714 | 0.49 | 0.08 | 0.000 |
| ≥11 | 79 | 0.32 | 0.15 | 0.028 |
| Heifer | | | | |
| aop | . | 0.01 | 0.00 | 0.000 |
| 2 | 622 | . | . | . |
| 3 | 518 | 0.04 | 0.07 | 0.610 |
| 4 | 434 | 0.09 | 0.08 | 0.258 |
| 5-10 | 1564 | 0.17 | 0.06 | 0.004 |
| ≥11 | 318 | 0.13 | 0.09 | 0.143 |
| Steer | | | | |
| aop | . | 0.01 | 0.00 | 0.002 |
| 2 | 90 | . | . | . |
| 3 | 95 | -0.10 | 0.18 | 0.591 |
| 4 | 88 | -0.01 | 0.18 | 0.975 |
| 5-10 | 370 | 0.07 | 0.14 | 0.619 |
| ≥11 | 118 | -0.07 | 0.17 | 0.667 |

[1]Age of dam levels contains 2, 3, 4, 5-10, ≥11 year of age
[2]Number of pulmonary arterial pressure records associated with each sex and age of dam level

APPENDIX 3.5 REGRESSION ESTIMATES OF SEX, AGE OF DAM AND AGE OF MEASUREMENT ON ALL, BULL, HEIFER AND STEER TWO-CATEGORY PULMONARY ARTERIAL PRESSURE PHENOTYPE

| Effect level[1] | Number of records[2] | Estimate | s.e | P-value |
|---|---|---|---|---|
| All | | | | |
| aop | . | 0.01 | 0.00 | 0.000 |
| Heifer | 3456 | . | . | . |
| Bull | 1392 | 0.51 | 0.07 | 0.000 |
| Steer | 761 | 0.26 | 0.11 | 0.020 |
| 2 | 963 | . | . | . |
| 3 | 780 | 0.25 | 0.08 | 0.003 |
| 4 | 703 | 0.23 | 0.09 | 0.007 |
| 5-10 | 2648 | 0.35 | 0.06 | 0.000 |
| ≥11 | 515 | 0.31 | 0.10 | 0.002 |
| Bull | | | | |
| aop | . | 0.01 | 0.00 | 0.000 |
| 2 | 251 | . | . | . |
| 3 | 167 | 0.58 | 0.15 | 0.000 |
| 4 | 181 | 0.58 | 0.15 | 0.000 |
| 5-10 | 714 | 0.67 | 0.12 | 0.000 |
| ≥11 | 79 | 0.50 | 0.21 | 0.017 |
| Heifer | | | | |
| aop | . | 0.01 | 0.00 | 0.000 |
| 2 | 622 | . | . | . |
| 3 | 518 | 0.14 | 0.11 | 0.209 |
| 4 | 434 | 0.12 | 0.11 | 0.284 |
| 5-10 | 1564 | 0.23 | 0.08 | 0.004 |
| ≥11 | 318 | 0.20 | 0.13 | 0.118 |
| Steer | | | | |
| aop | . | 0.00 | 0.00 | 0.354 |
| 2 | 90 | . | . | . |
| 3 | 95 | 0.35 | 0.32 | 0.273 |
| 4 | 88 | 0.23 | 0.33 | 0.494 |
| 5-10 | 370 | 0.45 | 0.25 | 0.074 |
| ≥11 | 118 | 0.47 | 0.29 | 0.113 |

[1]Age of dam levels contains 2, 3, 4, 5-10, ≥11 year of age
[2]Number of pulmonary arterial pressure records associated with each sex and age of dam level

APPENDIX 4.1 REGRESSION ESTIMATES OF SEX, AGE OF DAM AND AGE OF MEASUREMENT ON GROWTH PERFORMANCE TRAITS

| Effect level[1] | Number of records[2] | Estimate | s.e. | P-value | LSM[3] |
|---|---|---|---|---|---|
| BWT | | | | | |
| Intercept | . | 73.40 | 0.65 | <0.001 | . |
| Femal | 4315 | . | . | . | 76.18[a] |
| Male | 4709 | 5.28 | 0.21 | <0.001 | 81.45[b] |
| 2 | 1482 | . | . | . | 72.27[a] |
| 3 | 1276 | 6.78 | 0.38 | <0.001 | 79.04[b] |
| 4 | 1120 | 7.61 | 0.39 | <0.001 | 79.88[bc] |
| 5-10 | 4243 | 10.16 | 0.30 | <0.001 | 82.43[d] |
| ≥11 | 903 | 8.19 | 0.42 | <0.001 | 80.46[c] |
| WW | | | | | |
| Intercept | . | 2.44 | 7.68 | <0.001 | . |
| age | . | 2.00 | 0.03 | <0.001 | . |
| Heifer | 4009 | . | . | . | 448.74[a] |
| Bull | 1704 | 59.76 | 1.48 | <0.001 | 508.50[b] |
| Steer | 2615 | 12.48 | 1.26 | <0.001 | 461.22[c] |
| 2 | 1306 | . | . | . | 441.87[a] |
| 3 | 1190 | 29.87 | 2.20 | <0.001 | 471.74[b] |
| 4 | 1045 | 44.84 | 2.27 | <0.001 | 486.71[c] |
| 5-10 | 3957 | 56.04 | 1.87 | <0.001 | 497.92[d] |
| ≥11 | 830 | 24.00 | 2.48 | <0.001 | 465.87[b] |
| PWG | | | | | |
| Intercept | . | -55.03 | 14.43 | <0.001 | . |
| age | . | 0.50 | 0.04 | <0.001 | . |
| Heifer | 3304 | . | . | . | 323.02[a] |
| Bull | 1355 | 38.68 | 7.84 | <0.001 | 361.70[b] |
| Steer | 870 | -13.61 | 3.85 | <0.001 | 309.40[c] |
| 2 | 960 | . | . | . | 321.64[a] |
| 3 | 754 | 13.07 | 2.25 | <0.001 | 334.71[b] |
| 4 | 696 | 13.63 | 2.29 | <0.001 | 335.27[b] |
| 5-10 | 2613 | 10.63 | 1.89 | <0.001 | 332.27[b] |
| ≥11 | 506 | 11.34 | 2.56 | <0.001 | 332.98[b] |

| | | | | | |
|---|---|---|---|---|---|
| YW | | | | | |
| Intercept | . | -257.88 | 21.77 | <0.001 | . |
| age | . | 2.31 | 0.06 | <0.001 | . |
| Heifer | 3324 | . | . | . | 769.37[a] |
| Bull | 1363 | 93.19 | 11.83 | <0.001 | 862.56[b] |
| Steer | 882 | 4.88 | 5.78 | 0.399 | 774.25[a] |
| 2 | 970 | . | . | . | 764.14[a] |
| 3 | 760 | 38.42 | 3.40 | <0.001 | 802.56[b] |
| 4 | 700 | 53.08 | 3.45 | <0.001 | 817.22[c] |
| 5-10 | 2630 | 61.96 | 2.85 | <0.001 | 826.10[d] |
| ≥11 | 509 | 36.13 | 3.85 | <0.001 | 800.27[b] |

[1]Age of dam levels contains 2, 3, 4, 5-10, ≥11 year of age; BWT: birth weight (lb); WW: weaning weight (lb); PWG: post-weaning gain (lb); YW: yearling weight (lb).
[2]Number of pulmonary arterial pressure records associated with each sex and age of dam level
[3]LSM: least square mean for each effect level.
[abcd]Within the column, the least square mean without a common superscript differ ($P < 0.05$).

APPENDIX 5.1 SUMMARY OF GENOTYPED SAMPLES

| Year of birth | Number of samples | Year of genotyping |
|---|---|---|
| 1997 | 6 | 2013 |
| 1998 | 10 | 2013 |
| 1999 | 10 | 2013 |
| 2000 | 16 | 2013 |
| 2001 | 33 | 2013 |
| 2002 | 17 | 2013 |
| 2003 | 25 | 2013 |
| 2004 | 37 | 2013 |
| 2005 | 39 | 2013 |
| 2006 | 31 | 2013 |
| 2007 | 127 | 2013 |
| 2008 | 124 | 2013 |
| 2009 | 146 | 2013 |
| 2010 | 310 | 2013 |
| 2011 | 373 | 2013 |
| 2012 | 361 | 2013 |
| 2013 | 367 | 2014 |
| 2014 | 343 | 2014 |
| 2015 | 390 | 2015 |

APPENDIX 5.2 GENOMIC WINDOWS EXPLAIN >1% GENETIC VARIATION FROM THE GWAS RESULTS OF NON-TRANSFORMED, THREE-CATEGORY AND TWO-CATEGORY YEARLING PULMONARY ARTERIAL PRESSURE PHENOTYPES FROM ANGUS CATTLE AT HIGH ALTITUDE (ELEVATION AT 2,170 M)

| BTA_Mb | Gene[2] |
|---|---|
| 7_93 | *ARRDC3, G-protein coupled receptor 98 (ADGRV1), LOC104968987* |
| 8_83 | *FANCC, ERCC6L2, bta-mir-27b, U6* |
| 8_89 | *5S_rRNA* |
| 10_21 | *JPH4, AP1G2, THTPA, ZFHX2, NGDN, MYH7, MYH6, CMTM5, IL25, EFS, SLC22A17, PABPN1, BCL2L2, PPP1R3E, RNF212B, SLC7A8, CEBPE, ACIN1, CDH24, PSMB5, AJUBA, PRMT5, RBM23, REM2, LRP10, MMP14, MRPL52, SLC7A7, OXA1L, HOMEZ, PSMB11, U6* |
| 10_29 | *RYR3, TMCO5B, FMN1* |
| 10_36 | *DISP2, KNSTRN, IVD, BAHD1, RPUSD2, RAD51, RMDN3, GCHFR, DNAJC17, ZFYVE1, PPP1R14D, SPINT1, RHOV, VPS18, DLL4, CHAC1, INO80, EXD1, CHP1, OIP5, NUSAP1, NDUFAF1, CHST14, SNORA2, snoU89* |
| 11_4 | *TSGA10, C2orf15, LIPT1, MITD1, LYG2, TXNDC9, EIF5B, REV1, AFF3* |
| 11_86 | *LPIN1, NTSR2, E2F6, ROCK2, PQLC3, PDIA6, ATP6V1C2, NOL10, HIST1H4A, KCNF1* |
| 12_12 | *VWA8, DGKH, AKAP11, TNFSF11, FAM216B* |
| 12_25 | *CCNA1, SPG20, NBEA, MAB21L1* |
| 12_34 | *ATP8A2, NUP58, MTMR6, SPATA13, C1QTNF9, MIPEP, TNFRSF19, SACS, SGCG* |
| 12_42 | - |
| 12_57 | *bta-mir-1256* |
| 14_64 | *ODF1, UBR5, RRM2B, NCALD* |
| 15_59 | *BDNF, KIF18A, METTL15* |
| 19_5 | *TOM1L1, COX11, STXBP4, HLF, MMD, U6* |
| 20_4 | *NEURL1B, DUSP1, ERGIC1, RPL26L1, ATP6V0E1, CREBRF, BNIP1, NKX2-5, STC2, 5S_rRNA* |
| 20_34 | *5S_rRNA, U2* |
| 28_31 | *SAMD8, VDAC2, COMTD1, ZNF503, DUSP13* |
| 29_22 | *GAS2, SLC17A6, ANO5, FANCF* |
| X_110 | *TSPAN7, OTC, RPGR, SRPX, SYTL5* |

[1]Bovine chromosome and n[th] 1 Mb window on the same chromosome based on the UMD3.1 assembly
[2]Genens located in the windows

APPENDIX 6.1 WINDOWS FROM THE GWAS EXPLAINED > 1% GENETIC VARIATION OF GENETIC VARIATION OF PERFORMANCE TRAITS FROM ANGUS CATTLE AT HIGH ALTITUDE REGION (ELEVATION AT 2,170 M)[1]

| BTA_Mb[2] | Gene[3] |
|---|---|
| 1_2 | *PAXBP1, SYNJ1, EVA1C, URB1, MRAP, MIS18A, HUNK* |
| 3_85 | *NFIA, U1* |
| 5_19 | *DUSP6, POC1B, ATP2B1* |
| 6_114 | - |
| 7_4 | *MEF2B, TMEM161A, SLC25A42, ARMC6, SUGP2, HOMER3, DDX49, COPE, UPF1, COMP, CRTC1, U6, KLHL26, TMEM59L, CRLF1,C19orf60, KXD1, ELL, ISYNA1, SSBP4, LRRC25, GDF15, PGPEP1, LSM4, KIAA1683, PDE4C, RAB3A, MPV17L2, IFI30* |
| 7_93 | *ARRDC3, G-protein coupled receptor 98 (ADGRV1), LOC104968987* |
| 12_23 | *LHFP, NHLRC3, PROSER1, PROSER1, STOML3, FREM2, U6* |
| 12_88 | *MYO16, IRS2, COL4A1, 5S_rRNA* |
| 14_24 | *XKR4, TMEM68, TGS1, LYN, RPS20, MOS* |
| 14_25 | *SDR16C5, SDR16C6, PENK, IMPAD1, PLAG1, CHCHD7* |
| 14_35 | *SULF1, SLCO5A1, PRDM14, NCOA2* |
| 16_47 | *DNAJC11, THAP3, PHF13, KLHL21, ZBTB48, TAS1R1, NOL9, PLEKHG5, TNFRSF25, ESPN, HES2, ACOT7, GPR153, HES3, ICMT, RNF207* |
| 20_4 | *NEURL1B, DUSP1, ERGIC1, RPL26L1, ATP6V0E1, CREBRF, BNIP1, NKX2-5, STC2* |
| 23_32 | *SCGN, LRRC16A, GMNN, C6orf62, ACOT13, TDP2, KIAA0319, ALDH5A1, GPLD1, U6* |
| 23_41 | *DTNBP1, JARID2, U6, SNORD112* |
| 26_34 | *HABP2, NRAP, CASP7, PLEKHS1, DCLRE1A, NHLRC2, CCDC186, TDRD1, ADRB1* |
| 27_33 | *ASH2L, STAR, LSM1, BAG4, PLPP5, WHSC1L1, DDHD2, LETM2, FGFR1, TACC1, PLEKHA2, HTRA4, TM2D2, ADAM9, ADAM32, bta-mir-2400, U6* |
| X_7 | *GRIA3, THOC2, BIRC8, SNORA43, XIAP, STAG2* |
| X_49 | - |

[1]Performance traits included birth weight, weaning weight, maternal weaning weight, post-weaning gain and yearling weight

[2]Bovine chromosome and n$^{th}$ 1 Mb window on the same chromosome based on the UMD3.1 assembly

[3]Genens located in the windows

APPENDIX 7.1. SUMMARY OF ASSOCIATED WEIGHT MATRIX OF GENOME-WIDE ASSOCIATION ANALYSIS ON YEARLING PULMONARY ARTERIAL PRESSURE PHENOTYPES AND PERFORMANCE TRAITS IN ANGUS CATTLE

| Chromosome | Num. SNP ≤ 25000 bp of a gene | Num. SNP ≤ 25000 bp and < 1.5 Mb of a gene | Num. SNP ≥ 1.5 Mb from a range | Total |
|---|---|---|---|---|
| 1 | 33 | 83 | 0 | 116 |
| 2 | 31 | 63 | 0 | 94 |
| 3 | 36 | 65 | 1 | 102 |
| 4 | 34 | 64 | 0 | 98 |
| 5 | 23 | 52 | 0 | 75 |
| 6 | 26 | 73 | 0 | 99 |
| 7 | 29 | 61 | 0 | 90 |
| 8 | 25 | 62 | 0 | 87 |
| 9 | 18 | 48 | 2 | 68 |
| 10 | 34 | 47 | 0 | 81 |
| 11 | 19 | 43 | 0 | 62 |
| 12 | 23 | 77 | 0 | 100 |
| 13 | 27 | 37 | 0 | 64 |
| 14 | 27 | 60 | 0 | 87 |
| 15 | 22 | 44 | 2 | 68 |
| 16 | 32 | 47 | 0 | 79 |
| 17 | 16 | 33 | 0 | 49 |
| 18 | 21 | 46 | 0 | 67 |
| 19 | 34 | 32 | 0 | 66 |
| 20 | 17 | 51 | 0 | 68 |
| 21 | 17 | 57 | 0 | 74 |
| 22 | 31 | 21 | 0 | 52 |
| 23 | 20 | 32 | 0 | 52 |
| 24 | 13 | 28 | 0 | 41 |
| 25 | 7 | 14 | 0 | 21 |
| 26 | 9 | 15 | 0 | 24 |
| 27 | 17 | 40 | 0 | 57 |
| 28 | 8 | 30 | 0 | 38 |
| 29 | 11 | 49 | 0 | 60 |
| 30 | 13 | 53 | 0 | 66 |
| Total | 673 | 1427 | 5 | 2105 |

APENDIX 7.2 RESULTING ASSOCIATED WEIGHT MATRIX[1]

| Gene/SNP | RAW | CAT3 | CAT2 | BWT | WW | PWG | YW | MILK |
|---|---|---|---|---|---|---|---|---|
| ARS-BFGL-NGS-105604 | 0.05 | -0.02 | -0.01 | 0.00 | -0.01 | 0.01 | 0.01 | -1.66 |
| ARS-BFGL-NGS-27579 | 0.00 | 0.02 | 0.01 | -0.02 | -0.03 | 0.01 | -0.01 | 2.12 |
| BTA-91816-no-rs | 0.01 | -0.03 | -0.07 | -1.79 | -1.28 | -2.57 | -1.33 | 0.11 |
| BTB-01421892 | 0.03 | 0.03 | 0.11 | 4.27 | 0.56 | 0.80 | 0.55 | -0.19 |
| BTB-01487164 | -0.08 | -0.17 | -0.03 | -0.09 | -0.23 | -0.01 | -0.05 | -2.29 |
| ENSBTAG00000000061 | -0.86 | -1.91 | -0.38 | -0.04 | -0.05 | -0.02 | -0.03 | -0.13 |
| ENSBTAG00000000074 | -0.07 | -0.02 | -0.21 | -7.73 | -0.61 | -0.05 | -0.14 | -0.02 |
| ENSBTAG00000000138 | -0.03 | 0.01 | -0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 1.34 |
| ENSBTAG00000000146 | -1.23 | -0.06 | -0.12 | 0.04 | 0.05 | 0.07 | 0.06 | 0.02 |
| ENSBTAG00000000189 | -0.21 | -0.45 | -0.35 | -0.46 | -1.83 | -0.32 | -0.55 | 0.10 |
| ENSBTAG00000000207 | -0.09 | -2.12 | -0.04 | 0.03 | 0.03 | 0.01 | 0.01 | 0.08 |
| ENSBTAG00000000212 | 1.86 | 6.20 | 1.39 | 0.03 | 0.01 | 0.00 | 0.00 | 0.06 |
| ENSBTAG00000000219 | 3.75 | 0.26 | 0.06 | 0.00 | -0.03 | 0.00 | -0.03 | -0.02 |
| ENSBTAG00000000289 | -0.05 | -0.02 | 0.04 | 0.87 | 0.67 | 1.41 | 0.46 | 0.20 |
| ENSBTAG00000000559 | 0.01 | -0.08 | -0.02 | -0.09 | -1.03 | -0.33 | -0.47 | -0.05 |
| ENSBTAG00000000571 | 0.05 | -0.01 | 0.00 | -2.99 | -0.85 | -2.34 | -0.93 | -0.09 |
| ENSBTAG00000000575 | -0.23 | -0.22 | -0.02 | 0.03 | -0.01 | -0.01 | -0.02 | -1.34 |
| ENSBTAG00000000580 | -1.57 | -0.23 | -7.08 | -0.48 | -0.83 | -1.05 | -1.05 | -0.06 |
| ENSBTAG00000000588 | -0.35 | -1.20 | -0.20 | -0.22 | -0.04 | -0.03 | -0.03 | 0.02 |
| ENSBTAG00000000616 | 2.50 | 0.27 | 0.79 | 0.01 | 0.02 | -0.01 | 0.00 | -1.52 |
| ENSBTAG00000000655 | -0.21 | -9.98 | -0.08 | -0.27 | -0.03 | 0.00 | -0.01 | 0.08 |
| ENSBTAG00000000664 | 0.26 | 0.15 | 0.58 | 1.34 | 8.63 | 6.24 | 5.74 | 0.13 |
| ENSBTAG00000000698 | 0.13 | 2.09 | 0.18 | 0.01 | 0.00 | 0.02 | 0.02 | 0.01 |
| ENSBTAG00000000700 | -0.13 | -2.72 | -0.19 | -0.04 | -0.01 | -0.01 | 0.00 | -0.02 |
| ENSBTAG00000000706 | 2.50 | 0.29 | 0.43 | 0.02 | 0.04 | 0.05 | 0.05 | 1.45 |
| ENSBTAG00000000854 | -0.13 | -0.04 | 0.01 | 0.05 | 0.17 | 0.15 | 0.25 | 2.22 |
| ENSBTAG00000000873 | -0.04 | -0.01 | -0.04 | -0.25 | -0.99 | -0.10 | -0.22 | -0.07 |
| ENSBTAG00000000897 | -0.02 | -0.02 | 0.00 | -0.05 | -0.10 | -0.12 | -0.11 | 1.53 |
| ENSBTAG00000000953 | 0.06 | 2.34 | 0.61 | 0.14 | 0.33 | 1.06 | 0.44 | 0.32 |
| ENSBTAG00000001080 | 0.10 | 0.11 | 0.16 | 0.03 | 0.30 | 0.89 | 0.61 | 0.02 |
| ENSBTAG00000001132 | -0.17 | -0.01 | 0.03 | 1.72 | 1.57 | 2.96 | 2.34 | 0.08 |
| ENSBTAG00000001181 | 0.05 | 0.07 | 0.08 | 0.02 | 0.03 | -0.04 | 0.01 | 4.18 |
| ENSBTAG00000001290 | -0.01 | -0.04 | -0.01 | -2.61 | -0.07 | -0.02 | -0.08 | -0.06 |
| ENSBTAG00000001403 | 0.79 | 3.93 | 0.06 | 0.09 | 0.00 | -0.01 | 0.00 | -0.63 |
| ENSBTAG00000001406 | -0.49 | -0.13 | -1.54 | -0.68 | -1.34 | -1.61 | -1.39 | -0.05 |
| ENSBTAG00000001463 | -0.15 | -13.85 | -0.05 | -0.06 | -0.07 | -0.02 | -0.04 | 0.06 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000001545 | -9.62 | -0.67 | -9.97 | -0.18 | -0.14 | -0.04 | -0.05 | -0.16 |
| ENSBTAG00000001578 | -0.04 | -0.03 | -0.28 | -0.93 | -1.44 | -0.70 | -1.28 | -0.23 |
| ENSBTAG00000001589 | -0.03 | -0.03 | -0.22 | -0.07 | -1.61 | -11.10 | -4.35 | -0.04 |
| ENSBTAG00000001598 | -0.16 | -0.08 | -0.05 | -4.30 | -0.41 | -0.07 | -0.22 | 0.05 |
| ENSBTAG00000001602 | -0.04 | 0.00 | -0.07 | -0.30 | -3.13 | -0.45 | -1.23 | -0.08 |
| ENSBTAG00000001616 | 0.64 | 10.02 | 0.49 | 0.02 | 0.02 | -0.01 | 0.02 | -0.43 |
| ENSBTAG00000001627 | 1.89 | 0.54 | 0.59 | 0.10 | 0.07 | 0.02 | 0.03 | -0.04 |
| ENSBTAG00000001707 | -0.07 | -7.21 | -0.04 | 0.01 | 0.02 | 0.08 | 0.02 | -0.03 |
| ENSBTAG00000001710 | 0.05 | 0.23 | 0.03 | -0.02 | -0.05 | 0.01 | -0.01 | -0.89 |
| ENSBTAG00000001749 | 2.21 | 1.01 | 1.11 | 0.02 | 0.00 | -0.02 | -0.03 | 0.00 |
| ENSBTAG00000001803 | 0.20 | 0.00 | -0.01 | -0.03 | -0.14 | -0.27 | -0.15 | -10.50 |
| ENSBTAG00000001816 | -0.03 | 0.00 | 0.00 | 0.02 | -0.01 | 0.01 | -0.01 | -3.22 |
| ENSBTAG00000001826 | -0.04 | 0.06 | 0.08 | 1.06 | 0.06 | 0.05 | 0.05 | -0.07 |
| ENSBTAG00000001872 | -0.12 | -0.19 | -0.29 | -2.83 | -0.46 | -0.61 | -0.48 | 0.07 |
| ENSBTAG00000001927 | 0.91 | 1.19 | 0.13 | 0.02 | 0.04 | 0.01 | 0.03 | -0.02 |
| ENSBTAG00000001966 | -0.07 | -0.03 | -0.29 | -3.49 | -3.42 | -0.63 | -1.82 | -0.09 |
| ENSBTAG00000002020 | -0.18 | -1.79 | -0.09 | -0.01 | 0.00 | -0.05 | -0.03 | 0.13 |
| ENSBTAG00000002081 | -0.05 | -0.07 | -0.07 | -0.04 | -0.58 | -1.06 | -0.28 | -0.02 |
| ENSBTAG00000002115 | -0.04 | 0.03 | 0.00 | 0.12 | 0.08 | 0.05 | 0.07 | -1.86 |
| ENSBTAG00000002174 | -0.93 | -0.33 | -0.20 | 0.00 | 0.04 | 0.02 | 0.04 | 0.00 |
| ENSBTAG00000002181 | 0.03 | -0.04 | 0.06 | -0.01 | -0.06 | -0.07 | -0.07 | -12.02 |
| ENSBTAG00000002214 | 0.07 | 0.18 | -0.01 | -0.02 | -0.07 | -0.01 | -0.06 | -1.44 |
| ENSBTAG00000002316 | 1.06 | 3.16 | 9.90 | 0.21 | 0.01 | 0.00 | 0.01 | -0.14 |
| ENSBTAG00000002341 | -0.04 | -0.01 | 0.03 | 0.02 | 0.04 | 0.01 | 0.03 | 2.34 |
| ENSBTAG00000002356 | -0.04 | -0.02 | 0.08 | 0.60 | 1.51 | 0.60 | 0.62 | 0.41 |
| ENSBTAG00000002445 | 0.14 | 0.07 | 0.63 | 2.19 | 0.38 | 0.40 | 0.34 | -0.03 |
| ENSBTAG00000002452 | -0.27 | -0.71 | -0.43 | 0.01 | -0.01 | -0.01 | -0.02 | -1.21 |
| ENSBTAG00000002471 | -0.27 | -0.38 | -0.28 | -4.69 | -0.75 | -0.36 | -0.33 | -0.05 |
| ENSBTAG00000002485 | 1.67 | 1.93 | 0.57 | 0.02 | 0.03 | 0.05 | 0.05 | -0.05 |
| ENSBTAG00000002493 | -0.04 | 0.01 | 0.09 | 3.04 | 12.47 | 5.74 | 5.87 | 0.03 |
| ENSBTAG00000002586 | 0.10 | -0.03 | 0.14 | 0.01 | -0.01 | -0.01 | -0.01 | -27.53 |
| ENSBTAG00000002624 | -0.15 | -12.34 | -1.09 | -0.07 | -0.08 | -0.12 | -0.10 | -0.01 |
| ENSBTAG00000002678 | 0.11 | 0.14 | 0.09 | 3.12 | 0.73 | 0.22 | 0.22 | -0.92 |
| ENSBTAG00000002701 | -1.03 | -0.06 | -0.11 | -0.06 | -0.16 | -0.12 | -0.17 | -0.05 |
| ENSBTAG00000002730 | -1.11 | -4.16 | -1.54 | -0.36 | -0.09 | 0.00 | -0.02 | 0.09 |
| ENSBTAG00000002822 | -0.05 | -0.01 | -0.07 | -0.09 | -0.96 | -2.02 | -0.86 | 0.09 |
| ENSBTAG00000002865 | -0.07 | -0.20 | 0.01 | 0.07 | 0.11 | 0.04 | 0.06 | 1.39 |
| ENSBTAG00000002898 | 0.21 | 0.12 | 0.52 | 0.09 | 0.45 | 2.04 | 0.54 | 0.03 |
| ENSBTAG00000002914 | -0.06 | -0.07 | -0.02 | -0.25 | -1.05 | -0.04 | -0.22 | -0.51 |
| ENSBTAG00000002959 | 0.10 | 0.33 | 0.02 | 0.37 | 2.23 | 1.67 | 1.28 | 0.32 |
| ENSBTAG00000002997 | -0.15 | -0.20 | -0.11 | -0.03 | -0.06 | 0.00 | -0.03 | -0.89 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000003034 | -0.01 | 0.03 | -0.14 | -0.01 | -0.18 | -0.11 | -0.13 | -15.96 |
| ENSBTAG00000003069 | 0.01 | 0.03 | 0.00 | 0.04 | 0.09 | 0.16 | 0.08 | -1.38 |
| ENSBTAG00000003101 | 0.08 | 0.01 | 0.04 | 2.18 | 0.33 | 0.49 | 0.35 | 0.09 |
| ENSBTAG00000003128 | 1.10 | 0.10 | 0.75 | 0.01 | 0.00 | -0.02 | -0.02 | 0.11 |
| ENSBTAG00000003172 | -0.09 | 0.00 | -0.03 | -0.09 | -0.48 | -0.52 | -0.60 | -0.96 |
| ENSBTAG00000003218 | -0.01 | -0.32 | -0.12 | -1.42 | -0.88 | -0.18 | -0.54 | -0.05 |
| ENSBTAG00000003236 | 0.08 | 0.14 | 0.08 | 1.03 | 0.47 | 0.69 | 0.52 | 0.09 |
| ENSBTAG00000003301 | 0.00 | -0.04 | 0.01 | 0.94 | 0.44 | 0.08 | 0.13 | -0.43 |
| ENSBTAG00000003359 | -0.12 | -0.03 | -0.03 | -3.17 | -0.90 | -0.24 | -0.58 | -0.01 |
| ENSBTAG00000003418 | -2.53 | -0.60 | -0.17 | -0.03 | 0.01 | 0.04 | 0.03 | 0.18 |
| ENSBTAG00000003449 | 1.09 | 0.69 | 0.14 | -0.01 | 0.00 | -0.04 | -0.01 | 0.13 |
| ENSBTAG00000003455 | 1.27 | 0.07 | 1.07 | -0.03 | -0.03 | 0.01 | -0.02 | -0.06 |
| ENSBTAG00000003496 | 0.13 | 0.03 | 0.08 | 0.12 | 0.21 | 0.02 | 0.06 | -2.46 |
| ENSBTAG00000003509 | -0.02 | -0.10 | 0.02 | 0.08 | 0.47 | 0.25 | 0.40 | 43.54 |
| ENSBTAG00000003531 | 0.01 | -0.05 | -0.36 | -0.68 | -1.26 | -0.19 | -0.32 | -0.13 |
| ENSBTAG00000003555 | 0.12 | -0.02 | 0.03 | 0.20 | 1.03 | 2.19 | 1.98 | 0.13 |
| ENSBTAG00000003587 | -0.08 | -0.04 | -0.01 | 0.00 | 0.10 | 0.09 | 0.09 | 31.13 |
| ENSBTAG00000003594 | 1.12 | 0.50 | 0.07 | -0.04 | -0.06 | -0.13 | -0.08 | -0.01 |
| ENSBTAG00000003606 | 0.12 | 0.24 | 1.31 | 7.57 | 5.50 | 0.51 | 0.87 | -0.11 |
| ENSBTAG00000003610 | 0.05 | -0.05 | 1.76 | 0.01 | 0.01 | 0.03 | 0.03 | -3.61 |
| ENSBTAG00000003690 | -0.05 | 0.10 | 0.04 | 2.42 | 0.71 | 0.04 | 0.14 | -0.05 |
| ENSBTAG00000003701 | 0.01 | 0.00 | -0.01 | -0.03 | -0.04 | -0.01 | -0.05 | -11.78 |
| ENSBTAG00000003721 | -0.26 | -0.07 | -0.17 | -0.38 | -0.14 | -0.04 | -0.13 | -1.56 |
| ENSBTAG00000003749 | 0.15 | 0.07 | 0.42 | 0.36 | 0.81 | 0.29 | 0.51 | 3.56 |
| ENSBTAG00000003773 | 0.03 | 0.00 | 0.01 | -0.08 | -0.29 | -23.37 | -1.47 | -0.04 |
| ENSBTAG00000003808 | -0.05 | 0.06 | -0.03 | 0.02 | -0.01 | 0.04 | 0.01 | -1.19 |
| ENSBTAG00000003822 | 0.03 | 0.00 | -0.01 | -0.02 | -0.07 | 0.00 | -0.03 | -6.27 |
| ENSBTAG00000003825 | -0.03 | -0.02 | -0.14 | -0.79 | -1.01 | -0.39 | -0.55 | -0.07 |
| ENSBTAG00000003827 | 1.41 | 2.61 | 0.47 | 0.14 | 0.28 | 0.15 | 0.29 | 0.03 |
| ENSBTAG00000003895 | -0.16 | -0.03 | 0.02 | 0.70 | 1.13 | 0.72 | 0.91 | 0.02 |
| ENSBTAG00000004034 | 0.00 | -0.02 | -0.27 | -5.19 | -0.94 | -0.52 | -0.49 | -0.05 |
| ENSBTAG00000004066 | -3.33 | -0.14 | -2.57 | -0.02 | -0.02 | -0.04 | -0.04 | -0.48 |
| ENSBTAG00000004077 | 0.01 | 0.00 | 0.07 | 0.03 | 0.01 | -0.01 | 0.00 | 0.94 |
| ENSBTAG00000004081 | 1.36 | 0.22 | 0.25 | 0.00 | 0.04 | 0.01 | 0.02 | -0.04 |
| ENSBTAG00000004165 | -3.55 | -0.05 | -0.17 | 0.15 | 0.34 | 0.61 | 0.37 | 0.07 |
| ENSBTAG00000004238 | 0.00 | 0.08 | 0.00 | -0.02 | -0.15 | -0.29 | -0.22 | -33.01 |
| ENSBTAG00000004248 | 0.06 | 0.08 | 0.13 | 1.06 | 0.14 | 0.04 | 0.09 | 0.06 |
| ENSBTAG00000004279 | 0.03 | -0.04 | -0.11 | -0.17 | -0.26 | -1.24 | -0.40 | 0.07 |
| ENSBTAG00000004280 | 0.96 | 0.43 | 0.06 | 0.00 | 0.02 | 0.02 | 0.03 | 0.07 |
| ENSBTAG00000004287 | 0.02 | 0.03 | 0.07 | -0.02 | -0.01 | 0.02 | -0.01 | -3.44 |
| ENSBTAG00000004297 | -0.09 | -0.10 | -0.02 | -3.25 | -13.69 | -0.84 | -2.69 | 0.02 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000004351 | 2.08 | 0.13 | 0.27 | 0.02 | 0.02 | 0.01 | 0.02 | 0.07 |
| ENSBTAG00000004398 | 1.31 | 0.09 | 0.10 | -0.02 | -0.05 | -0.06 | -0.05 | 0.00 |
| ENSBTAG00000004407 | 1.19 | 0.60 | 0.08 | 0.01 | -0.06 | -0.04 | -0.07 | -0.33 |
| ENSBTAG00000004514 | -1.35 | -0.23 | -2.67 | -0.25 | 0.00 | 0.01 | 0.00 | 0.00 |
| ENSBTAG00000004555 | 0.01 | -0.19 | -0.20 | -1.44 | -0.88 | -0.42 | -0.59 | 0.02 |
| ENSBTAG00000004562 | 1.38 | 0.43 | 0.59 | 0.03 | 0.05 | 0.04 | 0.06 | -0.06 |
| ENSBTAG00000004587 | -0.07 | -1.81 | -0.01 | -0.04 | 0.00 | -0.08 | -0.06 | -0.11 |
| ENSBTAG00000004607 | -0.07 | -0.06 | -0.28 | -0.46 | -2.95 | -0.17 | -0.44 | -0.16 |
| ENSBTAG00000004723 | 0.33 | 1.04 | 0.01 | 0.01 | 0.00 | -0.04 | -0.02 | -0.03 |
| ENSBTAG00000004770 | 0.02 | 0.06 | 0.03 | 1.64 | 0.57 | 1.38 | 0.88 | -0.02 |
| ENSBTAG00000004907 | -0.80 | -1.61 | -1.69 | -0.01 | -0.01 | 0.01 | 0.00 | 0.03 |
| ENSBTAG00000004920 | -1.55 | -0.08 | -0.39 | 0.01 | -0.01 | -0.04 | -0.06 | -0.80 |
| ENSBTAG00000004931 | -4.22 | -0.86 | -3.55 | -0.03 | -0.02 | 0.00 | -0.02 | 0.05 |
| ENSBTAG00000005021 | -0.07 | -2.24 | -0.14 | -0.05 | -0.13 | -0.05 | -0.07 | -0.35 |
| ENSBTAG00000005083 | 0.26 | 0.91 | 0.06 | 0.01 | 0.01 | 0.00 | -0.01 | 0.05 |
| ENSBTAG00000005092 | 4.72 | 0.10 | 3.42 | 0.00 | 0.00 | 0.01 | 0.01 | 0.10 |
| ENSBTAG00000005104 | 0.09 | 1.25 | 0.28 | 0.30 | 0.97 | 1.22 | 0.83 | 0.16 |
| ENSBTAG00000005108 | -0.28 | -0.02 | -0.06 | -0.01 | -0.02 | -0.03 | -0.03 | 1.52 |
| ENSBTAG00000005246 | 0.05 | 0.06 | 0.01 | -1.22 | -1.52 | -0.39 | -0.98 | -0.26 |
| ENSBTAG00000005248 | 0.33 | 1.67 | 1.70 | -0.01 | -0.03 | 0.00 | -0.02 | -0.02 |
| ENSBTAG00000005321 | 0.26 | 1.67 | 0.30 | 0.05 | 0.04 | 0.25 | 0.07 | 0.36 |
| ENSBTAG00000005328 | -1.07 | -2.06 | -0.10 | 0.01 | 0.01 | 0.00 | 0.00 | -0.08 |
| ENSBTAG00000005349 | 0.00 | -0.03 | -0.03 | -0.15 | -2.23 | -0.35 | -1.25 | -0.15 |
| ENSBTAG00000005372 | -0.69 | -1.12 | -0.03 | -0.02 | -0.02 | 0.00 | -0.02 | 0.00 |
| ENSBTAG00000005481 | 0.07 | 0.03 | 2.47 | 1.08 | 0.75 | 0.15 | 0.39 | 0.34 |
| ENSBTAG00000005514 | 0.04 | 0.00 | -0.02 | -0.02 | -0.02 | -0.01 | -0.02 | -1.51 |
| ENSBTAG00000005533 | -0.38 | -0.12 | -0.36 | -0.72 | -5.64 | -0.78 | -2.13 | -0.02 |
| ENSBTAG00000005562 | -0.06 | -0.02 | -0.19 | -0.11 | -0.38 | -1.03 | -0.64 | -0.06 |
| ENSBTAG00000005633 | 0.94 | 0.03 | 0.01 | -0.07 | -0.60 | -1.05 | -0.45 | -0.08 |
| ENSBTAG00000005682 | -0.08 | -0.20 | -0.85 | -0.20 | -0.51 | -1.01 | -1.08 | 0.01 |
| ENSBTAG00000005738 | -0.42 | -0.21 | -1.57 | -0.01 | 0.01 | 0.02 | 0.03 | 1.25 |
| ENSBTAG00000005803 | -1.02 | -0.06 | -0.37 | -0.02 | -0.04 | -0.04 | -0.05 | -0.03 |
| ENSBTAG00000005810 | 0.06 | 0.11 | 0.03 | 2.51 | 0.80 | 0.05 | 0.17 | -0.12 |
| ENSBTAG00000005824 | -0.09 | -1.53 | 0.01 | -0.01 | -0.05 | -0.01 | -0.04 | 0.03 |
| ENSBTAG00000005827 | -0.08 | 0.01 | 0.13 | 0.19 | 0.43 | 1.27 | 0.55 | 0.14 |
| ENSBTAG00000005888 | -0.01 | -0.05 | 0.00 | -0.01 | -0.08 | -0.01 | -0.03 | -1.22 |
| ENSBTAG00000005913 | 1.92 | 0.66 | 3.74 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 |
| ENSBTAG00000005923 | 1.39 | 0.28 | 0.69 | 0.03 | 0.06 | -0.02 | 0.00 | 0.01 |
| ENSBTAG00000005973 | 2.03 | 0.16 | 0.19 | 0.00 | -0.01 | -0.01 | -0.01 | -0.08 |
| ENSBTAG00000006086 | 0.01 | -0.04 | -0.07 | -0.07 | -0.16 | -1.38 | -0.27 | -0.04 |
| ENSBTAG00000006129 | -0.03 | -0.02 | -0.08 | -0.36 | -1.25 | -0.14 | -0.60 | -0.01 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000006132 | 2.06 | 0.38 | 0.01 | 0.10 | 0.14 | 0.10 | 0.09 | 0.00 |
| ENSBTAG00000006188 | 1.21 | 0.42 | 0.03 | -0.07 | -0.06 | -0.02 | -0.04 | -0.03 |
| ENSBTAG00000006280 | -0.07 | -0.08 | -0.03 | -3.44 | -0.84 | -0.28 | -0.36 | 0.08 |
| ENSBTAG00000006419 | 0.11 | 2.00 | 0.06 | 0.10 | 0.09 | -0.01 | 0.03 | 0.09 |
| ENSBTAG00000006440 | -0.01 | 0.03 | -0.02 | -2.87 | -0.42 | -0.12 | -0.26 | 0.00 |
| ENSBTAG00000006466 | 0.01 | -0.05 | -0.14 | -6.13 | -1.88 | -0.34 | -0.38 | -0.05 |
| ENSBTAG00000006490 | -0.24 | -0.87 | -1.02 | -0.05 | -0.04 | 0.02 | -0.01 | 0.03 |
| ENSBTAG00000006618 | 0.27 | 0.16 | 0.07 | 0.12 | 0.06 | 0.24 | 0.17 | -0.92 |
| ENSBTAG00000006665 | 0.00 | 0.02 | -0.02 | 0.01 | 0.02 | 0.00 | 0.00 | 2.23 |
| ENSBTAG00000006712 | -1.74 | -0.22 | -0.05 | -0.02 | -0.02 | -0.03 | -0.03 | 0.27 |
| ENSBTAG00000006732 | 0.09 | 0.04 | 0.03 | 2.75 | 1.30 | 0.24 | 0.43 | 0.00 |
| ENSBTAG00000006747 | -0.05 | -0.15 | -0.03 | -0.58 | -16.58 | -2.63 | -4.17 | -0.53 |
| ENSBTAG00000006810 | 1.03 | 0.03 | 0.04 | -0.02 | -0.01 | -0.01 | -0.01 | -0.27 |
| ENSBTAG00000006947 | -0.04 | 0.05 | -0.03 | -0.02 | -0.05 | 0.00 | -0.03 | -1.49 |
| ENSBTAG00000007007 | -0.17 | -1.24 | -0.14 | 0.00 | 0.01 | 0.22 | 0.07 | -0.04 |
| ENSBTAG00000007013 | 5.10 | 4.74 | 35.56 | 0.21 | 0.20 | 0.17 | 0.12 | -0.07 |
| ENSBTAG00000007122 | 0.02 | -0.02 | 0.03 | 0.04 | 0.04 | 0.00 | 0.02 | 1.08 |
| ENSBTAG00000007141 | 0.05 | -0.01 | 0.02 | -0.01 | 0.00 | 0.02 | 0.01 | -1.27 |
| ENSBTAG00000007244 | 0.07 | -0.02 | -0.02 | -0.27 | -0.36 | -0.82 | -0.44 | -0.04 |
| ENSBTAG00000007305 | 0.64 | 0.91 | 0.51 | -0.01 | 0.08 | 0.03 | 0.08 | 0.96 |
| ENSBTAG00000007386 | 0.07 | 0.05 | 0.04 | 0.31 | 1.28 | 0.08 | 0.24 | -0.03 |
| ENSBTAG00000007473 | -2.64 | -0.16 | -0.57 | 0.01 | 0.04 | 0.03 | 0.04 | -0.04 |
| ENSBTAG00000007634 | 0.17 | 0.05 | 0.16 | 1.15 | 0.13 | 0.04 | 0.09 | -0.39 |
| ENSBTAG00000007635 | 0.32 | 0.11 | 0.17 | -2.10 | -0.27 | -0.07 | -0.16 | 0.02 |
| ENSBTAG00000007709 | 0.03 | -0.04 | 0.01 | -0.09 | -1.20 | -2.35 | -1.28 | 0.14 |
| ENSBTAG00000007732 | -3.14 | -0.28 | -0.19 | 0.00 | 0.00 | 0.01 | 0.00 | 0.22 |
| ENSBTAG00000007823 | -0.43 | -0.14 | -0.05 | -0.03 | -0.07 | -0.09 | -0.11 | -1.24 |
| ENSBTAG00000007867 | -1.03 | -0.44 | -0.28 | 0.03 | 0.06 | 0.00 | 0.01 | -0.06 |
| ENSBTAG00000007901 | 0.05 | 0.05 | -0.06 | -0.21 | -2.02 | -0.19 | -0.36 | -0.06 |
| ENSBTAG00000007962 | 1.43 | 0.09 | 0.21 | 0.01 | 0.02 | 0.03 | 0.05 | 0.01 |
| ENSBTAG00000008040 | -2.05 | -2.63 | -3.11 | -0.58 | -0.15 | -0.07 | -0.14 | -0.04 |
| ENSBTAG00000008093 | -0.02 | -0.05 | -0.03 | -8.92 | -3.53 | -0.42 | -0.81 | -0.15 |
| ENSBTAG00000008098 | 0.04 | 0.00 | 0.08 | 1.87 | 1.41 | 0.52 | 0.75 | 0.52 |
| ENSBTAG00000008125 | 0.02 | 0.04 | 0.15 | 0.19 | 1.24 | 0.16 | 0.69 | 0.12 |
| ENSBTAG00000008275 | -1.07 | -0.64 | -0.20 | 0.02 | 0.05 | 0.02 | 0.02 | 0.08 |
| ENSBTAG00000008338 | 0.08 | 0.07 | 0.01 | -0.80 | -1.71 | -0.96 | -1.14 | -0.39 |
| ENSBTAG00000008339 | -0.03 | -0.04 | 0.00 | -0.03 | -0.09 | -0.09 | -0.11 | -1.84 |
| ENSBTAG00000008389 | 0.13 | 0.00 | 0.23 | 0.04 | 0.03 | -0.01 | 0.00 | 2.38 |
| ENSBTAG00000008401 | 0.00 | -0.04 | 0.02 | 0.76 | 1.45 | 0.13 | 0.55 | 0.31 |
| ENSBTAG00000008420 | 2.60 | 11.73 | 3.21 | 0.06 | 0.19 | 0.32 | 0.21 | 0.00 |
| ENSBTAG00000008438 | 0.03 | -0.03 | -0.06 | -0.01 | -0.04 | -0.04 | -0.05 | -4.14 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000008442 | 0.02 | 0.01 | 0.03 | 1.64 | 1.39 | 0.35 | 0.58 | 0.70 |
| ENSBTAG00000008468 | -0.39 | -13.85 | -0.51 | -0.09 | 0.00 | -0.01 | 0.01 | 0.15 |
| ENSBTAG00000008470 | 0.13 | 0.94 | 0.20 | 0.02 | 0.01 | 0.01 | 0.02 | -0.06 |
| ENSBTAG00000008484 | 0.00 | -0.02 | -0.01 | -0.29 | -2.37 | -0.08 | -0.32 | -0.19 |
| ENSBTAG00000008509 | -1.60 | -0.25 | -0.03 | -0.01 | 0.01 | -0.02 | 0.01 | -0.02 |
| ENSBTAG00000008595 | 0.03 | 0.07 | 0.02 | 0.52 | 0.56 | 1.77 | 0.60 | 0.11 |
| ENSBTAG00000008605 | -0.84 | -1.43 | -1.15 | 0.01 | 0.00 | 0.01 | 0.00 | 0.00 |
| ENSBTAG00000008636 | 0.05 | 0.34 | 0.00 | 0.99 | 0.07 | 0.02 | 0.04 | -0.08 |
| ENSBTAG00000008687 | -0.01 | -0.02 | -0.04 | 0.01 | -0.01 | -0.01 | -0.02 | -2.80 |
| ENSBTAG00000008696 | -0.11 | -1.06 | -0.17 | -0.01 | -0.02 | -0.03 | -0.02 | 0.09 |
| ENSBTAG00000008708 | 1.44 | 0.39 | 0.42 | 0.02 | 0.01 | 0.03 | 0.03 | -0.25 |
| ENSBTAG00000008710 | -0.95 | -21.08 | -0.19 | -0.05 | -0.08 | -0.01 | -0.05 | -0.04 |
| ENSBTAG00000008718 | -3.07 | -0.06 | -0.02 | -0.03 | -0.02 | 0.02 | -0.01 | 0.03 |
| ENSBTAG00000008732 | -6.36 | -0.48 | -0.14 | 0.00 | 0.06 | 0.05 | 0.05 | 0.00 |
| ENSBTAG00000008783 | 1.14 | 0.22 | 0.08 | -0.01 | -0.03 | -0.05 | -0.04 | 0.01 |
| ENSBTAG00000008828 | 0.62 | 1.64 | 0.04 | 0.04 | 0.04 | 0.01 | 0.03 | 0.01 |
| ENSBTAG00000008840 | 0.02 | -0.02 | 0.00 | -0.01 | -0.01 | 0.00 | -0.01 | 3.78 |
| ENSBTAG00000008868 | 2.36 | 0.02 | 0.02 | -0.10 | -0.06 | 0.00 | -0.01 | 0.23 |
| ENSBTAG00000008888 | 1.23 | 4.19 | 0.86 | 0.08 | 0.04 | 0.00 | 0.02 | -0.01 |
| ENSBTAG00000008966 | 3.89 | 0.03 | 0.04 | 0.02 | 0.02 | -0.02 | 0.01 | 0.04 |
| ENSBTAG00000009076 | 0.01 | -0.01 | -0.01 | -0.88 | -0.30 | -0.16 | -0.23 | -0.05 |
| ENSBTAG00000009155 | 0.02 | 0.03 | 0.03 | 0.06 | 0.04 | 0.02 | 0.04 | -5.94 |
| ENSBTAG00000009182 | -0.03 | -0.01 | 0.04 | 3.68 | 0.30 | 0.05 | 0.07 | 0.04 |
| ENSBTAG00000009258 | 5.94 | 1.09 | 1.69 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 |
| ENSBTAG00000009306 | -3.61 | -7.11 | -0.34 | -0.01 | 0.00 | -0.04 | -0.01 | 0.37 |
| ENSBTAG00000009331 | -1.24 | -0.33 | -0.11 | 0.00 | -0.01 | -0.05 | -0.04 | -0.03 |
| ENSBTAG00000009362 | -0.05 | -0.03 | 0.00 | 0.04 | 0.00 | 0.01 | 0.00 | -0.96 |
| ENSBTAG00000009371 | 0.00 | -0.06 | -0.15 | -0.95 | -0.20 | -0.03 | -0.06 | 0.05 |
| ENSBTAG00000009394 | 0.37 | 1.49 | 1.33 | 0.09 | 0.28 | 0.15 | 0.25 | 0.12 |
| ENSBTAG00000009441 | 0.08 | -0.01 | 0.06 | 3.52 | 1.77 | 0.18 | 0.59 | -0.07 |
| ENSBTAG00000009446 | 0.96 | 0.05 | 0.03 | 0.00 | -0.02 | -0.09 | -0.06 | 0.11 |
| ENSBTAG00000009475 | -0.14 | -0.04 | -0.10 | -1.20 | -0.48 | -0.13 | -0.32 | -0.02 |
| ENSBTAG00000009481 | 0.01 | 0.06 | 0.01 | 0.94 | 1.50 | 0.21 | 0.66 | 0.02 |
| ENSBTAG00000009523 | 0.37 | 5.10 | 0.39 | 0.02 | 0.01 | 0.00 | 0.00 | -0.04 |
| ENSBTAG00000009575 | -2.45 | -1.68 | -0.15 | 0.07 | 0.06 | 0.00 | 0.02 | -0.20 |
| ENSBTAG00000009578 | 0.03 | 0.01 | 0.02 | 0.01 | -0.01 | -0.02 | -0.01 | -1.32 |
| ENSBTAG00000009622 | -0.01 | -0.07 | -0.03 | -0.92 | -0.50 | -0.51 | -0.45 | -0.11 |
| ENSBTAG00000009665 | -0.09 | -0.06 | -0.01 | 0.08 | 0.08 | 0.01 | 0.03 | -0.91 |
| ENSBTAG00000009760 | 0.07 | 0.03 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | -1.31 |
| ENSBTAG00000009777 | -1.36 | -0.13 | -8.34 | -0.05 | -0.11 | -0.09 | -0.12 | 0.06 |
| ENSBTAG00000009834 | 2.00 | 0.66 | 1.23 | -0.01 | 0.01 | -0.01 | 0.01 | -0.02 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000009835 | -0.07 | -0.09 | -1.03 | -2.32 | -0.39 | -0.36 | -0.31 | -0.05 |
| ENSBTAG00000009845 | -0.01 | -0.01 | -0.06 | -3.90 | -1.86 | -0.39 | -0.46 | 0.28 |
| ENSBTAG00000009903 | -0.12 | -5.97 | -0.02 | -0.08 | -0.11 | -0.16 | -0.15 | -0.08 |
| ENSBTAG00000009942 | -14.14 | -0.10 | -0.07 | 0.01 | 0.02 | 0.01 | 0.02 | -0.02 |
| ENSBTAG00000010030 | -2.79 | -0.10 | -1.35 | -0.01 | -0.01 | 0.00 | 0.00 | 0.28 |
| ENSBTAG00000010112 | 0.06 | 0.01 | 0.08 | 0.14 | 0.16 | 0.45 | 0.26 | -2.06 |
| ENSBTAG00000010126 | -0.01 | 0.06 | 0.00 | 0.12 | 0.91 | 0.33 | 0.40 | 0.00 |
| ENSBTAG00000010241 | -0.04 | -0.06 | 0.00 | -0.01 | 0.00 | -0.01 | 0.01 | 4.10 |
| ENSBTAG00000010244 | 0.00 | 0.00 | 0.02 | 1.43 | 0.29 | 0.16 | 0.21 | -0.02 |
| ENSBTAG00000010300 | 2.96 | 3.83 | 0.77 | 0.00 | 0.06 | 0.06 | 0.06 | 0.17 |
| ENSBTAG00000010343 | 2.81 | 0.53 | 0.45 | 0.06 | 0.26 | 0.14 | 0.13 | 0.13 |
| ENSBTAG00000010379 | -0.08 | -0.07 | -0.01 | -0.01 | 0.06 | 0.02 | 0.04 | 1.86 |
| ENSBTAG00000010380 | -4.94 | -0.42 | -0.59 | -0.05 | -0.07 | -0.02 | -0.05 | 0.04 |
| ENSBTAG00000010392 | -0.09 | -0.04 | 0.00 | 0.09 | 1.08 | 0.27 | 0.32 | 0.06 |
| ENSBTAG00000010571 | -1.12 | -3.76 | -0.06 | -0.05 | -0.01 | -0.02 | -0.01 | -0.02 |
| ENSBTAG00000010613 | 0.14 | 0.01 | 0.07 | 0.82 | 0.08 | 0.02 | 0.04 | -1.96 |
| ENSBTAG00000010661 | 0.10 | 0.55 | 6.98 | 0.12 | 0.16 | 0.11 | 0.19 | 8.81 |
| ENSBTAG00000010672 | -5.20 | -0.12 | -0.10 | -0.02 | 0.03 | 0.04 | 0.03 | -0.01 |
| ENSBTAG00000010689 | -0.47 | -1.31 | -0.05 | -0.06 | -0.02 | -0.04 | -0.03 | 0.23 |
| ENSBTAG00000010786 | -0.01 | -0.01 | -0.01 | 0.05 | 0.00 | -0.01 | -0.01 | -1.62 |
| ENSBTAG00000010837 | 0.08 | 0.10 | 0.19 | 0.87 | 0.06 | 0.04 | 0.05 | 0.33 |
| ENSBTAG00000010852 | -0.04 | -0.04 | -0.01 | -1.41 | -0.63 | -0.24 | -0.72 | 0.06 |
| ENSBTAG00000010865 | -3.93 | -0.36 | -0.53 | -0.16 | -0.12 | -0.05 | -0.07 | 0.02 |
| ENSBTAG00000010878 | -0.28 | -0.06 | -0.90 | -1.03 | -2.82 | -0.18 | -0.69 | -0.60 |
| ENSBTAG00000010944 | -0.05 | -0.01 | -0.12 | -0.28 | -2.19 | -0.21 | -0.64 | -0.04 |
| ENSBTAG00000011001 | 4.22 | 0.72 | 0.69 | 0.00 | -0.03 | -0.01 | -0.02 | 0.15 |
| ENSBTAG00000011032 | 0.02 | 0.32 | 0.04 | 0.10 | 0.56 | 0.94 | 0.47 | 0.00 |
| ENSBTAG00000011075 | 0.01 | 0.00 | 0.00 | 0.01 | 0.04 | 0.04 | 0.07 | 1.33 |
| ENSBTAG00000011076 | -0.01 | -0.04 | -0.16 | -1.20 | -4.35 | -0.21 | -0.83 | -0.10 |
| ENSBTAG00000011091 | 0.30 | 0.07 | 0.38 | 11.83 | 3.07 | 0.17 | 0.63 | 0.01 |
| ENSBTAG00000011217 | 0.27 | 0.04 | 0.00 | -6.92 | -3.28 | -8.23 | -4.38 | -0.02 |
| ENSBTAG00000011237 | 1.17 | 0.09 | 3.21 | 2.09 | 3.43 | 3.61 | 3.54 | 0.21 |
| ENSBTAG00000011267 | 1.03 | 15.02 | 2.41 | -0.03 | -0.04 | -0.05 | -0.04 | 0.00 |
| ENSBTAG00000011358 | 1.07 | 0.05 | 0.08 | 0.00 | -0.01 | 0.01 | 0.02 | 0.03 |
| ENSBTAG00000011367 | 0.23 | 0.13 | 0.09 | -0.32 | -0.03 | 0.00 | 0.00 | 1.00 |
| ENSBTAG00000011403 | -2.97 | -1.00 | -1.53 | -0.11 | -0.26 | -0.09 | -0.18 | -0.10 |
| ENSBTAG00000011431 | 1.72 | 0.41 | 1.69 | 0.01 | 0.02 | 0.01 | 0.03 | 0.09 |
| ENSBTAG00000011524 | -0.12 | 0.00 | 0.03 | -0.01 | 0.02 | 0.01 | 0.00 | 1.21 |
| ENSBTAG00000011527 | 0.16 | 1.74 | 0.21 | 0.16 | 0.74 | 0.08 | 0.15 | 0.05 |
| ENSBTAG00000011538 | 0.06 | 0.00 | 0.04 | -0.06 | -0.04 | -0.05 | -0.04 | 1.92 |
| ENSBTAG00000011582 | 2.56 | 0.27 | 0.17 | 0.00 | -0.01 | -0.02 | -0.01 | 0.02 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000011660 | -0.17 | -0.03 | -0.07 | 0.05 | 0.02 | 0.04 | 0.06 | -1.13 |
| ENSBTAG00000011693 | -6.42 | -0.80 | -2.59 | -0.23 | -0.55 | -0.21 | -0.26 | -0.21 |
| ENSBTAG00000011733 | -2.84 | -0.25 | -1.23 | -0.01 | 0.01 | 0.05 | 0.02 | -0.02 |
| ENSBTAG00000011741 | 1.20 | 1.96 | 1.36 | 0.00 | 0.01 | 0.01 | 0.00 | 0.04 |
| ENSBTAG00000011757 | 0.00 | 0.00 | 0.01 | 0.10 | 0.18 | 0.11 | 0.12 | -4.59 |
| ENSBTAG00000011766 | 2.01 | 3.72 | 0.65 | 0.02 | -0.02 | -0.06 | -0.06 | 0.03 |
| ENSBTAG00000011772 | -0.12 | -1.93 | -0.27 | -0.02 | 0.00 | 0.00 | 0.01 | 0.16 |
| ENSBTAG00000011804 | -0.02 | -0.04 | -0.06 | -1.38 | -0.34 | -0.04 | -0.16 | -0.04 |
| ENSBTAG00000011829 | 0.01 | -0.07 | -0.04 | -9.63 | -0.76 | -0.10 | -0.28 | -0.04 |
| ENSBTAG00000011833 | 0.02 | -0.03 | 0.11 | 3.43 | 1.00 | 2.22 | 2.82 | -0.01 |
| ENSBTAG00000011865 | -0.54 | -0.79 | -0.89 | -1.22 | -0.20 | -0.17 | -0.18 | -0.27 |
| ENSBTAG00000011899 | 0.09 | 1.33 | 0.63 | 0.32 | 0.18 | 0.01 | 0.05 | 0.04 |
| ENSBTAG00000011945 | 0.36 | 1.21 | 0.02 | 0.02 | 0.03 | 0.05 | 0.03 | -0.15 |
| ENSBTAG00000012039 | -0.08 | -0.01 | 0.03 | 0.06 | 0.53 | 1.73 | 0.49 | 0.06 |
| ENSBTAG00000012077 | -1.37 | -2.77 | -0.13 | 0.01 | 0.01 | 0.03 | 0.01 | -0.01 |
| ENSBTAG00000012111 | -1.35 | -0.49 | -0.11 | 0.01 | 0.04 | 0.05 | 0.06 | -0.01 |
| ENSBTAG00000012222 | 0.05 | 0.01 | 0.05 | 0.32 | 0.31 | 0.08 | 0.11 | -2.58 |
| ENSBTAG00000012305 | -2.42 | -0.60 | -4.26 | -0.09 | -0.10 | -0.01 | -0.02 | 0.07 |
| ENSBTAG00000012307 | -0.98 | -0.14 | -0.08 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 |
| ENSBTAG00000012475 | 1.43 | 1.76 | 1.45 | -0.02 | 0.01 | -0.01 | 0.00 | 0.01 |
| ENSBTAG00000012500 | -0.03 | -0.04 | -0.11 | -2.30 | -1.24 | -0.40 | -0.60 | -0.04 |
| ENSBTAG00000012558 | -0.35 | -0.29 | -0.04 | 0.02 | 0.03 | 0.04 | 0.03 | -1.73 |
| ENSBTAG00000012582 | 0.29 | 0.01 | 0.01 | -0.62 | -1.29 | -0.40 | -0.54 | -0.01 |
| ENSBTAG00000012585 | 0.17 | 0.01 | -0.01 | -0.83 | -0.46 | -0.10 | -0.17 | -0.04 |
| ENSBTAG00000012615 | -0.13 | -0.23 | -0.20 | -0.27 | -1.97 | -0.77 | -0.71 | 0.05 |
| ENSBTAG00000012632 | 0.25 | 0.04 | 0.12 | 0.01 | 0.04 | 0.07 | 0.05 | 0.94 |
| ENSBTAG00000012693 | -1.57 | -0.04 | -0.02 | -0.23 | -0.06 | -0.21 | -0.11 | 0.07 |
| ENSBTAG00000012700 | -0.24 | -0.08 | -0.03 | 0.02 | 0.00 | 0.01 | 0.00 | 1.22 |
| ENSBTAG00000012738 | 0.07 | 0.05 | 0.01 | -0.05 | -0.07 | -0.04 | -0.06 | 2.54 |
| ENSBTAG00000012800 | -0.01 | -0.50 | -0.07 | -1.11 | -0.53 | -0.04 | -0.14 | 0.00 |
| ENSBTAG00000012981 | 0.45 | 0.10 | 2.40 | 0.36 | 7.08 | 2.93 | 5.68 | 0.24 |
| ENSBTAG00000012988 | -0.17 | -0.12 | -0.02 | -1.05 | -0.33 | -0.04 | -0.07 | 0.36 |
| ENSBTAG00000013047 | -0.11 | -0.14 | -0.38 | -8.12 | -7.59 | -8.50 | -5.29 | -0.37 |
| ENSBTAG00000013048 | 0.03 | -0.01 | 0.11 | -0.01 | 0.01 | 0.00 | 0.02 | 1.46 |
| ENSBTAG00000013099 | -0.01 | -0.02 | 0.00 | 0.00 | 0.01 | 0.03 | 0.03 | -2.20 |
| ENSBTAG00000013100 | 0.00 | 0.01 | 0.00 | 0.00 | -0.01 | -0.06 | -0.03 | 2.55 |
| ENSBTAG00000013116 | -0.05 | -0.01 | -0.02 | -0.07 | -0.09 | -0.13 | -0.10 | -6.48 |
| ENSBTAG00000013117 | -0.12 | -0.03 | -0.02 | -0.24 | -0.12 | -0.06 | -0.07 | 14.06 |
| ENSBTAG00000013153 | 0.01 | -0.01 | 0.01 | 1.58 | 0.32 | 0.11 | 0.17 | 0.15 |
| ENSBTAG00000013203 | -0.01 | 0.00 | 0.01 | 4.51 | 0.10 | 0.06 | 0.10 | 0.09 |
| ENSBTAG00000013221 | -0.26 | -0.45 | -3.05 | -0.16 | -0.54 | -1.11 | -0.44 | -0.40 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000013226 | -0.03 | -0.01 | -0.08 | -2.05 | -1.82 | -0.18 | -0.39 | -0.13 |
| ENSBTAG00000013281 | 0.00 | 0.00 | 0.03 | 0.01 | 0.04 | 0.02 | 0.03 | 1.45 |
| ENSBTAG00000013407 | -0.29 | -0.91 | -0.46 | -1.09 | -0.13 | -0.02 | -0.04 | 0.07 |
| ENSBTAG00000013495 | 0.02 | -0.03 | -0.03 | -0.06 | -0.07 | -0.03 | -0.05 | -1.22 |
| ENSBTAG00000013551 | 0.06 | 0.05 | 0.05 | 0.06 | 0.01 | 0.00 | 0.02 | -1.33 |
| ENSBTAG00000013578 | -1.36 | -0.96 | -0.20 | -0.04 | -0.01 | -0.02 | -0.02 | 0.14 |
| ENSBTAG00000013629 | -1.11 | -0.25 | -0.17 | 0.03 | 0.03 | 0.02 | 0.02 | 0.38 |
| ENSBTAG00000013632 | 0.01 | 0.05 | 0.11 | 0.00 | 0.03 | 0.02 | 0.03 | 1.06 |
| ENSBTAG00000013730 | -0.06 | -0.02 | -0.05 | 0.36 | 0.18 | 1.27 | 0.47 | 0.11 |
| ENSBTAG00000013792 | -0.27 | -1.48 | -3.47 | -0.07 | -0.06 | -0.11 | -0.08 | -0.13 |
| ENSBTAG00000013856 | -0.08 | -0.04 | 0.02 | -0.19 | -0.24 | -1.00 | -0.77 | -0.01 |
| ENSBTAG00000013861 | 0.03 | -0.02 | 0.09 | 2.25 | 1.11 | 0.24 | 0.37 | -0.02 |
| ENSBTAG00000013869 | -9.66 | -0.30 | -0.69 | 0.04 | 0.10 | 0.34 | 0.12 | -0.01 |
| ENSBTAG00000013912 | -0.03 | -0.03 | -1.10 | -0.03 | -0.08 | -0.94 | -0.19 | 0.28 |
| ENSBTAG00000013935 | -0.01 | -0.02 | -0.49 | -0.56 | -2.24 | -0.25 | -0.39 | -0.24 |
| ENSBTAG00000014058 | -0.38 | -0.06 | -0.01 | -0.09 | -0.22 | -0.09 | -0.13 | -1.88 |
| ENSBTAG00000014063 | -0.08 | -1.38 | -1.00 | -0.11 | -0.13 | -0.04 | -0.09 | 0.10 |
| ENSBTAG00000014092 | 0.44 | 0.12 | 2.56 | 0.36 | 6.86 | 2.71 | 5.44 | 0.20 |
| ENSBTAG00000014124 | 0.77 | 0.44 | 0.22 | 2.06 | 1.52 | 0.45 | 0.64 | -0.04 |
| ENSBTAG00000014132 | -2.11 | -1.53 | -1.89 | -0.54 | -0.16 | -0.09 | -0.13 | -0.04 |
| ENSBTAG00000014225 | -0.03 | -0.01 | 0.00 | 0.06 | 0.01 | 0.04 | 0.04 | -1.35 |
| ENSBTAG00000014227 | -0.21 | -0.22 | -0.40 | -0.74 | -0.51 | -0.07 | -0.18 | -1.78 |
| ENSBTAG00000014284 | 0.06 | 0.57 | 0.98 | 0.13 | 0.05 | 0.07 | 0.04 | -1.26 |
| ENSBTAG00000014289 | 0.29 | 1.03 | 0.05 | 0.00 | 0.04 | 0.10 | 0.07 | -0.01 |
| ENSBTAG00000014295 | -26.84 | -1.33 | -5.82 | -0.56 | -0.38 | -0.14 | -0.33 | -0.04 |
| ENSBTAG00000014304 | -6.73 | -2.88 | -3.35 | -0.12 | -0.18 | -0.23 | -0.19 | -0.16 |
| ENSBTAG00000014306 | -0.21 | -0.03 | -0.11 | -0.02 | -0.05 | -0.13 | -0.10 | -1.59 |
| ENSBTAG00000014376 | 1.78 | 0.38 | 0.29 | 0.02 | 0.01 | 0.01 | 0.01 | -0.16 |
| ENSBTAG00000014418 | 1.73 | 5.17 | 0.15 | -0.08 | -0.25 | -0.18 | -0.18 | -0.14 |
| ENSBTAG00000014463 | -2.37 | -1.60 | -11.42 | -0.06 | -0.02 | 0.00 | 0.00 | 0.08 |
| ENSBTAG00000014476 | -0.17 | -1.35 | -0.18 | -0.22 | -0.09 | -0.10 | -0.08 | 0.22 |
| ENSBTAG00000014482 | -0.20 | -0.35 | -0.04 | -0.05 | -0.21 | -4.77 | -0.81 | 0.05 |
| ENSBTAG00000014495 | -0.15 | -0.03 | -0.05 | -1.14 | -0.48 | -1.38 | -0.35 | 0.03 |
| ENSBTAG00000014543 | 0.33 | 0.21 | 0.35 | 1.85 | 0.20 | 0.01 | 0.04 | -0.55 |
| ENSBTAG00000014551 | 0.10 | 0.04 | 0.01 | 0.00 | -0.02 | -0.03 | -0.05 | 1.26 |
| ENSBTAG00000014575 | -0.07 | -0.03 | 0.00 | 1.11 | 0.08 | 0.10 | 0.12 | -0.16 |
| ENSBTAG00000013117 | -0.12 | -0.03 | -0.02 | -0.24 | -0.12 | -0.06 | -0.07 | 14.06 |
| ENSBTAG00000013153 | 0.01 | -0.01 | 0.01 | 1.58 | 0.32 | 0.11 | 0.17 | 0.15 |
| ENSBTAG00000013203 | -0.01 | 0.00 | 0.01 | 4.51 | 0.10 | 0.06 | 0.10 | 0.09 |
| ENSBTAG00000013221 | -0.26 | -0.45 | -3.05 | -0.16 | -0.54 | -1.11 | -0.44 | -0.40 |
| ENSBTAG00000013226 | -0.03 | -0.01 | -0.08 | -2.05 | -1.82 | -0.18 | -0.39 | -0.13 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000013281 | 0.00 | 0.00 | 0.03 | 0.01 | 0.04 | 0.02 | 0.03 | 1.45 |
| ENSBTAG00000013407 | -0.29 | -0.91 | -0.46 | -1.09 | -0.13 | -0.02 | -0.04 | 0.07 |
| ENSBTAG00000013495 | 0.02 | -0.03 | -0.03 | -0.06 | -0.07 | -0.03 | -0.05 | -1.22 |
| ENSBTAG00000013551 | 0.06 | 0.05 | 0.05 | 0.06 | 0.01 | 0.00 | 0.02 | -1.33 |
| ENSBTAG00000013578 | -1.36 | -0.96 | -0.20 | -0.04 | -0.01 | -0.02 | -0.02 | 0.14 |
| ENSBTAG00000013629 | -1.11 | -0.25 | -0.17 | 0.03 | 0.03 | 0.02 | 0.02 | 0.38 |
| ENSBTAG00000013632 | 0.01 | 0.05 | 0.11 | 0.00 | 0.03 | 0.02 | 0.03 | 1.06 |
| ENSBTAG00000013730 | -0.06 | -0.02 | -0.05 | 0.36 | 0.18 | 1.27 | 0.47 | 0.11 |
| ENSBTAG00000013792 | -0.27 | -1.48 | -3.47 | -0.07 | -0.06 | -0.11 | -0.08 | -0.13 |
| ENSBTAG00000013856 | -0.08 | -0.04 | 0.02 | -0.19 | -0.24 | -1.00 | -0.77 | -0.01 |
| ENSBTAG00000013861 | 0.03 | -0.02 | 0.09 | 2.25 | 1.11 | 0.24 | 0.37 | -0.02 |
| ENSBTAG00000013869 | -9.66 | -0.30 | -0.69 | 0.04 | 0.10 | 0.34 | 0.12 | -0.01 |
| ENSBTAG00000013912 | -0.03 | -0.03 | -1.10 | -0.03 | -0.08 | -0.94 | -0.19 | 0.28 |
| ENSBTAG00000013935 | -0.01 | -0.02 | -0.49 | -0.56 | -2.24 | -0.25 | -0.39 | -0.24 |
| ENSBTAG00000014058 | -0.38 | -0.06 | -0.01 | -0.09 | -0.22 | -0.09 | -0.13 | -1.88 |
| ENSBTAG00000014063 | -0.08 | -1.38 | -1.00 | -0.11 | -0.13 | -0.04 | -0.09 | 0.10 |
| ENSBTAG00000014092 | 0.44 | 0.12 | 2.56 | 0.36 | 6.86 | 2.71 | 5.44 | 0.20 |
| ENSBTAG00000014124 | 0.77 | 0.44 | 0.22 | 2.06 | 1.52 | 0.45 | 0.64 | -0.04 |
| ENSBTAG00000014132 | -2.11 | -1.53 | -1.89 | -0.54 | -0.16 | -0.09 | -0.13 | -0.04 |
| ENSBTAG00000014225 | -0.03 | -0.01 | 0.00 | 0.06 | 0.01 | 0.04 | 0.04 | -1.35 |
| ENSBTAG00000014227 | -0.21 | -0.22 | -0.40 | -0.74 | -0.51 | -0.07 | -0.18 | -1.78 |
| ENSBTAG00000014284 | 0.06 | 0.57 | 0.98 | 0.13 | 0.05 | 0.07 | 0.04 | -1.26 |
| ENSBTAG00000014289 | 0.29 | 1.03 | 0.05 | 0.00 | 0.04 | 0.10 | 0.07 | -0.01 |
| ENSBTAG00000014295 | -26.84 | -1.33 | -5.82 | -0.56 | -0.38 | -0.14 | -0.33 | -0.04 |
| ENSBTAG00000014304 | -6.73 | -2.88 | -3.35 | -0.12 | -0.18 | -0.23 | -0.19 | -0.16 |
| ENSBTAG00000014306 | -0.21 | -0.03 | -0.11 | -0.02 | -0.05 | -0.13 | -0.10 | -1.59 |
| ENSBTAG00000014376 | 1.78 | 0.38 | 0.29 | 0.02 | 0.01 | 0.01 | 0.01 | -0.16 |
| ENSBTAG00000014418 | 1.73 | 5.17 | 0.15 | -0.08 | -0.25 | -0.18 | -0.18 | -0.14 |
| ENSBTAG00000014463 | -2.37 | -1.60 | -11.42 | -0.06 | -0.02 | 0.00 | 0.00 | 0.08 |
| ENSBTAG00000014476 | -0.17 | -1.35 | -0.18 | -0.22 | -0.09 | -0.10 | -0.08 | 0.22 |
| ENSBTAG00000014482 | -0.20 | -0.35 | -0.04 | -0.05 | -0.21 | -4.77 | -0.81 | 0.05 |
| ENSBTAG00000014495 | -0.15 | -0.03 | -0.05 | -1.14 | -0.48 | -1.38 | -0.35 | 0.03 |
| ENSBTAG00000014543 | 0.33 | 0.21 | 0.35 | 1.85 | 0.20 | 0.01 | 0.04 | -0.55 |
| ENSBTAG00000014551 | 0.10 | 0.04 | 0.01 | 0.00 | -0.02 | -0.03 | -0.05 | 1.26 |
| ENSBTAG00000014575 | -0.07 | -0.03 | 0.00 | 1.11 | 0.08 | 0.10 | 0.12 | -0.16 |
| ENSBTAG00000014643 | 0.02 | 0.25 | 0.16 | 3.84 | 0.91 | 0.85 | 0.57 | -0.21 |
| ENSBTAG00000014652 | -1.65 | -0.17 | -0.70 | -0.04 | -0.03 | 0.01 | -0.01 | -0.01 |
| ENSBTAG00000014784 | -2.21 | -1.46 | -1.90 | -0.15 | -0.02 | -0.01 | -0.02 | 0.00 |
| ENSBTAG00000014800 | 2.32 | 0.18 | 0.31 | 0.12 | 0.04 | -0.01 | 0.02 | 0.15 |
| ENSBTAG00000014823 | 0.02 | -0.02 | -0.01 | 0.00 | 0.01 | -0.03 | -0.02 | -2.80 |
| ENSBTAG00000014836 | -0.30 | -1.77 | -0.33 | 0.02 | 0.03 | 0.01 | 0.02 | 0.10 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000014913 | 1.38 | 0.21 | 0.14 | 0.22 | 0.13 | 0.09 | 0.10 | 0.11 |
| ENSBTAG00000014974 | -0.11 | -0.29 | -0.04 | -0.22 | -0.42 | -1.29 | -0.89 | -0.03 |
| ENSBTAG00000014981 | 0.06 | 0.05 | 0.04 | 7.95 | 0.55 | 0.25 | 0.23 | -0.94 |
| ENSBTAG00000015025 | -0.16 | -0.08 | -0.78 | -1.70 | -0.21 | -0.02 | -0.08 | -0.41 |
| ENSBTAG00000015058 | -0.05 | 0.02 | -0.01 | -0.21 | -0.37 | -1.24 | -0.49 | 0.01 |
| ENSBTAG00000015235 | 0.07 | 0.00 | 0.01 | -0.01 | -0.02 | 0.00 | -0.01 | -12.83 |
| ENSBTAG00000015273 | 2.62 | 0.72 | 2.12 | 0.35 | 0.68 | 0.12 | 0.25 | 0.01 |
| ENSBTAG00000015307 | 1.38 | 0.08 | 0.32 | -0.01 | 0.00 | -0.02 | -0.01 | 0.03 |
| ENSBTAG00000015311 | 0.12 | -0.03 | -0.01 | -1.34 | -0.32 | -0.35 | -0.25 | 0.27 |
| ENSBTAG00000015392 | -1.01 | -0.83 | -0.12 | -1.05 | -1.21 | -0.56 | -1.01 | 0.08 |
| ENSBTAG00000015413 | -0.01 | 0.11 | 0.03 | 0.23 | 0.44 | 1.33 | 0.41 | 0.01 |
| ENSBTAG00000015416 | -1.21 | -0.06 | -0.08 | -0.01 | -0.02 | -0.05 | -0.02 | 0.04 |
| ENSBTAG00000015427 | 0.02 | -0.01 | -0.03 | -0.01 | 0.01 | 0.02 | 0.01 | -4.52 |
| ENSBTAG00000015459 | 0.06 | 0.02 | 0.06 | 0.07 | 0.34 | 1.97 | 0.92 | 0.04 |
| ENSBTAG00000015512 | -0.46 | -1.61 | -0.31 | 0.01 | 0.00 | 0.00 | 0.01 | 0.01 |
| ENSBTAG00000015580 | 0.05 | 0.01 | 0.01 | 1.19 | 0.28 | 0.17 | 0.29 | 0.29 |
| ENSBTAG00000015596 | 0.05 | 0.05 | 0.01 | 0.07 | 2.20 | 1.06 | 1.44 | -0.02 |
| ENSBTAG00000015698 | 0.72 | 1.12 | 0.01 | 0.06 | 0.06 | 0.08 | 0.04 | 0.06 |
| ENSBTAG00000015732 | -6.33 | -0.14 | -0.02 | 0.06 | 0.05 | 0.01 | 0.02 | 0.15 |
| ENSBTAG00000015763 | 7.50 | 2.60 | 0.28 | 0.00 | 0.01 | -0.01 | 0.00 | 0.09 |
| ENSBTAG00000015782 | -0.06 | -0.04 | -0.06 | -0.85 | -0.10 | 0.00 | -0.04 | -0.05 |
| ENSBTAG00000015835 | 1.90 | 3.43 | 0.57 | -0.01 | 0.01 | 0.02 | 0.01 | 0.06 |
| ENSBTAG00000015839 | -0.09 | -0.14 | -0.10 | -1.48 | -0.31 | 0.02 | -0.04 | 0.03 |
| ENSBTAG00000015840 | 0.01 | -0.01 | -0.01 | 0.01 | 0.09 | 0.17 | 0.14 | 1.10 |
| ENSBTAG00000015880 | -0.27 | -0.26 | -0.02 | 0.01 | 0.01 | -0.01 | -0.01 | 2.24 |
| ENSBTAG00000015894 | -0.02 | -0.02 | 0.01 | 0.22 | 0.98 | 0.12 | 0.24 | -0.01 |
| ENSBTAG00000015904 | -0.04 | 0.01 | 0.00 | -0.01 | -0.04 | -0.02 | -0.04 | -1.24 |
| ENSBTAG00000015930 | -0.38 | -1.02 | -0.14 | -0.02 | -0.02 | -0.03 | -0.02 | -0.32 |
| ENSBTAG00000015955 | 0.95 | 23.54 | 80.83 | 90.10 | 120.44 | 118.19 | 140.37 | -0.07 |
| ENSBTAG00000015958 | 0.05 | -0.04 | -0.01 | -1.45 | -1.13 | -3.06 | -1.35 | -0.29 |
| ENSBTAG00000015974 | -0.41 | -5.59 | -0.05 | 0.00 | 0.01 | 0.01 | 0.00 | -0.08 |
| ENSBTAG00000016080 | 0.08 | 0.02 | 0.01 | -0.04 | -1.26 | -1.47 | -1.52 | -0.02 |
| ENSBTAG00000016208 | 0.81 | 0.01 | 0.04 | -1.14 | -0.61 | -0.78 | -0.54 | -0.07 |
| ENSBTAG00000016274 | -2.90 | -0.58 | -0.14 | 0.03 | 0.06 | 0.06 | 0.04 | -0.04 |
| ENSBTAG00000016277 | -0.07 | 0.01 | -0.01 | 0.21 | 1.24 | 2.56 | 1.26 | -0.08 |
| ENSBTAG00000016355 | 0.05 | 0.00 | 0.09 | 0.51 | 1.21 | 0.27 | 0.44 | -0.10 |
| ENSBTAG00000016368 | -0.11 | 0.01 | -0.03 | -0.07 | -0.39 | -0.92 | -0.82 | 0.03 |
| ENSBTAG00000016378 | -0.60 | -0.49 | -9.59 | -2.43 | -2.08 | -1.02 | -1.13 | -0.04 |
| ENSBTAG00000016387 | -1.71 | -0.63 | -3.37 | -0.20 | -0.64 | -0.10 | -0.29 | 0.03 |
| ENSBTAG00000016396 | 0.04 | 2.28 | 0.07 | 0.02 | 0.02 | 0.13 | 0.05 | 0.08 |
| ENSBTAG00000016456 | -1.75 | -0.30 | -1.05 | -0.14 | -0.05 | -0.02 | -0.06 | 0.03 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000016524 | 0.07 | 0.04 | 0.26 | 0.00 | 0.00 | 0.05 | 0.04 | -1.65 |
| ENSBTAG00000016546 | 1.10 | 0.24 | 0.46 | 0.02 | 0.00 | 0.00 | -0.01 | -0.19 |
| ENSBTAG00000016640 | 8.24 | 0.16 | 0.22 | -0.03 | 0.00 | -0.02 | -0.01 | -0.16 |
| ENSBTAG00000016662 | 0.02 | 0.05 | 0.05 | 0.00 | 0.01 | 0.03 | 0.03 | -1.07 |
| ENSBTAG00000016684 | 0.02 | 0.04 | 0.07 | -0.01 | -0.01 | 0.03 | 0.01 | -1.66 |
| ENSBTAG00000016782 | -0.14 | -0.96 | -0.02 | 0.00 | 0.02 | 0.06 | 0.04 | -0.17 |
| ENSBTAG00000016890 | 0.01 | 0.10 | 0.02 | -0.01 | 0.02 | 0.02 | 0.03 | 1.26 |
| ENSBTAG00000016915 | 1.91 | 0.26 | 0.18 | 0.05 | 0.06 | 0.05 | 0.08 | -0.04 |
| ENSBTAG00000016951 | -0.50 | -2.03 | -0.49 | -0.06 | -0.04 | -0.10 | -0.09 | 0.11 |
| ENSBTAG00000016984 | 2.19 | 4.05 | 2.42 | 0.04 | 0.03 | 0.02 | 0.02 | -0.06 |
| ENSBTAG00000017032 | 1.39 | 0.47 | 0.48 | 0.48 | 1.35 | 0.08 | 0.42 | 0.05 |
| ENSBTAG00000017051 | -0.09 | -0.07 | -0.07 | -1.24 | -0.23 | -0.01 | -0.08 | 0.28 |
| ENSBTAG00000017096 | 0.17 | 3.48 | 0.08 | 0.03 | 0.05 | 0.11 | 0.07 | 0.02 |
| ENSBTAG00000017133 | -0.18 | -0.04 | -0.10 | -3.51 | -3.52 | -1.33 | -2.79 | -0.04 |
| ENSBTAG00000017137 | -0.27 | -0.04 | 0.00 | 0.06 | 0.99 | 0.62 | 0.60 | -0.12 |
| ENSBTAG00000017165 | -0.04 | -0.01 | -0.35 | -1.49 | -0.55 | -0.15 | -0.40 | 0.13 |
| ENSBTAG00000017181 | -0.04 | -0.06 | -0.50 | -0.93 | -0.41 | -0.31 | -0.21 | -0.16 |
| ENSBTAG00000017195 | -0.98 | -0.85 | -1.21 | 0.00 | -0.01 | 0.00 | 0.00 | 0.02 |
| ENSBTAG00000017225 | -0.03 | 0.01 | 0.00 | 0.01 | -0.01 | 0.01 | 0.00 | -1.56 |
| ENSBTAG00000017239 | 0.12 | 0.03 | 0.04 | 1.73 | 0.24 | 0.06 | 0.09 | 0.02 |
| ENSBTAG00000017253 | -0.02 | -0.01 | -0.03 | -3.50 | -0.85 | -0.72 | -0.43 | 0.04 |
| ENSBTAG00000017310 | -0.11 | -0.24 | -0.06 | -0.02 | -0.13 | 0.00 | -0.03 | -1.61 |
| ENSBTAG00000017325 | -0.17 | -0.29 | 0.00 | -0.01 | -0.01 | 0.00 | 0.01 | 1.15 |
| ENSBTAG00000017350 | 0.53 | 0.17 | 0.28 | 2.02 | 1.06 | 0.15 | 0.28 | 0.30 |
| ENSBTAG00000017397 | 0.95 | 0.25 | 0.10 | 0.02 | 0.01 | 0.01 | 0.00 | -0.02 |
| ENSBTAG00000017458 | -1.15 | -2.40 | -0.08 | -0.02 | -0.08 | -0.03 | -0.09 | 0.02 |
| ENSBTAG00000017489 | -0.11 | -0.09 | -0.06 | -0.18 | -1.81 | -0.37 | -0.93 | -4.09 |
| ENSBTAG00000017537 | 0.44 | 8.97 | 2.81 | 0.04 | 0.09 | 0.14 | 0.10 | -0.02 |
| ENSBTAG00000017593 | 0.63 | 1.82 | 0.12 | -0.01 | 0.01 | 0.02 | 0.01 | 0.06 |
| ENSBTAG00000017661 | 2.62 | 2.64 | 0.18 | 0.00 | -0.04 | -0.01 | -0.02 | -0.06 |
| ENSBTAG00000017719 | -0.46 | -3.37 | -0.03 | 0.00 | 0.01 | 0.01 | 0.00 | 0.13 |
| ENSBTAG00000017731 | -0.17 | -0.02 | -0.17 | 0.00 | 0.01 | 0.00 | 0.01 | 2.98 |
| ENSBTAG00000017753 | -1.07 | -0.23 | -0.32 | -0.01 | 0.00 | -0.03 | -0.01 | 0.08 |
| ENSBTAG00000017764 | 0.21 | 0.11 | 0.25 | 3.71 | 2.53 | 0.60 | 1.34 | 0.14 |
| ENSBTAG00000017788 | 0.00 | -0.05 | -0.11 | 0.01 | -0.01 | 0.00 | 0.00 | -1.98 |
| ENSBTAG00000017808 | 1.14 | 0.75 | 0.29 | 0.03 | 0.01 | -0.03 | -0.01 | 0.00 |
| ENSBTAG00000017847 | 0.71 | 1.77 | 0.15 | 0.03 | 0.01 | -0.04 | 0.00 | 0.14 |
| ENSBTAG00000018012 | 2.33 | 0.44 | 0.23 | 0.01 | 0.01 | 0.03 | 0.00 | 0.00 |
| ENSBTAG00000018133 | -0.05 | -0.09 | -0.04 | -0.06 | -0.05 | -0.02 | -0.05 | -2.01 |
| ENSBTAG00000018138 | -0.08 | 0.03 | 0.01 | 0.00 | 0.00 | -0.02 | -0.01 | 1.72 |
| ENSBTAG00000018218 | 0.01 | -0.02 | 0.00 | -0.01 | 0.01 | 0.00 | 0.01 | 1.14 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000018260 | -0.51 | -4.57 | -1.34 | -0.06 | -0.02 | -0.01 | -0.02 | 0.17 |
| ENSBTAG00000018303 | -0.01 | -0.03 | -0.10 | -2.07 | -0.70 | -0.35 | -0.42 | -0.03 |
| ENSBTAG00000018404 | -0.36 | -2.09 | -0.22 | -0.15 | -0.13 | -0.15 | -0.11 | 0.12 |
| ENSBTAG00000018430 | -0.17 | -0.13 | -0.31 | -1.56 | -2.44 | -0.27 | -0.63 | 0.00 |
| ENSBTAG00000018465 | 1.93 | 4.18 | 0.22 | -0.01 | 0.01 | 0.00 | 0.01 | 0.17 |
| ENSBTAG00000018488 | 0.00 | -0.05 | -0.01 | 0.00 | 0.01 | 0.02 | 0.02 | -1.48 |
| ENSBTAG00000018501 | 2.19 | 4.41 | 0.93 | 0.01 | -0.01 | -0.02 | -0.01 | -0.02 |
| ENSBTAG00000018520 | 1.64 | 0.25 | 0.14 | 0.07 | 0.21 | 0.39 | 0.20 | 0.16 |
| ENSBTAG00000018540 | -1.06 | -0.51 | -1.26 | -0.01 | -0.03 | -0.01 | -0.01 | 0.25 |
| ENSBTAG00000018616 | 1.42 | 0.12 | 0.27 | -0.01 | -0.02 | -0.03 | -0.03 | -0.07 |
| ENSBTAG00000018629 | -0.05 | -0.05 | 0.00 | 0.12 | 0.27 | 1.10 | 0.67 | -0.03 |
| ENSBTAG00000018631 | -0.38 | -2.04 | -0.65 | -0.06 | -0.05 | 0.01 | -0.02 | -0.36 |
| ENSBTAG00000018657 | 0.33 | 0.09 | 0.24 | 24.06 | 3.16 | 0.73 | 1.55 | -0.12 |
| ENSBTAG00000018851 | -0.67 | -1.10 | -0.14 | -0.04 | -0.02 | 0.00 | -0.01 | 0.06 |
| ENSBTAG00000018854 | 0.00 | 0.10 | 0.05 | 0.00 | 0.02 | -0.02 | -0.01 | 2.29 |
| ENSBTAG00000018855 | 0.00 | -0.07 | -0.03 | 0.00 | -0.02 | 0.01 | 0.00 | -2.55 |
| ENSBTAG00000018965 | -1.34 | -0.72 | -0.25 | 0.00 | 0.08 | 0.08 | 0.12 | 0.21 |
| ENSBTAG00000019012 | 0.18 | 0.35 | 0.53 | 1.01 | 0.03 | 0.01 | 0.02 | 0.04 |
| ENSBTAG00000019041 | -0.31 | -2.27 | -0.12 | -0.04 | -0.02 | -0.01 | -0.03 | 0.03 |
| ENSBTAG00000019043 | -4.42 | -3.78 | -1.48 | -0.03 | -0.03 | -0.02 | -0.02 | -0.23 |
| ENSBTAG00000019052 | -1.19 | -0.65 | -0.03 | 0.03 | 0.03 | 0.05 | 0.03 | -0.03 |
| ENSBTAG00000019072 | -2.43 | -0.28 | -0.11 | -0.02 | 0.02 | 0.07 | 0.05 | 0.05 |
| ENSBTAG00000019121 | 2.62 | 1.26 | 0.25 | 0.01 | -0.06 | -0.13 | -0.07 | -0.27 |
| ENSBTAG00000019159 | 2.77 | 0.27 | 4.88 | 0.09 | 0.18 | 0.06 | 0.12 | -0.18 |
| ENSBTAG00000019302 | -0.16 | -1.17 | -0.47 | -0.03 | -0.01 | 0.00 | 0.00 | 0.00 |
| ENSBTAG00000019327 | -0.01 | 0.27 | 0.12 | 2.96 | 0.37 | 0.07 | 0.13 | 0.12 |
| ENSBTAG00000019350 | 0.08 | 0.08 | 0.72 | 0.06 | 0.04 | 0.04 | 0.03 | -1.14 |
| ENSBTAG00000019373 | 2.11 | 0.98 | 0.16 | 0.33 | 0.30 | 0.13 | 0.21 | 0.02 |
| ENSBTAG00000019382 | 2.50 | 0.35 | 0.37 | 0.07 | 0.10 | 0.06 | 0.09 | -0.03 |
| ENSBTAG00000019426 | -0.09 | -0.06 | -1.55 | -0.33 | -2.35 | -10.85 | -4.82 | -0.03 |
| ENSBTAG00000019458 | -1.08 | -0.92 | -1.02 | -0.12 | -0.01 | 0.03 | 0.00 | -0.52 |
| ENSBTAG00000019532 | -0.03 | 0.02 | 0.03 | 0.03 | 0.15 | 0.17 | 0.19 | 1.08 |
| ENSBTAG00000019545 | -1.13 | -23.38 | -0.95 | -0.06 | -0.04 | 0.00 | -0.01 | -0.06 |
| ENSBTAG00000019625 | -0.26 | -2.11 | -0.70 | -0.02 | 0.00 | -0.03 | -0.01 | 0.05 |
| ENSBTAG00000019644 | -0.44 | -0.54 | -0.42 | -0.61 | -0.74 | -0.56 | -0.47 | -1.94 |
| ENSBTAG00000019651 | -0.03 | -3.58 | -0.24 | -0.06 | -0.10 | 0.00 | -0.05 | -0.44 |
| ENSBTAG00000019719 | 0.02 | -0.09 | -0.05 | -0.13 | -0.69 | -5.51 | -0.90 | -0.37 |
| ENSBTAG00000019752 | 0.08 | 0.01 | 0.09 | 0.01 | -0.01 | -0.04 | 0.00 | -1.27 |
| ENSBTAG00000019761 | 0.05 | -0.01 | 0.12 | 1.14 | 0.36 | 0.08 | 0.15 | 0.30 |
| ENSBTAG00000019793 | -0.04 | -0.03 | -0.03 | -0.47 | -0.74 | -0.93 | -0.60 | 0.41 |
| ENSBTAG00000019794 | -1.62 | -0.16 | -0.37 | -0.16 | -0.08 | -0.01 | -0.04 | -0.75 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000019811 | -0.02 | -0.13 | -0.02 | -0.02 | -0.03 | -0.06 | -0.04 | -1.04 |
| ENSBTAG00000019821 | -0.05 | -0.34 | -0.19 | -1.08 | -0.19 | -0.04 | -0.10 | -0.01 |
| ENSBTAG00000019889 | -0.09 | 0.02 | -0.02 | -0.02 | -0.06 | 0.01 | -0.02 | -1.19 |
| ENSBTAG00000019915 | 1.21 | 0.28 | 0.32 | 0.00 | 0.01 | -0.02 | -0.01 | -0.16 |
| ENSBTAG00000019989 | -5.14 | -0.47 | -0.27 | -0.01 | 0.00 | 0.00 | 0.00 | 0.11 |
| ENSBTAG00000019997 | -0.01 | -0.01 | -0.01 | -1.42 | -0.34 | -0.09 | -0.13 | 0.02 |
| ENSBTAG00000020014 | 0.00 | -0.03 | -0.01 | -0.23 | -3.13 | -0.41 | -1.19 | -0.08 |
| ENSBTAG00000020048 | -0.10 | -0.01 | -0.02 | 0.02 | 0.01 | 0.02 | 0.01 | 2.77 |
| ENSBTAG00000020067 | -1.34 | -0.51 | -0.72 | -0.02 | -0.02 | 0.00 | -0.01 | -0.13 |
| ENSBTAG00000020096 | -1.45 | -0.16 | -0.15 | 0.01 | 0.03 | 0.05 | 0.05 | 0.09 |
| ENSBTAG00000020125 | 0.09 | 0.03 | 0.16 | 0.19 | 1.49 | 0.51 | 0.66 | -0.01 |
| ENSBTAG00000020225 | -0.89 | -0.96 | -8.40 | -0.17 | -0.02 | 0.00 | -0.01 | 0.29 |
| ENSBTAG00000020244 | -0.42 | -1.25 | -0.15 | 0.00 | -0.01 | -0.01 | -0.01 | -0.12 |
| ENSBTAG00000020407 | 0.02 | -0.01 | 0.04 | -0.04 | -0.05 | -0.05 | -0.07 | -0.99 |
| ENSBTAG00000020434 | -0.04 | -0.04 | 0.03 | 0.19 | 1.30 | 0.10 | 0.43 | 0.28 |
| ENSBTAG00000020455 | -0.09 | -0.07 | -0.35 | -1.12 | -0.84 | -0.26 | -0.49 | 0.02 |
| ENSBTAG00000020648 | 0.98 | 0.48 | 0.21 | 0.04 | 0.01 | 0.01 | 0.02 | 0.10 |
| ENSBTAG00000020654 | -1.31 | -1.83 | -0.35 | -0.03 | -0.17 | -0.04 | -0.06 | -0.31 |
| ENSBTAG00000020661 | 0.12 | 0.16 | 0.12 | 1.00 | 5.79 | 0.45 | 1.00 | 0.24 |
| ENSBTAG00000020671 | 0.05 | 0.04 | 0.00 | -3.99 | -0.22 | -0.19 | -0.14 | 0.61 |
| ENSBTAG00000020679 | 0.05 | 0.06 | 0.15 | 0.04 | 0.13 | 0.03 | 0.07 | 4.14 |
| ENSBTAG00000020726 | -0.07 | -0.04 | -0.02 | -0.02 | 0.04 | 0.13 | 0.08 | 2.90 |
| ENSBTAG00000020750 | 2.05 | 0.20 | 0.16 | -0.01 | 0.00 | 0.02 | 0.02 | -0.03 |
| ENSBTAG00000020769 | -0.04 | -0.14 | -0.02 | -0.69 | -0.59 | -0.97 | -0.63 | 0.06 |
| ENSBTAG00000020839 | -0.01 | -0.01 | 0.00 | 0.06 | 0.89 | 0.11 | 0.55 | 0.13 |
| ENSBTAG00000020858 | 0.00 | -0.02 | 0.00 | 0.23 | 1.54 | 1.03 | 1.46 | 0.01 |
| ENSBTAG00000020958 | -0.11 | -0.40 | -0.75 | -0.10 | -0.50 | -4.25 | -0.80 | -0.46 |
| ENSBTAG00000021029 | -0.04 | 0.05 | 0.08 | 5.42 | 10.58 | 2.54 | 1.81 | 4.53 |
| ENSBTAG00000021036 | 0.63 | 0.32 | 0.00 | -0.12 | -1.39 | -0.11 | -0.25 | -0.49 |
| ENSBTAG00000021064 | 1.36 | 0.46 | 0.52 | 0.04 | 0.07 | 0.16 | 0.12 | -0.10 |
| ENSBTAG00000021098 | 0.00 | -0.06 | -0.04 | -0.47 | -2.07 | -0.14 | -0.39 | 0.00 |
| ENSBTAG00000021133 | 1.49 | -0.01 | 0.03 | -0.08 | 0.00 | -0.05 | -0.03 | -0.31 |
| ENSBTAG00000021190 | -0.04 | -0.03 | -0.36 | -1.02 | -3.28 | -1.03 | -0.93 | -0.21 |
| ENSBTAG00000021209 | -0.96 | -1.05 | -0.22 | -0.03 | -0.05 | -0.04 | -0.08 | 0.14 |
| ENSBTAG00000021216 | 0.00 | -0.08 | -0.04 | -8.19 | -2.93 | -0.62 | -0.94 | -0.03 |
| ENSBTAG00000021217 | 0.94 | 0.16 | 1.57 | 0.38 | 0.30 | 0.35 | 0.36 | 0.07 |
| ENSBTAG00000021231 | 1.27 | 0.56 | 0.93 | 0.09 | 0.04 | 0.02 | 0.02 | 0.09 |
| ENSBTAG00000021291 | 0.09 | 0.08 | 0.02 | -0.01 | 0.00 | 0.00 | 0.00 | -1.35 |
| ENSBTAG00000021307 | -0.16 | -0.52 | -0.26 | -1.38 | -0.33 | -0.09 | -0.15 | 0.01 |
| ENSBTAG00000021365 | 2.71 | 1.72 | 0.70 | 1.14 | 0.91 | 0.14 | 0.36 | 0.44 |
| ENSBTAG00000021381 | -0.01 | -0.01 | -0.09 | -1.00 | -0.19 | -0.01 | -0.09 | 0.01 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000021416 | -0.04 | -5.62 | -0.14 | -0.16 | -0.04 | -0.03 | -0.02 | -0.19 |
| ENSBTAG00000021538 | -0.01 | -0.02 | -0.04 | -0.09 | -0.26 | -0.30 | -0.27 | -1.38 |
| ENSBTAG00000021543 | -0.73 | -0.14 | -0.05 | 0.07 | 0.31 | 2.08 | 0.78 | 0.00 |
| ENSBTAG00000021673 | 0.08 | 0.02 | 0.00 | -1.51 | -0.58 | -0.40 | -0.21 | 0.21 |
| ENSBTAG00000021841 | -2.50 | -1.77 | -0.89 | -0.03 | -0.09 | -0.05 | -0.11 | 0.04 |
| ENSBTAG00000021879 | 2.63 | 2.37 | 1.13 | 1.03 | 1.15 | 0.19 | 0.37 | 0.12 |
| ENSBTAG00000021880 | -0.04 | 0.00 | -0.02 | 0.05 | 0.15 | 0.02 | 0.08 | 1.50 |
| ENSBTAG00000021885 | -0.27 | -0.15 | -0.66 | -0.47 | -0.43 | -3.06 | -0.94 | -0.19 |
| ENSBTAG00000021902 | 0.00 | -0.01 | 0.00 | 8.58 | 23.43 | 1.46 | 5.46 | 0.06 |
| ENSBTAG00000021911 | -0.06 | 0.00 | -0.05 | -0.28 | -0.09 | -0.10 | -0.12 | -1.79 |
| ENSBTAG00000021919 | -0.05 | 0.03 | 0.08 | -0.01 | 0.00 | -0.03 | 0.00 | 1.96 |
| ENSBTAG00000021964 | 0.03 | -0.03 | 0.00 | -0.85 | -0.26 | -0.07 | -0.15 | -0.07 |
| ENSBTAG00000021978 | -0.06 | -0.02 | -0.09 | -3.53 | -1.00 | -0.11 | -0.33 | 0.07 |
| ENSBTAG00000021999 | -2.71 | -0.44 | -0.12 | -1.87 | -2.42 | -0.65 | -0.88 | -0.10 |
| ENSBTAG00000022004 | -1.61 | -0.41 | -0.09 | 0.02 | 0.03 | 0.00 | 0.02 | 0.03 |
| ENSBTAG00000022058 | 1.21 | 0.23 | 0.08 | 0.00 | 0.02 | 0.03 | 0.03 | 0.15 |
| ENSBTAG00000022169 | 0.09 | 0.48 | 0.03 | 0.07 | 0.19 | 0.79 | 0.22 | 0.05 |
| ENSBTAG00000022288 | -0.02 | -0.01 | -0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 1.03 |
| ENSBTAG00000022528 | -1.16 | -0.44 | -0.31 | -0.09 | -0.02 | -0.01 | -0.02 | -0.05 |
| ENSBTAG00000022588 | -1.84 | -0.06 | -0.14 | -0.01 | -0.03 | -0.10 | -0.04 | 0.12 |
| ENSBTAG00000022887 | 0.00 | -0.01 | -0.16 | -0.06 | -0.43 | -1.85 | -0.73 | -0.02 |
| ENSBTAG00000022920 | -0.05 | -0.03 | -0.05 | -0.26 | -3.68 | -2.24 | -2.87 | -0.06 |
| ENSBTAG00000022991 | -13.60 | -2.17 | -4.55 | -0.10 | -0.05 | -0.03 | -0.04 | -0.28 |
| ENSBTAG00000023216 | 4.61 | 0.15 | 0.07 | 0.02 | 0.00 | 0.01 | 0.02 | 0.01 |
| ENSBTAG00000023377 | -1.11 | -0.11 | -0.03 | 0.00 | 0.01 | 0.01 | 0.02 | -0.32 |
| ENSBTAG00000023718 | 0.06 | 0.09 | 0.07 | 0.03 | 0.12 | 0.00 | 0.06 | 0.91 |
| ENSBTAG00000023920 | -0.04 | -0.02 | 0.20 | 0.48 | 5.89 | 3.13 | 2.44 | 0.09 |
| ENSBTAG00000024015 | -0.02 | -0.03 | -0.03 | -2.88 | -0.19 | -0.05 | -0.09 | 0.16 |
| ENSBTAG00000024188 | 1.08 | 0.09 | 0.05 | 0.10 | 0.02 | 0.03 | 0.05 | 0.07 |
| ENSBTAG00000024420 | 2.78 | 0.09 | 0.05 | 0.00 | 0.00 | -0.03 | -0.02 | 0.01 |
| ENSBTAG00000024482 | 20.47 | 1.22 | 2.19 | 0.01 | -0.04 | -0.02 | -0.04 | -0.15 |
| ENSBTAG00000024545 | 0.04 | 0.01 | -0.11 | 0.00 | 0.02 | 0.03 | 0.04 | 1.43 |
| ENSBTAG00000025035 | 0.00 | 0.00 | 0.00 | -0.12 | -0.63 | -1.09 | -0.68 | 0.00 |
| ENSBTAG00000025078 | 0.07 | 0.00 | 0.03 | -0.34 | -0.92 | -0.24 | -0.34 | -5.99 |
| ENSBTAG00000025200 | -0.01 | 0.05 | 0.11 | 6.16 | 1.45 | 0.20 | 0.45 | 0.49 |
| ENSBTAG00000025450 | -0.01 | -0.06 | 0.01 | 0.05 | 0.01 | 0.02 | 0.03 | -1.01 |
| ENSBTAG00000025485 | -0.20 | -0.08 | -0.15 | -2.08 | -1.12 | -0.40 | -1.00 | -0.03 |
| ENSBTAG00000025642 | 0.02 | 0.00 | -0.06 | -0.10 | -0.30 | -1.09 | -0.60 | -0.12 |
| ENSBTAG00000025942 | -1.53 | -0.38 | -0.12 | -0.02 | -0.01 | 0.01 | 0.00 | -0.06 |
| ENSBTAG00000026133 | 1.77 | 0.63 | 0.23 | 0.04 | 0.07 | 0.07 | 0.06 | 0.01 |
| ENSBTAG00000026234 | -0.11 | -0.01 | -0.26 | -3.50 | -7.56 | -0.61 | -1.20 | 0.17 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000027064 | -1.90 | -4.16 | -0.41 | -0.05 | -0.03 | -0.03 | -0.02 | 0.12 |
| ENSBTAG00000027134 | -0.01 | 0.02 | -0.15 | -0.34 | -0.52 | -1.50 | -0.57 | 0.00 |
| ENSBTAG00000027173 | -31.18 | -60.99 | -0.87 | -0.03 | -0.04 | -0.04 | -0.06 | -0.06 |
| ENSBTAG00000027182 | -0.01 | 0.06 | 0.01 | 0.06 | 0.69 | 1.62 | 0.98 | 0.04 |
| ENSBTAG00000027327 | 0.01 | 0.05 | 0.05 | 0.18 | 1.63 | 0.15 | 0.35 | 0.11 |
| ENSBTAG00000027629 | -0.33 | -0.07 | -0.13 | -1.50 | -0.90 | -0.37 | -0.69 | -0.16 |
| ENSBTAG00000027665 | 0.04 | 0.04 | 0.05 | 0.03 | 0.09 | 0.06 | 0.08 | 1.31 |
| ENSBTAG00000030175 | 0.47 | 1.20 | 0.53 | 0.03 | 0.05 | 0.06 | 0.06 | 0.02 |
| ENSBTAG00000030366 | -0.93 | -3.43 | -0.29 | -0.07 | -0.02 | 0.00 | -0.01 | -0.04 |
| ENSBTAG00000030599 | -1.40 | -0.57 | -0.09 | 0.01 | 0.04 | 0.02 | 0.03 | 0.00 |
| ENSBTAG00000030669 | 0.13 | 1.60 | 0.06 | 0.00 | -0.01 | 0.00 | -0.01 | 0.01 |
| ENSBTAG00000030817 | 2.30 | 0.06 | 0.23 | 0.12 | 0.35 | 0.09 | 0.14 | -0.18 |
| ENSBTAG00000030990 | 0.16 | 0.09 | 0.53 | 0.10 | 1.37 | 4.54 | 1.28 | -0.14 |
| ENSBTAG00000031178 | -1.05 | -0.11 | -0.09 | -0.04 | -0.05 | -0.02 | -0.05 | -0.01 |
| ENSBTAG00000031335 | 1.52 | 0.54 | 0.09 | -0.01 | -0.05 | -0.05 | -0.06 | 0.17 |
| ENSBTAG00000031395 | -0.02 | 0.03 | 0.02 | 0.13 | 0.08 | 0.01 | 0.03 | 3.54 |
| ENSBTAG00000031561 | -0.06 | -0.04 | -0.01 | 1.46 | 0.23 | 0.07 | 0.13 | 0.01 |
| ENSBTAG00000031567 | -0.22 | -0.54 | -0.32 | -1.34 | -0.11 | -0.02 | -0.04 | 0.10 |
| ENSBTAG00000031654 | 2.38 | 0.41 | 4.59 | 0.10 | 0.08 | 0.09 | 0.11 | -0.04 |
| ENSBTAG00000031686 | -1.26 | -0.20 | -0.36 | 0.00 | -0.01 | -0.03 | -0.01 | 0.03 |
| ENSBTAG00000031697 | 4.81 | 0.86 | 3.46 | 0.03 | 0.03 | 0.00 | 0.01 | -0.06 |
| ENSBTAG00000031704 | 0.06 | 1.59 | 0.04 | 0.00 | 0.00 | 0.02 | 0.02 | 0.04 |
| ENSBTAG00000031707 | 0.59 | 0.05 | 0.25 | 0.00 | 0.01 | -0.01 | -0.01 | -2.47 |
| ENSBTAG00000031898 | 2.91 | 0.27 | 4.32 | -0.03 | 0.00 | 0.05 | 0.02 | 0.27 |
| ENSBTAG00000031967 | -1.52 | -0.65 | -0.10 | -0.01 | 0.00 | -0.03 | 0.00 | 0.00 |
| ENSBTAG00000032077 | 1.14 | 0.36 | 0.22 | 0.00 | 0.02 | 0.02 | 0.04 | 0.04 |
| ENSBTAG00000032121 | -0.09 | -0.16 | -0.07 | -0.03 | -1.38 | -0.14 | -0.31 | 0.00 |
| ENSBTAG00000032148 | 0.07 | -0.04 | 0.01 | -0.03 | -0.03 | -0.05 | -0.05 | 3.86 |
| ENSBTAG00000032485 | 1.52 | 1.27 | 0.90 | -0.03 | -0.02 | -0.03 | -0.01 | 0.20 |
| ENSBTAG00000032519 | -0.52 | -0.50 | -0.27 | -0.35 | -0.91 | -0.91 | -0.81 | -0.03 |
| ENSBTAG00000032544 | 0.02 | 0.02 | -0.01 | -3.32 | -2.99 | -0.80 | -0.94 | 0.11 |
| ENSBTAG00000032558 | 1.14 | 0.09 | 2.47 | 0.02 | 0.19 | 0.10 | 0.15 | -0.08 |
| ENSBTAG00000032603 | 0.11 | 0.07 | 0.27 | 0.05 | 0.48 | 0.35 | 0.44 | 0.92 |
| ENSBTAG00000032684 | 0.12 | 0.18 | 0.00 | 0.18 | 1.35 | 0.32 | 0.52 | -0.03 |
| ENSBTAG00000032719 | -0.03 | 0.01 | -0.02 | 0.00 | -0.19 | -0.06 | -0.12 | -1.84 |
| ENSBTAG00000032914 | -0.09 | -0.29 | -0.01 | -0.47 | -0.78 | -1.87 | -0.79 | -0.04 |
| ENSBTAG00000032951 | 0.11 | 2.53 | 3.18 | 0.09 | 0.18 | 0.30 | 0.19 | 0.13 |
| ENSBTAG00000033095 | -0.05 | -0.01 | -0.04 | -1.42 | -1.58 | -0.91 | -0.58 | 0.20 |
| ENSBTAG00000033137 | -0.27 | -1.26 | -0.07 | -0.06 | -0.03 | -0.02 | -0.03 | -0.03 |
| ENSBTAG00000033180 | -0.01 | -0.03 | -0.17 | -2.14 | -4.20 | -2.50 | -2.10 | -1.34 |
| ENSBTAG00000033182 | -0.02 | -0.02 | 0.00 | -0.04 | -0.01 | -0.07 | -0.03 | 2.73 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000033902 | 0.23 | 0.31 | 0.13 | 0.06 | 0.01 | 0.00 | 0.01 | -2.42 |
| ENSBTAG00000033983 | -5.54 | -0.22 | -0.30 | 0.01 | -0.02 | -0.05 | -0.02 | 0.07 |
| ENSBTAG00000034441 | 0.02 | -0.01 | 0.04 | 0.37 | 0.61 | 1.14 | 0.74 | 0.06 |
| ENSBTAG00000034827 | -0.04 | -0.01 | -0.02 | 0.12 | 0.21 | 1.08 | 0.50 | 0.04 |
| ENSBTAG00000035030 | -3.50 | -0.26 | -0.10 | 0.00 | 0.00 | 0.02 | 0.02 | 0.14 |
| ENSBTAG00000035084 | 0.04 | 0.07 | 0.51 | 1.58 | 5.49 | 1.05 | 0.87 | -0.04 |
| ENSBTAG00000036287 | -0.12 | -0.02 | -0.03 | -0.13 | -0.13 | -0.05 | -0.05 | 1.25 |
| ENSBTAG00000036349 | 0.23 | 1.82 | 0.56 | -0.08 | -0.04 | 0.00 | -0.02 | -0.07 |
| ENSBTAG00000037581 | -0.26 | -9.58 | -0.64 | -0.54 | -6.36 | -0.48 | -1.65 | -2.53 |
| ENSBTAG00000037717 | -0.37 | -1.52 | -0.03 | -0.02 | -0.04 | -0.06 | -0.03 | 0.20 |
| ENSBTAG00000037786 | 4.24 | 0.19 | 0.13 | -0.02 | -0.08 | -0.06 | -0.08 | 0.17 |
| ENSBTAG00000037972 | 0.83 | 0.91 | 0.14 | 0.03 | 0.00 | -0.01 | 0.00 | -0.04 |
| ENSBTAG00000037980 | 2.91 | 0.35 | 5.17 | 1.09 | 0.12 | 0.13 | 0.12 | -0.06 |
| ENSBTAG00000038180 | -4.72 | -0.16 | -1.04 | -0.06 | -0.22 | -0.15 | -0.21 | 0.10 |
| ENSBTAG00000038181 | 0.08 | -0.01 | 0.02 | 0.00 | -0.01 | 0.02 | 0.01 | -1.45 |
| ENSBTAG00000038251 | -1.60 | -0.27 | -1.05 | -0.02 | -0.04 | 0.01 | -0.02 | 0.04 |
| ENSBTAG00000038281 | 0.06 | 0.04 | 0.25 | 0.67 | 0.97 | 0.51 | 0.67 | -0.01 |
| ENSBTAG00000038333 | -0.71 | -0.63 | -13.32 | -2.78 | -2.28 | -0.97 | -1.10 | -0.04 |
| ENSBTAG00000038340 | -0.02 | -0.04 | -0.22 | -0.12 | -0.06 | -0.02 | -0.04 | 1.25 |
| ENSBTAG00000038347 | -1.79 | -0.82 | -30.93 | -0.03 | 0.00 | 0.00 | 0.01 | 0.22 |
| ENSBTAG00000038361 | 0.03 | -0.01 | -0.03 | -2.06 | -0.22 | -0.09 | -0.11 | 0.08 |
| ENSBTAG00000038495 | -4.57 | -5.04 | -2.54 | -0.06 | -0.09 | -0.03 | -0.04 | -0.10 |
| ENSBTAG00000038520 | 0.11 | -0.07 | -0.02 | -0.01 | -0.02 | -0.01 | -0.02 | -1.14 |
| ENSBTAG00000038650 | 14.98 | 3.16 | 0.52 | 0.00 | -0.02 | -0.04 | -0.06 | -0.27 |
| ENSBTAG00000038687 | -0.05 | 0.00 | -0.04 | -0.03 | -0.02 | -0.05 | -0.04 | 2.58 |
| ENSBTAG00000038716 | 3.71 | 6.88 | 0.31 | 0.01 | 0.00 | 0.04 | 0.01 | -0.36 |
| ENSBTAG00000038849 | 1.39 | 4.33 | 0.46 | 0.00 | -0.01 | -0.03 | -0.04 | -0.12 |
| ENSBTAG00000038920 | -1.91 | -0.51 | -0.06 | -0.01 | 0.00 | 0.01 | 0.00 | 0.06 |
| ENSBTAG00000039055 | -0.37 | -1.48 | -0.08 | -0.01 | 0.01 | 0.04 | 0.05 | 0.00 |
| ENSBTAG00000039080 | -4.17 | -2.64 | -2.74 | -0.06 | -0.03 | -0.01 | -0.01 | 0.26 |
| ENSBTAG00000039091 | 0.39 | 0.40 | 0.21 | 0.06 | 0.17 | 0.15 | 0.15 | 1.02 |
| ENSBTAG00000039197 | -0.03 | 0.05 | 0.01 | -0.02 | -0.01 | -0.03 | -0.03 | 3.68 |
| ENSBTAG00000040305 | 2.62 | 3.66 | 0.12 | -0.03 | -0.02 | -0.01 | -0.03 | 0.11 |
| ENSBTAG00000040496 | 0.13 | 3.53 | 0.26 | 0.03 | 0.01 | -0.04 | -0.01 | 0.03 |
| ENSBTAG00000042405 | -0.21 | -3.28 | -0.04 | 0.06 | 0.14 | 0.82 | 0.19 | -0.07 |
| ENSBTAG00000043312 | -2.22 | -0.72 | -2.54 | -0.01 | 0.02 | 0.06 | 0.06 | 0.02 |
| ENSBTAG00000043960 | -0.04 | 0.04 | 0.04 | 0.43 | 0.07 | 0.20 | 0.07 | 7.23 |
| ENSBTAG00000043993 | -0.07 | -0.27 | -0.20 | -0.03 | -0.30 | -0.01 | -0.09 | -31.44 |
| ENSBTAG00000044006 | -0.20 | -0.17 | -0.05 | -0.38 | -0.40 | -1.06 | -0.46 | 0.02 |
| ENSBTAG00000044038 | -0.66 | -1.25 | -3.75 | -0.11 | -0.12 | -0.07 | -0.09 | -0.08 |
| ENSBTAG00000044046 | -0.02 | -0.03 | -0.04 | -0.42 | -1.29 | -0.24 | -0.43 | -0.02 |

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|---|---|---|---|---|---|---|---|---|
| ENSBTAG00000044050 | -0.01 | -0.10 | -0.23 | -14.69 | -26.46 | -19.75 | -39.92 | -0.06 |
| ENSBTAG00000044119 | 0.07 | -0.02 | -0.07 | -1.12 | -0.46 | -0.25 | -0.44 | -0.08 |
| ENSBTAG00000044158 | -0.06 | -0.25 | -0.07 | -1.44 | -9.94 | -1.68 | -1.26 | -0.85 |
| ENSBTAG00000045359 | 2.70 | 0.77 | 1.25 | 0.24 | 0.27 | 0.16 | 0.27 | 0.15 |
| ENSBTAG00000045644 | 0.00 | -0.04 | -0.03 | -0.47 | -2.08 | -0.19 | -0.45 | 0.00 |
| ENSBTAG00000045645 | 0.03 | 0.03 | 0.05 | 2.71 | 0.24 | 0.13 | 0.17 | -0.06 |
| ENSBTAG00000045661 | 4.80 | 0.95 | 3.60 | 0.02 | 0.02 | 0.00 | 0.01 | -0.06 |
| ENSBTAG00000045699 | -2.31 | -1.52 | -1.38 | -0.02 | -0.02 | -0.05 | -0.03 | 0.11 |
| ENSBTAG00000045726 | -0.09 | -0.04 | -0.08 | -0.07 | -0.15 | -0.14 | -0.21 | -1.22 |
| ENSBTAG00000045868 | 0.10 | 0.06 | 0.07 | -0.01 | 0.02 | 0.01 | 0.01 | 1.17 |
| ENSBTAG00000046176 | 0.05 | 0.04 | 0.08 | 4.16 | 2.49 | 0.24 | 0.63 | -0.17 |
| ENSBTAG00000046199 | 1.42 | 0.05 | 0.04 | 0.00 | 0.00 | -0.02 | -0.02 | 0.09 |
| ENSBTAG00000046380 | 1.12 | 0.04 | 1.83 | 0.06 | 0.14 | 0.03 | 0.06 | 0.09 |
| ENSBTAG00000046462 | 0.10 | 0.06 | 0.00 | -0.04 | -0.02 | 0.01 | -0.02 | -2.13 |
| ENSBTAG00000046486 | -3.11 | -9.13 | -2.46 | -0.12 | -0.91 | -0.26 | -0.28 | 0.04 |
| ENSBTAG00000046602 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | -0.01 | -0.01 | 1.86 |
| ENSBTAG00000046723 | 1.97 | 0.41 | 0.19 | 0.00 | 0.02 | 0.02 | 0.03 | -0.01 |
| ENSBTAG00000046763 | 1.93 | 0.38 | 0.14 | -0.02 | -0.02 | -0.02 | -0.01 | -0.05 |
| ENSBTAG00000047450 | -0.10 | -0.10 | -0.01 | 0.03 | 0.03 | 0.06 | 0.07 | 0.96 |
| ENSBTAG00000047611 | -0.02 | 0.01 | 0.08 | 0.00 | -0.01 | 0.02 | -0.01 | -1.27 |
| ENSBTAG00000047667 | 0.00 | -0.04 | -0.02 | -1.48 | -1.53 | -0.40 | -0.79 | -0.09 |
| ENSBTAG00000047743 | -3.61 | -0.41 | -0.13 | -0.07 | -0.11 | -0.04 | -0.08 | -0.10 |
| ENSBTAG00000047834 | -0.06 | -0.02 | 0.01 | 0.28 | 0.05 | 0.04 | 0.04 | -2.74 |
| ENSBTAG00000048020 | 0.11 | 0.05 | 0.01 | 0.02 | 0.01 | -0.02 | 0.00 | 1.39 |
| ENSBTAG00000048113 | 0.05 | 0.08 | -0.01 | -0.01 | 0.00 | -0.03 | -0.02 | -0.92 |
| ENSBTAG00000048316 | 2.56 | 0.43 | 0.71 | 0.00 | -0.06 | -0.05 | -0.05 | -0.02 |

[1]RAW: non-transformed PAP score; CAT3: three-category phenotype; CAT2: two-category phenotype; BWT: birth weight; WW: weaning weight; PWG: post-weaning gain; YW: yearling weight; MILK: maternal weaning weight.

APPENDIX 7.3. SIGNIFICANT GENE ONTOLOGY (GO) TERMS FROM GENE ENRICHMENT ANALYSIS OF GENES IN AN ASSOCIATED WEIGHT MATRIX ON YEARLING PAP PHENOTYPES AND GROWTH PERFORMANCE TRAITS IN ANGUS CATTLE MANAGED AT HIGH ALTITUDE (ELEVATION AT 2,170 M)

| GO[1] | ID | P-value[2] | Name | Num. Genes[3] |
|---|---|---|---|---|
| BP | GO:0043087 | 0.02 | regulation of GTPase activity | 19 |
| BP | GO:0051260 | 0.02 | protein homooligomerization | 12 |
| BP | GO:0014009 | 0.03 | glial cell proliferation | 3 |
| BP | GO:1904158 | 0.01 | axonemal central apparatus assembly | 2 |
| BP | GO:0007214 | 0.01 | gamma-aminobutyric acid signaling pathway | 4 |
| BP | GO:0003013 | 0.05 | circulatory system process | 15 |
| BP | GO:0032787 | 0.01 | monocarboxylic acid metabolic process | 20 |
| BP | GO:0006631 | 0.02 | fatty acid metabolic process | 14 |
| BP | GO:0015800 | 0.05 | acidic amino acid transport | 3 |
| BP | GO:0006835 | 0.02 | dicarboxylic acid transport | 5 |
| BP | GO:0008150 | 0.00 | biological_process | 503 |
| BP | GO:1901362 | 0.02 | organic cyclic compound biosynthetic process | 103 |
| BP | GO:0019438 | 0.03 | aromatic compound biosynthetic process | 99 |
| BP | GO:0051171 | 0.00 | regulation of nitrogen compound metabolic | 107 |
| BP | GO:0009891 | 0.00 | positive regulation of biosynthetic process | 58 |
| BP | GO:0031326 | 0.00 | regulation of cellular biosynthetic process | 104 |
| BP | GO:0010033 | 0.02 | response to organic substance | 62 |
| BP | GO:0051173 | 0.00 | positive regulation of nitrogen compound metabolic process | 60 |
| BP | GO:0010771 | 0.02 | negative regulation of cell morphogenesis involved in differentiation | 7 |
| BP | GO:0048678 | 0.00 | response to axon injury | 6 |
| BP | GO:0006935 | 0.00 | chemotaxis | 22 |
| BP | GO:0071391 | 0.01 | cellular response to estrogen stimulus | 3 |
| BP | GO:0008344 | 0.03 | adult locomotory behavior | 7 |
| BP | GO:0006873 | 0.01 | cellular ion homeostasis | 18 |
| BP | GO:0021545 | 0.02 | cranial nerve development | 5 |
| BP | GO:1903827 | 0.02 | regulation of cellular protein localization | 20 |
| BP | GO:0019226 | 0.00 | transmission of nerve impulse | 11 |
| BP | GO:0010628 | 0.00 | positive regulation of gene expression | 57 |
| BP | GO:0090287 | 0.02 | regulation of cellular response to growth factor stimulus | 12 |
| BP | GO:0060070 | 0.04 | canonical Wnt signaling pathway | 12 |

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|---|---|---|---|---|
| BP | GO:0051056 | 0.01 | regulation of small GTPase mediated signal transduction | 13 |
| BP | GO:0051252 | 0.04 | regulation of RNA metabolic process | 87 |
| BP | GO:0006366 | 0.02 | transcription from RNA polymerase II promoter | 54 |
| BP | GO:0009301 | 0.01 | snRNA transcription | 3 |
| BP | GO:0006355 | 0.05 | regulation of transcription, DNA-templated | 83 |
| BP | GO:0045860 | 0.03 | positive regulation of protein kinase activity | 16 |
| BP | GO:0043405 | 0.03 | regulation of MAP kinase activity | 13 |
| BP | GO:1902667 | 0.05 | regulation of axon guidance | 3 |
| BP | GO:0043406 | 0.01 | positive regulation of MAP kinase activity | 10 |
| BP | GO:0051016 | 0.04 | barbed-end actin filament capping | 3 |
| BP | GO:2001238 | 0.01 | positive regulation of extrinsic apoptotic signaling pathway | 6 |
| BP | GO:2001241 | 0.03 | positive regulation of extrinsic apoptotic signaling pathway in absence of ligand | 3 |
| BP | GO:0099024 | 0.04 | plasma membrane invagination | 4 |
| BP | GO:1902774 | 0.03 | late endosome to lysosome transport | 2 |
| BP | GO:0000727 | 0.03 | double-strand break repair via break-induced replication | 2 |
| BP | GO:0071670 | 0.03 | smooth muscle cell chemotaxis | 2 |
| BP | GO:0034086 | 0.02 | maintenance of sister chromatid cohesion | 3 |
| BP | GO:0007063 | 0.05 | regulation of sister chromatid cohesion | 3 |
| BP | GO:0050890 | 0.03 | cognition | 11 |
| BP | GO:0030817 | 0.05 | regulation of cAMP biosynthetic process | 7 |
| BP | GO:0032228 | 0.05 | regulation of synaptic transmission, GABAergic | 3 |
| BP | GO:1903321 | 0.02 | negative regulation of protein modification by small protein conjugation or removal | 6 |
| BP | GO:0003199 | 0.03 | endocardial cushion to mesenchymal transition involved in heart valve formation | 2 |
| BP | GO:1902259 | 0.02 | regulation of delayed rectifier potassium channel activity | 3 |
| BP | GO:0007160 | 0.01 | cell-matrix adhesion | 11 |
| BP | GO:0048545 | 0.05 | response to steroid hormone | 11 |
| BP | GO:0048669 | 0.03 | collateral sprouting in absence of injury | 2 |
| BP | GO:0040007 | 0.00 | growth | 38 |
| BP | GO:0008152 | 0.00 | metabolic process | 304 |
| BP | GO:0002376 | 0.01 | immune system process | 59 |
| keg | KEGG:04024 | 0.05 | cAMP signaling pathway | 11 |
| keg | KEGG:04724 | 0.03 | Glutamatergic synapse | 8 |
| keg | KEGG:04510 | 0.00 | Focal adhesion | 13 |
| keg | KEGG:04713 | 0.04 | Circadian entrainment | 7 |
| keg | KEGG:04071 | 0.03 | Sphingolipid signaling pathway | 9 |
| keg | KEGG:04933 | 0.03 | AGE-RAGE signaling pathway in diabetic complications | 8 |

| | | | | |
|---|---|---|---|---|
| keg | KEGG:04918 | 0.01 | Thyroid hormone synthesis | 7 |
| keg | KEGG:04066 | 0.02 | HIF-1 signaling pathway | 8 |
| keg | KEGG:04728 | 0.02 | Dopaminergic synapse | 9 |
| keg | KEGG:04911 | 0.04 | Insulin secretion | 7 |
| keg | KEGG:04360 | 0.02 | Axon guidance | 11 |
| keg | KEGG:04726 | 0.00 | Serotonergic synapse | 10 |
| keg | KEGG:04340 | 0.03 | Hedgehog signaling pathway | 5 |
| keg | KEGG:04014 | 0.02 | Ras signaling pathway | 13 |
| keg | KEGG:04720 | 0.02 | Long-term potentiation | 6 |

[1]BP: Biological process; ke: KEGG pathway

[2] Benjamini-Honchberg FDR corrected P-Value

[3]Num.Genes: number of genes in gene ontology term