THE OVERALL USE OF GENETIC AND GENOMIC TECHNOLOGIES FOR BEEF CATTLE SYSTEMS FOR BEEF CATTLE HEALTH IMPROVEMENT

by

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ABSTRACT

As genetic and genomic technology continues to advance within the beef industry, there is an opportunity to improve beef cattle systems. Specifically, this may be achieved by evaluating genetics and environment of beef cattle simultaneously. As such, we conducted two independent experiments to 1) analyze host gene expression upon cattle arrival to a backgrounding system to determine predictive candidate biomarkers and genomic mechanisms related to respiratory disease risk and acquisition, and 2) evaluate production and carcass characteristics from the offspring of cloned sires to determine if meat quality and growth performance are replicable across a generation.

In experiment 1, bovine respiratory disease (BRD) remains the leading disease within the U.S. beef cattle industry. Marketing decisions made prior to backgrounding may shift BRD incidence into a different phase of production, and the importance of host gene expression on BRD incidence as it relates to marketing strategy is poorly understood. Our objective was to compare the influence of marketing on host transcriptomes measured on arrival at a backgrounding facility on the subsequent probability of being treated for BRD during a 45-day backgrounding phase. This study, through RNA-Seq analysis of blood samples collected on arrival, evaluated gene expression differences between cattle which experienced a commercial auction setting (AUCTION) versus cattle directly shipped to backgrounding from the cow–calf phase

(DIRECT); further analyses were conducted to determine differentially expressed genes (DEGs) between cattle which remained clinically healthy during backgrounding (HEALTHY) versus those that required treatment for clinical BRD within 45 days of arrival (BRD). A profound difference in DEGs (n = 2961) was identified between AUCTION cattle compared to DIRECT cattle, regardless of BRD development; these DEGs encoded for proteins involved in antiviral defense (increased in AUCTION), cell growth regulation (decreased in AUCTION), and inflammatory mediation (decreased in AUCTION). Nine and four DEGs were identified between BRD and HEALTHY cohorts in the AUCTION and DIRECT groups, respectively; DEGs between disease cohorts in the AUCTION group encoded for proteins involved in collagen synthesis and platelet aggregation (increased in HEALTHY). Our work demonstrates the clear influence marketing has on host expression and identified genes and mechanisms which may predict BRD risk.

In experiment 2, animal breeding and genetics have shifted significantly over the past several decades. Previously, genetic improvement of beef cattle was largely dependent on visual appearance. While this remains valuable in selecting cattle for breeding, current technology and performance determination contributes to modern genetic improvement strategies. As such, we have continued a unique crossbreeding project beginning with rare carcasses that exhibited a highly desirable yet antagonistic trait which includes being USDA Prime and yield grade 1. Sires (Alpha, Delta and AxG1) were produced and evaluated originally for high quality carcass characteristics, then bred accordingly in the summer of 2020. Our objective was to see if their offspring could replicate similar outcomes and produce quality carcasses and growth characteristics.

Here, thirty-five bull (n=24) and heifer (n=11) calf offspring were fed a commercial feedlot ration at the Palo Duro Consultation, Research & Feedlot in Canyon, TX for 68 days. Parentage results were tested to confirm sire, followed by weight gain, feed intake, and carcass ultrasound data collections. Significant differences were found (P<.05) for entire average daily gain and average intake, rib fat and backfat, and ribeye area and percent intramuscular fat for both SIRE and SEX. Spearmen's Rank correlations were found of (P<.05), with a coefficient of 0.59 for ADG and average intake, 0.42 for RF and BF both for SIRE. Spearmen's rank correlations for SEX found no significance for ADG and average intake but when evaluating RF and BF between sex on d68 significance was found (P< .05) with a coefficient of 0.39. Our results can help confirm the relationship between larger RF and BF with weight gain, as well as the relationship between carcass quality in the specific sex of beef cattle.

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CHAPTER I

INTRODUCTION

1.1. Introduction

The beef cattle industry within the United States is not placed into one single category, as there are several production stages such as cow-calf, stocker, seedstock, and feedlot systems. In contrast to other livestock systems, there is little to no vertical integration in the beef cattle industry (Araji, 1976; Hoar and Angelos, 2015). While other well-integrated animal production systems, such as in poultry or pork production, keep detailed health records across the lifespan of animals, it is uncommon for producers within the beef industry to have access to information from previous production stages. As a result, each stage of beef cattle production varies as beef cattle are raised under different management, environment, and production strategies. Additionally, cattle may be marketed in various ways, such as commercial auctioning or direct marketing system, in addition to seedstock replacement systems, owner–buyer interactions, and online sales (Dethloff et al., 2023). Moreover, these different production system factors possess several benefits and challenges faced daily by beef cattle producers.

Beef cattle production systems are defined as all cattle production systems where the purpose of the operation includes breeding, growing, and/or finishing cattle intended

for the consumption of beef (Herring, 2014). These beef cattle production systems can often be divided into four types of operations: cow-calf, stocker (also called “growers”), feedlot, and seedstock (Drouillard, 2018).

Two known factors that influence beef cattle production systems are cattle genetics and operation environment. Genetics is defined as the scientific study of genes and heredity and how certain qualities or traits are passed from parents to offspring (Durmaz et al., 2015). The environmental side encompasses any outside factors independent of genetics which includes climate, nutrition, infectious disease, and management, of which all are considered major stressors on animal health and production (Bova et al., 2014).

As technology utilized within the beef industry continues to advance, specifically in the areas of genetics and genomics, there is an opportunity to improve beef cattle systems within the United States. This may be achieved by evaluating genetics and environment of beef cattle simultaneously. Previous research and commercial advancements in beef cattle genetic and genomic programs over the past several decades have provided information to researchers regarding decision making and genetic factors, such as specific alleles and gene mutations, as certain traits of importance. These key findings have been provided by advancements in computer science, analytical methodology, statistics, and quantitative genetics (Golden et al., 2009). As our human population continues to grow, our world will have more limited resources of land and water, which means a large need for agriculture efficiency. There is a need to continue work in these fields, as genetic and genomic advancements may continue to improve animal health and production efficiency (Hickey et al., 2019). Topics such as cattle

marketing, disease risk, cattle background, and the advances in genetic and genomic technology, and their interconnectivity are imperative to evaluate, as research and development here may benefit the beef production industry by providing cattle producers an opportunity to raise higher quality and healthier beef cattle more efficiently.

This literature review provides an integrated look at beef cattle improvement with a systems and health-based approach, discussing the interrelationships of a broad range of aspects including beef cattle systems and health improvement with the overall goal of optimizing beef cattle production. By providing the background of systems, health advancements and genetics and genomics to allow cattle producers to match their production environments with genetic, management, and marketing opportunities for sustainable beef cattle production, we can better manage production in a more efficient manner globally. Efficient beef cattle management can be tailored to address specific regional challenges and opportunities worldwide that give beef cattle producers an opportunity to raise higher quality, healthier beef more efficiently, in order to contribute resources to an ever-growing population.

1.2. Literature Cited

Araji, A. A. (1976). The effect of vertical integration on the production efficiency of beef cattle operations. *American Journal of Agricultural Economics, 58*(1), 101-104. doi:10.2307/1238586

Bova, T. L., Chiavaccini, L., Cline, G. F., Hart, C. G., Matheny, K., Muth, A. M., . . . Memili, E. (2014). Environmental stressors influencing hormones and systems physiology in cattle. *Reproductive Biology and Endocrinology, 12*(1). doi:10.1186/1477-7827-12-58

Dethloff, N., Bir, C., & Jones, R. (2023, February 15). Cattle marketing plans: Traditional vs. direct to consumer - Oklahoma State University. Retrieved March 13, 2023, from https://extension.okstate.edu/fact-sheets/cattle-marketing-plans-traditional-vs-direct-to-consumer.html

Drouillard, J. S. (2018). Current situation and future trends for beef production in the United States of America — A Review. *Asian-Australasian Journal of Animal Sciences, 31*(7), 1007-1016. doi:10.5713/ajas.18.0428

Durmaz, A. A., Karaca, E., Demkow, U., Toruner, G., Schoumans, J., & Cogulu, O. (2015). Evolution of genetic techniques: Past, present, and beyond. *BioMed Research International, 2015*, 1-7. doi:10.1155/2015/461524

Golden, B. L., Garrick, D. J., & Benyshek, L. L. (2009). Milestones in beef cattle genetic evaluation. *Journal of Animal Science, 87*(Suppl_14). doi:10.2527/jas.2008-1430

Herring, A. D. (2014). *Beef cattle production systems*. Wallingford, Oxfordshire: CABI.

Hickey, L. T., N. Hafeez, A., Robinson, H., Jackson, S. A., Leal-Bertioli, S. C., Tester, M., . . . Wulff, B. B. (2019). Breeding crops to feed 10 billion. *Nature Biotechnology, 37*(7), 744-754. doi:10.1038/s41587-019-0152-9

CHAPTER II

REVIEW OF LITERATURE:

THE OVERALL USE OF GENETIC AND GENOMIC TECHNOLOGIES FOR BEEF CATTLE SYSTEMS FOR BEEF CATTLE HEALTH IMPROVEMENT

2.1. Marketing Strategies

There is an opportunity to market cattle in a variety of ways, depending on operational background, size, and economic feasibility. Two well-known marketing strategies for beef cattle include a “Direct Market” or “Auctioning” system (Popp and Parsch, 1998). Both are strongly associated in how cattle are sold and transported across the United States and in keeping the industry running in an efficient manner.

2.1.1. Direct Market

In a “Direct Market” these cattle are sold via directly from producer to buyer without going through any kind of intermediaries or holding (Munadi et al., 2021). Cattle that go through a direct market tend to be at lower relative risk for disease following sale transaction, because they are not facing the stress of an auction setting and are not commingling with other cattle, who could expose them to pathogens and stressors (Thomson and White, 2006; Ellis, 2013). They also are presented in a more uniform size,

have typically already been dehorned or castrated at the seller's operation with plenty of time allowed for healing prior to sale, weaning prior to sale, and are acclimated to feed bunks and water troughs. These mentioned practices are known as preconditioning and helps lower the risk of disease. (Lalman and Ward, 2005; Ives and Richeson, 2015).

2.1.2. Auction

Cattle that get sold through an "Auction" setting are those that are sent to a holding and selling facility. Auctions, sometimes referred to as "sale barns", are a means for establishing competitive bidding to attain the best prices for cattle (Robinson and Christley, 2007). Although auctions are convenient for buying, the cattle that are sold through them tend to be at a higher risk for disease. This is because when cattle go through an auction, they are typically dropped off and held in the facility before sale, where these cattle are comingling with other unknown livestock and are possibly exposed to various pathogens (Lovett, 2022; Groves, 2020). It is quite conflicting that many cattle buyers understand the risk of disease when cattle go through an auction setting, yet elect these high-risk systems due to potential economic advantages if morbidity and mortality rates are lower than anticipated and if cattle "catch up" to their low-risk counterparts because of compensatory weight gain later in the feeding system (Ives and Richeson, 2015; Smith, 2020).

2.2. Beef Cattle Systems

Beef cattle can have an upbringing in a variety of backgrounds. Beef cattle systems are defined as all cattle production systems where the purpose of the operation includes some or all of the breeding, rearing, and finishing of cattle intended for beef

consumption (Herring, 2014). Beef production systems can often be divided into four major types of operations: seedstock, cow-calf, stocker (also called “grower”), and feedlot (Drouillard, 2018). These four main systems are important in relation to beef cattle production globally.

2.2.1. Seedstock

A “seedstock” operation varies from the other three aforementioned sectors as its primary focus is producing high quality genetics and breeding animals rather that raising cattle solely for beef production (Herring, 2014). Seedstock operations produce registered (i.e., pedigree-documented) cattle typically for commercial groups or producers (Greenwood, 2021). These cattle have documented pedigrees to their respective breed and estimates of genetic merit, such as expected progeny differences or estimated breeding values (Hansen and Riley, 2006). An expected progeny difference (EPD) or estimated breeding value (EBV) is a ratio of an animal’s own records to those of relatives and progeny (Hammack and Paschal, 2009). These EPD’s also include heritability, the average part of the difference in a trait derived from transmittable genetic content (Hammack and Paschal, 2009). Producers use these values to determine breeding objectives they choose to select for (Hansen and Riley, 2006; Hammack and Paschal, 2009). The offspring from cattle purchased from seedstock herds are traditionally supplied to commercial systems, such as cow-calf operations, in order to provide specific genetically controlled characteristics into those systems (Greenwood, 2021).

2.2.2. Cow-Calf

Cow-calf operations are considered to be the first stage of the commercial beef production process (Herring, 2014). On cow-calf operations, the mothers, or "dams", are raised to produce an offspring that will eventually be sold and raised for beef production. Based on sexual maturity and breeding system and conception rates, it takes almost two-and-a-half years between the breeding of the dam, to the time the offspring is born, and finally to the time their offspring is ready for slaughter (Rae, 2006). Additionally, heifer calves that are born on the operation could be kept for herd expansion or replacements, sold to other producers as replacements, or sold to a feedlot system, fed out, and used for beef production (Felix and Freitas, 2020)

2.2.3. Stocker

A "stocker" or "grower" operation involves the weaned steer and heifer calves or yearlings which are turned out and grazed on roughages (Endres and Schwartzkopf-Genswein, 2018). These cattle are left here to gain during the growing seasons of roughages (Beck et al., 2013). After the growing season has ended, they are transported to feedlots and continue to the next stage of commercial production (Johnson et al., 2017).

2.2.4. Feedlot

The final stage of commercial beef cattle systems are "feedlots". Feedlot cattle are placed during their final stage of production, prior to shipment to an abattoir. The goal of feedlots in the United States market is to grow or fatten cattle until they reach the desired slaughter weight (Endres and Schwartzkopf-Genswein, 2018). Feedlot systems are commonly divided into two phases once the beef cattle arrive: the "backgrounding" and "finishing" phases (Endres and Schwartzkopf-Genswein, 2018; Boyles, 2019). In the

United States, the backgrounding phase is where cattle go when they first arrive at a feedlot, typically within the first 90 days after arrival (Boyles, 2019). Backgrounding specifically focuses on feeding high-forage/low-grain rations with the goal of maximizing growth while minimizing fat deposition. The finishing phase is typically the last 100 days after backgrounding, which specifically focuses on feeding high-grain/low-forage rations to calves or yearlings until they reach a prescribed finish or fat cover, then are marketed for slaughter (Phillips, 2001; Endres and Schwartzkopf-Genswein, 2018; McAllister et al., 2020).

2.2.5. Feed intake and efficiency

Feed intake and growth efficiency are important during the time that cattle are placed within a feedlot setting. Feed intake is a calculation of the amount of feedstuff consumed by an individual or pen of cattle over a specified amount of time (Lahart et al., 2020). Feed efficiency as a concept is feeding cattle in a manner that increases producer profitability and lowers the environmental footprint at the same time (Kenny et al., 2018). When evaluating feed efficiency and if cattle are consuming a ration efficiently, producers often calculate an individual calf's residual feed intake or RFI (Elolimy et al., 2018). A RFI is the difference between a calf's actual and predicted feed intake, based on the individual's weight and growth (Kenny et al., 2018). The RFI equation when calculated via a linear regression is used to determine the feed intake of a given group of animals on a given diet. The residual in the model, the RFI, finds differences in efficiency (Kenny et al., 2018). Animals with a negative value of RFI have an intake that is less than what is predicted for the group and are found to be more efficient having consumed less and still gained body weight (Martin et al., 2021). Cattle with a positive RFI are those

considered to be less efficient (Martin et al., 2021). Ultimately, RFI is an important tool for beef cattle systems because it directs producers as to which individuals or pens to finish choose to reproduce which are more efficient (Puillet et al., 2016).

2.3. Animal History

Depending on how cattle travel through these different commercial operations, they have a different likelihood of disease and virus risk throughout the systems, therefore cattle are put into the categories of high or low risk for exposure to any disease (Groves, 2020). When dealing with cattle and their background, typically it is divided into two categories: high risk and low risk cattle (Credille, 2022). Within these two risk categories, are the markets which they come from, and are strongly associated in what risk category they are classified as.

2.3.1. High-Risk Cattle

High-risk cattle are defined as individuals with little-to-no information as well as unknown vaccination backgrounds or history (Laborie, 2018). Along with this there are also risk factors that contribute to these high-risk associations such as bacterial and viral pathogens, the level of host immunity, and environmental conditions that favor transmission of pathogens and susceptibility from the conditions of their upbringing (Smith, 2020). These types of cattle are high risk as we do not know how they will react to types of environments since it is unknown if prevention could have occurred before cattle were obtained (Sjeklocha, 2019), because of this they are more likely to be exposed to pathogens and stressors, one of the most prevalent exposures in the industry being bovine respiratory disease (BRD) (Groves, 2020). These high-risk cattle will typically be

lighter weight upon arrival to feeding operations, compared to their low-risk cohorts who tend to gain more weight (Word et al., 2020). Calves with a stressed disposition have significantly less market value than docile calves, due largely to depressed performance and less carcass value (Smith, 2009). Although if they remain healthy after purchase, high-risk cattle may provide large economic returns due to maximizing feed conversion (Eibergen, 2022).

2.3.2. Low-Risk Cattle

Low-risk cattle are those less likely to be previously exposed to pathological components prior to arrival at a feeding operation. Broadly, low-risk cattle are placed in this categorization due to their prior knowledge regarding background, have been previously vaccinated against respiratory and *Clostridium*-based diseases, and have risk management strategies, such as feed bunk and water trough acclimation (Ellis, 2013). Low-risk cattle also include calves handled under minimum stress, or ones that have been weaned and backgrounded for more than 45 days in order to prepare calves for their next stage of production (Gaspers, 2021). Due to these preconditioning strategies and prior risk management systems, low risk cattle may be more expensive at the time of marketing when compared to their high-risk cohorts (Credille, 2022)

2.4. Disease Risk

Disease risk is an important factor to any segment of the beef cattle industry. This is because disease control must be part of any system in the industry not only where improved net returns from the cattle but also concern for animal welfare are the constant goal (Campbell, 1989). When beef cattle become infected with disease, the morbidity and

mortality rate of their pen mates or cohorts is going to drastically increase (Kelly and Janzen, 1986). When these rates increase, the profit of the producer's herd dramatically decreases (Tambi et al., 2006). This is directly due to the added costs of treatment for disease and, when occurring, mortality events. Along with the cost of disease, the concern for animal welfare is another factor as freedom from disease is an aspect of animal welfare (Stokstad et al., 2020; Hernandez et al., 2022).

2.4.1. Bovine Respiratory Disease

Regardless of risk categorization, cattle continue to face the challenge of BRD (Hilton, 2014; Credille, 2022). Today, BRD continues to be one of the most important yet least misdiagnosed diseases within the cattle industry (Lubbers and Hanzlicek, 2013). Ultimately, BRD within the United States it is extremely costly, dealing with estimated losses of almost $23.60 per treated head. (USDA, 2011). There are a vast number of factors that go into causing the disease, such as exposure to pathogenic components and environment conditions (Guadino, 2022).Furthermore, BRD is a multifactorial disease complex, with multiple pathogenic components, host risk factors, and component cases (Padalino et al., 2021). The primary viral pathogens associated with BRD include bovine herpesvirus type 1 (BHV-1), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), and parainfluenza-3virus (P13) (Czuprynski, 2009; Ellis, 2009; Taylor et al., 2010; Brodersen, 2010; Mosier, 2014). The primary bacterial pathogens of BRD include *Mannheimia haemolytica*, *Pasteurella multocida* , *Histophilus somni* and *Mycoplasma bovis* (Angen et al., 2009; Fulton et al., 2009; Hotchkiss et al., 2010). Additionally, these pathogens have been shown to interact with one another and weaken the animal's immune system which eventually culminates into severe clinical disease

(Smith and Sweet, 2014). Understanding cattle background can likely help detect where any beef cattle disease originated and how to treat it more effectively (Apley, 2014). As previously discussed, cattle derived from high-risk backgrounds are more likely to develop BRD, compared to their low-risk cohorts (Hilton, 2014). A study by Brualt et al. (2019), treatment and BRD incidence between high- and low-risk beef cattle on feedlots in Western Canada were compared, risk of BRD development was influenced antimicrobial drug exposures; this study found that 95% of cattle categorized as being high-risk for developing BRD receiving antimicrobial drugs compared to 59% of cattle categorized as being low-risk for developing BRD (Brualt, 2019). In relation to beef cattle health and welfare there are many genetic and genomic advances that can not only help improve herd health, but the herd's heritability of desired traits as well.

2.5. Genetic and Genomic Technologies

With the development of genetic and genomic resources, we can understand and utilize these beef cattle advances more efficiently. Genetic and genomic prediction is now an essential technology for genetic improvement in not only animal breeding, but in plant breeding and other areas of agriculture (Salgotra and Stewart, 2020). In the past, researchers have placed more focus on predicting the breeding values and heritability, but now being able to look at the non-additive genetic components, such as the interaction between genes, is becoming a larger interest (Su et al., 2012; Onogi et al., 2021). Significant advances in agriculture remain extremely important to increase production and quality to not only satisfy the global food demand, but also help contribute to the world's food production (Boyles et al., 2018). These advances in beef production are

achievable via genetic and genomic advancements and improvement (Hickey et al., 2019).

2.5.1. Assisted Reproductive Technologies and Genetics

There is an opportunity to improve beef cattle genetics using the given genetics or heritability from high quality bovine dams and sires. This is typically utilized by the seedstock industry, whose main goal is to multiply their elite genetics (Herring, 2014). Being able to multiply these genetics via reproduction techniques has become a large part of beef production systems. Embryo biotechnology has become one of the most prominent fields when pertaining to reproductive techniques (Ferré et al., 2020). This technology has evolved through three major times of production techniques. Embryo biotechnology, better known as the use of embryo transfers or somatic cell transfers, have widely been used in the beef cattle industry for the use of reproducing high quality traits or genetics (Smith, 1989). These production techniques include traditional embryo transfer which is typically done in vivo embryo production by donor superovulation (Bortoletto et al., 2018), *in vitro* embryo production via ovum pick up with *in vitro* fertilization, also known as IVF (First and Parrish, 2019), and cloning via somatic cell nuclear transfer and transgenic animal production (Wu and Zan, 2011).

2.5.2. Cloning

Cloning is specifically producing a twin of an animal at a different time (Shelton, 1990). This is often performed via somatic cell transfer, where somatic cell nuclear transfer offers the potential for relatively easy and low-cost production of clones while copying their DNA or deoxyribonucleic acid (Taylor-Robinson et al., 2014). Uses in

agriculture include many applications of the technology to improve certain areas, one of which includes making genetic copies of high-quality seedstock and/or show cattle. The reason for this is because breeders wish to replicate high-quality genetics or prize-winning cattle, in hopes to produce another (Faber et al., 2004). Other purposes may be to make a copy of a certain dam or sire that has sentimental value, similar to cloning of pets (Faber et al., 2004). The new opportunities with advancements given with cloning may provide for improvement in genetic gain. The ultimate goal of cloning has often been envisioned as a system for producing uniformity of the ideal beef cow (Van Vleck, 1999). Performing these methods can result in cloned offspring that are economically competitive or the same as other elite beef cattle that are produced by more traditional means (Faber et al., 2004). Although the cloned offspring may not be exactly the same due to difference in environment and phenotypical factors that the original individual was raised in, producers still hope for a similar outcome (Faber et al., 2004; Conover, 2019).

2.5.3. Genomics and RNA-Sequencing

Along with using DNA as improvement in beef cattle, we can utilize RNA and genomic sequencing to improve them as well. RNA sequencing (RNA-seq) can be performed to run transcriptome analyses on beef cattle and analyze their genomic factors. This is performed to determine what specific genes of cattle are being regulated and what biological pathways and mechanisms are influenced. RNA is a type of nucleic acid majorly involved in the gene expression, gene regulation, and coding of information. RNA is sequenced instead of DNA for transcriptome analysis as it lets us see what a cell organism is actually doing in that moment (Li et al., 2011). More specifically, the sequencing of mature messenger RNA (polyadenylated mRNA) allows for the relative

quantification of genes involved in the direct translation and eventual synthesis of specific proteins. It is found that approximately 4% of a sampled RNA pool consists of mRNA while the rest are considered to be non-coding RNAs (Buccitelli & Selbach, 2020; Chauhan, 2020). Messenger RNA has been the main focus of gene translation over the years, whereas the discovery of non-coding RNA for the same use is more recent (Dinger et al., 2008). There are beginning to be labs that study non-coding RNA and miRNA in beef cattle, yet still much is to be discovered in their reasoning to specific pathways and mechanisms (Kosinska-Selbi et al., 2020; Johnston et al., 2021). There are different methods in collecting RNA such as saliva, tissue, and whole blood collections (Pandit et al., 2013; Kukurba and Montgomery, 2015; Tan et al., 2018; Harrington et al., 2020). The opportunity to study entire transcriptomes in great detail using RNA sequencing has inspired and helped find many important discoveries, making it now an important and regular method in research for both humans and animals (Olsen and Baryawno, 2018). For example, in dairy cattle using RNA-Sequencing technology can provide measurements of transcript levels associated with the immune response to the infection of Mastitis (Asselstine et al., 2019).The analysis of beef cattle transcriptomes and its expressions are essential to extend the genetic information resources and more importantly support further studies on beef cattle which could help with the advancements in the industry and the animals health (Chen et al., 2014).

2.5.4. Transcriptome Analysis

We can use transcriptome analysis to determine gene expression related to certain molecular pathways and mechanisms in relation to disease outcomes (Wang et al., 2009, Huang et al., 2018). Investigations using transcriptomics and evaluating cattle acquiring

disease, in real-world settings, can help dictate the health status of livestock and can be done via gene expression (Raszek et al., 2016). The goal of evaluating differentially expressed genes (DEGs) is to determine which genes are expressed at different levels between conditions (McDermaid et al., 2018). These genes can offer biological insight into the different genes affected by the condition of interest. (Conesa et al., 2016). Lists of genes that differ between two sample sets are often provided by RNA-sequencing data analysis tools, or can be generated manually by statistical testing of data sets (Chen and Wong, 2019). Genomic advancements can help scientists understand topics such as animal health and genetics from a more detailed standpoint.

2.6. Conclusions

These discussed topics that include cattle marketing, disease risk, cattle background, and the advances in genetic and genomic technology, and how they are all related are important to understand for us and future research, as our industry progresses, challenges will continue to rise. The use of providing the background of systems, health improvements and genetics and genomics, can allow cattle producers to match their production environments with genetic, management, and marketing opportunities for sustainable beef production globally.

This logic and resulting considerations can then be tailored to address specific regional challenges and opportunities worldwide that give beef cattle producers an opportunity to raise higher quality, healthier beef more efficiently. In an ever-changing world, it is important to utilize these advancements to ensure that our human population is fed and that agriculture can stay sustainable for the many years to come.

2.7. Literature Cited

Angen, Ø, Thomsen, J., Larsen, L. E., Larsen, J., Kokotovic, B., Heegaard, P. M., & Enemark, J. M. (2009). Respiratory disease in calves: Microbiological investigations on trans-tracheally aspirated bronchoalveolar fluid and acute phase protein response. *Veterinary Microbiology, 137*(1-2), 165-171. doi:10.1016/j.vetmic.2008.12.024

Apley, M. (2014). The clinical syndrome of BRD: What it is and what it is not. *Animal Health Research Reviews, 15*(2), 135-137. doi:10.1017/s1466252314000152

Asselstine, V., Miglior, F., Suárez-Vega, A., Fonseca, P., Mallard, B., Karrow, N., . . . Cánovas, A. (2019). Genetic mechanisms regulating the host response during mastitis. *Journal of Dairy Science, 102*(10), 9043-9059. doi:10.3168/jds.2019-16504

Beck, P. A., Anders, M., Watkins, B., Gunter, S. A., Hubbell, D., & Gadberry, M. S. (2013). 2011 and 2012 Early Careers Achievement Awards: Improving the production, environmental, and economic efficiency of the Stocker Cattle Industry in the southeastern United States1,2. *Journal of Animal Science, 91*(6), 2456-2466. doi:10.2527/jas.2012-5873

Bortoletto, P., Bakkensen, J., & Anchan, R. M. (2018). Embryo transfer: Timing and techniques. *Minerva Endocrinology, 43*(1). doi:10.23736/s0391-1977.17.02649-9

Boyles, R. E., Brenton, Z. W., & Kresovich, S. (2018). Genetic and genomic resources of sorghum to connect genotype with phenotype in contrasting environments. *The Plant Journal, 97*(1), 19-39. doi:10.1111/tpj.14113

Boyles, S. (2019, August 26). Backgrounding; a phase of growing calves in preparation for the feedlot. Retrieved March 13, 2023, from https://u.osu.edu/beef/2019/09/04/backgrounding-a-phase-of-growing-calves-in-preparation-for-the-feedlot/

Brault, S. A., Hannon, S. J., Gow, S. P., Warr, B. N., Withell, J., Song, J., . . . Morley, P. S. (2019). Antimicrobial use on 36 beef feedlots in Western Canada: 2008–2012. *Frontiers in Veterinary Science, 6*. doi:10.3389/fvets.2019.00329

Brodersen, B. W. (2010). Bovine respiratory syncytial virus. *Veterinary Clinics of North America: Food Animal Practice, 26*(2), 323-333. doi:10.1016/j.cvfa.2010.04.010

Buccitelli, C., & Selbach, M. (2020). MRNAs, proteins and the emerging principles of gene expression control. *Nature Reviews Genetics, 21*(10), 630-644. doi:10.1038/s41576-020-0258-4

Campbell, R. (1989). Future challenges of disease in the beef cattle industry. *Australian Veterinary Journal, 66*(8), 252-257. doi:10.1111/j.1751-0813.1989.tb13583.x

Chauhan, D. (2020, January 06). RNA sequencing- principle, steps, methods and applications . Retrieved March 14, 2023, from https://geneticeducation.co.in/rna-sequencing-principle-steps-methods-and-applications/

Chen, D., Li, W., Du, M., Wu, M., & Cao, B. (2014). Sequencing and characterization of divergent marbling levels in the beef cattle (*longissimus dorsi* muscle) transcriptome. *Asian-Australasian Journal of Animal Sciences, 28*(2), 158-165. doi:10.5713/ajas.14.0394

Chen, L., & Wong, G. (2019). Transcriptome Informatics. *Encyclopedia of Bioinformatics and Computational Biology,* 324-340. doi:10.1016/b978-0-12-809633-8.20204-5

Cohen, J. E. (2003). Human population: The next half century. *Science, 302*(5648), 1172-1175. doi:10.1126/science.1088665

Conesa, A., Madrigal, P., Tarazona, S., Gomez-Cabrero, D., Cervera, A., McPherson, A., Michał Wojciech Szcześniak, Daniel J Gaffney, Laura L Elo, Xuegong Zhang, Ali Mortazavi (2016). A survey of best practices for RNA-seq data analysis. *Genome Biology, 17*(1). doi:10.1186/s13059-016-0881-8

Conover, E. (2019, February 07). Immune system shaped by environment more than genes. Retrieved March 29, 2023, from https://www.nih.gov/news-events/nih-research-matters/immune-system-shaped-environment-more-genes

Credille, B. (2022). High-risk cattle management and Stocker Calf Health. *Veterinary Clinics of North America: Food Animal Practice, 38*(2), 229-243. doi:10.1016/j.cvfa.2022.03.001

Czuprynski, C. J. (2009). Host response to bovine respiratory pathogens. *Animal Health Research Reviews, 10*(2), 141-143. doi:10.1017/s1466252309990181

Dinger, M. E., Pang, K. C., Mercer, T. R., & Mattick, J. S. (2008). Differentiating protein-coding and noncoding RNA: Challenges and ambiguities. *PLoS Computational Biology, 4*(11). doi:10.1371/journal.pcbi.1000176

Drouillard, J. S. (2018). Current situation and future trends for beef production in the United States of America — A Review. *Asian-Australasian Journal of Animal Sciences, 31*(7), 1007-1016. doi:10.5713/ajas.18.0428

Eibergen, C. (2022, September 01). Starting high risk cattle on feed - form-A-feed. Retrieved February 27, 2023, from https://formafeed.com/starting-high-risk-cattle/

Ellis, J. A. (2009). Update on viral pathogenesis in BRD. *Animal Health Research Reviews, 10*(2), 149-153. doi:10.1017/s146625230999020x

Ellis, R. (2013, July 2). BRD risk categorization for feeder calves: Part 1. Retrieved February 27, 2023, from https://www.thebeefsite.com/articles/3616/brd-risk-categorization-for-feeder-calves-part-1/

Elolimy, A. A., Abdelmegeid, M. K., McCann, J. C., Shike, D. W., & Loor, J. J. (2018). Residual feed intake in beef cattle and its association with carcass traits, ruminal solid-fraction bacteria, and epithelium gene expression. *Journal of Animal Science and Biotechnology, 9*(1). doi:10.1186/s40104-018-0283-8

Endres, M. I., & Schwartzkopf-Genswein, K. (2018). Overview of cattle production systems. *Advances in Cattle Welfare,* 1-26. doi:10.1016/b978-0-08-100938-3.00001-2

Faber, D., Ferre, L., Metzger, J., Robl, J., & Kasinathan, P. (2004). Review: Agro-economic impact of cattle cloning. *Cloning and Stem Cells, 6*(2), 198-207. doi:10.1089/1536230041372463

Ferré, L., Kjelland, M., Strøbech, L., Hyttel, P., Mermillod, P., & Ross, P. (2020). Review: Recent advances in bovine in vitro embryo production: Reproductive Biotechnology History and Methods. *Animal, 14*(5), 991-1004. doi:10.1017/s1751731119002775

First, N. L., & Parrish, J. J. (2019). In-vitro fertilization of ruminants. *Bioscientifica Proceedings*. doi:10.1530/biosciprocs.9.012

Fulton, R. W., Blood, K. S., Panciera, R. J., Payton, M. E., Ridpath, J. F., Confer, A. W., . . . Reck, A. (2009). Lung pathology and infectious agents in fatal feedlot pneumonias and relationship with mortality, disease onset, and treatments. *Journal of Veterinary Diagnostic Investigation, 21*(4), 464-477. doi:10.1177/104063870902100407

Gaspers, J. (2021, October 5). Receiving management for high-risk feedlot cattle. Retrieved March 13, 2023, from https://www.hubbardfeeds.com/blog/receiving-management-high-risk-feedlot-cattle

Gaudino, M., Nagamine, B., Ducatez, M. F., & Meyer, G. (2022). Understanding the mechanisms of viral and bacterial coinfections in bovine respiratory disease: A comprehensive literature review of experimental evidence. *Veterinary Research, 53*(1). doi:10.1186/s13567-022-01086-1

Greenwood, P. L. (2021). Review: An overview of beef production from pasture and feedlot globally, as demand for beef and the need for sustainable practices increase. *Animal, 15*, 100295. doi:10.1016/j.animal.2021.100295

Groves, J. T. (2020). Details to attend to when managing high-risk cattle. *Veterinary Clinics of North America: Food Animal Practice, 36*(2), 445-460. doi:10.1016/j.cvfa.2020.02.005

Hammack, S. P., & Paschal, J. C. (2009, September). Texas adapted genetic strategies for Beef Cattle VIII: Expected progeny difference (EPD). Retrieved February 27, 2023, from https://agrilifeextension.tamu.edu/asset-external/texas-adapted-genetic-strategies-for-beef-cattle-viii-expected-progeny-difference-epd/

Hansen, G. R., & Riley, D. G. (2006). Expected progeny differences (epds) in beef cattle. *EDIS, 2006*(20). doi:10.32473/edis-an164-2006

Harrington, C. A., Fei, S. S., Minnier, J., Carbone, L., Searles, R., Davis, B. A., . . . Choi, D. (2020). RNA-seq of human whole blood: Evaluation of globin RNA depletion on Ribo-Zero Library Method. *Scientific Reports, 10*(1). doi:10.1038/s41598-020-62801-6

Hernandez, E., Llonch, P., & Turner, P. V. (2022). Applied Animal Ethics in Industrial Food Animal Production: Exploring the role of the veterinarian. *Animals, 12*(6), 678. doi:10.3390/ani12060678

Herring, A. D. (2014). *Beef cattle production systems*. Wallingford, Oxfordshire: CABI.

Hickey, L. T., N. Hafeez, A., Robinson, H., Jackson, S. A., Leal-Bertioli, S. C., Tester, M., . . . Wulff, B. B. (2019). Breeding crops to feed 10 billion. *Nature Biotechnology, 37*(7), 744-754. doi:10.1038/s41587-019-0152-9

Hilton, W. (2014, April). Control and Prevention of Disease in feedlot cattle - management and Nutrition. Retrieved March 29, 2023, from https://www.msdvetmanual.com/management-and-nutrition/health-management-interaction-beef-cattle/control-and-prevention-of-disease-in-feedlot-cattle

Hilton, W. M. (2014). BRD in 2014: Where have we been, where are we now, and where do we want to go? *Animal Health Research Reviews, 15*(2), 120-122. doi:10.1017/s1466252314000115

Hoar, B., & Angelos, J. (2015). *Beef Cattle Production*. Reading.

Hotchkiss, E. J., Dagleish, M. P., Willoughby, K., McKendrick, I. J., Finlayson, J., Zadoks, R. N., . . . Hodgson, J. C. (2010). Prevalence of*pasteurella multocida*and other respiratory pathogens in the nasal tract of Scottish calves. *Veterinary Record, 167*(15), 555-560. doi:10.1136/vr.c4827

Huang, Q., Sun, M., & Yan, P. (2018). Pathway and network analysis of differentially expressed genes in transcriptomes. *Methods in Molecular Biology,* 35-55. doi:10.1007/978-1-4939-7710-9_3

Ives, S. E., & Richeson, J. T. (2015). Use of antimicrobial metaphylaxis for the control of bovine respiratory disease in high-risk cattle. *Veterinary Clinics of North America: Food Animal Practice, 31*(3), 341-350. doi:10.1016/j.cvfa.2015.05.008

Johnson, R. J., Doye, D., Lalman, D. L., Peel, D. S., & Raper, K. C. (2017, February 01). Stocker Cattle Production and Management Practices in Oklahoma - oklahoma State University. Retrieved February 27, 2023, from https://extension.okstate.edu/fact-sheets/stocker-cattle-production-and-management-practices-in-oklahoma.html

Johnston, D., Earley, B., McCabe, M. S., Kim, J., Taylor, J. F., Lemon, K., . . . Waters, S. M. (2021). Elucidation of the host bronchial lymph node MIRNA transcriptome response to bovine respiratory syncytial virus. *Frontiers in Genetics, 12*. doi:10.3389/fgene.2021.633125

Kelly, A. P., & Janzen, E. D. (1986, December 27). A Review of Morbidity and Mortality Rates and Disease Occurrence in North American Feedlot Cattle.

Kenny, D., Fitzsimons, C., Waters, S., & McGee, M. (2018). Invited review: Improving feed efficiency of beef cattle – the current state of the art and future challenges. *Animal, 12*(9), 1815-1826. doi:10.1017/s1751731118000976

Kosinska-Selbi, B., Mielczarek, M., & Szyda, J. (2020). Review: Long non-coding RNA in Livestock. *Animal, 14*(10), 2003-2013. doi:10.1017/s1751731120000841

Kosinska-Selbi, B., Mielczarek, M., & Szyda, J. (2020). Review: Long non-coding RNA in Livestock. *Animal, 14*(10), 2003-2013. doi:10.1017/s1751731120000841

Kukurba, K. R., & Montgomery, S. B. (2015). RNA sequencing and analysis. *Cold Spring Harbor Protocols, 2015*(11). doi:10.1101/pdb.top084970

Laborie, E. (2018, October 1). Recognizing and managing differences in health risk of incoming feeder cattle. Retrieved February 27, 2023, from https://beef.unl.edu/beefwatch/recognizing-and-managing-differences-health-risk-incoming-feeder-cattle

Lahart, B., Prendiville, R., Buckley, F., Kennedy, E., Conroy, S., Boland, T., & McGee, M. (2020). The repeatability of feed intake and feed efficiency in beef cattle offered high-concentrate, grass silage and pasture-based diets. *Animal, 14*(11), 2288-2297. doi:10.1017/s1751731120000853

Lalman, D., & Ward, C. E. (2005). Effects of preconditioning on health, performance and prices of weaned calves. *American Association of Bovine Practitioners Conference Proceedings,* 44-50. doi:10.21423/aabppro20054792

Li, M., Wang, I. X., Li, Y., Bruzel, A., Richards, A. L., Toung, J. M., & Cheung, V. G. (2011). Widespread RNA and DNA sequence differences in the human transcriptome. *Science, 333*(6038), 53-58. doi:10.1126/science.1207018

Lovett, J. (2022, December 19). Study shows no negative health effects on limit-fed diet in 'high-risk' cattle. Retrieved February 27, 2023, from https://aaes.uada.edu/news/limit-fed-diet-study/

Lubbers, B. V., & Hanzlicek, G. A. (2013). Antimicrobial multidrug resistance and coresistance patterns of *mannheimia haemolytica* isolated from bovine respiratory disease cases—a three-year (2009–2011) retrospective analysis. *Journal of Veterinary Diagnostic Investigation, 25*(3), 413-417. doi:10.1177/1040638713485227

Martin, P., Ducrocq, V., Faverdin, P., & Friggens, N. C. (2021). Invited review: Disentangling residual feed intake—insights and approaches to make it more fit for purpose in the modern context. *Journal of Dairy Science, 104*(6), 6329-6342. doi:10.3168/jds.2020-19844

McAllister, T. A., Stanford, K., Chaves, A. V., Evans, P. R., Eustaquio de Souza Figueiredo, E., & Ribeiro, G. (2020). Nutrition, feeding and management of beef cattle in intensive and extensive production systems. *Animal Agriculture,* 75-98. doi:10.1016/b978-0-12-817052-6.00005-7

McDermaid, A., Monier, B., Zhao, J., Liu, B., & Ma, Q. (2018). Interpretation of differential gene expression results of RNA-Seq Data: Review and Integration. *Briefings in Bioinformatics, 20*(6), 2044-2054. doi:10.1093/bib/bby067

Mosier, D. (2014). Review of BRD pathogenesis: The old and the new. *Animal Health Research Reviews, 15*(2), 166-168. doi:10.1017/s1466252314000176

Munadi, L., Rahim Aka, La Ode Muh Munadi, Razak Ali, Muhammad Amrullah Pagala, & Syamsuddin. (2021). Marketing analysis of beef cattle in Landono and Mowila subdistricts of South Konawe Regency. *International Journal of Science, Technology & Management, 2*(3), 747-754. doi:10.46729/ijstm.v2i3.194

National Animal Health Monitoring System. (n.d.). Retrieved February 21, 2023, from https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/monitoring-and-surveillance/nahms

Olsen, T. K., & Baryawno, N. (2018). Introduction to single-cell RNA sequencing. *Current Protocols in Molecular Biology, 122*(1). doi:10.1002/cpmb.57

Onogi, A., Watanabe, T., Ogino, A., Kurogi, K., & Togashi, K. (2021). Genomic prediction with non-additive effects in beef cattle: Stability of variance component

and genetic effect estimates against population size. *BMC Genomics, 22*(1). doi:10.1186/s12864-021-07792-y

Padalino, B., Cirone, F., Zappaterra, M., Tullio, D., Ficco, G., Giustino, A., . . . Pratelli, A. (2021). Factors affecting the development of bovine respiratory disease: A cross-sectional study in beef steers shipped from France to Italy. *Frontiers in Veterinary Science, 8*. doi:10.3389/fvets.2021.627894

Pandit, P., Cooper-White, J., & Punyadeera, C. (2013). High-yield RNA-extraction method for saliva. *Clinical Chemistry, 59*(7), 1118-1122. doi:10.1373/clinchem.2012.197863

Phillips C. J. C. (2001). Principles of cattle production. CABI Pub. Retrieved April 26 2023 from https://search.ebscohost.com/login.aspx?direct=true&scope=site&db=nlebk&db=nlabk&AN=121993.

Popp, M., & Parsch, L. (1998, January 1). Marketing practices of Arkansas beef cattle producers. Retrieved February 27, 2023, from https://scholarworks.uark.edu/aaesrb/39/

PSU. (2020, April 6). Beef cow-calf production. Retrieved February 27, 2023, from https://extension.psu.edu/beef-cow-calf-production

Puillet, L., Réale, D., & Friggens, N. C. (2016). Disentangling the relative roles of resource acquisition and allocation on animal feed efficiency: Insights from a dairy cow model. *Genetics Selection Evolution, 48*(1). doi:10.1186/s12711-016-0251-8

Rae, D. O. (2006). Assessing performance of cow-calf operations using epidemiology. *Veterinary Clinics of North America: Food Animal Practice, 22*(1), 53-74. doi:10.1016/j.cvfa.2005.11.001

Raszek, M. M., Guan, L. L., & Plastow, G. S. (2016). Use of genomic tools to improve cattle health in the context of infectious diseases. *Frontiers in Genetics, 7*. doi:10.3389/fgene.2016.00030

Robinson, S., & Christley, R. (2007). Exploring the role of auction markets in cattle movements within Great Britain. *Preventive Veterinary Medicine, 81*(1-3), 21-37. doi:10.1016/j.prevetmed.2007.04.011

Salgotra, R. K., & Stewart, C. N. (2020). Functional markers for Precision Plant Breeding. *International Journal of Molecular Sciences, 21*(13), 4792. doi:10.3390/ijms21134792

Shelton, J. (1990). Reproductive technology in Animal Production. *Revue Scientifique Et Technique De L'OIE, 9*(3), 825-845. doi:10.20506/rst.9.3.521

Sjeklocha, D. B. (2019, February). Receiving and managing high-risk cattle in the feedyard. *Receiving and Managing High-risk Cattle in the Feedyard*. American Association of Bovine Practitioners open access distribution

Smith, C. (1989). Cloning and genetic improvement of beef cattle. *Animal Science, 49*(1), 49-62. doi:10.1017/s0003356100004244

Smith, D. R. (2020). Risk factors for bovine respiratory disease in beef cattle. *Animal Health Research Reviews, 21*(2), 149-152. doi:10.1017/s1466252320000110

Smith, H., & Sweet, C. (2014). Cooperation between viral and bacterial pathogens in causing human respiratory disease. *Polymicrobial Diseases,* 199-212. doi:10.1128/9781555817947.ch11

Smith, R. A. (2009). North American cattle marketing and bovine respiratory disease (BRD). *Animal Health Research Reviews, 10*(2), 105-108. doi:10.1017/s1466252309990107

Stokstad, M., Klem, T. B., Myrmel, M., Oma, V. S., Toftaker, I., Østerås, O., & Nødtvedt, A. (2020). Using biosecurity measures to combat respiratory disease in cattle: The Norwegian control program for bovine respiratory syncytial virus and bovine coronavirus. *Frontiers in Veterinary Science, 7*. doi:10.3389/fvets.2020.00167

Su, G., Christensen, O. F., Ostersen, T., Henryon, M., & Lund, M. S. (2012). Estimating additive and non-additive genetic variances and predicting genetic merits using genome-wide dense single nucleotide polymorphism markers. *PLoS ONE, 7*(9). doi:10.1371/journal.pone.0045293

Tambi, N., Maina, O., Ndi, C., & Mewes, L. (2006). An estimation of the economic impact of contagious bovine pleuropneumonia in Africa. *OIE Scientific and Technical Review, 25*(3), 999-1011. doi:10.20506/rst.25.3.1710

Tan, P., Pepin, É, & Lavoie, J. L. (2018). Mouse adipose tissue collection and processing for RNA analysis. *Journal of Visualized Experiments,* (131). doi:10.3791/57026

Taylor, J. D., Fulton, R. W., Lehenbauer, T. W., Step, D. L., & Confer, A. W. (2010). The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors?. The Canadian veterinary journal = La revue veterinaire canadienne, 51(10), 1095–1102.

Taylor-Robinson, A. W., Walton, S., Swain, D. L., Walsh, K. B., & Vajta, G. (2014). The potential for modification in cloning and vitrification technology to enhance genetic progress in beef cattle in northern Australia. *Animal Reproduction Science, 148*(3-4), 91-96. doi:10.1016/j.anireprosci.2014.06.004

Thomson, D. U., & White, B. J. (2006). Backgrounding Beef Cattle. *Veterinary Clinics of North America: Food Animal Practice, 22*(2), 373-398. doi:10.1016/j.cvfa.2006.03.010

Vleck, L. D. (1999). Implications of cloning for breed improvement strategies: Are traditional methods of animal improvement obsolete? *Journal of Animal Science, 77*(Suppl_2), 111. doi:10.2527/1999.77suppl_2111x

Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-seq: A revolutionary tool for transcriptomics. *Nature Reviews Genetics, 10*(1), 57-63. doi:10.1038/nrg2484

Word, A., Wickersham, T., Trubenbach, L., Mays, G., & Sawyer, J. (2020). Effects of metaphylaxis on production responses and total antimicrobial use in high-risk beef calves. *Applied Animal Science, 36*(2), 265-270. doi:10.15232/aas.2019-01914

Wu, B., & Zan, L. (2011). Enhance beef cattle improvement by embryo biotechnologies. *Reproduction in Domestic Animals, 47*(5), 865-871. doi:10.1111/j.1439-0531.2011.01945.x

CHAPTER III

INFLUENCE OF THE AT-ARRIVAL HOST TRANSCRIPTOME ON BOVINE RESPIRATORY DISEASE INCIDENCE DURING BACKGROUNDING

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3.1. Abstract

Bovine respiratory disease (BRD) remains the leading disease within the U.S. beef cattle industry. Marketing decisions made prior to backgrounding may shift BRD incidence into a different phase of production, and the importance of host gene expression on BRD incidence as it relates to marketing strategy is poorly understood. Our objective was to compare the influence of marketing on host transcriptomes measured on arrival at a backgrounding facility on the subsequent probability of being treated for BRD during a 45-day backgrounding phase. This study, through RNA-Seq analysis of blood samples collected on arrival, evaluated gene expression differences between cattle which experienced a commercial auction setting (AUCTION) versus cattle directly shipped to backgrounding from the cow–calf phase (DIRECT); further analyses were conducted to determine differentially expressed genes (DEGs) between cattle which remained

clinically healthy during backgrounding (HEALTHY) versus those that required treatment for clinical BRD within 45 days of arrival (BRD). A profound difference in DEGs (n = 2961) was identified between AUCTION cattle compared to DIRECT cattle, regardless of BRD development; these DEGs encoded for proteins involved in antiviral defense (increased in AUCTION), cell growth regulation (decreased in AUCTION), and inflammatory mediation (decreased in AUCTION). Nine and four DEGs were identified between BRD and HEALTHY cohorts in the AUCTION and DIRECT groups, respectively; DEGs between disease cohorts in the AUCTION group encoded for proteins involved in collagen synthesis and platelet aggregation (increased in HEALTHY). Our work demonstrates the clear influence marketing has on host expression and identified genes and mechanisms which may predict BRD risk.

3.2. Introduction

Bovine respiratory disease (BRD) continues to be one of the leading disease complexes in cattle production in terms of cost, morbidity, and mortality. A previous USDA NAHMS surveillance study in 2011 on U.S. feedlots estimated that BRD costs producers $23.60 per treated animal, with over 16% of cattle placed in feedlots requiring at least one antimicrobial treatment for BRD (Dargatz, 2011; USDA, 2011). While BRD has been a priority area of health and disease research for the past several decades, it remains a persistent issue within the U.S. beef industry, with recent studies suggesting a worsening rate of morbidity despite advancements in management schemes, vaccination and therapeutic technologies, and field-level diagnostics (Snowder 2006; Wilson et al., 2017; Blakebrough-Hall et al., 2020). Consequently, the highly dynamic biology related

to BRD and inconsistencies in beef management systems across the U.S. creates difficulties in early detection and risk assessment (Woods et al., 1973; Ribble et al., 1995; Sanderson et al., 2008).

Bovine respiratory disease is often defined as an undifferentiated respiratory disease complex (McGill and Sacco, 2020). This is primarily due to the multifaceted biology of BRD, including pathogenic interactions, immunological response, and environmental conditions that impact the host (Mosier, 2014; Smith, 2020). Prominently, the beef cattle industry uses the nature and associated risk of these independent components, such as prior animal history, which includes previous administration of vaccines, sale and purchasing records, and commingling and sourcing status, to create a broad categorization of cattle populations into relative risk groups (Gummow and Mapham, 2000; Step, 2008) . Moreover, beef cattle producers often add value to calves by maintaining health records, adding weight prior to sale to a feedlot, improving uniformity within marketed groups, and preconditioning calves for a feedlot setting (Schneider et al., 2009; Taylor et al., 2010; Wilson et al., 2017). However, rates of BRD across these broad risk categories are highly variable, and the influence that management decisions such as marketing strategy on host immunity on later performance and BRD incidence is poorly understood. To better understand the influence that marketing strategy has on host metabolism, inflammation, and immunity, and to determine the ability of host genomic features to predict BRD risk and development, we evaluated at-arrival whole blood transcriptomes of newly weaned cattle that had experienced a commercial auction and order-buyer system prior to backgrounding or that were directly shipped from the cow–calf phase to backgrounding. Our primary objectives were to identify expressed

genes and associated biological mechanisms which may distinguish cattle that ultimately develop BRD and to explore whether we could identify the prior marketing history of individuals at backgrounding arrival. Our hypothesis was that gene expression patterns and identifiable associated mechanisms at arrival would identify cattle which would develop BRD during a 45-day period of backgrounding, and that gene expression on arrival may be leveraged to distinguish individuals derived from a commercial auction setting from those directly transported.

3.3. Materials and Methods

3.3.1. Animal Use and Study Enrollment

All animal use and procedures were approved by the Mississippi State University and West Texas A&M University Animal Care and Use Committee (IACUC protocols #19-169 and #2019.04.002, respectively). This study was carried out in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (Du et al., 2020). Eighty-four commercial cross-bred beef steers were randomly assigned into a whole-plot, split-plot design study to evaluate the effect of modified live viral (MLV) vaccination and commercial marketing strategy on health and performance. At the end of the calving season, cattle were selected for subcutaneous MLV vaccination and booster (2 mL SQ; Pyramid 5 (Boehringer Ingelheim Animal Health) during their cow–calf production phase in Mississippi (Prairie Research Unit, Prairie, MS, USA). Briefly, individuals were housed in six grass-lot pastures (n = 14 cow–calf pairs per pasture), grouped based on vaccination status (VAX (n = 3) or NOVAX (n = 3)) for a median time

of 217 days. Pasture groups contained some cow–calf pairs (n = 6 to 9 per group) not enrolled in the study; non-study calves in each pasture group received the same vaccination or not as study calves. At the end of the calving season, cattle were assigned to split-plot level treatment where they were either weaned and housed at the Prairie Research Unit for three days prior to direct shipment to Texas (Texas A&M AgriLife Bushland Research Feedlot, Bushland, TX, USA) (DIRECT, n = 7 calves per pasture group) or transported to a commercial auction market in north Mississippi, housed in pens for approximately six hours, then transported to a regional order-buyer facility for three days, and finally transported to Texas (AUCTION, n = 7 calves per pasture group). Between enrollment and transport to Texas, three calves were removed from the study: one calf from a VAX/AUCTION group was found acutely dead in the pasture approximately 6 weeks before weaning; necropsy revealed a colonic tear and hemoabdomen, likely due to trauma. A second calf from the same VAX/AUCTION group was removed from the study at weaning because it was much smaller (93.6 kg) than all other calves in its group (average weight 223.7 kg), and we concluded that the calf was at significant risk for injury if shipped with larger calves. The third calf, from a different VAX/AUCTION group, was removed from the study at weaning due to a chronic joint injury causing lameness that we determined also increased risk of injury to the calf during transport. These removals resulted in 81 study calves being transported to Texas. Cattle in DIRECT and AUCTION groups were transported to Texas on the same truck but in different compartments, with no contact between groups allowed. Upon arrival in Texas, whole blood was collected from all 81 steers (mean = 235.9 kg, s.d. = 35.6 kg) via jugular venipuncture into Tempus Blood RNA tubes (Applied Biosystems). Cattle were

then placed into one of twelve predetermined pens (n = 7 per pen), sorted based on vaccination and sale type status. All cattle were monitored daily for signs of clinical BRD over a 45-day backgrounding period by the same trained observer (SFC); all researchers and trained staff in Texas were blinded to treatment (vaccination, marketing strategy) during data collection. Cattle were assigned a clinical BRD score of 0–4 based on visual signs of disease (APPENDIX A-3). Cattle were considered BRD-positive and clinically treated if given a clinical score of 1 or 2 and a rectal temperature >40 ◦C, or if they were scored a 3 or 4, regardless of rectal temperature. At-arrival samples from all cattle having been treated for clinical BRD after arrival (n = 32) were prioritized for RNA sequencing, and randomly selected samples from clinically healthy cattle (n = 12) with equal proportion across marketing strategies were utilized (n = 6, DIRECT; n = 6, AUCTION). Cattle having been diagnosed with BRD were further categorized into marketing strategy groups (DIRECT, n = 20; AUCTION, n = 12). The overall median time to first treatment was 35 days, with BRD cattle within the AUCTION and DIRECT groups possessing median time to first treatment of 31 and 38 days, respectively. Information for all selected cattle is found in APPENDIX B-3.

3.3.2. Sample Processing, Next-Generation RNA Sequencing, and Bioinformatic Processing

Total blood RNA isolation, nucleic acid quality control, Stranded mRNA sequencing library preparation (Illumina, San Diego, CA, USA), and high-throughput shotgun sequencing was performed at the Texas A&M University Institute for Genome Sciences and Society (TIGSS; College Station, TX, USA), in conjunction with our previous work (Scott et al., 2022). Total RNA extraction was performed with Tempus

Spin RNA Isolation Kits (ThermoFisher Scientific; Waltham, MA, USA), following the manufacturer's instructions. Following extraction, total RNA from each sample was then analyzed for concentration and integrity with a Qubit 2.0 Fluorometer (ThermoFisher, Waltham, MA, USA) and an Agilent 2200 Bioanalyzer (Agilent, Santa Clara, CA, USA), respectively; RNA samples were of high quality (RIN: 8.3–9.2; mean = 8.8, s.d. = 0.2) and concentration (ng/μL: 6.4–284.0; mean = 191.3, s.d. = 58.7), with the exception of one sample (S.049.J009), from which we failed to extract RNA. Library preparation for mRNA was completed with the Stranded mRNA Prep Kit (Illumina), following the manufacturer's instructions. Paired-end sequencing for 150-base-pair read fragments was subsequently performed on an Illumina NovaSeq 6000 analyzer (v1.7+; S4 reagent kit v1.5) in one flow cell lane. Quality assessment of reads was performed with FastQC v0.11.9 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/, accessed on 11 April 2022) and MultiQC v1.12 (Ewels et al., 2016), and read-pair trimming for adaptors, undetermined base calling, and retained minimum read length of 28 bases were performed with Trimmomatic v0.39 (Bolger, 2014). Trimmed reads were then mapped and indexed to the bovine reference genome assembly ARSUCD1.2 with HISAT2 v2.2.1 (Kim et al., 2019). Sequence Alignment/Map (SAM) files were converted to Binary Alignment Map (BAM) files prior to transcript assembly via Samtools v1.14 (Li et al., 2009). Transcript assembly and gene-level expression estimation for differential expression analysis was performed with StringTie v2.1.7 (Kovaka et al., 2019), as described by Pertea and colleagues (Pertea et al., 2016). Three samples (S.054.J017, S.087.J113, and S.090.J123) were considered of low quality and technical outliers per the initial quality control assessment and were subsequently removed from further analysis.

All raw sequencing data produced in this study are available at the National Center for Biotechnology Information Gene Expression Omnibus (NCBI-GEO) under the accession number GSE218061.

3.3.3. Differential Gene Expression and Functional Enrichment Analyses

Gene-level raw count matrices were explored within RStudio, via R v4.1.2. Raw counts were processed and filtered by procedures previously described (Chen et al., 2016; Scott et al., 2022). Retained data were normalized for differential expression analysis with the Trimmed Mean of M-values method (TMM) (Robinson and Oshlack, 2010). Mixed effect statistical modeling was performed with edgeR v3.36.0 generalized linear model likelihood ratio testing (glmLRT), following tagwise dispersion estimate fitting. Specifically, analyses were performed in four steps: (1) HEALTHY (n = 10) versus BRD (n = 30) across all cattle (blocking for sale type), (2) AUCTION (n = 16) versus DIRECT (n = 24), (3) HEALTHY (n = 5) versus BRD (n = 11) within the AUCTION group, and (4) HEALTHY (n = 5) versus BRD (n = 19) within the DIRECT group; all testing was performed with additive models, accounting for vaccination (VAX) and pen order (MS_pasture) from when cattle were raised in Mississippi prior to transport to Texas. Genes were considered differentially expressed with a false discovery rate (FDR) of less than 0.05. Visual relationships of the genes identified by each analysis was performed with UpSetR v1.4.0 (Conway et al., 2017), utilizing the interactive interface Intervene (Khan and Mathelier, 2017) .

Identified differentially expressed genes (DEGs) were analyzed for enrichment of gene ontology terms, including biological processes, molecular functions, and cellular components, and pathways via the Reactome pathway database (Jassal et al., 2020) and

over-representation analysis through WebGestalt 2019 (WEB-based GEne SeT AnaLysis Toolkit) API (Liao et al., 2019). Over representation analysis parameters within WebGestalt 2019 included the Bos taurus genome as the reference set, between 2 and 2000 genes per category, Benjamini–Hochberg (BH) procedure for multiple hypothesis correction, FDR cutoff of 0.05 for significance, and a total of 10 expected reduced sets of the weighted set cover algorithm for redundancy reduction. Enriched gene ontology terms, specifically biological processes, and the Reactome pathways were evaluated for their directionality (increased or decreased) based on log2 fold changes of associated DEGs.

3.3.4. Data Visualization and Model-Based Unsupervised Clustering Analyses

To reduce the high dimensionality of the gene expression dataset and to identify potential correlations with clinical metadata (APPENDIX B-3), principal component analysis (PCA) was performed with the Bioconductor package PCAtools v2.10.0 (https://github.com/kevinblighe/PCAtools, accessed on 11 November 2022), utilizing a correlation matrix. Correlation matrix modeling was selected due to the uneven scale of variations generally found within gene expression data, where covariance matrix modeling tends to be less informative due to the skewness by the most variable and/or lowest abundant genes (Tipping and Bishop, 1999; Narasimhan and Shah, 2008; Lee and Han, 2022). Trimmed Mean of M-values normalized gene expression counts were log2-transformed after the addition of a (+1) pseudocount to prevent log-transformation of any zero counts, processed with mean-centering (“center = TRUE”) and variance-scaling (“scale = TRUE”), and the bottom 10% of genes with the lowest total variance across samples (“removeVar = 0.1”) were removed. A scree plot was used to determine the

number of principal components (PCs) to be retained for further analysis, employing the Elbow and Horn's parallel analysis methods, respectively (Horn, 1965). Spearman's rank correlations of the retained PCs were calculated with metadata components across all samples, specifically for average daily weight gain from birth until allocation to sale type (MSADG), age (in days) at time of Texas arrival (ArrivalAge), shrunk weight upon Texas arrival (ArrivalWt), binary coding if an individual was administered two modified live viral respiratory vaccines during the cow–calf phase in Mississippi (Vaccination; 0 = No, 1 = Yes), days at risk for BRD development during the backgrounding phase in Texas (Risk; maximum = 45 d), at-arrival fecal parasite egg counts per gram of feces calculated via the Modified McMaster technique on the same day of collection (EPG), the 25-acre pasture identity where individuals were housed in Mississippi during the cow–calf phase (Pasture), binary coding for the type of sale system the individual moved through prior to Texas arrival (Sale; 0 = AUCTION, 1 = DIRECT), the number of clinical treatments an individual received for BRD throughout the Texas backgrounding phase (Severity; min = 0, max = 2), and if an individual ever received treatment for clinical BRD throughout Texas backgrounding (Disease; 0 = BRD, 1 = Healthy). Spearman's correlations were considered to have significant associations with an FDR < 0.10. A PCA biplot was then constructed from the first PCs with significant correlations to metadata (PC2 and PC3) with the "encircle = TRUE" function selected to automatically depict a polygon around groups specified by SALE (top correlated feature; PC2). Lastly, to identify which genes were the primary drivers of the variation that was seen in each significantly correlated PC (PC2, PC3, and PC7), a loadings plot was generated with the top/bottom 1% of retained variables across each of the component loading range.

Following PCA, initial gene expression patterns within each analysis dataset (i.e., HEALTHY vs. BRD, AUCTION vs. DIRECT, etc.) were explored by applying multidimensional scaling (MDS) to each gene expression dataset after gene count filtering and TMM normalization, using the plotMDS function from the edgeR package. Visualizing differences in gene expression patterns via MDS is accomplished through unsupervised clustering of the root-mean-square average of the log-fold-changes for selected genes identified in each sample, allowing for the generalization of dissimilarities and potential batch effects within the dataset (Ritchie et al., 2015). Analysis via MDS was performed with "top = 500" to select the top 500 genes ranked on standard deviation for calculating distances, "gene.selection = common" to select the same genes for all comparisons, and "dim.plot = c(1,2)" to plot the first two principal components. Once DEGs were identified from each analysis, TMM-normalized counts were converted into log2 count-per-million (log2CPM) values for heatmap construction with the Bioconductor package pheatmap v1.0.12 (https://cran.r-project.org/web/packages/pheatmap/index.html, accessed on 11 November 2022). Data-centered and normalized z-scores from log2CPM counts were utilized for depiction and clustering of relative gene-wise variation of gene expression. Pearson correlation coefficients and Euclidean distances were calculated for clustering dissimilarities by column (sample) and row (gene), respectively. Color scaling for data visualization was performed with the Bioconductor package viridis v0.6.2 (Garnier et al., 2021), to allow for ease of visual interpretation for individuals affected with color blindness.

3.4. Results

Post-quality control and read trimming yielded a total of 1,431,561,514 filtered reads across all 43 samples (median = 33,259,429 reads per sample, s.d. = 2,938,551); mapping and alignment of trimmed reads to the Bos taurus reference genome assembly (ARS-UCD1.2) resulted in an average overall alignment rate of 95.9% (APPENDIX C-3). Post-alignment and gene-count matrix construction resulted in 33,310 unique annotated features. Following pre-processing and count filtering, 16,741 genes were retained for clustering and differential expression analyses. Prior to differential expression analysis, global gene expression patterns were evaluated through PCA. Using both the Horn's Parallel analysis and Elbow method, the first eight principal components were retained for downstream analysis, which accounted for 57.1% of the total variance (FIGURE 3.1A). Spearman's correlation of PCs with metadata components demonstrated significant correlations with Sale and PC2 (12.0% variance explained; $r = -0.82$, FDR < 0.01), ArrivalWt and PC3 (6.0% variance explained; $r = -0.48$, FDR < 0.05), MSADG and PC3 (6.0% variance explained; $r = -0.44$, FDR < 0.10) and ArrivalAge and PC7 (2.7% variance explained; $r = 0.55$, FDR < 0.01) (FIGURE 3.1B). A biplot of PC2 and PC3 was constructed to visualize the high relative dissimilarity of the samples, demonstrating a distinct pattern between individuals by Sale and no discernable pattern by Disease (FIGURE 3.1C). The top eight genes influencing these patterns within each PC (i.e., component loading) were CCDC146, EPSTI1, LOC101906463, LOC112443219, LOC507247, RTP4, SLFN11, and TRIM14 in PC2, and ANKRD34A, BOLA-DQA, CITA, DIRAS2, FFAR3, SKAP2, TMEM145, and TRABD2B in PC3. Those genes determined to be the drivers of variation within each of the three significantly correlated PCs are indicated by the Loadings Plot (FIGURE 3.1D). Genes

driving variation which was correlated specifically with AUCTION cattle (PC2) included ACOD1, ANKRD34A, ANKRD50, CCDC146, CCNF, CDCA8, DPYD, EPSTI1, LOC101906463, LOC104971363, LOC112442703, LOC112443219, LOC507247, rna-NR_031144.1, RTP4, SKAP2, SLFN11, SPC24, TARM1, TMEM145, and TROAP. Genes driving variation which was negatively correlated with average weight gain in Mississippi and shrunk weight at arrival (PC3) included the aforementioned genes, with the exclusion of LOC507247, rna-NR_031144.1, and SLFN11. The exact genes identified by PC2 were also found to be positively correlated with age at Texas arrival within PC7.

Differential expression analysis of all samples, evaluating HEALTHY versus BRD, resulted in one differentially expressed gene (DEG), BOLA-DQA5, which was decreased on arrival in cattle that remained healthy during backgrounding (APPENDIX D-3). As previously described by PCA, Sale possessed a significant influence on the gene expression dataset, and no discernable patterns were observed when evaluating for Disease or Severity. Subsequently, differential expression analysis was performed independently for AUCTION versus DIRECT, HEALTHY versus BRD within the AUCTION group, and HEALTHY versus BRD within the DIRECT group, which resulted in 2961 (1538 increased, 1423 decreased in DIRECT), 9 (all increased in HEALTHY), and 4 DEGs identified (3 increased, 1 decreased in HEALTHY), respectively (APPENDIX D-3). Visualization of the number and overlap of each DEG identified by each analysis are found in FIGURE 3.2.

Multidimensional scaling (MDS) and heatmap clustering was performed with three specific comparisons: (1) AUCTION versus DIRECT groups (including labeling for disease) (FIGURE 3.3), (2) HEALTHY versus BRD within the AUCTION group

(FIGURE 3.4), and (3.3) HEALTHY versus BRD within the DIRECT group (FIGURE 3.5). Euclidean distances based on the top 500 variable genes of all cattle demonstrated distinct clustering by sale type within the first principal component (x-axis) based on MDS, with little separation by disease status (FIGURE 3.3A). Further evaluation of the 2961 DEGs identified between sale types via heatmap clustering substantiated the distinction in gene expression patterns between the two sale type groups, with little division between disease status across all samples (FIGURE 3.3B). Visualization of gene expression variation within the AUCTION group via MDS demonstrated relative separation of disease groups within the first principal component (x-axis), which explained 9% of the total variance within the dataset (FIGURE 3.4 A). Hierarchical clustering of the nine DEGs identified by disease within the AUCTION group supported the findings of the aforementioned MDS plot, depicting separation of disease groups by the relative expression of these nine genes (FIGURE 3.4 B). Lastly, visualization of gene expression variation within the DIRECT group via MDS demonstrated no clear separation of disease groups (FIGURE 3.5A). Further hierarchical clustering of the four DEGs identified between disease cohorts within the DIRECT group did demonstrate separation of cattle that eventually developed BRD; however, two of the five analyzed HEALTHY cattle trended with BRD cattle, based on segmentation of the columns (samples) into two based on hierarchical patterns (FIGURE 3.5B).

Functional enrichment analysis was separately performed with DEGs identified within (1) AUCTION versus DIRECT and (2) HEALTHY versus BRD within the AUCTION group. Functional enrichment in HEALTHY versus BRD groups across all samples and HEALTHY versus BRD within the DIRECT group could not be performed

due to there being too few genes (n = 1 and n = 4, respectively). Analysis of the DEGs identified from AUCTION versus DIRECT revealed enrichment for 113 biological processes, 44 cellular components, 4 molecular functions, and 54 Reactome pathways (APPENDIX E-3). Biological processes identified within AUCTION versus DIRECT were related to the innate immune response (increased in AUCTION), protein metabolism and secretion (increased in AUCTION), viral response and type I interferon production (increased in AUCTION), response to interferon gamma (increased in AUCTION), response to external stimuli and cytokines (decreased in AUCTION), autophagy (increased in AUCTION), response to bacteria (increased in AUCTION), and fatty acid mobilization and metabolism (decreased in AUCTION). Cellular components identified from DEGs between AUCTION and DIRECT cattle involved the cytosol, nuclear lumen and nucleoplasm, both extracellular and intracellular vesicles, lipid droplets, ribosomes, and mRNA-editing complexes. Molecular functions identified between AUCTION and DIRECT cattle included structural constituent of ribosomes, pattern recognition receptor activity, and anion binding. The Reactome pathways enriched between AUCTION and DIRECT cattle included neutrophil degranulation (decreased in AUCTION); antiviral mechanisms by interferon, including ISG15-mediated antiviral activity (increased in AUCTION); antigen processing and cross-presentation, including MHC class I-mediated processing and presentation (increased in AUCTION); B-cell receptor signaling and activation of NF-κB (increased in AUCTION); MyD88-independent and TRIF-mediated toll-like receptor 4 signaling (increased in AUCTION); and p53-independent DNA damage response (increased in AUCTION).

Analysis of the DEGs identified from HEALTHY versus BRD within the AUCTION group revealed enrichment for 2 biological pathways, 8 cellular components, 2 molecular functions, and 21 Reactome pathways (APPENDIX E-3). Biological processes identified were protein heterodimerization (increased in HEALTHY) and skin morphogenesis (increased in HEALTHY). Cellular components identified were collagen type I and fibrillar collagen trimers, banded collagen fibril, and collagen-containing extracellular matrix components. Molecular functions identified were related to extracellular matrix structural constituents and platelet-derived growth factor binding. Reactome pathways identified were related to extracellular cellular matrix proteoglycans (increased in HEALTHY), platelet activation/aggregation and adhesion to exposed collagen (increased in HEALTHY), GP1b-IX-V activation signaling (increased in HEALTHY), collagen biosynthesis and formation (increased in HEALTHY), and immunoregulatory interactions between lymphoid and non-lymphoid cells (increased in HEALTHY).

3.5. Discussion

Broadly, research has demonstrated that cattle sold through a commercial auction setting and placed in novel commingling settings tend to be classified at higher risk for BRD development (Sanderson et al., 2008; Taylor et al., 2010). However, it is often difficult to separate out the impact of some of the other BRD-related risk factors that may accompany cattle marketed via an auction (lack of preconditioning, commingling, stress, social group disruption, pathogen exposure, abrupt or high stress weaning, lack of proper prior nutrition, unknown immunological status, etc.) (Martin et al., 1980; Galyean et al.,

1999; Gummow and Mapham, 2000; Sanderson et al., 2008; Step et al., 2008). Additionally, although we have identified some BRD-related risk factors, their exact direct influence on BRD development and the combined additive or multiplicative interactions among risk factors are relatively unknown and can be highly variable, as not every animal who moves through an auction has the same underlying experiences. The group of cattle evaluated in this study is unique in that their whole life history was known and every aspect of their management was planned and meticulously followed from the time of dam insemination to the end of backgrounding. This provided us a unique and invaluable opportunity to study the impact of marketing decisions without any potential confounding or effect modifying factors and to account for other factors.

Even so, the conditions that we raised these cattle under were not inclusive of all ways cattle are raised and all risk factors that cattle may experience prior to backgrounding. For our cattle, we controlled for prior vaccination with a modified live viral respiratory vaccine. We castrated all calves 69 days prior to abruptly weaning all animals. The type of auction market exposure we designed included a relatively short course to a local auction market, a short (~6 h) stay there as a group where they could have fence line contact with other cattle, and then a brief stay at a local order-buyer facility, where they remained for three days prior to shipment to Texas. Within the realm of "auction market" systems, there is certainly much variation in the time it takes to transport cattle to a market, e.g., the length of time animals spend at the actual market and how much or little they are commingled or exposed to pathogens there, the time and distance to their next destination, etc., that could result in more or less stress and risk of subsequent disease. Similarly, even cattle who are directly transported to the next phase

in the production cycle experience variations in prior management and transport time, distance, and conditions that may produce variation in outcomes. Therefore, our results may not be applicable to all cattle that move through a market system or that are directly transported; thus, further research exploring the variability in management and marketing is needed.

Our objective in this study was to identify differences in gene expression and associated host-driven biological systems that may be impacted by marketing decisions and how these influence eventual BRD morbidity after backgrounding arrival, in order to explore potential mechanisms of BRD development or resistance that may be leveraged in future studies. While both our research group and others have used the blood RNA-Seq methodology to identify potential predictive markers and mechanisms related to BRD (Sun et al., 2020; Hasankhani et al., 2021; Jiminez et al., 2021; Scott et al., 2020, 2021a, 2021b, 2022; Li et al., 2022), this study, to our knowledge, is one of the first to utilize said technologies to evaluate how marketing decisions, with relationship to BRD, influence inflammatory- and immune-mediated mechanisms in cattle. Importantly, exposure to an auction market setting and an order-buyer facility for only three days was associated with the differential expression of 2961 genes representing 113 biological processes. This striking difference in gene expression between the two groups of cattle that originated from the same herd illuminates the numerous immunologic and metabolic processes that can be affected by exposure to a marketing environment. It is notable that the cattle were never physically mixed with other cattle during auction market exposure, so commingling of cattle from outside sources was not a factor in the changes in gene expression observed.

Our initial results evaluating HEALTHY and BRD cattle at arrival yielded one DEG, BOLA-DQA5, that was decreased in HEALTHY cattle relative to BRD cattle. Members of the major histocompatibility complex class IIa region, of which BOLA-DQA5 is part of, have been researched in association with cattle diseases, such as viral infection and mastitis (Yoshida et al., 2011; Gowane et al., 2013; Hayashi et al., 2017). More recently, BOLA-DQA5 was a genotype target for genetic architecture research in Holsteins (Gelhaus et al., 1999; Fukunaga et al., 2020); however, its relationship with cattle health and disease development is largely unknown at this time. Moreover, the lack of significant findings associated with overt disease (i.e., between all samples used) can be attributed to the high amount of variance explained by marketing decision alone, shown in our dimensional reduction analyses (FIGURE 3.1 and FIGURE 3.3). Therefore, we first investigated what genes and mechanisms were driving this variation. Component loadings from our PCA determined that ACOD1, ANKRD34A, ANKRD50, CCDC146, CCNF, CDCA8, DPYD, EPSTI1, LOC101906463, LOC104971363, LOC112442703, LOC112443219, LOC507247, rna-NR_031144.1, RTP4, SKAP2, SLFN11, SPC24, TARM1, TMEM145, and TROAP were the primary drivers of variation in association with marketing decision. Further evaluation of genes detected in cattle distinguished by marketing decision identified a total of 2961 DEGs, of which all identified component loading genes overlapped. Several of these DEGs, including ACOD1, ANKRD34A, ANKRD50, LOC507247, RTP4, SLFN11, and TARM1, have been shown to be involved in macrophage-directed inflammatory mechanisms and antiviral response, specifically centered around type-I interferon production (Radjabova et al., 2015; Ren et al., 2016; Tallam et al., 2016; Luo et al., 2017; Malone et al., 2019; He et al., 2020; Lan et al.,

2020; Yang-Chun et al., 2020; Chen et al., 2022). Moreover, our functional enrichment analysis of these DEGs showed that they were largely involved in antiviral defense/type-I interferons (increased in AUCTION); cell growth regulation (decreased in AUCTION); immune activation, centered around toll-like receptor 4 activity and complement activity (increased in AUCTION); and inflammatory mediation and lipid metabolism (decreased in AUCTION). While a limitation of this study is the lack of respiratory metagenomic or viral identity information, our findings suggest that these cattle, only having been placed in a commercial auction setting for a relatively short period of time, were exposed and immunologically responded to a virulent virus or viruses (Tizioto et al., 2015; Behura et al., 2017; Johnston et al., 2021; Scott et al., 2021, 2022). Interestingly, these antiviral-related gene expression signatures were not necessarily associated with clinical BRD development and severity during backgrounding, as seen in previous RNA-Seq studies (Sun et al., 2020; Hasankhani et al., 2021; Scott et al., 2021, 2022). Future studies should pair RNA-Seq with host genetic and/or epigenetic evaluation and pathogen or microbiome identification methods to more clearly associate pathogen exposure and regulation with regards to these DEGs. While this finding does not negate the influence that viruses and prolonged inflammatory activity may have on BRD development, these type-I interferon-related gene expression patterns observed at backgrounding arrival may give us the ability to retrospectively identify cattle that may have experienced viral exposure and/or prior auction market exposure and may help us better categorize their risk status. Additional research is needed to see if we can refine this methodology to identify “stale” cattle who have spent more than several days in the marketing system and further pinpoint cattle at higher risk of disease or poor performance during

backgrounding or feeding. Furthermore, approximately 40% (32/81) of cattle entering this 45-day backgrounding period were subsequently diagnosed with BRD. While this relatively high rate of BRD is not uncommon in commercial beef production systems, the overall frequency of BRD treatment for this population is higher than cattle populations, especially those of the relatively same age and weight, as shown in our previous work (Scott et al., 2020, 2021, 2022). Potentially, these cattle, having been maintained and transported from a single-source, relatively low-risk environment, were exposed to pathological and environmental features novel to them, and/or our detection of clinical BRD was more rigorous compared to large commercial operations.

To account for the large amount of variation driven by marketing decisions, we further split the dataset by AUCTION or DIRECT, to identify at-arrival gene expression patterns and mechanisms which may indicate eventual clinical BRD development within each group. Beginning with the AUCTION group, we identified relative separation of disease groups based on expressional variation (FIGURE 3.4) and a total of nine DEGs between HEALTHY and BRD. These DEGs primarily are involved in collagen biosynthesis and modification and platelet adhesion and aggregation, which were relatively increased in HEALTHY calves. Recently, Johnston and colleagues discovered that COL1A1 and COL1A2, the genes driving the aforementioned mechanisms, were the most down-regulated genes in whole blood collected from BRSV-challenged calves compared to sham-control calves (Johnston et al., 2021). While the exact mechanism of how these type-I collagen-associated genes relate to viral exposure and subsequent BRD development is unknown at this time, they are involved in airway macrophage-driven cell clearance, metalloproteinase regulation, and fibrogenesis (Zhang et al., 2018; Tsitoura et

al., 2021; Li et al., 2022). Furthermore, platelet activity is linked to collagen exposure and is shown to increase both the adhesion capacity of lymphocytes and enhance T-cell differentiation (Hu et al., 2010; Nuyttens et al., 2011; Chebbo et al., 2021). Collectively, this may serve as a predictor of, and possible protective mechanism in, BRD development in auction-marketed, viral-infected cattle, which warrants future investigation.

Lastly, our analysis of cattle within the DIRECT group yielded no discernable patterns related to BRD outcome (FIGURE 3.5), with only four DEGs identified: EFEMP1, HELQ, LOC112445634, and LOC112446743. Due to the low number of DEGs identified, we were unable to ascertain unified functional enrichment within this analysis. To our knowledge, previous research has not identified nor linked these genes to infectious respiratory disease in mammals.

One key feature, and subsequent limitation, of our study was the timing to the first BRD treatment. These cattle possessed an overall median time to first treatment of 35 days, with BRD cattle in the AUCTION and DIRECT groups having median times of approximately 31 and 38 days, respectively (APPENDIX B-3). While discernable differences in gene expression were identified within the AUCTION group in relation to BRD development, the overall lack of DEGs identified related to BRD may be attributable to at-arrival gene expression patterns not being capable of representing BRD morbidity when disease occurs over four weeks post-sampling, as was the case for many of the cattle in this study. This is in contrast to the typical pattern of BRD in recently transported cattle, in which disease is expected (2–4 weeks post-arrival) (Snowder et al., 2006; White et al., 2015; Avra et al., 2017). Additionally, our study dependently evaluated

BRD from one clinical illness scoring system (APPENDIX A-3), while several concurrently exist in commercial production systems. In addition, visual assessment alone may not accurately identify all BRD cases. Moreover, not all cattle moving through a commercial auction setting may be exposed to a virus or viruses. As such, future research evaluating the host response with regards to different approaches of identifying and diagnosing BRD (e.g., lung ultrasonography, cytological evaluation of the airway, etc.), as well as the relationship with microbial exposure and/or upper respiratory microbiota, is imperative.

3.6. Conclusions

This study was conducted to explore whole blood gene expression profiles of newly received cattle at a backgrounding operation in order to determine patterns and specific genes and genomic mechanisms related to marketing decisions and BRD development during backgrounding. Here, we describe nearly 3000 differentially expressed genes with a distinction between cattle processed through a commercial auction setting compared with cattle directly shipped to backgrounding; these DEGs are hallmarked by genes related to type-I interferon production, toll-like receptor 4 activity, cell growth regulation, and lipid metabolism. While the prolonged time to BRD incidence may have influenced our inability to capture information related to the influence of marketing decisions on the diagnosis and treatment of BRD, key differences related to collagen formation and metabolism were identified within auctioned cattle that resisted or developed clinical BRD. These results, in accompaniment with the findings of previous RNA-Seq research, provide new information about gene expression pathways activated

by the process of auction market exposure. These results contribute to a growing body of knowledge regarding gene expression pathways related to management practices and BRD risk.

3.7. Literature Cited

Avra, T.D.; Abell, K.M.; Shane, D.D.; Theurer, M.E.; Larson, R.L.; White, B.J. A retrospective analysis of risk factors associated with bovine respiratory disease treatment failure in feedlot cattle1. J. Anim. Sci. 2017, 95, 1521–1527.

Behura, S.K.; Tizioto, P.C.; Kim, J.; Grupioni, N.V.; Seabury, C.M.; Schnabel, R.D.; Gershwin, L.J.; Van Eenennaam, A.L.; Toaff Rosenstein, R.; Neibergs, H.L.; et al. Tissue Tropism in Host Transcriptional Response to Members of the Bovine Respiratory Disease Complex. Sci. Rep. 2017, 7, 17938.

Blakebrough-Hall, C.; McMeniman, J.P.; González, L.A. An evaluation of the economic effects of bovine respiratory disease on animal performance, carcass traits, and economic outcomes in feedlot cattle defined using four BRD diagnosis methods. J. Anim. Sci. 2020, 98, skaa005.

Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 2014, 30, 2114–2120.

Chebbo, M.; Duez, C.; Alessi, M.C.; Chanez, P.; Gras, D. Platelets: A potential role in chronic respiratory diseases? Eur. Respir. Rev. 2021, 30, 210062.

Chen, F.; Elgaher, W.A.M.; Winterhoff, M.; Büssow, K.; Waqas, F.H.; Graner, E.; Pires-Afonso, Y.; Perez, L.C.; de la Vega, L.; Sahini, N.; et al. Citraconate inhibits ACOD1 (IRG1) catalysis, reduces interferon responses and oxidative stress, and modulates inflammation and cell metabolism. Nat. Metab. 2022, 4, 534–546.

Chen, Y.; Lun, A.T.L.; Smyth, G.K. From reads to genes to pathways: Differential expression analysis of RNA-Seq experiments using Rsubread and the edgeR quasi-likelihood pipeline. F1000Research 2016, 5, 1438.

Conway, J.R.; Lex, A.; Gehlenborg, N. UpSetR: An R package for the visualization of intersecting sets and their properties. Bioinformatics 2017, 33, 2938–2940.

Dargatz, D.A.; Lombard, J.E. Summary of BRD data from the 2011 NAHMS feedlot and dairy heifer studies. Anim. Health Res. Rev. 2014, 15, 123–125.

Du Sert, N.P.; Hurst, V.; Ahluwalia, A.; Alam, S.; Avey, M.T.; Baker, M.; Browne, W.J.; Clark, A.; Cuthill, I.C.; Dirnagl, U.; et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. Br. J. Pharmacol. 2020, 177, 3617–3624.

Ewels, P.; Magnusson, M.; Lundin, S.; Käller, M. MultiQC: Summarize analysis results for multiple tools and samples in a single report. Bioinformatics 2016, 32, 3047–3048.

Fukunaga, K.; Yamashita, Y.; Yagisawa, T. Copy number variations in BOLA-DQA2, BOLA-DQB, and BOLA-DQA5 show the genomic architecture and haplotype frequency of major histocompatibility complex class II genes in Holstein cows. Hla 2020, 96, 601–609.

Galyean, M.L.; Perino, L.J.; Duff, G.C. Interaction of cattle health/immunity and nutrition. J. Anim. Sci. 1999, 77, 1120–1134.

Garnier, S.; Ross, N.; Rudis, B.; Filipovic-Pierucci, A.; Galili, T.; Greenwell, B.; Sievert, C.; Harris, D.J.; Chen, J.J. Sjmgarnier/Viridis: Viridis 0.6.0 (Pre-CRAN Release). 2021. Available online: https://zenodo.org/record/4679424#.ZAqmOh9Bx7M (accessed on 11 November 2022).

Gelhaus, A.; Förster, B.; Wippern, C.; Horstmann, R.D. Evidence for an additional cattle DQA locus, BoLA-DQA5. Immunogenetics 1999, 49, 321–327.

Gowane, G.; Sharma, A.; Sankar, M.; Narayanan, K.; Das, B.; Subramaniam, S.; Pattnaik, B. Association of BoLA DRB3 alleles with variability in immune response among the crossbred cattle vaccinated for foot-and-mouth disease (FMD). Res. Veter.-Sci. 2013, 95, 156–163.

Gummow, B.; Mapham, P.H. A stochastic partial-budget analysis of an experimental Pasteurella haemolytica feedlot vaccine trial. Prev. Veter.-Med. 2000, 43, 29–42.

Hasankhani, A.; Bahrami, A.; Sheybani, N.; Fatehi, F.; Abadeh, R.; Farahani, H.G.M.; Behzadi, M.R.B.; Javanmard, G.; Isapour, S.; Khadem, H.; et al. Integrated Network Analysis to Identify Key Modules and Potential Hub Genes Involved in Bovine Respiratory Disease: A Systems Biology Approach. Front. Genet. 2021, 12, 753839.

Hayashi, T.; Mekata, H.; Sekiguchi, S.; Kirino, Y.; Mitoma, S.; Honkawa, K.; Horii, Y.; Norimine, J. Cattle with the BoLA class II DRB3*0902 allele have significantly lower bovine leukemia proviral loads. J. Vet. Med. Sci. 2017, 79, 1552–1555.

He, X.; Ashbrook, A.W.; Du, Y.; Wu, J.; Hoffmann, H.-H.; Zhang, C.; Xia, L.; Peng, Y.-C.; Tumas, K.C.; Singh, B.K.; et al. RTP4 inhibits IFN-I response and enhances experimental cerebral malaria and neuropathology. Proc. Natl. Acad. Sci. USA 2020, 117, 19465–19474.

Horn, J.L. A rationale and test for the number of factors in factor analysis. Psychometrika 1965, 30, 179–185.

Hu, H.; Zhu, L.; Huang, Z.; Ji, Q.; Chatterjee, M.; Zhang, W.; Li, N. Platelets enhance lymphocyte adhesion and infiltration into arterial thrombus. Thromb. Haemost. 2010, 104, 1184–1192.

Jassal, B.; Matthews, L.; Viteri, G.; Gong, C.; Lorente, P.; Fabregat, A.; Sidiropoulos, K.; Cook, J.; Gillespie, M.; Haw, R.; et al. The reactome pathway knowledgebase. Nucleic Acids Res. 2020, 48, D498–D503.

Jiminez, J.; Timsit, E.; Orsel, K.; van der Meer, F.; Guan, L.L.; Plastow, G. Whole-Blood Transcriptome Analysis of Feedlot Cattle With and Without Bovine Respiratory Disease. Front. Genet. 2021, 12, 627623.

Johnston, D.; Earley, B.; McCabe, M.S.; Kim, J.; Taylor, J.F.; Lemon, K.; Duffy, C.; McMenamy, M.; Cosby, S.L.; Waters, S.M. Messenger RNA biomarkers of Bovine Respiratory Syncytial Virus infection in the whole blood of dairy calves. Sci. Rep. 2021, 11, 9392.

Khan, A.; Mathelier, A. Intervene: A tool for intersection and visualization of multiple gene or genomic region sets. BMC Bioinform. 2017, 18, 287.

Kim, D.; Paggi, J.M.; Park, C.; Bennett, C.; Salzberg, S.L. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat. Biotechnol. 2019, 37, 907–915.

Kovaka, S.; Zimin, A.V.; Pertea, G.M.; Razaghi, R.; Salzberg, S.L.; Pertea, M. Transcriptome assembly from long-read RNA-seq alignments with StringTie2. Genome Biol. 2019, 20, 278.

Lan, L.; Xu, M.; Li, J.; Liu, L.; Xu, M.; Zhou, C.; Shen, L.; Tang, Z.; Wan, F. Mas-related G protein-coupled receptor D participates in inflammatory pain by promoting NF-κB activation through interaction with TAK1 and IKK complex. Cell. Signal. 2020, 76, 109813.

Lee, H.; Han, B. FastRNA: An efficient solution for PCA of single-cell RNA-sequencing data based on a batch-accounting count model. Am. J. Hum. Genet. 2022, 109, 1974–1985.

Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R.; 1000 Genome Project Data Processing Subgroup. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009, 25, 2078–2079.

Li, J.; Mukiibi, R.; Jiminez, J.; Wang, Z.; Akanno, E.C.; Timsit, E.; Plastow, G.S. Applying multi-omics data to study the genetic background of bovine respiratory disease infection in feedlot crossbred cattle. Front. Genet. 2022, 13, 1046192.

Li, W.; Duan, X.; Zhu, C.; Liu, X.; Jeyarajan, A.J.; Xu, M.; Tu, Z.; Sheng, Q.; Chen, D.; Zhu, C.; et al. Hepatitis B and Hepatitis C Virus Infection Promote Liver Fibrogenesis through a TGF-β1–Induced OCT4/Nanog Pathway. J. Immunol. 2022, 208, 672–684.

Liao, Y.; Wang, J.; Jaehnig, E.J.; Shi, Z.; Zhang, B. WebGestalt 2019: Gene Set Analysis Toolkit with Revamped UIs and APIs. Nucleic Acids Res. 2019, 47, W199–W205.

Luo, H.; Yang, H.; Lin, Y.; Zhang, Y.; Pan, C.; Feng, P.; Yu, Y.; Chen, X. LncRNA and mRNA profiling during activation of tilapia macrophages by HSP70 and Streptococcus agalactiae antigen. Oncotarget 2017, 8, 98455–98470.

Malone, D.; Lardelli, R.M.; Li, M.; David, M. Dephosphorylation activates the interferon-stimulated Schlafen family member 11 in the DNA damage response. J. Biol. Chem. 2019, 294, 14674–14685.

Martin, S.W.; Meek, A.H.; Davis, D.G.; Johnson, J.A.; Curtis, R.A. Factors associated with mortality and treatment costs in feedlot calves: The Bruce County Beef Project, years 1978, 1979, 1980. Can. J. Comp. Med. 1982, 46, 341–349.

McGill, J.L.; Sacco, R.E. The Immunology of Bovine Respiratory Disease. Veter.-Clin. N. Am. Food Anim. Pract. 2020, 36, 333–348.

Mosier, D. Review of BRD pathogenesis: The old and the new. Anim. Health Res. Rev. 2014, 15, 166–168.

Narasimhan, S.; Shah, S.L. Model identification and error covariance matrix estimation from noisy data using PCA. Control Eng. Pract. 2008, 16, 146–155.

Nuyttens, B.P.; Thijs, T.; Deckmyn, H.; Broos, K. Platelet adhesion to collagen. Thromb. Res. 2011, 127 (Suppl. S2), S26–S29.

Pertea, M.; Kim, D.; Pertea, G.M.; Leek, J.T.; Salzberg, S.L. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. Nat. Protoc. 2016, 11, 1650–1667.

Radjabova, V.; Mastroeni, P.; Skjødt, K.; Zaccone, P.; de Bono, B.; Goodall, J.C.; Chilvers, E.R.; Juss, J.K.; Jones, D.C.; Trowsdale, J.; et al. TARM1 Is a Novel Leukocyte Receptor Complex–Encoded ITAM Receptor That Costimulates Proinflammatory Cytokine Secretion by Macrophages and Neutrophils. J. Immunol. 2015, 195, 3149–3159.

Ren, K.; Lv, Y.; Zhuo, Y.; Chen, C.; Shi, H.; Guo, L.; Yang, G.; Hou, Y.; Tan, R.X.; Li, E. Suppression of IRG-1 Reduces Inflammatory Cell Infiltration and Lung Injury in Respiratory Syncytial Virus Infection by Reducing Production of Reactive Oxygen Species. J. Virol. 2016, 90, 7313–7322.

Ribble, C.S.; Meek, A.H.; Shewen, P.E.; Guichon, P.T.; Jim, G.K. Effect of Pretransit Mixing on Fatal Fibrinous Pneumonia in Calves. J. Am. Vet. Med. Assoc. 1995, 207, 616–619.

Ritchie, M.E.; Phipson, B.; Wu, D.; Hu, Y.; Law, C.W.; Shi, W.; Smyth, G.K. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015, 43, e47.

Robinson, M.D.; Oshlack, A. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol. 2010, 11, R25.

Sanderson, M.W.; Dargatz, D.A.; Wagner, B.A. Risk factors for initial respiratory disease in United States' feedlots based on producer-collected daily morbidity counts. Am. Jew. Hist. 2008, 49, 373–378.

Schneider, M.J.; Tait, R.G.; Busby, W.D.; Reecy, J.M. An evaluation of bovine respiratory disease complex in feedlot cattle: Impact on performance and carcass traits using treatment records and lung lesion scores. J. Anim. Sci. 2009, 87, 1821–1827.

Scott, M.A.; Woolums, A.R.; Swiderski, C.E.; Perkins, A.D.; Nanduri, B.; Smith, D.R.; Karisch, B.B.; Epperson, W.B.; Blanton, J.R., Jr. Whole blood transcriptomic analysis of beef cattle at arrival identifies potential predictive molecules and mechanisms that indicate animals that naturally resist bovine respiratory disease. PLoS ONE 2020, 15, e0227507.

Scott, M.A.; Woolums, A.R.; Swiderski, C.E.; Perkins, A.D.; Nanduri, B. Genes and regulatory mechanisms associated with experimentally-induced bovine respiratory disease identified using supervised machine learning methodology. Sci. Rep. 2021a, 11, 22916.

Scott, M.A.; Woolums, A.R.; Swiderski, C.E.; Perkins, A.D.; Nanduri, B.; Smith, D.R.; Karisch, B.B.; Epperson, W.B.; Blanton, J.R. Comprehensive at-arrival transcriptomic analysis of post-weaned beef cattle uncovers type I interferon and antiviral mechanisms associated with bovine respiratory disease mortality. PLoS ONE 2021b, 16, e0250758.

Scott, M.A.; Woolums, A.R.; Karisch, B.B.; Harvey, K.M.; Capik, S.F. Impact of preweaning vaccination on host gene expression and antibody titers in healthy beef calves. Front. Veter.-Sci. 2022a, 9, 1010039.

Scott, M.A.; Woolums, A.R.; Swiderski, C.E.; Finley, A.; Perkins, A.D.; Nanduri, B.; Karisch, B.B. Hematological and gene co-expression network analyses of high-risk beef cattle defines immunological mechanisms and biological complexes involved in bovine respiratory disease and weight gain. PLoS ONE 2022b, 17, e0277033.

Scott, M.A.; Woolums, A.R.; Swiderski, C.E.; Thompson, A.C.; Perkins, A.D.; Nanduri, B.; Karisch, B.B.; Goehl, D.R. Use of nCounter mRNA profiling to identify at-arrival gene expression patterns for predicting bovine respiratory disease in beef cattle. BMC Veter-Res. 2022c, 18, 77.

Smith, D.R. Risk factors for bovine respiratory disease in beef cattle. Anim. Health Res. Rev. 2020, 21, 149–152.

Snowder, G.D.; Van Vleck, L.D.; Cundiff, L.V.; Bennett, G. Bovine respiratory disease in feedlot cattle: Environmental, genetic, and economic factors. J. Anim. Sci. 2006, 84, 1999–2008.

Step, D.L.; Krehbiel, C.R.; DePra, H.A.; Cranston, J.J.; Fulton, R.W.; Kirkpatrick, J.G.; Gill, D.R.; Payton, M.E.; Montelongo, M.A.; Confer, A.W. Effects of

commingling beef calves from different sources and weaning protocols during a forty-two-day receiving period on performance and bovine respiratory disease1,2. J. Anim. Sci. 2008, 86, 3146–3158.

Sun, H.-Z.; Srithayakumar, V.; Jiminez, J.; Jin, W.; Hosseini, A.; Raszek, M.; Orsel, K.; Guan, L.L.; Plastow, G. Longitudinal blood transcriptomic analysis to identify molecular regulatory patterns of bovine respiratory disease in beef cattle. Genomics 2020, 112, 3968–3977.

Tallam, A.; Perumal, T.M.; Antony, P.M.; Jäger, C.; Fritz, J.V.; Vallar, L.; Balling, R.; del Sol, A.; Michelucci, A. Gene Regulatory Network Inference of Immunoresponsive Gene 1 (IRG1) Identifies Interferon Regulatory Factor 1 (IRF1) as Its Transcriptional Regulator in Mammalian Macrophages. PLoS ONE 2016, 11, e0149050.

Taylor, J.D.; Fulton, R.W.; Lehenbauer, T.W.; Step, D.L.; Confer, A.W. The Epidemiology of Bovine Respiratory Disease: What Is the Evidence for Predisposing Factors? Can. Vet. J. 2010, 51, 1095–1102.

Tipping, M.E.; Bishop, C.M. Probabilistic Principal Component Analysis. J. R. Stat. Soc. Ser. B Stat. Methodol. 1999, 61, 611–622.

Tizioto, P.C.; Kim, J.; Seabury, C.M.; Schnabel, R.D.; Gershwin, L.J.; Van Eenennaam, A.; Toaff-Rosenstein, R.; Neibergs, H.L.; Taylor, J.F. Bovine Respiratory Disease Complex Coordinated Agricultural Project Research Team Immunological Response to Single Pathogen Challenge with Agents of the Bovine Respiratory Disease Complex: An RNA-Sequence Analysis of the Bronchial Lymph Node Transcriptome. PLoS ONE 2015, 10, e0131459.

Tsitoura, E.; Trachalaki, A.; Vasarmidi, E.; Mastrodemou, S.; Margaritopoulos, G.A.; Kokosi, M.; Fanidis, D.; Galaris, A.; Aidinis, V.; Renzoni, E.; et al. Collagen 1a1 Expression by Airway Macrophages Increases In Fibrotic ILDs and Is Associated With FVC Decline and Increased Mortality. Front. Immunol. 2021, 12, 645548.

USDA. Part IV: Health and Health Management on U.S. Feedlots with a Capacity of 1000 or More Head; USDA-APHIS-VS-CEAH[1]NAHMS: Fort Collins, CO, USA, 2011. Available online: https://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_dr_PartIV_1.pdf (accessed on 14 November 2022).

White, B.J.; Amrine, D.E.; Goehl, D.R. Determination of value of bovine respiratory disease control using a remote early disease identification system compared with conventional methods of metaphylaxis and visual observations1. J. Anim. Sci. 2015, 93, 4115–4122.

Wilson, B.K.; Richards, C.J.; Step, D.L.; Krehbiel, C.R. Beef Species symposium: Best management practices for newly weaned calves for improved health and well-being1. J. Anim. Sci. 2017, 95, 2170–2182.

Woods, G.T.; E Mansfield, M.; Webb, R.J. A three year comparison of acute respiratory disease, shrink and weight gain in preconditioned and non-preconditioned Illinois beef calves sold at the same auction and mixed in a feedlot. Can. J. Comp. Med. 1973, 37, 249–255.

Yang-Chun, F.; Sen-Yu, W.; Yuan, Z.; Yan-Chun, H. Genome-Wide Profiling of Human Papillomavirus DNA Integration into Human Genome and Its Influence on PD-L1 Expression in Chinese Uygur Cervical Cancer Women. J. Immunol. Res. 2020, 2020, 6284960.

Yoshida, T.; Furuta, H.; Kondo, Y.; Mukoyama, H. Association of BoLA-DRB3 alleles with mastitis resistance and susceptibility in Japanese Holstein cows: BoLA-DRB3 alleles and cow mastitis. Anim. Sci. J. 2011, 83, 359–366.

Zhang, S.; Gong, Y.; Xiao, J.; Chai, Y.; Lei, J.; Huang, H.; Xiang, T.; Shen, W. A COL1A1 Promoter-Controlled Expression of TGF-β Soluble Receptor Inhibits Hepatic Fibrosis Without Triggering Autoimmune Responses. Dig. Dis. Sci. 2018, 63, 2662–2672.

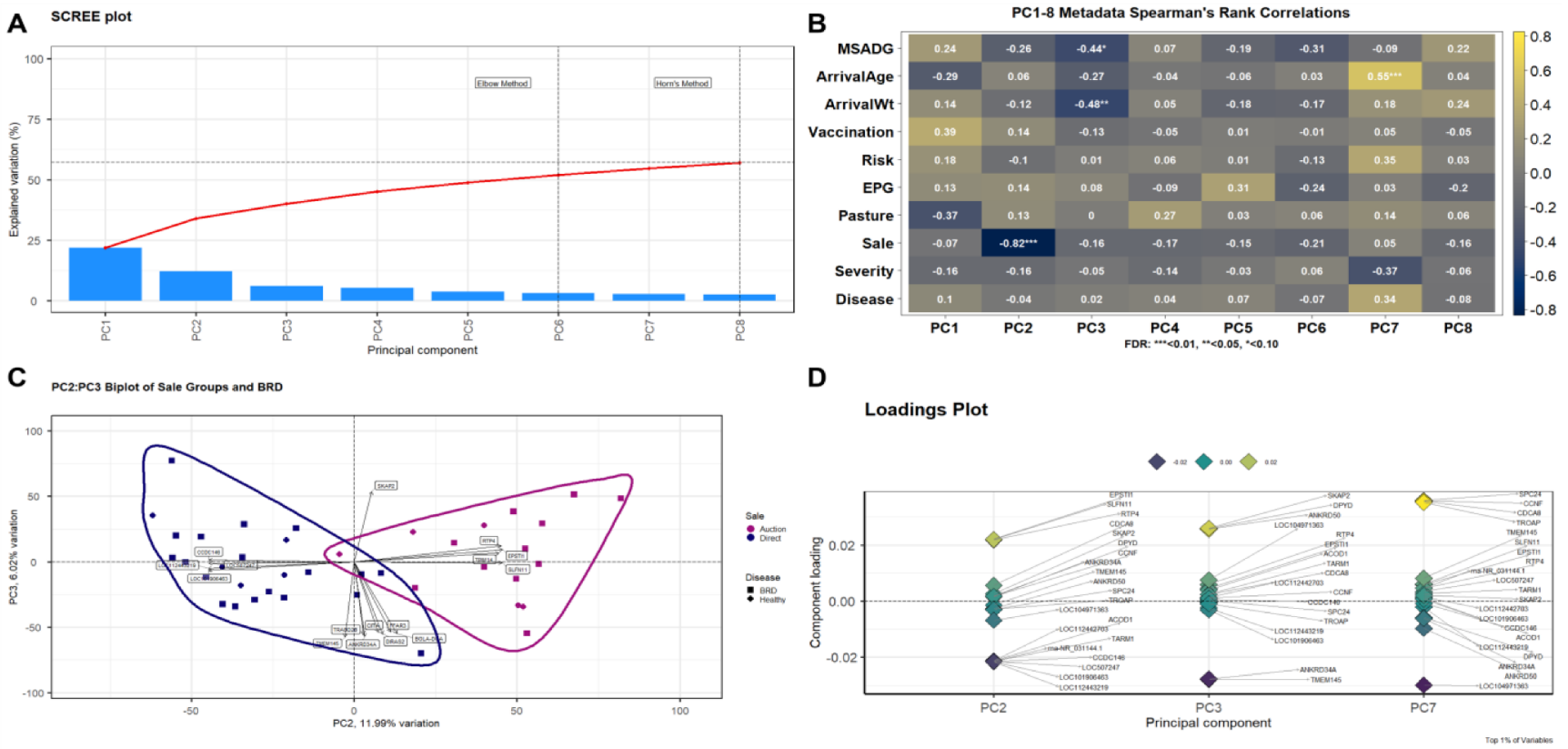
A
SCREE plot
Explained variation (%)
Elbow Method
Horn's Method
PC1
PC2
PC3
PC4
PC5
PC6
PC7
PC8
Principal component
B
PC1-8 Metadata Spearman's Rank Correlations
MSADG
ArrivalAge
ArrivalWt
Vaccination
Risk
EPG
Pasture
Sale
Severity
Disease
FDR: ***<0.01, **<0.05, *<0.10
C
PC2:PC3 Biplot of Sale Groups and BRD
PC3, 6.02% variation
PC2, 11.99% variation
Sale
Auction
Direct
Disease
BRD
Healthy
D
Loadings Plot
Component loading
Principal component
Top 1% of Variables

FIGURE 3.1. Principal component analysis (PCA) of the global gene expression data generated for all 40 samples utilized. (A) Scree plot depicting the first eight PCs retained for further PCA, which described over 57% of the total explainable variance. (B) Heatmap of Spearman's Rank correlation coefficients that were associated with metadata components within each of the eight retained PCs. Clinical metadata components are, in descending order, average daily weight gain from birth until allocation to sale type (MSADG), age (in days) at time of Texas arrival (ArrivalAge), shrunk weight upon Texas arrival (ArrivalWt), binary coding if an individual was administered two modified live viral respiratory vaccines during the cow–calf phase in Mississippi (Vaccination; 0 = No, 1 = Yes), days at risk for BRD development during the backgrounding phase in Texas (Risk; maximum = 45 d), at-arrival fecal parasite egg counts per gram of feces calculated via Modified McMaster technique on same day of collection (EPG), the 25-acre pen identity where individuals were housed in Mississippi during the cow–calf phase (Pasture), binary coding for the type of sale system the individual moved through prior to Texas arrival (Sale; 0 = AUCTION, 1 = DIRECT), the number of clinical treatments an individual received for BRD throughout the Texas backgrounding phase (Severity; min = 0, max = 2), and if an individual ever received treatment for clinical BRD throughout Texas backgrounding (Disease; 0 = BRD, 1 = Healthy). Color represents the R-value identified between each PC and metadata component; yellow/white cells represent a higher positive value, purple/black cells represent a lower negative value. Significance was calculated through FDR adjustments and is indicated by * FDR < 0.10, ** FDR < 0.05, or *** FDR < 0.01. (C) A biplot of PC2 and PC3, where samples were colored by sale type (purple = AUCTION, blue = DIRECT) and shaped by disease (square = BRD, diamond = Healthy). Individual plots (vectors) represent the PC score of the individual sample by gene expression, and vector distances along the x- and y-axes represent the total variational influence. Genes driving the explained variance for each PC are represented by arrows (directionality) and name. (D) Loadings plot with annotated genes driving associated variation and directionality (y-axis) of PC2, PC3, and PC7. The top 1% of genes identified by variance are seen as the most responsible for driving variation with each of the aforementioned PCs. Color (dark yellow to dark blue; positive to negative) demonstrates the corresponding directionality of expression and strength of influence for each gene within each PC.

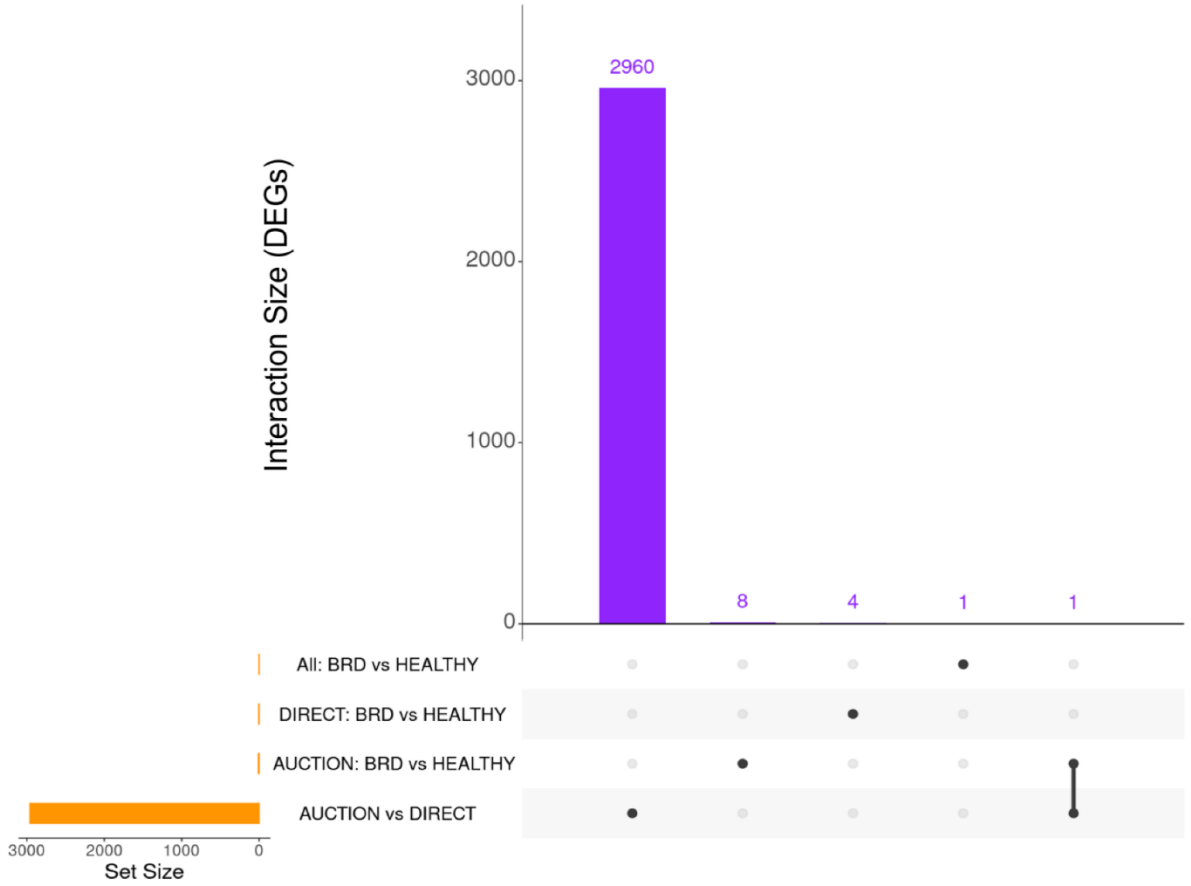

FIGURE 3.2 Upset plot representing the total number of DEGs identified by each analysis (Set Size) and the number of DEGs overlapping between analyses (Interaction Size). AUCTION versus DIRECT demonstrated the greatest number of unique DEGs (2960) across all analyses, with only one overlapping gene identified between any of the four analyses (RN18S1; AUCTION versus DIRECT and BRD versus HEALTHY in AUCTION cattle).

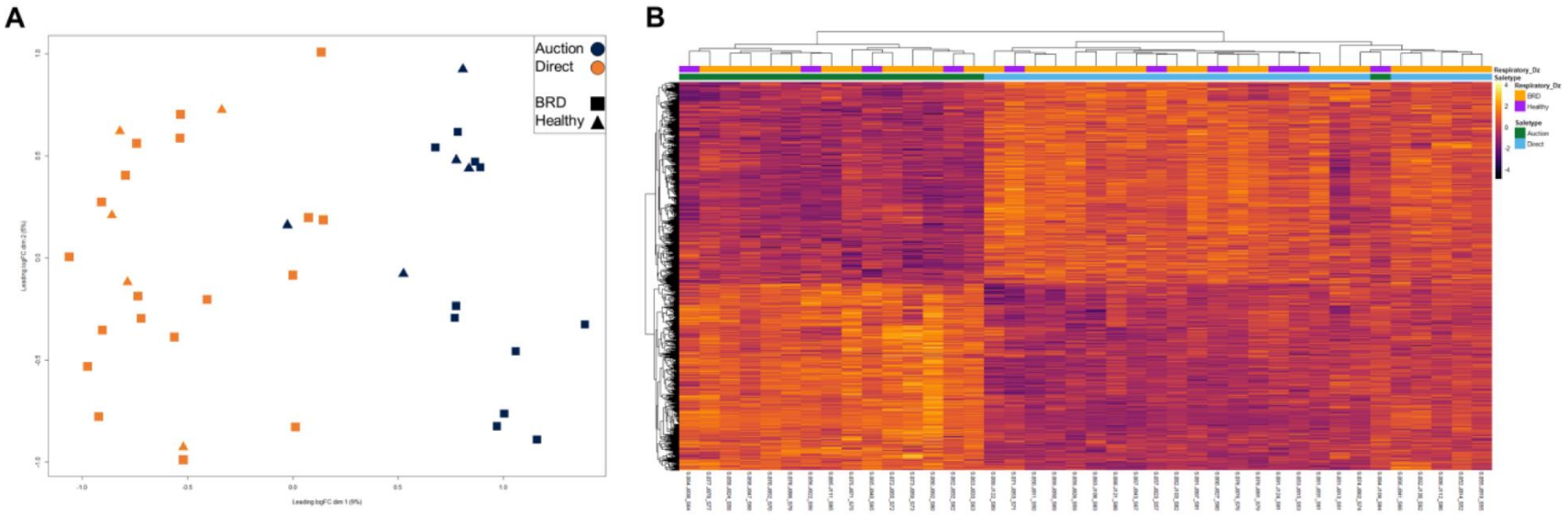

FIGURE 3.3 Multidimensional scaling (MDS) and unsupervised hierarchical clustering of gene expression between all AUCTION and DIRECT cattle at facility arrival. (A) Points within the MDS plot represent each sample and their transformed Euclidean distance in the first two principal components, observed as the leading log2-fold change between the common distances of the top 500 genes that best differentiate each sample. (B) Heatmap and hierarchical clustering of the 2961 DEGs identified between all AUCTION and DIRECT cattle. Gene expression values were scaled and normalized with z-scores calculated from log2 count-per-million transformed, Trimmed Mean of M-values (TMM)-normalized counts. Samples were labeled according to BRD acquisition (Respiratory_Dz) and method of sale (Saletype). Relative expression of each gene is depicted from high (yellow/white) to low (purple/black) within each sample.

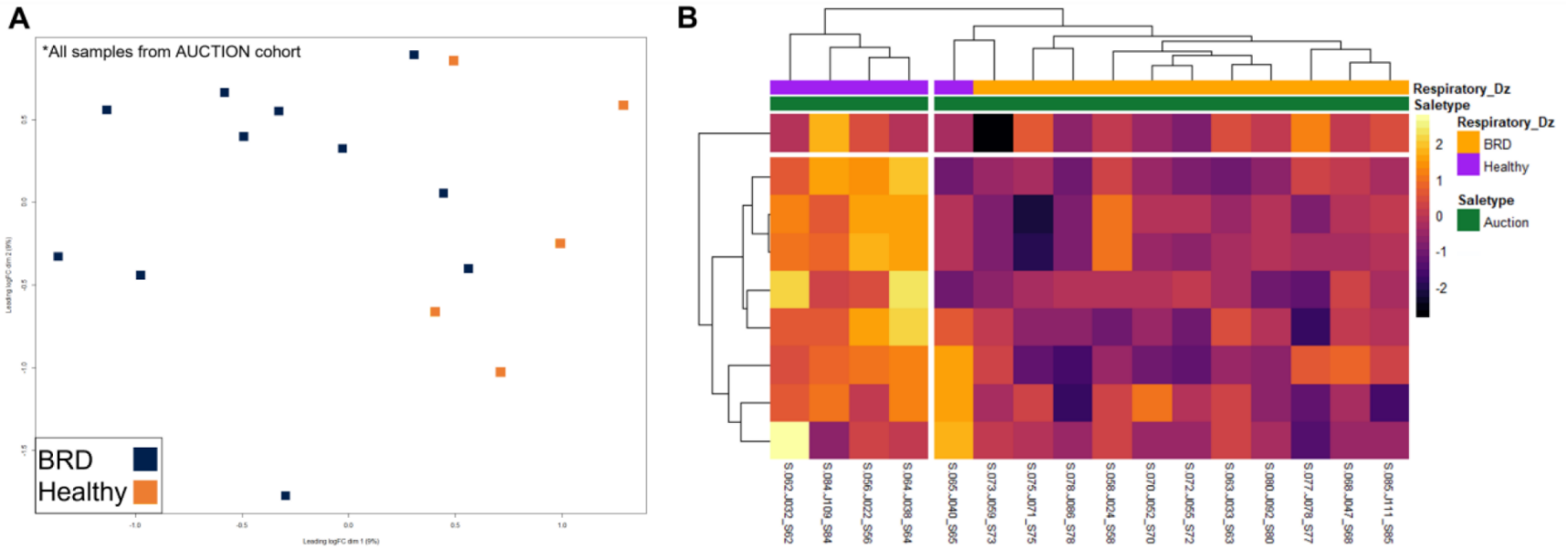

FIGURE 3.4 Multidimensional scaling (MDS) and unsupervised hierarchical clustering of gene expression between BRD and HEALTHY cattle within the AUCTION group at backgrounding arrival. (A) Points within the MDS plot represent each sample and their transformed Euclidean distance in the first two principal components, observed as the leading log2-fold change between the common distances of the top 500 genes that best differentiate each sample. (B) Heatmap and hierarchical clustering of the nine DEGs identified between disease cohorts within the AUCTION group. Gene expression values were scaled and normalized with z-scores calculated from log2 count-per-million transformed, Trimmed Mean of M-values (TMM)-normalized counts. The samples were labeled according to BRD acquisition (Respiratory_Dz). Relative expression of each gene is depicted from high (yellow/white) to low (purple/black) within each sample.

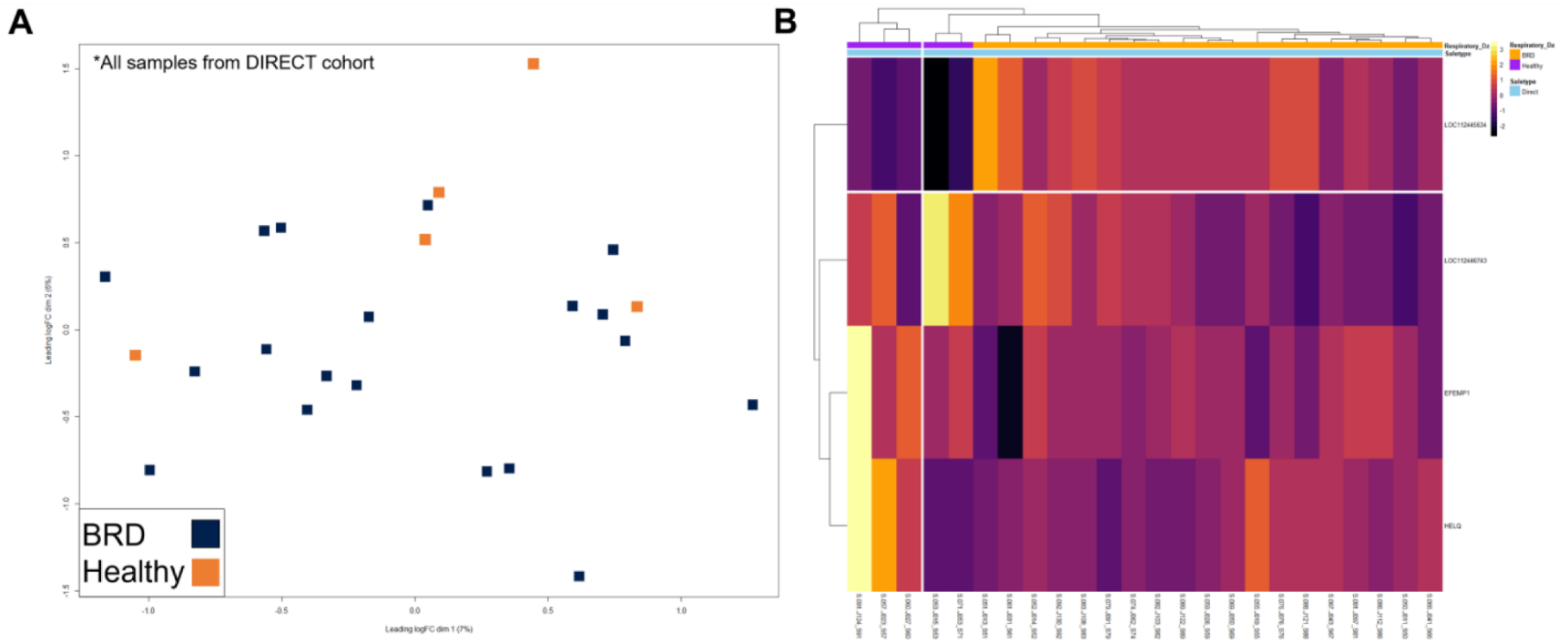

FIGURE 3.5 Multidimensional scaling (MDS) and unsupervised hierarchical clustering of gene expression between BRD and HEALTHY cattle within the DIRECT group at facility arrival. (A) Points within the MDS plot represent each sample and their transformed Euclidean distance in the first two principal components, observed as the leading log2-fold change between the common distances of the top 500 genes that best differentiate each sample. (B) Heatmap and hierarchical clustering of the four DEGs identified between disease cohorts within the DIRECT group. Gene expression values were scaled and normalized with z-scores calculated from log2 count-per-million transformed, Trimmed Mean of M-values (TMM)-normalized counts. The samples were labeled according to BRD acquisition (Respiratory_Dz). Relative expression of each gene is depicted from high (yellow/white) to low (purple/black) within each sample.

APPENDIX A-3

BRD Evaluation and Scoring System

Mollie M. Green, Amelia R. Woolums, Brandi B. Karisch, Kelsey M. Harvey, Sarah F. Capik, Matthew A. Scott

BRD Score	BRD Symptoms
0 = Normal	
1 = Mild BRD including one or more of the following signs:	• elevated respiratory rate for the environmental conditions. • mild to moderate gauntness • mild depressed attitude: not as alert as expected when viewed from a distance. becomes alert when animal sees human observer. • shallow or dry cough Cattle with score of 1 may also have cloudy, white, or yellow nasal discharge. Nasal discharge in the absence of any other abnormalities is not enough for a score of 1.
2 = Moderate BRD including one or more of the following signs:	• mild or moderate depression o lethargic but may look alert when approached. o head carriage lower than normal, but returns to normal when approached. o hiding behavior: tends to stay behind other cattle, relative to the observer. • mild to moderate muscle weakness o stepping slowly when walking, or mild incoordination o droopy ears • repeated coughing • moderate gauntness • breathing with mild to moderately increased abdominal effort Cattle with a score of 2 may also have: elevated respiratory rate for environmental conditions. clear, cloudy, white, or yellow nasal discharge.
3 = Severe BRD including one or more of the following signs:	• severe depression or weakness o lethargic and does not look more alert when approached. o low head carriage, does not return to normal when approached.

	o does not move away from examiner as expected when approached. o cross stepping • repeated deep cough. • severe breathing effort o open mouth breathing or panting. o moderately to markedly increased abdominal effort. • standing but does not move unless directly stimulated. o if the animal moves, it is very weak: drags feet, sways, stumbles, falls down. • eyes may be very sunken, abdomen may be very gaunt. Cattle with a score of 3 may also have: elevated respiratory rate for the environmental conditions. clear, cloudy, white, or yellow nasal discharge and/or moderate to extreme gauntness.
4 = Moribund (near death)	• Recumbent and does not rise when approached or directly stimulated. • Can rise but stumbles and falls repeatedly/is too weak to remain standing. Moribund animals may also have signs described for score of 1, 2, or 3. Coughing may be heard from animals with any score. NOTE: sometimes animals near death may act aggressively, trying to charge an observer. BRD Case Definition BRD score = 1 or 2 AND have a rectal temperature ≥104 ºF OR a BRD score ≥ 3 regardless of rectal temperature. WITH no other obvious signs of disease (lameness, diarrhea, swollen legs, strange behavior, etc.)

APPENDIX B-3

Clinical Metadata of all selected cattle

sample_ID	animal_ID	Treated TX	Retreat TX	Disease	Died	Severity	Vaccination	Sale	TX Pen
S.049.J009_S49	J009	N	N	Healthy	N	0	Y	Direct	7
S.050.J011_S50	J011	Y	N	BRD	N	1	N	Direct	6
S.051.J013_S51	J013	Y	N	BRD	N	1	N	Direct	6
S.052.J014_S52	J014	Y	N	BRD	N	1	Y	Direct	7
S.053.J015_S53	J015	N	N	Healthy	N	0	Y	Direct	9
S.054.J017_S54	J017	Y	N	BRD	N	1	Y	Direct	9
S.055.J019_S55	J019	Y	N	BRD	N	1	N	Direct	2
S.056.J022_S56	J022	N	N	Healthy	N	0	Y	Auction	10
S.057.J023_S57	J023	N	N	Healthy	N	0	Y	Direct	4
S.058.J024_S58	J024	Y	N	BRD	N	1	N	Auction	11
S.059.J026_S59	J026	Y	Y	BRD	N	2	N	Direct	2
S.060.J027_S60	J027	N	N	Healthy	N	0	N	Direct	2
S.061.J031_S61	J031	Y	N	BRD	N	1	N	Direct	1
S.062.J032_S62	J032	N	N	Healthy	N	0	N	Auction	11
S.063.J033_S63	J033	Y	N	BRD	N	1	N	Auction	11
S.064.J038_S64	J038	N	N	Healthy	N	0	N	Auction	8
S.065.J040_S65	J040	N	N	Healthy	N	0	N	Auction	5
S.066.J041_S66	J041	Y	N	BRD	N	1	N	Direct	1
S.067.J043_S67	J043	Y	N	BRD	N	1	N	Direct	6
S.068.J047_S68	J047	Y	N	BRD	N	1	Y	Auction	10
S.069.J050_S69	J050	Y	N	BRD	N	1	N	Direct	2
S.070.J052_S70	J052	Y	Y	BRD	N	2	N	Auction	8
S.071.J053_S71	J053	N	N	Healthy	N	0	N	Direct	1
S.072.J055_S72	J055	Y	N	BRD	N	1	N	Auction	11

S.073.J059_S73	J059	Y	N	BRD	N	1	N	Auction	11
S.074.J062_S74	J062	Y	Y	BRD	N	2	Y	Direct	4
S.075.J071_S75	J071	Y	N	BRD	N	1	N	Auction	5
S.076.J076_S76	J076	Y	N	BRD	Y	1	N	Direct	1
S.077.J078_S77	J078	Y	N	BRD	N	1	Y	Auction	10
S.078.J086_S78	J086	Y	N	BRD	N	1	N	Auction	5
S.079.J091_S79	J091	Y	N	BRD	N	1	N	Direct	2
S.080.J092_S80	J092	Y	N	BRD	N	1	Y	Auction	10
S.081.J097_S81	J097	Y	Y	BRD	N	2	N	Direct	1
S.082.J103_S82	J103	Y	N	BRD	N	1	Y	Direct	9
S.083.J108_S83	J108	Y	Y	BRD	N	2	N	Direct	1
S.084.J109_S84	J109	N	N	Healthy	N	0	Y	Auction	3
S.085.J111_S85	J111	Y	N	BRD	N	1	Y	Auction	10
S.086.J112_S86	J112	Y	N	BRD	N	1	N	Direct	1
S.087.J113_S87	J113	N	N	Healthy	N	0	Y	Auction	12
S.088.J121_S88	J121	Y	N	BRD	N	1	N	Direct	2
S.089.J122_S89	J122	Y	Y	BRD	N	2	Y	Direct	4
S.090.J123_S90	J123	Y	N	BRD	N	1	N	Auction	11
S.091.J124_S91	J124	N	N	Healthy	N	0	N	Direct	6
S.092.J130_S92	J130	Y	N	BRD	N	1	Y	Direct	9

MS_pasture	Dam ID	EPG	Time to first treatment	Time to second treatment	Risk
4	ID5538	0	N/A	N/A	45
5	B045	150	35	N/A	35

5	A030	1350	36	N/A	36
4	E006	100	39	N/A	39
2	F051	600	N/A	N/A	45
2	ID9589	300	33	N/A	33
6	X057	200	38	N/A	38
4	F117	2100	N/A	N/A	45
1	F532	200	N/A	N/A	45
5	E052	1050	32	N/A	32
6	ID9098	150	12	40	12
6	D537	12500	N/A	N/A	45
3	ID9063	3600	38	N/A	38
5	F562	2800	N/A	N/A	45
5	C257	750	32	N/A	32
6	F045	350	N/A	N/A	45
3	C177	450	N/A	N/A	45
3	Z061	350	37	N/A	37
5	ID9064	1200	35	N/A	35
4	Z012	550	36	N/A	36
6	ID9100	50	40	N/A	40
6	E066	1000	15	34	15
3	G042	1150	N/A	N/A	45
5	G021	900	24	N/A	24
5	C531	8400	32	N/A	32
1	C161	7550	19	38	19
3	D041	1050	11	N/A	11
3	C043	200	44	N/A	44
4	D104	350	25	N/A	25
3	D511	50	38	N/A	38
6	E020	1300	12	N/A	12
4	F633	650	45	N/A	45
3	Z032	300	38	45	38
2	D064	200	39	N/A	39
3	D538	3350	35	45	35
2	A066	82200	N/A	N/A	45
4	C067	250	29	N/A	29
3	C088	450	42	N/A	42
1	ID9612	2300	N/A	N/A	45
6	F046	1950	40	N/A	40
1	F102	150	22	35	22
5	E644	800	18	N/A	18
5	8012A	5800	N/A	N/A	45
2	G067	1600	40	N/A	40

Birthdate	Birthweight	ArrivalAge	ArrivalWt	MSADG
2/18/2021	70	239	675	2.5314
2/18/2021	81	239	637	2.3264
2/17/2021	94	240	571	1.9875
2/19/2021	101	238	718	2.5924
2/19/2021	87	238	526	1.8445
2/19/2021	83	238	568	2.0378
2/20/2021	100	237	577	2.0127
2/20/2021	97	237	566	1.9789
2/20/2021	70	237	433	1.5316
2/20/2021	98	237	565	1.9705
2/21/2021	102	236	630	2.2373
2/21/2021	73	236	540	1.9788
2/22/2021	73	235	590	2.2
2/22/2021	101	235	530	1.8255
2/23/2021	65	234	483	1.7863
2/24/2021	77	233	499	1.8112
2/25/2021	107	232	624	2.2284
2/25/2021	92	232	571	2.0647
2/26/2021	90	231	575	2.0996
2/27/2021	91	230	597	2.2
3/3/2021	93	226	545	2
3/4/2021	80	225	503	1.88

3/4/2021	70	225	476	1.8044
3/6/2021	84	223	450	1.6413
3/8/2021	78	221	402	1.4661
3/10/2021	84	219	562	2.1826
3/12/2021	86	217	584	2.2949
3/13/2021	85	216	549	2.1481
3/14/2021	77	215	487	1.907
3/15/2021	76	214	461	1.7991
3/16/2021	95	213	523	2.0094
3/16/2021	63	213	437	1.7559
3/18/2021	67	211	468	1.9005
3/21/2021	76	208	481	1.9471
3/24/2021	98	205	545	2.1805
3/24/2021	93	205	480	1.8878
3/25/2021	91	204	483	1.9216
3/27/2021	89	202	563	2.3465
3/27/2021	84	202	449	1.8069
4/1/2021	77	197	495	2.1218
4/2/2021	99	196	394	1.5051
4/2/2021	66	196	403	1.7194
4/5/2021	91	193	531	2.2798
4/3/2021	69	195	283	1.0974

APPENDIX C-3

RNA alignment rate of all 43 processes samples

Sample	Alignment Rate
S.050.J011_S50	95.66% overall alignment rate
S.051.J013_S51	95.33% overall alignment rate
S.052.J014_S52	94.87% overall alignment rate
S.053.J015_S53	95.65% overall alignment rate
S.054.J017_S54	96.14% overall alignment rate
S.055.J019_S55	95.53% overall alignment rate
S.056.J022_S56	95.59% overall alignment rate
S.057.J023_S57	96.14% overall alignment rate
S.058.J024_S58	96.01% overall alignment rate
S.059.J026_S59	95.94% overall alignment rate
S.060.J027_S60	96.27% overall alignment rate
S.061.J031_S61	96.01% overall alignment rate
S.062.J032_S62	96.57% overall alignment rate
S.063.J033_S63	96.44% overall alignment rate
S.064.J038_S64	96.66% overall alignment rate
S.065.J040_S65	94.65% overall alignment rate
S.066.J041_S66	94.94% overall alignment rate
S.067.J043_S67	95.36% overall alignment rate
S.068.J047_S68	94.59% overall alignment rate
S.069.J050_S69	94.72% overall alignment rate
S.070.J052_S70	95.00% overall alignment rate
S.071.J053_S71	94.95% overall alignment rate
S.072.J055_S72	95.74% overall alignment rate
S.073.J059_S73	96.77% overall alignment rate
S.074.J062_S74	96.26% overall alignment rate
S.075.J071_S75	96.68% overall alignment rate
S.076.J076_S76	96.22% overall alignment rate
S.077.J078_S77	96.91% overall alignment rate
S.078.J086_S78	95.96% overall alignment rate
S.079.J091_S79	96.55% overall alignment rate
S.080.J092_S80	96.94% overall alignment rate
S.081.J097_S81	95.36% overall alignment rate
S.082.J103_S82	95.57% overall alignment rate
S.083.J108_S83	95.69% overall alignment rate
S.084.J109_S84	95.37% overall alignment rate
S.085.J111_S85	94.59% overall alignment rate
S.086.J112_S86	95.93% overall alignment rate
S.087.J113_S87	97.07% overall alignment rate
S.088.J121_S88	96.11% overall alignment rate
S.088.J121_S88	96.11% overall alignment rate
S.089.J122_S89	96.37% overall alignment rate
S.090.J123_S90	96.73% overall alignment rate

S.091.J124_S91	97.09% overall alignment rate
S.092.J130_S92	96.49% overall alignment rate

APPENDIX D-3

Complete results from differential gene expression analyses (FDR<0.05).

Results from edgeR glmLRT analysis of all Healthy vs BRD cattle (FDR<0.05). Expressional direction (logFC) is in Healthy cattle as compared to BRD cattle.

Gene_ID	logFC	logCPM	LR	Pvalue	FDR
BOLA-DQA5	-8.1569754	4.722276933	23.86309366	1.03E-06	0.017316206

Results from edgeR glmLRT analysis of AUCTION vs DIRECT cattle (FDR<0.05) (TOP 10). Expressional direction (logFC) is in DIRECT cattle as compared to AUCTION cattle.

gene_ID	logFC	logCPM	LR	PValue	FDR
ZCCHC2	-1.53539048	6.358313263	160.6577387	8.13E-37	1.36E-32
RTP4	-2.498936162	6.458198049	156.7201138	5.89E-36	4.93E-32
KIAA1551	-0.985609596	9.712224519	149.8791202	1.84E-34	1.03E-30
LY6E	-1.162077141	8.329505924	149.0228733	2.83E-34	1.19E-30
CMTR2	-1.103723393	5.253776128	144.4838526	2.78E-33	9.32E-30
EIF2AK2	-1.534333517	8.614238981	137.8296203	7.94E-32	2.22E-28
EPSTI1	-1.331556246	7.975061435	136.2524087	1.76E-31	4.20E-28
LOC112443219	2.51928349	3.500029567	132.9532352	9.26E-31	1.94E-27
ZNFX1	-1.67297713	9.812324954	130.3104635	3.50E-30	6.52E-27
PSMF1	-1.017688642	6.750038851	124.2668553	7.36E-29	1.23E-25

Results from edgeR glmLRT analysis of AUCTION Healthy vs BRD cattle (FDR<0.05). Expressional direction (logFC) is in Healthy cattle as compared to BRD cattle.

gene_ID	logFC	logCPM	LR	PValue	FDR
COL1A1	3.246772053	2.153281538	26.789155	2.27E-07	0.002643211
COL1A2	3.04574045	1.01645632	26.1131153	3.22E-07	0.002643211
ARHGAP39	2.066997617	2.071834299	24.83970439	6.23E-07	0.003409521
SPARC	2.009585308	0.33689205	23.51637681	1.24E-06	0.005083629
LOC104973929	1.09249432	3.432634796	22.57581285	2.02E-06	0.006633263
MZF1	1.293629077	4.662123209	21.16966033	4.20E-06	0.011005112
LOC107132911	6.080723902	0.568212374	20.95901184	4.69E-06	0.011005112
LOC107132340	1.617532237	3.595466804	18.41749959	1.77E-05	0.036411245
RN18S1	0.953116968	8.823536569	17.95088618	2.27E-05	0.041351261

Results from edgeR glmLRT analysis of all DIRECT Healthy vs BRD cattle (FDR<0.05). Expressional direction (logFC) is in Healthy cattle as compared to BRD cattle.

gene_ID	logFC	logCPM	LR	PValue	FDR
EFEMP1	3.054431676	0.278151636	24.46351904	7.57E-07	0.012308438
LOC112445634	-2.259329763	-1.430700353	22.74813799	1.85E-06	0.014455795
HELQ	1.970697562	0.418257932	22.0414401	2.67E-06	0.014455795
LOC112446743	1.821455854	-1.079592093	19.33210373	1.10E-05	0.044632323

APPENDIX E-3

Complete functional enrichment results of identified DEGs (FDR<0.05)

Auction vs. Direct GO-BP (TOP 10)

geneSet	description	link	size	overlap	expect	enrichmentRatio	pValue	FDR
GO:0006952	defense response	http://amigo.geneontology.org/amigo/term/GO:0006952	673	155	91.7505438 7	**1.689363283**	**4.63E-12**	**4.75E-08**
GO:0002376	immune system process	http://amigo.geneontology.org/amigo/term/GO:0002376	1100	226	149.9637418	**1.507030948**	**1.01E-11**	**5.19E-08**
GO:0044248	cellular catabolic process	http://amigo.geneontology.org/amigo/term/GO:0044248	1113	223	151.7360406	**1.469657433**	**1.80E-10**	**4.74E-07**
GO:0045087	innate immune response	http://amigo.geneontology.org/amigo/term/GO:0045087	301	82	41.0355329 9	**1.998268184**	**1.85E-10**	**4.74E-07**
GO:0009056	catabolic process	http://amigo.geneontology.org/amigo/term/GO:0009056	1256	242	171.231327	**1.41329279**	**1.37E-09**	**2.81E-06**
GO:0006950	response to stress	http://amigo.geneontology.org/amigo/term/GO:0006950	1758	319	239.6693256	**1.331000533**	**2.09E-09**	**3.30E-06**
GO:0009605	response to external stimulus	http://amigo.geneontology.org/amigo/term/GO:0009605	1037	206	141.3749094	**1.457118529**	**2.25E-09**	**3.30E-06**

GO:0051707	response to other organism	http://amigo.geneontology.org/amigo/term/GO:0051707	446	105	60.8034807	**1.726874821**	**4.91E-09**	**6.30E-06**
GO:0006955	immune response	http://amigo.geneontology.org/amigo/term/GO:0006955	656	141	89.4329224	**1.57660061**	**7.10E-09**	**7.42E-06**
GO:0043207	response to external biotic stimulus	http://amigo.geneontology.org/amigo/term/GO:0043207	449	105	61.2124728	**1.715336682**	**7.23E-09**	**7.42E-06**

Auction vs. Direct GO-CC (TOP 10)

geneSet	**description**	**link**	**size**	**overlap**	**expect**	**enrichmentRatio**	**pValue**	**FDR**
GO:0005829	cytosol	http://amigo.geneontology.org/amigo/term/GO:0005829	1795	331	204.057232	1.622093939	0	0
GO:0022626	cytosolic ribosome	http://amigo.geneontology.org/amigo/term/GO:0022626	95	37	10.7996864	3.426025408	2.98E-12	2.01E-09
GO:0044445	cytosolic part	http://amigo.geneontology.org/amigo/term/GO:0044445	184	55	20.9172873	2.629404048	5.64E-12	2.54E-09
GO:0031981	nuclear lumen	http://amigo.geneontology.org/amigo/term/GO:0031981	1937	308	220.1999216	1.398728927	1.11E-11	3.73E-09
GO:0005654	nucleoplasm	http://amigo.geneontology.org/amigo/term/GO:0005654	1444	232	164.155233 2	1.413296399	3.73E-09	1.01E-06
GO:0022625	cytosolic large ribosom	http://amigo.geneontology.org/amigo/term/GO:0022625	50	22	5.68404547 2	3.870482759	5.30E-09	1.19E-06

	al subunit							
GO:0031982	vesicle	http://amigo.geneontology.org/amigo/term/GO:0031982	990	163	112.544100 4	1.448321142	2.59E-07	4.99E-05
GO:1990904	ribonucleoprotein complex	http://amigo.geneontology.org/amigo/term/GO:1990904	573	104	65.1391611 1	1.596581814	5.43E-07	9.17E-05
GO:1902494	catalytic complex	http://amigo.geneontology.org/amigo/term/GO:1902494	886	145	100.721285 8	1.439616253	1.86E-06	2.79E-04
GO:0031410	cytoplasmic vesicle	http://amigo.geneontology.org/amigo/term/GO:0031410	922	149	104.813798 5	1.421568554	2.77E-06	3.53E-04

Auction vs. Direct GO-MF

gene Set	**description**	**link**	**size**	**overlap**	**expect**	**enrichment Ratio**	**pValue**	**FDR**
GO:0003735	structural constituent of ribosome	http://amigo.geneontology.org/amigo/term/GO:0003735	148	40	20.11459027	1.988606253	9.91E-06	0.015448897
GO:0008329	signaling pattern recognition receptor activity	http://amigo.geneontology.org/amigo/term/GO:0008329	9	7	1.223184544	5.722766885	2.36E-05	0.015448897
GO:0038187	pattern recognition receptor activity	http://amigo.geneontology.org/amigo/term/GO:0038187	9	7	1.223184544	5.722766885	2.36E-05	0.015448897
GO:0043168	anion binding	http://amigo.geneontology.org/amigo/term/GO:0043168	1529	259	207.805463	1.246357994	2.49E-05	0.015448897

Auction vs. Direct Reactome (TOP 10)

geneSet	description	link	size	overlap	expect	enrichmentRatio	pValue	FDR
R-BTA-168256	Immune System	http://reactome.org/PathwayBrowser/#/R-BTA-168256	967	195	134.9302326	1.445191313	1.74E-09	2.60E-06
R-BTA-168249	Innate Immune System	http://reactome.org/PathwayBrowser/#/R-BTA-168249	557	124	77.72093023	1.595451825	1.06E-08	7.94E-06
R-BTA-6798695	Neutrophil degranulation	http://reactome.org/PathwayBrowser/#/R-BTA-6798695	293	71	40.88372093	1.736632537	8.52E-07	4.24E-04
R-BTA-1169410	Antiviral mechanism by IFN-stimulated genes	http://reactome.org/PathwayBrowser/#/R-BTA-1169410	17	11	2.372093023	4.637254902	2.02E-06	7.53E-04
R-BTA-1169408	ISG15 antiviral mechanism	http://reactome.org/PathwayBrowser/#/R-BTA-1169408	15	10	2.093023256	4.777777778	4.08E-06	0.001218963
R-BTA-913531	Interferon Signaling	http://reactome.org/PathwayBrowser/#/R-BTA-913531	35	16	4.883720 93	3.276190476	5.33E-06	0.001326881
R-BTA-1236975	Antigen processing-Cross presentation	http://reactome.org/PathwayBrowser/#/R-BTA-1236975	48	19	6.697674419	2.836805556	9.56E-06	0.002039455

R-BTA-450408	AUF1 (hnRNP D0) binds and destabilizes mRNA	http://reactome.org/PathwayBrowser/#/R-BTA-450408	37	16	5.162790698	3.099099099	1.29E-05	0.002401205
R-BTA-983170	Antigen Presentation: Folding, assembly and peptide loading of class I MHC	http://reactome.org/PathwayBrowser/#/R-BTA-983170	15	9	2.093023256	4.3	4.35E-05	0.007216215
R-BTA-1236978	Cross-presentation of soluble exogenous antigens (endosomes)	http://reactome.org/PathwayBrowser/#/R-BTA-1236978	33	14	4.604651163	3.04040404	5.84E-05	0.007928107

Auction BRD vs. Healthy GO-BP

geneSet	**description**	**link**	**size**	**overlap**	**expect**	**enrichmentRatio**	**pValue**	**FDR**
GO:0070208	protein heterotrimerization	http://amigo.geneontology.org/amigo/term/GO:0070208	5	2	0.002071895	965.3	1.29E-06	0.009912117
GO:0043589	skin morphogenesis	http://amigo.geneontology.org/amigo/term/GO:0043589	6	2	0.002486274	804.4166667	1.93E-06	0.009912117

Auction BRD vs. Healthy GO-CC

geneSet	description	link	size	overlap	expect	enrichmentRatio	pValue	FDR
GO:0005584	collagen type I trimer	http://amigo.geneontology.org/amigo/term/GO:0005584	2	2	7.84E-04	2551	1.15E-07	1.56E-04
GO:0005583	fibrillar collagen trimer	http://amigo.geneontology.org/amigo/term/GO:0005583	4	2	0.001568013	1275.5	6.91E-07	2.33E-04
GO:0098643	banded collagen fibril	http://amigo.geneontology.org/amigo/term/GO:0098643	4	2	0.001568013	1275.5	6.91E-07	2.33E-04
GO:0098644	complex of collagen trimers	http://amigo.geneontology.org/amigo/term/GO:0098644	4	2	0.001568013	1275.5	6.91E-07	2.33E-04
GO:0062023	collagen-containing extracellular matrix	http://amigo.geneontology.org/amigo/term/GO:0062023	79	3	0.030968248	96.87341772	1.78E-06	4.80E-04
GO:0044420	extracellular matrix component	http://amigo.geneontology.org/amigo/term/GO:0044420	16	2	0.006272025	318.875	1.38E-05	0.003106359
GO:0031012	extracellular matrix	http://amigo.geneontology.org/amigo/term/GO:0031012	167	3	0.065464524	45.826347 31	1.70E-05	0.003282103
GO:0005581	collagen trimer	http://amigo.geneontology.org/amigo/term/GO:0005581	39	2	0.015288122	130.8205128	8.50E-05	0.014343083

Auction BRD vs. Healthy GO-MF

geneSet	description	link	size	overlap	expect	enrichmentRatio	pValue	FDR
GO:0048407	platelet-derived growth factor binding	http://amigo.geneontology.org/amigo/term/GO:0048407	6	2	0.000266489	750.5	2.22E-06	0.005509994
GO:0005201	extracellular matrix structural constituent	http://amigo.geneontology.org/amigo/term/GO:0005201	16	2	0.007106374	281.4375	1.77E-05	0.022007344

Auction BRD vs. Healthy Reactome (TOP 10)

geneSet	**description**	**link**	**size**	**overlap**	**expect**	**enrichmentRatio**	**pValue**	**FDR**
R-BTA-3000178	ECM proteoglycans	http://reactome.org/PathwayBrowser/#/R-BTA-3000178	26	3	0.020324409	147.6057692	4.64E-07	6.94E-04
R-BTA-2214320	Anchoring fibril formation	http://reactome.org/PathwayBrowser/#/R-BTA-2214320	4	2	0.003126832	639.625	2.75E-06	0.002052363
R-BTA-75892	Platelet Adhesion to exposed collagen	http://reactome.org/PathwayBrowser/#/R-BTA-75892	5	2	0.00390854	511.7	4.58E-06	0.002052363
R-BTA-2243919	Crosslinking of collagen fibrils	http://reactome.org/PathwayBrowser/#/R-BTA-2243919	6	2	0.004690248	426.4166667	6.87E-06	0.002052363

R-BTA-430116	GP1b-IX-V activation signalling	http://reactome.org/PathwayBrowser/#/R-BTA-430116	6	2	0.00469024	426.4166667	6.87E-06	0.0020523
R-BTA-8948216	Collagen chain trimerization	http://reactome.org/PathwayBrowser/#/R-BTA-8948216	11	2	0.00859878	232.5909091	2.52E-05	0.0062629
R-BTA-8874081	MET activates PTK2 signaling	http://reactome.org/PathwayBrowser/#/R-BTA-8874081	12	2	0.00938049	213.2083333	3.02E-05	0.0064401
R-BTA-1442490	Collagen degradation	http://reactome.org/PathwayBrowser/#/R-BTA-1442490	16	2	0.01250732	159.90625	5.48E-05	0.0089683
R-BTA-3000171	Non-integrin membrane-ECM interactions	http://reactome.org/PathwayBrowser/#/R-BTA-3000171	17	2	0.01328903	150.5	6.21E-05	0.0089683
R-BTA-1474244	Extracellular matrix organization	http://reactome.org/PathwayBrowser/#/R-BTA-1474244	137	3	0.107094	28.01277372	7.37E-05	0.0089683

CHAPTER IV

PERFORMANCE DETERMINATION OF CLONED BEEF CATTLE

Mollie Green, David Lust, Matthew Scott, Tommy Perkins

4.1. Abstract

Animal breeding and genetics have shifted significantly over the past several decades. Previously, genetic improvement of beef cattle was largely dependent on visual appearance. While this remains valuable in selecting cattle for breeding, current technology and performance determination contributes to modern genetic improvement strategies. As such, we have continued a unique crossbreeding project beginning with rare carcasses that exhibited a highly desirable yet antagonistic trait which includes being USDA Prime and yield grade 1. Sires (Alpha, Delta and AxG1) were produced and evaluated originally for high quality carcass characteristics, then bred accordingly in the summer of 2020. Our objective was to see if their offspring could replicate similar outcomes and produce quality carcass and growth characteristics. Here, thirty-five bull (n=24) and heifer (n=11) calf offspring were fed a commercial feedlot ration at the Palo Duro Consultation, Research & Feedlot in Canyon, TX for 68 days. Parentage results were tested to confirm sire, followed by weight gain, feed intake and carcass ultrasound

data collections. . Significant differences were found (P<.05) for entire average daily gain and average intake, rib fat and backfat, and ribeye area and percent intramuscular fat for both SIRE and SEX. Spearmen's Rank correlations were found of (P<.05), with a coefficient of 0.59 for ADG and average intake, 0.42 for RF and BF both for SIRE. Spearmen's rank correlations for SEX found no significance for ADG and average intake but when evaluating RF and BF between sex on d68 significance was found (P< .05) with a coefficient of 0.39. Our results can help confirm the relationship between larger RF and BF with weight gain, as well as the relationship between carcass quality in the specific sex of beef cattle.

4.2. Introduction

In an ever-growing world, agriculture technology continues to advance with the need for more efficient food and production to feed our population. With the recent development of genetic and genomic resources, we can understand and utilize these resources to help advance the beef cattle industry and help it become more efficient. Genetic prediction is now an essential technology for improvement in not only animal breeding, but additionally in agronomy as well. A big part of genetic and genomic technology is researchers focusing on predicting the breeding values and heritability (Mateescu, 2020). Advances in agriculture, genetically improved crops, and animals, remain extremely important to increase production and quality to not only satisfy the global food demand, but also help contribute to the world's food production (Boyles et al., 2018; Sahebi et al., 2018, Hickey et al., 2019). Focusing on our world's food

production is important as our population continues to grow, comes a need for food, and less available natural resources, causing a need for agricultural advances.

When evaluating ways to improve beef cattle genetics, opportunity exists by the use of breeding genetics or improving heritability from high quality bovine dams and sires. Beef cattle genetic improvement is typically utilized by the seedstock industry, whose main goal is to multiply their elite genetics (Herring, 2014). Being able to multiply elite genetics via reproduction techniques has become a large part of the beef cattle system. Embryo biotechnology has become one of the prominent techniques when pertaining to reproductive techniques (Ferré et al., 2020). Beef cattle genetic technology has evolved through three major types of production techniques. Beef cattle production techniques include traditional embryo transfer which is typically done in vivo embryo production by donor superovulation (Bortoletto et al., 2018), in vitro embryo production by ovum pick up with in vitro fertilization, also known as IVF (First and Parrish, 2019), and lastly cloning by somatic cell nuclear transfer and transgenic animal production (Wu and Zan, 2011). Embryo biotechnology, better known as the use of embryo transfers or somatic cell transfers, have widely been used in the beef cattle industry for the use of reproducing high quality traits or genetics (Smith, 1989).

Cloning is a procedure in which produces a twin of given animal at a different time (Shelton, 1990). Cloning and making genetic copies of high-quality seedstock and or show cattle with excellent traits that others want to reproduce. Other purposes may be to make a copy of a certain dam or sire that has sentimental value, similar to cloning of pets (Faber et al., 2004). The new opportunities with advancements given with cloning may provide for improvement in genetic gain. The ultimate goal of cloning has often been

envisioned as a system for producing uniformity of the ideal beef cow, which is done via somatic cell transfer (Van Vleck, 1999). Somatic cell nuclear transfer offers the ability to preserve genetic material to produce clones by copying the DNA from that collected material (Taylor-Robinson et al., 2014). Performing these methods can result in cloned offspring that are economically competitive or the same as other elite beef cattle that are produced by more traditional means (Faber et al., 2004).

Drs. Lawrence and Hawkins, of West Texas A&M University, previously utilized cloning technology to produce live animals from three beef carcasses that were USDA Prime and yield grade 1, a highly sought-after carcass, (Lawrence and Hawkins, 2013). Muscle and fat are antagonists, so a Prime, yield grade 1 animal is inherently rare in beef production (Deng et al., 2017). Out of Lawrence and Hawkins study, cloned sires Alpha, Delta and AxG1 were produced and evaluated originally, then bred accordingly to reproduce offspring in hopes of finding these same carcass quality traits. The objective of our study was to evaluate the performance determination produced from these direct clones and their offspring. Parentage results were tested to confirm the sire, then each individual's weight gain, feed intake data and carcass ultrasound data was collected during this time period to evaluate the performance determination of these cloned beef cattle.

4.3. Materials and Methods

4.3.1. Animal Use and Study Enrollment

All animal use and procedures were approved by the West Texas A&M University Animal Care and Use Committee (IACUC protocol #2021.10.005). Three sires, Alpha, Delta, and AxG1, were produced and evaluated for their carcass and breeding characteristics, then bred accordingly to selected dam and recipients. An original total of 43 head were used in the study, although were removed for no parentage match to the three cloned sires enrolled in the study and must have been bred via clean up bull. So, thirty-five bull (n=24) and heifer (n=11) calf offspring from cloned sires were used in the study. The offspring were fed a standardized commercial feedlot ration, which contained approximately 4% additives 10% roughage and 86% milled grain (Forster, 2022), at the Palo Duro Consultation, Research & Feedlot in Canyon, TX for 78 days. Cattle enrolled in this study arrived on January 17,, 2022 and left the research feedlot on April 5, 2022. During their original arrival, on d0 of the study, temperatures were taken via rectal thermometer and treated for any temperature that was found to be ≥ 102.5°F. Treatment for qualified calves included an injection of a macrolide antibiotic (6mL SQ; Draxxin). All calves received subcutaneous modified-live viral vaccination (2 mL SQ; Pyramid 5 (Boehringer Ingelheim Animal Health)), per label instruction, during arrival to the research feedlot. During day zero of the trial all cattle were administered a topical dewormer (Vetrimec Ivermectin Pour-On), per label direction. Individuals were housed in two different pens, separated by sex (HEIFER and BULL), at the research feedlot. Both pens were equipped with monitoring systems taking raw intake feed data (RFI), measured by Vytelle Insight Beef Genetics: Feed Intake Report. During the calves sixty-eight (n=68) days on feed and were weighed on February 28th, 2022 (d28) and April 5th 2022 (d68). All cattle were given 10-day period to acclimate to feed bunks, therefore weight

recording (d0) began on January 27, 2022. Information for all selected cattle in the study can be found in APPENDIX A-4.

4.3.2. Parentage Testing, Feed Intake, and Carcass Scan Data

Offspring of the sires Alpha, Delta and AxG1 were confirmed via Neogen SeekSire parentage testing (APPENDIX B-4), which compares DNA markers from bulls or cows with DNA from calves to verify each calf's sire or dam (Van Eenennaam, 2019). Cut skin samples from the dorsal pinna were acquired from calves upon arrival to the Palo Duro Consultation, Research & Feedlot in Canyon, TX. Skin samples were sent to Neogen Beef Cattle, Inc. in Lincoln, NE for parent verification . DNA from skin tissue samples was extracted and evaluated through the Allflex tissue sampling tag applicator loaded with the Allflex DNA sampling unit.

At the Palo Duro Consultation, Research & Feedlot, all cattle were given electronic identification tags upon arrival, in addition to monitoring pens, to evaluate raw feed intake of each individual in the study. These monitoring systems known as Vytelle SENSE (previously known named GrowSafe pens) which are an individual animal data capture system. It records feed intake and in-pen weight gain measurements to help identify elite-performing animals expressing economically and environmentally important traits (Marston, 2021). These data were measured by the Vytelle Insight Beef Genetics: Feed Intake Report system which tracks multiple things including data measurement, changes in animal behavior and prediction of feed efficiency traits (Vytelle – Site Preparation Manual, 2020) to put together the intake data (APPENDIX C-4) of the cattle in this study.

Carcass scans of the ribeye area (REA), rib fat (BF), rib fat (RF), and percent intramuscular fat (IMF%). REA, in square inches, is measured between the 12th and 13th ribs and gives an estimate of the amount of muscle and lean product in the animal. BF (back fat), in inches, is also measured between the 12th and 13th ribs and is an estimate of the external fat on the animal. RF is an additional measure of external fat on the animal and is also measured in inches, which is measured at the rump of each individual. Lastly, IMF% is an objective measure of marbling in cattle (Vann et al., 2018). The individuals received scans of all listed scan areas which were taken before arrival of the research feedlot on January 3, 2022, by a certified technician. The cattle were scanned again before exiting the feedlot on March 28, 2022, in order to see the improvement of carcass quality while growing on the feedlot, done by the same ultrasound technician. The ultrasound technicians are required to meet a set of minimum standards for FIGURE quality, data accuracy and knowledge of ultrasound technology determined by the bylaws of the Ultrasound Guidelines Council (UGC (Ultrasound Guidelines Council, 2021). The results were sent back, found in APPENDIX D-4, and analyzed for each individual's carcass characteristics.

Once the feed period came to an end, the daily dry matter intake was calculated from the amount of feed consumed, along with the average daily gain and average body weight obtained for the time period to find the residual feed intake (RFI), originally discovered by Koch et al., (1963). The expected dry matter intake is obtained from linear regression of DMI and ADG. The formula used: $Y = \beta 0 + \beta 1\ X1 + \beta 2\ X2 + \varepsilon$ where Y is expected dry matter intake, β0 is the equation intercept, β1 and β2 are the coefficients of the equation, X1 is the metabolic body weight, X2 is the average daily gain, and ε is the

residual. The predicted feed intake of each animal is estimated using the equation. This prediction is the average or expected value for animals of similar weights and rates of gain. The actual feed intake minus the predicted feed intake corresponds to the RFI, which can be found in APPENDIX E-4.

4.3.3. Sire and Sex Analyses

SIRE and SEX within DELTA differences were explored within RStudio, via R v4.1.2 using generalized linear modeling (glm), with the R package lme4 to fit a linear mixed-effects model (LMM) to data, via REML or maximum likelihood (Bates et al, 2015). Next, an approximate F-test based on the Kenward-Roger approach, developed in 1977 (Kenward-Roger, 1977), was performed followed by an analysis of deviance with a type III chi-square test to determine significance between variables. Any comparison with a P-value < .05 was considered to be significantly different. Lastly, Spearman's Rank correlation coefficients were performed to identify variable relationships; correlations were considered significant having a P-value < .05 (Spearman, 1904).

Specifically, analyses were performed in two blocks. First, differences of factors that have potential to be different and significant of SIRE, evaluating the three variables (ALPHA n=2) (AG1 n=2) (DELTA n=29). Variables found in the metadata from this study(APPENDIX A-4) included average daily gain for entire feeding period in lbs/day (ADG Entire), average intake of each individual the entire feeding period in lbs/day (Avg_Intake), weights from exiting the feedlot (d68 Weights), carcass characteristics when exiting the feedlot, which further included, rump fat (RF d68), back fat (BF d68),

ribeye area (REA d68) and percent of intramuscular fat (IMF% d68). Second, difference of sexes (HEIFER n= 10) (BULL n=21) within the sire DELTA were performed due to the high number of individuals within this group compared to other SIRE groups. Variables that were compared between HEIFER and BULL in DELTA include average daily gain for entire feeding period in lbs/day (ADG Entire), average intake of each individual the entire feeding period in lbs/day (Avg_Intake), their weights from exiting the feedlot (d68 Weights), carcass characteristics when exiting the feedlot which include, rump fat (RF d68), back fat (BF d68), ribeye area (REA d68), and percent of intramuscular fat (IMF% d68).

4.4. Results

4.4.1. Descriptive Statistical Analysis Across SIRE and SEX

When evaluating initial analyses between the three sires, the following averages between the individuals were found. Averages of the d0 weight, d28 weight and d68 weight (TABLE 4.1). Average Daily Gains between the weight collections of d0 and d28, d28-d68, and d0-d68 were calculated (TABLE 4.2). During the conclusion of their days on feed, their entire average daily gain was found (FIGURE 4.1 A) and the entire feed intake averages were found (FIGURE 4.1 B).

Due to the increased number of individuals of DELTA, a statistical analysis were ran between HEIFER and BULL in the group of that sire. Initial results entailed the averages of the d0, d28, and d68 weights for the individuals between those two groups (TABLE 4.3). Average Daily Gains between the weight collections of d0 and d28, d28-d68, and d0-d68 were then calculated (TABLE 4.4). During the entire time on feed, the

average daily gain (FIGURE 4.2 A) and entire feed intake (FIGURE 4.2 B) was found between both groups.

4.4.2. Descriptive Analysis of D0 and D68Carcass Data Across SIRE and SEX

On d0, some results were not conclusive for this analysis as there were reader errors from the carcass scan found respectively in APPENDIX D-4, so carcass scan analyses from d68 were analyzed. The measurements of the individuals of each sires RF (FIGURE 4.3 A), BF (FIGURE 4.3 B), REA (FIGURE 4.3 C) IMF% (FIGURE 4.3 D) were determined. It was found that within the individuals that were sired by AG1possessed an average RF of .22in, BF of .22in, REA of 10.15in and IMF% of 4.82%. Within the individuals that were sired by ALPHA, it was found they possessed an average RF at .17in, BF of .17, REA of 9.9in and IMF% of 4.36%. Lastly, the individuals that were sired from DELTA possessed an average RF of .29in, BF of .27in, REA of 10.79in, and IMF% at 5.52%.

When evaluating the ultrasound carcass scans, there was difference between the group SEX within HEIFER and BULL. When comparing the scans after the groups had finished their days on feed (d68), HEIFER possessed an average of 0.334in for RF, and 0.27in for BULL (FIGURE 4.4 A). When comparing BF, there was an average of 0.331in for HEIFER and 0.24in for BULL (FIGURE 4.4 B). Next, evaluating REA, there was an average of 9.7in for HEIFER and 11.31in for BULL (FIGURE 4.4 C). Lastly when evaluating IMF%, 7.11% for HEIFER and 4.76% for the group BULL (FIGURE 4.4 D).

4.4.3 Results of RFI Calculations

Using the results of actual DMI and the predicted DMI that was collected for each individual, their RFI was then calculated (APPENDIX E-4). When evaluating within the group SIRE, the individuals of AG1 had an average RFI of -1.19, ALPHA had an average RFI of 0.77 and DELTA had an average RFI of 0.99. When analyzing the group SEX, the individuals within the group BULL had an RFI of 1.10, and those of HEIFER, 0.94. Although, there were no statistical differences found when comparing the calculated RFI to SIRE.

4.4.4. Results of Statistical Analysis within SIRE and SEX

Significant differences were found (P<.05) for entire ADG and average intake, RF and BF, and REA and IMF% for both SIRE and SEX. While evaluating the ran box and whisker plots of SIRE (FIGURE 4.3), when comparing correlations in the linear mixed model formulas, RF and BF was found to have significance between sire DELTA when compared to AG1 and ALPHA, for RF and BF, DELTA had a RF that was .07 in larger than AG1 and .12 in larger than ALPHA.DELTA had a back fat that was .05 in larger than AG1 and .10 larger than ALPHA. While evaluating the ran box and whisker plots of SEX (FIGURE 4.4), there were was significance in the carcass scan results as well. When looking at the group HEIFER, on average the individuals had a RF that was .064in and a BF that was .091in larger when compared to the group BULL. Spearman's Rank correlations were found of (P<.05), with a coefficient of 0.59 for ADG and average intake, 0.42 for RF and BF both for SIRE. Although when evaluating correlation of REA and IMF% no significance was found for SIRE. Spearmen's rank correlations for SEX found no significance for ADG and average intake but when evaluating RF and BF

between SEX on d68 significance was found (P< .05) with a coefficient of 0.39. Although, when comparing REA and IMF% on d68 no significance was found (P> .05).

4.5. Discussion

4.5.1. The Comparison of Sires

When evaluating the variables within SIRE (AG1, ALPHA, DELTA), it appears as if the individuals in the study who were sired by DELTA had numerically larger characteristics within the data set. Although there was a significantly larger number of individuals in this group, when compared to the others (n=29), the calves from DELTA still had the largest average of d0, d28 and d68 weight. When looking at the period gains and average daily gains, DELTA did not always perform the best in gains, although these individuals ended at the average highest weight. This can be confounding since they began at the average highest weight on d0 and required the smallest amount of weight gain when compared to the individuals from the other two sires. ALPHA had the highest period gain and average daily gain during the first feeding period, between d0 and d28. Although following this DELTA had the highest period gain and average daily gain between the feeding period of d28 and the final day on feed. This also helps argue for DELTA averaging the highest weight at the end of the feeding period. The individual's period gain and average daily gain (ADG) have a relationship, as when calculating the ADG you must take from the period gain for that individual (Flohr et al., 2018).

Although there were no statistical differences when comparing the calculated RFI to SIRE, ALPHA had negative average RFI measurement meaning the individuals of this group had the most efficient gains in comparison to their feed intake. Like previously mentioned, ALPHA had a numerically larger period gain and average gain during d0-d28

on the feedlot. This could be in correlation to the individuals within ALPHA having a negative RFI average measurement.

When evaluating carcass scans between SIRE, it was found that DELTA had a RF and BF that was statistically higher vs. AG1, ALPHA, although REA and %IMF were not statistically different across SIRE. This helps support the previous stated fact that there is a correlation between cattle weight and fat thickness. Similar findings were found in one study done by Pariacote et al., (1998), on Genetic and Phenotypic Parameters for Carcass Traits of American Shorthorn Beef Cattle. Their results indicate that heavier weights tend to have more fat thickness and a greater fraction of % Kidney Pelvic Heart fat when compared to cattle with lighter weights. A review titled, Breed differences and genetic parameters for body composition traits in beef cattle, done by Marshall (1994) also confirmed this relationship. These studies help argue the significance of DELTA obtaining the highest weight at each collection, as well as the thickest BF and RF.

4.5.2. The Comparison of the Sexes within Delta

When evaluating HEIFER and BULL within the sire, sex difference shows interesting data points too. Gender has an impact on performance as growth is highest for bulls, followed by steers and then heifers (Lehmkuhler, 2014). Steers have been shown to have about 15-20% per day greater daily gains compared to heifers (Brazle & Higgins, 1999). When comparing gender difference statements to the two groups found within DELTA, we can confirm that in the group BULL, the average weight, average daily gain and average feed intake was all higher when compared to the group HEIFER. Although, when looking at the carcass characteristics from the d0 and d68 scans, the group HEIFER, had significant quality carcass characteristic traits. It was found that in the

group HEIFER, there was a statistically higher average in d68 BF, RF, and %IMF when compared to the group BULL. The only characteristic where BULL had a numerically larger average, was in REA size. Heifers and cows both are reported to deposit more fat than steers and bulls (Bureš & Bartoň, 2012). Marbling greatly varies among cattle belonging to different sexes, and particularly, females have genetic makeup that efficiently controls deposition (Venkata Reddy et al., 2014). With female cattle having the ability to have quality carcass traits, heifers could possibly become a premium beef brand, that beef grown from steers currently takes a large part of market share across The U.S. (Drouillard, 2018). These results can help confirm the relationship between BF and RF with weight gain, as well as the relationship between carcass quality in the specific sex of beef cattle, to help the industry improve and understand more of how to utilize these traits.

4.6. Conclusions

When evaluating weight with BF and RF, the higher the live weight resulted in a larger fat thickness. Also, in the evaluation of sex, the heifers have genetic makeup that efficiently controls deposition. Although there was a small sample size and unequal comparisons across SIRE, results could help confirm the relationship between larger RF and BF with weight gain, as well as the relationship between carcass quality in the specific sex of beef cattle. Results are important to understand as we try to utilize a market and the beef cattle industry to produce more efficient product to the consumer. The objective of improvement in beef cattle continues to be important as the growth of our country continues to rise. Understanding the relationship between live cattle and their carcass value can help improve an imperative phase of beef production.

4.7. Literature Cited

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using**lme4**. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Bortoletto, P., Bakkensen, J., & Anchan, R. M. (2018). Embryo transfer: Timing and techniques. *Minerva Endocrinology*, *43*(1). https://doi.org/10.23736/s0391-1977.17.02649-9

Boyles, R. E., Brenton, Z. W., & Kresovich, S. (2018). Genetic and genomic resources of sorghum to connect genotype with phenotype in contrasting environments. *The Plant Journal*, *97*(1), 19–39. https://doi.org/10.1111/tpj.14113

Brazle, F. K., & Higgins, J. J. (1999). Effects of starting weight, body condition, and age on gain of cattle grazing native grass. *Kansas Agricultural Experiment Station Research Reports*, (1), 157–159. https://doi.org/10.4148/2378-5977.1872

Bureš, D., & Bartoň, L. (2012). Growth performance, carcass traits and meat quality of bulls and heifers slaughtered at different ages. *Czech Journal of Animal Science*, *57*(1), 34–43. https://doi.org/10.17221/5482-cjas

Cohen, J. E. (2003). Human population: The next half century. *Science*, *302*(5648), 1172–1175. https://doi.org/10.1126/science.1088665

Deng, B., Zhang, F., Wen, J., Ye, S., Wang, L., Yang, Y., Gong, P., & Jiang, S. (2017). The function of myostatin in the regulation of Fat Mass in mammals. *Nutrition & Metabolism*, *14*(1). https://doi.org/10.1186/s12986-017-0179-1

Drouillard, J. S. (2018). Current situation and future trends for beef production in the United States of America — A Review. *Asian-Australasian Journal of Animal Sciences*, *31*(7), 1007–1016. https://doi.org/10.5713/ajas.18.0428

Faber, D. C., Ferre, L. B., Metzger, J., Robl, J. M., & Kasinathan, P. (2004). Review: Agro-economic impact of cattle cloning. *Cloning and Stem Cells*, *6*(2), 198–207. https://doi.org/10.1089/1536230041372463

Ferré, L. B., Kjelland, M. E., Strøbech, L. B., Hyttel, P., Mermillod, P., & Ross, P. J. (2020). Review: Recent advances in bovine in vitro embryo production: Reproductive Biotechnology History and Methods. *Animal*, *14*(5), 991–1004. https://doi.org/10.1017/s1751731119002775

First, N. L., & Parrish, J. J. (2019). In-vitro fertilization of ruminants. *Bioscientifica Proceedings*. https://doi.org/10.1530/biosciprocs.9.012

Flohr, J. R., Dritz, S. S., Tokach, M. D., Woodworth, J. C., DeRouchey, J. M., & Goodband, R. D. (2018). Development of equations to predict the influence of floor space on average daily gain, average daily feed intake and gain : Feed ratio of finishing pigs. *Animal*, *12*(5), 1022–1029. https://doi.org/10.1017/s1751731117002440

Forster, S.-J. (2022, September 23). *Diet formulations*. Retrieved April 6, 2023, from https://futurebeef.com.au/resources/beef-cattle-feedlots-diet-formulations/#:~:text=Normal%20feedlot%20rations%20contain%20about%204%25%20additives,%28which%20are%20premixed%29%2C%2010%25%20roughage%20and%2086%25%20grain.

Herring, A. D. (2014). Introduction to beef cattle production systems. *Beef Cattle Production Systems*, 1–12. https://doi.org/10.1079/9781780645070.0001

Hickey, L. T., N. Hafeez, A., Robinson, H., Jackson, S. A., Leal-Bertioli, S. C., Tester, M., Gao, C., Godwin, I. D., Hayes, B. J., & Wulff, B. B. (2019). Breeding crops to feed 10 billion. *Nature Biotechnology*, *37*(7), 744–754. https://doi.org/10.1038/s41587-019-0152-9

Kenward, M. G., & Roger, J. H. (1997). Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics*, *53*(3), 983. https://doi.org/10.2307/2533558

Koch, R. M., Swiger, L. A., Chambers, D., & Gregory, K. E. (1963). Efficiency of feed use in beef cattle. *Journal of Animal Science*, *22*(2), 486–494. https://doi.org/10.2527/jas1963.222486x

Koch, R. M., Swiger, L. A., Chambers, D., & Gregory, K. E. (1963). Efficiency of feed use in beef cattle. *Journal of Animal Science*, *22*(2), 486–494. https://doi.org/10.2527/jas1963.222486x

Lawrence, T., & Hawkins, D. E. (2013, January). *From imagination to reality: Using DNA from an exceptional carcass to ...* Retrieved March 27, 2023, from https://beef.unl.edu/documents/18_modified_Hawkins_Proceedings_(3).pdf

Lehmkuhler, J. (2014). *Maximizing stocker gains on Pastures - University of Kentucky*. Retrieved April 3, 2023, from https://uknowledge.uky.edu/cgi/viewcontent.cgi?article=1014&context=ky_grazing

Marshall, D. M. (1994). Breed differences and genetic parameters for body composition traits in beef cattle. *Journal of Animal Science*, *72*(10), 2745–2755. https://doi.org/10.2527/1994.72102745x

Marston, J. (2021, September 29). *Vytelle raises $13.2m to help farmers pick the cream of the cattle crop*. Retrieved April 6, 2023, from https://agfundernews.com/vytelle-raises-13-2m-to-help-farmers-pick-the-cream-of-the-cattle-crop

Mateescu, R. G. (2020). Genetics and breeding of beef cattle. *Animal Agriculture*, 21–35. https://doi.org/10.1016/b978-0-12-817052-6.00002-1

Nour, A. Y., Thonney, M. L., Stouffer, J. R., & White, W. R. (1983). Changes in carcass weight and characteristics with increasing weight of large and small cattle. *Journal of Animal Science*, *57*(5), 1154–1165. https://doi.org/10.2527/jas1983.5751154x

Pariacote, F., Van Vleck, L. D., & Hunsley, R. E. (1998). Genetic and phenotypic parameters for carcass traits of American shorthorn beef cattle. *Journal of Animal Science*, *76*(10), 2584. https://doi.org/10.2527/1998.76102584x

Sahebi, M., Hanafi, M. M., Rafii, M. Y., Mahmud, T. M., Azizi, P., Osman, M., Abiri, R., Taheri, S., Kalhori, N., Shabanimofrad, M., Miah, G., & Atabaki, N. (2018). Improvement of drought tolerance in rice (*oryza sativa* L.): Genetics, genomic tools, and the WRKY gene family. *BioMed Research International*, *2018*, 1–20. https://doi.org/10.1155/2018/3158474

Shelton, J. N. (1990). Reproductive technology in Animal Production. *Revue Scientifique Et Technique De L'OIE*, *9*(3), 825–845. https://doi.org/10.20506/rst.9.3.521

Smith, C. (1989). Cloning and genetic improvement of beef cattle. *Animal Science*, *49*(1), 49–62. https://doi.org/10.1017/s0003356100004244

Spearman, C. (1904). The proof and measurement of association between two things. *The American Journal of Psychology*, *15*(1), 72. https://doi.org/10.2307/1412159

Taylor-Robinson, A. W., Walton, S., Swain, D. L., Walsh, K. B., & Vajta, G. (2014). The potential for modification in cloning and vitrification technology to enhance genetic progress in beef cattle in northern Australia. *Animal Reproduction Science*, *148*(3-4), 91–96. https://doi.org/10.1016/j.anireprosci.2014.06.004

Ultrasound Guidelines Council. (2021, December 30). *Home - Ultrasound Guidelines Council - By Laws*. Retrieved April 6, 2023, from https://ultrasoundguidelinescouncil.org/

Van Eenennaam, A. (2019, September 3). *How to get started with DNA testing*. Retrieved April 6, 2023, from https://beef-cattle.extension.org/how-to-get-started-with-dna-testing/

Vann, R. C., Parrish, J. A., & Karisch, B. B. (2018). *Using live animal carcass ultrasound information in beef cattle selection*. Retrieved April 6, 2023, from https://extension.msstate.edu/sites/default/files/publications/publications/p2802.pdf

Venkata Reddy, B., Sivakumar, A. S., Jeong, D. W., Woo, Y.-B., Park, S.-J., Lee, S.-Y., Byun, J.-Y., Kim, C.-H., Cho, S.-H., & Hwang, I. (2014). Beef quality traits of heifer in comparison with steer, Bull and cow at various feeding environments. *Animal Science Journal*, *86*(1), 1–16. https://doi.org/10.1111/asj.12266

Vleck, L. D. (1999). Implications of cloning for breed improvement strategies: Are traditional methods of animal improvement obsolete? *Journal of Animal Science*, *77*(suppl_2), 111. https://doi.org/10.2527/1999.77suppl_2111x

Vytelle. (2020). *Site Preparation Manual*. Retrieved April 6, 2023, from https://vytelle.com/tech-services/site-preparation-manual/

Wu, B., & Zan, L. (2011). Enhance beef cattle improvement by embryo biotechnologies. *Reproduction in Domestic Animals*, *47*(5), 865–871. https://doi.org/10.1111/j.1439-0531.2011.01945.x

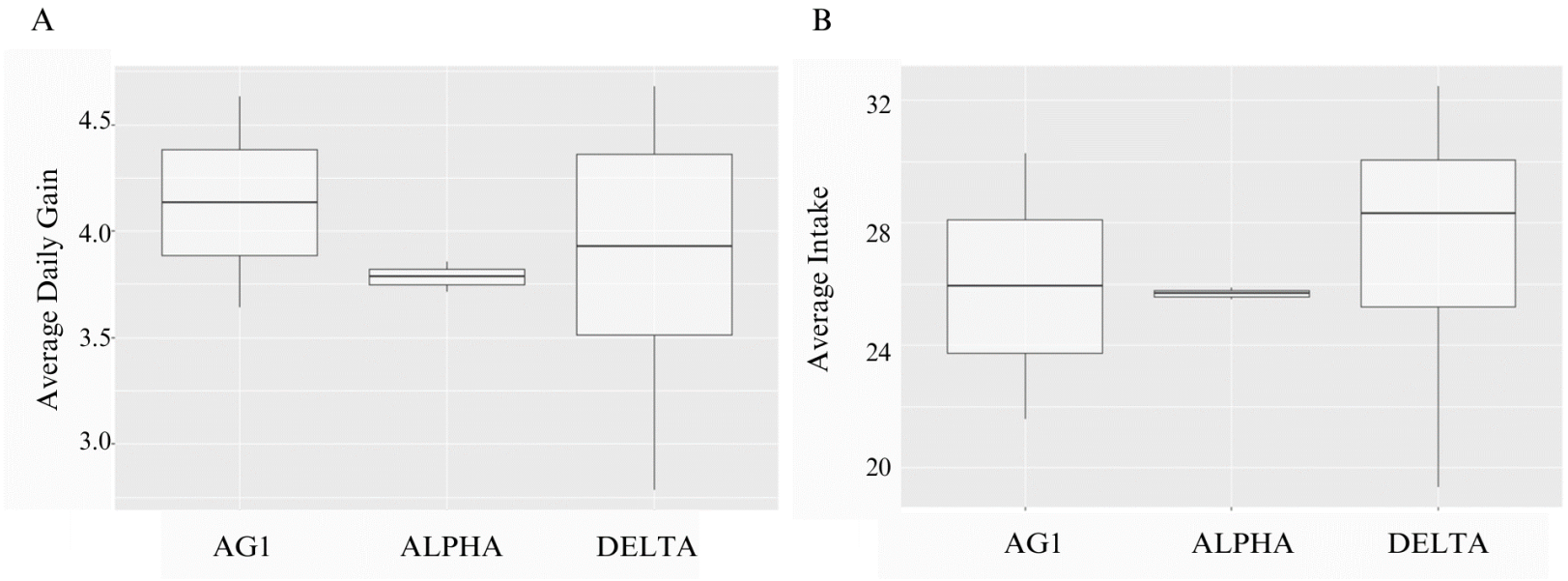

FIGURE 4.1. (A) The Entire Average Daily Gain for SIRE.). The average for the individuals sired by AG1 had an average daily gain of 4.14lbs, the individuals sired by ALPHA had an entire average daily gain of 3.79lbs and lastly, those sired by DELTA had an entire average daily gain of 3.88lbs. (B) The entire feed intake of SIRE. The average of the individuals sired by AG1 had an average feed intake of 25.93lbs daily. The average feed intake of the ALPHA individuals was 25.68lbs daily. Finally, the average feed intake of the individuals sired by DELTA was 27.38lbs daily.

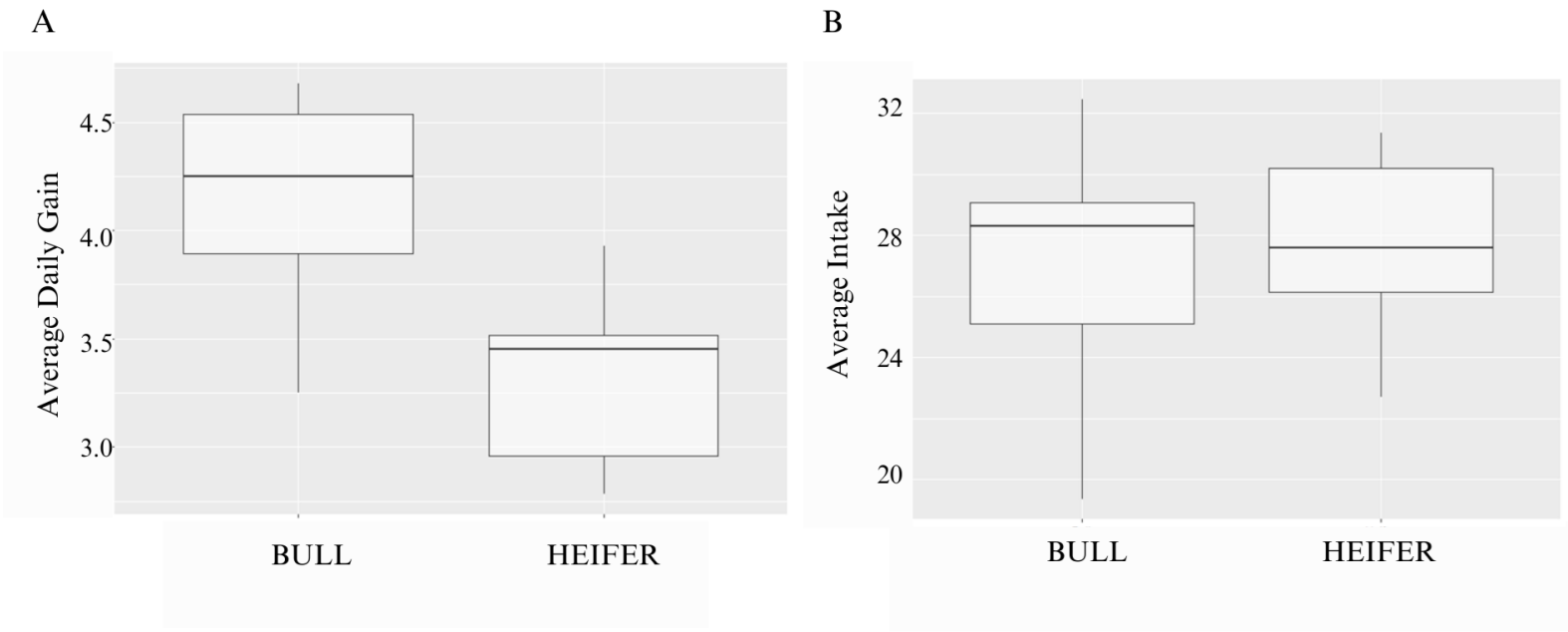

FIGURE 4.2. (A) The entire Average Daily Gain between SEX. For the group HEIFER was 3.33lbs and 4.17lbs for the group BULL (B) During the time on feed, the entire feed intake was a total average of 27.7lbs for HEIFER and a total average of 27.25lbs for the group BULL

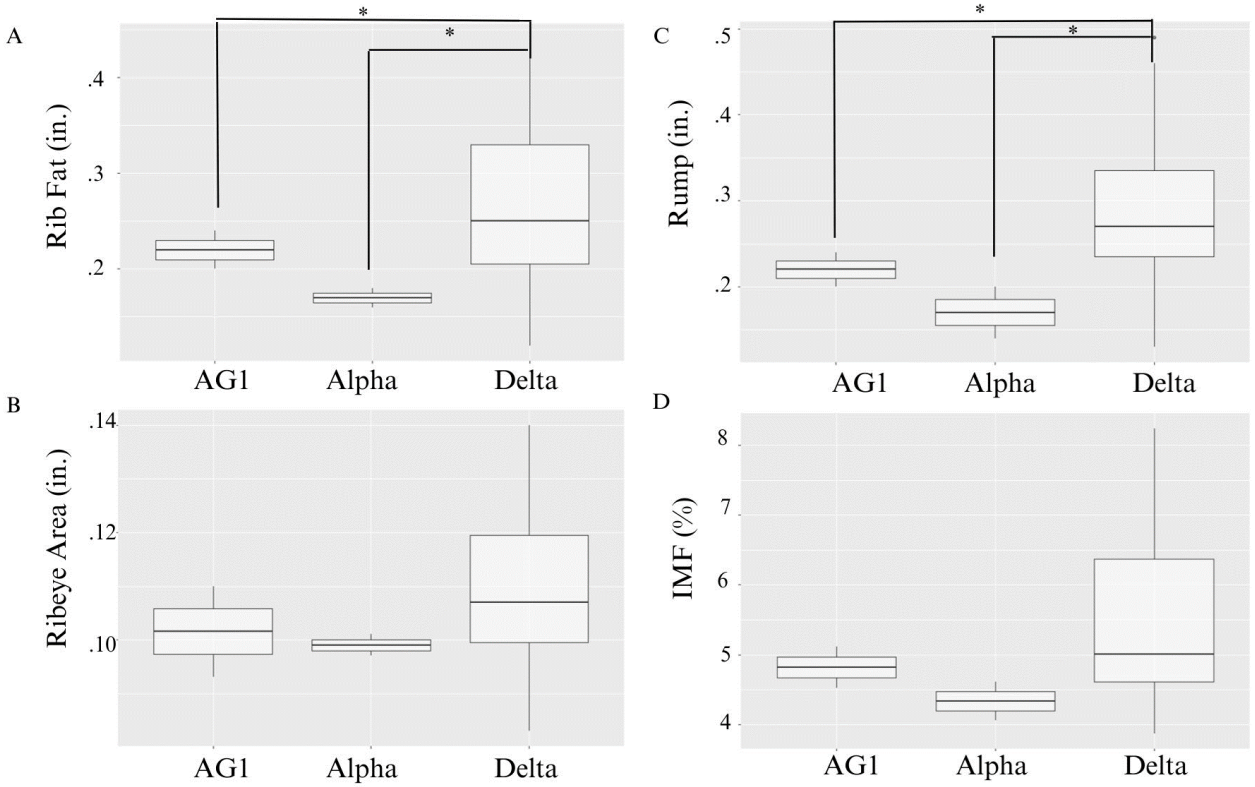

FIGURE 4.3 (A) Carcass Scan of RF after days on feed (d68) within SIRE. Within the sire AG1 there was an average for RF of .22in. Within ALPHA there was an average for RF of .17in. Within DELTA there was an average for RF of .29in. (B) Carcass Scan of BF after days on feed within SIRE. Within the sire AG1 there was an average for BF of .22 in. Within ALPHA there was an average of BF at .17in. Within DELTA there was an average of BF for .27in. (C) Carcass Scan of REA after days on feed within SIRE. Within the sire AG1 there was an average for REA for 10.15in. Within the sire ALPHA there was an average for REA of 9.9in. Within DELTA there was an average for REA of 10.79in. (D) Carcass Scan of % of IMF after days on feed within SIRE. Within the sire AG1 there was a % of IMF at 4.82. Within the sire ALPHA there was a % of IMF at 4.36. Within the sire DELTA there was a % of IMF at 5.52.

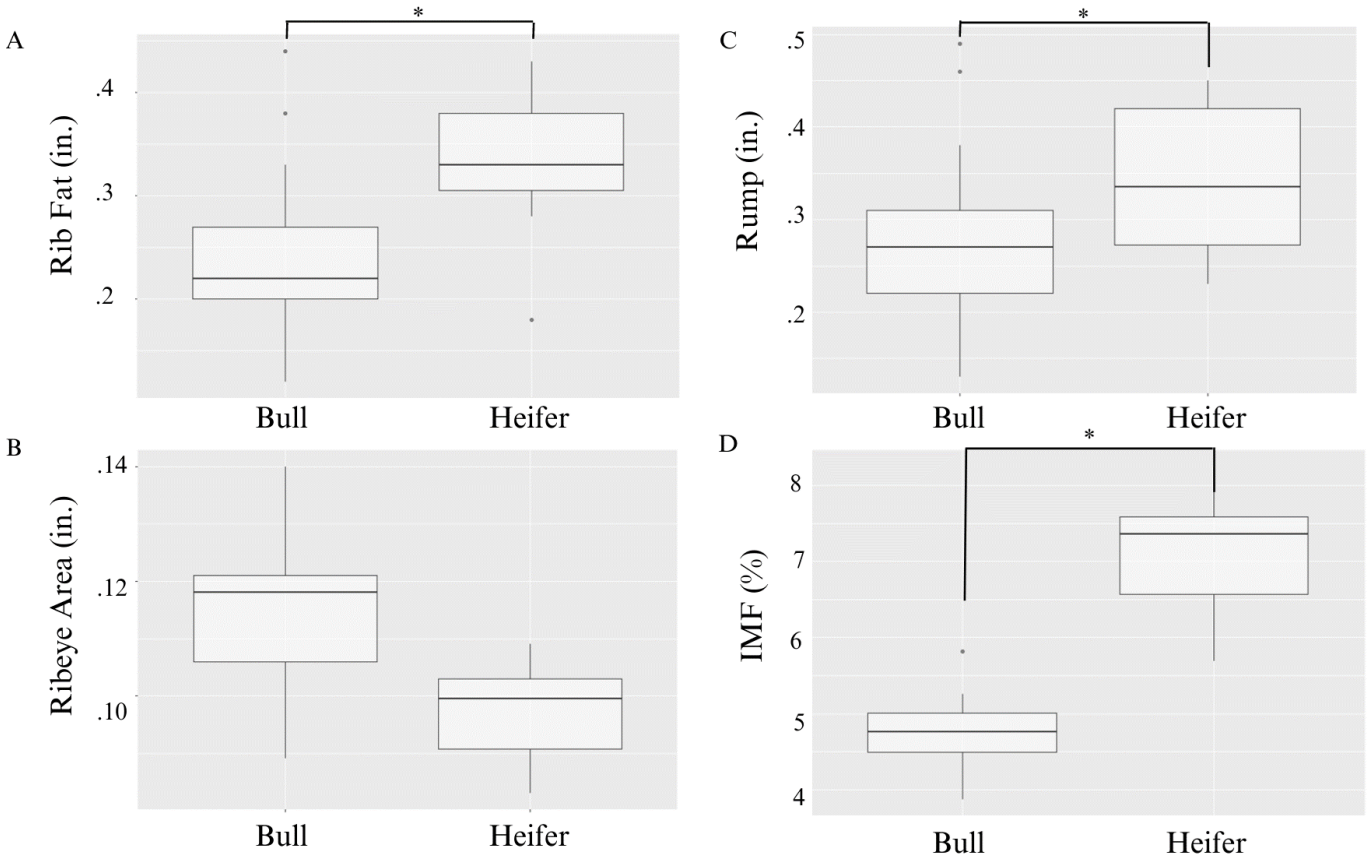

FIGURE 4.4. (A) After completion of days on feed (d68) Ultrasound scan of RF between SEX. . In the group HEIFER there was an average of 0.334in for RF, and 0.27in for BULL. (B) After Completion of Days on Feed Ultrasound Scan of BF between SEX. When comparing BF, there was an average of 0.331in for HEIFER and 0.24in for BULL. (C) After Completion of Days on Feed Ultrasound Scan of REA between SEX. . Next, looking at REA, there was an average of 9.7in for HEIFER and 11.31in for BULL (D) After Completion of Days on Feed Ultrasound Scan of IMF% between SEX. The average percent of IMF was 7.11% for HEIFER and 4.76% for the group BULL.

TABLE 4.1	AG1	ALPHA	DELTA
D0 WEIGHT	565lbs (σ = 91.924)	557.5lbs (σ = 20.506)	646.6lbs (σ = 89.766)
D28 WEIGHT	790lbs (σ = 134.350)	756.5lbs (σ = 34.648)	839lbs (σ = 97.474)
D68 WEIGHT	910.5lbs (σ = 153.442)	875.5lbs (σ = 28.991)	973lbs (σ = 103.457)

TABLE 4.1. Average weights from collections d0, d28 and d68 in comparison to the individuals within each sire.

TABLE 4.2	AG1	ALPHA	DELTA
D0-D28 ADG	**4.06lbs (σ = 0.713)**	**3.55lbs (σ = 0.252)**	**3.44lbs (σ = 0.796)**
D28-D68 ADG	**4.32lbs (σ = 0.682)**	**4.25lbs (σ = 0.202)**	**4.8lbs (σ = 0.969)**
D0-D68 ADG	**4.19lbs (σ = 0.701)**	**3.9lbs (σ = 0.101)**	**4.12lbs (σ = 0.561)**

TABLE 4.2. Average entire gain and ADG from time periods d0-d28, d28-d68, d0-d68 in comparison to the individuals within each sire.

TABLE 4.3	HEIFER	BULL
D0 WEIGHT	**670.7lbs (σ = 104.309)**	**634.75lbs (σ = 82.178)**
D28 WEIGHT	**827.9lbs (σ = 103.719)**	**844lbs (σ = 96.551)**
D68 WEIGHT	**947lbs (σ = 114.508)**	**985lbs (σ = 98.320)**

TABLE 4.3. The average weight gain at collections d0, d28, d68 within the individuals of sex within delta

TABLE 4.4	HEIFER	BULL
D0-D28 ADG	**2.86lbs (σ = 0.555)**	**3.75lbs (σ = 0.742)**
D28-D68 ADG	**4.25lbs (σ = 0.566)**	**5.03lbs (σ = 1.029)**
D0-D68 ADG	**3.33lbs (σ = 0.421)**	**4.17lbs (σ = 0.392)**

TABLE 4.4. Average entire gain and ADG from time periods d0-d28, d28-d68, d0-d68 in comparison to the individuals of sex within delta.

APPENDIX A-4

Metadata of the Individuals

WTAMU ID	ELECTRONIC ID	SEX	SIRE	D0 WEIGHT	D28 WEIGHT	ADG Day D0-D28	Period Gain D0-D28
2117	840032360264 00	Heifer	AG1	500	695	3.545	195
2175	840032360260 15	Bull	AG1	630	885	4.554	255
2190	840032360260 30	Bull	Alpha	572	781	3.732	209
2191	840032360260 31	Bull	Alpha	543	732	3.375	189
2103	840032360263 85	Heifer	Delta	855	955	1.818	100
2104	840032360263 86	Heifer	Delta	760	893	2.418	133
2105	840032360263 88	Heifer	Delta	715	905	3.455	190
2107	840032360263 90	Heifer	Delta	470	598	2.327	128
2108	840032360263 91	Heifer	Delta	585	735	2.727	150
2110	840032360263 93	Heifer	Delta	655	807	2.764	152
2111	840032360263 94	Heifer	Delta	720	910	3.455	190
2113	840032360263 96	Heifer	Delta	672	855	3.327	183
2114	840032360263 97	Heifer	Delta	610	795	3.364	185
2116	840032360263 99	Heifer	Delta	665	826	2.927	161
2164	840032360260 04	Bull	Delta	765	990	4.018	225
2165	840032360260 05	Bull	Delta	640	705	1.161	65
2166	840032360260 06	Bull	Delta	718	945	4.054	227
2167	840032360260 07	Bull	Delta	645	878	4.161	233
2170	840032360260 10	Bull	Delta	650	868	3.893	218
2171	840032360260 11	Bull	Delta	655	887	4.143	232
2172	840032360260 12	Bull	Delta	710	915	3.661	205

2173	98202808971960	Bull	Delta	745	950	3.661	205
2174	84003236026014	Bull	Delta	653	870	3.875	217
2176	84003236026016	Bull	Delta	430	636	3.679	206
2179	84003236026019	Bull	Delta	685	907	3.964	222
2182	84003236026022	Bull	Delta	560	818	4.607	258
2183	84003236026023	Bull	Delta	690	915	4.018	225
2184	84003236026024	Bull	Delta	550	702	2.714	152
2185	84003236026025	Bull	Delta	612	848	4.214	236
2186	84003236026026	Bull	Delta	695	936	4.304	241
2187	84003236026027	Bull	Delta	665	861	3.5	196
2188	84003236026028	Bull	Delta	490	693	3.625	203
2189	84003236026029	Bull	Delta	590	755	2.946	165
2192	84003236026032	Bull	Delta	583	795	3.786	212
2197	84003236026037	Bull	Delta	599	852	4.518	253

D 68 WEIGHT	ADG D28-D68	Period Gain D0-D68	ADG D0-D60	Pen	Avg_Intake	Wormed 1/17/22	Vaxed 1/17/22
802	3.821	107	3.639	pen 3	21.58	Yes	Yes
1019	4.786	134	4.631	pen 4	30.27	Yes	Yes
896	4.107	115	3.857	pen 4	25.49	Yes	Yes
855	4.393	123	3.714	pen 4	25.88	Yes	Yes
1090	4.821	135	2.831	pen 3	30.31	Yes	Yes
1005	4	112	2.952	pen 3	30.14	Yes	Yes
1038	4.75	133	3.892	pen 3	30.2	Yes	Yes
701	3.679	103	2.783	pen 3	22.71	Yes	Yes
832	3.464	97	2.976	pen 3	26.37	Yes	Yes
947	5	140	3.518	pen 3	24.76	Yes	Yes
1046	4.857	136	3.928	pen 3	31.35	Yes	Yes
963	3.857	108	3.506	pen 3	26.59	Yes	Yes

901	3.786	106	3.506	pen 3	26.06	Yes	Yes
947	4.321	121	3.398	pen 3	28.57	Yes	Yes
1105	4.107	115	4.048	pen 4	30.29	Yes	Yes
941	8.429	236	3.583	pen 4	26.15	Yes	Yes
1045	3.571	100	3.893	pen 4	29.96	Yes	Yes
1030	5.429	152	4.583	pen 4	27.87	Yes	Yes
997	4.607	129	4.131	pen 4	24.43	Yes	Yes
1029	5.071	142	4.452	pen 4	19.36	Yes	Yes
1070	5.536	155	4.286	pen 4	29.06	Yes	Yes
1130	6.429	180	4.583	pen 4	28.64	Yes	Yes
1010	5	140	4.25	pen 4	28.55	Yes	Yes
752	4.143	116	3.833	pen 4	20.08	Yes	Yes
1070	5.821	163	4.583	pen 4	30.56	Yes	Yes
953	4.821	135	4.679	pen 4	26.9	Yes	Yes
1075	5.714	160	4.583	pen 4	32.45	Yes	Yes
823	4.321	121	3.25	pen 4	25.04	Yes	Yes
969	4.321	121	4.25	pen 4	28.88	Yes	Yes
1068	4.714	132	4.44	pen 4	29.08	Yes	Yes
997	4.857	136	3.952	pen 4	25.37	Yes	Yes
835	5.071	142	4.107	pen 4	25.11	Yes	Yes
895	5	140	3.631	pen 4	24.85	Yes	Yes
910	4.107	115	3.893	pen 4	28.31	Yes	Yes
980	4.571	128	4.536	pen 4	31.13	Yes	Yes

RUMP 1/3/22	BF 1/3/22	REA 1/3/22	IMF% 1/3/22	RUMP 3/28/22	BF 3/28/22	REA 3/28/22	IMF% 3/28/22
0.11	0.1	R	4.51	0.24	0.24	9.3	5.11
0.07	0.07	6.4	3.86	0.2	0.2	11	4.52
0.07	0.08	7.5	3.45	0.14	0.16	10.1	4.61
0.06	0.07	5.6	2.94	0.2	0.18	9.7	4.06
0.16	0.19	6.8	5.53	0.39	0.43	9.9	8.24
0.14	0.08	7.4	4.75	0.33	0.39	10.5	7.49
0.07	0.1	7.1	4.36	0.26	0.33	10.9	6.19
0.11	0.06	5	5.34	0.23	0.28	8.5	7.23
0.16	0.12	6	4.81	0.43	0.4	8.3	5.69

0.2	0.13	7.7	5.7	0.34	0.32	10	6.56
0.12	0.07	R	4.48	0.44	0.33	10.4	7.6
0.09	0.09	7.3	5.44	0.26	0.18	10	8.01
0.24	0.06	6.8	5.04	0.45	0.3	8.9	7.53
0.11	0.11	7	4.59	0.31	0.35	9.6	6.58
0.04	0.09	7.1	4.07	0.46	0.33	12.3	4.51
0.04	0.07	6.9	4.68	0.22	0.21	11.2	4.95
0.08	0.08	7.6	4.61	0.27	0.22	12	5.01
0.06	0.08	6.8	4.47	0.3	0.17	11.9	4.61
0.12	0.09	R	R	0.32	0.27	10.7	5.22
0.11	0.11	R	4.16	0.33	0.31	10.4	5.02
0.04	0.08	8.2	3.19	0.27	0.2	12.7	4.49
0.1	0.12	8.3	5.61	0.3	0.38	12.4	5.25
0.07	0.1	7.2	4.12	0.19	0.22	12	5.05
0.05	0.07	5.5	4.13	0.13	0.12	8.9	4.98
0.14	0.15	6.5	5.04	0.49	0.44	11.8	5.82
0.1	0.08	R	4.28	0.27	0.22	10.9	4.95
0.1	0.09	7.1	2.4	0.31	0.3	12.1	4.37
0.04	0.09	5.7	4.13	0.19	0.19	9.4	4.76
0.08	0.09	7.2	3.65	0.24	0.22	11.8	4.19
0.09	0.11	7.8	3.42	0.23	0.23	12.2	4.31
0.1	0.1	6.9	4.53	0.27	0.2	11	4.68
0.08	0.07	5.5	3.5	0.16	0.17	9.1	3.87

0.07	0.09	6.3	3.95	0.21	0.16	10.1	4.61
0.09	0.08	6.7	2.95	0.25	0.24	10.6	4.38
0.05	0.08	6.7	2.99	0.38	0.25	14	4.9

Temperature	Treatment Type	Treatment Date
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A

104.5	DRAX 6SQ	1/17/2022
102.5	DRAX 6SQ	1/17/2022
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A

APPENDIX B-4

Neogen SeekSire Parentage Test Results

Order ID	Calf Barcode	Calf ID	Recip Dam	Dam ID	Dam ID	Dam Results	Comp	Match	Excl	Sire ID	Sire Barcode	Comp	Match	Result	Mating Trio
736493	840003236026386	2103	1715	1946	AG7	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236026387	2104	1873	1946	AG7	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236026388	2105	1754	1946	AG7	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236026390	2107	1158	1945	AG5	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236026393	2110	1837	1947	AG9	Qualified	209	208	1	Delta	41322022033	206	206	Qualified	Yes
736493	840003236026394	2111	1107	1945	AG5	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236026396	2113	1885	1947	AG9	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236026397	2114	1304	1945	AG5	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236026399	2116	1704	1947	AG9	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236026400	2117	1765							AG1	21518010503	205	205	Qualified	
736493	840003236262004	2164	1317	1944	AG3	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262005	2165	1878	1945	AG5	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262006	2166	1816	1945	AG5	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262007	2167	1828	1945	AG5	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262010	2170	1423	1946	AG7	Qualified	209	208	1	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262011	2171	1522	1946	AG7	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262012	2172	1764	1945	AG5	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
757842	1949	2173	1305	1943	Gamma	Qualified	209	208	1	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262014	2174	1869	1946	AG7	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262015	2175	1614	1942	Gamma	Qualified	209	209	0	AG1	21518010503	205	203	Qualified	Yes
736493	840003236262016	2176	1416	1945	AG5	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262022	2182	1832	1946	AG7	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262024	2184	1849	1945	AG5	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262025	2185	1741	1947	AG9	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
757842	1948	2186	1708	1943	Gamma	Qualified	209	208	1	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262027	2187	1430	1947	AG9	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262028	2188	1868	1947	AG9	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262029	2189	1827	1947	AG9	Qualified	209	209	0	Delta	41322022033	206	206	Qualified	Yes
736493	840003236262030	2190	1646	1942	Gamma	Qualified	209	209	0						
736493	840003236262031	2191	1771	1942	Gamma	Qualified	209	209	0						
736493	840003236262036	2196	1703							AG1	21518010503	205	205	Qualified	
736493	840003236262039	2199	1244							AG1	21518010503	205	205	Qualified	

Link for guidelines for cattle parentage verification based on SNP markers for “Comp” column:
https://www.isag.us/Docs/Guideline-for-cattle-SNP-use-for-parentage-2012.pdf

APPENDIX C-4

Vytelle Insight Beef Genetics: Feed Intake Report

ID	Pen	Avg_Intake
2103	pen 3	30.31
2104	pen 3	30.14
2105	pen 3	30.2
2107	pen 3	22.71
2108	pen 3	26.37
2110	pen 3	24.76
2111	pen 3	31.35
2113	pen 3	26.59
2114	pen 3	26.06
2116	pen 3	28.57
2117	pen 3	21.58
2164	pen 4	30.29
2165	pen 4	26.15
2166	pen 4	29.96
2167	pen 4	27.87
2170	pen 4	24.43
2171	pen 4	19.36
2172	pen 4	29.06
2173	pen 4	28.64
2174	pen 4	28.55
2175	pen 4	30.27
2176	pen 4	20.08
2179	pen 4	30.56
2182	pen 4	26.9
2183	pen 4	32.45
2184	pen 4	25.04
2185	pen 4	28.88
2186	pen 4	29.08
2187	pen 4	25.37
2188	pen 4	25.11
2189	pen 4	24.85
2190	pen 4	25.49
2191	pen 4	25.88
2192	pen 4	28.31
2197	pen 4	31.13

APPENDIX D-4

Ultrasound Carcass Data Results

	1/3/2022	1/3/2022	1/3/2022	1/3/2022	1/3/2022	Rump	BF	REA	IMF %	Weight
Scan ID	Rump	BF	REA	IMF %	Weight	3/28/2022	3/28/2022	3/28/2022	3/28/2022	3/28/2022
2101	0.19	0.15	08.5	05.14	740	0.49	0.42	12.7	07.33	1127
2102	0.27	0.21	07.3	06.07	810	0.56	0.51	11.1	07.20	1100
2103	0.16	0.19	06.8	05.53	723	0.39	0.43	09.9	08.24	1090
2104	0.14	0.08	07.4	04.75	670	0.33	0.39	10.5	07.49	1005
2105	0.07	0.10	07.1	04.36	655	0.26	0.33	10.9	06.19	1038
2107	0.11	0.06	05.0	05.34	430	0.23	0.28	08.5	07.23	0701
2108	0.16	0.12	06.0	04.81	560	0.43	0.41	08.3	05.69	0832
2110	0.20	0.13	07.7	05.70	665	0.34	0.32	10.0	06.56	0947
2111	0.12	0.07	R	04.48	670	0.44	0.33	10.4	07.60	1046
2113	0.09	0.09	07.3	05.44	665	0.26	0.18	10.0	08.01	0963
2114	0.24	0.06	06.8	05.04	585	0.45	0.30	08.9	07.53	0901
2116	0.11	0.11	07.0	04.59	635	0.31	0.35	09.6	06.58	0947
2117	0.11	0.10	R	04.51	515	0.24	0.24	09.3	05.11	0802
2162	0.12	0.14	R	R	730	0.24	0.23	10.7	05.83	1085
2163	0.09	0.09	08.1	04.65	850	0.25	0.20	11.8	05.10	1170
2164	0.04	0.09	07.1	04.07	740	0.46	0.33	12.3	04.51	1105
2165	0.04	0.07	06.9	04.68	630	0.22	0.21	11.2	04.95	0941
2166	0.08	0.08	07.6	04.61	726	0.27	0.22	12.0	05.01	1045
2167	0.06	0.08	06.8	04.47	680	0.30	0.17	11.9	04.61	1030
2169	0.08	0.08	07.8	04.29	780	0.30	0.28	12.3	04.42	1225
2170	0.12	0.09	R	R	650	0.32	0.27	10.7	05.22	0997
2171	0.11	0.11	R	04.16	665	0.33	0.31	10.4	05.02	1029
2172	0.04	0.08	08.2	03.19	680	0.27	0.20	12.7	04.49	1070
2173	0.10	0.12	08.3	05.61	745	0.30	0.38	12.4	05.25	1130

2174	0.07	0.10	07.2	04.12	630	0.19	0.22	12.0	05.05	1011
2175	0.07	0.07	06.4	03.86	625	0.20	0.20	11.0	04.52	1019
2176	0.05	0.07	05.5	04.13	475	0.13	0.12	08.9	04.98	0752
2179	0.14	0.15	06.5	05.04	685	0.49	0.44	11.8	05.82	1070
2181	0.10	0.09	R	03.35	665	0.38	0.29	10.8	03.67	1040
2182	0.10	0.08	R	04.28	600	0.27	0.22	10.9	04.95	0953
2183	0.10	0.09	07.1	02.40	670	0.31	0.30	12.1	04.37	1075
2184	0.04	0.09	05.7	04.13	518	0.19	0.19	09.4	04.76	0823
2185	0.08	0.09	07.2	03.65	595	0.24	0.22	11.8	04.19	0969
2186	0.09	0.11	07.8	03.42	680	0.23	0.23	12.2	04.31	1068
2187	0.10	0.10	06.9	04.53	654	0.27	0.20	11.0	04.68	0997
2188	0.08	0.07	05.5	03.50	530	0.16	0.17	09.1	03.87	0835
2189	0.07	0.09	06.3	03.95	650	0.21	0.16	10.1	04.61	0895
2190	0.07	0.08	07.5	03.45	628	0.14	0.16	10.1	04.61	0896
2191	0.06	0.07	05.6	02.94	530	0.20	0.18	09.7	04.06	0855
2192	0.09	0.08	06.7	02.95	598	0.25	0.24	10.6	04.38	0910
2196	0.11	0.09	07.4	04.09	645	0.28	0.28	12.1	04.91	1021
2197	0.05	0.08	06.7	02.99	604	0.22	0.23	11.5	04.04	0980
2199	0.07	0.06	08.6	02.22	670	0.38	0.25	14.0	04.90	1128

APPENDIX E-4

Residual Feed Intake Data

WTAMU ID	SEX	SIRE	RFI	DMI	Predicted DMI
2117	Heifer	AG1	-1.78255	17.4	20.51586523
2175	Bull	AG1	-0.59474	19.9	20.09535663
2190	Bull	Alpha	0.557568	26.6	20.08617592
2191	Bull	Alpha	0.976949	18.4	18.76642567
2103	Heifer	Delta	-3.11587	19.5	17.02823779
2104	Heifer	Delta	-0.19536	16.9	14.72135535
2105	Heifer	Delta	6.513824	18.8	16.30767149
2107	Heifer	Delta	-0.36643	15.6	16.52685987
2108	Heifer	Delta	2.471762	15.6	17.04355411
2110	Heifer	Delta	2.178645	20.4	16.96373418
2111	Heifer	Delta	2.492329	14.3	16.08254555
2113	Heifer	Delta	-0.92686	13.1	16.90283783
2114	Heifer	Delta	-1.44355	18.8	14.31557619
2116	Heifer	Delta	3.436266	19.1	17.60522728
2164	Bull	Delta	-3.80284	13.3	15.65049841
2165	Bull	Delta	4.484424	16	17.20242478
2166	Bull	Delta	1.494773	17.4	15.15117471
2167	Bull	Delta	-2.3505	15.5	15.82937575
2170	Bull	Delta	-1.20242	14.6	15.48743309
2171	Bull	Delta	2.248825	16.7	16.36580525
2172	Bull	Delta	-0.32938	15.8	16.39473802
2173	Bull	Delta	-0.88743	17.1	15.68448198
2174	Bull	Delta	0.334195	15.9	14.98905056
2176	Bull	Delta	1.415518	19.3	12.95764729
2179	Bull	Delta	0.910949	16.1	15.65356262
2182	Bull	Delta	6.342353	18.5	13.92960245
2183	Bull	Delta	0.446437	19	15.74026645
2184	Bull	Delta	4.570398	13.6	15.33217703
2185	Bull	Delta	3.259734	17.1	15.20651098
2186	Bull	Delta	-1.73218	17.2	16.1181064
2187	Bull	Delta	1.893489	17.2	16.19179469
2188	Bull	Delta	1.081894	14	13.44243245
2189	Bull	Delta	1.008205	16.4	15.42305135
2192	Bull	Delta	1.329792	16.4	15.07020755
2197	Bull	Delta	-0.84359	13.9	14.74358873

CHAPTER V

CONCLUSIONS

Our human population continues to grow causing a need for genetic and genomic advancements that may continue to improve animal health and production efficiency. The reason for this is to continue to provide efficient product to consumer as our resources become more limited.

With our findings we can help contribute to the belief of beef cattle efficiency and improvement. Being able to use genomics and determine disease, pathogens and stressors of beef cattle before they ever show clinical signs is one way we can improve animal health and efficiency. Comparing the genetics of cloned cattle, and determining differences between performance of chosen sires, and sex within the offspring's is another way to improve animal health and efficiency. The use of these genetic and genomic technologies and resulting considerations can then be tailored to address specific regional challenges and opportunities worldwide that give beef cattle producers an opportunity to raise higher quality, healthier beef more efficiently.