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Integration of Molecular Techniques for the Investigation of Bovine Respiratory Disease

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INTEGRATION OF MOLECULAR TECHNIQUES FOR THE INVESTIGATION OF BOVINE RESPIRATORY DISEASE

BY

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This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

INTEGRATION OF MOLECULAR TECHNIQUES FOR THE INVESTIGATION OF BOVINE RESPIRATORY DISEASE

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Bovine respiratory disease (BRD) is the leading cause of morbidity and mortality in the cattle industry. The complexity of host, pathogen, and environmental factors contributing to the incidence of BRD necessitate a multifaceted approach to investigate BRD. A greater understanding of pathogenic and genetics factors associated with BRD would improve prevention and treatment of BRD. Due to the complexity of BRD, genetic technologies have been limited in their ability to identify a genetic basis for BRD. Pooling of DNA samples prior to extraction can increase the ability to conduct genotyping studies of complex traits. Once generated, new information and management methods must be disseminated to the livestock industry. Higher education provides the opportunity to train future livestock producers and promote self-directed learning skills. Therefore, our objectives were to 1) investigate the upper nasal microbiome in BRD affected calves prior to weaning, 2) evaluate the accuracy of pooling samples based on equalized white blood cell counts, and 3) determine the effect of classroom assessment techniques in an introductory level animal science course. Nasal swabs were collected from calves prior to, during a BRD outbreak, and following the outbreak. Analysis by sequencing a variable region of the 16s rRNA gene revealed differences in microbial abundance and community diversity associated with BRD incidence. To evaluate accuracy of blood pooling, whole blood samples were collected from 10 bovine animals

and pools were constructed based on number of white blood cells, spectrophotometric readings, spectrofluorometric readings, extracted DNA volume, and whole blood volume. The outcome of this study indicates that pooling based on white blood cell count is an accurate pooling method and has less variability among pools compared to all other methods. Finally, a classroom assessment technique (**CAT**) was administered in an introductory animal science course. Students completed an assessment form at the conclusion of each class and the instructor provided feedback based on the responses at the beginning of the next class period. While there were no differences in student grades, students perceived that the CAT increased their ability to learn and their engagement in the class.

CHAPTER 1: Review of Literature

Introduction

Bovine respiratory disease (**BRD**) complex is the most prominent disease affecting the cattle industry. Bovine respiratory disease is a multi-faceted disease resulting from complex host, pathogen, and environmental interactions. Although BRD occurs most often in the feedlot, cattle in all stages of production can develop BRD. The prevalence and severity of BRD is further confounded by a range of genetic, environmental, and management components. These factors include; breed, age, sex, extreme weather changes, cold, stress, transportation, dust, and social interactions (Taylor et al., 2010a). The complex nature of this disease makes it highly difficult to prevent and manage. Despite the improvement and widespread use of vaccines and management techniques aimed at preventing BRD, the prevalence of the disease has not declined (Gagea et al., 2006).

As molecular technology continues to advance, the ability to study BRD and similarly complex traits improves. This technology includes culture-independent methods for characterizing whole microbial communities, metagenomic analysis, and large-scale genome wide association studies that can be done economically on larger groups of individuals to study genetic aspects of complex traits. Previously, it was not always practical to complete genome wide association studies (**GWAS**) for complex traits which required thousands of DNA samples to be individually extracted and genotyped. Pooling DNA samples and genotyping the pools emerged as a viable approach to reduce the cost, time, and labor while still being able to detect difference between case and control groups (Macgregor et al., 2008). This technique is frequently used for large scale GWAS studies

but is still expensive and time consuming since DNA must be extracted from each individual sample. Furthermore, inconsistency in DNA quantification can introduce variation and errors during pool construction. Pooling samples prior to DNA extraction offers a way to further reduce the cost, time, and potentially avoid pooling error associated with DNA quantification. As pooling methods improve in accuracy and feasibility, the capacity for studying complex traits expands, along with the potential for commercial application of DNA testing.

The field of molecular genetics continues to advance and generate information and tools that can be utilized by livestock producers. However, if producers lack the awareness, knowledge, or skills to correctly apply emerging technology, their operation and the industry itself will not benefit. Education has been cited as a common factor influencing the propensity of livestock producers to seek out information and adopt new technology or management practices (Dorfman, 1996; Ward et al., 2008). Higher education offers the opportunity to provide the next generation of livestock producers with the knowledge and skill set to successfully utilize emerging technology and management practices that will promote long term success and efficiency for individual operations and the industry as a whole. It is especially important that those students become self-directed learners so that in the future they continue to seek out information and critically evaluate management strategies and various tools that are available to cattle producers. Classroom assessment techniques (**CATs**) engage students in the learning process and assist instructors in identifying gaps in learning and understanding prior to high steaks exams (Angelo and Cross, 1993). By shifting the focus on learning, students are encouraged to monitor their own progress and become self-directed learners and more

engaged in the classroom. Using CATs in the classroom can foster student's success both within and beyond the classroom setting. Promoting self-directed learning habits in the classroom and providing the tools and knowledge to seek out and apply emerging technologies in the livestock industry will greatly contribute to the future success of the livestock industry, especially when addressing complex issues like BRD.

Prevalence of Bovine Respiratory Disease in the Cattle Industry

Bovine respiratory disease is the leading cause of natural death in the cattle industry and has a global impact on all segments of cattle production (Hay et al., 2016; Timsit et al., 2016b; Murray et al., 2017; Wang et al., 2018a). A USDA (2008) study that included 87.8% of all U.S. beef cows reported that 31.4% of death loss in calves age three weeks to weaning was due to BRD. While calves are highly susceptible to BRD, the percent of death loss in feedlot cattle attributed to BRD is even greater than preweaned calves. Controlling BRD among newly received cattle remains the biggest challenge facing the feedlot industry. Bovine respiratory disease has been cited as the primary cause of morbidity (70-80%) and death loss (45-55%) in the feedlot (Smith, 1998; NAHMS, 2011). The estimated percent of cattle that will contract BRD during the feedlot phase ranges from 14.4% – 21.2% (Edwards, 2010; NAHMS, 2011). Additional studies have reported observing lung lesions in 29.7% to 77% of cattle in the feedlot, and that many affected animals go undetected (Wittum et al., 1996; Thompson et al., 2006; Schneider et al., 2009b). The occurrence of lung lesions in animals not previously diagnosed with BRD indicates that the prevalence of BRD is actually greater than reported due to undetected subclinical cases of BRD.

Economic Impact of Bovine Respiratory Disease

Bovine respiratory disease is the most costly disease effecting the cattle industry. It has been estimated that economic loss due to BRD is over $1 billion annually and can escalate to upwards of $3 billion annually when considering the combined cost of prevention and treatment (Griffin, 1997; Miles, 2009). Wang et al. (2018b) reported that the median cost of medicine and labor to treat preweaned calves at $26 per calf based on a survey data from beef producers in Nebraska, South Dakota, and North Dakota. Additional studies simulating the cost of BRD in preweaned calves, spread across the entire US beef cow inventory, found that the cost to cow-calf producers was a median of $5.63 per cow each year (Wang et al., 2018a). Expenditures related to BRD are often higher during the transition period from nursing to weaning since this is typically a stressful time for calves, increasing their susceptibility to BRD (Griffin, 1997). Due to the complexity of factors contributing to the development of BRD, there is often inconsistency in the occurrence of BRD during the transition period from nursing to weaning regardless of preventative measures taken. This unpredictability in morbidity can create doubt for feedlot managers regarding vaccination history of the cattle, resulting in the adoption of a policy to assume that all newly received cattle are at risk of developing BRD and a mass treatment approach during initial processing. The estimated processing cost during the transition period associated with BRD ranges from $5 to $15 (Griffin, 1997). Economic loss associated with BRD during the feedlot phase has been predicted at $13,895 per 1000 animals when considering reduced weight gain, direct treatment, labor, and death loss.

Factors Contributing to Bovine Respiratory Disease

Cattle are often exposed to viral and bacterial pathogens throughout their lifespan, many of which have been associated with BRD. There are many factors that influence the change from normal microbial populations inhabiting the respiratory tract to the manifestation of BRD. Most common factors causing BRD act synergistically and occur in combination rather than as a single causative problem (Callan and Garry, 2002). Bovine respiratory disease typically develops after an initial viral infection followed by a secondary bacterial infection. However, the complex interaction between host and pathogen is further confounded by epidemiological and environmental factors (Duff and Galyean, 2007; Edwards, 2010) (Figure 1.1)

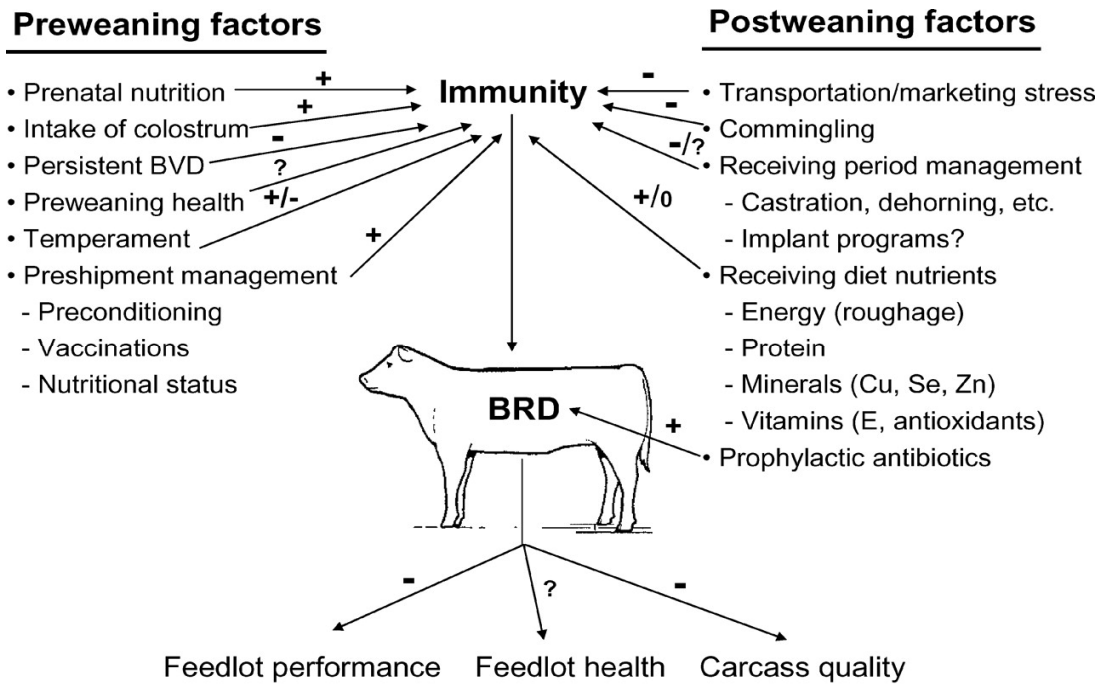

Figure 1.1. Pre- and postweaning factors affecting bovine respiratory disease (BRD) in beef cattle and the resulting outcomes of the disease. + = decreased incidence or consequence; − = increased incidence or consequence; ? = effects not fully understood based on the available data. BVD = bovine viral diarrhea virus (Duff and Gaylean, 2007).

Epidemiologic factors contributing to BRD include; microbial agents, mode of transmission, parasite density, infectious period, latent and carrier periods, and virulence (Ellis, 2009; Caswell, 2014). Factors that predispose cattle to BRD can be related to the host or environment. Predisposing host factors include, but are not limited to, age, weight, sex, breed, immunological background, and genetics (Muggli-Cockett et al., 1992; Dixit et al., 2001; Snowder et al., 2005, 2006). Examples of environmental predisposing factors include ambient temperature, humidity, and management practices that increase stress, such as weaning, transportation, handling, surgical procedures (dehorning and castration), comingling, and nutritional changes (Callan and Garry, 2002). Although there is a body of literature supporting the association between BRD and these predisposing factors, there are many challenges to effective field research surrounding BRD that make it difficult to establish causal relationships.

Increased stress resulting from transportation is one of the leading contributors to the incidence of BRD. Transportation is the greatest identified non-infectious risk factor leading to BRD and the reason that BRD is often referred to as “shipping fever”. Various aspects of transportation (e.g. loading and unloading, the duration of transport, and method of transport) have been studied in an attempt to identify which component of transportation has the greatest impact (Martin et al., 1988; Dixit et al., 2001).

While BRD affects cattle of all ages, there are three timepoints when it is most prominent: 1) when passive immunity is waning in calve (approximately 3-4 month of age); 2) at weaning, and 3) at entrance into the feedlot (Muggli-Cockett et al., 1992; Callan and Garry, 2002). Lightweight cattle (< 250 kg) entering the feedlot may have an increased risk for BRD (Sanderson et al., 2008). Weight may not be a direct factor but

rather an indicator for age. Younger calves are more susceptible to disease as they have lower immunity and tend to experience greater stress during transportation. The incidence of BRD was reported as greater in male calves during both preweaning and feedlot periods (Muggli-Cockett et al., 1992). Similarly, Snowder et al. (2006) reported a higher BRD occurrence in steer calves compared to heifers. However, it was suggested that this difference was due to castration practices in steer calves rather than sex.

Variation in BRD susceptibility between breed type supports a genetic component to BRD (Muggli-Cockett et al., 1992; Snowder et al., 2005, 2006). Muggli-Cockett et al. (1992) found that Gelbvieh, Simmental, Hereford, and MARC II (a composite of Charolais, Limousin, Hereford, Braunvieh, and Angus), had lower preweaning frequencies of BRD throughout a six-year study. There was an effect of location for the study, preventing the direct comparisons between all breeds. The same study reported differences between breeds postweaning which were not influenced by location, allowing for comparisons between all breeds. Angus, Gelbvieh, Charolais, Limousin, and two of the composite breeds, MARC I (a composite of Hereford, Angus, Gelbvieh, and Simmental) and MARC II had lower incidence of BRD while Hereford and Pinzgauer had the greatest occurrence of BRD postweaning (Snowder et al., 2006). Differences in BRD risk related to breed type have been identified but not clearly elucidated.

Clinical and Subclinical Diagnosis of Bovine Respiratory Disease

Bovine respiratory disease is a common occurrence in the cattle industry. Because it is generally accepted that a high percentage of cattle industry wide will become infected with BRD, accurate and early diagnosis is crucial. Multiple diagnostic

approaches have been developed to detect BRD. The most common method for BRD detection is the use of visual appraisal to identify sick cattle. Perino and Apley (1998) defined a clinical scoring system of: 0 = normal animal; 1 = noticeable depression without apparent signs of weakness; 2 = marked depression with moderate signs of weakness without a significantly altered gait; 3 = severe depression with signs of weakness such as a significantly altered gait; and 4 = moribund and unable to rise. According to this protocol, animals with a rectal temperature of ≥40°C (104°F) and a clinical score of ≥1 should receive therapeutic treatment. The ease and little to no cost of visual appraisal makes it the most conventional method for BRD detection; however, given the subjective nature of identification through visualization, it is not always the most reliable or accurate approach.

Many cattle do not display noticeable signs of BRD and go undetected until the observation of pulmonary lesions at slaughter. Multiple studies have reported the occurrence of lung lesions in cattle not previously treated for BRD at greater than 60% (Bryant et al., 1999). This high percentage of undiagnosed cases of BRD indicates that visual appraisal methods alone are not adequate to identify all cases of BRD in the herd. The presence or absence of pulmonary lesions has been proposed as an accurate method for BRD diagnosis (Bryant et al., 1999; Galyean et al., 1999). Systems for visual scoring lung lesions have been investigated by multiple research groups (Bryant et al., 1999; Schneider et al., 2009a). Although a lung lesion scoring system offers a more accurate approach to BRD detection, the timing of the diagnosis does not allow for treatment of BRD as it is already occurring and is not a commonly used method in commercial cattle herds.

Several laboratory methods for BRD detection are available. These methods include identifying the causative viral or bacterial pathogen through the use of culture, immunohistochemistry, antigen capture ELISA, and PCR assays (Duff and Galyean, 2007; Urban-Chmiel and Grooms, 2012). Laboratory diagnostic methods are useful to confirm cases of BRD and guide treatment procedures; however, the cost and time required to complete the laboratory analysis are often prohibitive for widespread use in commercial herds. Additional proposed methods for BRD detection include; ultrasound, radio frequency identification-associated thermography, lung biopsy, and rumen temperature boluses (Schaefer et al., 2007; Rose-Dye et al., 2011; Abutarbush et al., 2012; Burgess et al., 2016). Many of these approaches offer a greater ability to detect BRD compared to visual methods, but cost, labor, and practicality prevent widespread use in the cattle industry.

Pathogenesis of Bovine Respiratory Disease Complex

The pathogenesis of BRD often involves complex interactions between the environment, the pathogens, and the animal. Viral infections reduce host defenses and cause nasopharyngeal dysbiosis (Caswell, 2014). The most frequently documented viral pathogens associated with BRD are bovine herpesvirus 1 (**BHV-1**), bovine viral diarrhea virus (**BVDV**), bovine parainfluenza type 3 virus (**PI3V**), and bovine respiratory syncytial virus (**BRSV**) (Ellis, 2009; Ng et al., 2015; Hay et al., 2016). Both BRSV and PI3V are principally respiratory pathogens while BHV-1 and BVDV can affect multiple systems (Fulton, 2009; Hay et al., 2016). These BRD-associated viruses replicate in epithelial cells of the respiratory tract, causing mucosal inflammation and allowing for

adhesion and replication by pathogenic bacterial species (Caswell, 2014). Additional viruses that are thought to play a minor role in BRD include bovine adenovirus 3 (**BAdSV**) and bovine corona virus (**BoCV**) (Ng et al., 2015; Murray et al., 2016). Recent studies have utilized metagenomics to further characterize the virome of cattle afflicted with BRD. Ng et al. (2015) reported that in addition to previously documented viruses, bovine influenza D virus and bovine rhinitis A virus were also associated with BRD (Ng et al., 2015; Murray et al., 2016). Although BAdSV, BoCV, bovine influenza D virus, and bovine rhinitis A virus were initially considered to be minor players in BRD, these viruses may actually have a pathogenic role rather than strictly commensal in nature (Murray et al., 2016).

Traditional models for BRD pathogenesis describe a primary viral infection followed by opportunistic bacterial infection. It is thought that the viral infection induces immunosuppression and damage to the respiratory epithelial, leading to a secondary bacterial infection and clinical signs of BRD (Ellis, 2009; Hay et al., 2016). However, this viewpoint has been challenged as being overly simplistic as resent research has demonstrated a greater role of certain pathogens previously considered minor or secondary contributors to BRD (Murray et al., 2016). The pathogenesis of BRD has expanded to include conditions (such as stress) that allow pathogenic bacteria to proliferate in the respiratory tract and gain access to the lungs through inhalation in the absence of a viral infection.

The bacterial pathogens most commonly associated with BRD are *Mannheimia haemolytica (*formally *Pasteurella haemolytica), Pasteurella multocida, Haemophilus somnus, Mycoplasma spp, Chlamydia spp* (Fulton, 2009). Although strains of these

bacteria are considered opportunistic pathogens and are often associated with BRD and morbidity in cattle, they are common inhabitants of the bovine respiratory tract in both healthy and diseased animals (Caswell, 2014; Timsit et al., 2016a; Zeineldin et al., 2017).

Historically, most research surrounding bovine respiratory bacteria focused on pathogens and utilized a culture-based method for identification and characterization of bacteria (Holman et al., 2015a). Recent studies using culture-independent methods have facilitated a more complete characterization of the total nasopharyngeal microbiota. This is especially useful for investigating bacterial community dynamics and the role of the bacterial community in regulating specific microbial populations in the respiratory tract. Although *Mycoplasma, Moraxella, Mannheimia, Pasteurela,* and *Haemophilus* are among the most documented genera associated with BRD, recent studies have detected a higher relative abundance of *Acinetobater* in nasopharyngeal samples of affected BRD calves compared to healthy calves. While these lesser known bacteria have been implemented in a range of diseases, little is known about their specific role in respiratory microbial communities in livestock.

There is increasing evidence to support the role of community diversity and stability in the development of BRD (Holman et al., 2015a). Several studies have reported a decrease in microbial community diversity and stability in BRD affected cattle (Holman et al., 2015a; Timsit et al., 2016a; Timsit et al., 2018). It has been hypothesized that greater microbial community diversity facilitates the suppression of pathogenic bacterial colonization in the bovine respiratory tract. Further research is warranted to determine the specific mechanism and key interactions within nasopharyngeal bacterial communities that result in the development of BRD.

Preventative and Management Strategies

Because the manifestation of BRD is a result of complex interactions between environmental, pathogen and host related factors, preventative measures and best management practices also require a multifaceted approach to reduce BRD prevalence. Preconditioning, vaccination protocols, nutritional status, cattle temperament, castration, dehorning, and general cattle handling practices should all be considered when developing strategies for the prevention of BRD.

The benefits of preconditioning cattle prior to entry in the feedlot is not a new concept, yet industry wide adoption of this practice has been slow (Dhuyvetter et al., 2005). Only 32.4% of all feedlots surveyed by USDA-APHIS (2000) received information regarding the previous history of the cattle and if they were part of a preconditioning program. The term preconditioning refers to a planned preventative health management program occurring before shipping cattle to the feedlot. Preconditioning programs ensure that animals have been weaned for a specific amount of time, typically 30-45 days, vaccinated (detailed below), treated with anthelmintics, castrated, dehorned, and acclimated to feed bunks and water (Duff and Galyean, 2007). The reduction in morbidity, gain in feed efficiency, and premiums offered by many feedlots offset expenses associated with preconditioning and increase value of those calves entering the feedlot making preconditioning programs economically feasible for producers (Macartney et al., 2003; Dhuyvetter et al., 2005).

Vaccination against the various pathogens involved in BRD is an important part of any BRD prevention program. In the United States, vaccines against the viral

pathogens IRB, BVD, PI-3, BRSV and the bacterial pathogens *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus sommus* are readily available (Urban-Chmiel and Grooms, 2012). Vaccines against viral pathogens can be found in different combinations and in both killed and live forms, but most vaccines used are killed due to restrictions on the use of modified-live vaccines on calves nursing pregnant cows (Fulton, 2009). It is recommended that calves be vaccinated prior to weaning followed by revaccination 4 to 6 weeks later at weaning (Duff and Galyean, 2007). If preweaning vaccination is not feasible, it is recommended to vaccinate at weaning and again 14 to 21 days after (Fulton et al., 2004).

Additional management practices that have been associated with the development of BRD include nutritional management and cattle handling. Nutritional status of the herd prior to a BRD challenge plays a crucial role in the percentage and outcome of animals infected. While it is generally accepted that nutrition plays a role in the development of BRD, there is not a clear consensus on the specific effects of different concentrations of dietary energy or crude protein in the diet (Duff and Galyean, 2007; Taylor et al., 2010b). Copper, selenium, zinc, and vitamins A, E and B complex have also been investigated to determine their role in BRD and potential as tools for prevention and treatment. Similar to dietary energy and protein, some studies support a reduced occurrence of BRD while others fail to detect a difference between specific vitamin and mineral concentrations and BRD risk (Galyean et al., 1999; Cusack et al., 2009; Taylor et al., 2010b). It is likely that this inability to consistently support a definitive link between nutritional factors and BRD may be due to confounding factors within the studies such as the source of the cattle and time between arrival and processing.

Cattle handling and cattle temperament are important considerations when discussing BRD management as both impact stress levels of cattle. There is strong evidence to support the negative effect of stress on cattle health and performance. Utilizing low-stress cattle handling practices can reduce the risk for BRD (Hodgson et al., 2005). Fell et al. (1999) evaluated differences in BRD treatments in cattle that were designated as “calm” or “nervous”. They reported that cattle in the nervous group required a greater number of treatments for BRD compared to the calm group. Furthermore, Oliphint (2006) determined that cattle temperament may influence the response to vaccinations.

Although environmental and management stressors play a major role in the incidence of BRC, there is an increasing body of evidence to support that BRD susceptibility is partially subject to genetic control. Selection of animals that are less susceptible to BRD offers a viable method for reducing the prevalence of BRD. Heritability estimates for BRD susceptibility range from low (0.04) to moderately (0.26) heritable (Muggli-Cockett et al., 1992; Snowder et al., 2005; Schneider et al., 2009b; Neibergs et al., 2014). Furthermore, Snowder et al. (2006, 2007) reported that the incidence of BRD was heritable for genetic variation within and between breeds. The continued advancements in molecular genetic technology has enhanced the ability to investigate the influence of genetics in complex diseases such as BRD (Casas and Snowder, 2008; Hayes et al., 2010). Molecular technology can be applied to identify regions of the genome associated with BRD susceptibility that can ultimately be used to select breeding stock that are less susceptible to BRD.

DNA Pooling

Investigating the genetic basis for complex traits often requires genotyping many individuals, making it cost prohibitive research. Pooling DNA samples offers a cost-effective alternative to individual genotyping. Pooling of individual DNA samples provides a means to reduce the expense of genotyping studies without compromising the ability to detect causative allele associations. Rather than genotyping each individual in case and control cohorts, stringently quantified equimolar DNA pools derived from all case and all control animals are constructed separately and then genotyped (Macgregor et al., 2008). This strategy allows allele frequencies to be measured between groups of individuals using far fewer PCR reactions and genotyping assays (Sham et al., 2002). Utilizing DNA pooling can significantly reduce the cost, time, and labor required for large scale studies, especially those investigating complex traits (Pearson et al., 2007). Barratt et al. (2002) demonstrated a 60-fold reduction in DNA usage and 30-fold savings in cost compared to individual genotyping. Macgregor et al. (2006) suggested that the future of genome-wide association studies will be limited by the available sample size rather than cost, since very few arrays are required to extract the majority of information and there is high concordance between individual genotyping and pooling.

Pooling is not a novel concept and was first used in genetics for a case-control association study of HLA class II DR and DQ alleles in type I diabetes mellitus (Arnheim et al., 1985). Since then pooling has been applied to a variety of research including linkage studies, homozygosity mapping of recessive disease in inbred populations and mutation detection (Michelmore et al., 1991; Nystuen et al., 1996; Sheffield et al., 1997;

Amos et al., 2000). Livestock research has utilized pooling techniques for many studies, including those investigating complex traits such as reproduction, and disease (McDaneld et al., 2014; Strillacci et al., 2014; Keele et al., 2015).

DNA Quantification Methods

Pooling DNA samples for genetic analysis offers a promising way to reduce the cost of genetic studies. However, the ability to reduce sampling error is largely contingent on accurate DNA quantification methods. The two most commonly used DNA quantification methods are spectrofluorometry and spectrophotometry. Spectrophotometry is the most frequently used DNA quantification technique, measuring absorbance of ultraviolet (**UV**) light absorbance of a sample at a specific wavelength (Yu et al., 2017). Ultraviolet absorbance at 260 nm measures nucleic acids while absorbance at 280 nm measures contaminating protein in the sample; these ratios are used to evaluate purity of the sample (Boesenberg-Smith et al., 2012). Spectrophotometry is widely used in molecular labs since it is relatively simple, inexpensive, and quick to use, does not require a large amount of the sample, and can assess the purity of a sample (Haque et al., 2003; Li et al., 2014; Yu et al., 2017). Despite the advantages of spectrophotometry quantitation, there are several limitations to this technology. Li et al. (2014) reported that spectrophotometry overestimated the sample concentration and was more susceptible to contaminants in the sample compared to spectrofluorometry measurements.

In contrast to spectrophotometry, fluorometric methods of DNA quantification are among the most sensitive measurement approaches available (Rengarajan et al., 2002). This method uses an intercalating dye, such as PicoGreen, which selectively binds to

double-stranded DNA and fluoresces when excited. The intensity of this signal can be measured to determine the DNA concentration of a sample. Singer et al. (1997) demonstrated the ability of PicoGreen to detect nucleic acid concentration from a range of 1pg/ml to 1µg/ml. In addition to the high sensitivity of fluorescent quantitation, it is less susceptible to contaminants and has the ability to differentiate intact DNA from degraded DNA (Li et al., 2014). Despite the superior sensitivity of fluorometric methods compared to UV absorbance methods, fluorometric methods have several drawbacks that likely impede its use. Fluorometric methods require costly equipment and reagent kits necessary to obtain fluorometric readings. There is also added time required to prepare standards and incubate the dye. Although it is less susceptible to contaminants, the signal intensity of PicoGreen is decreased in the presence of organic solvents, influencing measurement accuracy (Li et al., 2014). Furthermore, fluorometric methods are unable to determine purity of a sample. Although spectrophotometry and spectrofluorometry quantification techniques are widely utilized, discrepancies often occur between the two methods (Holden et al., 2009; Li et al., 2014; Yu et al., 2017). It has been suggested that the optimal method of DNA quantification is dependent upon the type of sample and desired downstream applications (Haque et al., 2003; Li et al., 2014).

Pooling prior to DNA extraction

Pooling samples prior to DNA extraction could be a way to mitigate potential bias introduced due to inconsistency in DNA quantification methods. Pool construction based on individually extracting, quantifying, and pooling each sample is labor-intensive and requires sophisticated DNA quantification procedures. Pooling samples prior to DNA

extraction would significantly decrease the cost of large-scale genomics studies. Figure 1.2 demonstrates the time and cost associated with individual genotyping, DNA pooling, and pooling prior to DNA extraction using whole blood as an example.

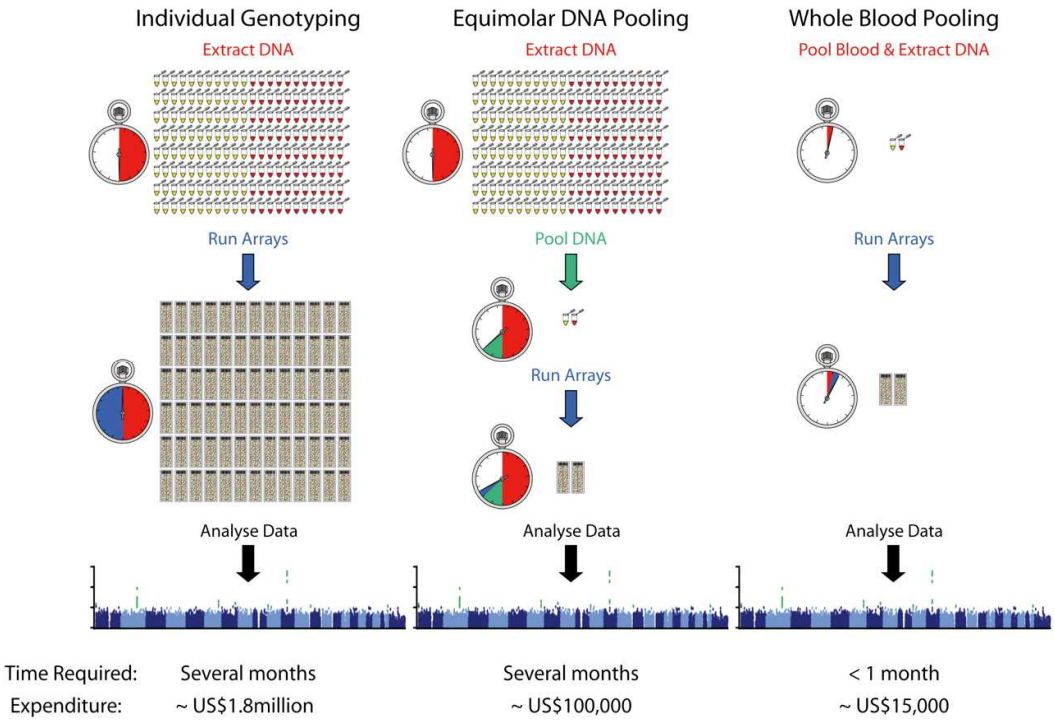

Figure 1.2. Flowchart comparing available options for performing genome-wide association studies (Craig et al., 2009).

Several studies have evaluated the accuracy of pooling prior to DNA extraction using lung tissue and whole blood (Craig et al., 2009; Keele et al., 2015). A study by Craig et al. (2009) evaluated the concept of pooling samples based on equal volumes of unquantified whole blood aliquots from case and control cohorts prior to DNA extraction. The pooling of whole blood by volume was corrected for typical cell count values to reduce error associated with volume. Three pools comprised of 100 Caucasian individuals in each were constructed based on blue eyes, brown eyes, and

pseudoexfoliation. Using this pooled blood method Craig et al. (2009) identified associations between previously identified genes for eye color, skin pigmentation, and age-related macular degeneration.

Pooling prior to DNA extraction has also been used to study lung lesions and liver abscesses in beef cattle (Keele et al., 2015; Keele et al., 2016). Both genome-wide association studies were completed using pools constructed from lung tissue collected from case and control animals. From each individual sample, one cylindrical core of equal size was collected and placed in a pool. Each pool contained 96 individual animals and a total of 120 pools (60 case, 60 control) for the lung lesion study and 24 pools (12 case, 12 control) for the liver abscess study. Based on the genotyped lung tissue pools, 85 SNP were significantly associated with lung lesions and 35 SNP associate with liver abscess.

The accuracy and efficacy of pooling samples prior to DNA extraction has been demonstrated for several types of samples. Further research into pooling based on different sample or tissue types is warranted and would benefit both research groups and the livestock industry by reducing the time, cost, and labor of genotyping analysis. As large-scale DNA testing becomes more economically feasible, this increases the likelihood of commercial application and utilization.

Importance of Education to the Livestock Industry

Developing and improving molecular genetic tools is important to the future success of the livestock industry. However, advancements can only be achieved if these genetic techniques are being implemented and correctly utilized in livestock production.

Higher education has been associated with the adoption of new technologies in the livestock industry (Pruitt et al., 2015). A study by Kim et al. (2005) found that education level positively affected the likelihood of cow-calf producers to adopt best management practices in Louisiana. Ward et al. (2008) reported that education was significant to the adoption of forage and reproductive management practices by cow-calf producers in Oklahoma. Similarly, survey data indicated that producers with a college degree were more likely to adopt breeding technology in the swine industry (Gillespie et al., 2015). Higher education offers an opportunity to train producers in best management practices and provide them with the knowledge and abilities to utilize emerging technology in the livestock industry.

While a college degree can greatly benefit producers and prepare them for a career in the livestock industry, the adjustment to higher education can be challenging and overwhelming for first year students. They often find it intimidating to communicate with instructors and are tasked with developing study habits that will allow them to succeed in a university setting (Mulvey, 2009). The use of classroom assessment methods helps to address these challenges and monitor student learning progress.

Assessment in Higher Education

According to Angelo and Cross (1993), "learning can take place in the absence of teaching, but there is no such thing as effective teaching in the absence of learning". But how do instructors know if they are teaching students or just talking at them? Assessment provides valuable insight on the teaching and learning processes. The term assessment describes the systematic collection, review, and use of information about educational

programs undertaken for the purpose of improving student learning and development (Palomba and Banta, 1999). Various types and styles of assessments are necessary to accurately determine student learning and overall achievement. Assessments can be divided into two main categories; summative and formative. Although there can be overlap between these categories, summative assessments are the most common evaluation method used in education (Black et al., 2004). Summative assessments typically occur at the end of a unit or semester and are used to evaluate student learning, knowledge, proficiency, or success at the conclusion of an instructional period. While summative assessment methods are a necessary and beneficial component of higher education, the information gained from summative assessments is not always timely enough to make adjustments and provide adequate feedback (Black and Wiliam, 1998).

Formative assessment occurs throughout the semester and includes a variety of assessment tools aimed at identifying gaps in knowledge and guiding improvements in teaching and learning (Dunn and Mulvenon, 2009). Works by Angelo and Cross (1993), particularly their book *Classroom Assessment Techniques: A handbook for college teachers*, are attributed with the formal development and advancement of formative assessment techniques, which they referred to as Classroom Assessment Techniques or "CATs".

Classroom Assessment Techniques

Continual evaluation of the teaching and learning process is paramount for successful instruction. Effective instructors understand the need to proactively assess student learning informally prior to exams, finals, or high stakes assignments. Classroom

assessment techniques are quick, low risk (ungraded) assessments that provide timely feedback to the instructor and allow educators to monitor student learning and adjust material or teaching methodology as needed (Angelo and Cross, 1993). Classroom assessment techniques are designed to gather information on the student and instructor to provide feedback loops for both parties (Cross and Palese, 2015). This information can range from assessing prior knowledge to evaluating the level of mastery in critical thinking tasks.

While the primary focus of CATs has been their value to student learning through increased student engagement, metacognition, and performance, studies have identified additional ways that CATs can benefit the learning process. Classroom assessment techniques can guide course design, enhance instructor teaching effectiveness, and promote a student-centered teaching environment (Angelo and Cross, 1993; Cottell Jr and Harwood, 1998; Byon, 2005; Goldstein, 2007; Cross and Palese, 2015).

Classroom Assessment Techniques and Student Learning and Engagement

According to Angelo and Cross (1993), CATs are learner-centered, teacher-directed, mutually beneficial, formative, and context specific approaches firmly rooted in good practice. Classroom assessment techniques focus the primary attention of teachers and students on observing and improving learning, rather than on observing and improving teaching. This focus on learning encourages students to monitor their own progress, become self-directed learners, and be more engaged in the classroom. The feedback provided through CATs is a critical component in the teaching and learning process; this helps both parties to monitor their learning and make necessary adjustments

(Angelo and Cross, 1993). This increased awareness allows students to identify gaps in their knowledge and form connections between prior knowledge or experience and the new information (Steadman, 1998).

Increasing student engagement and ownership in the learning process are commonly cited benefits of using CATs (Steadman, 1998; Hogan and Daw, 2014; Cross and Palese, 2015). A study by Steadman (1998) surveyed 56 instructors from multiple community colleges in Northern California on their use and perspectives of CATs. When asked about the advantages of using CATs in the classroom, the most frequently mentioned response was the ability to "tune into students' voices". Instructors elaborated that this increased student satisfaction in the course because the CAT demonstrated care for student's learning on the part of the instructor. This encouraged student investment in the outcome of the class and increased involvement in their own learning. Instructor perceptions were supported by student surveys. Students positively responded to the opportunity to express their opinions in the classroom and were appreciative when instructors acknowledged or made changes based on student comments.

Students feeling that they have a voice and the instructor cares about their individual learning experience creates an engaging and collaborative classroom environment and encourages students to take possession of the learning process engage in self-directed learning habits. Cross and Palese (2015) reported increased participation in response to implementing CATs in an online class as demonstrated by an increase in average posting frequency in a discussion forum. Furthermore, Henderson (2001) reported that the implementation of CATs not only increased student participation but also generated "deeper" discussion threads.

Feedback

A primary advantage of CATs compared to summative assessment methods is the ability to correct misconceptions prior to high stakes situations through continual feedback. The establishment of a continual feedback loop between instructors and students is a key component to the effectiveness and success of CATs. Students often benefit from the act of participating in a CAT activity. However, if little is done with the information collected from the students, gains in students learning will be minimal. Instructors must "close the feedback loop" by communicating the results to the students. Angelo and Cross (1993) modeled feedback as a cylindrical process in which instructors collect information regarding the effectiveness of instruction, the learning process, or other data relevant to the course, then convey the results of the assessment back to the students and provide suggestions for improving learning. Once adjustments have been made, these are then reevaluated through additional use of CATs and the cycle continues.

Feedback is not only useful for identifying and addressing misconceptions or gaps in student learning but can also increase student confidence in the subject matter. Butler and Roediger (2008) investigated the effect of feedback on final exam correct responses for previously incorrect answer or low-confidence correct answers. Student completed an initial multiple-choice test which also required them to rate their confidence for each response. Following this, some students were provided with feedback while other students were not. After this, the students completed a final test which included questions identical to the initial multiple-choice exam. Of the two groups of students, those that received feedback had greater improvement of initially incorrect responses, maintained previously correct responses, and increased in confidence on the final test for previously

low-confidence correct questions. In contrast, students that did not received feedback often changed low-confidence correct answers to incorrect answers or omitted them on the final test.

Classroom Assessment Techniques and Student Performance

While classroom assessment techniques are often promoted as tools to increase student performance, the evidence directly connecting CATs to an improvement in student grades is lacking. Multiple studies have reported that CATs increase student performance on quizzes, exams, and overall course grades (Angelo and Cross, 1993; Holbeck et al., 2014; Cross and Palese, 2015). However, Cottell Jr and Harwood (1998) reported no difference in grade performance between control and CAT groups at two universities. Similarly, Simpson-Beck (2011) and Bullock et al. (2018) did not detect a difference in student learning between groups that did or did not participate in a Muddiest Point activity.

Despite decades of implementation, whether CATs to have a direct effect on student learning or direct effect on teaching remains unclear. Classroom assessment techniques may not directly impact academic performance but rather serve to guide instruction, increase student engagement, and strengthen the feedback loop between instructors and students (Simpson-Beck, 2011; Bullock et al., 2018). Another possibility is that this discrepancy is a result of confounding factors in the study design that are preventing the detection of grade difference.

There are several common experimental design related challenges that could be influencing the outcome and preventing the detection of true differences in student performance. Inadequate sample size, sample bias, contamination bias, and improper

CAT design for the course could all be contributing to the mixed outcomes of CATs on student performance (Simpson-Beck, 2011). A major barrier to achieving clear, well-defined results is the inability of many studies to control for selection effects within educational research designs. Educational settings are inherently complex, creating a challenge between maintaining scientific integrity and preserving student well-being (Graesser, 2009). In research, the "golden standard" of experimental design is randomization. However, in educational research, true randomization is often neither feasible or ethical (Adelson, 2013). This results in the issue of selection effect (bias) in many educational research studies.

Additionally, it can be challenging to measure the difference between students that memorize material for an exam versus students that have a deeper understanding of the content. Long term retention of material may be a more accurate measure of the association between CATs and student performance. Since CATs promote self-directed learning and metacognitive development, it is possible that comparing variation in grades does not actually measure how well a student learned the material. A final exam grade does not necessarily separate students that have memorized the material versus those that acquired a greater understanding of the material through self-awareness of their learning as a result of CATs. Classroom assessment techniques have been shown to promote long term gains in the form of increased critical thinking abilities, facilitation of self-directed learning, and forming of bridges between content (Angelo and Cross, 1993; Cottell Jr and Harwood, 1998; Byon, 2005). Therefore, assessment of short-term memory in the form of quizzes or exams may not be adequate to detect the true benefit of CATs on student performance. Furthermore, one of the challenges to evaluating the effects of CATs on

student performance is that many CATs are utilized informally and there is a greater amount of antidotal information available compared to empirical evidence (Simpson-Beck, 2011).

Factors Limiting the Use of Classroom Assessment Techniques

Although there is a body of research supporting the positive attributes of CATs, some instructors remain hesitant to use them in the classroom. When instructors were questioned as to why they refrained from using CATs in their classroom, some of the most common responses included time constraints, negative student perceptions, complexity, course structure, and unfamiliarity (Steadman, 1998). While all responses are valid concerns, most are rooted in misconception. Limited time available both inside and outside of the classroom is a challenge faced by almost all instructors throughout their teaching careers. When considering implementation of CATs in the classroom, there is often a concern that CATs will take away form class time needed to deliver material or will add a great burden on their time and workload outside of the classroom. While some CATs require additional time of the instructor in order to review the responses, such as those that ask students to write reflections or points of clarification (Minute paper, muddiest point, double journal, etc.), many CATs require minimal class time to complete and do not require evaluation by the instructor outside of the class period (e.g. think-pair-share, group polls, creating concept maps as a class, etc.,) (Angelo and Cross, 1993). Furthermore, even though CATs may take time to complete during the class period, using CATs to monitor learning as it is happening and making necessary adjusts can save time in the long term by preventing the need for review or allowing the instructor to progress

more quickly if a topic is well understood by students. Cottell Jr and Harwood (1998) reported that students in classes that used CATs felt that the class time was appropriate to cover the material and that the instructor adequately answered their questions more often compared to the control groups, suggesting that the addition of CATs did not negatively impact the presentation of course material.

Students' perceptions of their instructors teaching ability and course satisfaction can be a barrier to the utilization of CATs. Faculty have voiced concern that students will not recognize the benefits of CATs and that implementing CATs in their classroom could negatively impact their course ratings by students (Steadman, 1998). Additionally, it can be difficult or off putting for faculty to receive negative comments from students. One of the key components of CATs is the collection of teaching related information. Faculty who have taken CAT-derived student remarks and applied this information to guide improvements to their teaching methodology or course structure have reported recognition of this effort by students on subsequent assessments and overall positive responses on end of semester reviews (Cottell and Harwood, 1998).

Some instructors abstain from using CATs because they think that their class is structured in a way that is not suitable for CATs, including class size, delivery format, or subject matter. This may be true if implemented incorrectly; however, benefits of CATs include their variety and flexibility (Henderson, 2001). There are numerous CATs available, descriptions of several commonly used CATs are providing in Table 1.1. With the range of documented CATs, it is a matter of finding the one that is best suited for the course structure and content. In recent years there has been a greater focus on evaluating different CATs within the context of specific subject matter or course delivery platform

(Goldstein, 2007; Cohen, 2008; Isaksson, 2008; Holbeck et al., 2014; Cross and Palese, 2015). Classroom assessment techniques have been evaluated in traditional classrooms settings, flipped classrooms, and online courses. Studies have evaluated the effect of specific CATs on statistics, calculus, pharmacy, foreign language, biology, physics, and chemistry, among others. Additional discipline-specific studies for CATs could provide useful information and aid in selecting the optimal CAT for individual courses.

Conclusion

Bovine respiratory disease affects cattle producers involved in all segments of industry in the U.S. and globally. Despite improvements in prevention and management, BRD remains prominent. The complicated pathogenesis of this disease makes it especially difficult to infer causal relationship between environment, associated pathogens, predisposing factors, genetics, and the occurrence of BRD. The primary mechanism for BRD infection is initial immunosuppression caused by a stress event (viral or environmental), followed by a secondary bacterial infection. Further research is warranted to understand the fundamental role of nasopharyngeal bacterial communities and in BRD development and suppression. Furthermore, insights into the genetic component of BRD could provide cattle producers with tools to enhance selection against BRD susceptibility. However, understanding the genetic basis for complex disease is labor intensive and extremely costly. Pooling samples prior to DNA extraction is an emerging technique that could greatly reduce the time and economic barriers to investigating complex diseases such as BRD. However, new technology and management techniques are only beneficial if livestock producers are willing to adopt and correctly

use them. Higher education offers the opportunity to provide future producers with the knowledge and skills to utilize emerging tools in the livestock industry. Along with this knowledge base, students must become self-directed learners with a desire to seek out and learn about innovative technology and management techniques throughout their careers in the cattle industry.

REFERENCES

Abutarbush, S. M., C. M. Pollock, B. K. Wildman, T. Perrett, O. C. Schunicht, R. K. Fenton, S. J. Hannon, A. R. Vogstad, G. K. Jim, and C. W. Booker. 2012. Evaluation of the diagnostic and prognostic utility of ultrasonography at first diagnosis of presumptive bovine respiratory disease. Canadian Journal of Veterinary Research 76(1):23-32.

Adelson, J. L. 2013. Educational research with real-world data: Reducing selection bias with propensity scores. Practical Assessment, Research & Evaluation 18(15):2.

Amos, C. I., M. L. Frazier, and W. Wang. 2000. DNA pooling in mutation detection with reference to sequence analysis. The American Journal of Human Genetics 66(5):1689-1692.

Angelo, T. A., and K. P. Cross. 1993. Classroom assessment techniques : a handbook for college teachers. San Francisco : Jossey-Bass Publishers, San Francisco.

Barratt, B. J., F. Payne, H. E. Rance, S. Nutland, J. A. Todd, and D. G. Clayton. 2002. Identification of the sources of error in allele frequency estimations from pooled DNA indicates an optimal experimental design. Ann Hum Genet 66(Pt 5-6):393-405. doi: 10.1017/S0003480002001252

Black, P., C. Harrison, C. Lee, B. Marshall, and D. Wiliam. 2004. Working inside the black box: Assessment for learning in the classroom. Phi delta kappan 86(1):8-21.

Black, P., and D. Wiliam. 1998. Assessment and classroom learning. Assessment in Education: principles, policy & practice 5(1):7-74.

Boesenberg-Smith, K. A., M. M. Pessarakli, and D. M. Wolk. 2012. Assessment of DNA Yield and Purity: an Overlooked Detail of PCR Troubleshooting. Clinical Microbiology Newsletter 34(1):1-6. doi: 10.1016/j.clinmicnews.2011.12.002

Bryant, L., L. Perino, D. Griffin, A. Doster, and T. Wittum. 1999. A method for recording pulmonary lesions of beef calves at slaughter, and the association of lesions with average daily gain. The Bovine Practitioner (33):163-173.

Bullock, K. C., C. Gibson, M. Howard, J. Liu, A. Tatachar, and W. C. Yuet. 2018. Use of the Muddiest Point Technique as an exam review in an integrated pharmacotherapy course. Currents in Pharmacy Teaching and Learning 10(9):1295-1302.

Burgess, J. K., T. Mauad, G. Tjin, J. C. Karlsson, and G. Westergren□Thorsson. 2016. The extracellular matrix–the under□recognized element in lung disease? The Journal of pathology 240(4):397-409.

Butler, A. C., and H. L. Roediger. 2008. Feedback enhances the positive effects and reduces the negative effects of multiple-choice testing. Memory & Cognition 36(3):604-616.

Byon, A. S. 2005. Classroom assessment tools and students' affective stances: KFL classroom settings. Language and Education 19(3):173-193.

Callan, R. J., and F. B. Garry. 2002. Biosecurity and bovine respiratory disease. The Veterinary Clinics of North America. Food Animal Practice 18(1):57-77.

Casas, E., and G. D. Snowder. 2008. A putative quantitative trait locus on chromosome 20 associated with bovine pathogenic disease incidence. J Anim Sci 86(10):2455-2460. doi: 10.2527/jas.2008-0933

Caswell, J. 2014. Failure of respiratory defenses in the pathogenesis of bacterial pneumonia of cattle. Veterinary pathology 51(2):393-409.

Cohen, M. 2008. Participation as assessment: political science and classroom assessment techniques. PS: Political Science & Politics 41(3):609-612.

Cottell Jr, P. G., and E. M. Harwood. 1998. Using classroom assessment techniques to improve student learning in accounting classes. Issues in accounting education 13(3):551.

Cottell, P., and E. Harwood. 1998. Do Classroom Assessment Techniques (CATs) Improve Student Learning? New Directions for Teaching and Learning 75:37-46.

Craig, J. E., A. W. Hewitt, A. E. McMellon, A. K. Henders, L. Ma, L. Wallace, S. Sharma, K. P. Burdon, P. M. Visscher, G. W. Montgomery, and S. MacGregor. 2009. Rapid inexpensive genome-wide association using pooled whole blood. Genome Res 19(11):2075-2080. doi: 10.1101/gr.094680.109

Cross, T., and K. Palese. 2015. Increasing learning: Classroom assessment techniques in the online classroom. American Journal of Distance Education 29(2):98-108.

Cusack, P., N. McMeniman, A. Rabiee, and I. Lean. 2009. Assessment of the effects of supplementation with vitamin E on health and production of feedlot cattle using meta-analysis. Prev Vet Med 88(4):229-246. doi: 10.1016/j.prevetmed.2008.12.002

Dhuyvetter, K. C., A. Bryant, and D. Blasi. 2005. Case study: Preconditioning beef calves: Are expected premiums sufficient to justify the practice? The Professional Animal Scientist 21(6):502-514.

Dixit, V., M. Marahrens, and N. Parvizi. 2001. Transport stress modulates adrenocorticotropin secretion from peripheral bovine lymphocytes. Journal of animal science 79(3):729-734.

Dorfman, J. H. 1996. Modeling Multiple Adoption Decisions in a Joint Framework. American Journal of Agricultural Economics 78(3):547-557. doi: 10.2307/1243273

Duff, G. C., and M. L. Galyean. 2007. Board-invited review: recent advances in management of highly stressed, newly received feedlot cattle. Journal of Animal Science 85(3):823-840.

Dunn, K. E., and S. W. Mulvenon. 2009. A critical review of research on formative assessment: The limited scientific evidence of the impact of formative assessment in education. Practical assessment, research & evaluation 14(7):1-11.

Edwards, T. A. 2010. Control Methods for Bovine Respiratory Disease for Feedlot Cattle. Veterinary Clinics of North America: Food Animal Practice 26(2):273-284. doi: 10.1016/j.cvfa.2010.03.005

Ellis, J. A. 2009. Update on viral pathogenesis in BRD. Animal health research reviews 10(2):149-153.

Fell, L. R., I. G. Colditz, K. H. Walker, and D. L. Watson. 1999. Associations between temperament, performance and immune function in cattle entering a commercial feedlot. Australian Journal of Experimental Agriculture 39(7)doi: 10.1071/ea99027

Fulton, R. W. 2009. Bovine respiratory disease research (1983–2009). Animal health research reviews 10(2):131-139.

Fulton, R. W., R. E. Briggs, M. E. Payton, A. W. Confer, J. T. Saliki, J. F. Ridpath, L. J. Burge, and G. C. Duff. 2004. Maternally derived humoral immunity to bovine viral diarrhea virus (BVDV) 1a, BVDV1b, BVDV2, bovine herpesvirus-1, parainfluenza-3 virus bovine respiratory syncytial virus, Mannheimia haemolytica and Pasteurella multocida in beef calves, antibody decline by half-life studies and effect on response to vaccination. Vaccine 22(5-6):643-649.

Gagea, M. I., K. G. Bateman, T. van Dreumel, B. J. McEwen, S. Carman, M. Archambault, R. A. Shanahan, and J. L. Caswell. 2006. Diseases and Pathogens Associated with Mortality in Ontario Beef Feedlots. Journal of Veterinary Diagnostic Investigation 18(1):18-28. doi: 10.1177/104063870601800104

Galyean, M., L. Perino, and G. Duff. 1999. Interaction of cattle health/immunity and nutrition. Journal of Animal Science 77(5):1120-1134.

Gillespie, J. M., C. G. Davis, and N. C. Rahelizatovo. 2015. Factors Influencing the Adoption of Breeding Technologies in U.S. Hog Production. Journal of Agricultural and Applied Economics 36(1):35-47. doi: 10.1017/s1074070800021842

Goldstein, G. S. 2007. Using classroom assessment techniques in an introductory statistics class. College Teaching 55(2):77-82.

Graesser, A. C. 2009. Inaugural editorial for Journal of Educational Psychology.

Griffin, D. 1997. Economic impact associated with respiratory disease in beef cattle. Veterinary Clinics: Food Animal Practice 13(3):367-377.

Haque, K. A., R. M. Pfeiffer, M. B. Beerman, J. P. Struewing, S. J. Chanock, and A. W. Bergen. 2003. Performance of high-throughput DNA quantification methods. BMC Biotechnology 3(1):20. (journal article) doi: 10.1186/1472-6750-3-20

Hay, K. E., J. M. Morton, M. L. Schibrowski, A. C. A. Clements, T. J. Mahony, and T. S. Barnes. 2016. Associations between prior management of cattle and risk of bovine respiratory disease in feedlot cattle. Preventive Veterinary Medicine 127:37-43. doi: 10.1016/j.prevetmed.2016.02.006

Hayes, B. J., J. Pryce, A. J. Chamberlain, P. J. Bowman, and M. E. Goddard. 2010. Genetic architecture of complex traits and accuracy of genomic prediction: coat colour, milk-fat percentage, and type in Holstein cattle as contrasting model traits. PLoS Genet 6(9):e1001139. doi: 10.1371/journal.pgen.1001139

Henderson, T. 2001. Classroom assessment techniques in asynchronous learning networks. The Technology Source

Hodgson, P., P. Aich, A. Manuja, K. Hokamp, F. Roche, F. Brinkman, A. Potter, L. Babiuk, and P. Griebel. 2005. Effect of stress on viral–bacterial synergy in bovine respiratory disease: novel mechanisms to regulate inflammation. Comparative and functional genomics 6(4):244-250.

Hogan, A., and J. Daw. 2014. Mode of Delivery: A Classroom Assessment Technique Comparison between Verbal and Non-Verbal Communication. Journal of Instructional Research 3:21-26.

Holbeck, R., E. Bergquist, and S. Lees. 2014. Classroom assessment techniques: Checking for student understanding in an introductory university success course. Journal of Instructional Research 3:38-42.

Holden, M. J., R. J. Haynes, S. A. Rabb, N. Satija, K. Yang, and J. R. Blasic, Jr. 2009. Factors affecting quantification of total DNA by UV spectroscopy and PicoGreen fluorescence. J Agric Food Chem 57(16):7221-7226. doi: 10.1021/jf901165h

Holman, D. B., T. A. McAllister, E. Topp, A.-D. G. Wright, and T. W. Alexander. 2015. The nasopharyngeal microbiota of feedlot cattle that develop bovine respiratory disease. Veterinary microbiology 180(1-2):90-95.

Isaksson, S. 2008. Assess as you go: the effect of continuous assessment on student learning during a short course in archaeology. Assessment & Evaluation in Higher Education 33(1):1-7.

Keele, J., L. Kuehn, T. McDaneld, R. Tait, S. Jones, T. Smith, S. Shackelford, D. King, T. Wheeler, A. Lindholm-Perry, and A. McNeel. 2015. Genomewide association study of lung lesions in cattle using sample pooling 1,2. Journal of Animal Science 93(3):956-964. doi: 10.2527/jas.2014-8492

Keele, J. W., L. A. Kuehn, T. G. McDaneld, R. G. Tait, S. A. Jones, B. N. Keel, and W. M. Snelling. 2016. Genomewide association study of liver abscess in beef cattle. J Anim Sci 94(2):490-499. doi: 10.2527/jas.2015-9887

Kim, S.-A., J. Gillespie, and K. Paudel. 2005. The effect of socioeconomic factors on the adoption of best management practices in beef cattle production. Journal of Soil and Water Conservation 60(3):111-120.

Li, X., Y. Wu, L. Zhang, Y. Cao, Y. Li, J. Li, L. Zhu, and G. Wu. 2014. Comparison of three common DNA concentration measurement methods. Anal Biochem 451:18-24. doi: 10.1016/j.ab.2014.01.016

Macartney, J. E., K. G. Bateman, and C. S. Ribble. 2003. Health performance of feeder calves sold at conventional auctions versus special auctions of vaccinated or

conditioned calves in Ontario. Journal of the American Veterinary Medical Association 223(5):677-683. doi: 10.2460/javma.2003.223.677

Macgregor, S., P. M. Visscher, and G. Montgomery. 2006. Analysis of pooled DNA samples on high density arrays without prior knowledge of differential hybridization rates. Nucleic Acids Res 34(7):e55. doi: 10.1093/nar/gkl136

Macgregor, S., Z. Z. Zhao, A. Henders, M. G. Nicholas, G. W. Montgomery, and P. M. Visscher. 2008. Highly cost-efficient genome-wide association studies using DNA pools and dense SNP arrays. Nucleic Acids Res 36(6):e35. doi: 10.1093/nar/gkm1060

Martin, S., G. Darlington, K. Bateman, and J. Holt. 1988. Undifferentiated bovine respiratory disease (shipping fever): Is it communicable? Preventive Veterinary Medicine 6(1):27-35.

McDaneld, T. G., L. A. Kuehn, M. G. Thomas, W. M. Snelling, T. P. Smith, E. J. Pollak, J. B. Cole, and J. W. Keele. 2014. Genomewide association study of reproductive efficiency in female cattle. J Anim Sci 92(5):1945-1957. doi: 10.2527/jas.2012-6807

Michelmore, R. W., I. Paran, and R. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proceedings of the national academy of sciences 88(21):9828-9832.

Miles, D. G. 2009. Overview of the North American beef cattle industry and the incidence of bovine respiratory disease (BRD). Animal Health Research Reviews 10(2):101-103.

Muggli-Cockett, N., L. Cundiff, and K. Gregory. 1992. Genetic analysis of bovine respiratory disease in beef calves during the first year of life. Journal of animal science 70(7):2013-2019.

Mulvey, M. E. 2009. Characteristics of Under-prepared Students: Who Are" The Under-prepared"? Research and Teaching in Developmental Education 25(2):29-58.

Murray, G. M., S. J. More, D. Sammin, M. J. Casey, M. C. McElroy, R. G. O'neill, W. J. Byrne, B. Earley, T. A. Clegg, H. Ball, C. J. Bell, and J. P. Cassidy. 2017. Pathogens, patterns of pneumonia, and epidemiologic risk factors associated with respiratory disease in recently weaned cattle in Ireland. Journal of Veterinary Diagnostic Investigation 29(1):20-34. doi: 10.1177/1040638716674757

Murray, G. M., R. G. O'Neill, S. J. More, M. C. McElroy, B. Earley, and J. P. Cassidy. 2016. Evolving views on bovine respiratory disease: An appraisal of selected key pathogens - Part 1. Vet J 217:95-102. doi: 10.1016/j.tvjl.2016.09.012

NAHMS. 2011. Feedlot 2011 Part IV: Health and Health Management on U.S. Feedlots with a Capacity of 1,000 or More Head, National Animal Health Monitoring System.

Neibergs, H., J. Neibergs, A. Wojtowicz, J. Taylor, C. Seabury, and J. Womack. 2014. Economic benefits of using genetic selection to reduce the prevalence of bovine respiratory disease complex in beef feedlot cattle. In: Proceedings of the Beef Improvement Federation Annual Meeting and Convention. p 82-86.

Ng, T. F. F., N. O. Kondov, X. Deng, A. Van Eenennaam, H. L. Neibergs, and E. Delwart. 2015. A metagenomics and case-control study to identify viruses associated with bovine respiratory disease. Journal of virology 89(10):5340-5349.

Nystuen, A., P. J. Benke, J. Merren, E. M. Stone, and V. C. Sheffield. 1996. A cerebellar ataxia locus identified by DNA pooling to search for linkage disequilibrium in an isolated population from the Cayman Islands. Human molecular genetics 5(4):525-531.

Oliphint, R. A. 2006. Evaluation of the inter-relationships of temperament, stress responsiveness and immune function in beef calves, Texas A&M University.

Palomba, C. A., and T. W. Banta. 1999. Assessment Essentials: Planning, Implementing, and Improving Assessment in Higher Education. Higher and Adult Education Series. ERIC.

Pearson, J. V., M. J. Huentelman, R. F. Halperin, W. D. Tembe, S. Melquist, N. Homer, M. Brun, S. Szelinger, K. D. Coon, V. L. Zismann, J. A. Webster, T. Beach, S. B. Sando, J. O. Aasly, R. Heun, F. Jessen, H. Kolsch, M. Tsolaki, M. Daniilidou, E. M. Reiman, A. Papassotiropoulos, M. L. Hutton, D. A. Stephan, and D. W. Craig. 2007. Identification of the genetic basis for complex disorders by use of pooling-based genomewide single-nucleotide-polymorphism association studies. Am J Hum Genet 80(1):126-139. doi: 10.1086/510686

Perino, L. J., and M. D. Apley. 1998. Clinical trial design in feedlots. Veterinary Clinics: Food Animal Practice 14(2):343-365.

Pruitt, J. R., J. M. Gillespie, R. F. Nehring, and B. Qushim. 2015. Adoption of Technology, Management Practices, and Production Systems by U.S. Beef Cow-Calf Producers. Journal of Agricultural and Applied Economics 44(2):203-222. doi: 10.1017/s1074070800000274

Rengarajan, K., S. M. Cristol, M. Mehta, and J. M. Nickerson. 2002. Quantifying DNA concentrations using fluorometry: A comparison of fluorophores. Molecular Vision 8(43):416-421.

Rose-Dye, T., L. Burciaga-Robles, C. Krehbiel, D. Step, R. Fulton, A. Confer, and C. Richards. 2011. Rumen temperature change monitored with remote rumen temperature boluses after challenges with bovine viral diarrhea virus and Mannheimia haemolytica. Journal of animal science 89(4):1193-1200.

Sanderson, M. W., D. A. Dargatz, and B. A. Wagner. 2008. Risk factors for initial respiratory disease in United States' feedlots based on producer-collected daily morbidity counts. The Canadian Veterinary Journal 49(4):373.

Schaefer, A., N. Cook, J. Church, J. Basarab, B. Perry, C. Miller, and A. Tong. 2007. The use of infrared thermography as an early indicator of bovine respiratory disease complex in calves. Research in Veterinary Science 83(3):376-384.

Schneider, M., R. Tait Jr, W. Busby, and J. Reecy. 2009a. An evaluation of bovine respiratory disease complex in feedlot cattle: Impact on performance and carcass traits using treatment records and lung lesion scores. Journal of animal science 87(5):1821-1827.

Schneider, M., R. Tait, W. Busby, and J. Reecy. 2009b. An evaluation of bovine respiratory disease complex in feedlot cattle: Impact on performance and carcass traits using treatment records and lung lesion scores1,2. Journal of Animal Science 87(5):1821-1827. doi: 10.2527/jas.2008-1283

Sham, P., J. S. Bader, I. Craig, M. O'Donovan, and M. Owen. 2002. DNA Pooling: a tool for large-scale association studies. Nat Rev Genet 3(11):862-871. doi: 10.1038/nrg930

Sheffield, V. C., M. E. Pierpont, D. Nishimura, J. S. Beck, T. L. Burns, M. A. Berg, E. M. Stone, S. R. Patil, and R. M. Lauer. 1997. Identification of a complex congenital heart defect susceptibility locus by using DNA pooling and shared segment analysis. Human molecular genetics 6(1):117-121.

Simpson-Beck, V. 2011. Assessing classroom assessment techniques. Active Learning in Higher Education 12(2):125-132.

Singer, V. L., L. J. Jones, S. T. Yue, and R. P. Haugland. 1997. Characterization of PicoGreen reagent and development of a fluorescence-based solution assay for double-stranded DNA quantitation. Anal Biochem 249doi: 10.1006/abio.1997.2177

Smith, R. 1998. Impact of disease on feedlot performance: A review. Journal of Animal Science 76(1):272-274. doi: 10.2527/1998.761272x

Snowder, G., L. D. Van Vleck, L. Cundiff, and G. Bennett. 2005. Influence of breed, heterozygosity, and disease incidence on estimates of variance components of respiratory disease in preweaned beef calves. Journal of animal science 83(6):1247-1261.

Snowder, G., L. D. Van Vleck, L. Cundiff, and G. Bennett. 2006. Bovine respiratory disease in feedlot cattle: environmental, genetic, and economic factors. Journal of animal science 84(8):1999-2008.

Steadman, M. 1998. Using classroom assessment to change both teaching and learning. New Directions for Teaching and Learning 1998(75):23-35.

Strillacci, M. G., E. Frigo, F. Schiavini, A. B. Samoré, F. Canavesi, M. Vevey, M. C. Cozzi, M. Soller, E. Lipkin, and A. Bagnato. 2014. Genome-wide association study for somatic cell score in Valdostana Red Pied cattle breed using pooled DNA. BMC genetics 15(1):106.

Taylor, J. D., R. W. Fulton, T. Lehenbauer, D. L. Step, and A. Confer. 2010a. The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors? Can. Vet. J.-Rev. Vet. Can. 51(10):1095-1102.

Taylor, J. D., R. W. Fulton, T. Lehenbauer, D. L. Step, and A. Confer. 2010b. The epidemiology of bovine respiratory disease: What is the evidence for preventive measures? Can. Vet. J.-Rev. Vet. Can. 51(12):1351-1359.

Thompson, P., A. Stone, and W. Schultheiss. 2006. Use of treatment records and lung lesion scoring to estimate the effect of respiratory disease on growth during early and late finishing periods in South African feedlot cattle. Journal of animal science 84(2):488-498.

Timsit, E., M. Workentine, A. B. Schryvers, D. B. Holman, F. van Der Meer, and T. W. Alexander. 2016a. Evolution of the nasopharyngeal microbiota of beef cattle from weaning to 40 days after arrival at a feedlot. Veterinary Microbiology 187:75-81. doi: 10.1016/j.vetmic.2016.03.020

Timsit, E., M. Workentine, A. B. Schryvers, D. B. Holman, F. van der Meer, and T. W. Alexander. 2016b. Evolution of the nasopharyngeal microbiota of beef cattle from weaning to 40 days after arrival at a feedlot. Vet Microbiol 187:75-81. doi: 10.1016/j.vetmic.2016.03.020

Timsit, E., M. Workentine, F. van der Meer, and T. Alexander. 2018. Distinct bacterial metacommunities inhabit the upper and lower respiratory tracts of healthy feedlot cattle and those diagnosed with bronchopneumonia. Vet Microbiol 221:105-113. doi: 10.1016/j.vetmic.2018.06.007

Urban-Chmiel, R., and D. Grooms. 2012. Prevention and control of bovine respiratory disease. Journal of Livestock Science 3:27-36.

USDA-APHIS. 2000. Attitudes Towards Pre-Arrival Processing in U.S. Feedlots., USDA-APHIS.

USDA. 2008. Beef 2007-2008. PART IV: reference of beef cow-calf managment practices in the United States, National Animal Health Monitoring System.

Wang, M., L. G. Schneider, K. J. Hubbard, D. M. Grotelueschen, R. F. Daly, G. S. Stokka, and D. R. Smith. 2018a. Beef producer survey of the cost to prevent and treat bovine respiratory disease in preweaned calves. J Am Vet Med Assoc 253(5):617-623. doi: 10.2460/javma.253.5.617

Wang, M., L. G. Schneider, K. J. Hubbard, and D. R. Smith. 2018b. Cost of bovine respiratory disease in preweaned calves on US beef cow–calf operations (2011–2015). Journal of the American Veterinary Medical Association 253(5):624-631.

Ward, C. E., M. K. Vestal, D. G. Doye, and D. L. Lalman. 2008. Factors affecting adoption of cow-calf production practices in Oklahoma.

Wittum, T., N. Woollen, L. Perino, and E. Littledike. 1996. Relationships among treatment for respiratory tract disease, pulmonary lesions evident at slaughter, and

rate of weight gain in feedlot cattle. Journal of the American Veterinary Medical Association 209(4):814-818.

Yu, S., Y. Wang, X. Li, F. Yu, and W. Li. 2017. The factors affecting the reproducibility of micro-volume DNA mass quantification in Nanodrop 2000 spectrophotometer. Optik 145:555-560. doi: 10.1016/j.ijleo.2017.08.031

Zeineldin, M., J. Lowe, M. de Godoy, N. Maradiaga, C. Ramirez, M. Ghanem, Y. Abd El-Raof, and B. Aldridge. 2017. Disparity in the nasopharyngeal microbiota between healthy cattle on feed, at entry processing and with respiratory disease. Vet Microbiol 208:30-37. doi: 10.1016/j.vetmic.2017.07.006

Table 1.1. Description and application of commonly use classroom assessment techniques.

Name	Description	Purpose
Minute paper	Ask students to answer: "What is the most important point you learned today?"; and, "What point remains least clear to you?". The purpose is to elicit data about students' comprehension of a particular class session.	Review responses and note any useful comments. During the next class periods emphasize the issues illuminated by your students' comments.
Background Knowledge Probe	Short, simple questionnaires prepared by instructors for use at the beginning of a course or at the start of new unitS.	Can serve as a pretest and guide direction of depth of coverage on a topic.
Memory matrix	Students fill in cells of a two-dimensional diagram for which instructor has provided labels.	Demonstration of student's ability to remember and classify key concepts.
Directed paraphrasing	Ask students to write a layman's "translation" of something they have just learned -- geared to a specified individual or audience.	Assess student's ability to comprehend and transfer concepts.
One-sentence summary	Students summarize knowledge of a topic by constructing a single sentence that answers the questions "Who does what to whom, when, where, how, and why?"	The purpose is to require students to select only the defining features of an idea.
Teacher-designed Feedback Forms	Students respond to specific questions through a focused feedback form about the effectiveness of a particular class session.	Can be adapted to meet specific needs or goals for a course.

CHAPTER 2: Evaluating Microbial Communities Associated with Bovine Respiratory Disease Prior to Weaning

ABSTRACT

The microbiome of the nasal cavity has been associated with disease incidence in cattle. Therefore, an understanding of interactions of respiratory pathogens in the upper respiratory tract would provide important information on the role of these pathogens on the incidence of respiratory disease in cattle. This study aimeds to characterize bacterial papulations associated with pre-weaning calves during two outbreaks of bovine respiratory disease (**BRD**). Nasal swabs from the upper nasal cavity were collected at the time of the outbreaks of BRD that occurred 1 and 2 weeks prior to preconditioning (approximately 13 days of age). To evaluate and compare the microbiome, the variable region (approximately 600bp; variable regions 1-3) along the 16s ribosomal RNA gene was amplified by polymerase chain reaction (**PCR**). This amplified product was then sequenced using next generation sequencing (Illumina MiSeq) and sequence reads were processed and compared to the GreenGenes data base in MICCA to identify bacterial taxa present. Overall, bacteria profile differed ($P < 0.002$) between calves during the BRD outbreak and calves sampled prior to and after the outbreak. Calves treated for BRD had less diverse microbial communities compared to healthy calves. There was an increase ($P < 0.0001$) in abundance of bacteria belonging to *Proteobacteia, Firmicutes, Bacteroides,* and *Fusobacteria* phyla were enriched among BRD affected animals.

Analysis of microbial community dynamics prior to weaning will provide insight into the relationship of microbial profiles in animals that are associated with the development of respiratory disease.

INTRODUCTION

Bovine respiratory disease (**BRD**) complex is the most prominent disease in the cattle industry. Despite improvements in vaccination and management practices, BRD continues to be the leading cause of morbidity and mortality in the feedlot. It has been estimated that economic loss due to BRD is over $1 billion annually and upwards of $3 billion annually when considering the combined cost of prevention and treatment (Griffin, 1997; Miles, 2009). The multifaceted nature of BRD creates challenges for prevention, diagnosis, and treatment of this disease. The occurrence of BRD is dependent on complex interactions between host, pathogens, and environmental factors (Duff and Galyean, 2007; Edwards, 2010). Included in these factors are age, breed, weight, dramatic changes in temperature, humidity, and management practices that increase stress (weaning, transportation, handling, dehorning, castration, comingling, nutritional changes, etc.) (Muggli-Cockett et al., 1992; Dixit et al., 2001; Callan and Garry, 2002; Snowder et al., 2005, 2006).

Bovine respiratory disease develops after the occurrence of a primary viral infection or stress event, followed by a secondary bacterial infection and clinical signs of BRD (Ellis, 2009; Hay et al., 2016). The initial viral infection or stress event reduces host defenses, resulting in immunosuppression and damage to the respiratory epithelial and, leading to dysbiosis of the nasopharyngeal microbial communities (Caswell, 2014). The

most common bacterial pathogens associated with BRD are *Mannheimia haemolytica* (formally *Pasteurella haemolytica), Pasteurella multocida, Haemophilus somnus, Mycoplasma spp,* and *Chlamydia spp* (Fulton, 2009). Many bacterial species associated with BRD are considered common inhabitants of the bovine respiratory tract in healthy animals (Timsit et al., 2016a; Zeineldin et al., 2017). It is likely that diverse microbial communities in healthy animals have the ability to suppress pathogenic bacterial from colonizing in the bovine respiratory tract, but when this symbiosis is disrupted, the opportunistic pathogenic bacteria are able to proliferate (Holman et al., 2015a; Timsit et al., 2016a; Timsit et al., 2018).

Research surrounding bacterial pathogens associated with BRD has primarily focused on outbreak associated with the feedlot period after weaning (Holman et al., 2015a; Timsit et al., 2016a; Zeineldin et al., 2017; McMullen et al., 2018; Timsit et al., 2018). While BRD is most prevalent during the feedlot period, animals may be predisposed to BRD based on bacterial inhabitants of the microbiome of the upper nasal cavity. Therefore, characterizing the upper nasal microbiome during a BRD outbreak pre-weaning could provide insight into the role of microbial diversity and have implications at feedlot entry and provide a greater understanding of pathogenic bacterial interactions prior to the development of BRD. This study aims to characterize the nasal microbiome of calves at the U.S. Meat Animal Research Center (**USMARC**) during a pre-weaning BRD outbreak by specifically comparing microbial characteristics prior to and after the BRD outbreak.

MATERIALS AND METHODS

Animal Population. All animal use was approved by the U.S. Meat Animal Research Center Animal Care and Use Committee. Data were collected in 2016 from cattle in the USMARC GPE herd (Germplasm Evaluation Program; Schiermiester et al., 2015), Clay Center, Nebraska. This particular GPE subset of approximately 800 animals each year, was a product of multiple-sire matings of crossbred cows to F1 bulls of varying breed composition. The cattle used within this study consisted of a variable fraction of 18 breeds: Angus, Hereford, Red Angus, Brahman, Charolais, Gelbvieh, Limousin, Simmental, Brangus, Beefmaster, Shorthorn, Maine Anjou, Santa Gertrudis, Chiangus, Salers, Braunvieh, South Devon, and Tarentaise. For 2016, approximately 800 animals were evaluated that originated from and were managed in separate locations (location 1, location 2 and location 3) at USMARC. These calves were raised under similar management conditions, receiving standardized vaccinations and diets as described by Workman et al. (2019). All claves received an initial vaccination of Bovi-Shield Gold One Shot (Zoetis) and Vision 7 with Spur (Merck) followed by a booster of booster Bovi-Shield Gold One Shot (Zoetis) and Vision 8 (Merck) during preconditioning processing. Calves at any one location never had direct contact with calves at the other locations until weaning. In 2016, locations 1, 2, and 3 included 376, 256, and 162 calves, respectively. Animals treated for BRD were at location 2, and those results are presented and discussed herein. Animals at location 2 were separated into 3 breeding groups, housed in separate pastures. Calves in one breeding group never had direct contact with calves in another breeding group until weaning.

Nasal Swab Collection. Nasal swab samples were collected from the upper nasal cavity of calves using 6-inch nasal swabs at the time of the outbreak. Samples were also collected at all three locations at initial vaccination (approximately 40 d of age), preconditioning (approximately 130 d of age), and weaning (approximately 150 d of age) as described by McDaneld et al. (2018). Briefly, the 6-inch nasal swab was gently inserted into the nasal cavity at an approximate depth of 6 in. The nasal swab was than rotated and removed. After collection of the sample, all swabs were placed in buffered peptone water with 12% glycerol, drop frozen in liquid nitrogen directly after collection and stored at -80^{0}C.

BRD Outbreak. At approximately 51 d of age, calves in one breeding group (n=93 study calves) were mass treated for BRD following the observation by the attending veterinarian that approximately 15-20% of the calves were displaying clinical signs of BRD, including: cough, nasal discharge, increased respiratory rate, lethargy, and anorexia. Calves were individually restrained in a squeeze chute for sample collection (as described above) and treatment (Draxxin (tulathromycin; macrolide), Zoetis) then returned to their pasture. At approximately 58 d of age, a second breeding group (n=142 study calves) was mass treated with a different antibiotic (Zuprevo (tildipirosin; macrolide), Merck) following the observation that 25-30% of the calves in that pasture were displaying clinical signs of BRD. They were similarly sampled, treated, and returned to their pasture. All treatment decisions were made by the attending veterinarians and carried out according to SOP.

DNA Extraction and Library Preparation. Total DNA was extracted from each swab using a commercial kit (PowerSoil DNA Isolation Kit; MoBio, Carlsbad, CA, USA) following the manufacturer's instructions. Swabs were thawed and placed into a new 2mL microfuge tube along with 350µL of the freezing solution and 650µL buffered peptone water (**BPW**). Extracted DNA samples were then quantified by the DeNovix DS-11 FX+ spectrophotometer (DeNovix Inc., Wilmington, DE, USA). Equal amounts of DNA from each swab were then pooled based on rectal temperature (Table 2.1). Amplicon library preparation was performed by PCR amplification of the V1–V3 region of 16S rRNA gene, using modified universal primers 27F (5'- Adapter / Index / AGAGTTTGATCCTGGCTCAG) and 519R (5' Adapter / Index / GTATTACCGCGGCTGCTG) including TruSeq adapter sequences and indices, as well as AccuPrime Taq high fidelity DNA Polymerase (Life Technologies, Carlsbad, CA). Amplification consisted of 20 cycles, with an annealing temperature of 58° C. Products were purified using AmPure bead purification (Agencourt, Beverly, MA) and all libraries were quantified by the NEBNext Library Quant Kit (New Egland BioLabs, Ipswich, MA, USA). The PCR amplicon libraries were sequenced using the 2x300, v3 600-cycle kit and the Illumina MiSeq sequencing platform (Illumina, San Diego, CA).

Sequence Processing. Reads were pre-processed using the MICCA pipeline (v. 1.7.2) (Albanese et al., 2015). All read files were merged, primers trimmed, and quality filtering applied using a maximum error rate of 75%. De novo sequence clustering, chimera filtering and taxonomy assignment were performed by micca-otu-de novo (parameters -s 0.97 -c). Operational taxonomic units (**OTU**s) were assigned by clustering the sequences with a threshold of 97% pair-wise identity, and their representative

sequences were classified using 97% similarity against the Greengenes database (DeSantis et al., 2006).

Statistical analysis. All statistical analysis was done in R 3.6.0 (Core Team, 2019) primarily using the phyloseq 1.3.2 (McMurdie and Holmes, 2013), vegan 2.5-5 (Oksanen et al., 2010), and DESeq2 1.24.0 (Love et al., 2014) packages. Plots were created with ggplot2 3.2.0 (Wickham, 2016). Alpha diversity was assessed with the Shannon Diversity Index. Group means were compared using the Wilcoxon Rank Sum test ($\alpha < 0.05$). A filtered copy of the OTU table was created for further analysis by removing all taxa with < 5 counts for all samples and OTU that were "unclassified" at the phylum level. The filtered file was used to estimate beta diversity by creating a principal coordinates analysis (**PCoA**) based on computed Bray-Curtis distances (Bray and Curtis, 1957). The effects of health status (healthy, moderately sick, or severely sick) and timepoint (initial vaccination, mass treatment, preconditioning, and weaning) on community structure were investigated using a permutational multivariate analysis of variance (PERMANOVA) using the adonis() function of the R package vegan with 9999 permutations. The DEseq2 package was used to normalize counts and estimate differences in taxa abundance for treatment groups and timepoints using a Bonferroni correction and ($\alpha < 0.01$).

RESULTS AND DISCUSSION

Amplification and sequencing of the V1-V3 region of the 16s rRNA gene generated a total of 129,671,64 sequences assigned to 60 sample pools. The mean number of sequences per pool was 216,119 ± 215,434 (range, 685 to 12,967,164).

A total of 40 phyla were observed across all samples. After filtering low count taxa (<5 counts across samples) and unclassified phyla, there were a total of 21 remaining

phyla (Table 2.3). There were 7 phyla with relative abundance > 1%. Tenericutes was the most prominent phylum across sampling days (33.96%), followed by Firmicutes (30.5%), Bacteroidetes (16.2%), proteobacteria (10.7%), actinobacteria (3.1%), Fusobacteria (1.7%), and Acidobacteria (1.3%).

Based on alpha diversity, there were differences in species richness and stability between sampling timepoints ($P < 0.001$) but not between animals that were treated for BRD and those that were not ($P = 0.122$) (Figure 2.2). However, when considering species richness between moderate, severe, and healthy animals, both timepoint and group were significant ($P = 0.011$) (Figure 2.3). Regardless of the group comparison (i.e. treated versus non treated or health status), species richness and stability was lowest when calves were diagnosed with BRD and mass treated. When comparing the two treatment groups, the intra community diversity was lower for group 2 at the initial vaccination, mass treatment, and preconditioning time points compared to the moderate group. The moderately sick group had the greater species diversity at the initial vaccination timepoint followed by a significant decrease in species richness at mass treatment and a return to greater diversity at initial vaccination. Similarly, treatment group two had greater intra community microbial diversity at initial vaccination followed by a significant decline at mass treatment and an increase in diversity by preconditioning. Interestingly, the moderate group had greater species richness at the initial vaccination and preconditioning timepoints compared to the non-treated group. By weaning, there were no differences between beta diversity between treated and non-treated animals.

There was a distinct shift in microbial communities between groups at the different timepoints (Figure 2.4 and 2.5). Based on the PERMANOVA, the treatment

group and timepoint were significant sources of variation, with timepoint accounting for a greater portion of the variation compared to treatment group. When comparing treated to untreated animals over four timepoints, the unadjusted R^2 was 0.038 ($P < 0.002$) for group and 0.276 for timepoint ($P < 0.001$). When considering each treatment group separately (healthy, moderate, severe), the unadjusted R^2 increased to 0.038 ($P < 0.001$). While treatment group, health status, and timepoint were all significant sources of variation, individual sample variation was large for both treatment groups (treatment group $R^2 = 0.686$; health status $R^2 = 0.657$). Significant variability among bacterial communities of individual animals has been previously reported in animals housed in similar environments (Holman et al., 2015a; Zeineldin et al., 2017). This is expected since the upper respiratory tract is continually exposed to many and various bacteria from the surrounding environment (Holman et al., 2015b).

Bacterial communities of the upper nasal tract were less diverse in animals that had been treated for respiratory disease, especially for the more severe cases of BRD. The present study supports a growing body of literature indicating that low diversity or an imbalance in nasal microbial communities is an important risk factor in the development of BRD (Holman et al., 2015a; Timsit et al., 2016a; Zeineldin et al., 2017; Timsit et al., 2018). It is likely that a less diverse bacterial community has lower capability to resist colonization by pathogenic bacteria or resident pathogens (de Steenhuijsen Piters et al., 2015).

Differences in taxa abundance for treatment groups over time are reported in Table 2.6 - Table 2.9. There was a significant difference in microbial abundance between treatment groups at the initial vaccination timepoint for 7 taxa, with 4 of these increasing

in abundance and three decreased in animals treated for BRD. Taxa with significant differences in abundance at the preconditioning timepoint largely increased in abundance compared to non-treated animals, with only one taxa having lower abundance. At weaning, animals previously treated in response to BRD had a decrease in abundance of 11 taxa and increase in 6 taxa compared to non-treated animals. Overall, bacteria belonging to *Proteobacteia, Firmicutes, Bacteroides,* and *Fusobacteria* phyla were enriched in BRD affected animals in the present study. *Proteobacteia, Firmicutes, Bacteroides,* and *Fusobacteria* are commonly associated with the occurrence of BRD (Caswell, 2014; Holman et al., 2015a; Timsit et al., 2016a; Zeineldin et al., 2017).

While previous research associated with bacterial pathogens has primarily focused on a small number of causative pathogens, it is likely that microbial community dynamics are a major risk factor in the development of BRD. Although certain bacteria are considered opportunistic pathogens and are often associated with BRD and morbidity in cattle, they are common inhabitants of the bovine respiratory tract in both healthy and diseased animals (Caswell, 2014; Timsit et al., 2016a; Zeineldin et al., 2017). It is likely that microbial communities play a major role in suppressing the pathogenic bacteria from colonizing in the bovine respiratory tract. Bacterial species belonging to the genus *Lactococcus* have been reported in greater abundance in healthy cattle compared to their BRD affected pen mates (Timsit et al., 2018). Similarly Holman et al. (2015a) found that bacteria belonging to the *Lactobacillaceae* family were absent in BRD affected calves but present in healthy calves at the time of sampling.

CONCLUSION

Previous research characterizing microbial communities associated with BRD has largely focused on the feedlot phase. Less is known regarding microbial community dynamics prior to the feedlot phase and to our knowledge, this is the first study evaluate a large group of calves during a BRD outbreak and timepoints before and after the outbreak. Overall, we were able to demonstrate variation in bacterial communities during a BRD outbreak pre-weaning. There were distinct changes in microbial abundance and diversity of the upper nasal microbiome between healthy and BRD affected animals. Further evaluation of changes in the upper nasal microbiome prior to weaning will improve our understanding of the relationship between the microbiome and the incidence of BRD.

REFERENCES

Albanese, D., P. Fontana, C. De Filippo, D. Cavalieri, and C. Donati. 2015. MICCA: a complete and accurate software for taxonomic profiling of metagenomic data. Scientific reports 5:9743.

Bray, J. R., and J. T. Curtis. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecological monographs 27(4):325-349.

Callan, R. J., and F. B. Garry. 2002. Biosecurity and bovine respiratory disease. The Veterinary Clinics of North America. Food Animal Practice 18(1):57-77.

Caswell, J. 2014. Failure of respiratory defenses in the pathogenesis of bacterial pneumonia of cattle. Veterinary pathology 51(2):393-409.

de Steenhuijsen Piters, W. A., E. A. Sanders, and D. Bogaert. 2015. The role of the local microbial ecosystem in respiratory health and disease. Philosophical Transactions of the Royal Society B: Biological Sciences 370(1675):20140294.

DeSantis, T. Z., P. Hugenholtz, N. Larsen, M. Rojas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu, and G. L. Andersen. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72(7):5069-5072.

Dixit, V., M. Marahrens, and N. Parvizi. 2001. Transport stress modulates adrenocorticotropin secretion from peripheral bovine lymphocytes. Journal of animal science 79(3):729-734.

Duff, G. C., and M. L. Galyean. 2007. Board-invited review: recent advances in management of highly stressed, newly received feedlot cattle. Journal of Animal Science 85(3):823-840.

Edwards, T. A. 2010. Control Methods for Bovine Respiratory Disease for Feedlot Cattle. Veterinary Clinics of North America: Food Animal Practice 26(2):273-284. doi: 10.1016/j.cvfa.2010.03.005

Ellis, J. A. 2009. Update on viral pathogenesis in BRD. Animal health research reviews 10(2):149-153.

Fulton, R. W. 2009. Bovine respiratory disease research (1983–2009). Animal health research reviews 10(2):131-139.

Griffin, D. 1997. Economic impact associated with respiratory disease in beef cattle. Veterinary Clinics: Food Animal Practice 13(3):367-377.

Hay, K. E., J. M. Morton, M. L. Schibrowski, A. C. A. Clements, T. J. Mahony, and T. S. Barnes. 2016. Associations between prior management of cattle and risk of bovine respiratory disease in feedlot cattle. Preventive Veterinary Medicine 127:37-43. doi: 10.1016/j.prevetmed.2016.02.006

Holman, D. B., T. A. McAllister, E. Topp, A.-D. G. Wright, and T. W. Alexander. 2015a. The nasopharyngeal microbiota of feedlot cattle that develop bovine respiratory disease. Veterinary microbiology 180(1-2):90-95.

Holman, D. B., E. Timsit, and T. W. Alexander. 2015b. The nasopharyngeal microbiota of feedlot cattle. Scientific reports 5:15557.

Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biology 15(12):550.

McDaneld, T. G., L. A. Kuehn, and J. W. Keele. 2018. Evaluating the microbiome of two sampling locations in the nasal cavity of cattle with bovine respiratory disease complex (BRDC). Journal of animal science 96(4):1281-1287.

McMullen, C., K. Orsel, T. Alexander, F. van Der Meer, G. Plastow, and E. Timsit. 2018. The evolution of the nasopharyngeal bacterial community in beef steer calves from spring processing to 40 days after arrival at the feedlot. Journal of Animal Science 96:171-171.

McMurdie, P. J., and S. Holmes. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS one 8(4):e61217.

Miles, D. G. 2009. Overview of the North American beef cattle industry and the incidence of bovine respiratory disease (BRD). Animal Health Research Reviews 10(2):101-103.

Muggli-Cockett, N., L. Cundiff, and K. Gregory. 1992. Genetic analysis of bovine respiratory disease in beef calves during the first year of life. Journal of animal science 70(7):2013-2019.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, R. O'hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2010. Vegan: community ecology package. R package version 1.17-4. http://cran. r-project. org>. Acesso em 23:2010.

Schiermiester, L. N., R. Thallman, L. Kuehn, S. D. Kachman, and M. L. Spangler. 2015. Estimation of breed-specific heterosis effects for birth, weaning, and yearling weight in cattle. Journal of animal science 93(1):46-52.

Snowder, G., L. D. Van Vleck, L. Cundiff, and G. Bennett. 2005. Influence of breed, heterozygosity, and disease incidence on estimates of variance components of respiratory disease in preweaned beef calves. Journal of animal science 83(6):1247-1261.

Snowder, G., L. D. Van Vleck, L. Cundiff, and G. Bennett. 2006. Bovine respiratory disease in feedlot cattle: environmental, genetic, and economic factors. Journal of animal science 84(8):1999-2008.

Team, R. C. 2019. R: A language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria.

Timsit, E., M. Workentine, A. B. Schryvers, D. B. Holman, F. van der Meer, and T. W. Alexander. 2016. Evolution of the nasopharyngeal microbiota of beef cattle from weaning to 40 days after arrival at a feedlot. Vet Microbiol 187:75-81. doi: 10.1016/j.vetmic.2016.03.020

Timsit, E., M. Workentine, F. van der Meer, and T. Alexander. 2018. Distinct bacterial metacommunities inhabit the upper and lower respiratory tracts of healthy feedlot cattle and those diagnosed with bronchopneumonia. Vet Microbiol 221:105-113. doi: 10.1016/j.vetmic.2018.06.007

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. Springer.

Zeineldin, M., J. Lowe, M. de Godoy, N. Maradiaga, C. Ramirez, M. Ghanem, Y. Abd El-Raof, and B. Aldridge. 2017. Disparity in the nasopharyngeal microbiota between healthy cattle on feed, at entry processing and with respiratory disease. Vet Microbiol 208:30-37. doi: 10.1016/j.vetmic.2017.07.006

Table 2.1. Rectal temperature scoring system used to assign animals to pools.

Temperature Score	**Number of animals per score**
Treatment Group 1: Moderate	
3 > or = 103ºF	n = 22
2 = 102-102.9 ºF	n = 56
1 = 101-101.9 ºF	n = 13
0 = 100-100.9 ºF	n = 2
Treatment Group 2: Severe	
3 > or = 103 ºF	n=62
2 = 102-102.9 ºF	n=48
1 = 101-101.9 ºF	n=25
0 = 100-100.9 ºF	n=6

Table 2.2. Timeline of nasal swab sample collection and the average age (days, d) of calves sampled for 2016. Calves sampled in outbreak 1 were mass treated for BRD following the observation that approximately 15-20% of the calves were displaying clinical signs of BRD. Calves included in outbreak 2 were mass treated for BRD following the observation that approximately 25-30% of the calves were displaying clinical signs of BRD.

Year	Number of Animals	Initial vaccination (Prebreeding)	Outbreak 1	Outbreak 2	Preconditioning	Weaning
2016	794	36.6 d	50.6 d	57.6 d	124.2 d	145.3 d

Table 2.3. Relative abundance by phylum across all samples after filtering low count (< 5) and unclassified phylum.

Phylum	Mean Relative Abundance
Tenericutes	33.96%
Firmicutes	30.52%
Bacteroidetes	16.17%
Proteobacteria	10.65%
Actinobacteria	3.06%
Fusobacteria	1.73%
Acidobacteria	1.32%
Chloroflexi	0.95%
Cyanobacteria	0.44%
Gemmatimonadetes	0.31%
Planctomycetes	0.21%
Verrucomicrobia	0.16%
TM7	0.15%
Lentisphaerae	0.15%
Spirochaetes	0.05%
[Thermi]	0.05%
Fibrobacteres	0.03%
Nitrospirae	0.02%
Armatimonadetes	0.02%
GN02	0.02%
Chlorobi	0.01%
Elusimicrobia	0.01%

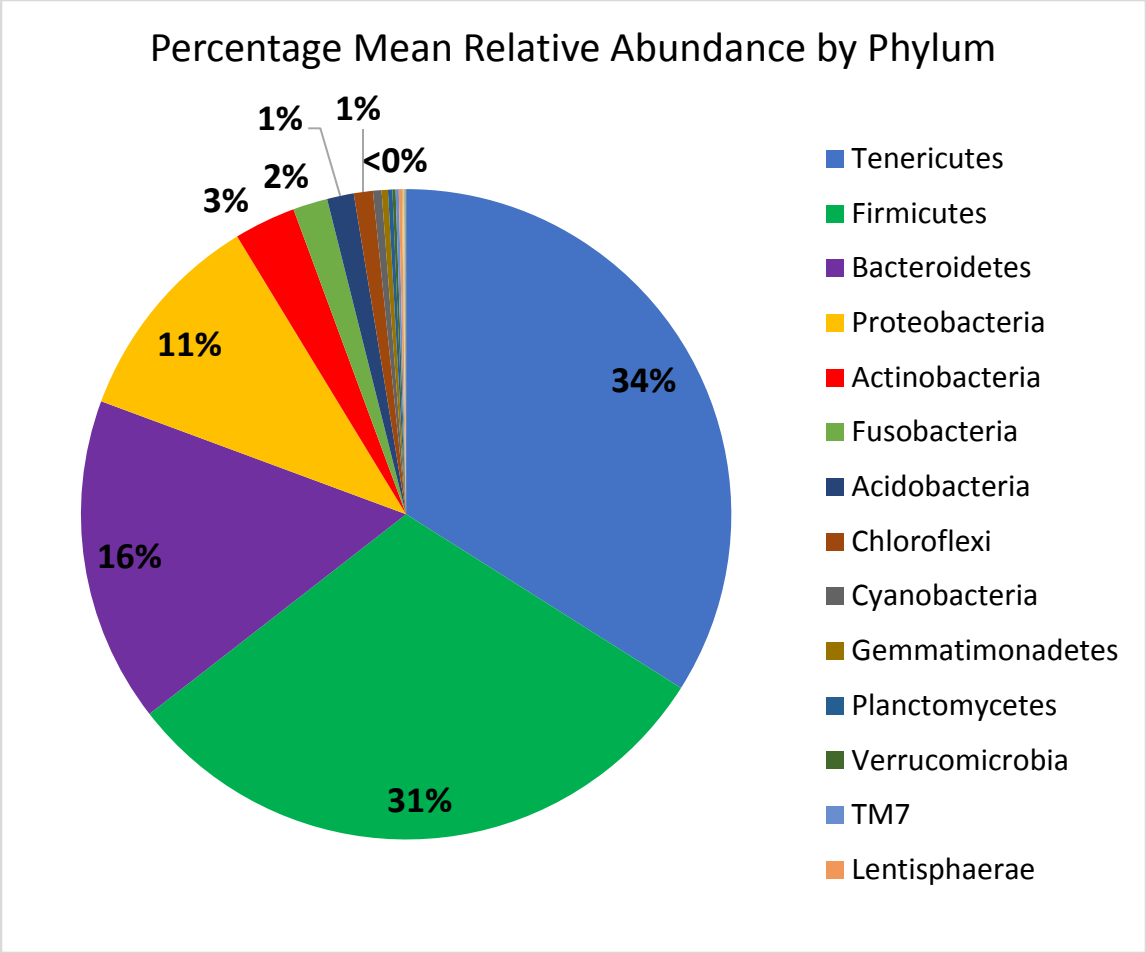

Figure 2.1. Mean relative abundance by phylum across all samples after filtering low count (< 5) and unclassified phylum.

Table 2.4. Alpha diversity by Shannon index comparing treatment group (treated for bovine respiratory disease (BRD) vs non-treated), health status (healthy, moderate BRD outbreak, and severe BRD outbreak groups), and timepoint.

	Df	Sum Sq	Mean Sq	F value	*P*-values
Treatment group	1	7.895	7.895	2.473	0.122
Residuals	58	185.172	3.193		
Health Status	2	20.5179	20.518	6.897	0.011
Residuals	57	172.548	2.975		
Timepoint	3	67.3017	67.302	31.038	<0.0001
Residuals	56	125.765	2.1684		

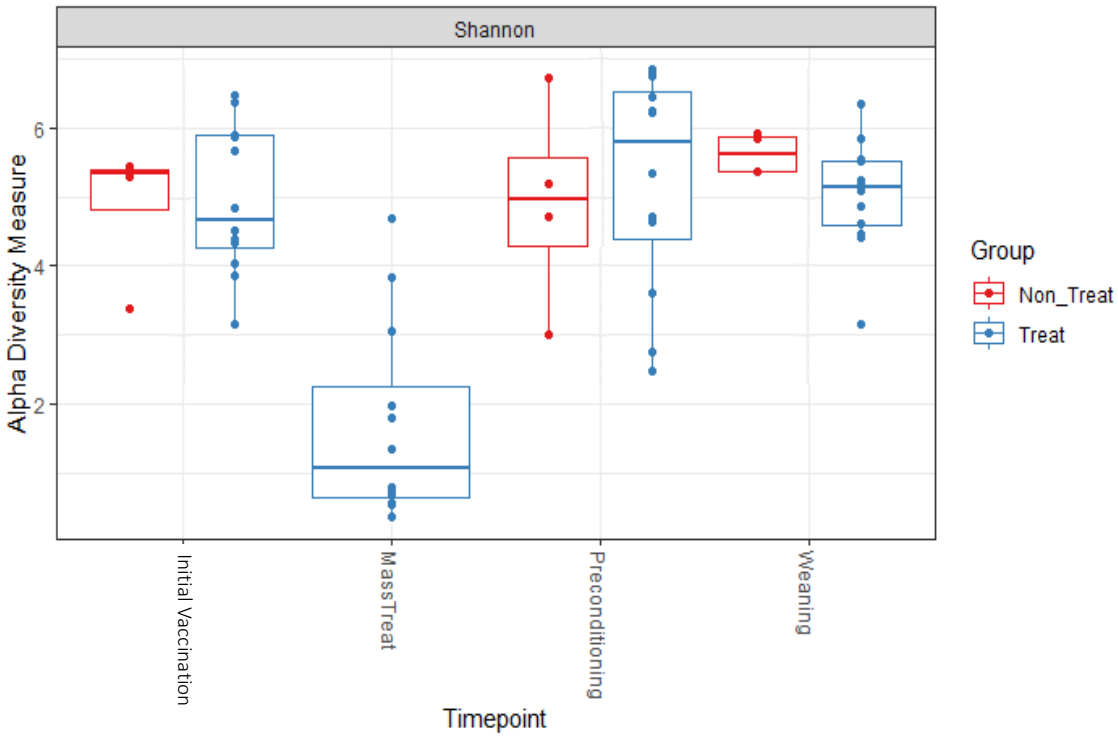

Figure 2.2. Boxplot of alpha diversity by Shannon index comparing treatment group (treated for bovine respiratory disease (BRD) vs non-treated) at each timepoint.

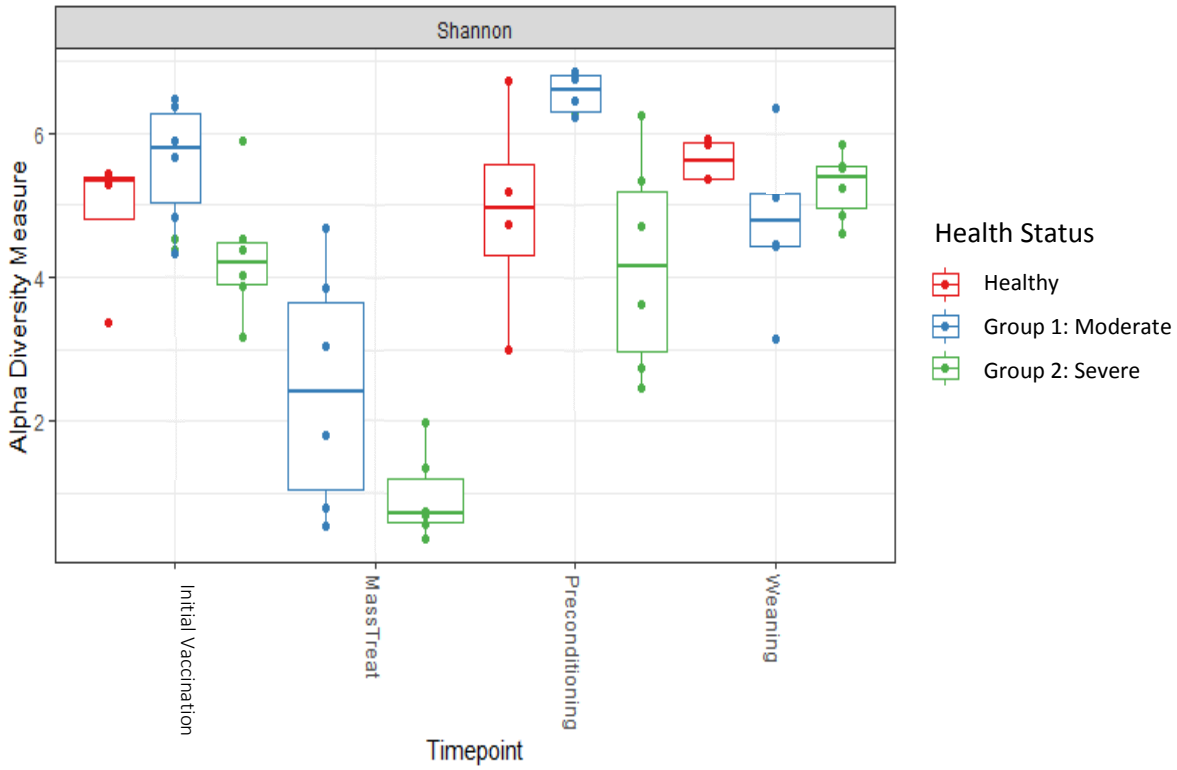

Figure 2.3. Boxplot of alpha diversity by Shannon index comparing health status (healthy, moderate BRD outbreak, and severe BRD outbreak groups) at each timepoint.

Table 2.5. Beta Diversity calculated using a permutational multivariate analysis of variance (PERMANOVA). Adonis model includes the terms included in the PERMANOVA estimate for treatment group (treated for bovine respiratory disease (BRD) vs non-treated), health status (healthy, moderate BRD outbreak, and severe BRD outbreak groups), and timepoint (Initial Vaccination, BRD outbreak, Preconditioning, and Weaning).

Adonis model	Term	df	*F*-value	R^2 value	% variation attributed to term	*P*-value
TreatmentGroup + Timepoint	Treatment Group	1	3.027	0.0378	3.78%	0.002
TreatmentGroup + Timepoint	Timepoint	3	7.374	0.276	27.6%	<0.001
TreatmentGroup + Timepoint	Residuals	55		0.687	68.7%	
HealthStatus + Timepoint	Health Status	2	2.734	0.067	6.67%	<0.001
HealthStatus + Timepoint	Timepoint	3	7.558	0.277	27.6%	<0.001
HealthStatus + Timepoint	Residuals	54		0.657	65.7%	

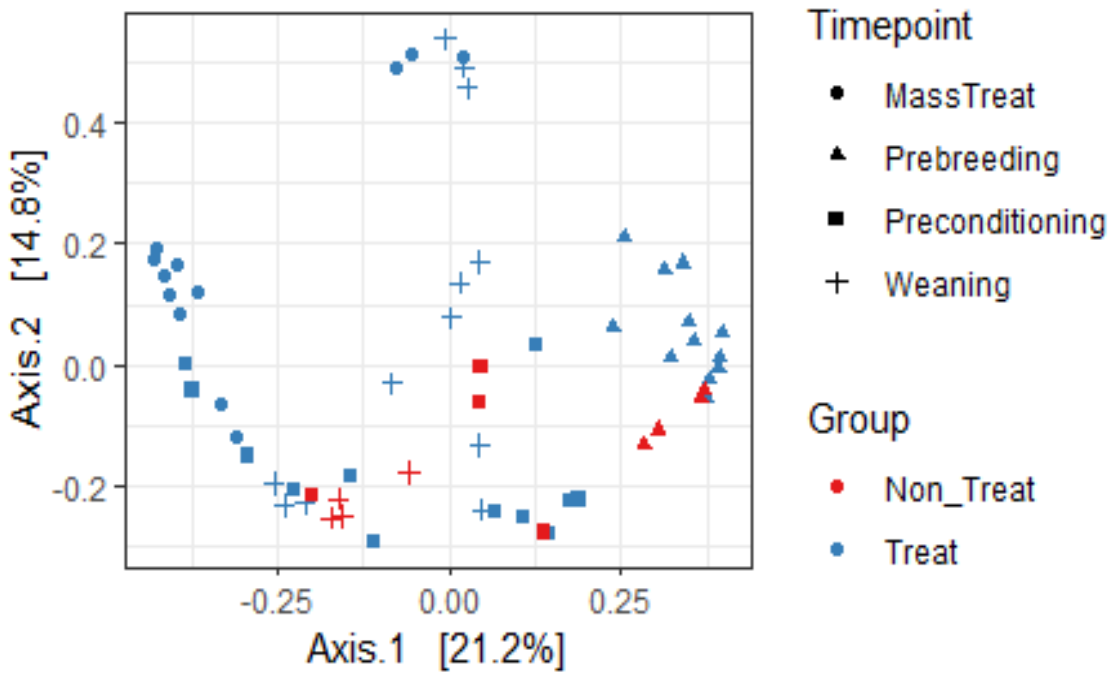

Figure 2.4. Principal coordinates analysis (PCoA) based on computed Bray-Curtis distances comparing treatment group (treated for bovine respiratory disease vs non-treated) and timepoint.

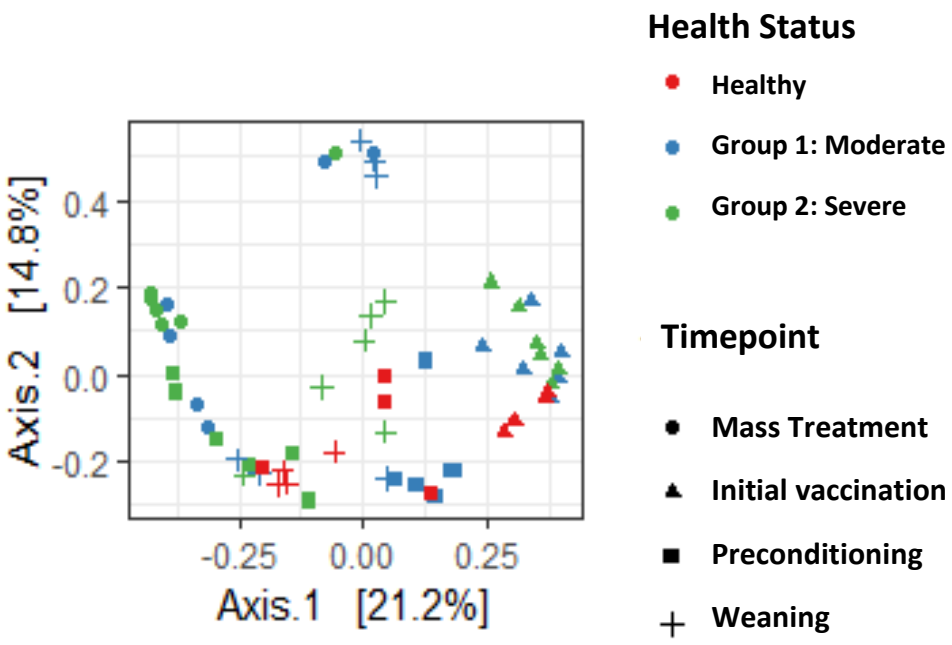

Figure 2.5. Principal coordinates analysis (PCoA) based on computed Bray-Curtis distances comparing timepoint and health status (treated for moderate bovine respiratory disease outbreak (BRD), treated for severe BRD outbreak, vs non-treated healthy animals).

Table 2.6. Taxa with significantly different abundances in animals treated for bovine respiratory disease compared to animals that were not at the initial vaccination timepoint.

Order	Family	Genus	Base Mean	log2Fold Change	*P*-value[1]
Increase Abundance					
Flavobacteriales	Weeksellaceae	Chryseobacterium	11.727	24.01	<0.0001
Pasteurellales	Pasteurellaceae	Pasteurella	1533.32	20.90	<0.0001
Neisseriales	Neisseriaceae	Kingella	41.87	19.54	<0.0001
Clostridiales	Lachnospiraceae	Unclassified	11.95	5.83	0.0025
Decrease Abundance					
Bacteroidales	Prevotellaceae	Prevotella	42.46	-17.84	<0.0001
Lactobacillales	Aerococcaceae	Unclassified	6.87	-12.40	0.0013
Bacteroidales	Rikenellaceae	Unclassified	47.51	-11.64	<0.0001

[1]*P*-value determined using a Bonferroni correction and ($\alpha < 0.01$)

Table 2.7. Taxa with significantly different abundances in animals treated for bovine respiratory disease compared to animals that were not at the preconditioning timepoint.

Order	Family	Genus	Base Mean	log2 Fold Change	*P*-value[1]
Increase Abundance					
Flavobacteriales	Weeksellaceae	Chryseobacterium	11.73	24.28	<0.0001
Bacillales	Planococcaceae	Sporosarci	7.16	21.08	<0.0001
Pasteurellales	Pasteurellaceae	Aggregatibacter	1746.29	16.01	<0.0001
Pasteurellales	Pasteurellaceae	Pasteurella	1533.32	14.23	<0.0001
Pseudomonadales	Moraxellaceae	Moraxella	113.01	11.36	<0.0001
Pseudomonadales	Moraxellaceae	Moraxella	809.85	10.79	<0.0001
Pseudomonadales	Moraxellaceae	Moraxella	136.80	10.27	<0.0001
Pseudomonadales	Moraxellaceae	Moraxella	272.58	10.11	<0.0001
Bacteroidales	Porphyromonadaceae	Porphyromonas	30.70	9.67	<0.0001
Lactobacillales	Streptococcaceae	Streptococcus	1916.71	8.36	<0.0001
Lactobacillales	Unclassified	Unclassified	1273.71	7.76	<0.0001
Pseudomonadales	Moraxellaceae	Moraxella	2882.41	7.39	<0.0001
Decrease Abundance					
JG30-KF-CM45	Unclassified	Unclassified	5.619	-16.411	<0.0001

[1]*P*-value determined using a Bonferroni correction and ($\alpha < 0.01$)

Table 2.8. Taxa with significantly different abundances in animals treated bovine respiratory disease compared to animals that were not at the weaning timepoint.

Order	Family	Genus	Base Mean	log2Fold Change	*P*-value[1]
Increase Abundance					
Fusobacteriales	Fusobacteriaceae	Fusobacterium	34.62	24.99	<0.0001
Flavobacteriales	Weeksellaceae	Chryseobacterium	11.73	23.94	<0.0001
Bacteroidales	Barnesiellaceae	Unclassified	4.90	20.33	<0.0001
Pseudomonadales	Moraxellaceae	Moraxella	113.01	9.18	0.0028
Lactobacillales	Unclassified	Unclassified	1273.71	8.51	<0.0001
Pseudomonadales	Moraxellaceae	Moraxella	136.80	6.91	0.0072
Decrease Abundance					
Bacillales	Planococcaceae	Rummeliibacillus	8.40	-25.56	<0.0001
Erysipelotrichales	Erysipelotrichaceae	Unclassified	31.68	-22.66	<0.0001
SBR1031	A4b	Unclassified	5.13	-21.25	0.0006
Clostridiales	Lachnospiraceae	Coprococcus	40.22	-20.31	<0.0001
Clostridiales	Clostridiaceae	Clostridium	5.18	-20.19	<0.0001
Actinomycetales	Corynebacteriaceae	Corynebacterium	9.16	-19.42	<0.0001
Clostridiales	Ruminococcaceae	Faecalibacterium	95.97	-7.73	0.0028
Clostridiales	Ruminococcaceae	Faecalibacterium	183.56	-7.64	0.0019
Opitutales	Opitutaceae	Opitutus	25.01	-7.24	0.0051
RB41	Ellin6075	Unclassified	28.18	-7.24	0.0030
Actinomycetales	Nocardioidaceae	Unclassified	13.80	-6.50	0.0066

[1]*P*-value determined using a Bonferroni correction and ($\alpha < 0.01$)

Table 2.9. Taxa with significantly different abundances in animals treated for bovine respiratory disease that were severely sick compared to treated animals that were moderately sick.

Order	Family	Genus	Base Mean	log2Fold Change	*P*-value[1]
Increase Abundance					
Lactobacillales	Lactobacillaceae	Lactobacillus	333.97	2.49	0.009
Bacteroidales	Bacteroidaceae	5-7N15	241.78	2.67	0.009
Clostridiales	Lachnospiraceae	Unclassified	157.85	2.97	0.004
Clostridiales	Lachnospiraceae	Unclassified	838.26	3.20	<0.0001
Lactobacillales	Lactobacillaceae	Lactobacillus	1033.12	3.70	<0.0001
Bacteroidales	Rikenellaceae	Unclassified	47.38	5.21	0.007
Clostridiales	Ruminococcaceae	Unclassified	24.63	6.23	0.002
Bacteroidales	Paraprevotellaceae	CF231	152.22	7.19	<0.0001
Clostridiales	Lachnospiraceae	Unclassified	9.95	14.40	<0.0001
Neisseriales	Neisseriaceae	Kingella	41.87	16.37	<0.0001
Erysipelotrichales	Erysipelotrichaceae	Unclassified	31.68	18.14	<0.0001
Decrease Abundance					
Bacteroidales	Rikenellaceae	Unclassified	47.51	-40.85	<0.0001
Streptophyta	Unclassified	Unclassified	14.86	-21.71	<0.0001
Xanthomonadales	Sinobacteraceae	Steroidobacter	13.09	-19.94	<0.0001
Burkholderiales	Comamonadaceae	Methylibium	7.95	-17.14	<0.0001
Bacillales	Planococcaceae	Sporosarcina	7.16	-16.48	<0.0001

[1]*P*-value determined using a Bonferroni correction and ($\alpha < 0.01$)

CHAPTER 3: Evaluating accuracy of DNA pool construction based on white blood cell counts versus two common DNA quantification methods

ABSTRACT

Pooling individual samples prior to DNA extraction can be used to mitigate the cost of genotyping; however, these methods need to accurately generate equal representation of individuals within pools. The objective of this study was to determine accuracy of pool construction based on white blood cell counts compared to two common DNA quantification methods. Ten individual bovine blood samples were collected, and then pooled with two different individuals represented in each pool. Pools were constructed with the target of equal representation of the 2 animals based on number of white blood cells, spectrophotometric readings, spectrofluorometric readings, extracted DNA volume, and whole blood volume for a total of 25 pools. Pools and individual samples that comprised the pools were genotyped using the a commercially available genotyping array. Representation of individuals in each pool was estimated by non-negative least squares on array genotypes. Each pool was tested against all 10 individuals to ensure that only the two individual animals represented in the pool had nonzero representation. Square root of mean square differences (min, max) between observed and expected sample representations were 0.013 (0.008, 0.018) for white blood cell counts, 0.036 (0.016, 0.050) for spectrofluorometric DNA concentration, 0.022 (0.009, 0.044) for spectrophotometric DNA concentration, 0.023 (0.014, 0.042) for whole blood volume, and 0.033 (0.011, 0.062) for DNA volume. White blood cell count was more predictive of sample representation compared to pooling based on DNA concentration. Therefore,

constructing pools using white blood cell counts prior to DNA extraction may reduce cost associated with genotyping and improve representation of individuals in a pool.

INTRODUCTION

Determining the genetic basis of complex traits requires genotyping a large number of individuals in order to achieve replicable findings. While the use of genotyping panels with hundreds of thousands of single nucleotide polymorphisms (**SNPs**) has provided the capability to scan genomic regions for genetic markers associated with a trait or disease, the cost of these studies can be prohibitive. Pooling genomic DNA samples offers a way to substantially reduce the cost of large-scale genotyping studies, providing an economic approach to investigate the genetic bases for many traits and diseases (Macgregor et al., 2008). This approach reduces the overall cost of association studies by replacing individual genotyping with genotyping of pooled genomic DNA and has been successfully applied in multiple case-control association studies (Huang et al., 2010; McDaneld et al., 2014; Strillacci et al., 2014). This approach utilizes allele frequency estimations from pooled DNA samples to identify regions of association that can be targeted for further investigation (Barratt et al., 2002).

Detection of true regions of association using pooled DNA methods is influenced by variance in allele frequency estimates resulting from quantitative errors introduced at different stages of the experimental process (Barratt et al., 2002). One such source of error can occur during DNA quantification and pool construction. Previous research has demonstrated disagreement and inconsistency between prominent DNA quantification methods including spectrofluorometry and spectrophotometry (Holden et al., 2009; Li et

al., 2014; Yu et al., 2017). Spectrofluorometry based quantification using intercalating dye, such as PicoGreen, is highly sensitive, less susceptible to contaminants, and can differentiate intact DNA from degraded DNA (Rengarajan et al., 2002; Li et al., 2014). However, fluorometric methods require costly equipment and reagent kits, require additional time to generate standard and incubate samples, do not provide purity measurements, and often underestimated the DNA concentration of a sample. Spectrophotometry based quantification using ultraviolet absorption is the most popular method because it is relatively simple, inexpensive, and quick to use, does not require a large amount of the sample, and can assess the purity of a sample (Haque et al., 2003; Li et al., 2014; Yu et al., 2017). Despite its advantages, spectrophotometry is sensitive to contaminants in the sample and tends to overestimated DNA concentrations (Li et al., 2014). Because of the differences in abilities and limitations of each approach, selecting the most appropriate method depends on the sample type and desired downstream analysis (Haque et al., 2003; Li et al., 2014). Although previous studies have compared various DNA quantification methods, none have specifically evaluated which method is most appropriate for DNA pooling.

While DNA pooling has made large scale association studies more feasible, pooling samples prior to DNA extraction could further mitigate the cost of genotyping. Craig et al. (2009) demonstrated that pooling whole blood samples prior to DNA extraction substantially reduced the time, cost, and labor required for large-scale genotyping studies. Pooling samples prior to DNA extraction has also been successful using lung tissue samples for the study of liver abscesses and lung lesions in cattle (Keele et al., 2015; Keele et al., 2016). Furthermore, studies evaluating sample pooling prior to

DNA extraction have not reported increased variation in allele frequency, suggesting that pooling samples prior to DNA extraction is a viable method without introducing additional error associated with pooling samples. Blood samples are relatively simple and inexpensive to obtain and are a commonly collected sample in the livestock industry. Because white blood cells contain equal amounts of DNA, and because they are the main source of DNA in whole blood, pooling samples based on equal white blood cells counts should result in an equal contribution from each individual sample DNA within a pool. Furthermore, since individual samples are added to the pool based on white blood cell count rather than DNA concentration, the variation within pools may actually be lower compared to pools constructed from florescence or UV absorption quantification methods. Therefore, the objective of this study was to determine variation in pools constructed based on white blood cell counts compared to the two primary DNA quantification methods.

MATERIALS AND METHODS

Sample Collection. All animal use was approved by the U.S. Meat Animal Research Center (**USMARC)** Institutional Animal Care and Use Committee. Samples were collected from 10 steers at the USMARC feedlot in Clay Center, Nebraska. Blood samples were collected via jugular venipuncture into 9-ml Sarstedt Monovette blood collection tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant (Sarstedt AG & Co., Numbrecht, Germany).

Sample Processing and Pool Construction. Pools were constructed using five different methods, each with two individuals represented in each pool for a total of 25

pools. The five methods of pooling were based on equal amounts of 1) white blood cell count, 2) DNA concentrations determined by spectrophotometer, 3) DNA concentrations determined by spectrofluorometer, 4) extracted DNA based on volume, and 5) whole blood based on volume. The same pairs of animals were used in each of the five pools across all pooling methods.

Pool Construction Based on White Blood Cell Dilutions. Whole blood with ETDA was transferred to a 2-ml screw cap vial and mixed for approximately 10 min prior to white blood cell analysis using an Element HT5 Veterinary Hematology Analyzer (Heska, Loveland, CO, USA). Samples were analyzed in triplicate and values averaged for white blood cell count. Once an average cell count was calculated for each sample, whole blood samples were diluted in phosphate buffer solution (**PBS**) to obtain white blood cell concentrations of 5.0×10^3 in a total of 500 µL. Diluted and whole blood samples were frozen at -20^{o}C prior to DNA extraction and pooling. Equal volumes (100 µL) of diluted blood samples from two individuals were combined in a 1.5 microcentrifuge tube for a total of 5 pools. DNA was then extracted from each of the pools using the QiAamp DNA Mini Kit following the manufacturer's instructions (Qiagen, Santa Clarita, CA, USA). Quality of DNA was evaluated using gel electrophoresis to ensure high molecular weight DNA was present and intact.

Pool Construction Determined by Spectrophotometer. DNA was extracted from individual whole blood samples using previously described methods. Extracted DNA samples were then quantified by the DeNovix DS-11 FX+ spectrophotometer (DeNovix Inc., Wilmington, DE, USA) using 2 µL of sample and the dsDNA setting.

DNA Quantification and Pool Construction Determined by Spectrofluorometer. DNA was extracted from individual whole blood samples using previously described methods. Extracted DNA was quantified using the Quantifluor® dsDNA System following the manufacturer's instructions (Promega, Madison, Wisconsin, USA). Quantification was completed using the DeNovix DS-11 FX+ spectrophotometer sing the Quantifluor dsDNA setting.

Pool Construction Based on DNA Volume. DNA was extracted from individual whole blood samples using previously described methods. Pools were assembled by adding 25µL of extracted DNA from two individuals to a pool for a total of 50 µL per pool. In this case if the true concentration of DNA in the extract samples varied then the representation of animals to the pool would also vary. This is essentially constructing pools as if DNA concentration is constant among samples.

Pool Construction Based on Whole Blood Volume. Whole blood pools were generated by adding 100 µL of whole blood from two individuals to a pool. DNA was then extracted from pools using previously described methods.

Genotyping. All individual animals and pools were genotyped with the Illumina (San Diego, CA) Bovine GGP 50K SNP array by Neogen Corporation (Lincoln, NE, USA)

Statistical Analysis. Pooling allele frequency (**PAF**; Peiris et al., 2011) was computed as $x/(x+y)$, where x is normalized red intensity and y is normalized green intensity. Therefore, PAF is a pooling estimate of the frequency of the A allele. Representation of individuals in each pool was estimated by non-negative least squares using the nnls function (Mullen and van Stokkum, 2012) in R (Version 3.6.0; R Core

Team, 2019). Each pool was tested against all 10 individuals to ensure that only the two individual animals represented in the pool had nonzero representation.

RESULTS

Representation of individual animals within each pool is shown in Figure 1 Each pool represented two different individual animals, with all other individuals being absent. The square root of mean square differences between observed and expected sample representations are shown in Figure 2. Pools constructed from equalized white blood cell counts resulted in the lowest variability within pools compared to all other methods. Pools generated from spectrophotometric quantified DNA samples were less accurate for predicting equal sample representation within pools when compared to pools from equalized white blood cell counts. However, both spectrophotometer and equalized white blood cell counts were more accurate compared to pooling methods based on whole blood volume, DNA volume, and spectrofluorometry.

In comparing equalized white blood cell count to spectrofluorometric and spectrophotometric methods for pooling construction, the square root of mean squared difference was 0.013 with a range of 0.008, 0.018 for pools based on white blood cell count, 0.036 (0.016, 0.050) for spectrofluorometric DNA representation, 0.022 (0.009, 0.044) for spectrophotometric DNA representation, 0.023 (0.014, 0.042) for whole blood volume, and 0.033 (0.011, 0.062) for DNA volume (Table 1).

DISCUSSION

Pooling samples prior to DNA extraction could further mitigate the cost of genotyping if these methods can accurately generate equal representation of individuals within pools. Based on the outcome of this study, white blood cell count is a viable approach for pool construction and was more predictive of sample representation compared to all other pooling methods evaluated. While current DNA quantification methods are adequate to generate pools for genotyping, the pools constructed from equalized white blood cell counts were more likely to have equal sample representation of DNA from each individual compared to pools constructed based on both spectrofluorometric and spectrophotometric DNA quantification methods. Obtaining white blood cell counts via hematology analyzer offers a method to accurately quantify samples for pool construction. The lower variability among pools constructed from diluted blood samples suggest that generating pools based on quantification using the hematology analyzer is more accurate compared to common DNA quantification methods.

Comparison of pools constructed based the DNA quantification methods resulted in spectrofluorometry having greater variability compared to the pools based on spectrophotometry. Pools constructed based on spectrofluorometry also resulted in greater variation compared to pools constructed from equal amount of DNA by volume. It is possible that there were contaminants within the pools or that the DNA extracted for the spectrofluorometry based pools was damaged during the freeze/thaw process prior to DNA extraction. However, as spectrofluorometry is less influenced by contaminants or degraded DNA, this process should have had minimal to no impact on the ability of the

spectrofluorometry method to accurately measure DNA concentrations in the samples (Singer et al., 1997; Li et al., 2014). While the variability of sample representation within spectrofluorometry based pools was somewhat unexpected, a lack of agreement between spectrofluorometry and spectrophotometry methods for measuring DNA concentration has been previously documented (Holden et al., 2009; Li et al., 2014; Yu et al., 2017). Although results from the present study were more favorable for spectrophotometry quantification methods over spectrofluorometer quantification, caution should be taken before concluding that one DNA quantification methods is more accurate for pool construction compared to another, as accuracy of both methods can be influenced by the quality of the DNA present, impurities in the sample, and structure of the DNA (Li et al., 2014). It is possible that utilizing white blood cell counts yields a more equal sample representation within pools because this approach is based on the relative constant DNA content in individual white blood cells and is not sensitive to DNA quality or structure in an extracted sample.

As expected, pools constructed based on whole blood volume or DNA volume resulted in greater variability in individual sample representation compared to all other pooling methods evaluated because white blood cell density varied by sample. A previous study by Craig et al. (2009) demonstrated that pooling whole blood samples by volume was successful in identifying associated genes in a case/control study. However, the authors acknowledged that pooling whole blood by volume would result in unequal sample representation within pools. Therefore, pooling by blood volume may not be an accurate approach when completing genotyping studies for complex traits, especially

disease related traits since variation in individual white blood cell counts would be expected due to immune response.

Pooling samples based on equalized white blood cell counts offers many benefits in terms of reduced cost, labor, and time, there are several challenges that should be considered before utilizing this method. First, the ability to quantify white blood cell concentration prior to freezing samples. Once frozen, the cells will lyse and white blood cell count can no longer be an accurate determinant of DNA concentration. Second, since DNA is not extracted from individual samples, this prohibits further exploration of individual genotypes. However, this is not necessarily a limiting factor since subsequent stages of genotyping studies are often done using a population independent from the discovery study. Furthermore, blood samples that were counted prior to storage can be used at a later time if needed.

CONCLUSION

A major factor limiting the ability to complete large-scale genotyping is the expense, labor, and time required to individually genotype many individuals. DNA pooling methods can mitigate this as pooling requires fewer genotyping arrays to measure allele frequencies in groups of individuals. While DNA pooling is an effective way to reduce the cost of genotyping studies, pooling prior to DNA extraction would further minimize the cost, time, and labor associated with extracting DNA from each individual sample. Pooling blood samples based on equalized white blood cell counts is a viable method to generate pools without compromising accuracy. Since collecting blood is a

relatively simple and minimally invasive means of sampling, pooling based on white blood cell counts offers great potential for future research and commercial applications.

REFERENCES

Barratt, B. J., F. Payne, H. E. Rance, S. Nutland, J. A. Todd, and D. G. Clayton. 2002. Identification of the sources of error in allele frequency estimations from pooled DNA indicates an optimal experimental design. Ann Hum Genet 66(Pt 5-6):393-405. doi: 10.1017/S0003480002001252

Craig, J. E., A. W. Hewitt, A. E. McMellon, A. K. Henders, L. Ma, L. Wallace, S. Sharma, K. P. Burdon, P. M. Visscher, G. W. Montgomery, and S. MacGregor. 2009. Rapid inexpensive genome-wide association using pooled whole blood. Genome Res 19(11):2075-2080. doi: 10.1101/gr.094680.109

Haque, K. A., R. M. Pfeiffer, M. B. Beerman, J. P. Struewing, S. J. Chanock, and A. W. Bergen. 2003. Performance of high-throughput DNA quantification methods. BMC Biotechnology 3(1):20. (journal article) doi: 10.1186/1472-6750-3-20

Holden, M. J., R. J. Haynes, S. A. Rabb, N. Satija, K. Yang, and J. R. Blasic, Jr. 2009. Factors affecting quantification of total DNA by UV spectroscopy and PicoGreen fluorescence. J Agric Food Chem 57(16):7221-7226. doi: 10.1021/jf901165h

Huang, W., B. Kirkpatrick, G. Rosa, and H. Khatib. 2010. A genome□wide association study using selective DNA pooling identifies candidate markers for fertility in Holstein cattle. Animal genetics 41(6):570-578.

Keele, J., L. Kuehn, T. McDaneld, R. Tait, S. Jones, T. Smith, S. Shackelford, D. King, T. Wheeler, A. Lindholm-Perry, and A. McNeel. 2015. Genomewide association study of lung lesions in cattle using sample pooling 1,2. Journal of Animal Science 93(3):956-964. doi: 10.2527/jas.2014-8492

Keele, J. W., L. A. Kuehn, T. G. McDaneld, R. G. Tait, S. A. Jones, B. N. Keel, and W. M. Snelling. 2016. Genomewide association study of liver abscess in beef cattle. J Anim Sci 94(2):490-499. doi: 10.2527/jas.2015-9887

Li, X., Y. Wu, L. Zhang, Y. Cao, Y. Li, J. Li, L. Zhu, and G. Wu. 2014. Comparison of three common DNA concentration measurement methods. Anal Biochem 451:18-24. doi: 10.1016/j.ab.2014.01.016

Macgregor, S., Z. Z. Zhao, A. Henders, M. G. Nicholas, G. W. Montgomery, and P. M. Visscher. 2008. Highly cost-efficient genome-wide association studies using DNA pools and dense SNP arrays. Nucleic Acids Res 36(6):e35. doi: 10.1093/nar/gkm1060

McDaneld, T. G., L. A. Kuehn, M. G. Thomas, W. M. Snelling, T. P. Smith, E. J. Pollak, J. B. Cole, and J. W. Keele. 2014. Genomewide association study of reproductive efficiency in female cattle. J Anim Sci 92(5):1945-1957. doi: 10.2527/jas.2012-6807

Mullen, K. M., and I. H. van Stokkum. 2012. The Lawson–Hanson algorithm for nonnegative least squares (NNLS), Technical report, CRAN. http://cran. r-proje ct. org/web/packa ges/nnls/nnls ….

Rengarajan, K., S. M. Cristol, M. Mehta, and J. M. Nickerson. 2002. Quantifying DNA concentrations using fluorometry: A comparison of fluorophores. Molecular Vision 8(43):416-421.

Singer, V. L., L. J. Jones, S. T. Yue, and R. P. Haugland. 1997. Characterization of PicoGreen reagent and development of a fluorescence-based solution assay for double-stranded DNA quantitation. Anal Biochem 249doi: 10.1006/abio.1997.2177

Strillacci, M. G., E. Frigo, F. Schiavini, A. B. Samoré, F. Canavesi, M. Vevey, M. C. Cozzi, M. Soller, E. Lipkin, and A. Bagnato. 2014. Genome-wide association study for somatic cell score in Valdostana Red Pied cattle breed using pooled DNA. BMC genetics 15(1):106.

Team, R. C. 2019. R: A language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria.

Yu, S., Y. Wang, X. Li, F. Yu, and W. Li. 2017. The factors affecting the reproducibility of micro-volume DNA mass quantification in Nanodrop 2000 spectrophotometer. Optik 145:555-560. doi: 10.1016/j.ijleo.2017.08.031

Table 3.1. Square root of mean square differences between observed and expected sample representations for pools constructed based on white blood cell count and two common DNA quantification methods.

Pool	Mean	Min, Max
White Blood Cell Count	0.013	0.008, 0.018
Spectrofluorometer Quantified DNA	0.022	0.009, 0.044
Spectrophotometer Quantified DNA	0.036	0.016, 0.050
Whole Blood Volume	0.023	0.014, 0.042
DNA Volume	0.033	0.011, 0.062

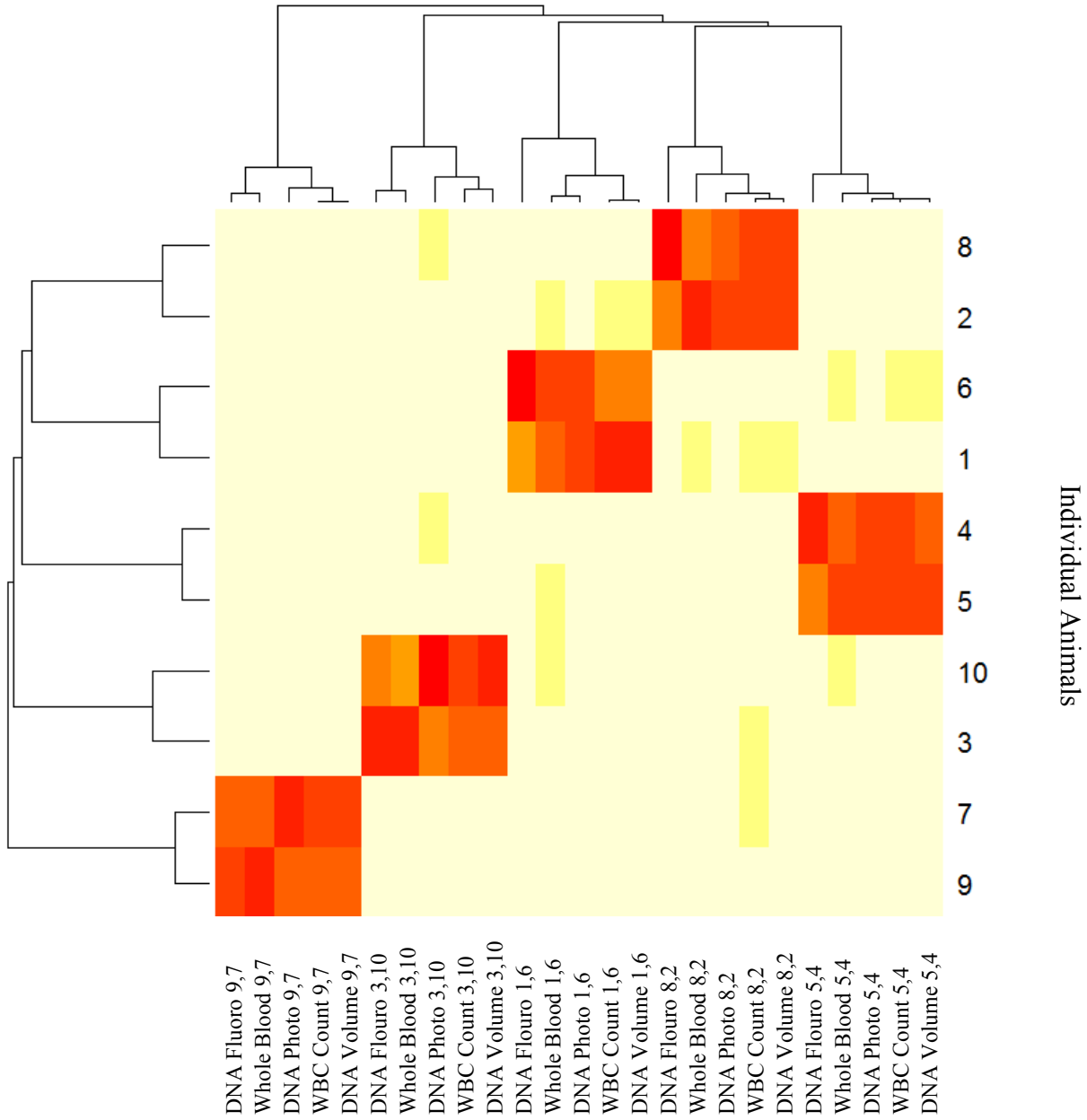

Figure 3.1. Heat map of individual sample representation within pools by method of pooling. DNA Flouro = pools constructed from DNA concentrations determined by spectrofluorometer. Whole Blood = pools constructed from whole blood based on volume. DNA Photo = pools constructed from DNA concentrations determined by spectrophotometer. WBC count = pools constructed from white blood cell count. DNA Volume = pools constructed from extracted DNA based on volume.

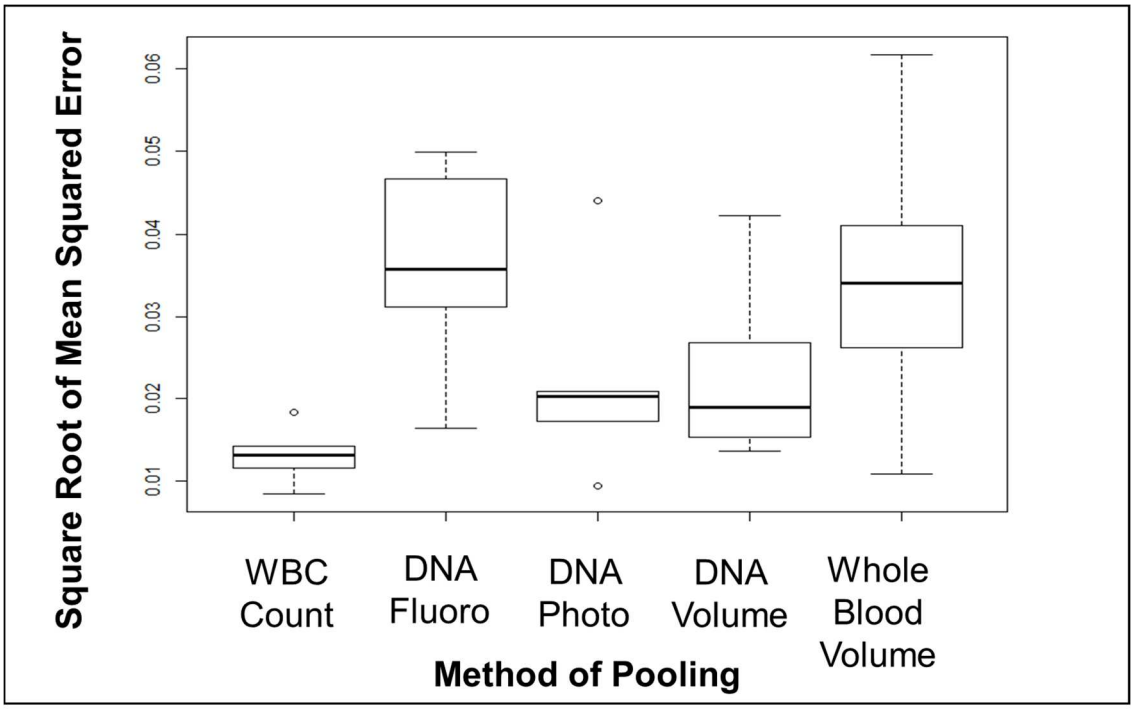

Figure 3.2. Square root of mean squared difference by method of pooling. WBC count = pools constructed from white blood cell count. DNA Flouro = pools constructed from DNA concentrations determined by spectrofluorometer. DNA Photo = pools constructed from DNA concentrations determined by spectrophotometer. DNA Volume = pools constructed from extracted DNA based on volume. Whole Blood Volume = pools constructed from whole blood based on volume.

CHAPTER 4: Implementing a classroom assessment technique to improve student engagement, communication, and performance in an introductory animal science laboratory course

ABSTRACT

Classroom assessment techniques (CATs) are valuable for engaging students in the learning process, monitoring students' learning, and establishing a communication feedback loop between students and instructors. The effectiveness of CATs in traditional Animal Science curriculum has not been formally evaluated. The objective of this study was to determine if a CAT affected final exam grades, weekly quiz scores, and perception of learning among Animal Science students. The study was conducted over the Fall 2017 and Fall 2018 semesters, and consisted of students (n = 161 Fall 2017; n = 95 Fall 2018) enrolled in laboratory sections of an Introduction to Animal Science course at South Dakota State University. Half of the sections were randomly selected to complete a CAT at the conclusion of each laboratory period. Assessment forms encouraged students to reflect, seek clarification, and evaluate their confidence on that day's material. A rubric was used to evaluate quality of the assessment responses throughout the semester. Feedback was provided at the beginning of the next week's laboratory period to clarify material that students had expressed difficulty in understanding. Final exam grades were compared between assessment and control groups using a Students *t*-test. Data were collected on student perception of classroom engagement, communication, and learning abilities through a Likert scale survey administered at the end of the semester. Surveys administered to students that participated in the CAT included additional questions to evaluate assessment effectiveness. Survey data was analyzed using a chi-square test to

evaluate differences between laboratory sections for general questions and within laboratory sections for assessment-specific questions. No differences ($P > 0.05$) were observed for final exam and weekly quiz grades for both semesters. Participating students reported that the CAT had a positive impact on their ability to learn, provided guidance for study material, and was an effective use of classroom time ($P < 0.05$). Although the CAT was viewed favorably by students who completed the assessment activity, CAT participation was not associated with increased final exam grades or weekly quiz scores.

INTRODUCTION

Assessment is a vital part of the teaching and learning process. To provide effective instruction, teachers must regularly monitor student learning. Ideal classroom assessment techniques are quick, low risk (ungraded) assessments that provide timely feedback to the instructor and allow educators to monitor student learning and adjust material or teaching methodology as needed (Angelo and Cross, 1993). Classroom assessment techniques focus the primary attention of teachers and students on observing and improving learning, rather than on observing and improving teaching. Learner-centered assessment is more than an assessment program-it is a paradigm shift from the traditional teacher-centered, lecture style teaching. While the traditional lecture style model is not ineffective, shifting to a learner-centered approach has been shown to enhance student learning (Steadman, 1998; Terenzini et al., 2001). Furthermore, the use of CATs in the classroom increases student engagement and metacognition, guides course design, enhances instructor teaching effectiveness, and strengthens communication between instructors and students by providing a continuous feedback

loop (Angelo and Cross, 1993; Cottell and Harwood, 1998; Goldstein, 2007; Butler and Roediger, 2008).

Some instructors argue that students are indifferent to feedback and only care about a grade (Wojtas, 1998). However, this argument is not supported in the literature. Although summative assessment methods are useful and necessary to measure student learning, often too much time elapses before feedback can be provided to students. The perceived lack of quality and discontent with the feedback process regarding summative assessment based feedback has been well documented (Hounsell et al., 2008; Ferguson, 2011). In a survey by Scott (2006), of students across 14 Australian Universities, 90% of respondents reported that feedback was in need of improvement, citing that the feedback typically received was poor quality, low quantity, and was not received in a timely manner. This sentiment regarding the lack of quality, quantity, and efficiency has been reiterated across several studies (Higgins et al., 2001; Hounsell et al., 2008; Ferguson, 2011).

The recognition of student dissatisfaction in and ineffectiveness of summative assessment methods that has caused a resurgence of interest in formative assessment methods (Higgins et al., 2001; Hounsell et al., 2008). The establishment of a consistent feedback loop between instructors and students is a key component to the effectiveness and success of CATs. Because of their variety and versatility, many CATs can be used in a manner that allow instructors to provide immediate, quality feedback.

There are many different CATs available. Some of the most common include Muddiest Point, Minute Paper, Misconception/Preconception Check, One Sentence Summary, and Background Probe (Angelo and Cross, 1993). Greater focus has been

placed on evaluating the ideal CAT or combination of CATs for specific disciplines or classroom structure (Cottell Jr and Harwood, 1998; Simpson-Beck, 2011; Mansson, 2013; Hogan and Daw, 2014). There is no evidence in the literature that CATs have been formally evaluated in Animal Science classes.The objective of this study was to evaluate if the use of a CAT affected final exam grades, weekly quiz scores, and students' perception of learning among Animal Science students in an Introductory to Animal Science Laboratory course.

MATERIALS AND METHODS

All data collection was approved by the South Dakota State University Institutional Review Board.

Introduction to Animal Science 101 Laboratory Course. The Introduction to Animal Science course at South Dakota State University is a first year (100 level) course designed to introduce students to the broad sectors of the animal science industry and provide a basic understanding of common principles and practices in animal science through lecture and laboratory class periods. While laboratories are associated with concurrent lectures and students must be enrolled in both, grades are assigned separately for the lecture and laboratory sections of this course. The laboratory portion of the course is divided into smaller sections with approximately 28 students per section. The laboratory class periods are structured similarly with each class beginning with a quiz over the previous week's material. After completion, the quiz answers are immediately reviewed before introducing the new material that will be covered in the current class period. After a brief introduction to the new material, students participate in an activity

(e.g.,piglet processing, carcass grading, live animal evaluation). The activities are guided by a worksheet that students must complete and submit at the end of class.

Each section is typically taught by a different instructor. With the exception of the laboratory coordinator (the primary instructor for the concurrent lectures), the laboratory instructors are graduate students within the Animal Science department. While the material covered during each laboratory period is nearly identical, each instructor is responsible for creating and grading quizzes and exams for their own respective laboratory section. At the conclusion of the semester, students complete a comprehensive final exam involving practical and written portions.

Fall 2017 Pilot Study Design. Students participating in the study were enrolled in the Introduction to Animal Science Course during the Fall 2018 semester at South Dakota State University. The course was separated into six laboratory sections with 26-29 students per section for a total of 161 students. All laboratory sections were taught by a different graduate student instructor with varying experience (number of semesters) teaching the AS101 laboratory course. Of these six laboratory sections, three were randomly selected to participate in the CAT. The remaining 3 laboratory sections served as controls throughout the semester.

Fall 2017 Pilot Study Classroom Assessment Technique Design. Laboratory sections participating in the CAT activity completed weekly assessments and received subsequent feedback. The assessment forms were instructor designed and resembled a combination of a One Sentence Summary, Application Card, and Muddiest Point (Angelo and Cross, 1993). The assessment forms included three open ended, short answer questions and two Likert-scale questions which asked students to rate their confidence

with the material pre- and post-class period. The assessment form was designed for students to reflect on that day's material, apply it beyond the classroom, and seek needed clarification material. Following the class period, assessment forms were evaluated to identify the main points that needed clarification. This information was then addressed at the beginning of the subsequent class period, prior to administering the quiz.

At the conclusion of the semester, all laboratory sections were administered a survey to gather information related to the CAT (Figures 4.8 and 4.9). All surveys included questions asking students to rate their confidence in their ability to retain and apply the information beyond AS101. The surveys complete by students in the CAT laboratory sections included additional questions specific to the CAT to ascertain student's perception of the assessment and feedback on classroom engagement, communication, and learning abilities.

Fall 2018 Design. Based on the outcome of the Fall 2017 pilot study (see results), the classroom assessment technique was altered and reimplemented in the Fall 2018. The main changes that occurred between the Fall 2017 and Fall 2018 semesters were: 1) the questions included on the end of class assessment forms; 2) how the feedback was delivered; 3) fewer instructors teaching more laboratory sections; and 4) enrollment structure. There were also several minor alterations made to the end of semester surveys.

Students participating in the study were enrolled in the Introduction to Animal Science Course Fall 2018 semester at South Dakota State University. The course was divided into four laboratory sections, with approximately 24 students per laboratory section, for a total of 95 students. Enrollment during the laboratories was stratified in attempt to evenly distribute early and late enrollment student and control for potential

bias between the laboratory sections. It was hypothesized that early enrollment students tended to be more highly motivated compared to late enrollment students. The early enrollment students typically selected laboratory sections that are earlier in the day, leaving the last section of the day as the only option for the late enrollment students and potentially causing introduction to selection bias. Maximum enrollment in all laboratory sections was limited to 15 students per section to distribute early enrollment students, which are typically students that have had success in their high school academic careers. Once the initial maximum enrollment was achieved for all lab sections, each section was reopened, and the maximum limit increased to 28 students per section.

The four laboratory sections were taught by two instructors; both were graduate students in the Animal Science department with previous experience teaching the AS101 laboratory course. Each instructor taught two consecutive laboratory sections, with one of the two laboratory sections being randomly selected to participate in the CAT while the remaining section served as a control. For one instructor, the first weekly laboratory section taught was the CAT group while the second section served as the control; the other instructor's first weekly laboratory section was the control and the second weekly section taught was the CAT group.

Fall 2018 Assessment Form and Feedback. Students participating in the CAT were allotted approximately 5 min at the end of each laboratory period to complete an assessment form. The assessment form was made up of four questions (Figure 4.6). The questions asked students to reflect on that classes material, rate their confidence on the main learning objectives, explain their confidence rating, and identify topics that needed additional clarification. The greatest change in this form between the Fall 2017 and Fall

2018 studies was the replacement of the application question with questions that were related to student confidence of the material. Students were asked to rate their confidence regarding each of the main topics from that laboratory period and explain why they selected that confidence rating (e.g., they were very confident grading beef carcasses because they had been a member of an FFA meat judging team; they were not confident at all because they have no experience with that topic and struggled to grasp the related concepts). After each class period, the assessment forms were graded using a rubric to evaluate quality of student responses over the semester (Figure 4.7). The rubric categorized assessment responses into beginner, proficient, and advanced for the categories of content, confidence explanation, and quality of questions asked. Based on the assessment responses, feedback was provided to the students at the beginning of the next laboratory period.

Because the students in the Fall 2017 pilot study perceived that the feedback component the most valuable aspect of the CAT to the learning process, and there is existing literature to support the importance of quality feedback, the feedback was redesigned to be more structured and interactive in the Fall 2018 study. Depending on the material, various active teaching techniques were used to review material that was confusing to students. The activities included: think-pair-share, concept mapping, and clicker questions. For example, students stated that they needed clarification on egg grading, at the beginning of the next class period, students were given concept map and ask to complete with their neighbor it by filling in characteristics of each grade category. Students were encouraged to work together during the activities while the instructor

moved throughout the room and interacted with students. The feedback activities lasted 3-10 minutes depending on the difficulty of the material and availability of class time.

End of Semester Survey. At the conclusion of the semester, students in all laboratory sections were administered a survey to gather information related to student perception of various aspect of the laboratory course (Figures 4.8 and 4.9). All surveys included seven questions focused on student interaction with their instructor and peers, perceived ability to understand course material, and engagement in the course. There were three additional questions asking students to rate their confidence in their ability to retain and apply the information beyond AS101. The surveys completed by students in the CAT laboratory sections included 17 additional questions specific to the CAT to ascertain student's perception of the assessment form and feedback on classroom engagement, communication, and learning abilities. Survey data was not analyzed until final grades were submitted to encourage students to provide authentic answers without concern for repercussion.

Statistical Analysis. Statistical analysis was completed using R (Version 3.6.0) and RStudio (Version 1.2.1335; R core team. 2019). The packages 'dyplr' (v0.8.1; Wickham et al., 2019) and 'psych' (v1.8.12; Revelle, 2018) were used to calculate descriptive data, Student's *T*=test, and Chi-squared analyses. Laboratory final exam grades and average weekly quiz scores were compared between the CAT and control groups using a Student's *t*-test. Likert scale survey data were analyzed using a Chi-squared test in R to determine response differences between students that did or did not complete weekly assessments. Likert scale response graphs were generated using the 'likert' package (v1.3.5; Bryer et. al., 2016).

RESULTS

Fall 2017 Pilot Study. Of the 161 students enrolled in the AS101 Laboratory course, 4 students withdrew from the class prior to the final exam and were removed from the data set. There were 157 students total (n= 78 CAT; 79 Control) that completed the final exam and end of semester survey. No differences ($P > 0.05$) were observed for final exam grades or average weekly quiz scores between students in laboratory sections that did or did not complete weekly assessments. The average final exam grade for students in the CAT and control groups were 80.0 points and 78.2 points (out of 100 points), respectively. Average weekly quiz scores were 8.29 points (out of 10 points) for students in the CAT group and 8.62 points for students in the control group. When comparing the survey questions that were common between the CAT and control groups, there were no differences ($P > 0.05$) in student responses, including the questions regarding student's confidence of their ability for short or long-term retention of the material.

Those students that participated in the CAT did report a positive overall perception of the CAT. Students rated the assessment-based feedback component higher for its impact on learning ability ($P = 0.014$) and classroom engagement ($P = 0.01$) compared to only completing the assessment form, indicating that students perceived greater value in the feedback versus the reflection aspect of the CAT. Approximately 73% of students agreed or strongly agreed that completing the CAT at the end of each class was an effective use of class time. Additionally, the end of semester survey administered to students in the CAT groups included an open-ended question asking

students for suggested changes to the CAT. There were multiple responses advising that the assessment forms should be altered each week to ask questions specific to each laboratory topic. Despite an even distribution for instructor experience level between the CAT and control groups, the variation in motivation and academic standards between instructors proved to be a confounding factor. The results from the pilot study were used to guide changes to the CAT prior to reimplantation in Fall 2018 (previously described).

Fall 2018 Study. No differences ($P < 0.05$) were detected between students in the Fall 2018 CAT and Control groups for final exam grades or weekly quiz scores (figure 4.1). The average final exam score was 88.85 points for students in the CAT group and 85.67 points for students in the control group. When comparing survey questions in common between the CAT and Control groups, there were no differences ($P > 0.05$) in student responses, including the questions regarding student's confidence of their ability for short or long-term retention of the material.

Results from the surveys administered to the CAT and control groups were are shown in figure 4.1 through figure 4.5. As with the previous semester, students had an overall positive perception of the CAT. When specifically questioned about the impact of completing the assessment form at the end of each class period, 83% agreed or strongly agreed that filling out the form helped them identify topics from the current day's laboratory that they did not understand but needed to learn and encouraged them to seek clarification. Approximately 85 percent of students indicated that the form provided direction on what they needed to study outside of class; however, only 74% responded that the form encouraged them to study outside of class. Students responded that completing the form positively impacted their ability to learn (76%) and their

engagement during laboratory (74%). Lastly, 74% of students indicated that the filling out the assessment form was an effective use of class time. When questioned about the feedback component of the CAT, responses were especially positive with over half of the students moderately agreeing or strongly agreeing with each prompt. Approximately 98% of students indicated that the feedback activity gave them the opportunity to ask questions prior to the quiz, and 96% indicated that the feedback activity itself addressed the material for which they had questions.

In reference to classroom communications, 91% of students agreed that the feedback activity encouraged interaction with peers and their instructor. Approximately 93 percent of students indicated that the feedback activity positively impacted their ability to learn and engage during class. Although the feedback activity was more time consuming compared to filling out the assessment, 98% of students agreed that it was an effective use of time. As in the pilot study, students were given the opportunity to make suggestions regarding the CAT. The most common response was that students wanted more time to complete the assessment at the end of the class period. Some students explained that they felt rushed because they needed to get to their next class. Others felt that if more time and depth was required for responses, their peers would put in more effort and there would be a greater benefit for for the students as a whole. When asked their opinion on the design of the assessment form, several students suggested that the form should include a question that allows students to provide suggestions on improvements or share aspects that they really enjoyed. Overall the responses were positive, and many students felt that the CAT should not be altered and should continue to be utilized in subsequent semesters.

DISCUSSION

Because CATs are promoted as a tool to enhance student learning, this can lead to an expectation that utilizing a CAT in the classroom will improve student grades. Despite the adjustments made to the study design following the Fall 2017 pilot study, neither Fall 2018 or Fall 2017 studies were able to detect a difference between students in CAT and control groups for final exam grades or weekly quiz scores. There are several possibilities that could have influenced this outcome. Academic research is inherently plagued with the issue of selection bias. It is often difficult or unethical to control for confounding factors, such as self-selection, instructor bias, previous experience, and socioeconomic factors (Showalter and Mullet, 2017). This can create noise in the study, preventing the detection of differences due to the treatment. In an effort to partially account for self-selection between laboratory sections, a stratified approach was applied for enrollment to more evenly distribute early and late enrollment students. Instructor bias was also addressed in the Fall 2018 study after the Fall 2017 pilot study showed a large variation in laboratory performance due to instructor differences. While there are additional factors that could be preventing the establishment of causal relationships between CATs and academic performance, it is also a likely possibility that a direct relationship does not actually exist. Despite studies by Bullock et al. (2018) and Cross and Palese (2015) which reported an increase student performance in response to using CATs, research on the direct relationship between CATs and student grades is limited and has failed to establish a clear improvement in academic performance in response to CATs. However, this does

not mean that CATs lack merit in the classroom but rather support the view that the true value of CATs lies in the ability to shift the focus from teaching to learning.

Interestingly, 98% of students in the control group felt that they could easily identify the main topics covered during that class and what they were expected to know compared to 89% of students in the CAT group. However, both groups were almost identical in their response to identifying topics that they did not understand and needed to review. Although not statistically signficant, the 9% difference between groups in their ability to identify the main topics may indicate a greater self-awareness in the CAT group. Encouraging students to reflect on the material and evaluate their knowledge level was one of the core objectives of incorporating this CAT in the AS101 laboratory class. Completing reflective and self-evaluation activities can help students identify gaps in their own content knowledge. The CAT form that students completed in the present study was a combination of One-Sentence Summary, Course-Related Self-Confidence Survey, and Muddiest Point CATs. The main purpose of these specific CATs are for students to reflect on their own learning and demonstrate understanding of the material (Angelo and Cross, 1993). Furthermore, Byon (2005) demonstrated that the use of a Minute Paper in combination with a One-Sentence Summary to ask students to reiterate the lesson in their own words allowed students the chance to verbalize specific parts of the lesson that they needed to study more and raise metacognitive awareness regarding their own self learning processes. It is possible that filling out the assessment form at the conclusion of each class made students more aware of what information they actually retained and understood compared to what they thought they had retained during the class period.

The outcome of this study aligns with a large body of literature reporting a positive perception of CATs by students and instructors. Overall, students felt that the CAT positively influenced their engagement in the classroom and ability to learn. One common justification by instructors for not implementing CATs in their classroom is time constraints (Goldstein, 2007). There is a concern that incorporating a CAT will require a significant amount of time and reduce the amount of material that can be covered during a class period. If applied in the correct manner, this concern is unwarranted and CATs can even improve teaching and learning efficiency in the classroom. Students in the current study indicated that the CAT was an appropriate use of time, with 74% agreeing or strongly agreeing that completing the assessment form was an effective use of time and the time spent completing the feedback activity was supported by 98% of students.

Overall, students in the CAT group indicated that at the beginning of the semester they felt more comfortable using the assessment form to ask for clarification on material that they were struggling with as opposed to directly speaking with the instructor. However, by midway through the semester, students stated that they were more likely to seek help by directly talking to their instructor rather than using the form. While this difference could indicate a failure to appropriately address student needs through the assessment form, it is unlikely since the students perceived that the assessment-based feedback sufficiently addressed the desired material. Furthermore, students agreed that they were more comfortable posing questions and interacting with their instructor once they became more familiar with them and that the CAT encouraged them to interact with their instructor. Cross and Palese (2015) reported an increase in mean posting frequency

in a discussion form in an online class after implementing CATs. Similarly, Steele and Dyer (2014) reported increased participation in a discussion forum for students that using a CAT compared to students that did not. Furthermore, Henderson (2001) reported that the implementation of CATs not only increased student participation but also generated "deeper" discussion threads. Although data related to frequency of student interactions in class was not collected to validate this perception, the combined survey information suggested that CATs may be especially useful at the beginning of a course when students may be more hesitant to pose questions or actively seek help.

Although not directly measured and based solely on anecdotal information, both instructors noted that their CAT laboratory sections were more interactive and engaging compared to the control laboratory sections. The overall instructor experience was more rewarding while teaching students in the CAT groups. This was somewhat surprising considering that one of the CAT sections was the last laboratory section of the day, which is historically perceived as one of the lower energy, less motivated sections. Instructors felt that they received more questions from students in the CAT groups, and that class periods were more conversational in the delivery of the subject content. While this perceived difference could be due to random selection and that students in the CAT groups happened to be more assertive by nature, the instructors perceptions are supported by studies that have demonstrated that CATs promote a more interactive and positive classroom environment. Adams (2004) and Byon (2005) suggested that CATs create a community centered environment and increase student satisfaction.

CONCLUSION

The use of a CAT in the Introduction to Animal Science Laboratory course was positively perceived by students. Based on the outcome of the study, students highly valued the feedback component of the CAT, especially when provided in a structured and interactive manner. Time limitations are often a concern when implementing a CAT; however, students indicated that the CAT was an effective use of time and did not require too much class time to complete. Additional value in the CAT may be realized early in the course in the form of increased communication between students and instructors. However, since this assumption is based on student perception, further research is warranted to investigate if CATs increase initial communication between students and instructors. Although there were no differences in final exam grades or weekly quiz scores between students in the CAT versus control groups, the outcome of this study indicated that classroom assessment techniques guide instruction, increase student engagement, and strengthen the feedback loop between instructors and students.

REFERENCES

Adams, P. 2004. Classroom assessment and social welfare policy: Addressing challenges to teaching and learning. Journal of Social Work Education 40(1):121-142.

Angelo, T. A., and K. P. Cross. 1993. Classroom assessment techniques : a handbook for college teachers. San Francisco : Jossey-Bass Publishers, San Francisco.

Bryer, J., and K. Speerschneider. 2016. likert: Analysis and Visualization Likert Items.

Bullock, K. C., C. Gibson, M. Howard, J. Liu, A. Tatachar, and W. C. Yuet. 2018. Use of the Muddiest Point Technique as an exam review in an integrated pharmacotherapy course. Currents in Pharmacy Teaching and Learning 10(9):1295-1302.

Butler, A. C., and H. L. Roediger. 2008. Feedback enhances the positive effects and reduces the negative effects of multiple-choice testing. Memory & Cognition 36(3):604-616.

Byon, A. S. 2005. Classroom assessment tools and students' affective stances: KFL classroom settings. Language and Education 19(3):173-193.

Cottell Jr, P. G., and E. M. Harwood. 1998. Using classroom assessment techniques to improve student learning in accounting classes. Issues in accounting education 13(3):551.

Cottell, P., and E. Harwood. 1998. Do Classroom Assessment Techniques (CATs) Improve Student Learning? New Directions for Teaching and Learning 75:37-46.

Cross, T., and K. Palese. 2015. Increasing learning: Classroom assessment techniques in the online classroom. American Journal of Distance Education 29(2):98-108.

Ferguson, P. 2011. Student perceptions of quality feedback in teacher education. Assessment & Evaluation in Higher Education 36(1):51-62.

Goldstein, G. S. 2007. Using classroom assessment techniques in an introductory statistics class. College Teaching 55(2):77-82.

Henderson, T. 2001. Classroom assessment techniques in asynchronous learning networks. The Technology Source

Higgins, R., P. Hartley, and A. Skelton. 2001. Getting the message across: the problem of communicating assessment feedback. Teaching in higher education 6(2):269-274.

Hogan, A., and J. Daw. 2014. Mode of Delivery: A Classroom Assessment Technique Comparison between Verbal and Non-Verbal Communication. Journal of Instructional Research 3:21-26.

Hounsell, D., V. McCune, J. Hounsell, and J. Litjens. 2008. The quality of guidance and feedback to students. Higher Education Research & Development 27(1):55-67.

Mansson, D. 2013. Assessing student learning in intercultural communication: Implementation of three classroom assessment techniques. College Student Journal 47(2):343-351.

Revelle, W. 2018. psych: Procedures for Personality and Psychological Research, Northwestern University, Evanston, Illinois, USA,.

Scott, G. 2006. Accessing the student voice: A higher education innovation program project. Canberra: Department of

Showalter, D. A., and L. B. Mullet. 2017. Sniffing Out the Secret Poison: Selection Bias in Educational Research. Mid-Western Educational Researcher 29(3)

Simpson-Beck, V. 2011. Assessing classroom assessment techniques. Active Learning in Higher Education 12(2):125-132.

Steadman, M. 1998. Using classroom assessment to change both teaching and learning. New Directions for Teaching and Learning 1998(75):23-35.

Steele, J., and T. Dyer. 2014. Use of KWLs in the Online Classroom as It Correlates to Increased Participation. Journal of Instructional Research 3:8-14.

Team, R. C. 2019. R: A language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria.

Terenzini, P. T., A. F. Cabrera, C. L. Colbeck, J. M. Parente, and S. A. Bjorklund. 2001. Collaborative learning vs. lecture/discussion: Students' reported learning gains. Journal of Engineering Education 90(1):123-130.

Wickham, H., R. François, L. Henry, and K. Müller. 2019. dplyr: A Grammar of Data Manipulation.

Wojtas, O. 1998. Feedback? No, just give us the answers. Times Higher Education Supplement 25(7)

Table 4.1. Average final exam and weekly quiz scores for the Fall 2017 (n = 161) and Fall 2018 (n = 95) semesters of the AS101 laboratory course.

Assessment (Number of points)	Group				*P*-values
	CAT		CON		
	Mean Score (%)	SEM	Mean Score (%)	SEM	
Fall 2018 Final Exam (100 points)	88.85	1.36	85.67	1.86	0.166
Fall 2017 Final Exam (100 points)	80.0	1.39	78.2	1.34	0.745
Fall 2018 Quiz (10 points)	8.92	0.18	8.49	0.17	0.092
Fall 2017 Quiz (10 points)	8.29	0.18	8.62	0.21	0.234

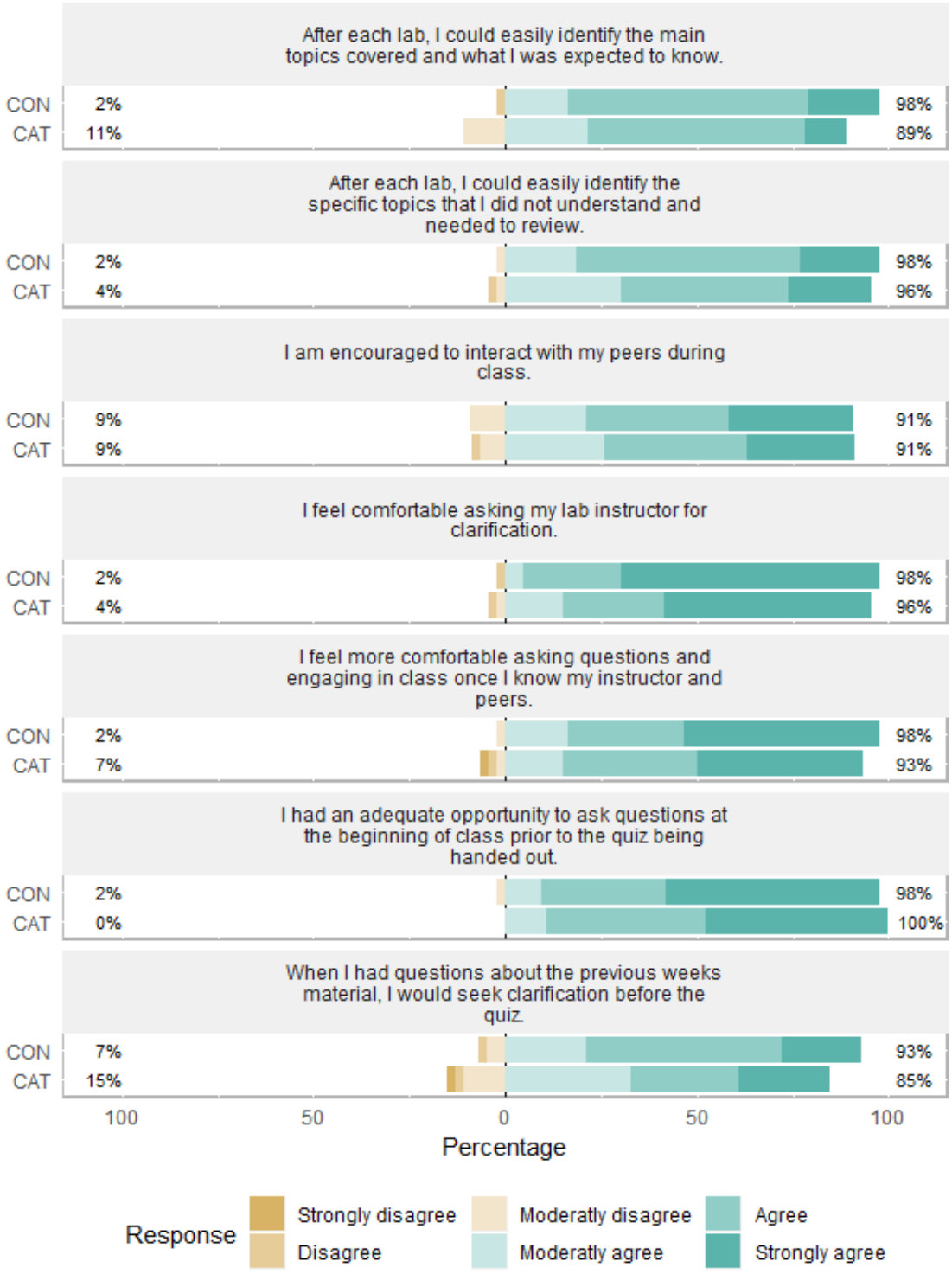

Figure 4.1. End of semester survey responses for general course related questions that were common between the sections that participated in the control sections (CON) and the classroom assessment technique (CAT). Percentage values to the right of the bar represents students with a response of agree to strongly agree. Percentage values to the left of the bar represents students with a response of disagree to strongly disagree.

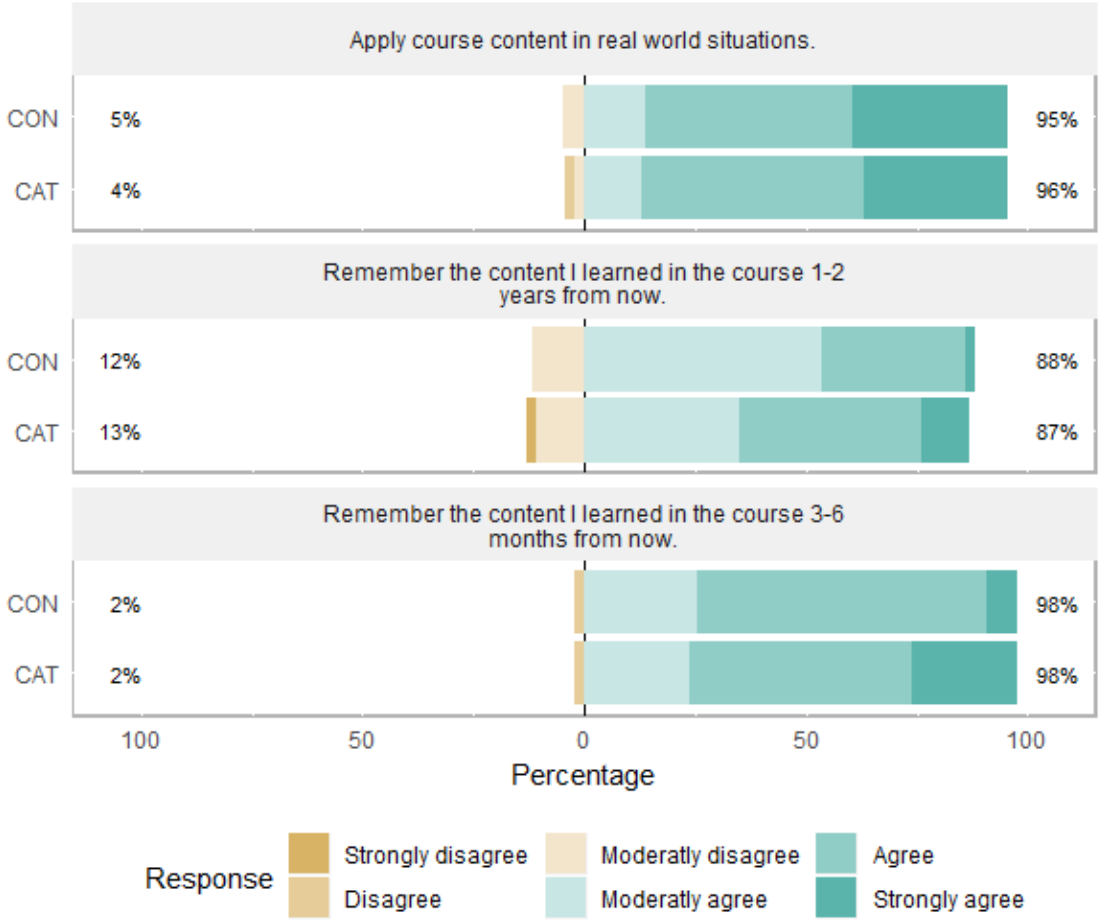

Figure 4.2. End of semester survey responses for application and retention related questions that were common between the sections that participated in the control sections (CON) and the classroom assessment technique (CAT) based on the prompt "I am confident in my ability to….". Percentage values to the right of the bar represents students with a response of agree to strongly agree. Percentage values to the left of the bar represents students with a response of disagree to strongly disagree.

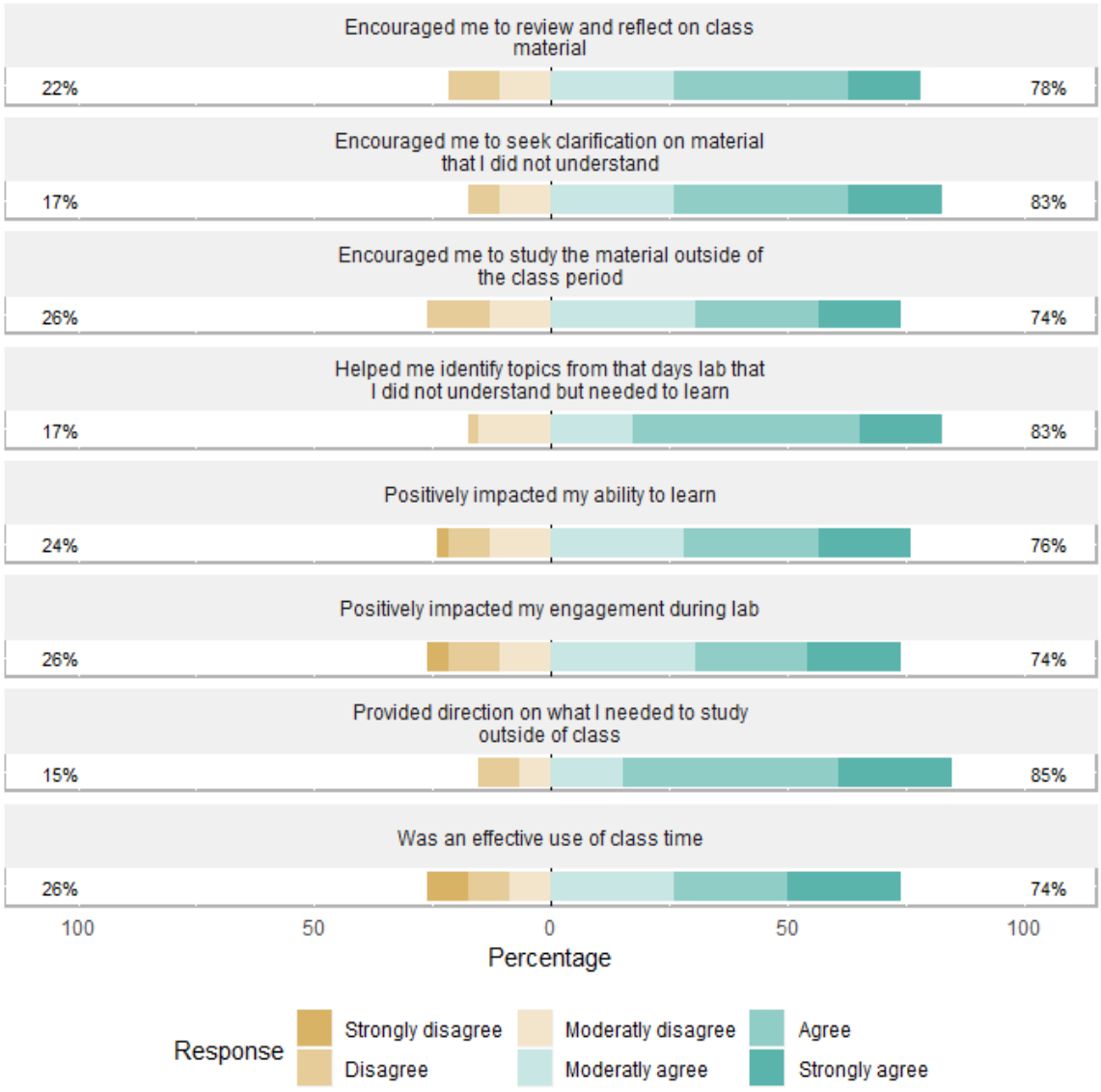

Figure 4.3. End of semester survey responses specific to filling out the assessment form for the sections that participated in the classroom assessment technique (CAT) based on the prompt "Filling out the assessment form at the end of each class period...". Percentage values to the right of the bar represents students with a response of agree to strongly agree. Percentage values to the left of the bar represents students with a response of disagree to strongly disagree.

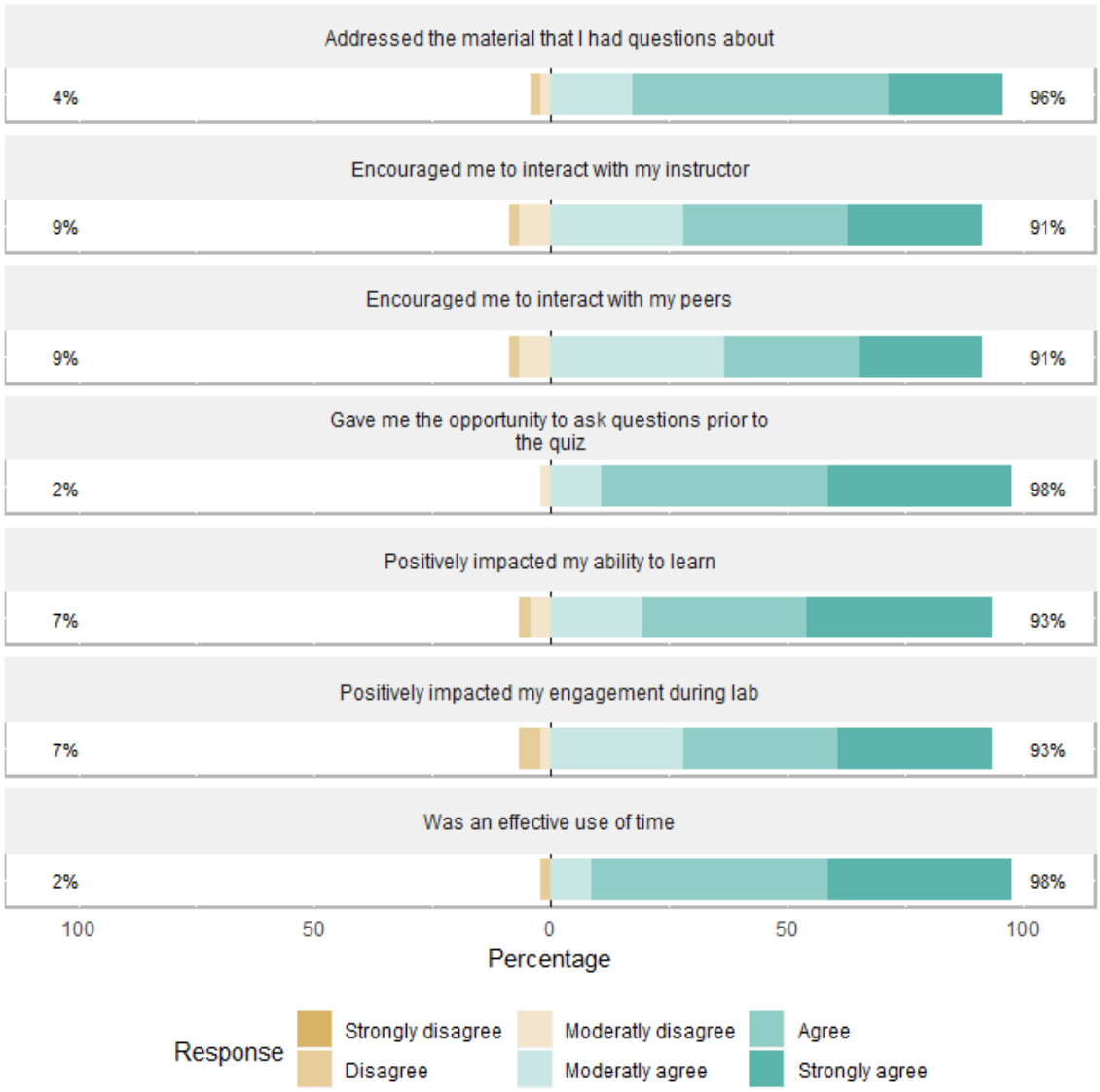

Figure 4.4. End of semester survey responses to the assessment-based feedback activity for the sections that participated in the classroom assessment technique (CAT) based on the prompt "Using information from the assessment forms, your instructor provided feedback and additional information at the beginning of each period. This feedback and information...". Percentage values to the right of the bar represents students with a response of agree to strongly agree. Percentage values to the left of the bar represents students with a response of disagree to strongly disagree.

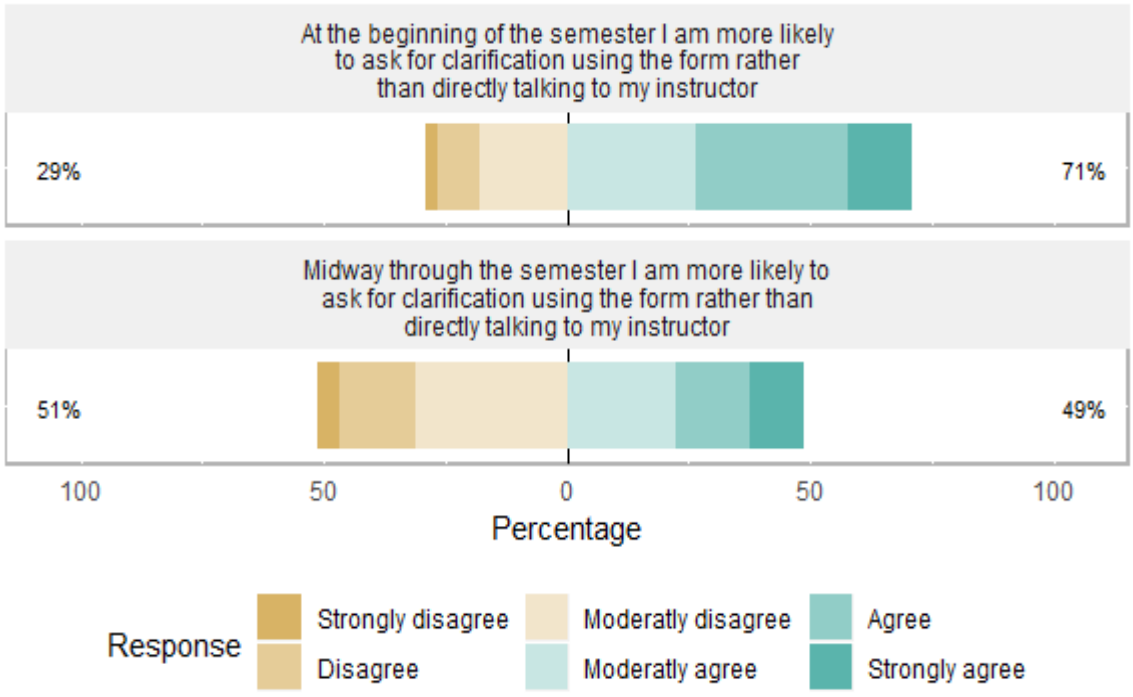

Figure 4.5. End of semester survey responses for the sections that participated in the classroom assessment technique (CAT) evaluating the mostly likely source of help that students will seek at the beginning and middle of the semester. Percentage values to the right of the bar represents students with a response of agree to strongly agree. Percentage values to the left of the bar represents students with a response of disagree to strongly disagree.

AS 101 LAB ASSESSMENT

1. In 2-4 sentences, how would you summarize what you learned in today's lab?

2. How confident are you that could explain the following topics from todays lab to one of your animal science peers?

    i. Learning outcome one
        a. Very confident
        b. Somewhat confident
        c. Not very confident
        d. Not confident at all

    ii. Learning outcome two
        a. Very confident
        b. Somewhat confident
        c. Not very confident
        d. Not confident at all

    iii. Learning outcome three
        a. Very confident
        b. Somewhat confident
        c. Not very confident
        d. Not confident at all

3. Explain why you did or did not feel confident on the topics above.

4. What aspect of today's lab did you have the most difficulty understanding?

Figure 4.6. Weekly Assessment form for Fall 2018 semester of AS101 Laboratory course.

CAT Weekly Assessment Form Rubric

Main topics/big ideas for this lesson:

	Beginner **(1)**	**Proficient** **(2)**	**Advanced** **(3)**
Content (Question 1) No Response = 0	-Identify only big ideas but not specifics. - For a multi-part lab, may only focus on one aspect. - Information provided is inaccurate.	-Identify more than one, but not all big ideas and provides some additional specifics.	-Identify all big ideas and provides additional specifics.
Confidence (Question 3) No Response = 0	-Unable to explain why they were or were not confident in the material. -Confidence rating did not match their confidence explanation (i.e. They rated themselves as not very confident but then stated they were confident because of previous experience). - Response of "I just need more practice" but no additional explanation or does not match a confidence rating of somewhat confident. - Response of "It was explained well" but does not match with very confident rating. - "I am confident"	-Were able to provide some explanation regarding their confidence but not fully explain reason for their confidence selection. -Provided general idea (i.e. "previous experience") but no specifics. - Explanation closely but not fully matched confidence ratings. (i.e. Rated themselves somewhat confident but only explained why they were confident and not why they were somewhat rather than very confident) -Response of "I just need more practice" and additional explanation. Matches somewhat confident rating. -Response of "It was explained well" matches very confident.	-Were able to clearly identify and justify their confidence. -Provided specifics as to justify their confidence. - Confidence explanation matched their confidence ratings.
Feedback (Question 4) No Response = 0	-Very general, unsure what questions to ask. -Not useful for providing specific feedback.	-More specific in their questions but still somewhat vague. -Can provide limited feedback.	-Very specific question. -Can provide, guided and detailed feedback.

Total score: Beginner = 0-4 Proficient = 5-7 Advanced = 8-9

Figure 4.7. Rubric applied to weekly assessment responses.

Figure 4.8. Fall 2018 AS101 Laboratory course end of semester survey for the classroom assessment technique group.

AS 101 Lab Student Assessment Form Survey – Fall 2018

Throughout the semester, you completed a form after each lab and participated in an activity at the beginning of the following lab. We would like your feedback to help guide improvements for future semesters……

	Strongly Disagree	Disagree	Moderately Disagree	Moderately Agree	Agree	Strongly Agree
After each lab, I could easily identify the main topics covered and what I was expected to know.	1	2	3	4	5	6
After each lab, I could easily identify the specific topics that I did not understand and needed to review.	1	2	3	4	5	6
I feel comfortable asking my lab instructor for clarification.	1	2	3	4	5	6
I feel more comfortable asking questions and engaging in class once I know my instructor and peers.	1	2	3	4	5	6
I am encouraged to interact with my peers during class	1	2	3	4	5	6
I had an adequate opportunity to ask questions at the beginning of class prior to the quiz being handed out.	1	2	3	4	5	6
When I had questions about the previous weeks material, I would seek clarification before the quiz.	1	2	3	4	5	6

Filling out the assessment form at the end of each class period...

	Strongly Disagree	Disagree	Moderately Disagree	Moderately Agree	Agree	Strongly Agree
Encouraged me to review and reflect on class material.	1	2	3	4	5	6
Helped me identify topics from that day's lab that I did not understand but needed to learn.	1	2	3	4	5	6

Encouraged me to seek clarification on material that I did not understand.	1	2	3	4	5	6
Provided direction on what I needed to study outside of class.	1	2	3	4	5	6
Encouraged me to study the material outside of the class period.	1	2	3	4	5	6
Was an effective use of class time	1	2	3	4	5	6
Positively impacted my ability to learn.	1	2	3	4	5	6
Positively impacted my engagement during lab.	1	2	3	4	5	6

Using information from the assessment forms, your instructor provided feedback and additional information at the beginning of each period. This feedback and information...

	Strongly Disagree	Disagree	Moderately Disagree	Moderately Agree	Agree	Strongly Agree
Addressed the material that I had questions about.	1	2	3	4	5	6
Gave me the opportunity to ask questions prior to the quiz.	1	2	3	4	5	6
Was an effective use of time.	1	2	3	4	5	6
Encouraged me to interact with my peers.	1	2	3	4	5	6
Encouraged me to interact with my instructor.	1	2	3	4	5	6
Positively impacted my ability to learn.	1	2	3	4	5	6
Positively impacted my engagement during lab.	1	2	3	4	5	6

At the BEGINNING of the semester, I am more likely to…

	Strongly Disagree	Disagree	Moderately Disagree	Moderately Agree	Agree	Strongly Agree

Ask for clarification using the form rather than directly talking to my instructor.	1	2	3	4	5	6

Midway through the semester, I am more likely to…

	Strongly Disagree	Disagree	Moderately Disagree	Moderately Agree	Agree	Strongly Agree
Ask for clarification using the form rather than directly talking to my instructor.	1	2	3	4	5	6

I am confident in my ability to….

	Strongly Disagree	Disagree	Moderately Disagree	Moderately Agree	Agree	Strongly Agree
Remember the content I learned in the course 3-6 months from now.	1	2	3	4	5	6
Remember the content I learned in the course 1-2 years from now.	1	2	3	4	5	6
Apply course content in real world situations.	1	2	3	4	5	6

Background information

What livestock experience did you have prior to this course? (select all that apply)

- ☐ Grew up on a farm/ranch
- ☐ Raised small number of livestock as a hobby
- ☐ Participated in 4-H/FFA/
- ☐ Showed livestock
- ☐ Previous animal science college courses
- ☐ Other (please explain)
- ☐ None

What livestock species did you have experience with prior to the course? (select all that apply)

- ☐ Beef Cattle

- [ ] Dairy Cattle
- [ ] Swine
- [ ] Sheep
- [ ] Poultry
- [ ] Equine
- [ ] Other (explain):

Did you attend one of the AS 101 Lab final review sessions?

[ ] Yes [ ] No

What suggestions do you have for improving the **assessments forms** used in this course?

What suggestions do you have for improving the **feedback activity** used in this course?

What suggestions do you have for improving this **lab course**?

Figure 4.9. Fall 2018 AS101 Laboratory course end of semester survey for the control group.

AS 101 Lab General Information Survey – Fall 2017

We would like your feedback to help guide improvements for future semesters……

	Strongly Disagree	Disagree	Moderately Disagree	Moderately Agree	Agree	Strongly Agree
After each lab, I could easily identify the main topics covered and what I was expected to know.	1	2	3	4	5	6
After each lab, I could easily identify the specific topics that I did not understand and needed to review.	1	2	3	4	5	6
I feel comfortable asking my lab instructor for clarification.	1	2	3	4	5	6
I feel more comfortable asking questions and engaging in class once I know my instructor and peers	1	2	3	4	5	6
I am encouraged to interact with my peers during class	1	2	3	4	5	6
I had an adequate opportunity to ask questions at the beginning of class prior to the quiz being handed out.	1	2	3	4	5	6
When I had questions about the previous weeks material, I would seek clarification before the quiz.	1	2	3	4	5	6

I am confident in my ability to….

	Strongly Disagree	Disagree	Moderately Disagree	Moderately Agree	Agree	Strongly Agree
Remember the content I learned in the course 3-6 months from now.	1	2	3	4	5	6

Remember the content I learned in the course 1-2 years from now.	1	2	3	4	5	6
Apply course content in real world situations.	1	2	3	4	5	6

Background information

What livestock experience did you have prior to this course? (select all that apply)

- ☐ Grew up on a farm/ranch
- ☐ Raised small number of livestock as a hobby
- ☐ Participated in 4-H/FFA/
- ☐ Showed livestock
- ☐ Previous animal science college courses
- ☐ Other (please explain)
- ☐ None

What livestock species did you have experience with prior to the course? (select all that apply)

- ☐ Beef Cattle
- ☐ Dairy Cattle
- ☐ Swine
- ☐ Sheep
- ☐ Poultry
- ☐ Equine
- ☐ Other (explain):

Did you attend one of the AS 101 Lab final review sessions?

☐ Yes ☐ No

What suggestions do you have for improving this lab course?