

**IMPACTS OF COMMINGLING CATTLE FROM DIFFERENT SOURCES ON THEIR
PHYSIOLOGICAL, HEALTH, AND PERFORMANCE RESPONSES DURING
FEEDLOT RECEIVING**

A Thesis

by

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ABSTRACT

This experiment compared physiological, health, and performance responses of beef heifers assigned to different commingling schemes (1, 2, or 4 sources/pen) during a 56-d feedlot receiving period. Ninety-six recently weaned Angus-influenced heifers were obtained from an auction facility. Heifers originated from 4 cow-calf ranches and were reared in the same herd within each ranch since birth. Heifers were loaded into 2 livestock trailers at the auction yard (2 sources/trailer; d -2), arranged in 2 sections of each trailer according to source, and transported for 10 h to stimulate the stress of a long-haul. Heifers were not mixed with cohorts from other sources prior to and at the auction yard. Upon arrival (d -2), shrunk body weight (**BW**) was recorded and heifers were maintained in 4 paddocks by source with ad libitum access to a complete starter feed and water for 36 h. On d 0, heifers were ranked by source and shrunk BW and allocated to 1 of 24 drylot pens (4 heifers/pen) containing: 1) heifers from a single source (**1SRC**, n = 8), 2) heifers from 2 sources (**2SRC**, n = 8), or 3) heifers from 4 sources (**4SRC**, n = 8). From d 0 to 55, heifers had free-choice access to the complete starter feed and water. Heifers were assessed daily for symptoms of bovine respiratory disease (**BRD**), and feed intake was recorded from each pen daily. Blood samples were collected on d 0, 6, 13, 27, 41, and 55, and shrunk BW (after 16 h of water and feed withdrawal) was recorded on d 56 for average daily gain (**ADG**). No treatment differences were noted ($P \geq 0.56$) for heifer ADG (mean \pm SE = 0.853 ± 0.043 kg/d), final shrunk BW, feed intake, and feed efficiency. No treatment differences were noted ($P \geq 0.27$) for plasma concentrations of cortisol and haptoglobin, and serum concentrations of antibodies against BRD viruses and *Mannheimia haemolytica*. No treatment differences were noted ($P \geq 0.17$) for incidence of BRD (mean \pm SE = 59.3 ± 5.0 %) or mortality. The proportion of heifers diagnosed with BRD that required three antimicrobial treatments to regain health increased linearly ($P = 0.03$) according to the

number of sources (0.0, 12.3, and 20.8% of 1SRC, 2SRC, and 4SRC heifers, respectively; SEM = 7.0). Hence, commingling heifers from different sources did not impact performance, physiological responses, and BRD incidence during a 56-d receiving period, although recurrence of BRD after the second antimicrobial treatment increased according to commingling level.

DEDICATION

This thesis is dedicated to my wife Farah and sons John and Brock. I could not have done this without your unending love and adoration.

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First and foremost, I would like to express my eternal love and gratitude to my wife Farah. If it were not for her love and support in every way this venture would never have been possible. During the initial three months of this program while I was in New Mexico conducting this research she stayed home in Utah and took care of our 18-month-old son, worked full time, and all this in her third trimester of pregnancy. Then coordinated and moved our household goods to College Station a few weeks prior to her due date, gave birth to our second son, and over the subsequent year put our boys and myself first so that I could put the required time and effort into completing this program.

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Contributors

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Research has shown that the use of modern production practices, including concentrated feeding operations, are essential to economic viability and environmental responsibility (Capper, 2012). The U.S. beef industry has drastically improved efficiency in production and in the last four decades reduced feed intake by 19%, used 12% less water, 33% less land, and demonstrated a 16% reduction in the carbon footprint per unit of beef produced (Capper, 2011). Capper (2012) showed that in order to produce the same amount of beef, in terms of hot carcass weight, conventional cattle systems that use growth-enhancing technology and high-energy concentrated feed required 43.7% fewer head of cattle, 24.5% amount of water, 55.3% of the land, 71.4% of fossil fuel energy, and had a reduced carbon footprint compared to “natural” and “grass-fed” operations.

In 2018, U.S. beef exports exceeded eight billion dollars (NCBA, 2019) in pursuit of a rising global market demand as the world population races to a projected 9.5 billion people by the year 2050 and a 70% increase in food requirements (FAO, 2009). Elevations in per capita income in emerging nations (Tilman et al., 2002) and higher standards of living are ensuring a continuing escalation in global demand for animal protein. As competition for natural resources increase feedlot operations remain essential to keeping beef an economically and environmentally sustainable food source (Capper, 2012).

The beef industry can improve upon previous advances in efficiency and ensure sustainability by continuing to identify and mitigate critical stressors that leave cattle vulnerable to microbial challenges, such as bovine respiratory disease (**BRD**), that negatively

impact growth performance due to morbidity, reduced feed efficiency, mortality, and increased pharmaceutical expenses (Loerch and Fluharty, 1999).

Literature Review

The beef value chain

Beef production systems in the U.S. are characterized by a wide range of climates, environmental conditions, animal phenotypes, management practices, and a variety of nutritional inputs. These are predominantly pastoral-based systems, with climates spanning from tropical savannahs to temperate plains and mountain pastures (Drouillard, 2018). The U.S. beef industry is classified into three discrete phases of operations before cattle are harvested and fabricated into end-products available to the consumer. These three phases include cow/calf operations, stocker and backgrounding operations, and feedlot finishing operations. Cattle are born and raised in all 50 states; however, over 11 million of the 14.4 million head of cattle on feed in the U.S. were housed in just 14% of the nation's commercial feedyards in 2019, which are heavily clustered in the central plains region and the highest concentration in Texas (Lowe and Gereffi, 2009). This suggests an inevitability that the majority of beef cattle in the U.S. supply chain will face a long-haul relocation at some point in their life that in conjunction with weaning, processing, receiving, and commingling will pose a psychological, physiological, and physical stressors response (Swanson and Morrow-Tesch, 2001; Araujo et al., 2010; Cooke et al., 2017). The consequential stress associated with these events elicits a disruption in endocrine and neuro-endocrine function as characterized by the activation of the hypothalamic-pituitary-adrenal axis (Carroll and Forsberg, 2007) and a subsequent inflammatory and acute-phase response from the innate immune system (Cooke, 2017) that negatively impacts growth performance and increase expenses resulting in

diminished margins and profit loss. It stands to reason that through an understanding of the adverse effects of these stressors producers can adopt or evolve management practices that mitigate or reduce the culminating impact of stress during this period in production.

Stress

Stress is defined as an interruption of homeostasis in the body resulting from the summation of biologic reactions to physical, emotional, or mental stimuli (Carroll and Forsberg, 2007). Hans Selye first introduced the concept of stress in the medical community over 70 years ago. He proposed that an organism responds similarly to different types of stressors in an attempt to maintain homeostasis (Selye, 1973). Understanding of stress has evolved to advocate that an organism does not activate an absolute response to stressors, rather prompts a proportionate physiological reaction necessary to return the body to a homeostatic state (Carroll and Forsberg, 2007). The extent of the physiologic consequences of stress have still not been fully revealed (Pacak and Palkovits, 2001); however, it is widely accepted that stressors impact the function of the immune system while eliciting different responses within the body through activation of the hypothalamic-pituitary-adrenal (**HPA**) axis and the sympathetic nervous system (Elenkov et al., 2000).

The segmented nature of the U.S. beef value chain renders cattle vulnerable to stress-induced compromises to their immune system during transitions in phases of production. Beef calves are exposed to several extremely stressful events in a relatively short period of time around six to nine months of age when they are weaned and shipped from the pasture to backgrounding or stocker operations (Araujo et al., 2010). In a matter of a few weeks, calves face the psychological stresses of being separated from their mothers while adjusting to new herd dynamics and compounded by the physical stress of being handled through processing facilities and the thermal stress, fatigue, and feed and water deprivation endured during

transportation (Cooke, 2017). As a result, endocrine or neuroendocrine function (physiologic stress) is disrupted as indicated by the activation of the hypothalamic-pituitary-adrenocortical axis (HPA, Carroll and Forsberg, 2007) and as a consequence negatively impacts cattle immune function and health (Blecha, 2000). Performance is directly affected by reduced feed and nutrient intake in stressed calves (Galyean et al, 1999) that further exacerbates the negative impact of stress on immunocompetence, and the pathological challenges met during the receiving period in the feed yard (Duff and Galyean, 2007).

The Hypothalamic-Pituitary-Adrenocortical Axis

In response to internal or external stressors, the sympathetic nervous system activates the HPA axis to support a fight or flight response. Neurotransmitters in the hypothalamus signal the secretion of corticotropin-releasing hormone (**CRH**) and vasopressin (**VP**) that bind with their respective receptors in the anterior lobe of the pituitary gland (Carroll and Forsberg, 2007). Although there is a suggested biological interaction by the presence of CRH and VP, both peptides can independently stimulate the release of adrenocorticotrophic hormone (**ACTH**) into the blood stream (Carroll et al, 2007). An increase in ACTH concentration in blood plasma arouses the release of glucocorticoids from the zona fasciculata in the adrenal cortex.

Glucocorticoids are species specific and evoke numerous biologic effects in the body ranging from the adjustment of reproductive and growth axes to regulation of the stress response, suppression of inflammation, and overall function of the immune system (citation here). Proper balance of glucocorticoids is essential for the balance of homeostasis and overall survival (Carroll and Forsberg, 2007).

Cortisol

Cortisol is the primary glucocorticoid produced in cattle (Salak-Johnson and McGlone, 2007) and plays an important role in gluconeogenesis during the fight or flight response by inciting the liver to metabolize fats and proteins into pyruvate, lactate, glycerol, and amino acids which are in turn converted into glucose for energy (Carroll and Forsberg, 2007). Cortisol, in conjunction with CRH, boosts the concentration of catecholamines, epinephrine, and norepinephrine which elevate heart rate, blood pressure, pupil dilation, tightening of the skin and gut, and improves blood flow to locomotive muscles (Fricchione et al., 2016). As previously mentioned, HPA activation is not a binary “on/off” function. Different stressors, such as thermal stress or stress from confinement, stimulate different regions of the paraventricular nucleus (**PVN**) of the hypothalamus stimulating the release of CRH (Sawchenko et al, 1996) and eliciting the release of appropriate proportions of cortisol and catecholamines (Salak-Johnson and McGlone, 2007).

Although cortisol concentrations are elevated during periods of extremis, the hormone is also an essential component necessary to maintain homeostasis. Cortisol provides a negative feedback signal to neurons in the PVN which inhibit CRH and catecholamine synthesis (Fulford and Harbuz, 2005) and control the magnitude and duration of the neuroendocrine stress response (Carroll and Forsberg, 2007). When the animal is relaxed and not in a stressful state, CRH secretion is restrained by basal levels of cortisol (Waselus et al., 2005). Chronic stimulation of the stress response and excessive concentrations of cortisol are extremely damaging to the animal causing excessive protein catabolism, hyperglycemia, immunosuppression (Carroll and Forsberg, 2007) and can ultimately result in death (Munck et al., 1984).

Hair Cortisol

Analyzing blood cortisol levels in cattle presents a unique set of challenges. Even low-stress cattle handling practices can trigger an undesired cortisol release masking the underlying effects and clouding the accuracy of a sample. Considering the detrimental effects chronic exposure to elevated blood cortisol levels has on the immune system and performance metrics (Dobson and Smith, 2000), it is essential to find a reliable approach to quantify stress. Traditional methods for cortisol level analysis have previously been blood, saliva, or fecal samples; however, these measurements can easily be compromised by the stress response resulting from common cattle handling and management practices. Alternatively, hair samples clipped from the tail switch of cattle (Burnett et al., 2014) to assess long-term changes in cortisol levels and chronic stress have proved an increased measure of reliability. Hair sampling for cortisol analysis has also proven successful in a variety of species including humans (Sauve et al., 2007) and dogs (Bennett and Hayssen, 2010). Cortisol accumulates gradually in tail hair through passive diffusion from blood into the growing hair follicle (Combs, 1987) as well as from the apocrine and sebaceous glands after the hair shaft is formed (Cone, 1996) resulting in a reliable metric for measuring periods of stress as evident by HPA activity.

Effects of stress on immunocompetence in the innate immune system

Cortisol is the most widely accepted biomarker of a neuroendocrine stress response in beef cattle (Sapolsky et al., 2000) regardless of the impetus for HPA activation (Crookshank et al., 1979; de Kloet et al., 2005; Carroll et al., 2007) and currently is regarded as the most accurate link between the effects of elevated stress on immune function (Glaser and Kiecolt-Glaser, 2005). As first shown by Cooke and Bohnert (2011) while simulating natural HPA activation in response to chronic stress through the intravenous injection of CRH in visibly

healthy beef cattle, acute increases in circulating cortisol levels during a stress event elicit transient increases in proinflammatory cytokines and a subsequent temporary immune response represented by an inflammatory reaction. However, prolonged periods of stress incite abnormally high levels of circulating cortisol that actually promote an anti-inflammatory response resulting in immunosuppression by decreasing immune cell synthesis of proinflammatory cytokines (Kelley, 1988). This suggests that stressors can also activate the acute-phase response in animals without the presence of a pathogen and establish the connection between proinflammatory cytokines and an acute phase response, as both mutually crucial components of the innate immune system (Murata et al., 2004).

Although both pro- and anti-inflammatory mechanisms are necessary for prompt homeostatic restoration following pathogenic infection (Kushner, 1982), when induced by external stressors these reactions may be superfluous and have detrimental effects by continually suppressing the immune system. It remains to be seen whether circulating levels of cytokines and acute phase proteins (**APP**) are causal or an effect but the presence of both have corroborated a positive correlation with bovine respiratory disease (**BRD**) in feedlot cattle (Young et al., 1996; Berry et al., 2004). Araujo et al (2010) reported a negative correlation ($r \leq -0.50$) between circulating concentrations of APP with dry matter intake (DMI) and average daily gain (**ADG**) in receiving cattle, providing further evidence that both proinflammatory cytokines and acute-phase responses vie for substantial amounts of organic resources in the animal to sustain vigorous innate and adaptive immune systems, which can result in decreased growth performance, increased maintenance requirements, and a decrease nutrient intake (Elsasser et al., 1997; Cooke, 2017).

Acute Phase Proteins

Acute phase proteins are a collection of serum proteins generated in the liver to support an acute phase response (Horadagoda et al., 1999) and have become a widely accepted biomarker in blood serum to detect inflammation, injury, and disease in cattle (Kushner and Rzewnicki, 1994; Baumann and Gauldie, 1994). In support of an acute-phase response to an immunological challenge, muscle protein is activated, and amino acids are absorbed into the liver to produce APPs (Moriel et al., 2015). Previous research has demonstrated that haptoglobin and ceruloplasmin are the most prominent APPs in cattle when presented with a CRH challenge, or stress response generated from events such as feedlot entry (Alsemgeest et al., 1994; Arthington et al., 2005; Arthington et al., 2008; Cooke and Bohnert, 2011, Cooke 2012b). Unlike in other species, haptoglobin is an accurate quantitative indicator to diagnosis and monitor an acute phase response in ruminants (Eckersall and Bell, 2010). Haptoglobin can increase in plasma concentrations 50 to 100 times normal detectable levels during an acute phase response (Conner et al., 1988, 1989; Gruys et al., 1993; Godson et al., 1996). Heegaard et al. (2000) modeled “strong and reproducible” haptoglobin concentrations correlated with the magnitude of a bovine respiratory syncytial virus (**BRSV**) infection even if the infection did not lead to pathological changes and associated tissue damage (Collins et al., 1996). For these reasons haptoglobin has become an extremely reliable indicator for exposing the sub-clinical presence of the bovine respiratory disease complex.

Bovine Respiratory Disease

The innate response in cattle to the physiological, psychological, and physical stressors during transitions in production phases, particularly entry into the feedlot, are major predisposing contributors to BRD (Duff and Galyean, 2007; Taylor et al., 2010). The BRD

complex is a multi-factorial infectious respiratory disease that is initiated by a viral infection that further compromises an animal's defenses complicated by a current or subsequent bacterial or mycoplasma infection that deteriorates lung function leading to pneumonia and death (Snowder et al., 2006).

Anatomically and physiologically speaking, cattle are highly susceptible to viral and bacterial respiratory infections during phases of compromised immunity, particularly due to their relatively small lung capacity compared with other livestock species. In contrast to horses with lungs of similar size and dimensions, cattle have approximately 30% less lung capacity (Gallivan et al., 1989) despite having an oxygen requirement 2.6 times greater than that of horses of a similar size (Hinchcliff et al., 2008). In addition, cattle have a relatively narrow trachea and a lung system that is partitioned into isolated lobes and lobules connected by interlobular septa that are capable of harboring aggressive bacterial pathogens and protracting respiratory infections (Cooper and Brodersen, 2010).

Pathogens associated with BRD are present in everyday life in the nasal cavity of feedlot cattle although research has shown a larger and more diverse population of opportunistic bacteria appearing in calves under stress (Algammal et al., 2020). In healthy cattle, the epithelial surface of the respiratory tract provides a "mechanical, chemical, and microbiological" barrier to BRD pathogens (Griffin et al., 2010). The epithelial cilia in the trachea are in continuous motion pushing mucus up to remove pathogens (Satir and Sleight, 1990). When bacteria are inhaled into the lungs and attempt to colonize the epithelial surface a mucus layer is secreted to prevent the bacteria from adhering to the infected surface (Caswell, 2014). A shielding layer of defensin peptides and surfactant proteins A and D6 render potential pathogens more susceptible to eradication from defensive macrophages and neutrophils, and ultimately, within four hours cattle with uncompromised immune systems are capable of eliminating 90% of inhaled bacteria (Griffin et al., 2010).

The most prominent viral pathogens associated with the BRD complex are bovine herpesvirus-1 (**BHV-1**), parainfluenza-3 virus (**PI₃**), bovine respiratory syncytial virus (**BRSV**), and bovine diarrhea viruses (**BVDV**; Srikumaran et al., 2007). These viruses can work individually or interactively and can be characterized by immunosuppression and an upper respiratory tract infection that paralyze mucosa, ciliary, and phagocytosis defensive functions. Although, PI₃ is generally an asymptomatic condition that can predispose lung tissue to a secondary bacterial infection (Veljovic et al., 2014), BHV-1 and BRSV typically have an incubation time of two to six days and include high fever, coughing, nasal inflammation and discharge, unwarranted salivation, increased respiratory rate, and conjunctivitis (Jones and Chowdhury, 2010; Raaperi et al., 2012;). How about BVDV? Add a statement here for the contribution of BVDV to the BRD complex (type I and type II BVDV)

A viral infection typically runs its course in four or five days (Grissett et al., 2015); however, a compromised immune system allows for the penetration of virulent bacterial pathogens found in the upper respiratory tract of healthy cattle into the lower respiratory tract, which causes bronchopneumonia and completing the typical BRD complex scenario (citation here). The most prominent bacteria linked to acute and chronic BRD are *Mannheimia haemolytica*, *Pasturella multocida*, *Haemophilus somnus*, and *Mycoplasma spp* (Callan and Garry, 2002; Hodgson and Manuja, 2005).

Economic impact of BRD on the U.S. beef industry

Bovine respiratory disease is the most pervasive and costly diseases in commercial feedlots across North America (Griffin, 1997). In 2017, the USDA reported that respiratory disease accounted for 23.9% and 26.9% of all non-predator death loss in cattle and calves, respectively (Peel, 2020). While BRD can be empirically estimated to be around a \$1 billion

detriment to the beef industry annually, it is estimated that annual prevention and microbial treatment costs surpass \$3 billion (Wang et al., 2018).

Lung lesions and necrosis attributed to BRD impairs growth performance as noted by reduced average daily gain and feed efficiency (Galyean et al., 1999). Although depression, appetite loss, and changes in respiratory rate can be observable signs of BRD (Griffin, 2014), Kiser et al. (2017) reported that lung lesions and abnormalities were prevalent in over 65% of cattle that appeared healthy, suggesting a far greater economic toll from sub-clinical BRD than previously thought (Kiser et al., 2017). Buchanan et al (2016) stated that cattle receiving one anti-microbial treatment in response to BRD symptoms had 1.3% to 5.3% lighter hot-carcass weight, a 7.5% to 26.5% lower yield grade, and a 2.4% to 5.3% reduction in marbling score compared with healthy cattle (Buchanan, 2016). Loss in carcass value as a result in BRD was further represented by Schneider et al. (2009), who reported a decline in carcass value of \$23.23 for cattle treated once, \$30.15 for cattle treated twice, and \$54.01 for cattle treated three or more times as compared to cattle that did not show symptoms requiring treatment. Cattle requiring subsequent anti-microbial treatments to fully recover continues to prove costly as depicted by Fulton et al. (2002), who reported an overall reduction in net return of \$40.64, \$58.35, and \$291.93 per head for cattle receiving one, two, or three treatments, respectively.

Social bonds in cattle

Cattle are a gregarious species that form very dynamic, complex, and stable matriarchal groups in typical grazing conditions, consisting of 40 to 50 head comprised mainly of mature cows and young heifers, their calves, and a few males (Sowell et al., 1999). These groups will graze a very distinct home range while maintaining separation from other groups (Roath and Krueger, 1982). Smaller and less formal groups will split off and shift in

size and composition as terrain and environment dictate, however, always fusing back together in the larger matrilineal group (Sowell et al., 1999).

Research has shown that cattle form fervent and long-lasting relationships through visual, vocal, olfactory, and physical interaction at a very early age (Reinhardt and Reinhardt, 1981; Bouissou et al., 2001). A mother cow will separate herself from the herd a few hours prior to giving birth. During the first few days of life a calf will remain hidden in the vegetation within a few meters of his/her mother. After four or five days the mother will rejoin remain on the periphery of the grazing herd with her calf staying close (Sato et al., 1987). Calves can be seen venturing away from their mothers 10 days after birth. Aside from a mid-morning suckling event and another later in the afternoon, calves will spend the majority of the day with calves of similar age from day 11 to 40 after birth (Vitale et al., 1986). At six weeks of age, calves will spend their days in kindergarten groups made up of between 20 and 25 of their peers supervised by only one or two cows (citation). Although group size may decrease by 5 to 6 months of age as calves spend more time grazing, this behavior is evidence of powerful social bonds and the strength of these relationships correlated to being established at an earlier age (Sato et al., 1987; Raussi et al., 2010). Accordingly, Niskanen et al. (2008) reported that calves that were born together tended to treat each other less aggressively than those calves that entered the group at a later age (Niskanen et al., 2008).

Commingling

Commingling is widely accepted to be a critical stressor in feedlot systems and is characterized as the destabilization of a social hierarchy when mixing cattle from multiple sources of origin (Duff and Galyean, 2007). Commingling provokes an acute and/or chronic stress response depending on how long it takes for a social structure to be restored and can be

amplified due to its proximity stresses associated with weaning, shipping, receiving, processing, and long-haul transportation (Cooke, 2017). Cattle form intricate smaller social groups within the herd and grouping cattle according to the ranch or even the pasture of origin does not guarantee that a social hierarchy has not been destabilized (Broom, 2003). Stress associated with social destabilization is often compounded by changes in environment and it can take anywhere from 3 to 7 days for social structures to stabilize and reform (Grant and Albright, 2001).

Economic circumstances force feedlot managers to feed large numbers of cattle in high spatial density conditions that infringe upon natural social distancing zones, and inadvertently spark agonistic interaction that is detrimental to growth performance (Kondo et al., 1989). During the first 48 hours after commingling, interactions can be intense and are overwhelmingly physical with displays of agonistic behavior gradually receding over the ensuing days (Cook and Nordlund, 2004). Larger group sizes require longer time periods to restore social stabilization, as cattle have difficulty remembering large numbers of individuals and their social status resulting in repeated physical and non-physical interactions in order to establish dominance in the social order (Kondo and Hurnik, 1990). While observing grazing dairy cows, Rind and Phillips (1999) reported that cows in groups of 16 or more, as compared to smaller group sizes, were more aggressive, maintained greater distances between groups, and displayed behaviors of underlying stress, alluding to a continued state of inter-cow competition (Rind and Phillips, 1999). In the majority of commercial feedlot environments, lower-order cattle are forced to continuously violate the space of dominate animals inciting confrontation and a continuous state of stress and disorder (Donaldson et al., 1971).

Our research objective

There has been extensive epidemiological research associating the adverse effect of commingling stress on the bovine immune system. Ribble et al. (1998) surveyed receiving yards that commingle cattle and reported that pens with fewer cattle sources had reduced incidence of BRD compared with pens containing cattle from a larger number of sources (Ribble et al., 1998). These authors, however, did not quantify number of sources nor evaluated performance and immune responses to commingling. In fact, no experimental research has investigated if number of cattle sources being commingled impact resultant stress, immunocompetence, and productive responses of receiving cattle. To fulfill this lack in knowledge, we hypothesized that commingling will elicit stress responses that impact immunocompetence and performance of feedlot receiving cattle, and such outcomes intensify according to the number of cattle sources mixed within the receiving pen. Therefore, a research experiment compared physiological, health, and performance responses of beef heifers assigned to different commingling schemes (1, 2, or 4 sources/pen) during a 56-d feedlot receiving period.

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CHAPTER II

**IMPACTS OF COMMINGLING CATTLE FROM DIFFERENT SOURCES ON
THEIR PHYSIOLOGICAL, HEALTH, AND PERFORMANCE RESPONSES
DURING FEEDLOT RECEIVING¹**

Introduction

Feedlot receiving is one of the most challenging phases within the beef production cycle, when cattle are exposed to a multitude of stressors that impair their immunocompetence and growth (Duff and Galyean, 2007). Commingling is recognized as a critical stressor during feedlot receiving, and typically occurs shortly after major stressful events such as weaning and road transport (Cooke, 2017). When cattle from various sources are commingled in the same pen, social hierarchy is destabilized and psychological stress reactions are provoked until social structure is re-established (Loerch and Fluharty, 1999). Commingling can be perceived by cattle as an acute or chronic stressor depending upon how much time is required for social structures to reform and stabilize (Grant and Albright, 2001).

Several epidemiological studies recognized commingling as a risk factor for bovine respiratory disease (**BRD**) in feedlots (Taylor et al., 2010). Nonetheless, few experimental research trials have attempted to examine the magnitude of commingling-induced stress and its consequences to immunocompetence and productivity of receiving cattle. Step et al. (2008) reported reduced performance and increased BRD incidence in receiving pens containing steers from multiple sources compared to pens with single source steers. Ribble et al. (1998) surveyed receiving yards that commingled receiving cattle and reported that pens with fewer cattle sources had reduced BRD incidence compared with pens with cattle from a larger number of

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sources. These research efforts, however, did not quantify number of cattle sources in commingled pens nor evaluated stress and physiological responses to commingling.

To our knowledge, no experimental research has investigated if number of cattle sources being commingled impact resultant stress, immune, and productive responses of receiving cattle. We hypothesized that commingling will elicit stress responses that influence cattle immunocompetence and growth, and such outcomes intensify according to the number of cattle sources mixed within the receiving pen. Therefore, this experiment compared physiological, health, and performance responses of beef heifers assigned to different commingling schemes (1, 2, or 4 sources/pen) during a 56-d feedlot receiving period.

Materials and Methods

This experiment was conducted at the New Mexico State University - Clayton Livestock Research Center (Clayton, NM). All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the New Mexico State University - Institutional Animal Care and Use Committee (#2018-028).

Animals and treatments

Ninety-six recently weaned Angus-influenced heifers were obtained from a commercial auction facility (Cattlemen's Livestock Commission Company, Dalhart, TX) and utilized in this experiment. Heifers originated from 4 cow-calf ranches and were reared in the same herd within each ranch since birth. Besides origin, no additional heifer management history was available. Heifers were not mixed with cohorts from other sources prior to and at the auction yard. On the day of purchase (d -2; 0800 h), heifers were loaded into two commercial livestock trailers (Legend 50' cattle liner; Barrett LLC., Purcell, OK), arranged in two sections of each trailer according to source (being 2 sources/trailer), and transported for 700 km (10 h) to stimulate the stress of a long-haul (Cooke, 2017). On d -2 (1800 h), heifers were unloaded and

immediately weighed [initial shrunk body weight (**BW**) = 239 ± 2 kg], and maintained in 4 paddocks according to source with ad libitum access to water and a complete starter feed (RAMP; Cargill Corn Milling, Blair, NE; Schneider et al., 2017) for a 36-h rest period.

On d 0 of the experiment, heifers were ranked by source and initial shrunk BW and allocated to 1 of 24 drylot pens (10×5 m; 4 heifers/pen) containing: 1) heifers from a single source (**1SRC**, n = 8), 2) heifers from 2 sources (**2SRC**, n = 8), or 3) heifers from 4 sources (**4SRC**, n = 8). Heifers were assigned to pens in a manner that initial shrunk BW was equivalent across pens and treatments, following the design illustrated in Figure 1. A solid construction tarp was placed on the sides of all pens, on top of the original metal pipe fencing, to minimize interaction of heifers from differing pens. All tarps were firmly secured using industrial-strength nylon ties to prevent tarp movement that would influence heifer behavior.

On d 0 of the experiment, heifers were vaccinated against *Clostridium* (Covexin 8; Merck Animal Health, Madison, NJ), *Mannheimia haemolytica*, *bovine respiratory syncytial virus*, *bovine herpesvirus-1*, *bovine viral diarrhea virus 1* and *2*, and *parainfluenza-3 virus* (Vista Once SQ; Merck Animal Health), administered an anthelmintic (Safe-Guard, Merck Animal Health), and received a growth-promoting implant (Synovex-H; Zoetis, Florham Park, NJ). Heifers had free-choice access to water and the aforementioned starter feed (RAMP; Cargill Corn Milling) from d 0 to 55, which was fed once daily (0800 h) in a manner to yield 10% residualorts (Colombo et al., 2019)

Sampling

Samples of starter feed were collected weekly, pooled across weeks, and analyzed for nutrient content (Dairy One Forage Laboratory, Ithaca, NY). Feed intake (dry matter basis) was evaluated from d 0 to 55 from each pen by collecting and weighing offered and non-consumed feed daily. Samples of offered and non-consumed feed were dried for 96 h at 50°C in forced-air ovens for dry matter calculation. Feed intake of each pen was divided by the

number of heifers within each pen, and expressed as kg per heifer/day. Heifer shrunk BW was recorded again on d 56, after 16 h of water and feed withdrawal. Shrunk BW values from d -2 and 56 were used to calculate heifer average daily gain (**ADG**) during the experiment. Total BW gain and feed intake of each pen were used for feed efficiency calculation. Blood samples were collected from all heifers on d 0, 6, 13, 27, 41, and 55 into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing either no additive or freeze-dried sodium heparin for serum and plasma collection, respectively. Hair samples were collected from the tail switch on d 0, 13, 27, 41, and 55 as in Schubach et al. (2017).

Heifers were observed daily for symptoms of BRD according to the DART system (Zoetis) and Sousa et al. (2019), using rectal temperature $\geq 40.0^{\circ}\text{C}$ (GLM-500, GLA Agricultural Electronics, San Luis Obispo, CA) as clinical criterion, and received antimicrobial treatment similar to Lopez et al. (2018). More specifically, heifers diagnosed with BRD received florfenicol with flunixin meglumine (Resflor Gold, Merck Animal Health) at 1 mL/7.6 kg of BW subcutaneously as the first antimicrobial administered, followed by a 5-d moratorium. Heifers diagnosed with BRD after first antimicrobial treatment were administered ceftiofur crystalline free acid (Excede; Zoetis) at 1 mL/30.3 kg of BW, followed by another 5-d moratorium. Heifers diagnosed with BRD after the second antimicrobial treatment were administered oxytetracycline (Bio-Mycin 200; Boehringer Ingelheim, Ridgefield, CT) at 1 mL/10 kg of BW. Heifers diagnosed with BRD after the third antimicrobial treatment would be removed from the experiment; however, none of the heifers were diagnosed with BRD after the third treatment. Heifer mortality was observed daily.

Laboratorial analyses

Feed samples were analyzed by wet chemistry procedures for concentrations of crude protein (method 984.13; AOAC, 2006), acid detergent fiber (method 973.18 modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp., Fairport, NY; AOAC, 2006), and

neutral detergent fiber using α -amylase and sodium sulfite (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp.). Net energy for maintenance and gain were calculated using the equations proposed by NRC (2000). Nutrient profile of the starter feed was (dry matter basis) 22.1% crude protein, 38.3% neutral detergent fiber, 19.1% acid detergent fiber, 1.83 Mcal/kg of net energy for maintenance, and 1.20 Mcal/kg of net energy for gain.

After collection, all blood samples were placed immediately on ice, centrifuged (2,500 \times g for 30 min; 4°C) for plasma or serum harvest, and stored at -80°C on the same day of collection. All plasma samples were analyzed for concentrations of cortisol (radioimmunoassay kit #07221106, MP Biomedicals, Santa Ana, CA; Colombo et al., 2019) and haptoglobin (Cooke and Arthington, 2013). Serum samples collected on d 0, 13, 27, 41, and 55 were analyzed for antibodies against *bovine respiratory syncytial virus* (#P00651-2; IDEXX Switzerland AG, Liebefeld-Bern, Switzerland), *bovine herpesvirus-1* (#99-41459; IDEXX), *parainfluenza-3 virus* (#P0652-2; IDEXX), *bovine viral diarrhea viruses* types I and II (#99-44000; IDEXX) and *Mannheimia haemolytica* (BIOK139 Monoscreen AbELISA; Bio-X Diagnostics S.A., Rochefort, Belgium). Only samples from heifers not diagnosed with BRD were analyzed for antibodies against BRD pathogens to ensure that this response was associated with vaccine efficacy rather than pathogenic infection (Callan, 2001). The intra- and inter-assay CV were, respectively, 5.5 and 6.8% for haptoglobin, 5.8 and 4.6% for cortisol, 3.1 and 5.7% for *bovine respiratory syncytial virus*, 1.0 and 4.1% for *bovine respiratory syncytial virus*, 3.6 and 3.5% for *bovine herpesvirus-1*, 2.8 and 4.9% for *bovine viral diarrhea viruses*, and 1.6 and 2.4% for *M. haemolytica*, Hair samples were analyzed for cortisol concentrations as in Schubach et al. (2017), with an intra- and inter-assay CV of 3.7 and 6.1%, respectively.

Statistical analysis

All data were analyzed using pen as the experimental unit, and Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. Quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), whereas binary data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.). All models included heifer source as independent fixed variable in addition to pen(treatment) and heifer(pen) as random variables, but for feed intake and efficiency that used pen(treatment) as random variable without heifer source as fixed variable. Model statements for BW parameters, feed efficiency, and morbidity-related results contained the effects of treatment. Model statements for feed intake, cumulative BRD incidence, blood and hair variables contained the effects of treatment, day, and the resultant interaction. Plasma, serum, and hair variables were analyzed using results from d 0 as independent covariate. The specified term for all repeated statements was day, with pen(treatment) as subject for feed intake and efficiency, and heifer(pen) as subject for all other analyses. The covariance structure used was first-order autoregressive, which provided the smallest Akaike information criterion and hence the best fit for all variables analyzed. All results are reported as least square means, or covariately-adjusted least square means for blood and hair variables. Orthogonal contrasts were tested to determine if number heifer sources within a pen yielded linear or quadratic responses. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10 . Repeated measures are reported according to main treatment effects if the treatment \times day interaction was $P > 0.10$.

Results

As designed, initial shrunk BW (d -2) did not differ ($P \geq 0.98$) among treatments (Table 1). Average daily gain and final BW also did not differ ($P \geq 0.60$) among treatments (Table 1).

No treatment differences were noted ($P \geq 0.56$) for feed intake and feed efficiency during the experiment (Table 1).

No treatment differences were detected ($P \geq 0.68$) for concentrations of plasma cortisol, plasma haptoglobin, and hair cortisol (Table 2), whereas day effects were detected ($P < 0.01$) for all these variables (Table 3). No treatment differences were detected ($P \geq 0.27$) for serum antibodies against BRD viruses and *M. haemolytica* (Table 2), which increased (day effects; $P < 0.01$) across treatments with the advance of the experiment (Table 3).

No treatment differences were detected ($P \geq 0.24$) for incidence of BRD (Table 4; Figure 2). Within heifers diagnosed with BRD during the experiment, no treatment differences were noted ($P \geq 0.12$) in the proportion of heifers that required one or two antimicrobial treatments to regain health (Table 4). However, the proportion of heifers that required three antimicrobial treatments to regain health increased linearly ($P = 0.03$) according to the number of sources within the receiving pen (Table 4). No treatment differences were detected ($P \geq 0.17$) for mortality rate during the experiment (Table 4).

Discussion

The heifers used in this experiment were considered high-risk as their management and health history were not fully known (Wilson et al., 2017; Sousa et al., 2019). All heifers were exposed to the stress of transport, initial processing, and exposure to a new environment within a 48-h period, whereas the combination of these stressors impact physiological and immune responses in cattle (Duff and Galyean, 2007; Cooke, 2017). The day effects noted for cortisol (plasma and hair) and haptoglobin concentrations across treatments corroborate that heifers experienced the adrenocortical and acute phase protein reactions provoked by road transport and feedlot entry (Cooke et al., 2013; Lippolis et al., 2017). These stress-induced physiological and inflammatory responses impair cattle immunity, corroborating the substantial incidence of

BRD observed in this experiment (Cooke, 2017). Therefore, this experimental model represented the stress and health challenges typically experienced by high-risk cattle during feedlot receiving (Duff and Galvayan, 2007).

Commingling is one of the main stressors experienced by receiving cattle, and considered a major predisposing cause for BRD in feedlots (Loerch and Fluharty, 1999; Taylor et al., 2010). Step et al. (2008) reported that average daily gain was reduced (1.25 vs. 1.34 kg/d) and BRD incidence was increased (41.9 vs. 11.1%) during a 42-d receiving period in pens containing steers from multiple sources compared to pens containing steers that originated from a single source. However, these authors acknowledged several shortcomings in their research design, including the contribution of previous steer management and genetic potential to research outcomes, different transportation distances, and an unknown number of cattle sources used in the multiple-sourced pens. Arthington et al. (2003) compared performance and acute-phase protein responses of single-sourced newly weaned calves assigned to a 2×2 factorial design, including road-transport and commingling with auction-originated calves as main factors. Commingling did not impact acute-phase and performance responses, but authors implied that previous management of auction-originated calves may have biased the commingling treatment. Ribble et al. (1998) reported a positive association between BRD incidence and number of cattle sources within receiving pens, but without quantifying number of sources nor evaluating cattle performance traits. Based on these gaps in knowledge, this experiment was designed to examine productive, physiological and immunological implications of commingling, while exploring different levels of commingling and balancing experimental treatments according to cattle source.

We hypothesized that commingling would heighten the cortisol and haptoglobin responses that cattle experience during feedlot receiving, which in turn would reduce performance responses and increase BRD incidence. Both adrenocortical and acute-phase

protein reactions are known to impair productive responses and immunocompetence in cattle, particularly when these reactions are elicited by stressors (Berry et al., 2004; Carroll and Forsberg, 2007; Cooke, 2017). However, heifer performance, physiological responses, and overall BRD incidence were not affected by commingling during the 56-d receiving period. Acquired immunity against BRD pathogens were also not impacted by commingling, as serum concentrations of antibodies against these antigens increased similarly across treatments during the 56-d receiving period (Richeson et al., 2008). These findings corroborate Arthington et al. (2003), although these authors did not report morbidity or BRD incidence in their study. Nevertheless, all the 1SRC heifers diagnosed with BRD regained health without the need for a third treatment, whereas this response increased linearly according to number of heifer sources within the receiving pen. Number of antimicrobial treatments to BRD is often associated negatively with ADG and feed efficiency in receiving cattle (Thompson et al., 2012; Blakebrough-Hall et al., 2020). These latter outcomes partially support our hypothesis as level of commingling impacted heifer competence to recover from BRD upon antimicrobial treatments, but without any benefits to heifer performance nor changes in the physiological responses measured herein.

Collectively, this experiment did not observe major negative impacts of commingling on performance and health responses of feedlot heifers during a 56-d receiving period. This is the first research investigating different levels of commingling and accounting for cattle source. To achieve our objectives, heifers were originated from 4 cow-calf ranches and housed in pens with 4 heifers, in a manner that commingling treatments and pens were balanced for heifer source. Either 1 or 2 heifers from the same source (4SCR and 2SCR, respectively) were housed together in commingled pens. Cattle are social animals and may form group sizes containing 20 to 100 individuals in free-living populations (Bouissou et al., 2001), whereas young cattle may form subgroups up to 25 individuals (Rankine and Donaldson, 1968; Sato et al., 1987).

Cattle also form intricate smaller social groups within the herd, and grouping cattle according to the ranch of origin may result in either conservation or disruption of social hierarchy (Hagen and Broom, 2003). Epidemiological studies reporting increased BRD in commingled cattle surveyed feedlots with large pen sizes (i.e. ≥ 50 animals/pen; Alexander et al., 1989; Ribble et al., 1998), whereas Step et al. (2008) evaluated commingling effects in receiving pens with 15 calves/pen. Therefore, the lack of substantial commingling effects noted herein can be associated with the number of heifers from the same source assigned to 2SRC and 4SRC pens, which may have limited the occurrence and subsequent disruption of pre-existing social groups during feedlot receiving.

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Table 1. Performance parameters of beef heifers commingled (**2SRC** = 2 sources; n = 8; **4SRC** = 4 sources, n = 8) or not (**1SRC** = single source, n = 8) with cohorts from different cow-calf sources during a 56-d feedlot receiving period.¹

Item	1SRC	2SRC	4SRC	SEM	Contrasts (<i>P</i> -value) ²	
					Linear	Quadratic
Initial body weight, kg	240	239	240	7	0.98	0.97
Final body weight, kg	287	290	286	7	0.85	0.72
Average daily gain, kg/d	0.849	0.887	0.813	0.076	0.66	0.60
Feed intake (kg/day)	6.48	6.55	6.32	0.23	0.56	0.67
Feed efficiency (g/kg)	132	136	127	9	0.66	0.67

¹ Reprinted with permission from “Impacts of commingling cattle from different sources on their physiological, health, and performance responses during feedlot receiving” by Wiegand et al., 2020. *Translational Animal Science*; Volume 4, Issue 4. Copyright 2020 by Translational Animal Science. Heifer shrunk body weight was recorded on d -2 (initial; after 10-h road transport) and d 56 (final; after 16 h of water and feed withdrawal), and used for average daily gain calculation. Heifers received a complete starter feed (RAMP; Cargill Corn Milling, Blair, NE) for ad libitum consumption from d 0 to 55. Feed intake was recorded daily measuring offer and refusals from each pen, divided by the number of heifers within each pen, and expressed as kg per heifer/d. Feed efficiency was calculated using total body weight gain (in grams), and total feed intake (kg of dry matter) of each pen during the experimental period.

² Orthogonal contrasts were tested to determine if number of cow-calf sources within a pen affected performance responses linearly or quadratically.

Table 2. Physiological responses from beef heifers commingled (**2SRC** = 2 sources; n = 8; **4SRC** = 4 sources, n = 8) or not (**1SRC** = single source, n = 8) with cohorts from different cow-calf sources during a 56-d feedlot receiving period.^{1,2}

Item	1SRC	2SRC	4SRC	SEM	Contrasts (<i>P</i> -value) ³	
					Linear	Quadratic
Hormones and metabolites						
Plasma cortisol, ng/mL	22.0	21.1	21.3	1.7	0.82	0.75
Plasma haptoglobin, mg/mL	0.848	0.844	0.896	0.089	0.68	0.86
Hair cortisol, pg/mg of hair	3.85	3.80	3.84	0.18	0.99	0.82
Serum antibodies against respiratory viruses						
<i>Parainfluenza-3 virus</i>	73.4	63.7	63.0	8.7	0.34	0.46
<i>Bovine respiratory syncytial virus</i>	84.2	81.7	80.7	16.5	0.88	0.94
<i>Bovine viral diarrhea viruses type I and II</i>	51.4	74.1	79.9	14.1	0.21	0.40
<i>Bovine herpesvirus-1</i>	182	175	195	22	0.59	0.63
<i>Maenhemia haemolytica</i>	56.1	49.7	65.3	8.26	0.28	0.33

¹ Reprinted with permission from “Impacts of commingling cattle from different sources on their physiological, health, and performance responses during feedlot receiving” by Wiegand et al., 2020. Translational Animal Science; Volume 4, Issue 4. Copyright 2020 by Translational Animal Science. Blood samples were collected on d 0, 6, 13, 27, 41, and 55. Hair samples were collected on 0, 13, 27, 41, and 56 as in Schubach et al. (2017). Results from d 0 were used as covariate in each respective analysis.

² Heifers received vaccination against respiratory pathogens on d 0 (Vista Once SQ; Merck Animal Health, Madison, NJ). Samples collected on d 0, 13, 27, 41, and 55 were analyzed and results expressed as sample:positive control ratio (%) as in Cooke et al. (2020). Results from d 0 was used as covariate in each respective analysis.

³ Orthogonal contrasts were tested to determine if number of cow-calf sources within a pen affected performance responses linearly or quadratically.

Table 3. Serum concentrations of antibodies against *parainfluenza-3 virus (PI3)*, *bovine respiratory syncytial virus (BRSV)*, *bovine viral diarrhea viruses types I and II (BVD-1)*, *bovine herpesvirus-1 (BHV)*, and *Maenhemia haemolytica (MH)*, plasma concentrations of cortisol (ng/mL) and haptoglobin (mg/dL), and concentrations of cortisol in tail-switch hair (**HC**, pg/mg of hair) from beef heifers during a 56-d feedlot receiving period.¹

Day	Serum antibodies against respiratory pathogens					Hormones and metabolites		
	PI3	BRSV	BVDV	BHV	MH	Cortisol	Haptoglobin	HC
0	27.6 ^c	36.5 ^b	20.6 ^d	92.0 ^b	30.3 ^c	19.4 ^d	0.878 ^c	3.51 ^b
6	-	-	-	-	-	9.22 ^f	1.57 ^a	-
13	41.7 ^b	63.6 ^a	34.8 ^d	123 ^b	46.2 ^b	14.5 ^e	1.06 ^b	3.75 ^b
27	69.5 ^a	79.6 ^a	52.9 ^c	192 ^a	47.7 ^b	24.9 ^c	0.736 ^{cd}	3.62 ^b
41	70.3 ^a	75.4 ^a	82.5 ^b	200 ^a	61.5 ^a	28.1 ^b	0.636 ^d	4.48 ^a
55	67.6 ^a	76.9 ^a	101.6 ^a	204 ^a	64.8 ^a	31.0 ^a	0.330 ^e	3.47 ^b
SEM	5.9	10.3	9.0	14	6.8	1.47	0.075	0.18
<i>P-value</i>	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

¹ Reprinted with permission from “Impacts of commingling cattle from different sources on their physiological, health, and performance responses during feedlot receiving” by Wiegand et al., 2020. *Translational Animal Science*; Volume 4, Issue 4. Copyright 2020 by Translational Animal Science. Within columns, values with different superscripts differ ($P \leq 0.05$). Serum antibodies results expressed as sample:positive control ratio (%) as in Cooke et al. (2020). Heifers received vaccination against respiratory pathogens on d 0 (Vista Once SQ; Merck Animal Health, Madison, NJ).

Table 4. Health responses from beef heifers commingled (**2SRC** = 2 sources; n = 8; **4SRC** = 4 sources, n = 8) or not (**1SRC** = single source, n = 8) with cohorts from different cow-calf sources during a 56-d feedlot receiving period.¹

Item	1SRC	2SRC	4SRC	SEM	Contrasts (<i>P</i> -value) ²	
					Linear	Quadratic
Heifers treated for respiratory disease, %	53.1	68.7	56.2	9.7	0.99	0.24
One treatment required	73.8	66.9	70.6	11.0	0.90	0.66
Two treatment required	31.7	20.9	8.88	9.89	0.12	0.78
Three treatments required	0.00	12.3	20.8	7.0	0.03	0.32
Mortality, %	9.37	0.00	3.12	3.54	0.35	0.17

¹ Reprinted with permission from “Impacts of commingling cattle from different sources on their physiological, health, and performance responses during feedlot receiving” by Wiegand et al., 2020. *Translational Animal Science*; Volume 4, Issue 4. Copyright 2020 by Translational Animal Science. Heifers were observed daily for symptoms of BRD according to the DART system (Zoetis, Florham Park, NJ), and received antimicrobial treatment as in Lopez et al. (2018).

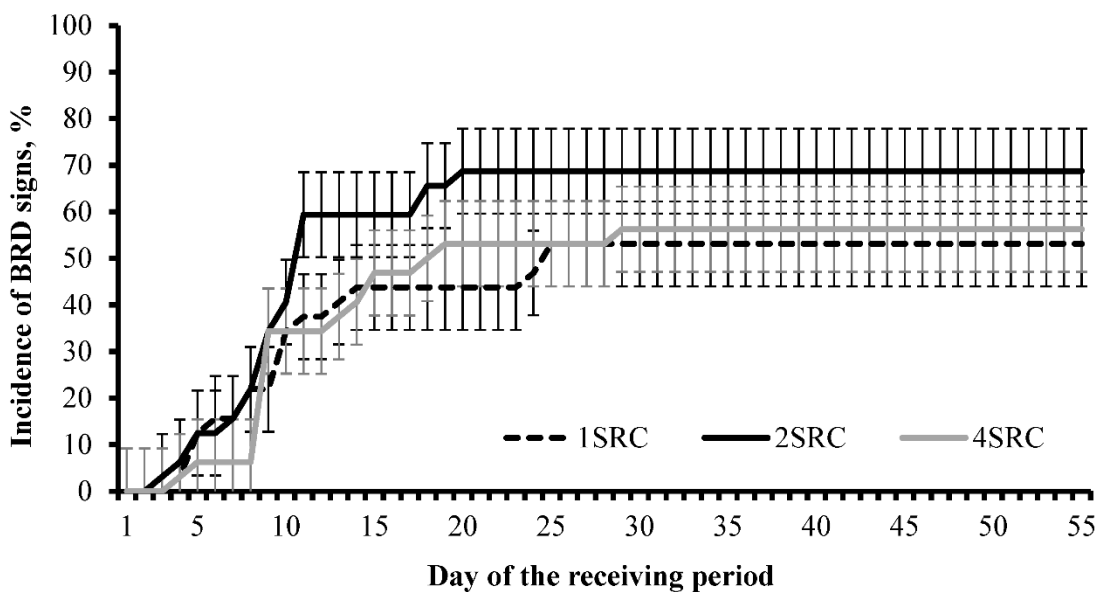
² Orthogonal contrasts were tested to determine if number of cow-calf sources within a pen affected performance responses linearly or quadratically.

Figure 1. Arrangement of heifers and pens according to cow-calf sources (A, B, C, or D) and treatments (**1SRC** = 1 source; **2SRC** = 2 sources; **4SRC** = 4 sources). Each pen contained 4 heifers, and each treatment contained 8 pens.¹

Pen 1	Pen 2	Pen 3	Pen 4	Pen 5	Pen 6	Pen 7	Pen 8	Pen 9	Pen 10	Pen 11	Pen 12
1SRC	2SRC	4SRC	1SRC	2SRC	4SRC	1SRC	2SRC	4SRC	1SRC	4SRC	2SRC
A	A	A	B	A	A	C	B	A	D	A	B
A	A	B	B	A	B	C	B	B	D	B	B
A	B	C	B	C	C	C	D	C	D	C	C
A	B	D	B	C	D	C	D	D	D	D	C
West ----- East orientation											
1SRC	2SRC	4SRC	1SRC	2SRC	4SRC	1SRC	2SRC	4SRC	1SRC	2SRC	4SRC
B	C	A	A	B	A	D	A	A	C	A	A
B	C	B	A	B	B	D	A	B	C	A	B
B	D	C	A	D	C	D	D	C	C	C	C
B	D	D	A	D	D	D	D	D	C	C	D
Pen 24	Pen 23	Pen 22	Pen 21	Pen 20	Pen 19	Pen 18	Pen 17	Pen 16	Pen 15	Pen 14	Pen 13

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Figure 2. Cumulative incidence of bovine respiratory disease (**BRD**) symptoms beef heifers commingled (**2SRC** = 2 sources; n = 8; **4SRC** = 4 sources, n = 8) or not (**1SRC** = single source, n = 8) with cohorts from different cow-calf sources during a 56-d feedlot receiving period. Heifers were observed daily for symptoms of BRD according to the DART system (Zoetis, Florham Park, NJ) and Sousa et al. (2019), and received antimicrobial treatment as described in Lopez et al. (2018). No treatment differences nor the treatment × day interaction were detected ($P \geq 0.24$).¹



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CHAPTER III

Research Conclusion¹

Commingling heifers from 2 or 4 different cow-calf sources did not impact performance and overall BRD incidence during a 56-d receiving period, despite increasing the need for antimicrobial treatments for BRD. Perhaps the number of heifers assigned to commingled pens, and resultant pre-existing social groups, was not sufficient to provoke major stress reactions from social disruption. Group size is directly associated with the time required for social stabilization, given that cattle have difficulty remembering large numbers of individuals and their social status to establish hierarchy (Kondo and Hurnik, 1990; Rind and Phillips, 1999). Although the daily change in plasma haptoglobin supported our prediction that the cattle would elicit an acute phase response to the stressors in which they were exposed, our model was unable to simulate an imbalance in defined social groups that would generate the antagonistic behavior that would clearly mark subsequent stress response as manifested by the occurrence of BRD.

In light of this, we speculate that the necessary social disruption can be obtained with a model that is more closely representative to the conditions encountered in a commercial feedlot. Therefore, experimental research to further explore the impacts of commingling receiving cattle are warranted, particularly designs using large pen and groups sizes typical of commercial feed yards (Alexander et al., 1989; Ribble et al., 1998). It is typical for feeder cattle to be transported to commercial feedlots in truckloads of 60 to 100 head and sorted and grouped into pens of upwards of 300 animals. It is our speculation that sizes with a greater number of sources and more

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than 10 head per source coupled with an uneven number of animals per source would be a logical next step to create the anticipated effect.

There may be further opportunity to isolate the weight of social stress on cattle immunocompetence through a study of cattle that are resorted during the last half of their time in the feed yard to obtain a more even distribution of frame size and composition prior to being shipped to the packing facility. This is a common practice in the southern and western plains regions; particularly with cattle of Mexican origin that exposed to longer than normal periods on feed.

We believe that as the first experimental approach to investigate the correlation between the number of sources with bovine immunocompetence, this body of work will serve as an important steppingstone to expose the further reaching implications of one of the costliest economic detriments on cattle feeding operations today.

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