THESIS

EVALUATION OF PROPATH FOR CONTROL OF LIVER ABSCESSES, PULMONARY LESIONS, AND HEAT STRESS OF FEEDLOT BEEF CATTLE MANAGED UNDER A NATURAL FEEDING PROTOCOL

Submitted by

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ABSTRACT

EVALUATION OF PROPATH FOR CONTROL OF LIVER ABSCESSES, PULMONARY LESIONS, AND HEAT STRESS OF FEEDLOT BEEF CATTLE MANAGED UNDER A NATURAL FEEDING PROTOCOL

Thirty-two pens housing from 249 – 282 beef cattle each were used to evaluate the efficacy of a novel trace mineral source for control of liver abscesses, heat stress, and lung lesions in a feedlot setting. Arrival date and sex were used as blocking factors for a randomized complete block experiment design, with a total of 11 blocks of steers and five blocks of heifers. Trace minerals of interest were Co, Cu, I, Mn, Se, and Zn. The two treatments that were used were: 1) Control (n = 16 pens), with inorganic sources for all trace minerals of interest; and 2) Test (n = 16 pens), with ProPath (Zinpro Corporation, Eden Prairie, MN, USA) used to provide additional AA complexes of Zn and Mn, complexed Co, and ruminally-protected folic acid to basal control diet. All cattle within both treatments were fed to meet JBS "Aspen Ridge" beef labeling requirements. Cattle were not administered any ionophores, antimicrobials, β-andrenergic agonists, or growth-promoting implants. Cattle identified as sick and pulled from pens for administration of antimicrobials were removed from the study. Cattle were fed for approximately 180d at a commercial feedlot in Eastern Colorado. Liver abscesses were scored using the Elanco Liver Check System (Elanco, Greenfield, IN, USA). Lungs of harvested cattle were evaluated for presence of lesions tags using the system described by Tennant et al (2014). To evaluate heat stress, cattle were observed twice monthly from June – September. Three observations per observation day were made at these times: 1) 0700 – 1000; 2) 1015 – 1315; 3)

1430 – 1700 (all times ± 30 min). Hide temperatures were observed caudal to left glenohumeral joints of 10 black-hided and, when available, 10 non-black-hided animals per pen. Within each pen, surface temperatures were observed at 3 locations on the cement bunk apron and 7 locations on the dirt surface. Temperatures were observed using a Fluke VT04 visual infrared thermometer (Fluke Corporation, Everett, WA, USA). Performance data were collected and evaluated on all cattle. Hide temperatures were greater on black-hided cattle than non-black-hided cattle ($P < 0.0001$) and on steers than heifers ($P < 0.0001$). Hide temperatures on Test cattle were greater ($P = 0.0008$) than temperatures on Control cattle, but this effect was small (0.251° C) and inconsistent across observation days (treatment within date interaction: $P < 0.0001$). Pen-surface temperatures were greater in Time 2 than Time 1 ($P < 0.0001$), but not different between Time 2 and Time 3 ($P = 0.37$). Hide temperatures on all cattle were correlated with pen-surface temperature ($R^2 = 0.43$). There were no differences between treatments for cattle observed open-mouth breathing (OMB, $P = 0.22$). Percentages of cattle observed OMB was different across all time points ($P < 0.01$). No differences were observed between sexes in Time A ($P = 0.50$) or Time B ($P = 0.36$), but percentages of heifers observed OMB were greater than percentages of steers observed OMB in Time C ($P = 0.01$; time point-by-sex interaction $P < 0.01$). There was also a significant time point-by-date interaction ($P < 0.01$). Based on these data, infrared hide temperature observed caudal to the glenohumeral joint is not likely to be a useful measurement of heat stress. ProPath did not lessen observed incidence of open-mouth breathing compared to inorganic sources of trace minerals in these cattle fed under a natural-feeding protocol. No treatment differences were observed for percent of livers containing any ($P = 0.62$), A+ ($P = 0.14$), A ($P = 0.88$), A- abscesses ($P = 0.63$). No significant differences were observed for sex for all liver abscesses ($P = 0.32$), A+ liver abscesses ($P = 0.82$), A liver abscesses ($P = 0.72$), or A-

liver abscesses ($P = 0.18$). No treatment differences were observed for percent of cattle with mild ($P = 0.64$), moderate ($P = 0.86$), or severe ($P = 0.30$) pulmonary lesions. For percentage of cattle observed with any lung lesions, no differences were found between treatments ($P = 0.51$) or between sexes ($P = 0.39$). A sex-by-treatment interaction was observed for cattle with severe lung lesions ($P < 0.01$). Control animals achieved higher ADG than Test cattle on both a deads-and fallouts-in ($P = 0.01$) and deads-and fallouts-out ($P = 0.03$) basis. Control cattle achieved higher G:F than Test cattle when analyzed on a deads-and fallouts-in basis ($P = 0.02$), but not on a deads-and fallouts-out basis ($P = 0.92$). Control cattle achieved greater HCW ($P = 0.03$), FT ($P = 0.04$), and marbling score ($P = 0.05$). No other differences were found in carcass metrics between treatments ($P > 0.05$).

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CHAPTER I: INTRODUCTION

Feedlot cattle are highly prone to incidence of liver damage, principally inflicted by liver abscess. Incidence of liver abscess is widely believed to be attributable to presence of *Fusobacterium nucrophorum ssp. necrophorum* that enters the liver through lesions in the rumen wall. This incidence is traditionally referred to as the "Rumenitis-Liver Abscess Complex" (Jensen et al., 1954; Nagaraja and Chengappa, 1998). Liver abscess incidence is associated with decreased ADG, G:F, HCW, and carcass yield (Nagaraja and Chengappa, 1998; Rezac et al., 2014; Amachawadi and Nagaraja, 2016). Additionally, liver abscesses are the cause most commonly associated with condemnation of livers of feedlot cattle in the United States (Nagaraja and Lechtenberg, 2007). Control of liver abscesses in cattle has historically been done through the use of feed-grade antimicrobials, with tylosin phosphate being the most commonly utilized antimicrobial (Nagaraja and Chengappa, 1998; Nagaraja and Lechtenberg, 2007). Tylosin has consistently been shown to be the most effective antimicrobial available in controlling liver abscess (Brown et al., 1973; Brown et al., 1975; Potter et al., 1985; Tan et al., 1994; Weinroth et al., 2019). Recent studies have indicated that *F. necrophorum* may not be the causative agent in incidence of liver abscess in feedlot cattle; rather, it may be only a vector for the causative agent commonly associated with liver abscess (Weinroth et al., 2017; Weinroth et al., 2019). It seems apparent that liver abscess is a multi-factorial disease of cattle. Given this, and given the increased public scrutiny surrounding the use of medically important antibiotics in livestock (Clark et al., 2012), development and testing of alternative methods of control of liver abscess incidence will likely be beneficial to feedlot producers in the future.

Feedlot cattle are often exposed to harmful viruses and bacteria. The impact of these pathogens often results in the common ailment of cattle known as Bovine Respiratory Disease Complex (BRD) (Lillie, 1974). This sickness is widely accepted as the most common and costly disease of feedlot cattle (Blakebrough-Hall et al., 2020). It is particularly problematic because diagnosis in live animals has been repeatedly shown to be inaccurate and/or simply miss symptoms in morbid cattle (White and Renter, 2009). Additionally, cattle experiencing subclinical levels of BRD often present no symptoms at all (Griffin, 2014). Evaluation of pulmonary lesions at harvest has been shown to be a more accurate means of diagnosis of BRD but is only possible after the animal is already dead. It has been repeatedly reported that rates of pulmonary lesion presence in cattle far outpaces rates of BRD diagnoses in live animals (Gardner et al., 1999; Schneider et al., 2009a; Leruste et al., 2012), thus indicating the need for better methods of control of BRD. Metaphylactic use of antimicrobials has been consistently shown to reduce incidence of BRD (Dennis et al., 2018). However, increasing scrutiny surrounding the use of antimicrobials in livestock production necessitates the finding of alternative methods of control. Nutritional management for improved health outcomes represents a potential management solution for BRD, particularly that which goes undiagnosed.

Environmental stress – particularly related to heat – has been shown to impact livestock performance through reduction in DMI, ADG, and G:F (Ray, 1989; O'Brien et al., 2010; Broadway et al., 2020). While management of heat stress has often relied on mechanical methods (e.g. provision of shade and/or fans), nutritional management through provision of feed additives or alternative ingredients has shown promise in reducing the negative impacts of heat stress (Mader et al., 2010b; Hales et al., 2014; Young et al., 2017). Provision of alternative

sources of trace minerals may mitigate performance and/or physiological responses to heat stress.

The literature reviewed herein encompasses different impacts, causes, and management strategies of hepatic abscesses, pulmonary lesions, and heat stress in feedlot cattle. Additionally, a general overview of different types of trace mineral sources is provided. The study reported herein is an evaluation of the efficacy of a novel trace mineral product for control of hepatic abscesses, pulmonary lesions, and heat stress in feedlot cattle.

CHAPTER II: REVIEW OF LITERATURE

2.1 LIVER ABSCESSES

2.1.1 Prevalence of Liver Abscesses

Liver abscess is the most common cause of liver condemnation of cattle in the United States (Nagaraja and Lechtenberg, 2007). It is well-accepted that liver abscess is the sequelae of rumen acidosis and rumenitis. This was first described as the "Rumenitis-Liver Abscess Complex" by Jensen et al (1954). Smith (1944) first published a report hypothesizing that liver abscess incidence in cattle is associated with rumenitis and lesions of the reticulo-rumen.

Liver damage is described in cattle carcasses as appearing in many different forms, including liver abscess, cirrhosis, liver flukes, and many others. It has been widely reported that liver abscess is the most common and most costly of liver abnormalities in terms of carcass quality and carcass value of cattle. Additionally, presence of severe liver abscesses is associated with diminished feedlot performance. This has been repeatedly shown in feedlot trials, audits of beef carcass plants, and surveys of beef producers (Roberts, 1982; Brink et al., 1990; Rezac et al., 2014).

Liver abscesses have been reported to be present in 12-32% of cattle on average (Nagaraja and Chengappa, 1998; Nagaraja and Lechtenberg, 2007), though prevalence of liver abscesses in cattle has been reported to be as high as 95% in some herds. Recent research has indicated that prevalence of liver abscess is related to region of origin of cattle (Weinroth et al., 2019). In addition, it has been often reported that different management strategies in feedlots (e.g. feeding times, feedbunk management, use of antimicrobials, etc.) are associated with

different rates of liver abscess incidence (Tan et al., 1994; Nagaraja and Chengappa, 1998; Tedeschi and Gorocica-Buenfil, 2018).

2.1.2 Evaluation of Liver Abscess

Liver abscesses are commonly assessed and scored using the Elanco Liver Check System (Elanco, Greenfield, IN, USA). The scoring system is as follows: 0 – no abscesses present; A- - 1-2 small abscesses and/or inactive abscess scars; A – 1-2 moderately-sized abscesses or >4 small abscesses; and A+ - 1 or more large abscesses and/or adherence of the liver to other abdominal tissue. Severely abscessed livers – that is, those with liver scores of A or A+ - are widely associated with significant economic losses in cattle (Brink et al., 1990; Brown and Lawrence, 2010).

2.1.3 Economic Impact of Liver Abscesses

Studies and surveys of slaughtered cattle have attempted to quantify the economic impact of liver abscess incidence in cattle. True estimates of economic impact of condemnation of livers due to damage is difficult, as comparison of discounts and economic losses across time leads to inaccurate use of dollar figures. However, it has repeatedly been shown that severe liver abscesses result in economic losses due to diminished feedlot performance, reduced carcass yield, reduced HCW, diminished G:F, and loss of carcass weight due to disposal of damaged livers (Brink et al., 1990; Nagaraja and Lechtenberg, 2007; Brown and Lawrence, 2010). Stock et al (1990) found that cattle with severe (A+) liver abscesses saw dressing percentages diminished by 1.2% when compared with their cohorts with other liver scores.

Estimates of incidence of liver abscess in feedlot cattle seem to most commonly be placed in the range of 10-20% of carcasses, and condemnation of severely abscessed livers is

widely reported to result in 2-3% loss in HCW (Johnson, 1991). These losses are estimated to translate to loss of $7M-$15M annually (Brown and Lawrence, 2010).

2.1.4 Etiology of Liver Abscesses

Since the first association of rumenitis with liver abscess was hypothesized by Smith in 1944, research into etiology of liver abscesses has commonly focused on understanding the causative agents. The "Rumenitis Liver Abscess Complex" has been widely associated with presence of *Fusobacterium necrophorum* since it was first described (Jensen et al., 1954). It has been commonly accepted that presence of *F. necrophorum* in the rumen wall increases as presence of lactic acid increases in the rumen (Elam, 1976; Nagaraja and Lechtenberg, 2007). It follows, then, that increase in presence of *F. necrophorum* is a sequela of episodes of ruminal lactic acidosis. Indeed, the connection between rumenitis resulting from ruminal acidosis and incidence of liver abscess caused by *F. necrophorum* has been made since Jensen et al. first described the "Rumenitis Liver Abscess Complex". This bacterium is particularly problematic because it has been shown to adhere to the ruminal epithelium and lesions left over from episodes of ruminal acidosis (Kanoe and Iwaki, 1987; Weinroth et al., 2017).

It is believed that rumenitis – inflammation of the rumen wall – results in parakeratosis of the rumen, ruminal lesions, and ruminal ulcers (Elam, 1976; Owens et al., 1998). Each of these conditions provides a pathway for *F. necrophorum* and other pathogenic agents to enter the portal system of the bloodstream. Portal blood is carried to the liver through the portal venous system, and these pathogens will often cause hepatic abscesses. Abscesses have been reported to disappear within 50-70 d of appearance, leaving behind inactive, fibrous scar tissue (Rezac et al., 2014).

The pathogenicity of *F. necrophorum* has been assumed to be due to a leukotoxin produced by the bacteria. This *F. necrophorum* leukotoxin has been presumed to be the causative agent and has commonly been the target of attempts to create a vaccine against the effectiveness of *F. necrophorum* (Nagaraja and Lechtenberg, 2007). Garcia et al. (1974) reported that lipopolysaccharide in the cell wall *of F. necrophorum* is likely the main causative agent in development of *F. necrophorum* leukotoxin.

Rumenitis is most commonly associated with rumen acidosis due to increased content of rapidly fermentable starch in ruminant diets (Owens et al., 1998). Rumen acidosis is associated with elevated levels of lactic acid in the rumen. Because of this, and the observation that *F. necrophorum* populations rapidly increase in the presence of lactic acid, it is believed that the presence of *F. necrophorum* is simply a matter of opportunity; it readily uses lactic acid as an energy source, but it does not ferment starch or sugars, therefore it grows rapidly in an acidic ruminal environment (Nagaraja and Lechtenberg, 2007). Since it is a facultative anaerobe that readily adheres to the epithelial cells of the rumen wall, it is able to grow rapidly in the right environment and easily find ulcerations of the rumen wall.

Trueperella pyogenes is the second most common bacterium found in bovine hepatic abscesses. Since it is not normally found by itself, and it is also not found in all liver abscesses, it is not believed to be a primary causative agent of liver abscesses. Additionally, *T. pyogenes* is more commonly found in abscesses from dairy cattle than beef cattle (Nagaraja and Lechtenberg, 2007). It is believed to a companion to *F. necrophorum*. However, *T. pyogenes* has been shown to be in higher concentration in abscesses of cattle fed tylosin than cattle not fed tylosin (Nagaraja et al., 1999). *Salmonella enterica* has recently been found in abscesses from Holstein

cattle, though its role as a causative agent has not been determined (Amachawadi and Nagaraja, 2015).

It is important to note that the rumenitis liver abscess complex is not only caused by episodes of ruminal acidosis, and the rumen is not the only bovine organ that *carries F. necrophorum*. *Fusobacterium necrophorum* has been found in the lung and spleen, though it has not been observed to produce severe abscesses in those organs (Abe et al., 1976a). The presence of *F. necrophorum* in other organs led to use of mice as a model for bovine hepatic abscesses, though the usefulness of this model is not clear (Abe et al., 1976b). Grooming behavior in cattle has been described as a cause of ruminal lesions, as hair has been found in bovine rumen walls and folds of the omasum (Brent, 1976; Bartle and Preston, 1992). It is interesting to note that sheep have not been found to have ruminal lesions caused from wool, though they have been shown to develop ruminal lesions when subjected to insertion of bovine hair into the rumen (Brent, 1976).

Finally, hepatic abscesses in cattle have been demonstrated to be more prevalent in some breeds than others. In particular, Holstein cattle fed for beef are more commonly found to have liver abscesses on inspection of their carcasses (Reinhardt and Hubbert, 2015). This is believed to be due to Holstein cattle being introduced to feedlots at lighter weights and being subjected to high grain diets for a longer period than typical beef breeds. It should be noted that percentage of Holstein cattle observed during the 2011 National Beef Quality Audit was 5.5% (Mckeith et al., 2012), while the percentage of Holstein cattle observed during the 2016 National Beef Quality Audit was 20.4% (Eastwood et al., 2017).

2.1.5 Control of Liver Abscesses

Control of hepatic abscesses in cattle has most often been reliant on antimicrobials in feed. Six antimicrobial products are approved for use as control mechanisms for liver abscesses: bacitracin, chlortetracycline, neomycin/oxytetracycline, oxytetracycline, tylosin phosphate, and virginiamycin. Tylosin is the only antimicrobial approved to be used in combination with the commonly fed ionophore monensin (Feed Additive Compendium, 2020). Tylosin has repeatedly been shown to be the most effective available antimicrobial in control of liver abscesses (Brown et al., 1973; Brown et al., 1975; Potter et al., 1985; Baba et al., 1989; Rogers et al., 1995; Nagaraja and Lechtenberg, 2007; Weinroth et al., 2019). However, it has never been shown to completely eliminate incidence of liver abscess, indicating that liver abscess incidence is a multi-factorial problem.

Incidence of liver abscess has often been attributed to development of ruminal acidosis due to feeding of diets high in rapidly fermentable starch. Length and management of the step-up period in cattle diets has received attention as a potential factor in development of liver abscesses in cattle. None of the literature reviewed here has shown an association with the length of the step-up period and incidence or severity of liver abscesses. The lowest level of concentrate in a starter diet reported in this literature was 48% DMB. Cattle that remain on high grain diets for longer periods of time have been repeatedly shown to exhibit more active liver abscesses at slaughter (Nagaraja and Lechtenberg, 2007). This could explain the reported increased levels of liver abscesses in Holstein cattle fed for beef when compared to rates of liver abscess incidence in traditional beef breeds. Intermittently feeding tylosin has been shown to have no difference in effectiveness compared to continuously feeding tylosin for the duration of the feeding period (Müller et al., 2018a), but this constitutes off-label use and therefore cannot be done under the

Veterinary Feed Directive. Laidlomycin propionate was reported by Galyean et al (1992) to show no difference in effectiveness in control of liver abscesses when compared with the combination of tylosin and monensin.

Attempts have been made to develop a vaccine against *F. necrophorum* leukotoxin. Vaccines have been found to be effective; however, they are not commercially available (Nagaraja and Lechtenberg, 2007). Additionally, vaccines for controlling *F. necrophorum* have been shown to be much more effective at higher ruminal pH on higher roughage diets, potentially limiting their usefulness in controlling liver abscesses related to the rumenitis-liver abscess complex (Checkley et al., 2005).

Recent attempts have been made to develop alternative means beyond antimicrobials for control of liver abscesses. Use of *Saccaromyces cerevisiae* fermentation products (SCFP) showed no difference when compared with use of no control method in formation of liver abscesses (Huebner et al., 2019). Zilpaterol hydrochloride was shown to reduce incidence of A-liver abscesses, but had no effect on larger, more severe abscesses (Montgomery et al., 2009). Essential oils and essential oil mixes have been thought to have antimicrobial properties. However, use of essential oils has not been shown to be useful in control of liver abscess incidence (Meyer et al., 2009). Addition of organic acids in feedlot diets was shown to not lessen incidence of ruminal acidosis, though malic acid did shorten the time that rumen pH dropped below 6.2 (Vyas et al., 2015). Supplementing antioxidants – α-tocopherol acetate, either alone or in combination with crystalline ascorbate – did not have any impact on liver abscess incidence or severity (Müller et al., 2018b)

Outside of antimicrobial use, the only control measure that has consistently been shown to decrease incidence of liver abscess has been increased roughage in the diet. Roughage sources

are generally more expensive than concentrates on a per-unit energy basis (Bartle and Preston, 1991), however, and increased roughage level has been shown to lessen feedlot performance (Birkelo et al., 1991; Bartle et al., 1994). The effectiveness of a given roughage source may be dependent upon the energy source used (Mader et al., 1991b). The effectiveness of roughage as a control mechanism is likely due to its capacity to stimulate buffering of the rumen by increasing production of saliva through increased rumination. Additionally, processing of grains and roughages has been shown to increase incidence of ruminal acidosis and has been associated with increased levels of liver abscess incidence (Johnson, 1991; Mader et al., 1991a; Kim et al., 2016).

2.1.6 Direction of Future Research

It seems apparent that the microbiome research and DNA sequencing will likely lead future discussions on control of liver abscesses. Increased understanding of the microbiome of the rumen, fecal microbiota, and microbial populations across different geographic regions is an area of emerging research, and it could potentially have profound impacts on scientific understanding of etiology and control of liver abscesses.

An approach that may be of more interest to the industry of cattle feeding would be to re-examine the structure and length of the step-up period on rumen health. It seems clear from nearly a century of scientific literature that rumen health plays a key role in the development of liver abscesses. Given this, it may be useful to focus on improving the health and development of the ruminal environment. Researching the effects of different roughage and concentrate levels in starter diets, length of the step-up period, and relative increases in concentrate levels at different points in the step-up period could prove beneficial in practical control of liver abscess.

Cattle receiving no anaphylactic or metaphylactic treatment for liver abscesses have been reported to have two-to-three times the rate of liver abscess incidence as cattle receiving tylosin (Brown and Lawrence, 2010; Weinroth et al., 2019). Given this and given concerns over use of medically necessary antimicrobials in livestock production, it is apparent that future attention must be directed toward developing new and better control mechanisms that do not include use of antimicrobials. Better understanding of etiological processes leading to development of liver abscesses in cattle will likely allow for better control techniques. The traditional understanding of *F. necrophorum* as the primary causative agent is ripe for re-investigation, as recent studies have indicated that it may just be opportunistic. That is, *F. necrophorum* may just happen to be well-adapted to thriving in an environment with high concentrations of lactic acid and is therefore available to migrate through the rumen wall and into the portal system. Better understanding of the ruminal microbiome may lead to more understanding of the development of ruminal lesions.

Management practices in feedlots should likely be a focus of future efforts at control of liver abscesses. However, this may be difficult to target, as Weinroth et al. (2019) clearly demonstrated that there is a significant geographical effect that plays a role in the development of liver abscesses. Better understanding of soil microbiota, effect of heat and cold stress, and climate impacts may lead to better understanding of etiology of liver abscesses.

Finally, it is apparent that length of time on high-concentrate diets is a key factor in development of liver abscesses. Recent trends toward keeping cattle on feed for longer periods and growing cattle to ever-larger sizes and weights likely play a key role in development of liver abscesses. These practices should be a target for scrutiny, as they do not necessarily place animal welfare at the forefront of management decisions in feedlots and packing plants. Additionally,

the beef industry as a whole should be very cautious about not selecting for a narrow range of genetic traits (e.g. rapid growth, large frame size, marbling, etc.), as other sectors of the livestock industry have shown that selecting for a small number of traits tends to lead to unforeseen health problems. It seems reasonable to assume that genetics likely play a key role in development of liver abscesses, and better understanding of pre-disposing traits can help lead to better breeding programs and more control mechanisms.

2.2 PULMONARY LESIONS

2.2.1 General Overview of Bovine Respiratory Disease Complex

It has long been widely accepted that the illness responsible for a majority of cases of morbidity in feedlot cattle is bovine respiratory disease (BRD). Unfortunately, BRD is a catch-all term referring to many different ailments that present similar clinical symptoms and are caused by many different etiologic agents. In general, it is believed that most cases of BRD begin with a viral challenge followed by a subsequent bacterial attack (Duff and Galyean, 2007; McGill and Sacco, 2020). This is referred to as the bovine respiratory disease complex (BRDC) (Lillie, 1974), and has typically been classified into three different categories of respiratory disease: 1) enzootic, referring to region-specific or seasonally-specific disease; 2) shipping fever, referring to respiratory challenge in the early feeding period; and 3) acute interstitial pneumonia, generally referring to disease in the later feeding period.

Acute interstitial pneumonia (AIP; formerly referred to as atypical interstitial pneumonia) is often fatal and can be particularly economically devastating. It is commonly found on necropsy in later feedlot deaths, and it is problematic to control. It is most often found in cattle that have been on feed for longer than 45 days (Loneragan et al., 2001; Panciera and Confer,

2010; Woolums, 2015). Its etiology is not well-understood, although it is a frequent target of research.

Shipping fever refers to onset of BRD early in the feeding period and is the most common form of BRDC. Shipping fever has received the bulk of attention in BRD research, and it is widely associated with decreased performance throughout the feeding period. Majority of BRD illness has been shown to occur in the first week after arrival at a feedlot, owing to numerous factors. In particular, energy balance state, stress of shipping and handling, and commingling at auction barns with cattle from different sources render animals susceptible to viral and bacterial challenges (Lofgreen et al., 1975). Elevated cortisol levels have been reported to be associated with shipping (Aich et al., 2007), suggesting a link between stress and increased susceptibility to shipping fever-related BRD.

Bacterial challenge is a key component of BRDC, particularly in shipping fever-related BRD. It is thought that bacteria are easily spread among animals in close-proximity with one another. In general four bacteria are most often found in lungs and airways of animals identified as suffering from BRD, either while alive or at harvest: 1) *Pasteurella multicida;* 2) *Manheimma haemolytica;* 3) *Mycoplasma bovis*; and 4) *Histophilus somni* (Dabo et al., 2008; Griffin et al., 2010). *M. haemolytica* is the bacterium most commonly identified in lungs of calves and sheep with pneumonia (Booker et al., 2008; Rice et al., 2008). It remains unclear whether this is due to *M. haemolytica* being the main causative agent or if it just happens to be readily available. Many different bacterial pathogens are commonly identified in nasopharyngeal pathways of cattle of all ages and are thus readily able to cause infections following some type of challenge or stressor (Murray et al., 2016a).

Bacteria are not generally thought to be effective BRD pathogens without an initial viral attack. Four viruses are generally thought to be the major causes of viral challenges associated with BRD (Lillie, 1974; Krehbiel, 2020): 1) infectious bovine rhinotracheitis (IBR); 2) bovine viral diarrhea virus (BVDV; more common in young calves); 3) parainfluenza virus type 3 (PI-3); and 4) bovine respiratory syncytial virus (BRSV). It has long been known that these four viruses are frequently exposed to virtually all been cattle in the United States (Kahrs, 1974). Other viral agents have been identified, but are not generally thought to be important in the US (Murray et al., 2016a).

Management at the feedlot level has generally relied upon use of anti-viral and antimicrobial pharmaceuticals. Use of these products for control and treatment of BRD is reviewed later in this paper. Managing against BRD at a feedlot is particularly challenging owing to many factors, not least of which is the commonly accepted belief that nursing through weaning time is the highest risk time for BRD (Griffin, 1997). Given that this generally occurs prior to arrival at a feedlot, and that BRD challenge at any point can have long term effects on animal performance, it is apparent that management at the feedlot level can only be of limited use. Additionally, herd level management, while the only practical method of management, is very difficult owing to variation in individual animal responses to viral and bacterial challenges. It is reasonable to assume that different individuals respond differently and show different symptoms to the same illnesses. Indeed, differentiated genetic (mRNA) responses across individual animals have been documented (Eitam et al., 2010). In their study, they reported that young calves with elevated levels of beta-glycan after shipping were more likely to have normal (i.e. no lesions) lungs at harvest. They further reported nearly 100% predictive power of BRD risk based on β-glycan level after shipping. This study was conducted on only 4d-old Holstein

bulls, with only 12 individuals. Its predictive power on different breeds or on a large scale is unclear. In assessing lung lesions at harvest, Kiser et al (2017) reported different pathogens associated with specific types of lung abnormalities (e.g. fibrin tissue, lung consolidation, hyperinflation), further indicating inherent difficulties in combating BRDC. Lillie (1974) was particularly prescient when he instructed veterinarians, "We must take care not to seek solutions to diseases only in the barrel of a syringe." Investigation of non-pharmaceutical management techniques will be useful.

2.2.2 Economic Impact of BRD

Assessing economic impacts of any ailment is challenging, owing to fluctuation in value of money and cattle over time. However, incidence of BRD is widely associated with decreased performance and carcass value. Lung lesions present at slaughter have been reported to be associated with decreased carcass value (Jaja et al., 2016). Cattle treated even once for BRD and/or having lung lesions present at harvest have often been reported to have decreased live weight, HCW, ADG, and marbling scores (Smith, 1998; Gardner et al., 1999; Reinhardt et al., 2009; Schneider et al., 2009b; Tennant et al., 2014; Krehbiel, 2020). This decrease in value has been shown to be a linear effect as the number of treatments increases from 0 to 1 to 2 to 3 (Holland et al., 2010; Blakebrough-Hall et al., 2020). Blakebrough-Hall et al (2020) reported that 73.3% of mortalities in their economic evaluation owed to BRD, underlining the particularly devastating effect of this disease complex. With even one pull for treatment, cattle were noted to have up to a 35% decrease in economic return on investment. Brooks et al (2011) reported similar findings in terms of decreasing returns as number of pulls for treatment increased from 0 to 1 to 2 to 3. This finding is not unique to feedlots, as decreased ADG associated with morbidity

related to undifferentiated BRD has been reported in stocker cattle on pasture (Pinchak et al., 2004).

This decrease in performance as measured by various metrics has been repeatedly demonstrated. Of particular note are repeated observations that performance impacts have been shown in cattle that were never identified as being affected with active BRD at the feedlot. Gardner et al (1999) noted that performance of cattle is more closely associated with presence of lung lesions at harvest than traditional clinical symptoms of BRD. Rezac et al (2014) reported decreases in ADG and HCW in cattle with pulmonary lesions identified at harvest, with particularly severe decreases in cattle with severe lesions. Alternatively, Jim et al (1993) reported no performance differences in cattle that had been treated ("sick") prior to processing and cattle that had not ("well"), though they also noted unexpectedly low incidence of disease that may have confounded results. This suggests that identification of disease at the feedlot level is unreliable as a tool to combat BRD. Subclinical (undiagnosed) BRD has been estimated to be worth nearly $1/hd/percentage point of cattle being in subclinical BRD; in other words, if 20% of cattle in a feedlot were subclinical, average carcass values would be decreased by $19.44 per animal (Griffin, 2014).

Performance is not the only driver of economic losses related to BRD, as treatment and prevention represent significant costs at the feedlot. Processing costs have been estimated to make up 2-6% of total cost of production during the feeding period (Griffin, 1997). In processing, metaphylactic use of antibiotics is estimated to add $538.2 million in value to feedlot cattle annually owing to decreased morbidity and mortality. Removal of metaphylaxis as a tool for BRD control is estimated to mean a 0.92% reduction in feedlot revenues (Dennis et al., 2018). These dollar figures and percentages were higher ($679.56 million; 1.17%) when

researchers used proprietary feedlot data rather than USDA data in their estimations. This analysis also assumed that no alternative treatment options would be used, which underlines the need for development of treatment alternatives that are both effective and cost effective.

In any estimate of economic value, it is important to note that performance losses related to BRD, or potential performance improvements owing to improved treatment of BRD, has economic impacts beyond simply the value of the cattle. Decreasing levels of BRD would have financial impacts on beef cattle producers, feedlots, processors, consumers, and competing industries such as poultry, lamb, and pork (Johnson and Pendell, 2017). These impacts should be considered in any economic analysis.

2.2.3 Subclinical BRD

Subclinical and/or undetected BRD has been reported to be very common (Blakebrough-Hall et al., 2020). Detection of BRD in a feedlot is generally performed by trained feedlot personnel on horseback ("pen riders") who observe cattle from within pens for nasal and ocular discharge, signs of depression, anorexia, elevated respiratory rates, and rectal temperature. These signs are commonly referred to as DART (Griffin, 2014). These observation methods are often unreliable, as cattle are prey animals and will often hide signs of weakness. The act of entering the pen by a pen rider is likely to put cattle "on alert" and cause them to hide any sign of discomfort, rendering diagnosis based upon clinical signs particularly difficult. In reviewing and analyzing available published data, diagnosis based on DART was found to have 61.8% sensitivity and 62.8% specificity (White and Renter, 2009). This means that both positive and negative diagnoses are estimated to have nearly 40% fail rates. They further estimated diagnosis of BRD based on pulmonary lesions at harvest to have 77.8% sensitivity and 89.7% specificity. While imperfect, use of pulmonary lesions represents a much better alternative to diagnosis.

However, it is only possible once the animal is already dead. This indicates a need for better controls than simply identifying and treating BRD cases. Only post-weaning phase cattle were evaluated in their study, so it is difficult to determine when illness occurred. In a study of Holstein steers raised for veal, Leruste and colleagues (2012) reported much higher incidence of pulmonary lesions at slaughter than clinical signs while the animals were alive. Gardner et al (1999) reported almost equal percentages of lungs showing lesions at harvest from cattle treated for BRD (37%) and those not treated for BRD (29%). Schneider et al (2009) reported a 8.17% of cattle being observed in active BRD, while 61.9% of cattle presented with pulmonary lesions at harvest. Leach et al (2013) summed up results by saying that lung lesions and feedlot health records are "very poor predictors of each other". It is clear, then, that undiagnosed BRD is far more prevalent than diagnosed BRD, indicating a need for better methods of control.

It has been noted that even among cattle identified as being in active BRD, treatment is very difficult due to unknown location in the lung and/or pathogenesis (e.g. viral, bacterial, or both). Even use of pulmonary lesions at the abattoir is unreliable, as lesions may heal (Buczinski and Pardon, 2020). Additionally, identification based on DART or other clinical observation methods may occur too late for treatment to be effective. Use of technology to track feeding behavior and plotting behaviors using statistical process control methods has been reported to detect BRD up to 4d earlier than pen riders (Quimby et al., 2001). More recent investigation has supported anorexia as an earlier indicator of BRD than clinical signs. Wolfger and colleagues (2015) found meal times, frequency of meals, and time between meals 7d before visual detection of BRD to be associated with BRD status. Febrile response when measured by rectal temperature loggers was shown to be an even earlier indicator of respiratory distress (Toaff-Rosenstein and

Tucker, 2018). These results highlight the inherent difficulty in identification of BRD, as well as the need for methods of earlier identification.

Many methods of scoring of pulmonary lesions at harvest have been utilized. The system described by Tennant et al (2014) is often used, with scoring categories as follows:

NORM – Normal lung

FIB – Presence of fibrin tags or adhesions between pulmonary lobes

5CON – Less than 5% consolidation of tissue of mycoplasma-like lesion

15CON – Greater than or equal to 5%, but less than 15%, consolidation of tissue or mycoplasma-like lesion

50CON – Greater than or equal to 15%, but less than 50% consolidation of tissue, pleural adhesion less than 50%, or less than 50% of lung missing

ALLCON – Greater than 50% consolidation of tissue, pleural adhesion greater than 50%, or greater than 50% of lung missing

2.2.4 Methods of Control of BRD

Control of BRD has traditionally relied of metaphylactic use of antimicrobials. This has been repeatedly shown to be effective and is not regulated by the Veterinary Feed Directive (VFD) (Dennis et al., 2018). In a 2011 survey, metaphylaxis was reported to be used to prevent shipping fever at 59.8% of feedlots, with 92.8% of feedlots with 8000 or more animals reporting use of metaphylaxis on at least some cattle (USDA, 2013). Risk for BRD has been shown to increase as in-transit shrink of cattle increases (Cernicchiaro et al., 2012a; Cernicchiaro et al., 2012b). This indicates that shipping distance may be a useful tool for identifying risk of BRD.

Indeed, BRD risk has been estimated to increase by 10% for every 160 km of transport distance (Sanderson et al., 2008). Richeson et al (2013) reported higher incidence of BRD in cattle as shipping distance increases, as well as when cattle of mixed sexes are shipped together. They further noted an increased risk when cattle arrive at the feedlot as bulls rather than steers. This may be due to added stress of castration in initial processing. Commingling cattle of mixed origins has been shown to increase risk for BRD (Sanderson et al., 2008; Step et al., 2008). This makes sense, as commingled cattle are likely to be shipped to an auction barn and mixed before further shipping to the feedlot. These added stressors are likely to increase susceptibility to BRD. Given these realities, use of pharmaceuticals for control of BRD on arrival at the feedlot is a sensible management decision.

Interpreting published literature can be difficult, but among widely-used antimicrobials, tulathromycin and tilmicosin appear to the most effective for lessening incidence of BRD (Wellman and O'Connor, 2007; Abell et al., 2017). Tilmicosin was shown to be effective in lessening incidence of BRD and improving feedlot performance (Brazle, 1997). This effect was reported whether tilmicosin was administered to all cattle on arrival or only administered to those cattle presenting elevated rectal temperatures (≥ 39.7 degrees C) at processing. This suggests that mass medication is likely an unnecessary management practice (Galyean et al., 1995a), though improvements in G:F and ADG were inconsistent in their 3 trials. Tulathromycin used at processing, followed after a 14d treatment moratorium by as-needed ceftiofur was shown to be more effective than tilmicosin followed after a 3d moratorium by enroflaxacin for control of BRD (Stegner et al., 2013). As antimicrobial resistance increases, effectiveness of antimicrobials changes. Currently, tulathromycin and tilmicosin are both widely used for reduction of BRD incidence.

Increased public scrutiny around use of medically important antimicrobials is likely to lead to diminished use of antimicrobials in livestock production in the future. Much of the national conversation surrounding use of antimicrobials in livestock is focused on bacterial antimicrobial resistance and unclear benefits of use of antimicrobials on all livestock rather than only those animals identified to be at risk. Antimicrobials, particularly those used for control of *M. haemolytica,* are not always effective owing to poor diagnosis, unclear causes of BRD, and antimicrobial resistance (Rice et al., 2008; Portis et al., 2012), indicating that other management interventions are warranted. It has been said that every time an antimicrobial is used, the user is selecting for resistance. Indeed, resistance to antimicrobials has been identified within populations of all the major identified bacterial pathogens considered important in BRD (Murray et al., 2016b). In analysis done in 2009, 5% of *M. haemolytica* isolates were found to be resistant to 5 or more antimicrobials; that percentage increased to 35% in 2011 (Lubbers and Hanzlicek, 2013). Mechanisms of resistance are unclear, but clearly problematic. It should be noted that the authors left tulathromycin out of their final analysis, but resistance to oxytetracycline and tilmicosin was very common and associated with resistance to 1 or more other antimicrobials. Compounding the issue of increasing resistance is poor diagnosis of the classes of BRDC diseases. Treatment for AIP has long been known to be difficult owing to unclear etiology and failures of trials to identify effective treatments (Curtis et al., 1979). Antimicrobials are often used, however, to little effect. Given these realities, management tools to improve BRD diagnostics and curtail the use of antimicrobials are warranted and will likely be a target for investigation for some time.

Despite thousands of published articles, respiratory diseases across species remain poorly understood. Thus, treatment of active respiratory illness is challenging. Injection of

antimicrobials on identification of active BRD is a strategy that is often used. This may be effective, though post-treatment intervals are unclear. Effectiveness is difficult to evaluate given natural immune responses in animals. Whether medicine or the animal's natural immune system is more responsible for recovery is often unclear (Apley, 2015). Babcock et al (2009) found that time between treatment and harvest mattered in terms of recovery of performance; the longer the interval, the heavier the HCW. They noted, however, that responses differed among individuals and different weights of cattle at both arrival and at time of first treatment, further illustrating the challenge of herd-level management of BRD. Interestingly, they noted decreased ADG for cattle treated further from harvest. This suggests that respiratory challenge in the early feeding period is particularly problematic. Booker and colleagues (2008) reported bacterial findings that were associated with one specific virus in most cases (e.g. BVDV associated with *M. haemolytica*), illustrating the difficulty in "identify and treat" programs. They noted many different combinations of viral and bacterial pathogens present on the same feedlot, illustrating the difficulty of targeting antimicrobial use for one disease. To combat this difficulty, use of more targeted metaphylaxis by focusing on higher risk cattle (e.g. cattle of auction/unknown origin, long transit distance, bulls, lighter calves, commingled cattle) has been suggested (Ives and Richeson, 2015). Additionally, cattle that have not been pre-conditioned (e.g. short weaning period or directly off pasture) are believed to be at higher risk for development of BRD. Purchasing only pre-conditioned cattle for placement in feedlots has been suggested as a solution, but it may be more expensive than simply using whole-herd metaphylaxis (Griffin et al., 2010).

Emerging technologies and disease modeling have shown promise for identifying high risk cattle. Babcock et al (2013) developed models that predicted BRD morbidity within 5% of

observed data, but this predictive effect was inconsistent throughout the year. In particular, the models' predictive power was diminished in fall. The authors suggested this decrease in predictive value owed to relatively higher percentage of cattle arriving from unknown origins (e.g. auction barns). They noted, however, that after as little as two days on feed for cattle the models' predictive value increased, indicating the need for continual monitoring of health status of animals. Use of a remote early disease identification (REDI) system to identify cattle that are likely to be challenged with BRD has been evaluated. Identification criteria used by REDI are based on individual animal behavior algorithms and have been reported to result in earlier identification and decreased overall use of antimicrobials than utilizing pen-riders to identify sick cattle (White et al., 2015). The cost of such a system must be weighed versus the cost of different control programs.

Richeson et al (2013) evaluated the use of complete blood counts (CBC) as a tool to identify high risk cattle. While many different components of CBC were identified as significant in their prediction model, actual use may be difficult owing to individual variation in blood parameters. However, high RBC count was consistently shown to be useful as a predictor. High RBC counts are often an indicator of dehydration, which can be an added stressor of the shipping process. Serum haptoglobin concentration has also been evaluated as a tool for making early treatment decisions. Cattle with elevated serum haptoglobin at processing were reported to have higher odds of being treated 3 times for BRD during their stay at the feedlot, but showed no difference in performance (Holland et al., 2011). However, Brooks et al (2011) reported serum haptoglobin concentrations having no effect on net returns or predictive use for number of BRD treatments. Evaluation of blood and/or serum parameters may be useful, but cost and/or time of testing may render their implementation unfeasible.

Use of ultrasonography as a tool for identification of high-risk cattle. Its utility as a predictor is unclear, however, as finding pulmonary lesions in cattle on arrival at a feedlot has been shown to not be predictive of future health outcomes (Abutarbush et al., 2012). Additionally, they reported nearly 10 minutes/animal in the squeeze chute, which may be untenable. Use of thoracic ultrasonography has been reported to be unable to differentiate between lung lesions from chronic and acute cases of pulmonary lesions from BRD (Buczinski et al., 2018). It was noted that this may be more useful as a tool to assist with culling of animals at dairies than identification of BRD risk on feedlots. Baruch et al (2019) found strong associations between many BRD diagnostic tools and lung scores and necropsy, but that was after inoculation with IBR and *M. haemolytica.* While useful, this information does little to inform treatment of subclinical or undiagnosed BRD. They further reported that increases in rectal and facial temperatures were useful for identification of BRD, but did nothing to identify the cause, whether viral, bacterial, or both. This further illustrates the inherent difficulty in treating BRD.

2.2.5 Direction of Future Research

It is clear from literature reviewed here that control of BRD is at best challenging due to the multifactorial nature of the disease complex. Future research is likely to involve many different control mechanisms. Utilization of genetic selection tools to identify disease-resistant animals may hold promise and is likely to be investigated by geneticists. Unclear, however, is the heritability of disease resistance as a trait.

Utilization of "chute-side" – that is, rapid-response technologies that can be employed during processing at the squeeze chute – diagnostic tools to identify those cattle at highest risk for BRD should, and likely will, receive attention in the future. This concept has been around for some time, as serum was evaluated as a predictor of BRD over 30 years ago (Martin and

Lumsden, 1987). They reported inconsistent results, however, and their results have proven difficult to validate. More recent investigations such as those conducted by Richeson et al (2013) indicate that chute-side diagnostics may be promising. However, diagnostic tools must be economical and rapid enough to make their employment on a large scale feasible.

Finally, given the clear evidence that subclinical and/or undiagnosed BRD is extremely prevalent, it seems apparent that nutritional strategies to combat BRD should receive investigation. Better management of ruminal health, improved energy status on arrival, and nutritional enhancement of immune systems all hold potential as strategies to combat BRD. Formulation of rations for higher concentrations of minerals and energy in starter diets is a common suggestion to compensate for low DMI early in the feeding period (Galyean et al., 1999; Duff and Galyean, 2007; Krehbiel, 2020). Lofgreen et al (1975) reported improved DMI and performance when starter rations contained 72% concentrate compared with rations containing 55% or 90% concentrate. While hardly a new concept, nutrition's role in animal health is clear. Nutritional manipulation may be the most feasible and easiest tool to implement on a large scale. Thus, extensive investigation of nutritional tools to combat BRD is warranted.

2.3 HEAT STRESS IN FEEDLOT CATTLE

2.3.1 Evaluation of Heat Stress

Evaluation of all kinds of environmental stress in feedlot cattle has been a target for research for many decades. This is due to repeated observations that heat stressed cattle exhibit lower ADG, depressed feed intake, and decreased G:F when compared with non-heat-stressed cattle (Ray, 1989; Gaughan and Mader, 2009; Blaine and Nsahlai, 2011; Broadway et al., 2020). Additionally, increased scrutiny surrounding animal welfare concerns in food animal production

have led to interest in researching methods of control of heat stress over the past 25 years. In general, cattle that can withstand extreme heat with little or no effect on traditional production metrics are said to be more heat tolerant.

Various measurement techniques have been evaluated and adapted to evaluate environmental stress and heat tolerance in cattle. Bianca (1961) defined heat tolerance as, "The ability of the body to endure the impact of a hot environment without suffering ill-effects," and noted that measuring body temperature appeared to be the best method to measure heat tolerance. Previous attempts to correlate respiration rate with body temperature found little or no correlation or use of respiration rate as a predictive tool for body temperature (Vernon et al., 1959). Brown-Brandl et al (2005) asserted, however, that respiratory rate is an acceptable measure of heat stress due to their observation that normal respiration rate exhibited little variation across individuals. They also noted that this indicates the necessity of commercial feedlot studies to determine long term impacts of heat stress. Heat tolerance has been found to be breed dependent, and different genotypes are better equipped for performance under heat stress conditions (Howard et al., 2013; Lees et al., 2018). *Bos indicus* cattle are generally more heat tolerant than *Bos taurus* cattle (Hahn, 1985; Finch, 1986; Blackshaw and Blackshaw, 1994; Lees et al., 2018), though Gaughan et al (2010) noted the challenges in making broad generalizations relative to heat tolerance owing to variation across individual animals. Within *B. taurus,* it is generally accepted that black-hided cattle are less heat tolerant than non-black-hided cattle. Busby and Loy (1996) reported increased death loss in black cattle on feedlots in Iowa compared with cattle of other hide colors. Black-hided cattle have also been shown to exhibit increased tympanic temperatures and panting scores when compared with non-black-hided cattle (Mader et al., 2002; Davis et al., 2003; Mader et al., 2006). This is an increasingly challenging point to

manage, as the percentage of black-hided bulls in the United States is reported to be as high as 65% and increasing (Corah, 2016).

It is thought that heavier animals are more susceptible to heat stress (Busby and Loy, 1996). In a study of Holstein bulls, Dikmen et al (2012) found that the heavier group of bulls consumed less dry matter per day than the lighter group of bulls. They further reported that heavier bulls spent more time standing during hot temps than lighter bulls. Interestingly, the heavier group of bulls preferred to eat and drink at night, while the lighter group preferred to eat and drink during the day when temperatures were high. This suggests that these bulls modified behavior to produce less metabolic heat during the hottest time of day. Daytime temperature has been reported to be the main driver of DMI in heat stressed situations (Koknaroglu et al., 2008), suggesting a lagged effect of temperature on DMI. Indeed, decreases in DMI due to heat stress events have been reported to lag behind onset of high temperatures for up to five days (Gaughan et al., 2010; Curtis et al., 2017). Gaughan et al (2010) noted, though, that these results were inconsistent, as some cattle responded to heat by decreasing DMI on the day of the heat event.

Optimal temperatures for livestock performance are generally referred to as being within the thermoneutral zone, which is bounded by the lower critical temperature (LCT) and the upper critical temperature (UCT). Temperatures outside of the thermoneutral zone have been shown to impact dry matter intake (NRC, 1981). The upper critical temperature for beef cattle has been shown to be approximately 25°C (Kelly et al., 1959; Lefcourt and Adams, 1996; Hahn, 1999). Temperatures above the UCT for beef cattle restrict the animal's ability to dissipate heat. In general, as the temperature outside the animal's body reaches a point closer to the animal's internal temperature, heat can no longer be dissipated through convection (Finch, 1986). This effect is further compounded in times of high humidity, as the animal's capacity for evaporative

cooling is restricted (Bianca, 1961). The Temperature Humidity Index (THI) (Thom, 1959; Mader et al., 2010a) was developed using ambient temperature (Ta) and relative humidity (RH) as to calculate one index. It has been used for many decades as an indicator of heat stress and is commonly calculated using the following equation:

Calculated THI = (0.8 * Ta) + [(RH * 0.01) * (Ta – 14.4)] + 46.4

Using this calculation, three levels of THI related to heat stress have been identified (Hahn, 1985; Mader et al., 2006; Hagenmaier et al., 2016):

1) THI ≤ 74 = Alert

2) 74 < THI < 79 = Danger

3) 79 ≤ THI < 84 = Emergency

Use of THI as an indicator of heat stress is limited, however, as it only accounts for Ta and RH. While these have been found to be major drivers of heat stress, other environmental factors such as wind speed (WS) and solar radiation as represented by black globe temperature (BG) are known to play a role in heat stress. These factors were utilized by Gaughan et al (2008b) to calculate the Heat Load Index (HLI) with the following equations:

$HLI_{BG > 25} = 8.62 + (0.38 * RH) + (1.55 * BG) – (0.25 * WS) + e^{(2.4 – WS)}$, and

$HLI_{BG < 25} = 10.66 + (0.28 * RH) + (1.3 * BG) – WS$, where e is the base of the natural logarithm. It should be noted that both THI and HLI are used extensively in recent literature as worthwhile measures of environmental heat stressors.

Negative effects of high temperatures on cattle performance are thought accumulate over time. Brown-Brandl et al (2005) found that yesterday's temperature has a major impact on

today's heat stress. This lagged effect has been known for some time, as Bianca (1961) noted that increases in body temperature (Tb) due to increases in Ta were not immediate, as well as that heat tolerance appears to lessen at the end of the summer. It has been reported that temperature at midnight is the second biggest driver of DMI behind daytime temperature (Koknaroglu et al., 2008). This is in agreement with previous work that showed that nighttime cooling is necessary for the animal to offload stored heat (Hahn, 1999; Gaughan et al., 2008a). Without a sufficient drop in temperature overnight, cattle are unable to offload all of the heat absorbed during the day. This stored – or "accumulated" – heat is added to the following day's heat load. This effect is referred to as Accumulated Heat Load (AHL) (Gaughan et al., 2008b). This can be an especially difficult challenge to manage, as offloading of accumulated heat requires more energy than absorption of heat (Parkhurst, 2010). This indicates that equal amounts of time of cool temperatures and hot temperatures will not be sufficient to avoid accumulation of heat. Cattle that are unable to sufficiently offload accumulated heat at night have been found to reach higher BT the following day when measured using tympanic measuring devices (Mader et al., 1999; Mader et al., 2010b).

Research into impacts of heat stress on feedlot cattle often requires some visual assessment of heat stress. Classic signs of heat stress in cattle include bunching, increased salivation, elevated respiratory rates, panting, and open-mouth breathing (OMB). Bunching is a curious reaction to heat stress, as cattle that are bunched together seem to have a diminished ability to dissipate heat. Nevertheless, bunching has been observed during periods of high heat (Wieman et al., 1992). This behavior is also thought to be a way to lessen the impacts of biting flies, so its utility as a heat stress indicator is unclear. Most often, respiratory rates and panting are used as visual signs of heat stress. Panting is often scored on a scale of 0 – 4 (Mader et al.,

2006; Brown-Brandl et al., 2010; Sullivan et al., 2011; Mader, 2014; Unruh et al., 2017), using the following scores as guide:

0 = normal respiration

1 = increased respiration

2 = moderate panting

3 = OMB

4 = OMB with tongue out

These are referred to as "panting scores" (PS) and are generally thought to be a useful way to assess heat load (Gaughan and Mader, 2014). Panting scores have repeatedly been shown to be higher in afternoon than in morning regardless of morning body temperature or hide color (Gaughan and Mader, 2014; Unruh et al., 2017). These PS can be challenging to evaluate in a large scale, commercial setting, however, as pen stocking densities and sizes make reasonable estimations of PS difficult. Other literature has evaluated heat stress solely on the basis of OMB, defined as mouth open and tongue exposed (Johnson et al., 2010; Hagenmaier et al., 2016). In a commercial setting, evaluating heat stress with this metric is more reasonable, as simply counting the observations of OMB in a pen can be more readily accomplished.

2.3.2 Economic Impact of Heat Stress

Severe economic impacts of heat stress in beef cattle have been reported due to both losses in performance and losses of livestock during heat waves. Performance losses are reasonable to expect, as temperatures above the UCT have been repeatedly shown to decrease DDMI, ADG, and G:F. Ray (1989) noted 18% decrease in ADG, 9% decrease in DDMI, and an

increased NEm requirement in summer of 9% compared to other seasons. Heifers subjected to heat stress recently again exhibited a decrease in DMI (Broadway et al., 2020). While decreasing DMI may lead to improvements in diet digestion by decreasing passage rate (Beede and Collier, 1986), it is reasonable to conclude that decreases in DMI are a primary driver of decreased performance during episodes of heat stress. Indeed, Holstein bull calves subjected to heat stress were found to achieve lower ADG and G:F, with decreased DMI described as the main causative factor (O'Brien et al., 2010).

Economic losses due to heat stress are difficult to quantify across time owing to the dynamic nature of the value of money and prices of cattle. Attempts have been made to quantify losses, however. These attempts have generally focused on heat wave events in specific years. In one particularly bad heat wave, survey results indicated mortality of 2.3% of cattle on feed in Iowa in one week (Busby and Loy, 1996). In attempting to quantify the annual economic impacts of heat stress, St-Pierre et al (2003) estimated losses to be $2.4 billion annually across all US livestock species, with beef cattle specifically accounting for $369 million in annual losses. It is reasonable to conclude that losses are significant, as decreases in feed efficiency add to the costs of production for livestock.

2.3.3. Methods of Control of Heat Stress

Various methods have been proposed to control all kinds of environmental stress in livestock. Evaluation of control methods has proven to be difficult for many reasons, particularly the demonstrated ability of livestock to adapt to heat stress situations over time and compensate for losses during episodes of heat stress (Mader and Davis, 2004; Brown-Brandl et al., 2005; Sullivan et al., 2011). In general, two main areas of focus have been evaluated for control of heat stress in feedlot cattle: 1) environmental adaptation; and 2) nutritional management. Provision of

shade, sprinkling of pen surfaces and cattle, feed additives, and time of feeding have all shown promise as methods of control of heat stress.

2.3.3.1 Provision of Shade

Impacts of provision of shade on livestock performance are controversial. Shade has often been proposed to improve performance, and indeed has been demonstrated on many occasions to improve performance of feedlot cattle, though it is hardly considered a standard provision on cattle feedlots. In a recent survey of feedlots in the High Plains region of the United States, only 17% reported providing shade in pens (Simroth et al., 2017), likely owing to the high initial cost of installing shade structures. For those feedlots providing shade, it was not reported what percentage of pens included shade structures. Shade has been reported to dramatically decrease death loss in extreme heat events (Busby and Loy, 1996), indicating that animal welfare may be improved through adequate provision of shade.

Provision of shade has often been evaluated in order to improve DMI during heat stress events. Impacts of shade on DMI are unclear, however, as mixed results have been reported. In some cases, shaded cattle have been reported to achieve higher DMI than unshaded cattle (Mitlöhner et al., 2001; Mitlöhner et al., 2002; Hagenmaier et al., 2016). Other studies, however, have not shown this effect (Blaine and Nsahlai, 2011; Sullivan et al., 2011; Lees et al., 2018). In particular, Sullivan et al (2011) reported higher DDMI for unshaded cattle than for shaded cattle. In their study, unshaded cattle dramatically reduced intakes on very hot days, but followed those decreases with seven days of enhanced intakes. This is in agreement with other reports of compensatory behaviors after heat stress events.

Other performance metrics have often demonstrated benefits of utilizing shade in a feedlot setting to combat heat stress. Final BW and ADG have been reported to be higher in shaded cattle versus unshaded cattle (Mitlöhner et al., 2001; Mitlöhner et al., 2002), though these studies have not reported a change in G:F through provision of shade. Even in studies where no positive impact on DMI was reported, provision of shade was shown to have performance benefits, including improved ADG and G:F (Blaine and Nsahlai, 2011; Sullivan et al., 2011; Lees et al., 2018). Mitlöhner et al (2002) reported a higher percentage of heifer carcasses grading choice when shaded due to lesser amount of dark cutters on carcasses compared to unshaded heifers.

Lees et al (2018) reported fewer cattle observed lying down in unshaded pens during heat stress events. This may be due to increased surface temperatures in unshaded pens. Soil surface temperature in pens is an additional stressor in heat events and is related to Ta (Mader et al., 2010b; Brown-Brandl et al., 2017). This suggests that provision of shade may reduce heat from underneath the animals in addition to mitigating the effects of solar radiation. Rumen motility may be improved by use of shade, as animals lying down at rest experience more rumen contractions.

2.3.3.2 Sprinkling

Wetting of pen surfaces and/or of cattle as a means of heat abatement has been investigated. Much of the work that has been reported has focused on effects of sprinkling on PS and Tb, with less focus on traditional performance metrics. Mader and Davis (2004) reported a tendency for cattle to consume more feed when sprinkling of pen surface occurred in the morning rather than the afternoon, but this stimulation only occurred under mild heat stress. They noted no differences in intake during periods of severe heat stress. Interestingly, they

reported higher marbling scores in cattle from pens that were not sprinkled versus those pens that were sprinkled. Reasons for this difference are unclear.

It seems apparent that there exist animal welfare benefits to well-timed sprinkling of pen surfaces and/or cattle when heat stress events are expected. Sprinkling of pen surfaces has been shown to lower THI (Mader et al., 2007), though it should be noted that sprinkling after the onset of heat stress may not be beneficial. Sprinkling of both cattle and pen surfaces was shown to decrease tympanic temperature of cattle if sprinkling occurred prior to the hottest part of the day (Davis et al., 2003). Gaughan et al (2008a) suggested sprinkling at night rather than during the day when heat stress is expected, as they noted that rapid changes in environmental temperature can be difficult for cattle to cope with. Given this observation, and given the observation by Mader et al (2007) that sprinkling of pen surfaces lowered THI, it is reasonable to expect nighttime sprinkling of pen surfaces would allow for great offloading of accumulated heat by heat stress cattle, thereby lessening the effects of daytime heat.

Wetting of cattle during handling has been investigated as a means to combat increases in BT after handling (Brown-Brandl et al., 2010). They noted average increases in BT of 1.13°C when cattle were moved for processing. Cattle that were wetted at the squeeze chute reached lower peak BT, had a shorter recovery time before returning to normal BT, and had lower PS than cattle that were not wetted. They suggested that wetting of cattle during processing may be a good welfare practice, and it may lower incidence of odor emissions associated with wetting of pen surface.

2.3.3.3 Time of Feeding

Altering of feeding times has shown promise in combating heat stress, particularly in well-conditioned, grain-fed, feedlot cattle. It is known that digestion and fermentation of feedstuffs results in metabolic heat production. This "heat increment" represents not only lost energy, but also an increase in heat load, particularly when the Ta is sufficiently high to prevent cattle from dissipating heat. Thus, altering feeding times such that peak heat production is reached during the cooler period of the day has been investigated.

It has been noted that feeding of animals at any point during the day stimulates feed intake. Feeding cattle in the late afternoon was reported to result in increased heat production during the cooler hours of the day when compared to feeding late in the morning or early in the afternoon (Brosh et al., 1998). This was later supported when Davis et al (2003) found that feeding cattle in the afternoon rather than the morning resulted in lower tympanic temperatures of cattle during the hottest part of the day. This was particularly true when feed bunks were kept empty for a few hours prior to feeding. Limiting of feed intakes has been shown to result in lower peak tympanic temperature when compared to ad libitum feeding (Mader et al., 2002). This suggests a mitigating effect of altering the time and/or amount of feeding on heat stress. More investigation into effect of altering feeding times and amounts on PS and performance metrics is warranted.

2.3.3.4 Diet Manipulation

Manipulating diets through the use of alternative feed ingredients and feed additives has is thought to have an impact on reducing heat stress, both due to reduction of heat increment and replacement of electrolytes (Blackshaw and Blackshaw, 1994; Sullivan et al., 2011). Heat

production from high fiber, roughage-based diets, is traditionally thought to produce more heat than feeding high concentrate diets due to increased fermentation times. Indeed, on a per-unit-energy basis, high roughage diets produce more heat (Sullivan and Mader, 2018). Interestingly, though, feeding diets higher in roughage has been shown to increase DMI during periods of extreme heat (Mader et al., 1999). This was true when cattle were fed diets with 40% roughage versus those fed diets with 25% and 10% roughage DMB. This may be due simply to an energy deficit in cattle fed high roughage diets.

Evaporative cooling is one method cattle use to combat the effects of high heat. This is done primarily through sweating when RH is sufficiently low to allow for evaporation. While a useful cooling method, ruminant sweat contains electrolytes, including Na, Mg, Ca, Cl, and particularly high levels of K (Beede and Collier, 1986). Systemic deficiency of K may partially explain the increased levels of DMI reported by Mader et al (1999) in heat stress situations. Manipulating the dietary cation-anion difference (DCAD) to be more positive – say, 15 – 30 mEq/100g DM – in summer time has been suggested as a potential method to avoid excessive release of electrolytes in sweat (Mader et al., 2010a).

Feeding increased levels of fat is thought to lessen the impacts of heat stress, as digestion of fat is widely believed to emit a lower heat increment than digestion of protein or carbohydrate. Supplementing extra fat with no mechanical (e.g. fans and/or sprinkling) methods of cooling was not reported to impact DMI, but supplementing fat in addition to fans and sprinkling increased DMI in heat stress situations (Gaughan et al., 2008a). Gaughan and Mader (2009) reported depressed DMI and increased daily water intake when cattle were supplemented with higher levels of salt and fat in severe heat situations. This may suggest that supplementation of fat and salt leads to more cooling behaviors, however whether salt or fat alone increase water intake is

unclear. Water's utility as a cooling agent, as well as the physiological response to high heat of increasing voluntary water intake, has been known for some time (Winchester and Morris, 1956).

Common feed additives have shown promise in combating the negative effects of heat stress on behavior and performance. Heifers fed melengestrol acetate were reported to have less death loss compared with all other classes of cattle in a severe heat wave event (Busby and Loy, 1996). Supplementation of ionophores when THI was high appeared to lower maintenance needs, as intake was not reduced but performance was improved (Barreras et al., 2013). Boyd et al (2015) reported lower intraruminal temperatures in cattle supplemented with zilpaterol hydrochloride (ZH) compared with those not supplemented with ZH. This may be due to less heat of fermentation, as DMI was lower in ZH-supplemented cattle. Previous work, however, showed no difference in respiration rates or PS in cattle fed ZH (Hales et al., 2014), so its potential as a heat stress mitigation tool is unclear.

Feeding of alternative feed ingredients has shown potential as a heat stress mitigation tool. Feeding of yeast and yeast cell walls was reported to result in lower intravaginal temperatures in heifers, with greater effect during a heat stress event (Broadway et al., 2020). They also reported that heifers supplemented with yeast drank more water than those not supplemented with yeast, illustrating again the cooling effect of water consumption. This agrees with earlier results reported by Young et al (2017) that showed supplementing yeast cell walls from *Saccharomyces cerevisiae* during moderate-to-severe heat events in the early feeding period resulted in increased ADG and DMI. This indicates that the early feeding period is critical to combating the effects of heat stress, perhaps due to stress of shipping and processing. Supplementation of an immunomodulatory feed ingredient resulted in lower observed intravaginal temperatures in the afternoon in heifers exposed to heat stress situations (Colombo

et al., 2019). Gamma-aminobutyric acid supplementation was reported to result in lower BT when measured with a rectal thermometer in the afternoon (Guo et al., 2018), but no impact was observed on respiratory rate in heat stressed cattle. They did, however, report increased DMI, G:F, and ADG.

Studies reviewed here suggest potential benefits of utilizing novel sources of trace minerals in heat stressed situations. These potential benefits may include altered immune function, better retention of nutrients, increase in daily water intake, and/or decreased loss of electrolytes through sweating. Investigation of these effects is warranted.

2.3.4 Use of Infrared Thermography in Evaluating Heat Stress

Evaluation of heat stress has often included some measure of body temperature as a response. Most often, measurement devices have included thermistors implanted at the base of the ear to measure tympanic temperature, temperature loggers and/or probes to measure rectal temperature, intraruminal temperature loggers, and intravaginal temperature loggers as representative measurements of core temperatures. Less invasive methods of observing body temperatures are warranted. Infrared thermography (IR) has recently received attention as a potential tool to observe BT from a distance. Thermographic temperatures taken from around the eyes and muzzle in sheep and cattle have been reported to be correlated with core temperature as measured using rectal and vaginal thermometers (George et al., 2014). These correlations were stronger in sheep than in cattle and only reported in females. It is unclear how this translates to males, though strong correlation ($r = 0.98$) has been reported between tympanic and intravaginal temperatures (Howard et al., 2014). Giro et al (2019) also reported that IR temperature on the eyes was correlated with rectal temperature.

Internal rumen temperature has been reported to be correlated (r = 0.55) with rectal temperature (Lees et al., 2019a), but IR thermography was not used in their study. In evaluating different locations on the body for collection of IR temperatures, Peng et al (2019) reported a lower correlation coefficient (r = 0.43) between flank temperature and rectal temperature, while noting that flank temperature may be a good tool to use in heat stress evaluations due to heat generated from the rumen. It appears that collection of flank temperatures may be useful for illustrating extreme temperatures experienced by cattle when Ta is high and solar radiation is unblocked.

Use of IR thermography has illustrated the role that time of day plays in heat stress. Body temperatures as measured by IR thermography have been reported to be greater in afternoon than in morning, though use of IR thermography in the morning was of little additive value in predicting heat stress in the afternoon when compared to morning temperature and humidity alone (Unruh et al., 2017). This observation of morning temperature and humidity as useful predictors of afternoon heat stress supports work discussed earlier indicating the lagged effect of severe heat on physiological responses to heat stress.

2.3.5 Implications for Feedlot Management

Careful planning should be undertaken to mitigate the impacts of heat stress in feedlot settings. Installation of shades appears to be a useful method to combat the impacts of heat and solar radiation on body temperature and pen surface temperature, but substantial initial costs may render their installation a non-starter for many operations. Thus, different management techniques have been suggested. Dietary manipulation to avoid loss of electrolytes may be useful. Black-hided cattle on full feed have been found to be more susceptible to heat stress than other classes of feedlot cattle, especially when nearly finished and in higher than average body

condition (Sullivan and Mader, 2018), indicating that it may be best to avoid feeding black-hided cattle through the summer, if possible. However, premiums paid for black-hided cattle that achieve QG of choice or better make this a difficult decision. Perhaps having as few heavy cattle on feed as possible through the summer is a viable alternative, as cattle have been shown to acclimate to heat as summer goes on (Brown-Brandl et al., 2005). Compensatory gain effects at the conclusion of heat episodes will make up for any performance losses through the summer.

Planning of facilities to include physical alterations including mounds and sprinklers may be useful. It should be noted that surrounding areas may also impact heat stress. Nienaber et al (2003) suggested that planting of tall crops such as corn next to cattle pens could reduce air flow to pens, thus making offloading of heat more difficult. They suggested planting other crops, such as alfalfa, next to pens. Alternatively, inclusion of mounds in feedlot pens to allow cattle access to improved air flow can be useful.

Timing of handling activities is also important. Brown-Brandl et al (2010) noted increased body temperatures associated with handling. It is reasonable to expect this effect to be magnified in periods of high heat. Thus, planning of activities such as loading trucks and processing of cattle to occur prior to the hottest part of the day is a simple management technique to use to mitigate effects of heat stress. Nienaber et al (2003) suggested not planning any animal handling activities if heat waves are forecast.

2.3.6 Direction of Future Research

Given increased scrutiny of confinement animal operations surround animal welfare concerns, as well as potential for rising global temperatures in the future, research into techniques to mitigate heat stress will likely be warranted for some time. Additionally, the trend

of increasing live weights of cattle (Corah, 2016) is unlikely to change any time soon. Given the repeated observations reviewed here that heavier cattle are more prone to heat stress, it is apparent that heat stress is a problem that is not likely to abate without improved facilities, handling, and feeding techniques.

Food animal research in general is likely to focus on improvements in health, well-being, and welfare. Additionally, research into methods of reducing waste on livestock operations – waste generated through feed, use of fossil fuels, and water in particular – will lead the day. These two interests – conservation of resources and better lives for livestock – can and should work in conjunction with one another. It seems reasonable from literature reviewed here that promoting better utilization of feedstuffs through longer retention and improved digestion can both mitigate heat stress by producing less metabolic heat and decrease waste through less wasted feed. It is interesting to note that many papers reviewed herein utilized DMI as the main response variable. Dry matter intake alone may not be the best performance metric to chase. It is true that cattle – and all livestock – can only utilize the amount of energy they consume. The Law of Conservation of Energy has not yet been shown to be wrong. However, finding ways to more efficiently utilize energy provided such that there is less waste may be a better technique to combat heat stress while remaining cognizant of environmental and other concerns. It seems reasonable, then, that future research into nutritional strategies to combat heat stress should focus on efficiency of utilization of nutrients.

Use of less invasive techniques to evaluate heat stress – particularly as measured through body temperature – should also be a target of future research. Infrared thermography has shown promise as a tool to evaluate and identify signs of heat stress. Use of tools such as this may be useful in determining better feeding practices or facility design. Perhaps, for example, use of

infrared thermography could determine the optimal time of day and amount of water to wet pen surfaces to combat effects of ambient temperature, solar radiation, and pen surface temperature on cattle. Such technologies have the added benefit of being able to be used from outside the pen, thereby potentially reducing stress of cattle. Infrared is but one technology available that should likely be further evaluated.

Finally, genetic selection tools to find and select for more heat tolerant cattle should be continually evaluated. It is likely that genetics plays a key role in determining growth and performance potential of cattle in a variety of situations. Given this, investigation and utilization of better selection tools by cattle breeders are warranted.

2.4 MINERAL SOURCES

2.4.1 General Overview of Supplemental Mineral Sources

Supplementation of macro- and micro-minerals in livestock and poultry diets is ubiquitous in industry. In feedlot cattle, over 90% of nutritionists have reported providing vitamins and minerals in some form of supplement (Vasconcelos and Galyean, 2007). Mineral status of livestock has been shown to impact marbling development throughout the stay at the feedlot, as well as impacting health, growth, and DMI of animals, particularly in the initial post-transit period (Genther and Hansen, 2014). Accounting for mineral content of the total diet is key, as trace mineral status can be managed through adequate inclusion rates of minerals. Genther and Hansen (2014) reported mineral deficiencies in livers of steers after 71 d on feed with a mineral-deficient diet. It should be noted that Zn was shown to be in adequate status in the liver but was fed at 33.9 mg total Zn/kg DM, in excess of NRC recommendations (National Academy of Sciences, 2016). Management of mineral status is further complicated by

antagonistic interactions among different minerals (Hansen et al., 2008). Dry matter intake is known to generally decrease for a period following transportation stress (Hutcheson and Cole, 1986); therefore, strategies to combat mineral deficiencies in this period are warranted. Strategies to combat mineral deficiencies may include increased concentration of minerals, particularly in starting diets, or utilizing trace minerals from relatively more bioavailable sources.

In general, three categories of mineral supplements are used in beef production: 1) inorganic sources, such as -sulfate or -oxide minerals; 2) organic sources, including chelated or complexed minerals; and 3) hydroxy crystal mineral forms. This review focuses on functional differences between organic and inorganic sources of minerals. Mineral sources that are chelated or complexed, particularly those complexed with various AA, have been shown to be relatively more bioavailable than inorganic sources (Henry et al., 1992; Cao et al., 2000; Guo et al., 2001; Wright and Spears, 2004; Hansen et al., 2008; Pal et al., 2010). Other researchers, however, have found inconsistent results when measuring the bioavailability of organic and inorganic minerals. Kegley and Spears (Kegley and Spears, 1994) reported that copper-lysine (**CuLys**) was more soluble and bioavailable than copper-oxide (**CuO**), but not different from $CuSO_4$. Similarity in digestion *in vitro* between CuLys and $CuSO_4$ has been reported (Ward and Spears, 1993). Copper-oxide has been found to be essentially unavailable to cattle (Spears, 2003). Rojas et al (1996) reported no difference in bioavailability between zinc-methionine (**ZnMet**) and inorganic Zn sources when feeding high levels of dietary Zn (> 200 mg Zn/kg DM). Nockels et al (1993) observed greater retention of Cu from CuLys than $CuSO_4$ but no difference in Zn retention when feeding Zn from $ZnSO_4$ and ZnMet.

Observed differences in bioavailability of different mineral sources may be explained by the observation that minerals from organic sources appear to be metabolized differently both pre-

and post-absorption than minerals from inorganic sources (Spears, 1989). As well, complexed minerals are likely to escape digestion in the rumen and be absorbed further downstream in the digestive process. Copper from organic sources has been shown to have no impact on liver Zn and Fe status in rats, suggesting a different mode of absorption compared with $CuSO_4$ (Du et al., 1996). How this compares to ruminants is uncertain. Supplementing Co from cobalt-propionate (**CoPro**) was reported to result in decreased acetate:propionate in steers compared to supplementation with $CoCO_3$, which may result in improved yield and/or marbling (Tiffany et al., 2003).

Investigation of effects of organic mineral sources have often focused on the highly stressful post-transit, early feeding period, with the initial 28 d in the feedlot receiving the most attention. Many studies have reported early effects in mitigation of stressors, but no differences in feedlot performance. As such, performance effects from organic and inorganic mineral sources – particularly those supplying Cu, Zn, and Mn – are controversial. Ahola et al (2005) monitored effects of supplementation with different sources of Zn, Mn, and Cu from pre-partum through slaughter. They reported improved G:F in cattle supplemented organic minerals compared to inorganic in all phases of the study, but greater G:F in the finishing phase in cattle not supplemented with any Zn, Mn, and Cu. Other researchers have also reported no effects on performance when comparing Zn, Mn, and Cu from various organic and inorganic sources (Malcolm-Callis et al., 2000; Rhoads et al., 2003; Wagner et al., 2008; Ryan et al., 2015). Still others have reported improved HCW, dressing percentage, and ADG from feeding organic sources of Zn, Mn, and/or Cu (Greene et al., 1988; Spears and Kegley, 2002; Dorton et al., 2006). Mixed results may be due to accepted industry practices of feeding minerals at levels far in excess of NRC recommendations (Vasconcelos and Galyean, 2007; Samuelson et al., 2016).

In particular, Zn is often reported to be included in feedlot diets at more than 90 mg Zn/kg DM, over triple the NRC requirement. Feeding Zn in excess of NRC requirements has been shown to decrease morbidity, particularly related to BRD (Galyean et al., 1995b; Carmichael et al., 2018), which likely explains excessive levels of supplementation. Thus, simulating industry practices in research has shown mixed effects.

Supplementing CuLys in the 28 d receiving period has been reported to decrease ADG and DMI, as well as appearing to have a favorable effect on Zn supplementation later in the feeding period (Galyean et al., 1995b). Other researchers have reported no performance effects related to Cu source (Engle et al., 2000; Engle and Spears, 2000). Engle and Spears (2000) did note improved DMI and G:F in first 84 d of the trial for organic Cu source compared to $CuSO_4$ but no effect over total study period. Shorter repletion time after Zn deficiency in cattle fed ZnMet than those fed ZnLys or $ZnSO_4$ has been demonstrated (Engle et al., 1997). Pal et al (2010) reported improved G:F in ewes fed CuMet and ZnMet versus those supplemented with $CuSO_4$ and $ZnSO_4$. Overall, as results are presently controversial, it can be concluded that feeding of chelated or complexed minerals as performance enhancers is likely an economic question. They may be useful, but they must be priced sufficiently low to make them cost effective compare to inorganic mineral forms (Goff, 2018).

2.4.2 Minerals and Immune Function

Many minerals are known to play a role in immune function. In particular, Zn, Se, Fe, Cu, and folate are important immune regulators (Erickson et al., 2000). However, assessing of mineral status in ruminants is difficult, as antagonistic relationships among different minerals that inhibit absorption and/or metabolism of minerals render interpretation of assays challenging (Ward and Spears, 1993; Spears, 2000; Spears, 2003). Zinc deficiency has been associated with

increases in blood parasites and bacteria escaping the mucosa or epithelium of the digestive tract in various species (Goswami et al., 2005). One function that may be attributed to minerals is in preserving the integrity of epithelial tight junctions. Epithelial tight junctions in all kinds of cells are believed to act as a barrier to diffusion of solutes or molecules between body systems (e.g. digestive and circulatory) (Gumbiner, 1987). Should their junctions be weakened, bacteria are readily able to diffuse into the bloodstream. Particularly in the case of liver abscess incidence, given the accepted etiology of the rumenitis-liver abscess complex, it is reasonable to believe that preservation of tight junctions within the rumen represents an avenue of liver abscess control. Investigation of different Zn sources may be warranted. Wagner et al (2008) reported no differences in liver abscess incidence in cattle supplemented with liquid ZnMet versus those supplemented with inorganic sources of all minerals of interest, though tylosin phosphate was used for liver abscess control. Investigation of mineral sources for control of liver abscess in non-tylosin-fed cattle is likely warranted.

Use of organic mineral sources for control of BRD, and thus pulmonary lesions, has also been investigated with mixed results. Dorton et al (2003) concluded that feeding AA-complexed Cu may improve immune response compared with feeding $CuSO_4$. Ahola et al (2005) noted changes in liver concentrations of Cu and Mn from feeding different sources, but concluded that impacts on health and/or immune function were unlikely. Others, however, have reported improved immune responses and increased antibody titers following vaccination in cattle fed organic sources of Zn, Mn, Cu, and/or Co (George et al., 1997; Chirase and Greene, 2001). Earlier research had demonstrated a reduction in days where DMI was reduced due to IBR challenge in cattle fed ZnMet compared with those fed ZnO (Chirase et al., 1991; Chirase et al., 1994). Given observations that BRD challenge is particularly common in the early feeding

period, feeding of organic minerals may be warranted in the first 28-40 d on feed. Decreases in morbidity have been reported from feeding AA-chelated minerals versus AA-complexed minerals (Goodall and Schuetze, 2019).

2.4.3 Direction of Future Research

With public scrutiny of antibiotics increasing, alternative solutions to combat all kinds of morbidity in feedlot animals are needed. Investigation into impacts of various sources of trace minerals are, and will likely remain, warranted. Additionally, increasing environmental concerns related to excess N and P in feedlot waste are not likely to diminish without interventions. Supplementation in feeds to maximize digestibility and retention represents one avenue of waste minimization. Additionally, further investigation into biological requirements for various vitamins and minerals represents opportunities to minimize waste through avoidance of over-feeding of minerals.

It is likely that these two areas will drive research decisions and financing for some time into the future. However, as noted by Goff (2018), investigation and marketing of mineral sources must be done with thin profit margins of feedlots in mind. As well, livestock producers will need to adapt to use of new technologies that may negatively impact their bottom lines. Mineral supplementation is a complicated subject that encompasses animal health, producer and consumer finance, foreign trade, and many other facets of agriculture. As such, research into new mineral products has potentially far-reaching impacts.

CHAPTER 3: LIVER ABSCESS, PULMONARY LESION, AND PERFORMANCE RESULTS

3.1 OVERVIEW

Nutritional methods of control of common feedlot ailments are becoming increasingly important as use of antimicrobials receives added scrutiny. The objectives of this study were to evaluate a novel trace mineral product (ProPath, Zinpro Corporation, Eden Prairie, MN, USA) as a means of controlling liver abscess incidence and pulmonary lesion incidence in feedlot cattle fed under a natural-feeding protocol. Additionally, performance metrics were evaluated. Thirty-two lots (249 – 282 animals/lot) of beef cattle were purchased from various sources and enrolled in a randomized complete block design. Blocking factors used were date of arrival at the feedlot and sex of cattle. Upon enrollment cattle were randomly assigned at processing to one of two treatments: 1) Control (n = 16), with inorganic sources for all trace minerals of interest; and 2) Test (n = 16), with ProPath used to provide additional AA complexes of Zn and Mn, complexed Co, and ruminally-protected folic acid to the basal control diet. A total of ten blocks of steers and six blocks of heifers were enrolled. All cattle were fed a traditional step-up diet regimen, with a total of three diets used per treatment. Cattle were not administered any antimicrobials, ionophores, β-andrenergic agonists, or growth-promoting implants. Cattle were fed for an average of 223 d (range: 186 – 270). At harvest, cattle were observed for presence of liver abscesses using the Elanco Liver Check System (Elanco, Greenfield, IN, USA) and pulmonary lesions using the system described by Tennant et al (2014). No treatment differences were observed for percent of livers containing any ($P = 0.62$), A+ ($P = 0.14$), A ($P = 0.88$), A- abscesses ($P = 0.63$). No significant differences were observed for sex for all liver abscesses ($P = 0.32$), A+ liver abscesses ($P = 0.82$), A liver abscesses ($P = 0.72$), or A- liver abscesses ($P =$

0.18). No treatment differences were observed for percent of cattle with mild ($P = 0.64$), moderate ($P = 0.86$), or severe ($P = 0.30$) pulmonary lesions. For percentage of cattle observed with any lung lesions, no differences were found between treatments ($P = 0.51$) or between sexes ($P = 0.39$). A sex-by-treatment interaction was observed for cattle with severe lung lesions ($P < 0.01$). Control animals achieved higher ADG than Test cattle on both a deads-and fallouts-in ($P = 0.01$) and deads-and fallouts-out ($P = 0.03$) basis. Control cattle achieved higher G:F than Test cattle when analyzed on a deads-and fallouts-in basis ($P = 0.02$), but not on a deads-and fallouts-out basis ($P = 0.92$). Control cattle achieved greater HCW ($P = 0.03$), FT ($P = 0.04$), and marbling score ($P = 0.05$). No other differences were found in carcass metrics between treatments ($P > 0.05$).

3.2 INTRODUCTION

Use of antimicrobials in livestock production has recently received increasingly intense scrutiny, as concerns about ethics and antimicrobial resistance have come to the forefront in recent years. Cattle are often administered antimicrobials for control of liver abscesses and bovine respiratory disease (BRD). Indeed, feeding of the antimicrobial tylosin phosphate has consistently been shown to reduce incidence of liver abscess in feedlot cattle (Brown et al., 1975; Potter et al., 1985; Baba et al., 1989; Rogers et al., 1995; Nagaraja and Lechtenberg, 2007). Additionally, metaphylactic use of antimicrobials has been shown to be effective in control of BRD (Dennis et al., 2018). Since the majority of cases of BRD go undetected (Gardner et al., 1999; Leruste et al., 2012), and diagnoses in feedlots based on clinical signs are inconsistent and often unreliable (White and Renter, 2009), antimicrobials are often administered to all cattle on arrival at the feedlot. As this practice is receiving increased scrutiny, alternative control methods are needed.

Feeding of amino acid chelated or complexed sources of minerals has been shown to improve performance and health outcomes for feedlot cattle (Greene et al., 1988; George et al., 1997; Chirase and Greene, 2001; Spears and Kegley, 2002; Dorton et al., 2006). Wagner et al (2008) reported no decrease in liver abscess incidence from feeding Zn-methionine, but tylosin was fed in that trial. Increased bioavailability of complexed minerals (Spears, 2003) may decrease bacterial escape from the rumen through strengthening of epithelial tight junctions, thereby lessening liver abscess incidence. The objective of this study, then, was to evaluate ProPath for control of liver abscesses and pulmonary lesions in cattle fed under a natural-feeding protocol, as well as evaluating its performance in traditional feedlot performance metrics.

3.3 MATERIALS AND METHODS

3.3.1 Cattle Population and Processing Protocol

This study protocol was approved by the Five Rivers Research Committee, and the study was conducted according to the standard operating procedures for humane handling of Five Rivers Cattle Feeding, Johnstown, CO.

Thirty-two pens of beef cattle from various sources around the United States were enrolled in the study program for feeding at a commercial feedlot in the Eastern Colorado. Cattle were of mixed breeds and sexes. A total of 8,635 cattle were initially enrolled in the study. Study cattle were purchased and arrived at the feedlot over a period of 15 days from May 9, 2019, through May 23, 2019. Cattle were fed for an average of 222.6 days, with a range from 186 – 258 days on feed.

All cattle were fed a step-up feeding program according to the standard operating procedures of the feedlot. Cattle enrolled in both treatments were fed and managed to meet

requirements for “Aspen Ridge” (JBS USA, Greeley, CO) beef labeling. Cattle were not administered any antimicrobials, ionophores, β-andrenergic agonists, or growth promoting implants of any kind. Cattle requiring antibiotic treatment at any point during the study were removed from the study. A total of 7,678 cattle finished the study.

3.3.2 Treatments

Cattle were randomly assigned to one of two treatments using a randomized complete block experimental design. Arrival date and sex were used as blocking factors. A total of 16 complete blocks were used, with ten blocks consisting entirely of steers and six blocks consisting entirely of heifers. Trace minerals identified as being of interest for the study were cobalt, copper, iodine, manganese, selenium, and zinc.

Treatments were identified as 1) Control; and 2) Test. Cattle assigned to the Control group were fed a diet that included all trace minerals of interest from inorganic sources according to the standard diet program at the feedlot. Cattle assigned to the Test group were fed a diet that included ProPath (Zinpro Corporation, Eden Prairie, MN, USA) to provide additional amino acid complexes of zinc and manganese, complexed cobalt, and ruminally-protected folic acid to the basal control diet. Composition of treatment finishing diets is presented in Table 3.1.

3.3.3 Observation of Liver Abscess Incidence

Cattle were harvested at a commercial abattoir over a period of three months from mid-November of 2019 through mid-February of 2020. All cattle within a block were harvested on the same day.

All liver observations were made by the same individual. Observations were made by inspection of livers at the abattoir on the day of harvest. Livers were observed for presence,

number, and severity of abscesses, presence of liver cirrhosis, presence of liver flukes, and telangiectasis. Livers were scored using the Elanco Liver Check System (Elanco, Greenfield, IN, USA). If a liver was determined to fall into a score category (e.g. A-, A, A+, etc.), it was counted as one liver in a lot. The total number of livers observed in each category from each lot were divided by the total number of animals harvested from that lot, then multiplied by 100 to determine percentage of livers from that lot falling into each category.

3.3.4 Observation of Pulmonary Lesions

Lungs of harvested cattle were observed at the abattoir on the day of harvest. All observed lungs were observed by the same individual. Lungs from seven blocks were unable to be observed owing to difficulties at the abattoir (e.g. incorrect ear tag identification) and were thus excluded from the final data set. Evaluation of pulmonary lesions was performed according to the system described by Tennant et al (2014), and lungs were classified into four categories: 1) normal (e.g. no visible lesions); 2) mild (e.g. presence of fibrin tags or 5CON); 3) moderate (e.g. 15CON); and 4) severe (e.g. 50CON or ALLCON). If a lung fell into a certain category, the animal that that lung was from was counted as being in that category. Number of animals in each category was then divided by the total number of animals observed in that lot and multiplied by 100 to obtain the percent of animals in that lot falling into that category. Percentage of animals in each category was used as the response variable for analysis.

3.3.5 Performance Metrics

Performance data were collected on all cattle harvested. If an individual animal was unable to be identified at the abattoir (e.g. ear tag missing or mismatched), that animal was excluded from the final count. Traditional feedlot performance metrics were collected at the

feedlot and included ADG, G:F, initial BW, final BW (4% pencil shrink included), and DDMI. Results were calculated on both a deads and rejects in and deads and rejects out basis. Weight metrics were recorded in pounds and converted to kg by multiplying by 0.45359237.

Carcass metrics were collected at the abattoir, and included dressing percentage, HCW, KPH, QG, REA, and fat depth at 12 – 13th rib (FT). Ribeye area was recorded in square inches and converted to square centimeters by multiplying by 6.4516 (2.54 x 2.54). Fat thickness was recorded in inches and converted to cm by multiplying by 2.54. Weight metrics were recorded in pounds and converted to kg by multiplying by 0.45359237. For QG and YG, total number of animals within a lot falling into each category (e.g. YG2) was divided by total number of animals harvested in that lot. Percentage of animals in each category was used as the response variable for analysis.

3.3.6 Statistical Analysis

3.3.6.1 Liver Abscess Incidence and Severity

Analysis was performed using ANOVA of linear mixed-effects regression models fitted by the lmer function in R. Models were fitted for four different response variables. Response variables of interest were: 1) percent of livers observed displaying any abscesses; 2) percent of livers observed displaying A+ abscesses; 3) percent of livers observed displaying A abscesses; and 4) percent of livers observed displaying A abscesses. Each model included fixed effects of treatment, sex, and the interaction between treatment and sex. Block was included in each model as a random effect. All terms were left in each model regardless of significance. Pairwise comparisons of estimated marginal means were performed using the emmeans function. Lot (n =

16) was treated as the experimental unit. Significance was declared at $P < 0.05$, and tendencies were declared at $0.05 \leq P \leq 0.10$.

3.3.6.2 Pulmonary Lesions

Analysis was performed using ANOVA of linear mixed-effects regression models fitted by the lmer function in R. Models were fitted for four different response variables. Response variables of interest were: 1) percent of lungs observed displaying any lesions; 2) percentage of lungs scored as mild; 3) percent of lungs scored as moderate; and 4) percent of livers scored as severe. Each model included fixed effects of treatment, sex, and the interaction between treatment and sex. Block was included in each model as a random effect. All terms were left in each model regardless of significance. Pairwise comparisons of estimated marginal means were performed using the emmeans function. Lot (n = 9) was treated as the experimental unit. Significance was declared at $P < 0.05$, and tendencies were declared at $0.05 \leq P \leq 0.10$.

3.3.6.3 Performance Metrics

Analysis was performed using ANOVA of linear mixed-effects regression models fitted by the lmer function in R. Models were fitted for many different response variables. Response variables of interest were: 1) initial BW; 2) final BW; 3) ADG, deads and rejects out; 4) ADG, deads and rejects in; 5) G:F, deads and rejects out; 6) G:F, deads and rejects in; 7) HCW; 8) dressing percentage; 9) FT; 10) REA; 11) percent of carcasses grading prime; 12) percent of carcasses grading choice; 13) percent of carcasses grading upper 2/3 choice; 14) percent of carcasses grading choice or greater; 15) percent of carcasses grading select; 16) average marbling score; 17) percentage of carcasses in each USDA YG; 18) percentage of YG1 and YG2 carcasses; and 19) percentage of YG4 and YG5 carcasses. Each model included fixed effects of

treatment, sex, and the interaction between treatment and sex. Block was included in each model as a random effect. All terms were left in each model regardless of significance. Pairwise comparisons of estimated marginal means were performed using the emmeans function. Lot (n = 16) was treated as the experimental unit. Significance was declared at $P < 0.05$, and tendencies were declared at $0.05 \leq P \leq 0.10$.

3.4 RESULTS

3.4.1 Liver Abscess Incidence

No treatment differences were observed for percent of livers containing any abscesses ($P = 0.62$), percent of livers presenting A+ abscesses ($P = 0.14$), percent of livers containing A abscesses ($P = 0.88$), or percent of livers containing A- abscesses ($P = 0.63$). No significant differences were observed for sex for all liver abscesses ($P = 0.32$), A+ liver abscesses ($P = 0.82$), A liver abscesses ($P = 0.72$), or A- liver abscesses ($P = 0.18$). No significant interaction was found between sex and treatment ($P > 0.10$) for any of the response variables. Results are summarized in Table 3.2.

3.4.2 Pulmonary Lesion Incidence

No treatment differences were observed for percent of cattle in mild ($P = 0.64$), moderate ($P = 0.86$), or severe ($P = 0.30$). For percentage of cattle observed with any lung lesions, no differences were found between treatments ($P = 0.51$) or between sexes ($P = 0.39$). No interaction between sex and treatment was observed ($P = 0.29$).

An interaction was observed between sex and treatment in the severe category ($P < 0.01$). There was a tendency ($P = 0.05$) for more heifers in the Test treatment to be in the severe category than steers in the Test treatment. Within heifers, more cattle in the Test treatment fell in

the severe category than those in the Control treatment ($P < 0.01$). However, more steers in the Control treatment fell in the severe category than steers in the Test treatment ($P = 0.01$). Overall, the effect of sex was not found to be significant in the severe category ($P = 0.30$).

A tendency for an interaction between sex and treatment was found in both the mild ($P = 0.06$) and moderate ($P = 0.10$) categories. Test steers tended ($P = 0.05$) to be more likely to fall into the mild category than Control steers, but no treatment differences were observed in heifers ($P = 0.32$). Within the Test treatment, heifers tended ($P = 0.08$) to be more likely to fall into the mild category than steers. This tendency was not observed in the Control treatment ($P = 0.33$). Within the Control treatment, heifers tended ($P = 0.08$) to be more likely than steers to fall into the moderate category. This tendency was not found in the Control treatment.

These results are summarized in Table 3.3.

3.4.3 Performance Results

No differences existed for initial BW between treatments ($P = 0.82$). Control animals achieved higher ADG than Test cattle on both a deads and fallouts in ($P = 0.01$) and deads and fallouts out ($P = 0.03$) basis. Control cattle achieved higher G:F than Test cattle when analyzed on a deads and rejects in basis ($P = 0.02$), but this difference did not exist when deads and rejects were excluded ($P = 0.92$). These results are summarized in Table 3.4.

Control cattle achieved greater HCW ($P = 0.03$), FT ($P = 0.04$), and marbling score ($P = 0.05$). No other differences were found in carcass metrics between treatments ($P > 0.05$). These results are summarized in Table 3.5.

3.5 DISCUSSION

Control of liver abscess incidence in feedlot cattle is a challenging proposition. For many years, reports have indicated that use of feed-grade antimicrobials is an effective method of control (Brown et al., 1973; Rogers et al., 1995; Weinroth et al., 2019). However, increasing scrutiny surrounding the use of antimicrobials – particularly feed-grade antimicrobials fed at sub-therapeutic levels – has led to renewed interest in development of control methods for liver abscesses. Additionally, economic realities of feeding cattle disincentivize feeding higher levels of roughage in diets. While effective as a control mechanism, increasing roughage levels leads to increased costs (Bartle and Preston, 1991; Birkelo et al., 1991; Bartle et al., 1994). As such, it is unlikely that roughage levels will substantially increase in feedlot diets. Development of other nutritional control methods represents a potential avenue for liver abscess control.

Observations in this study indicate limited efficacy of the ProPath product for control of liver abscess incidence in natural-fed cattle. This may be viewed in the wider context of challenges in demonstrating success in controlling liver abscesses through the use of feed additives. *Saccharomyces cerevisiae* fermentation products (Huebner et al., 2019), antioxidants (Müller et al., 2018b), and essential oils (Meyer et al., 2009) have all been investigated and shown limited promise as control mechanisms. It may be that these ingredients do not improve rumen health; it may also be that rumenitis-liver abscess complex is difficult to control without more conservative approaches to feeding. Vyas et al (2015) reported a decrease in time that rumen pH was below 6.2 through supplementation of malic acid, but no effect on acidosis. This illustrates the difficulty in controlling ruminal lesions.

These results are mixed when analyzed for control of pulmonary lesions in feedlot cattle. The observation of fewer heifers having severe lesions when fed the Test diet versus the basal

Control diet may suggest a benefit to feeding heifers different finishing diets than steers. It should be noted, however, that only three of six blocks of heifers were observed for pulmonary lesions owing to difficulties at the abattoir. Given that no differences were observed overall between treatments for any category of lesions, it is possible that more observations may have removed this effect. However, there may be a benefit to supplementing ProPath to heifers.

Performance results indicate no benefit to feeding ProPath throughout the finishing period. No differences were observed for USDA quality and yield grade categories. Absent improved performance in carcass categories, any feeding decisions are likely to rely on economics. Larger cattle generally generate higher revenues. As such, the study results here would suggest more performance benefit to supplementing inorganic forms of trace minerals. Previous studies have indicated improved performance and health outcomes from feeding complexed forms of minerals in the post-transit, early feeding period (Engle and Spears, 2000; Ahola et al., 2005). This may suggest that investigation of ProPath in starting diets is warranted. It should further be noted that both finishing diets used in this study provided Zn at over 90 mg Zn/kg DM. There may be no health benefits to increased supplementation of Zn.

It should be noted that the manufacturer indicated after the trial an error in the batch of folic acid included in the ProPath product for this experiment, resulting in a product that may not have been protected from rumen degradation. Ruminally-protected folic acid has been reported to improve growth and performance metrics in Holstein steers (La et al., 2019). Impacts of this error in production are unclear but should be noted in any interpretation of these data. It is possible that complete rumen protection in manufacturing may have improved performance.

3.6 CONCLUSIONS

Supplementing ProPath in this feedlot's basal finishing diet did not reduce incidence of liver abscess or pulmonary lesions in natural-fed cattle. These data suggest no performance benefits from supplementation of ProPath in the basal finishing diet. Given errors in manufacturing, it is unclear what conclusions can be drawn from this study.

3.6 TABLES AND FIGURES

Table 3.1. Dry matter ingredient and chemical composition for finishing diets averaged over feeding period.

Ingredient	Control	Test
Steam-flaked corn	43.2	43.2
Steam-flaked wheat	23.9	23.9
Corn silage	10.2	10.2
Cereal hay	3.9	3.9
DDG	9.2	9.2
Whey delactose permeate	2.5	2.5
Supplement	3.9	3.9
Vegetable oil	3.2	3.2
Feed additive[1]		
Chemical Composition[2]		
Crude Protein, %	14.3	14.6
NDF, %	17.4	16.8
Ca, %	0.5	0.5
P, %	0.5	0.5
Cu, mg/kg	13.9	14.6
Zn, mg/kg	90.1	125.6
Mn, mg/kg	54.0	71.2

[1]ProPath fed at 3.5 g/d for Test treatment.
[2]As determined by Servi-tech Laboratories (Hastings, NE, USA).

Table 3.2. Estimated marginal means of liver abscess prevalence across treatments.

Category[1]	Control	Test	*P*-Value	SEM
	Percent of all livers observed			
A-minus	11.5	12.0	0.63	1.35
Heifers	13.3	13.8	0.78	2.14
Steers	9.8	10.3	0.68	1.66
A	11.7	11.5	0.88	0.76
Heifers	12.3	11.4	0.54	1.20
Steers	11.1	11.7	0.59	0.93
A-plus	19.6	18.3	0.14	1.09
Heifers	20.2	18.2	0.16	1.73
Steers	19.0	18.4	0.54	1.34
All	42.8	41.9	0.62	2.34
Heifers	45.8	43.3	0.44	3.71
Steers	39.9	40.4	0.84	2.87

1. Liver abscess evaluations made using Elanco Liver Check System (Elanco, Greenfield, IN, USA)

Table 3.3. Estimated marginal means of pulmonary lesion prevalence across treatments.

Category[1]	Control	Test	*P*-Value	SEM
	Percent of all lungs observed			
Mild	4.55	5.52	0.64	1.69
Heifers	5.70	2.28	0.33	2.76
Steers	3.40	8.75	0.05	1.95
Moderate	17.8	18.2	0.86	2.20
Heifers	21.9	18.0	0.33	3.59
Steers	13.7	18.5	0.11	2.54
Severe	11.3	12.4	0.30	1.94
Heifers	11.07	16.89	0.01	3.17
Steers	11.61	7.99	0.01	2.24
Any Lesions	33.7	36.2	0.51	3.73
Heifers	38.7	37.1	0.80	6.09
Steers	28.7	35.2	0.16	4.30

1. Evaluations made using the system described by Tennant et al (2014): Mild = FIB or 5CON; Moderate = 15CON; Severe = 50CON or ALLCON

Table 3.4. Estimated marginal means of growth performance across treatments.

Trait[a]	Control	Test	*P*-Value	SEM
Number of replicates (pens)	16	16	-	-
Animals enrolled	4,425	4,431	-	-
Animals sold from original lot	3,951	3,844	-	-
Initial BW, kg	378	379	0.82	8.14
Live-Basis				
Final BW, kg[b]	623	616	0.03	4.18
ADG[ab] Deads & Rejects Out[c]	1.25	1.23	0.03	0.018
G:F[ab] Deads & Rejects Out[c]	0.12	0.12	0.92	0.001
ADG[ab] Deads & Rejects Included[d]	0.99	0.91	0.01	0.045
G:F[ab] Deads & Rejects Included[d]	0.10	0.09	0.02	0.004

[a]ADG = average daily gain, kg; BW = body weight; G:F = gain:feed ratio, DM basis.

[b]Gross weights decreased by 4% to represent a standard industry shrink. This represents the average weight of animals sold.

[c](Average sale weight of non-fallouts - average starting weight of all animals enrolled)/average days-on-feed.

[d](Total sale weight of non-fallouts - total starting weight of all animals enrolled)/head-days.

Table 3.5. Estimated marginal means of carcass characteristics across treatments.

Trait[a]	Control	Test	*P*-Value	SEM
Number of replicates (lots)	16	16	-	-
HCW, kg	409	406	0.03	1.41
Dressing percentage	62.4	62.3	0.11	0.17
Camera Grading and Measurements				
Prime, %	35.7	34.7	0.58	2.93
Choice, %	62.8	64.0	0.51	2.88
Upper 2/3 Choice, %	24.4	22.7	0.29	1.92
Choice or greater, %	98.5	98.6	0.62	0.24
Select, %	1.21	1.11	0.64	0.22
Marbling score[b]	614	607	0.05	7.98
YG 1, %	0.19	0.37	0.16	0.09
YG 2, %	6.83	7.58	0.51	1.09
YG 1 and 2, %	7.02	7.95	0.43	1.13
YG 3, %	64.9	62.2	0.22	2.99
YG 4, %	24.8	25.6	0.70	2.68
YG 5, %	3.26	4.21	0.22	1.00
YG 4 and 5, %	28.1	29.8	0.49	3.52
Fat thickness, cm	0.67	0.66	0.04	0.01
Ribeye area, cm^2	87.0	87.1	0.84	0.45

[a]HCW = hot carcass weight; ADG = average daily gain; YG = yield grade.

[b]Marbling scores: 200=Traces^0, 300=Slight^0, 400=Small^0, 500=Modest^0, 600=Moderate^0, 700=Slightly Abundant^0, 800=Moderately Abundant^0.

CHAPTER IV: HEAT STRESS RESULTS

4.1 OVERVIEW

Mitigation of environmental stress in feedlot cattle is a difficult and important challenge. In particular, heat stress has been shown to diminish feedlot performance, thereby negatively impacting feedlot profits. Additionally, animal welfare related to heat stress and environmental management is an area of increasing scrutiny. The objective of the current study was to evaluate a novel trace mineral product (ProPath, Zinpro Corporation, Eden Prairie, MN, USA) as a means to mitigate impacts of heat stress in feedlot cattle as measured by percentage of cattle observed open-mouth breathing. Thirty-two lots (249 – 282 animals/lot) of beef cattle were purchased from various sources and enrolled in a randomized complete block design. Blocking factors used were date of arrival at the feedlot and sex of cattle. Upon enrollment, cattle were randomly assigned at processing to one of two treatments: 1) Control (n = 16), with inorganic sources for all trace minerals of interest; and 2) Test (n = 16), with ProPath used to provide additional AA complexes of Zn and Mn, complexed Co, and ruminally-protected folic acid to the basal control diet. A total of ten blocks of steers and six blocks of heifers were enrolled. All cattle were fed a traditional step-up diet regimen, with a total of three diets used per treatment. Cattle were not administered any antimicrobials, ionophores, β-andrenergic agonists, or growth-promoting implants. Cattle were fed for an average of 223 d (range: 186 – 270). Cattle were observed twice monthly throughout the summer months on the following dates: 21 Jun 2019; 24 Jun 2019; 3 Jul 2019; 17 Jul 2019; 14 Aug 2019; 21 Aug 2019; 3 Sep 2019; and 17 Sep 2019. Within each observation date, observations were made at the following times: Time 1) 0700 – 1000; Time 2) 1015 – 1315; and Time 3) 1430 – 1700 (all times ± 30 min). At each observation, both pens

within a block were evaluated consecutively. At each observation, number of cattle within a pen observed open-mouth breathing were counted and divided by the total number of cattle within that pen and multiplied by 100 to determine percentage of cattle open-mouth breathing in that pen. At each observation, hide temperatures were collected caudal to left glenohumeral joints on ten black-hided and, when available, ten non-black-hided animals/per pen. At each observation, pen-surface temperatures were collected at ten locations within each pen. All temperature observations were performed using a Fluke VT 04 Visual Infrared Thermometer (Fluke Corporation, Everett, WA, USA). Hide temperatures were greater on black-hided cattle than non-black-hided cattle ($P < 0.01$) and on steers than heifers ($P < 0.01$). Hide temperatures on Test cattle were greater than hide temperatures on Control cattle ($P < 0.01$), but this effect was inconsistent across observation days (time point-by-date interaction $P < 0.01$). Hide temperatures were correlated with pen-surface temperatures ($R^2 = 0.43$). There were no differences between treatments for cattle observed open-mouth breathing ($P = 0.22$). Percentages of cattle observed OMB was different across all time points ($P < 0.01$). No differences were observed between sexes in Time A ($P = 0.50$) or Time B ($P = 0.36$), but percentages of heifers observed OMB were greater than percentages of steers observed OMB in Time C ($P = 0.01$; time point-by-sex interaction $P < 0.01$). There was also a significant time point-by-date interaction ($P < 0.01$). Based on these data, infrared hide temperature observed caudal to the glenohumeral joint is not likely to be a useful measurement of heat stress. ProPath did not lessen observed incidence of open-mouth breathing compared to inorganic sources of trace minerals in these cattle fed under a natural-feeding protocol.

4.2 INTRODUCTION

Environmental stress, particularly heat stress, is an important factor in performance of feedlot cattle. Heat stress has been repeatedly shown to decrease DMI, G:F, and ADG in feedlot cattle (Ray, 1989; O'Brien et al., 2010; Blaine and Nsahlai, 2011; Broadway et al., 2020). This effect is particularly problematic in *Bos taurus* cattle common in the US beef herd, as *B. taurus* have been shown to be less heat tolerant than their *Bos indicus* counterparts (Hahn, 1985; Finch, 1986; Lees et al., 2019b). Different strategies have been evaluated to mitigate negative impacts of heat stress on feedlot cattle. Examples of these strategies include provision of shade, sprinkling of animals and/or pen surfaces, provision of fans, and altering of feeding times. Many of these strategies are costly, and results are controversial. In particular, provision of shade has been reported to improve DMI (Mitlöhner et al., 2001; Mitlöhner et al., 2002; Hagenmaier et al., 2016), have no impact on DMI (Lees et al., 2018), and decrease DMI (Sullivan et al., 2011) in heat-stressed cattle. Diet manipulation through provision of feed additives and/or alternative ingredients, however, has shown promising results in increasing DMI and/or decreasing body temperatures in heat-stressed cattle (Young et al., 2017; Colombo et al., 2019; Broadway et al., 2020). Diet manipulation represents a potential simple and cost-effective tool for combating the effects of heat stress. The objective of the current study, then, was to evaluate the efficacy of ProPath in reducing incidence of OMB in feedlot cattle fed under a natural-feeding protocol.

4.3 MATERIALS AND METHODS

4.3.1 Cattle Population and Processing Protocol

This study protocol was approved by the Five Rivers Research Committee, and the study was conducted according to the standard operating procedures for humane handling of Five Rivers Cattle Feeding, Johnstown, CO.

Thirty-two pens of beef cattle from various sources around the United States were enrolled in the study program for feeding at a commercial feedlot in the Eastern Colorado. Cattle were of mixed breeds and sexes. A total of 8,635 head of cattle were initially enrolled in the study. Study cattle were purchased and arrived at the feedlot over a period of 15 days from May 9, 2019, through May 23, 2019. Cattle were fed for an average of 222.6 days, with a range from 186 – 258 days on feed.

All cattle were fed a step-up feeding program according to the standard operating procedures of the feedlot. Cattle enrolled in both treatments were fed and managed to meet requirements for “Aspen Ridge” (JBS USA, Greeley, CO) beef labeling. Cattle were not administered any antimicrobials, ionophores, β-andrenergic agonists, or growth promoting implants of any kind. Cattle requiring antibiotic treatment at any point during the study were removed from the study. A total of 7,678 cattle finished the study.

4.3.2 Treatments

Cattle were randomly assigned to one of two treatments using a randomized complete block experimental design. Arrival date and sex were used as blocking factors. A total of 16 complete blocks were used, with ten blocks consisting entirely of steers and six blocks consisting

entirely of heifers. Trace minerals identified as being of interest for the study were cobalt, copper, iodine, manganese, selenium, and zinc.

Treatments were identified as 1) Control; and 2) Test. Cattle assigned to the Control group (n = 16 pens) were fed a diet that included all trace minerals of interest from inorganic sources according to the standard diet program at the feedlot. Cattle assigned to the Test group (n = 16 pens) were fed a diet that included ProPath (Zinpro Corporation, Eden Prairie, MN, USA) to provide additional amino acid complexes of zinc and manganese, complexed cobalt, and ruminally-protected folic acid to the basal control diet.

4.3.3 Observation and Evaluation of Heat Stress

Cattle were observed for hide temperature and incidence of open-mouth breathing as an indicator of heat stress over a period of three months from June – September 2019. Observations were made twice per month. Personnel used for heat stress observations were blinded to study treatments. All temperature observations were made using a Fluke VT04 visual infrared thermometer (Fluke Corporation, Everett, WA, USA). Cattle were observed three times per observation day at the following times: Time 1) 0700 – 1000; Time 2) 1015 – 1315; and Time 3) 1430 – 1700 (all times ± 30 minutes). Pens within a block were evaluated consecutively to minimize effects of changing weather.

In each pen, attempts were made to observe hide temperatures on ten black-hided and ten non-black-hided cattle at each observation time. Hide temperatures were observed at various locations throughout the pen, and attempts were made to observe hide temperatures on cattle that were exhibiting visible signs of heat stress, as well as those that were not observed exhibiting visible signs of heat stress. In the event that personnel were unable to observe temperatures on

ten animals of each category in a pen – e.g. there were no non-black-hided animals in the pen – observations were made on as many animals in that category as possible.

Cattle were also observed for visible signs of heat stress at each observation time. Open-mouth breathing was used as the visible indicator of heat stress. Observation personnel observed cattle prior to entering the pen to avoid disturbing the cattle. Cattle were counted as being in visible heat stress when they were observed breathing with mouth open and tongue visible according to the system described by Hagenmeier et al (2016). The number of cattle observed open-mouth breathing in a pen was counted at each observation.

Pen surface temperatures were also collected at each observation time. Observation personnel entered each pen and collected surface temperatures at ten locations. These ten locations were: 1) three locations on the cement bunk apron, including one location adjacent to the water tank in each pen, and 2) seven locations on the dirt pen surface. See Figure 3.1 for a diagram of pen surfaces. Temperatures were observed by holding the thermometer approximately one meter above the ground and aiming it directly at the ground away from the observer's shadow.

Weather data were collected from two weather stations approximately one km from the feedlot. Data collected included hourly ambient temperature, daily high temperature, daily low temperature, and daily humidity, previous day high temperature, previous day low temperature, and previous day hourly temperature. Data were used to calculate Temperature Humidity Index (THI) (Thom, 1959).

4.3.4 Statistical Analysis

4.3.4.1 Hide temperatures

To evaluate differences in hide temperatures between treatments, hide colors, and sexes, a linear mixed-effects regression model was fitted using the lmer function in the statistical software R and evaluated using the anova function for analysis of variance. The mixed model included a random effect of block and fixed effects of date, time, treatment, sex, hide color, average pen surface temperature, date-by-time interaction, and date-by-treatment interaction. The response variable was average infrared hide temperature, with pen serving as the experimental unit. Relevant pairwise comparisons of estimated marginal means were performed using the emmeans function in R. Significance was declared at $P < 0.05$ and tendencies were declared at $0.05 \leq P \leq 0.10$.

4.3.4.2 Open mouth breathing

To evaluate differences in rates of OMB, a linear mixed-effects regression model was fit using the lmer function in R. A full model was fitted and analyzed using the anova function. Model selection was then performed using backward elimination by hand, starting with the least significant (e.g. highest *P*-value) predictor. Predictors with $P \leq 0.10$ were included in the final model. Treatment was left in the model regardless of significance. The final model included a random effect of block and fixed effects of date, time point, treatment, total average pen surface temperature, date-by-time interaction, and time-by-sex interaction. The response variable was percent of all cattle observed OMB, with pen serving as the experimental unit (n = 16). Relevant pairwise comparisons of estimated marginal means were evaluated using the emmeans function in R. Significance was declared at $P < 0.05$ and tendencies were declared at $0.05 \leq P \leq 0.10$.

Correlation coefficients between the response variable and various predictors were calculated using the cor.test function in R.

4.4 RESULTS

4.4.1 Hide and Pen-Surface Temperatures

Observed hide temperatures were greater ($P < 0.01$) on Test cattle than on Control cattle, though this difference was not observed on all observation days or at all observation times within all observation days (treatment within date interaction $P < 0.01$). Hide temperatures were observed to be greater on black-hided cattle than on non-black-hided cattle ($P < 0.01$). Hide temperatures were observed to be greater on steers than on heifers ($P < 0.01$).

Across all observation days, average pen surface temperatures were greater in Time 2 than in Time 1 ($P < 0.01$), but pen-surface temperatures were not different between Time 2 and Time 3 ($P = 0.37$). Hide temperatures were positively correlated with pen-surface temperatures ($R^2 = 0.43$). These results are summarized in Table 4.1.

4.4.2 OMB Data

No differences were observed between treatments for incidence of OMB ($P = 0.22$). This similarity between treatments was consistent across all time points. Percentages of cattle observed OMB was different across all time points ($P < 0.01$). There was a significant time point-by-sex interaction ($P < 0.01$). No differences were observed between sexes in Time A ($P = 0.50$) or Time B ($P = 0.36$), but percentages of heifers observed OMB were greater than percentages of steers observed OMB in Time C ($P = 0.01$). There was also a significant time point-by-date interaction ($P < 0.01$). These data are summarized in Table 4.2 and Table 4.3. Total average surface temperature was found to be a significant predictor of OMB ($P < 0.01$) but

was weakly correlated with observed rates of OMB (r = 0.46). See Table 4.4 for correlation coefficients of all observed predictors.

4.5 DISCUSSION

Quantification of heat stress in a commercial setting is a difficult task under the best of circumstances owing to variation in heat tolerance between individual animals. Additionally, cattle are likely to hide any symptom of weakness when an unfamiliar human is nearby. This behavioral modification increases difficulty in observing cattle in distress. It is known, however, that environmental stress is associated with declines in productivity and increases in expenses in feedlot cattle (Ray, 1989; Busby and Loy, 1996; St-Pierre et al., 2003; O'Brien et al., 2010; Broadway et al., 2020). Previous studies have sought to evaluate heat stress using panting scores as the outward sign of heat stress (Mader et al., 2006; Unruh et al., 2017). In general, these studies have relied on a 0 – 4 scoring scale that is difficult to evaluate on a commercial scale. Panting has, however, been accepted as a useful means of assessing heat load (Gaughan and Mader, 2014) in feedlot cattle.

Observations in the present study that panting incidence is different across time points was expected, as THI generally increases as the day goes on. In general, THI reaching higher levels should be expected to result in higher incidence of OMB. It was interesting, then, that THI was not found to be a significant predictor of OMB. Previous studies have found that daily high and low ambient temperatures, THI, and/or heat load index were significant predictors of heat stress (Brown-Brandl et al., 2005; Koknaroglu et al., 2008). It has also been shown that night-time cooling is an important factor in reducing effects of heat stress (Mader et al., 1999; Mader et al., 2010b). These studies have often relied on DDMI as the response variable, which was not used in the present study. Analyses of pen-level DDMI and other performance data may be more

useful than OMB as tools for evaluating impacts of heat stress. It is likely that difficulties inherent in observing a pen housing 250 animals for individuals OMB restricted observations, thus making data difficult to interpret.

Based on these observations, feeding the ProPath product is unlikely to mitigate impacts of heat stress. It should be noted that trace mineral concentrations in the present study, particularly levels of Zn, were far in excess of NRC recommendations (NAS, 2016). It is possible that different results may be obtained by feeding levels of minerals closer to NRC recommendations, though particularly in the case of Zn, levels investigated here more closely reflected industry norms (Vasconcelos and Galyean, 2007). It should also be noted that the manufacturer alerted investigators to problems with this batch of folic acid at the conclusion of the present study. The folate may not have been fully protected from ruminal degradation. Impacts of these manufacturing errors are unknown.

Observations of differences in OMB, hide temperatures, and pen-surface temperatures across different time points in the present study largely agreed with previous studies. In the present study, pen-surface temperatures increased rapidly in the morning, but levelled off in the middle of the day. This suggests that mitigation attempts should be initiated either the night before or early in the day when high temperatures are expected, supporting earlier reports (Davis et al., 2003; Gaughan et al., 2008a).

The present study was conducted in Eastern Colorado, an area with a temperate climate. Ambient temperatures may reach 40°C C for brief periods during the day, but often cool down below 20°C at night and remain relatively cool through the morning hours. This night-time cooling effect is believed to be an important factor in heat tolerance (Hahn, 1999; Gaughan et al., 2008a). Thus, cattle utilized in this study may not have experienced a significant accumulation of

heat throughout the summer. It is unlikely that this affected results indicating no difference between treatments, as all cattle within a block experienced the same cooling effect. However, results may be different if a similar study were to be conducted in an area with a different summer climate. It is worth noting that the highest single pen-surface temperature observed here was 68.7°C, and the highest hide temperature observed here was 57.2°C.

4.6 CONCLUSIONS

Based on these data, trace mineral source had an effect on hide temperature, but this effect was not correlated with visible signs of heat stress. It is unlikely that incidence of OMB was influenced by trace mineral source. Further investigation is warranted to determine best practices for observation and mitigation of heat stress in a commercial setting.

4.7 TABLES AND FIGURES

Table 4.1. Mean observed infrared temperatures of hides of black-hided and non-black-hided (NB) cattle, pen-surface locations, and treatments across time points.

Category	Time Point (All times ± 30 min)					
	Time 1 (0700 – 1000)		Time 2 (1015 – 1315)		Time 3 (1430 – 1700)	
	Mean Temp, Degrees C	SEM	Mean Temp, Degrees C	SEM	Mean Temp, Degrees C	SEM
Black	35.4	0.419	36.6	0.415	38.2	0.413
Steers	36.8	0.450	38.0	0.447	39.6	0.451
Heifers	34.0	0.511	35.2	0.506	36.8	0.501
NB	33.9	0.422	35.1	0.418	36.7	0.417
Steers	35.3	0.453	36.5	0.451	38.1	0.455
Heifers	32.5	0.513	33.7	0.509	35.3	0.504
Surface	25.0	0.139	37.8	0.127	38.0	0.130
Dirt	27.6	0.143	40.4	0.131	40.6	0.134
Apron	22.4	0.173	35.2	0.163	35.4	0.165
Test[1]	34.8	0.420	36.0	0.416	37.6	0.415
Control[2]	34.5	0.420	35.8	0.416	37.3	0.415

1. ProPath (Zinpro Corporation, Eden Prairie, MN) used to provide additional AA complexes of Zn and Mn, complexed Co, and ruminally-protected folic acid to basal control diet
2. Trace minerals of interest provided by inorganic sources

Table 4.2. Estimated marginal mean percentages of cattle observed open-mouth breathing (OMB)[1]

Category	Time Point (All times ± 30 min)					
	Time 1 (0700 – 1000)		Time 2 (1015 – 1315)		Time 3 (1430 – 1700)	
	Percent observed OMB	SEM	Percent observed OMB	SEM	Percent observed OMB	SEM
Heifers[2]	0.38[a]	0.138	0.06[b]	0.121	0.97[c]	0.118
Steers[2]	0.48[a]	0.120	0.20[b]	0.094	0.54[c]	0.095
Test[3]	0.47[a]	0.107	0.17[b]	0.08	0.79[c]	0.083
Control[3]	0.39[a]	0.108	0.09[b]	0.083	0.72[c]	0.082

1. Observations made on a pen basis
2. Significant Time:Sex interaction ($P < 0.01$)
3. Effect of time significant ($P < 0.01$); effect of treatment not significant ($P = 0.22$)

[a,b,c]: Means within a row lacking a common superscript differ ($P < 0.05$)

Table 4.3. Estimated marginal means of cattle observed open-mouth breathing (OMB)[1].

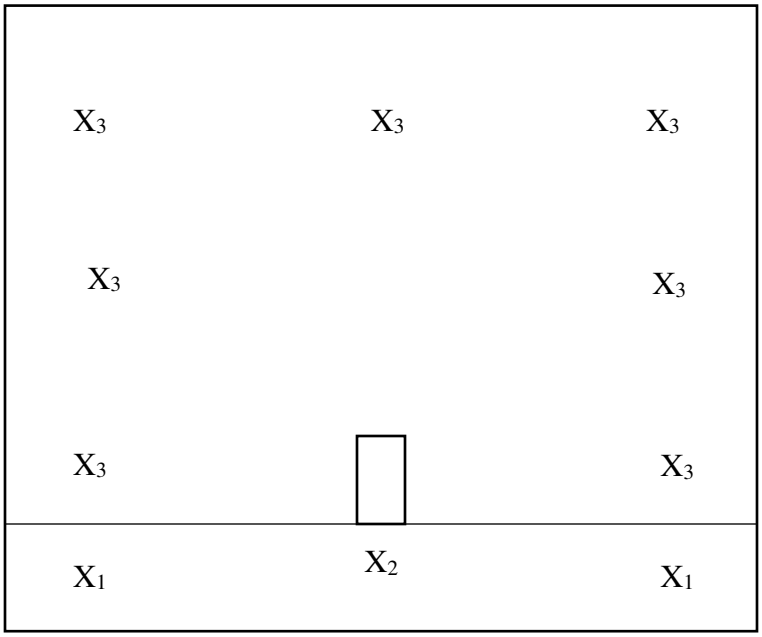
Time Point	Control	Test	*P*-Value	SEM
	Percent of all cattle observed			
A[2]	0.39	0.47	0.22	0.107
B[2]	0.09	0.17	0.22	0.083
C[2]	0.72	0.79	0.22	0.082

1. Observations made a pen basis
2. Effect of time significant ($P < 0.01$)

Table 4.4. Correlation coefficients between total percent of cattle observed OMB and various predictor variables.

Predictor Variable	r
Average Hide Temperature	0.44
Average Surface Temperature	0.46
Previous Day Average THI[a]	0.18
Previous Day Low THI[a]	0.17
Previous Day High THI[a]	0.12
Same Day Average THI[a]	0.29
Same Day Low THI[a]	0.24
Same Day High THI[a]	0.28

a. Temperature Humidity Index (Thom, 1959)



1. Location on cement bunk apron
2. Location on cement next to pen water tank
3. Location on dirt surface of pen

Figure 4.1. General diagram of pen surface with locations of pen temperature observations.

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Performance, Liver Abscess, and Pulmonary Lesion Analysis R Code

WT Nelson

July 28, 2020

```
Livers <- read.csv("C:/Users/WilliamNelson/Dropbox/R Analysis for Thesis/R Analysis Code and Data/LiverAbscessPercentagesCSVForR.csv")
Livers$Lot <- as.factor(Livers$Lot)
Livers$Block <- as.factor(Livers$Block)
str(Livers)
```

```
## 'data.frame':    32 obs. of  18 variables:
##  $ Lot                           : Factor w/ 32 levels "20971","20972",..: 1 2 3 4 5 6 7 8 9 10 ...
##  $ Treatment                     : Factor w/ 2 levels "Control","Zinpro": 2 1 1 2 1 2 1 2 2 1 ...
##  $ Block                         : Factor w/ 16 levels "1","2","3","4",..: 1 1 2 2 3 3 4 4 5 5 ...
##  $ Sex                           : Factor w/ 2 levels "Heifer","Steer": 2 2 2 2 2 2 2 2 2 2 ...
##  $ Normal                        : num  55.9 56.5 50 54.2 52.5 ...
##  $ Aminus                        : num  7.76 7.69 9.62 9.23 10.74 ...
##  $ A                             : num  14.3 13.5 10.4 13.1 13.6 ...
##  $ Aplus                         : num  10.61 11.15 16.92 10.38 9.92 ...
##  $ APlusAdheredOpen              : num  2.86 3.08 3.08 1.92 4.55 ...
##  $ APlusAdhered                  : num  2.86 2.31 1.92 4.23 1.65 ...
##  $ APlusOpen                     : num  4.49 2.31 6.54 5 4.55 ...
##  $ TotalAPlusPercentofTotalLivers: num  20.8 18.8 28.5 21.5 20.7 ...
##  $ Cirrhosis                     : num  0.408 1.538 0.769 0.769 0.413 ...
##  $ Fluke                         : num  0.408 0 0 0 0 ...
##  $ Telang                        : num  0.408 1.923 0.769 1.154 2.066 ...
##  $ TotalAbscess                  : num  42.9 40 48.5 43.8 45 ...
```

```
##  $ TotalAPlusPercentofAbscess    : num  38.1 41.3 45.2 37.7 35.8 ..
.
##  $ Total                         : int  100 100 100 100 100 100 100
100 100 100 ...
```

```
library(lme4)
```

```
## Loading required package: Matrix
```

```
library(lmerTest)
```

```
## Warning: package 'lmerTest' was built under R version 3.6.3
```

```
##
## Attaching package: 'lmerTest'
```

```
## The following object is masked from 'package:lme4':
##
##     lmer
```

```
## The following object is masked from 'package:stats':
##
##     step
```

```
library(pbkrtest)
library(emmeans)
```

```
## Welcome to emmeans.
## NOTE -- Important change from versions <= 1.41:
##     Indicator predictors are now treated as 2-level factors by defa
ult.
##     To revert to old behavior, use emm_options(cov.keep = character
(0))
```

```
TotalPercent <- lmer(TotalAbscess ~ Treatment*Sex + (1|Block), data =
Livers)
anova(TotalPercent, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Treatment      6.9846  6.9846     1    14  0.2529 0.6229
## Sex           29.5755 29.5755     1    14  1.0707 0.3183
## Treatment:Sex 15.8917 15.8917     1    14  0.5753 0.4607
```

```
EMTotal1 <- emmeans(TotalPercent, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMTotal1
```

```
## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     42.8 2.34 19.4     37.9     47.7
##  Zinpro      41.9 2.34 19.4     37.0     46.8
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    0.965 1.92 14 0.503   0.6229
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
EMTotal2 <- emmeans(TotalPercent, pairwise ~ Sex)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMTotal2
```

```
## $emmeans
##  Sex    emmean   SE df lower.CL upper.CL
##  Heifer   44.6 3.38 14     37.3     51.8
##  Steer    40.1 2.62 14     34.5     45.7
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast       estimate   SE df t.ratio p.value
##  Heifer - Steer     4.43 4.28 14 1.035   0.3183
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
```

```
EMTotal3 <- emmeans(TotalPercent, pairwise ~ Sex|Treatment)
EMTotal3
```

```
## $emmeans
## Treatment = Control:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   45.8 3.71 19.4     38.0     53.5
##  Steer    39.9 2.87 19.4     33.9     45.9
##
```

```
## Treatment = Zinpro:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   43.3 3.71 19.4     35.6     51.1
##  Steer    40.4 2.87 19.4     34.4     46.4
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = Control:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer     5.88 4.69 19.4 1.255   0.2245
##
## Treatment = Zinpro:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer     2.97 4.69 19.4 0.634   0.5338
##
## Degrees-of-freedom method: kenward-roger
```

```
EMTotal4 <- emmeans(TotalPercent, pairwise ~ Treatment|Sex)
EMTotal4
```

```
## $emmeans
## Sex = Heifer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     45.8 3.71 19.4     38.0     53.5
##  Zinpro      43.3 3.71 19.4     35.6     51.1
##
## Sex = Steer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     39.9 2.87 19.4     33.9     45.9
##  Zinpro      40.4 2.87 19.4     34.4     46.4
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = Heifer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    2.421 3.03 14  0.798  0.4383
##
## Sex = Steer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro   -0.491 2.35 14 -0.209  0.8377
##
## Degrees-of-freedom method: kenward-roger
```

```r
APlusAbscess <- lmer(TotalAPlusPercentofTotalLivers ~ Treatment*Sex +
(1|Block), data = Livers)
anova(APlusAbscess, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Treatment     12.9102 12.9102     1    14  2.4009 0.1436
## Sex            0.3011  0.3011     1    14  0.0560 0.8164
## Treatment:Sex  3.2009  3.2009     1    14  0.5953 0.4532
```

```r
EMAPlus <- emmeans(APlusAbscess, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```r
EMAPlus
```

```
## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     19.6 1.09 18.8     17.3     21.9
##  Zinpro      18.3 1.09 18.8     16.0     20.6
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate    SE df t.ratio p.value
##  Control - Zinpro     1.31 0.847 14 1.549   0.1436
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```r
EMAPlus1 <- emmeans(APlusAbscess, pairwise ~ Sex)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```r
EMAPlus1
```

```
## $emmeans
##  Sex    emmean   SE df lower.CL upper.CL
##  Heifer   19.2 1.59 14     15.8     22.6
##  Steer    18.7 1.23 14     16.1     21.4
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
```

```
##  contrast       estimate   SE df t.ratio p.value
##  Heifer - Steer    0.477 2.02 14 0.237   0.8164
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
```

```
EMAPlus2 <- emmeans(APlusAbscess, pairwise ~ Sex|Treatment)
EMAPlus2
```

```
## $emmeans
## Treatment = Control:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   20.2 1.73 18.8     16.6     23.8
##  Steer    19.0 1.34 18.8     16.2     21.8
##
## Treatment = Zinpro:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   18.2 1.73 18.8     14.6     21.8
##  Steer    18.4 1.34 18.8     15.6     21.2
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = Control:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer    1.130 2.19 18.8  0.517  0.6112
##
## Treatment = Zinpro:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer   -0.176 2.19 18.8 -0.081  0.9366
##
## Degrees-of-freedom method: kenward-roger
```

```
EMAPlus3 <- emmeans(APlusAbscess, pairwise ~ Treatment|Sex)
EMAPlus3
```

```
## $emmeans
## Sex = Heifer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     20.2 1.73 18.8     16.6     23.8
##  Zinpro      18.2 1.73 18.8     14.6     21.8
##
## Sex = Steer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     19.0 1.34 18.8     16.2     21.8
##  Zinpro      18.4 1.34 18.8     15.6     21.2
```

```
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = Heifer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    1.965 1.34 14 1.468   0.1642
##
## Sex = Steer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    0.659 1.04 14 0.635   0.5355
##
## Degrees-of-freedom method: kenward-roger
```

```
AMinusAbscess <- lmer(Aminus ~ Treatment*Sex + (1|Block), data = Liver
s)
anova(AMinusAbscess, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Treatment      1.8381  1.8381     1    14  0.2385 0.6328
## Sex           15.1026 15.1026     1    14  1.9596 0.1833
## Treatment:Sex  0.0077  0.0077     1    14  0.0010 0.9752
```

```
EMAMinus <- emmeans(AMinusAbscess, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMAMinus
```

```
## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     11.5 1.35 18.4     8.69     14.4
##  Zinpro      12.0 1.35 18.4     9.19     14.9
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro   -0.495 1.01 14 -0.488  0.6328
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
EMAMinus1 <- emmeans(AMinusAbscess, pairwise ~ Sex)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMAMinus1
```

```
## $emmeans
##  Sex    emmean   SE df lower.CL upper.CL
##  Heifer   13.5 1.98 14     9.28     17.8
##  Steer    10.0 1.54 14     6.72     13.3
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast       estimate   SE df t.ratio p.value
##  Heifer - Steer     3.51 2.51 14 1.400   0.1833
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
```

```
EMAMinus2 <- emmeans(AMinusAbscess, pairwise ~ Sex|Treatment)
EMAMinus2
```

```
## $emmeans
## Treatment = Control:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer  13.30 2.14 18.4     8.81     17.8
##  Steer    9.76 1.66 18.4     6.28     13.2
##
## Treatment = Zinpro:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer  13.76 2.14 18.4     9.28     18.3
##  Steer   10.28 1.66 18.4     6.81     13.8
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = Control:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer     3.55 2.71 18.4 1.310   0.2063
##
## Treatment = Zinpro:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer     3.48 2.71 18.4 1.286   0.2143
##
## Degrees-of-freedom method: kenward-roger
```

```
EMAMinus3 <- emmeans(AMinusAbscess, pairwise ~ Treatment|Sex)
EMAMinus3
```

```
## $emmeans
## Sex = Heifer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control    13.30 2.14 18.4     8.81     17.8
##  Zinpro     13.76 2.14 18.4     9.28     18.3
##
## Sex = Steer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     9.76 1.66 18.4     6.28     13.2
##  Zinpro     10.28 1.66 18.4     6.81     13.8
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = Heifer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro   -0.463 1.60 14 -0.289  0.7769
##
## Sex = Steer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro   -0.527 1.24 14 -0.425  0.6776
##
## Degrees-of-freedom method: kenward-roger
```

```
AAbscess <- lmer(A ~ Treatment*Sex + (1|Block), data = Livers)
anova(AAbscess, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Treatment     0.1645  0.1645     1    14  0.0254 0.8756
## Sex           0.8605  0.8605     1    14  0.1329 0.7208
## Treatment:Sex 4.4496  4.4496     1    14  0.6874 0.4209
```

```
EMA <- emmeans(AAbscess, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMA
```

```
## $emmeans
##  Treatment emmean    SE   df lower.CL upper.CL
##  Control     11.7 0.756 26.4    10.13     13.2
##  Zinpro      11.5 0.756 26.4     9.98     13.1
##
```

```
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate    SE df t.ratio p.value
##  Control - Zinpro    0.148 0.929 14 0.159   0.8756
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger

EMA1 <- emmeans(AAbscess, pairwise ~ Sex)

## NOTE: Results may be misleading due to involvement in interactions

EMA1

## $emmeans
##  Sex    emmean    SE df lower.CL upper.CL
##  Heifer   11.8 0.944 14     9.81     13.9
##  Steer    11.4 0.731 14     9.83     13.0
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast       estimate   SE df t.ratio p.value
##  Heifer - Steer    0.435 1.19 14 0.365   0.7208
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger

EMA2 <- emmeans(AAbscess, pairwise ~ Sex|Treatment)
EMA2

## $emmeans
## Treatment = Control:
##  Sex    emmean    SE   df lower.CL upper.CL
##  Heifer   12.3 1.196 26.4     9.83     14.7
##  Steer    11.1 0.926 26.4     9.18     13.0
##
## Treatment = Zinpro:
##  Sex    emmean    SE   df lower.CL upper.CL
##  Heifer   11.4 1.196 26.4     8.91     13.8
##  Steer    11.7 0.926 26.4     9.80     13.6
##
```

```
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = Control:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer    1.206 1.51 26.4  0.797  0.4326
##
## Treatment = Zinpro:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer   -0.335 1.51 26.4 -0.221  0.8265
##
## Degrees-of-freedom method: kenward-roger
```

```
EMA3 <- emmeans(AAbscess, pairwise ~ Treatment|Sex)
EMA3
```

```
## $emmeans
## Sex = Heifer:
##  Treatment emmean    SE   df lower.CL upper.CL
##  Control     12.3 1.196 26.4     9.83     14.7
##  Zinpro      11.4 1.196 26.4     8.91     13.8
##
## Sex = Steer:
##  Treatment emmean    SE   df lower.CL upper.CL
##  Control     11.1 0.926 26.4     9.18     13.0
##  Zinpro      11.7 0.926 26.4     9.80     13.6
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = Heifer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    0.918 1.47 14  0.625  0.5419
##
## Sex = Steer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro   -0.622 1.14 14 -0.547  0.5931
##
## Degrees-of-freedom method: kenward-roger
```

```
NormalLiver <- lmer(Normal ~ Treatment*Sex + (1|Block), data = Livers)
anova(NormalLiver, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
```

```
## Treatment      3.4462  3.4462     1    14  0.1291 0.7247
## Sex           22.2942 22.2942     1    14  0.8351 0.3763
## Treatment:Sex 18.2622 18.2622     1    14  0.6841 0.4221

EMNorm <- emmeans(NormalLiver, pairwise ~ Treatment)

## NOTE: Results may be misleading due to involvement in interactions

EMNorm

## $emmeans
##  Treatment emmean   SE df lower.CL upper.CL
##  Control     55.1 2.38 19     50.1     60.1
##  Zinpro      55.8 2.38 19     50.8     60.8
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro   -0.678 1.89 14 -0.359  0.7247
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger

EMNorm1 <- emmeans(NormalLiver, pairwise ~ Sex)

## NOTE: Results may be misleading due to involvement in interactions

EMNorm1

## $emmeans
##  Sex    emmean   SE df lower.CL upper.CL
##  Heifer   53.5 3.46 14     46.0     60.9
##  Steer    57.4 2.68 14     51.7     63.2
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast       estimate   SE df t.ratio p.value
##  Heifer - Steer       -4 4.38 14 -0.914  0.3763
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
```

```
EMNorm2 <- emmeans(NormalLiver, pairwise ~ Sex|Treatment)
EMNorm2
```

```
## $emmeans
## Treatment = Control:
##  Sex    emmean   SE df lower.CL upper.CL
##  Heifer   52.3 3.77 19     44.4     60.2
##  Steer    57.9 2.92 19     51.8     64.0
##
## Treatment = Zinpro:
##  Sex    emmean   SE df lower.CL upper.CL
##  Heifer   54.6 3.77 19     46.7     62.5
##  Steer    57.0 2.92 19     50.9     63.1
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = Control:
##  contrast       estimate   SE df t.ratio p.value
##  Heifer - Steer    -5.56 4.77 19 -1.167  0.2578
##
## Treatment = Zinpro:
##  contrast       estimate   SE df t.ratio p.value
##  Heifer - Steer    -2.44 4.77 19 -0.512  0.6147
##
## Degrees-of-freedom method: kenward-roger
```

```
EMNorm3 <- emmeans(NormalLiver, pairwise ~ Treatment|Sex)
EMNorm3
```

```
## $emmeans
## Sex = Heifer:
##  Treatment emmean   SE df lower.CL upper.CL
##  Control     52.3 3.77 19     44.4     60.2
##  Zinpro      54.6 3.77 19     46.7     62.5
##
## Sex = Steer:
##  Treatment emmean   SE df lower.CL upper.CL
##  Control     57.9 2.92 19     51.8     64.0
##  Zinpro      57.0 2.92 19     50.9     63.1
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
```

```
## Sex = Heifer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro   -2.238 2.98 14 -0.750  0.4655
##
## Sex = Steer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    0.883 2.31 14  0.382  0.7082
##
## Degrees-of-freedom method: kenward-roger
```

```
Lungs <- read.csv("C:/Users/WilliamNelson/Dropbox/R Analysis for Thesi
s/R Analysis Code and Data/PulmonaryLesionPercentagesCSVForR.csv")
Lungs$Lot <- as.factor(Lungs$Lot)
Lungs$Block <- as.factor(Lungs$Block)
str(Lungs)
```

```
## 'data.frame':    32 obs. of  12 variables:
##  $ Lot           : Factor w/ 32 levels "20971","20972",..: 1 2 3 4
5 6 7 8 9 10 ...
##  $ Treatment     : Factor w/ 2 levels "Control","Zinpro": 2 1 1 2 1
2 1 2 2 1 ...
##  $ Block         : Factor w/ 16 levels "1","2","3","4",..: 1 1 2 2
3 3 4 4 5 5 ...
##  $ Sex           : Factor w/ 2 levels "Heifer","Steer": 2 2 2 2 2 2
2 2 2 2 ...
##  $ InitialHdCount: int  280 279 280 280 277 279 280 279 280 280 ...
##  $ DOF           : int  249 249 214 214 241 241 199 199 227 227 ...
##  $ Normal        : num  71.4 62.9 NA NA 64.3 ...
##  $ Mild          : num  1.24 5.79 NA NA 5.22 ...
##  $ Moderate      : num  14.1 13.9 NA NA 19.6 ...
##  $ Severe        : num  13.3 17.4 NA NA 10.9 ...
##  $ TotalLesion   : num  28.6 37.1 NA NA 35.7 ...
##  $ Total         : int  100 100 NA NA 100 100 NA NA 100 100 ...
```

```
NormLung <- lmer(Normal ~ Treatment*Sex + (1|Block), data = Lungs)
anova(NormLung, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Treatment     24.923  24.923     1     7  0.4888 0.5070
## Sex           42.385  42.385     1     7  0.8312 0.3922
## Treatment:Sex 65.879  65.879     1     7  1.2920 0.2931
```

```
EMNL <- emmeans(NormLung, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMNL
```

```
## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     66.3 3.73 10.8     58.1     74.5
##  Zinpro      63.8 3.73 10.8     55.6     72.0
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro      2.5 3.57  7 0.699   0.5070
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
EMNL1 <- emmeans(NormLung, pairwise ~ Sex)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMNL1
```

```
## $emmeans
##  Sex    emmean   SE df lower.CL upper.CL
##  Heifer   62.1 5.34  7     49.4     74.7
##  Steer    68.0 3.78  7     59.1     77.0
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast       estimate   SE df t.ratio p.value
##  Heifer - Steer    -5.97 6.54  7 -0.912  0.3922
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
```

```
EMNL2 <- emmeans(NormLung, pairwise ~ Sex|Treatment)
EMNL2
```

```
## $emmeans
## Treatment = Control:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   61.3 6.09 10.8     47.9     74.7
##  Steer    71.3 4.30 10.8     61.8     80.8
##
```

```
## Treatment = Zinpro:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   62.9 6.09 10.8     49.4     76.3
##  Steer    64.8 4.30 10.8     55.3     74.3
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = Control:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer   -10.02 7.45 10.8 -1.345  0.2062
##
## Treatment = Zinpro:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer    -1.91 7.45 10.8 -0.256  0.8029
##
## Degrees-of-freedom method: kenward-roger
```

```
EMNL3 <- emmeans(NormLung, pairwise ~ Treatment|Sex)
EMNL3
```

```
## $emmeans
## Sex = Heifer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     61.3 6.09 10.8     47.9     74.7
##  Zinpro      62.9 6.09 10.8     49.4     76.3
##
## Sex = Steer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     71.3 4.30 10.8     61.8     80.8
##  Zinpro      64.8 4.30 10.8     55.3     74.3
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = Heifer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    -1.56 5.83  7 -0.268  0.7965
##
## Sex = Steer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro     6.55 4.12  7  1.590  0.1559
##
## Degrees-of-freedom method: kenward-roger
```

```r
MildLung <- lmer(Mild ~ Treatment*Sex + (1|Block), data = Lungs)
anova(MildLung, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value  Pr(>F)
## Treatment      3.742   3.742     1     7  0.2383 0.64038
## Sex            9.159   9.159     1     7  0.5832 0.47003
## Treatment:Sex 76.742  76.742     1     7  4.8860 0.06276 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
EMMildLung <- emmeans(MildLung, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```r
EMMildLung
```

```
## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     4.55 1.69 12.8    0.896     8.20
##  Zinpro      5.52 1.69 12.8    1.863     9.17
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro   -0.967 1.98  7 -0.488  0.6404
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```r
EMMildLung2 <- emmeans(MildLung, pairwise ~ Sex)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```r
EMMildLung2
```

```
## $emmeans
##  Sex    emmean   SE df lower.CL upper.CL
##  Heifer   3.99 2.23  7    -1.29     9.27
##  Steer    6.08 1.58  7     2.35     9.81
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
```

```
## $contrasts
##  contrast       estimate   SE df t.ratio p.value
##  Heifer - Steer    -2.09 2.73  7 -0.764  0.4700
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
```

```
EMMildLung3 <- emmeans(MildLung, pairwise ~ Sex|Treatment)
EMMildLung3
```

```
## $emmeans
## Treatment = Control:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   5.70 2.76 12.8   -0.271    11.66
##  Steer    3.40 1.95 12.8   -0.815     7.62
##
## Treatment = Zinpro:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   2.28 2.76 12.8   -3.684     8.25
##  Steer    8.75 1.95 12.8    4.532    12.97
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = Control:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer     2.29 3.38 12.8  0.679  0.5094
##
## Treatment = Zinpro:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer    -6.47 3.38 12.8 -1.916  0.0781
##
## Degrees-of-freedom method: kenward-roger
```

```
EMMildLung4 <- emmeans(MildLung, pairwise ~ Treatment|Sex)
EMMildLung4
```

```
## $emmeans
## Sex = Heifer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     5.70 2.76 12.8   -0.271    11.66
##  Zinpro      2.28 2.76 12.8   -3.684     8.25
##
## Sex = Steer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     3.40 1.95 12.8   -0.815     7.62
```

```
##  Zinpro      8.75 1.95 12.8    4.532    12.97
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = Heifer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro     3.41 3.24  7  1.055  0.3266
##
## Sex = Steer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    -5.35 2.29  7 -2.337  0.0521
##
## Degrees-of-freedom method: kenward-roger
```

```
ModerateLung <- lmer(Moderate ~ Treatment*Sex + (1|Block), data = Lung
s)
anova(ModerateLung, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value  Pr(>F)
## Treatment      0.724   0.724     1     7  0.0340 0.85903
## Sex           22.894  22.894     1     7  1.0732 0.33468
## Treatment:Sex 77.592  77.592     1     7  3.6373 0.09817 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
EMMod <- emmeans(ModerateLung, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMMod
```

```
## $emmeans
##  Treatment emmean  SE   df lower.CL upper.CL
##  Control     17.8 2.2 11.7     13.0     22.6
##  Zinpro      18.2 2.2 11.7     13.4     23.0
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro   -0.426 2.31  7 -0.184  0.8590
##
```

```
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger

EMMod1 <- emmeans(ModerateLung, pairwise ~ Sex)

## NOTE: Results may be misleading due to involvement in interactions

EMMod1

## $emmeans
##  Sex    emmean   SE df lower.CL upper.CL
##  Heifer   20.0 3.05  7     12.7     27.2
##  Steer    16.1 2.16  7     11.0     21.2
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast       estimate   SE df t.ratio p.value
##  Heifer - Steer     3.87 3.74  7 1.036   0.3347
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger

EMMod2 <- emmeans(ModerateLung, pairwise ~ Sex|Treatment)
EMMod2

## $emmeans
## Treatment = Control:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   21.9 3.59 11.7    14.10     29.8
##  Steer    13.7 2.54 11.7     8.13     19.2
##
## Treatment = Zinpro:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   18.0 3.59 11.7    10.13     25.8
##  Steer    18.5 2.54 11.7    12.96     24.0
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = Control:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer    8.274 4.39 11.7  1.884  0.0847
##
```

```
## Treatment = Zinpro:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer   -0.535 4.39 11.7 -0.122  0.9052
##
## Degrees-of-freedom method: kenward-roger
```

```
EMMod3 <- emmeans(ModerateLung, pairwise ~ Treatment|Sex)
EMMod3
```

```
## $emmeans
## Sex = Heifer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     21.9 3.59 11.7    14.10     29.8
##  Zinpro      18.0 3.59 11.7    10.13     25.8
##
## Sex = Steer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     13.7 2.54 11.7     8.13     19.2
##  Zinpro      18.5 2.54 11.7    12.96     24.0
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = Heifer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro     3.98 3.77  7  1.055  0.3264
##
## Sex = Steer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    -4.83 2.67  7 -1.811  0.1130
##
## Degrees-of-freedom method: kenward-roger
```

```
SevereLung <- lmer(Severe ~ Treatment*Sex + (1|Block), data = Lungs)
anova(SevereLung, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value   Pr(>F)
## Treatment      4.869   4.869     1     7  1.2716 0.296636
## Sex            4.743   4.743     1     7  1.2385 0.302512
## Treatment:Sex 89.345  89.345     1     7 23.3307 0.001899 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
EMSevere <- emmeans(SevereLung, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions

EMSevere

## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     11.3 1.94 7.94     6.85     15.8
##  Zinpro      12.4 1.94 7.94     7.96     16.9
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate    SE df t.ratio p.value
##  Control - Zinpro     -1.1 0.978  7 -1.128  0.2966
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger

EMSevere1 <- emmeans(SevereLung, pairwise ~ Sex)

## NOTE: Results may be misleading due to involvement in interactions

EMSevere1

## $emmeans
##  Sex    emmean   SE df lower.CL upper.CL
##  Heifer   14.0 3.07  7     6.72     21.2
##  Steer     9.8 2.17  7     4.66     14.9
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast       estimate   SE df t.ratio p.value
##  Heifer - Steer     4.18 3.76  7 1.113   0.3025
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger

EMSevere2 <- emmeans(SevereLung, pairwise ~ Sex|Treatment)
EMSevere2

## $emmeans
## Treatment = Control:
##  Sex    emmean   SE   df lower.CL upper.CL
```

```
##  Heifer  11.07 3.17 7.94     3.74     18.4
##  Steer   11.61 2.24 7.94     6.43     16.8
##
## Treatment = Zinpro:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer  16.89 3.17 7.94     9.57     24.2
##  Steer    7.99 2.24 7.94     2.81     13.2
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = Control:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer   -0.543 3.88 7.94 -0.140  0.8924
##
## Treatment = Zinpro:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer    8.910 3.88 7.94  2.294  0.0512
##
## Degrees-of-freedom method: kenward-roger
```

```
EMSevere3 <- emmeans(SevereLung, pairwise ~ Treatment|Sex)
EMSevere3
```

```
## $emmeans
## Sex = Heifer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control    11.07 3.17 7.94     3.74     18.4
##  Zinpro     16.89 3.17 7.94     9.57     24.2
##
## Sex = Steer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control    11.61 2.24 7.94     6.43     16.8
##  Zinpro      7.99 2.24 7.94     2.81     13.2
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = Heifer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    -5.83 1.60  7 -3.648  0.0082
##
## Sex = Steer:
##  contrast         estimate   SE df t.ratio p.value
```

```
##  Control - Zinpro     3.62 1.13  7  3.206  0.0149
##
## Degrees-of-freedom method: kenward-roger
```

```
TotalLung <- lmer(TotalLesion ~ Treatment*Sex + (1|Block), data = Lung
s)
anova(TotalLung, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Treatment     24.923  24.923     1     7  0.4888 0.5070
## Sex           42.385  42.385     1     7  0.8312 0.3922
## Treatment:Sex 65.879  65.879     1     7  1.2920 0.2931
```

```
EMTL <- emmeans(TotalLung, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMTL
```

```
## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     33.7 3.73 10.8     25.5     41.9
##  Zinpro      36.2 3.73 10.8     28.0     44.4
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro     -2.5 3.57  7 -0.699  0.5070
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
EMTL1 <- emmeans(TotalLung, pairwise ~ Sex)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMTL1
```

```
## $emmeans
##  Sex    emmean   SE df lower.CL upper.CL
##  Heifer   37.9 5.34  7     25.3     50.6
##  Steer    32.0 3.78  7     23.0     40.9
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
```

```
## Confidence level used: 0.95
##
## $contrasts
##  contrast       estimate   SE df t.ratio p.value
##  Heifer - Steer     5.97 6.54  7 0.912   0.3922
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
```

```
EMTL2 <- emmeans(TotalLung, pairwise ~ Sex|Treatment)
EMTL2
```

```
## $emmeans
## Treatment = Control:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   38.7 6.09 10.8     25.3     52.1
##  Steer    28.7 4.30 10.8     19.2     38.2
##
## Treatment = Zinpro:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   37.1 6.09 10.8     23.7     50.6
##  Steer    35.2 4.30 10.8     25.7     44.7
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = Control:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer    10.02 7.45 10.8 1.345   0.2062
##
## Treatment = Zinpro:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer     1.91 7.45 10.8 0.256   0.8029
##
## Degrees-of-freedom method: kenward-roger
```

```
EMTL3 <- emmeans(TotalLung, pairwise ~ Treatment|Sex)
EMTL3
```

```
## $emmeans
## Sex = Heifer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     38.7 6.09 10.8     25.3     52.1
##  Zinpro      37.1 6.09 10.8     23.7     50.6
##
## Sex = Steer:
```

```
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     28.7 4.30 10.8     19.2     38.2
##  Zinpro      35.2 4.30 10.8     25.7     44.7
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = Heifer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro     1.56 5.83  7  0.268  0.7965
##
## Sex = Steer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    -6.55 4.12  7 -1.590  0.1559
##
## Degrees-of-freedom method: kenward-roger
```

```
Performance <- read.csv( "C:/Users/WilliamNelson/Dropbox/R Analysis fo
r Thesis/R Analysis Code and Data/PerformanceMetricCSVForR.csv")
Performance$Trt <- as.factor(Performance$Trt)
Performance$Rep <- as.factor(Performance$Rep)
Performance$Lot <- as.factor(Performance$Lot)
str(Performance)
```

```
## 'data.frame':    32 obs. of  45 variables:
##  $ Lot               : Factor w/ 32 levels "20971","20972",..: 1 2
3 4 5 6 7 8 9 10 ...
##  $ Trt               : Factor w/ 2 levels "1","2": 2 1 1 2 1 2 1 2
2 1 ...
##  $ Rep               : Factor w/ 16 levels "1","2","3","4",..: 1 1
2 2 3 3 4 4 5 5 ...
##  $ Sex               : Factor w/ 2 levels "H","S": 2 2 2 2 2 2 2 2
2 2 ...
##  $ Initial_BW        : num  367 367 412 415 360 ...
##  $ Final_BW_DRIN     : num  637 646 660 673 617 ...
##  $ Total_Gain_DRIN   : num  242 265 220 227 211 ...
##  $ ADG_DRIN          : num  1.03 1.11 1.08 1.11 0.94 0.74 1.07 1.07
1.04 1.08 ...
##  $ DMI_DRIN          : num  9.81 10.2 10.52 10.48 9.9 ...
##  $ FG_DRIN           : num  9.48 9.18 9.79 9.43 10.49 ...
##  $ GF_DRIN           : num  0.105 0.109 0.102 0.106 0.095 0.075 0.1
06 0.104 0.102 0.107 ...
##  $ Final_BW_DRO      : num  664 673 684 691 650 ...
##  $ Total_Gain_DRO    : num  297 306 272 276 290 ...
##  $ ADG_DRO           : num  1.19 1.23 1.27 1.29 1.21 1.19 1.19 1.27
```

```
1.29 1.27 ...
##  $ DMI_DRO           : num  9.81 10.2 10.52 10.48 9.9 ...
##  $ FG_DRO            : num  8.22 8.3 8.29 8.11 8.2 8.32 8.47 8.1 7.
89 7.97 ...
##  $ GF_DRO            : num  0.122 0.12 0.121 0.123 0.122 0.12 0.118
0.123 0.127 0.125 ...
##  $ Final_BW_RO       : num  664 673 684 691 650 ...
##  $ Total_Gain_RO     : num  287 294 263 272 279 ...
##  $ ADG_RO            : num  1.12 1.16 1.19 1.24 1.1 1.04 1.12 1.23
1.2 1.17 ...
##  $ DMI_RO            : num  9.81 10.2 10.52 10.48 9.9 ...
##  $ FG_RO             : num  8.72 8.8 8.87 8.45 9.03 9.53 9 8.33 8.5
2 8.66 ...
##  $ GF_RO             : num  0.115 0.114 0.113 0.118 0.111 0.105 0.1
11 0.12 0.117 0.115 ...
##  $ ADJFinal_BW_DRIN  : num  645 658 649 661 626 ...
##  $ Total_ADJGain_DRIN: num  278 291 237 246 267 ...
##  $ ADJADG_DRIN       : num  1.19 1.22 1.16 1.21 1.19 1.19 1.18 1.26
1.23 1.2 ...
##  $ ADJFG_DRIN        : num  8.25 8.36 9.1 8.68 8.3 8.32 8.5 8.13 8.
3 8.43 ...
##  $ ADJGF_DRIN        : num  0.121 0.12 0.11 0.115 0.121 0.12 0.118
0.123 0.121 0.119 ...
##  $ ADJFinal_BW_DRO   : num  674 686 672 679 660 ...
##  $ DJTotal_Gain_DRO  : num  306 319 260 264 301 ...
##  $ ADJADG_DRO        : num  1.23 1.28 1.22 1.23 1.25 1.23 1.24 1.28
1.28 1.27 ...
##  $ ADJFG_DRO         : num  7.98 7.98 8.66 8.49 7.93 8.04 8.09 8.02
7.97 7.97 ...
##  $ ADJGF_DRO         : num  0.125 0.125 0.116 0.118 0.126 0.124 0.1
24 0.125 0.125 0.125 ...
##  $ ADJFinal_BW_RO    : num  673 686 672 679 661 ...
##  $ ADJTotal_Gain_RO  : num  296 307 251 260 290 ...
##  $ ADJADG_RO         : num  1.19 1.23 1.17 1.21 1.2 1.18 1.2 1.26 1
.24 1.22 ...
##  $ ADJFG_RO          : num  8.25 8.27 8.97 8.63 8.23 8.41 8.41 8.12
8.22 8.29 ...
##  $ ADJGF_RO          : num  0.121 0.121 0.111 0.116 0.121 0.119 0.1
19 0.123 0.122 0.121 ...
##  $ HCW               : num  420 428 419 423 412 ...
##  $ Inwt_Tatum        : num  215 215 246 247 210 ...
##  $ HCW_ADG_Tatum     : num  0.82 0.85 0.81 0.82 0.84 0.82 0.83 0.85
0.86 0.85 ...
##  $ Dress_Perc        : num  0.628 0.633 0.613 0.612 0.627 ...
##  $ BFAT              : num  1.74 1.71 1.61 1.62 1.57 ...
##  $ REA               : num  86.2 86.6 84.1 84.2 81.7 ...
```

```
##  $ MARB              : int  626 652 578 586 621 621 567 573 617 619
...

InitialBW <- lmer(Initial_BW ~ Trt*Sex + (1|Rep), data = Performance)
anova(InitialBW, ddf = "Kenward-Roger")

## Type III Analysis of Variance Table with Kenward-Roger's method
##          Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Trt     0.07130 0.07130     1    14  0.0510 0.8246
## Sex     2.77788 2.77788     1    14  1.9874 0.1804
## Trt:Sex 0.02067 0.02067     1    14  0.0148 0.9049

EMInit <- emmeans(InitialBW, pairwise ~ Trt)

## NOTE: Results may be misleading due to involvement in interactions

EMInit

## $emmeans
##  Trt emmean   SE df lower.CL upper.CL
##  1      378 8.14 14      361      396
##  2      379 8.14 14      361      396
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate    SE df t.ratio p.value
##  1 - 2     -0.0975 0.432 14 -0.226  0.8246
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger

FBWDRIN <- lmer(Final_BW_DRIN ~ Trt*Sex + (1|Rep), data = Performance)
anova(FBWDRIN, ddf = "Kenward-Roger")

## Type III Analysis of Variance Table with Kenward-Roger's method
##          Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## Trt      404.09  404.09     1    14  5.5075 0.0341707 *
## Sex     1625.44 1625.44     1    14 22.1540 0.0003368 ***
## Trt:Sex   13.73   13.73     1    14  0.1872 0.6718693
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

EMFBWDRIN <- emmeans(FBWDRIN, pairwise ~ Trt)

## NOTE: Results may be misleading due to involvement in interactions
```

```
EMFBWDRIN

## $emmeans
##  Trt emmean   SE   df lower.CL upper.CL
##  1      623 4.18 18.4      615      632
##  2      616 4.18 18.4      607      625
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate   SE df t.ratio p.value
##  1 - 2        7.34 3.13 14 2.347   0.0342
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger

EMFBWDRIN1 <- emmeans(FBWDRIN, pairwise ~ Sex)

## NOTE: Results may be misleading due to involvement in interactions

EMFBWDRIN1

## $emmeans
##  Sex emmean   SE df lower.CL upper.CL
##  H      601 6.13 14      588      615
##  S      638 4.75 14      628      648
##
## Results are averaged over the levels of: Trt
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate   SE df t.ratio p.value
##  H - S       -36.5 7.76 14 -4.707  0.0003
##
## Results are averaged over the levels of: Trt
## Degrees-of-freedom method: kenward-roger

EMFBWDRIN2 <- emmeans(FBWDRIN, pairwise ~ Sex|Trt)
EMFBWDRIN2

## $emmeans
## Trt = 1:
##  Sex emmean   SE   df lower.CL upper.CL
##  H      606 6.61 18.4      592      620
##  S      641 5.12 18.4      630      652
```

```
##
## Trt = 2:
##  Sex emmean   SE   df lower.CL upper.CL
##  H      597 6.61 18.4      583      611
##  S      635 5.12 18.4      624      646
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Trt = 1:
##  contrast estimate   SE   df t.ratio p.value
##  H - S       -35.2 8.36 18.4 -4.203  0.0005
##
## Trt = 2:
##  contrast estimate   SE   df t.ratio p.value
##  H - S       -37.9 8.36 18.4 -4.527  0.0002
##
## Degrees-of-freedom method: kenward-roger
```

```
EMFBWDRIN3 <- emmeans(FBWDRIN, pairwise ~ Trt|Sex)
EMFBWDRIN3
```

```
## $emmeans
## Sex = H:
##  Trt emmean   SE   df lower.CL upper.CL
##  1      606 6.61 18.4      592      620
##  2      597 6.61 18.4      583      611
##
## Sex = S:
##  Trt emmean   SE   df lower.CL upper.CL
##  1      641 5.12 18.4      630      652
##  2      635 5.12 18.4      624      646
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = H:
##  contrast estimate   SE df t.ratio p.value
##  1 - 2        8.69 4.95 14 1.758   0.1006
##
## Sex = S:
##  contrast estimate   SE df t.ratio p.value
##  1 - 2        5.99 3.83 14 1.563   0.1404
```

```
##
## Degrees-of-freedom method: kenward-roger

ADGDRIN <- lmer(ADG_DRIN ~ Trt*Sex + (1|Rep), data = Performance)
anova(ADGDRIN, ddf = "Kenward-Roger")

## Type III Analysis of Variance Table with Kenward-Roger's method
##           Sum Sq  Mean Sq NumDF DenDF F value   Pr(>F)
## Trt     0.051047 0.051047     1    14  9.9431 0.007046 **
## Sex     0.000021 0.000021     1    14  0.0041 0.949992
## Trt:Sex 0.000047 0.000047     1    14  0.0091 0.925230
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

EMADGDRIN <- emmeans(ADGDRIN, pairwise ~ Trt)

## NOTE: Results may be misleading due to involvement in interactions

EMADGDRIN

## $emmeans
##  Trt emmean    SE   df lower.CL upper.CL
##  1    0.991 0.045 16.6    0.896     1.09
##  2    0.908 0.045 16.6    0.813     1.00
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate     SE df t.ratio p.value
##  1 - 2      0.0825 0.0262 14 3.153   0.0070
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger

GtoFDRIN <- lmer(GF_DRIN ~ Trt*Sex + (1|Rep), data = Performance)
anova(GtoFDRIN, ddf = "Kenward-Roger")

## Type III Analysis of Variance Table with Kenward-Roger's method
##             Sum Sq    Mean Sq NumDF DenDF F value  Pr(>F)
## Trt     0.00035535 0.00035535     1    14  7.3202 0.01707 *
## Sex     0.00000218 0.00000218     1    14  0.0448 0.83534
## Trt:Sex 0.00000060 0.00000060     1    14  0.0124 0.91291
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

EMGtoF <- emmeans(GtoFDRIN, pairwise ~ Trt)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMGtoF
```

```
## $emmeans
##  Trt emmean      SE   df lower.CL upper.CL
##  1   0.0974 0.00447 16.4   0.0880    0.107
##  2   0.0906 0.00447 16.4   0.0811    0.100
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate      SE df t.ratio p.value
##  1 - 2     0.00688 0.00254 14 2.706   0.0171
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
ADGDRO <- lmer(ADG_DRO ~ Trt*Sex + (1|Rep), data = Performance)
anova(ADGDRO, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##            Sum Sq   Mean Sq NumDF DenDF F value  Pr(>F)
## Trt     0.0028519 0.0028519     1    14  5.9636 0.02848 *
## Sex     0.0005148 0.0005148     1    14  1.0765 0.31707
## Trt:Sex 0.0018019 0.0018019     1    14  3.7679 0.07265 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
EMADGDRO <- emmeans(ADGDRO, pairwise ~ Trt)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMADGDRO
```

```
## $emmeans
##  Trt emmean     SE   df lower.CL upper.CL
##  1     1.25 0.0177 15.5     1.21     1.29
##  2     1.23 0.0177 15.5     1.19     1.27
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate      SE df t.ratio p.value
```

```
##  1 - 2      0.0195 0.00799 14 2.442   0.0285
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
EMADGDRO1 <- emmeans(ADGDRO, pairwise ~ Sex|Trt)
EMADGDRO1
```

```
## $emmeans
## Trt = 1:
##  Sex emmean     SE   df lower.CL upper.CL
##  H     1.27 0.0280 15.5     1.21     1.33
##  S     1.22 0.0217 15.5     1.18     1.27
##
## Trt = 2:
##  Sex emmean     SE   df lower.CL upper.CL
##  H     1.24 0.0280 15.5     1.18     1.30
##  S     1.22 0.0217 15.5     1.17     1.26
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Trt = 1:
##  contrast estimate     SE   df t.ratio p.value
##  H - S      0.0513 0.0354 15.5 1.448   0.1675
##
## Trt = 2:
##  contrast estimate     SE   df t.ratio p.value
##  H - S      0.0203 0.0354 15.5 0.574   0.5745
##
## Degrees-of-freedom method: kenward-roger
```

```
EMADGDRO2 <- emmeans(ADGDRO, pairwise ~ Trt|Sex)
EMADGDRO2
```

```
## $emmeans
## Sex = H:
##  Trt emmean     SE   df lower.CL upper.CL
##  1     1.27 0.0280 15.5     1.21     1.33
##  2     1.24 0.0280 15.5     1.18     1.30
##
## Sex = S:
##  Trt emmean     SE   df lower.CL upper.CL
##  1     1.22 0.0217 15.5     1.18     1.27
##  2     1.22 0.0217 15.5     1.17     1.26
##
```

```
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = H:
##  contrast estimate      SE df t.ratio p.value
##  1 - 2       0.035 0.01263 14 2.772   0.0150
##
## Sex = S:
##  contrast estimate      SE df t.ratio p.value
##  1 - 2       0.004 0.00978 14 0.409   0.6887
##
## Degrees-of-freedom method: kenward-roger
```

```
GtoFDRO <- lmer(GF_DRO ~ Trt*Sex +(1|Rep), data = Performance)
anova(GtoFDRO, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##             Sum Sq    Mean Sq NumDF DenDF F value  Pr(>F)
## Trt     1.8800e-08 1.8800e-08     1    14  0.0097 0.92278
## Sex     6.4852e-06 6.4852e-06     1    14  3.3690 0.08777 .
## Trt:Sex 1.5769e-05 1.5769e-05     1    14  8.1916 0.01255 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
EMGtoFDRO <- emmeans(GtoFDRO, pairwise ~ Trt)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMGtoFDRO
```

```
## $emmeans
##  Trt emmean      SE df lower.CL upper.CL
##  1    0.122 0.00136 15     0.12    0.125
##  2    0.122 0.00136 15     0.12    0.125
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate       SE df t.ratio p.value
##  1 - 2       5e-05 0.000507 14 0.099   0.9228
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
EMGtoFDRO1 <- emmeans(GtoFDRO, pairwise ~ Sex)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMGtoFDRO1
```

```
## $emmeans
##  Sex emmean      SE df lower.CL upper.CL
##  H    0.125 0.00212 14    0.120    0.129
##  S    0.120 0.00164 14    0.116    0.124
##
## Results are averaged over the levels of: Trt
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate      SE df t.ratio p.value
##  H - S     0.00492 0.00268 14 1.835   0.0878
##
## Results are averaged over the levels of: Trt
## Degrees-of-freedom method: kenward-roger
```

```
EMGtoFDRO2 <- emmeans(GtoFDRO, pairwise ~ Sex|Trt)
EMGtoFDRO2
```

```
## $emmeans
## Trt = 1:
##  Sex emmean      SE df lower.CL upper.CL
##  H    0.126 0.00216 15    0.121    0.130
##  S    0.119 0.00167 15    0.116    0.123
##
## Trt = 2:
##  Sex emmean      SE df lower.CL upper.CL
##  H    0.124 0.00216 15    0.120    0.129
##  S    0.121 0.00167 15    0.117    0.124
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Trt = 1:
##  contrast estimate      SE df t.ratio p.value
##  H - S     0.00637 0.00273 15 2.335   0.0338
##
## Trt = 2:
##  contrast estimate      SE df t.ratio p.value
##  H - S     0.00347 0.00273 15 1.272   0.2229
##
## Degrees-of-freedom method: kenward-roger
```

```
EMGtoFDRO3 <- emmeans(GtoFDRO, pairwise ~ Trt|Sex)
EMGtoFDRO3
```

```
## $emmeans
## Sex = H:
##  Trt emmean      SE df lower.CL upper.CL
##  1    0.126 0.00216 15    0.121    0.130
##  2    0.124 0.00216 15    0.120    0.129
##
## Sex = S:
##  Trt emmean      SE df lower.CL upper.CL
##  1    0.119 0.00167 15    0.116    0.123
##  2    0.121 0.00167 15    0.117    0.124
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = H:
##  contrast estimate       SE df t.ratio p.value
##  1 - 2      0.0015 0.000801 14  1.873  0.0822
##
## Sex = S:
##  contrast estimate       SE df t.ratio p.value
##  1 - 2     -0.0014 0.000620 14 -2.256  0.0406
##
## Degrees-of-freedom method: kenward-roger
```

```
HCWModel <- lmer(HCW ~ Trt*Sex + (1|Rep), data = Performance)
anova(HCWModel, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##         Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## Trt      45.85   45.85     1    14  6.0230   0.02782 *
## Sex     545.80  545.80     1    14 71.6996 7.026e-07 ***
## Trt:Sex   6.80    6.80     1    14  0.8939   0.36047
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
EMHCW <- emmeans(HCWModel, pairwise ~ Trt)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMHCW
```

```
## $emmeans
##  Trt emmean   SE df lower.CL upper.CL
##  1      409 1.41 18      406      412
```

```
##  2      406 1.41 18      403      409
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate   SE df t.ratio p.value
##  1 - 2        2.47 1.01 14 2.454   0.0278
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
EMHCW1 <- emmeans(HCWModel, pairwise ~ Sex)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMHCW1
```

```
## $emmeans
##  Sex emmean   SE df lower.CL upper.CL
##  H      396 2.08 14      392      401
##  S      419 1.61 14      415      422
##
## Results are averaged over the levels of: Trt
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate   SE df t.ratio p.value
##  H - S       -22.3 2.63 14 -8.468  <.0001
##
## Results are averaged over the levels of: Trt
## Degrees-of-freedom method: kenward-roger
```

```
EMHCW2 <- emmeans(HCWModel, pairwise ~ Sex|Trt)
EMHCW2
```

```
## $emmeans
## Trt = 1:
##  Sex emmean   SE df lower.CL upper.CL
##  H      398 2.23 18      393      403
##  S      419 1.72 18      416      423
##
## Trt = 2:
##  Sex emmean   SE df lower.CL upper.CL
##  H      395 2.23 18      390      399
```

```
##  S      418 1.72 18      414      422
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Trt = 1:
##  contrast estimate   SE df t.ratio p.value
##  H - S       -21.3 2.82 18 -7.568  <.0001
##
## Trt = 2:
##  contrast estimate   SE df t.ratio p.value
##  H - S       -23.2 2.82 18 -8.245  <.0001
##
## Degrees-of-freedom method: kenward-roger
```

```
EMHCW3 <- emmeans(HCWModel, pairwise ~ Trt|Sex)
EMHCW3
```

```
## $emmeans
## Sex = H:
##  Trt emmean   SE df lower.CL upper.CL
##  1      398 2.23 18      393      403
##  2      395 2.23 18      390      399
##
## Sex = S:
##  Trt emmean   SE df lower.CL upper.CL
##  1      419 1.72 18      416      423
##  2      418 1.72 18      414      422
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = H:
##  contrast estimate   SE df t.ratio p.value
##  1 - 2        3.42 1.59 14 2.150   0.0495
##
## Sex = S:
##  contrast estimate   SE df t.ratio p.value
##  1 - 2        1.52 1.23 14 1.232   0.2383
##
## Degrees-of-freedom method: kenward-roger
```

```
REACM <- lmer(REA ~ Trt*Sex + (1|Rep), data = Performance)
anova(REACM, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##         Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## Trt      0.027   0.027     1    14  0.0416    0.8414
## Sex     37.888  37.888     1    14 57.6910 2.492e-06 ***
## Trt:Sex  1.162   1.162     1    14  1.7698    0.2047
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
EMREA <- emmeans(REACM, pairwise ~ Trt)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMREA
```

```
## $emmeans
##  Trt emmean    SE   df lower.CL upper.CL
##  1     87.0 0.454 17.3     86.1       88
##  2     87.1 0.454 17.3     86.1       88
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate    SE df t.ratio p.value
##  1 - 2     -0.0603 0.296 14 -0.204  0.8414
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
EMREA1 <- emmeans(REACM, pairwise ~ Sex)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMREA1
```

```
## $emmeans
##  Sex emmean    SE df lower.CL upper.CL
##  H     90.3 0.678 14     88.9     91.8
##  S     83.8 0.525 14     82.7     84.9
##
## Results are averaged over the levels of: Trt
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate    SE df t.ratio p.value
##  H - S        6.51 0.858 14 7.595   <.0001
```

```
##
## Results are averaged over the levels of: Trt
## Degrees-of-freedom method: kenward-roger
```

```
EMREA2 <- emmeans(REACM, pairwise ~ Sex|Trt)
EMREA2
```

```
## $emmeans
## Trt = 1:
##  Sex emmean    SE   df lower.CL upper.CL
##  H     90.5 0.717 17.3     89.0     92.0
##  S     83.6 0.556 17.3     82.4     84.7
##
## Trt = 2:
##  Sex emmean    SE   df lower.CL upper.CL
##  H     90.1 0.717 17.3     88.6     91.6
##  S     84.0 0.556 17.3     82.8     85.2
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Trt = 1:
##  contrast estimate    SE   df t.ratio p.value
##  H - S        6.91 0.907 17.3 7.614   <.0001
##
## Trt = 2:
##  contrast estimate    SE   df t.ratio p.value
##  H - S        6.12 0.907 17.3 6.746   <.0001
##
## Degrees-of-freedom method: kenward-roger
```

```
EMREA3 <- emmeans(REACM, pairwise ~ Trt|Sex)
EMREA3
```

```
## $emmeans
## Sex = H:
##  Trt emmean    SE   df lower.CL upper.CL
##  1     90.5 0.717 17.3     89.0     92.0
##  2     90.1 0.717 17.3     88.6     91.6
##
## Sex = S:
##  Trt emmean    SE   df lower.CL upper.CL
##  1     83.6 0.556 17.3     82.4     84.7
##  2     84.0 0.556 17.3     82.8     85.2
##
## Degrees-of-freedom method: kenward-roger
```

```
## Confidence level used: 0.95
##
## $contrasts
## Sex = H:
##  contrast estimate    SE df t.ratio p.value
##  1 - 2       0.333 0.468 14  0.712  0.4879
##
## Sex = S:
##  contrast estimate    SE df t.ratio p.value
##  1 - 2      -0.454 0.362 14 -1.253  0.2308
##
## Degrees-of-freedom method: kenward-roger
```

```
MarbScore <- lmer(MARB ~ Trt*Sex + (1|Rep), data = Performance)
anova(MarbScore, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##          Sum Sq Mean Sq NumDF DenDF F value  Pr(>F)
## Trt     305.602 305.602     1    14  4.4842 0.05259 .
## Sex       2.048   2.048     1    14  0.0301 0.86484
## Trt:Sex  12.352  12.352     1    14  0.1812 0.67678
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
EMMarbScore <- emmeans(MarbScore, pairwise ~ Trt)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMMarbScore
```

```
## $emmeans
##  Trt emmean   SE df lower.CL upper.CL
##  1      614 7.98 15      596      631
##  2      607 7.98 15      590      624
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate   SE df t.ratio p.value
##  1 - 2        6.38 3.01 14 2.118   0.0526
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
EMMarbScore2 <- emmeans(MarbScore, pairwise ~ Sex)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMMarbScore2
```

```
## $emmeans
##  Sex emmean   SE df lower.CL upper.CL
##  H      612 12.4 14      585      638
##  S      609  9.6 14      588      630
##
## Results are averaged over the levels of: Trt
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate   SE df t.ratio p.value
##  H - S        2.72 15.7 14 0.173   0.8648
##
## Results are averaged over the levels of: Trt
## Degrees-of-freedom method: kenward-roger
```

```
EMMarbScore3 <- emmeans(MarbScore, pairwise ~ Sex|Trt)
EMMarbScore3
```

```
## $emmeans
## Trt = 1:
##  Sex emmean    SE df lower.CL upper.CL
##  H      616 12.62 15      589      642
##  S      612  9.77 15      591      632
##
## Trt = 2:
##  Sex emmean    SE df lower.CL upper.CL
##  H      608 12.62 15      581      635
##  S      606  9.77 15      586      627
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Trt = 1:
##  contrast estimate SE df t.ratio p.value
##  H - S        4.00 16 15 0.251   0.8055
##
## Trt = 2:
##  contrast estimate SE df t.ratio p.value
##  H - S        1.43 16 15 0.090   0.9296
##
## Degrees-of-freedom method: kenward-roger
```

```
EMMarbScore4 <- emmeans(MarbScore, pairwise ~ Trt|Sex)
EMMarbScore4
```

```
## $emmeans
## Sex = H:
##  Trt emmean    SE df lower.CL upper.CL
##  1      616 12.62 15      589      642
##  2      608 12.62 15      581      635
##
## Sex = S:
##  Trt emmean    SE df lower.CL upper.CL
##  1      612  9.77 15      591      632
##  2      606  9.77 15      586      627
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = H:
##  contrast estimate   SE df t.ratio p.value
##  1 - 2        7.67 4.77 14 1.609   0.1300
##
## Sex = S:
##  contrast estimate   SE df t.ratio p.value
##  1 - 2        5.10 3.69 14 1.381   0.1888
##
## Degrees-of-freedom method: kenward-roger
```

```
Grades <- read.csv("C:/Users/WilliamNelson/Dropbox/R Analysis for Thes
is/R Analysis Code and Data/USDAGradesCSVforR.csv")
Grades$Lot <- as.factor(Grades$Lot)
Grades$Block <- as.factor(Grades$Block)
str(Grades)
```

```
## 'data.frame':    32 obs. of  26 variables:
##  $ Lot               : Factor w/ 32 levels "20971","20972",..: 1 2
3 4 5 6 7 8 9 10 ...
##  $ Treatment         : Factor w/ 2 levels "Control","Zinpro": 2 1
1 2 1 2 1 2 2 1 ...
##  $ Block             : Factor w/ 16 levels "1","2","3","4",..: 1 1
2 2 3 3 4 4 5 5 ...
##  $ Sex               : Factor w/ 2 levels "Heifer","Steer": 2 2 2
2 2 2 2 2 2 2 ...
##  $ YG1               : num  0.402 0 0.385 0 0 ...
##  $ YG2               : num  5.62 6.54 8.08 8.88 5.81 ...
##  $ YG3               : num  64.7 56.9 78.5 83.8 62.7 ...
```

```
##  $ YG4                 : num  25.7 31.15 13.08 7.34 30.29 ...
##  $ YG5                 : num  3.61 5.38 0 0 1.24 ...
##  $ Total               : int  100 100 100 100 100 100 100 100 100 10
0 ...
##  $ YG1and2             : num  6.02 6.54 8.46 8.88 5.81 ...
##  $ YG4and5             : num  29.32 36.54 13.08 7.34 31.54 ...
##  $ PrimePlus           : num  2.41 0.769 0 0.386 0 ...
##  $ Prime               : num  8.03 13.08 2.69 3.86 6.64 ...
##  $ PrimeMinus          : num  21.7 24.2 31.9 30.9 30.7 ...
##  $ ChoicePlus          : num  31.3 30 17.3 14.7 21.2 ...
##  $ Choice              : num  28.1 20 29.2 31.7 19.5 ...
##  $ ChoiceMinus         : num  6.83 10.38 18.46 18.53 21.58 ...
##  $ Select              : num  1.606 1.538 0 0 0.415 ...
##  $ Standard            : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ DarkCutter          : num  0 0 0.385 0 0 ...
##  $ Commercial          : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Condemed            : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ TotalPrime          : num  32.1 38.1 34.6 35.1 37.3 ...
##  $ TotalChoice         : num  66.3 60.4 65 64.9 62.2 ...
##  $ TotalChoiceandPrime: num  98.4 98.5 99.6 100 99.6 ...
```

```
USDAPrime <- lmer(TotalPrime ~ Treatment*Sex + (1|Block), data = Grade
s)
anova(USDAPrime, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Treatment     8.0229  8.0229     1    14  0.3296 0.5750
## Sex           0.2066  0.2066     1    14  0.0085 0.9279
## Treatment:Sex 3.2775  3.2775     1    14  0.1347 0.7191
```

```
EMPrime <- emmeans(USDAPrime, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMPrime
```

```
## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     35.7 2.93 16.9     29.5     41.9
##  Zinpro      34.7 2.93 16.9     28.5     40.8
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
```

```
##  contrast         estimate  SE df t.ratio p.value
##  Control - Zinpro     1.03 1.8 14 0.574   0.5750
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
USDAChoice <- lmer(TotalChoice ~ Treatment*Sex + (1|Block), data = Gra
des)
anova(USDAChoice, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Treatment     10.0669 10.0669     1    14  0.4466 0.5148
## Sex            0.0833  0.0833     1    14  0.0037 0.9524
## Treatment:Sex  4.0979  4.0979     1    14  0.1818 0.6763
```

```
EMChoice <- emmeans(USDAChoice, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMChoice
```

```
## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     62.8 2.88 16.8     56.8     68.9
##  Zinpro      64.0 2.88 16.8     57.9     70.1
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    -1.16 1.73 14 -0.668  0.5148
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
USDAChoicePrime <- lmer(TotalChoiceandPrime ~ Treatment*Sex +(1|Block)
, data = Grades)
anova(USDAChoicePrime, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value  Pr(>F)
## Treatment     0.11585 0.11585     1    14  0.2502 0.62469
## Sex           2.06690 2.06690     1    14  4.4643 0.05305 .
## Treatment:Sex 0.04577 0.04577     1    14  0.0989 0.75784
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

EMChoicePrime <- emmeans(USDAChoicePrime, pairwise ~ Treatment)

## NOTE: Results may be misleading due to involvement in interactions

EMChoicePrime

## $emmeans
##  Treatment emmean     SE    df lower.CL upper.CL
##  Control    98.51 0.2359 23.37    98.03    99.00
##  Zinpro     98.64 0.2359 23.37    98.15    99.13
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate    SE df t.ratio p.value
##  Control - Zinpro   -0.124 0.248 14 -0.500  0.6247
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger

EMChoicePrime1 <- emmeans(USDAChoicePrime, pairwise ~ Sex)

## NOTE: Results may be misleading due to involvement in interactions

EMChoicePrime1

## $emmeans
##  Sex    emmean     SE df lower.CL upper.CL
##  Heifer  98.15 0.3170 14    97.47    98.83
##  Steer   99.00 0.2455 14    98.47    99.53
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast       estimate    SE df t.ratio p.value
##  Heifer - Steer   -0.847 0.401 14 -2.113  0.0531
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
```

```
USDAHighChoice <- lmer(ChoicePlus ~ Treatment*Sex + (1|Block), data =
Grades)
anova(USDAHighChoice, ddf = "Kenward-Roger")

## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Treatment     21.3415 21.3415     1    14  1.2203 0.2879
## Sex            1.4666  1.4666     1    14  0.0839 0.7764
## Treatment:Sex  0.3097  0.3097     1    14  0.0177 0.8960

EMHighChoice <- emmeans(USDAHighChoice, pairwise ~ Treatment)

## NOTE: Results may be misleading due to involvement in interactions

EMHighChoice

## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     24.4 1.92 19.1     20.4     28.4
##  Zinpro      22.7 1.92 19.1     18.7     26.7
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro     1.69 1.53 14 1.105   0.2879
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger

USDASelect <- lmer(Select ~ Treatment*Sex + (1|Block), data = Grades)
anova(USDASelect, ddf = "Kenward-Roger")

## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Treatment     0.08001 0.08001     1    14  0.2302 0.6388
## Sex           0.75244 0.75244     1    14  2.1650 0.1633
## Treatment:Sex 0.01991 0.01991     1    14  0.0573 0.8143

EMSelect <- emmeans(USDASelect, pairwise ~ Treatment)

## NOTE: Results may be misleading due to involvement in interactions

EMSelect

## $emmeans
##  Treatment emmean   SE df lower.CL upper.CL
```

```
##  Control     1.21 0.22 22    0.756     1.67
##  Zinpro      1.11 0.22 22    0.653     1.57
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate    SE df t.ratio p.value
##  Control - Zinpro    0.103 0.215 14 0.480   0.6388
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
YieldGrade1 <- lmer(YG1 ~ Treatment*Sex + (1|Block), data = Grades)
```

```
## boundary (singular) fit: see ?isSingular
```

```
anova(YieldGrade1, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                 Sum Sq  Mean Sq NumDF DenDF F value Pr(>F)
## Treatment     0.239557 0.239557     1    14  2.1524 0.1644
## Sex           0.144729 0.144729     1    14  1.3004 0.2733
## Treatment:Sex 0.002041 0.002041     1    14  0.0183 0.8942
```

```
EMYG1 <- emmeans(YieldGrade1, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMYG1
```

```
## $emmeans
##  Treatment emmean     SE df lower.CL upper.CL
##  Control    0.192 0.0861 28   0.0158    0.369
##  Zinpro     0.371 0.0861 28   0.1945    0.547
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate    SE df t.ratio p.value
##  Control - Zinpro   -0.179 0.122 14 -1.467  0.1644
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
YieldGrade2 <- lmer(YG2 ~ Treatment*Sex + (1|Block), data = Grades)
anova(YieldGrade2, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Treatment     4.2409  4.2409     1    14  0.4508 0.5129
## Sex           0.2209  0.2209     1    14  0.0235 0.8804
## Treatment:Sex 0.4629  0.4629     1    14  0.0492 0.8277
```

```
EMYG2 <- emmeans(YieldGrade2, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMYG2
```

```
## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     6.83 1.09 22.9     4.58     9.08
##  Zinpro      7.58 1.09 22.9     5.33     9.83
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro   -0.752 1.12 14 -0.671  0.5129
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
YieldGrade1and2 <- lmer(YG1and2 ~ Treatment*Sex +(1|Block), data = Gra
des)
anova(YieldGrade1and2, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Treatment     6.4964  6.4964     1    14  0.6673 0.4277
## Sex           0.0553  0.0553     1    14  0.0057 0.9410
## Treatment:Sex 0.5264  0.5264     1    14  0.0541 0.8195
```

```
EM1and2 <- emmeans(YieldGrade1and2, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EM1and2
```

```
## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
```

```
##  Control     7.02 1.13 22.6     4.69     9.36
##  Zinpro      7.95 1.13 22.6     5.62    10.29
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro   -0.931 1.14 14 -0.817  0.4277
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
YieldGrade3 <- lmer(YG3 ~ Treatment*Sex + (1|Block), data = Grades)
anova(YieldGrade3, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                Sum Sq Mean Sq NumDF DenDF F value  Pr(>F)
## Treatment      52.820  52.820     1    14  1.6203 0.22378
## Sex            55.212  55.212     1    14  1.6937 0.21412
## Treatment:Sex 230.510 230.510     1    14  7.0713 0.01869 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
EMYG3 <- emmeans(YieldGrade3, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMYG3
```

```
## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     64.9 2.99 17.8     58.6     71.2
##  Zinpro      62.2 2.99 17.8     55.9     68.5
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro     2.65 2.08 14 1.273   0.2238
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
YieldGrade4 <- lmer(YG4 ~ Treatment*Sex +(1|Block), data = Grades)
anova(YieldGrade4, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value  Pr(>F)
## Treatment      4.545   4.545     1    14  0.1593 0.69585
## Sex           42.591  42.591     1    14  1.4925 0.24201
## Treatment:Sex 142.244 142.244     1    14  4.9845 0.04242 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
EMYG4 <- emmeans(YieldGrade4, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMYG4
```

```
## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     24.8 2.68 18.2     19.2     30.5
##  Zinpro      25.6 2.68 18.2     20.0     31.2
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro   -0.778 1.95 14 -0.399  0.6958
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
EMYG4A <- emmeans(YieldGrade4, pairwise ~ Sex)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMYG4A
```

```
## $emmeans
##  Sex    emmean   SE df lower.CL upper.CL
##  Heifer   28.3 3.95 14     19.8     36.7
##  Steer    22.2 3.06 14     15.6     28.7
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
```

```
## $contrasts
##  contrast       estimate SE df t.ratio p.value
##  Heifer - Steer     6.11  5 14 1.222   0.2420
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
```

```
EMYG4B <- emmeans(YieldGrade4, pairwise ~ Sex|Treatment)
EMYG4B
```

```
## $emmeans
## Treatment = Control:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   25.7 4.24 18.2     16.8     34.6
##  Steer    23.9 3.29 18.2     17.0     30.8
##
## Treatment = Zinpro:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   30.8 4.24 18.2     21.9     39.7
##  Steer    20.4 3.29 18.2     13.5     27.3
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = Control:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer     1.75 5.37 18.2 0.327   0.7475
##
## Treatment = Zinpro:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer    10.46 5.37 18.2 1.949   0.0669
##
## Degrees-of-freedom method: kenward-roger
```

```
EMYG4C <- emmeans(YieldGrade4, pairwise ~ Treatment|Sex)
EMYG4C
```

```
## $emmeans
## Sex = Heifer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     25.7 4.24 18.2     16.8     34.6
##  Zinpro      30.8 4.24 18.2     21.9     39.7
##
## Sex = Steer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     23.9 3.29 18.2     17.0     30.8
```

```
##  Zinpro      20.4 3.29 18.2     13.5     27.3
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = Heifer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    -5.13 3.08 14 -1.664  0.1182
##
## Sex = Steer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro     3.58 2.39 14  1.497  0.1566
##
## Degrees-of-freedom method: kenward-roger
```

```
YieldGrade5 <- lmer(YG5 ~ Treatment*Sex +(1|Block), data = Grades)
anova(YieldGrade5, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value  Pr(>F)
## Treatment     6.6923  6.6923     1    14  1.6289 0.22262
## Sex           2.1209  2.1209     1    14  0.5162 0.48427
## Treatment:Sex 15.8521 15.8521     1    14  3.8585 0.06966 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
EMYG5 <- emmeans(YieldGrade5, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMYG5
```

```
## $emmeans
##  Treatment emmean SE   df lower.CL upper.CL
##  Control     3.26  1 18.3     1.16     5.36
##  Zinpro      4.21  1 18.3     2.11     6.31
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro   -0.945 0.74 14 -1.276  0.2226
##
```

```
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger

EMYG5A <- emmeans(YieldGrade5, pairwise ~ Sex)

## NOTE: Results may be misleading due to involvement in interactions

EMYG5A

## $emmeans
##  Sex    emmean   SE df lower.CL upper.CL
##  Heifer   4.40 1.47 14    1.249     7.56
##  Steer    3.07 1.14 14    0.623     5.51
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast       estimate   SE df t.ratio p.value
##  Heifer - Steer     1.34 1.86 14 0.718   0.4843
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger

EMYG5B <- emmeans(YieldGrade5, pairwise ~ Sex|Treatment)
EMYG5B

## $emmeans
## Treatment = Control:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   3.20 1.58 18.3   -0.117     6.53
##  Steer    3.32 1.23 18.3    0.749     5.89
##
## Treatment = Zinpro:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   5.60 1.58 18.3    2.281     8.92
##  Steer    2.81 1.23 18.3    0.239     5.38
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = Control:
##  contrast       estimate SE   df t.ratio p.value
##  Heifer - Steer   -0.117  2 18.3 -0.059  0.9540
##
```

```
## Treatment = Zinpro:
##  contrast       estimate SE   df t.ratio p.value
##  Heifer - Steer    2.790  2 18.3  1.394  0.1801
##
## Degrees-of-freedom method: kenward-roger
```

```
EMYG5C <- emmeans(YieldGrade5, pairwise ~ Treatment|Sex)
EMYG5C
```

```
## $emmeans
## Sex = Heifer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     3.20 1.58 18.3   -0.117     6.53
##  Zinpro      5.60 1.58 18.3    2.281     8.92
##
## Sex = Steer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     3.32 1.23 18.3    0.749     5.89
##  Zinpro      2.81 1.23 18.3    0.239     5.38
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = Heifer:
##  contrast         estimate    SE df t.ratio p.value
##  Control - Zinpro   -2.398 1.170 14 -2.050  0.0596
##
## Sex = Steer:
##  contrast         estimate    SE df t.ratio p.value
##  Control - Zinpro    0.509 0.906 14  0.562  0.5832
##
## Degrees-of-freedom method: kenward-roger
```

```
YieldGrade4and5 <- lmer(YG4and5 ~ Treatment*Sex +(1|Block), data = Gra
des)
anova(YieldGrade4and5, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##               Sum Sq Mean Sq NumDF DenDF F value  Pr(>F)
## Treatment     22.268  22.268     1    14  0.4963 0.49269
## Sex           57.189  57.189     1    14  1.2746 0.27788
## Treatment:Sex 253.067 253.067     1    14  5.6401 0.03239 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
EM45 <- emmeans(YieldGrade4and5, pairwise ~ Treatment)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EM45
```

```
## $emmeans
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     28.1 3.52 17.8     20.7     35.5
##  Zinpro      29.8 3.52 17.8     22.4     37.2
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    -1.72 2.45 14 -0.704  0.4927
##
## Results are averaged over the levels of: Sex
## Degrees-of-freedom method: kenward-roger
```

```
EM45A <- emmeans(YieldGrade4and5, pairwise ~ Sex)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EM45A
```

```
## $emmeans
##  Sex    emmean   SE df lower.CL upper.CL
##  Heifer   32.7 5.21 14     21.5     43.9
##  Steer    25.2 4.04 14     16.6     33.9
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast       estimate  SE df t.ratio p.value
##  Heifer - Steer     7.45 6.6 14 1.129   0.2779
##
## Results are averaged over the levels of: Treatment
## Degrees-of-freedom method: kenward-roger
```

```
EM45B <- emmeans(YieldGrade4and5, pairwise ~ Sex|Treatment)
EM45B
```

```
## $emmeans
## Treatment = Control:
##  Sex    emmean   SE   df lower.CL upper.CL
```

```
##  Heifer   28.9 5.56 17.8     17.2     40.6
##  Steer    27.3 4.31 17.8     18.2     36.3
##
## Treatment = Zinpro:
##  Sex    emmean   SE   df lower.CL upper.CL
##  Heifer   36.4 5.56 17.8     24.7     48.1
##  Steer    23.2 4.31 17.8     14.1     32.2
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = Control:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer     1.64 7.03 17.8 0.233   0.8186
##
## Treatment = Zinpro:
##  contrast       estimate   SE   df t.ratio p.value
##  Heifer - Steer    13.25 7.03 17.8 1.884   0.0760
##
## Degrees-of-freedom method: kenward-roger
```

```
EM45C <- emmeans(YieldGrade4and5, pairwise ~ Treatment|Sex)
EM45C
```

```
## $emmeans
## Sex = Heifer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     28.9 5.56 17.8     17.2     40.6
##  Zinpro      36.4 5.56 17.8     24.7     48.1
##
## Sex = Steer:
##  Treatment emmean   SE   df lower.CL upper.CL
##  Control     27.3 4.31 17.8     18.2     36.3
##  Zinpro      23.2 4.31 17.8     14.1     32.2
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Sex = Heifer:
##  contrast         estimate   SE df t.ratio p.value
##  Control - Zinpro    -7.53 3.87 14 -1.948  0.0718
##
## Sex = Steer:
##  contrast         estimate   SE df t.ratio p.value
```

```
##  Control - Zinpro     4.09 3.00 14  1.364  0.1941
##
## Degrees-of-freedom method: kenward-roger
```

Heat Stress Full Analysis R Markdown

WT Nelson

July 27, 2020

```
library(lme4)

## Loading required package: Matrix

library(lmerTest)

## Warning: package 'lmerTest' was built under R version 3.6.3

##
## Attaching package: 'lmerTest'

## The following object is masked from 'package:lme4':
##
##     lmer

## The following object is masked from 'package:stats':
##
##     step

library(pbkrtest)
library(emmeans)

## Welcome to emmeans.
## NOTE -- Important change from versions <= 1.41:
##     Indicator predictors are now treated as 2-level factors by defa
ult.
##     To revert to old behavior, use emm_options(cov.keep = character
(0))

FullData <- read.csv("C:/Users/WilliamNelson/Dropbox/Will and Sadie/Gr
ad School/Five Rivers Cattle Study/Heat Stress Work/Final Thesis Analy
sis Materials/Source Data For R Final CSV.csv")

FullData$Pen <- as.factor(FullData$Pen)
FullData$Lot <- as.factor(FullData$Lot)
FullData$Block <- as.factor(FullData$Block)
FullData$Treatment <- as.factor(FullData$Treatment)
str(FullData)
```

```
## 'data.frame':    512 obs. of  24 variables:
##  $ Date               : Factor w/ 8 levels "6/21/2019","6/24/2019",
..: 1 1 1 1 1 1 1 1 1 ...
##  $ Time               : Factor w/ 3 levels "A","B","C": 3 2 1 3 2 1
3 3 2 1 ...
##  $ Pen                : Factor w/ 40 levels "309","311","350",..: 3
5 35 35 33 33 33 33 38 38 38 ...
##  $ Lot                : Factor w/ 32 levels "20971","20972",..: 1 1
1 2 2 2 2 3 3 3 ...
##  $ Block              : Factor w/ 16 levels "1","2","3","4",..: 1 1
1 1 1 1 1 2 2 2 ...
##  $ Treatment          : Factor w/ 2 levels "1","2": 2 2 2 1 1 1 1 1
1 1 ...
##  $ Sex                : Factor w/ 2 levels "Hfr","Str": 2 2 2 2 2 2
2 2 2 2 ...
##  $ HeadCount          : int  269 269 269 272 272 272 272 270 270 27
0 ...
##  $ PercentNonBlack    : int  5 5 5 2 2 2 2 2 2 2 ...
##  $ CountBlackOMB      : int  0 0 0 1 0 0 0 0 0 0 ...
##  $ CountNonBlackOMB   : int  0 0 0 0 0 0 0 0 0 0 ...
##  $ PercentOMBTotal    : num  0 0 0 0.368 0 ...
##  $ PercentOMBBlack    : num  0 0 0 0.375 0 ...
##  $ PercentOMBNonBlack : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ AvgHideTempBlk     : num  29.6 31.6 29 26.8 32.1 ...
##  $ AvgHideTempNB      : num  28.2 26.6 26.5 25 29.4 ...
##  $ AvgHideTempTotal   : num  29.1 29.6 27.8 26.4 31.3 ...
##  $ AvgSurfaceTempTotal: num  27.2 30.9 15.5 21.4 29.2 ...
##  $ AvgTHI             : num  57.9 57.9 57.9 57.9 57.9 ...
##  $ HighTHI            : num  61 61 61 61 61 ...
##  $ LowTHI             : num  55 55 55 55 55 ...
##  $ YesterdayHighTHI   : num  69.2 69.2 69.2 69.2 69.2 69.2 69.2 69.
2 69.2 69.2 ...
##  $ YesterdayLowTHI    : num  56.5 56.5 56.5 56.5 56.5 ...
##  $ YesterdayAvgTHI    : num  62.9 62.9 62.9 62.9 62.9 ...
```

```
FullModel <- lmer(PercentOMBTotal ~ AvgTHI + YesterdayAvgTHI + Yesterd
ayHighTHI + YesterdayLowTHI + AvgSurfaceTempTotal + AvgHideTempTotal +
(1|Block)*Date*Sex*Treatment*Time, data = FullData)
```

```
## fixed-effect model matrix is rank deficient so dropping 4 columns /
coefficients
```

```
anova(FullModel, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                         Sum Sq Mean Sq NumDF  DenDF F value    Pr(>
F)
```

```
## AvgTHI                    0.103  0.1031     1 401.78  0.2082 0.64841
26
## YesterdayAvgTHI           0.206  0.2058     1 402.59  0.4155 0.51953
60
## YesterdayHighTHI          0.053  0.0526     1 406.39  0.1062 0.74472
89
## YesterdayLowTHI           0.114  0.1143     1 404.26  0.2307 0.63127
77
## AvgSurfaceTempTotal      13.150 13.1503     1 412.30 26.5464 3.997e-
07 ***
## AvgHideTempTotal          0.560  0.5599     1 405.40  1.1302 0.28836
74
## Date                     10.159  3.3864     3 409.87  6.8360 0.00016
69 ***
## Sex                       0.191  0.1913     1  17.18  0.3862 0.54248
17
## Treatment                 0.351  0.3509     1 399.27  0.7084 0.40048
77
## Time                     21.920 10.9601     2 404.79 22.1251 7.603e-
10 ***
## Date:Sex                  4.564  0.6519     7 409.88  1.3160 0.24101
58
## Date:Treatment            1.568  0.2240     7 399.15  0.4522 0.86855
51
## Sex:Treatment             1.063  1.0627     1 399.20  2.1452 0.14380
66
## Date:Time                34.377  2.4555    14 400.26  4.9570 1.568e-
08 ***
## Sex:Time                  6.036  3.0179     2 401.74  6.0922 0.00247
47 **
## Treatment:Time            0.636  0.3180     2 399.08  0.6420 0.52680
68
## Date:Sex:Treatment        1.887  0.2696     7 399.18  0.5442 0.80081
74
## Date:Sex:Time             8.298  0.5927    14 399.64  1.1965 0.27510
56
## Date:Treatment:Time       1.779  0.1271    14 399.13  0.2565 0.99731
74
## Sex:Treatment:Time        0.357  0.1786     2 399.09  0.3605 0.69753
55
## Date:Sex:Treatment:Time   1.983  0.1417    14 399.11  0.2860 0.99520
10
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
FullModel <- update(FullModel, ~ . -Date:Sex:Treatment:Time)
```

```
## fixed-effect model matrix is rank deficient so dropping 4 columns /
coefficients

## boundary (singular) fit: see ?isSingular

## Warning: Model failed to converge with 6 negative eigenvalues: -1.9
e-01 -1.4e+01
## -7.0e+01 -2.8e+02 -2.4e+03 -4.8e+03

anova(FullModel, ddf = "Kenward-Roger")

## Type III Analysis of Variance Table with Kenward-Roger's method
##                       Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## AvgTHI               0.00626 0.00626     1 247.58  0.1008  0.751158
## YesterdayAvgTHI      0.02287 0.02287     1 248.01  0.3682  0.544542
## YesterdayHighTHI     0.00697 0.00697     1 261.81  0.1122  0.737906
## YesterdayLowTHI      0.01301 0.01301     1 255.87  0.2095  0.647558
## AvgSurfaceTempTotal 1.13396 1.13396     1 371.85 18.2532 2.460e-05
***
## AvgHideTempTotal     0.01943 0.01943     1 371.86  0.3128  0.576332
## Date                 0.68913 0.22971     3  62.75  3.6970  0.016236
*
## Sex                  0.02651 0.02651     1  17.97  0.4267  0.521863
## Treatment            0.06726 0.06726     1  18.79  1.0827  0.311305
## Time                 1.69990 0.84995     2  58.11 13.6643 1.368e-05
***
## Date:Sex             0.30262 0.04323     7  62.05  0.6951  0.675828
## Date:Treatment       0.39094 0.05585     7  63.90  0.8980  0.513672
## Sex:Treatment        0.18821 0.18821     1  18.99  3.0295  0.097941
.
## Date:Time            2.40012 0.17144    14 126.62  2.7570  0.001356
**
## Sex:Time             0.43470 0.21735     2  38.75  3.4983  0.040116
*
## Treatment:Time       0.19698 0.09849     2  29.89  1.5853  0.221626
## Date:Sex:Treatment   0.44871 0.06410     7  63.24  1.0303  0.419148
## Date:Sex:Time        0.69360 0.04954    14 123.60  0.7963  0.671574
## Date:Treatment:Time 0.41511 0.02965    14 136.21  0.4771  0.942006
## Sex:Treatment:Time   0.14514 0.07257     2  28.79  1.1679  0.325323
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

FullModel <- update(FullModel, ~ . -Date:Treatment:Time)

## fixed-effect model matrix is rank deficient so dropping 4 columns /
coefficients

## boundary (singular) fit: see ?isSingular
```

```
## Warning: Model failed to converge with 6 negative eigenvalues: -4.1
e-02 -3.7e-01
## -1.4e+00 -1.1e+02 -5.5e+02 -2.8e+03
```

```
anova(FullModel, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                       Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## AvgTHI               0.00243 0.00243     1 213.74  0.0395  0.842670
## YesterdayAvgTHI      0.01632 0.01632     1 218.56  0.2651  0.607137
## YesterdayHighTHI     0.00433 0.00433     1 240.93  0.0704  0.791017
## YesterdayLowTHI      0.00821 0.00821     1 228.88  0.1335  0.715206
## AvgSurfaceTempTotal 1.13042 1.13042     1 383.42 18.3689 2.305e-05
***
## AvgHideTempTotal     0.02302 0.02302     1 387.22  0.3740  0.541191
## Date                 0.68361 0.22787     3  62.72  3.7022  0.016139
*
## Sex                  0.02655 0.02655     1  17.95  0.4314  0.519638
## Treatment            0.07171 0.07171     1  18.61  1.1652  0.294172
## Time                 1.68301 0.84150     2  57.99 13.6570 1.380e-05
***
## Date:Sex             0.30030 0.04290     7  62.06  0.6964  0.674817
## Date:Treatment       0.39120 0.05589     7  63.61  0.9071  0.506802
## Sex:Treatment        0.18626 0.18626     1  19.06  3.0266  0.098029
.
## Date:Time            2.36511 0.16894    14 126.67  2.7426  0.001433
**
## Sex:Time             0.42642 0.21321     2  38.74  3.4642  0.041294
*
## Treatment:Time       0.29410 0.14705     2  27.01  2.3892  0.110810
## Date:Sex:Treatment   0.44113 0.06302     7  63.41  1.0226  0.424328
## Date:Sex:Time        0.68526 0.04895    14 123.84  0.7943  0.673773
## Sex:Treatment:Time   0.16795 0.08397     2  27.23  1.3643  0.272489
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
FullModel <- update(FullModel, ~ . -Date:Sex:Time)
```

```
## fixed-effect model matrix is rank deficient so dropping 4 columns /
coefficients
```

```
## boundary (singular) fit: see ?isSingular
```

```
## Warning: Model failed to converge with 4 negative eigenvalues: -1.8
e-01 -1.4e+00
## -4.0e+01 -1.9e+02
```

```
anova(FullModel, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                        Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## AvgTHI                0.02857 0.02857     1 186.45  0.4758   0.49117
## YesterdayAvgTHI       0.05111 0.05111     1 178.96  0.8513   0.35742
## YesterdayHighTHI      0.01987 0.01987     1 170.27  0.3310   0.56586
## YesterdayLowTHI       0.03448 0.03448     1 176.27  0.5743   0.44955
## AvgSurfaceTempTotal 1.31119 1.31119     1 402.49 21.8409 4.047e-06
***
## AvgHideTempTotal      0.03459 0.03459     1 397.25  0.5761   0.44829
## Date                  0.65706 0.21902     3  62.35  3.6477   0.01724
*
## Sex                   0.02224 0.02224     1  17.76  0.3705   0.55042
## Treatment             0.07117 0.07117     1  18.61  1.1855   0.29014
## Time                  2.18317 1.09159     2  50.18 18.1506 1.172e-06
***
## Date:Sex              0.30682 0.04383     7  60.67  0.7289   0.64807
## Date:Treatment        0.38206 0.05458     7  63.62  0.9082   0.50600
## Sex:Treatment         0.18613 0.18613     1  19.05  3.1004   0.09432
.
## Date:Time             2.86740 0.20481    14 138.61  3.4094 9.455e-05
***
## Sex:Time              0.57773 0.28887     2  30.33  4.8104   0.01533
*
## Treatment:Time        0.28487 0.14244     2  27.02  2.3724   0.11240
## Date:Sex:Treatment    0.42681 0.06097     7  63.35  1.0141   0.43007
## Sex:Treatment:Time    0.16732 0.08366     2  27.24  1.3933   0.26540
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
FullModel <- update(FullModel, ~ . -Date:Sex:Treatment)
```

```
## fixed-effect model matrix is rank deficient so dropping 4 columns /
coefficients
```

```
## boundary (singular) fit: see ?isSingular
```

```
## Warning: Model failed to converge with 5 negative eigenvalues: -1.8
e-01 -2.2e-01
## -2.7e+01 -4.0e+01 -1.5e+02
```

```
anova(FullModel, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                        Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## AvgTHI                0.05262 0.05262     1 169.80  0.9131   0.34066
## YesterdayAvgTHI       0.06462 0.06462     1 159.24  1.1213   0.29125
## YesterdayHighTHI      0.02724 0.02724     1 142.17  0.4726   0.49291
```

```
## YesterdayLowTHI     0.04690 0.04690     1 153.46  0.8139   0.36838
## AvgSurfaceTempTotal 1.33864 1.33864     1 412.51 23.2284 2.025e-06
***
## AvgHideTempTotal    0.06206 0.06206     1 416.85  1.0768   0.30001
## Date                0.62107 0.20702     3  62.27  3.5917   0.01843
*
## Sex                 0.02117 0.02117     1  17.64  0.3674   0.55215
## Treatment           0.16190 0.16190     1  15.04  2.8094   0.11437
## Time                2.18536 1.09268     2  50.24 18.9264 7.473e-07
***
## Date:Sex            0.30072 0.04296     7  60.96  0.7446   0.63527
## Date:Treatment      0.37736 0.05391     7  69.37  0.9347   0.48564
## Sex:Treatment       0.21334 0.21334     1  14.93  3.7019   0.07362
.
## Date:Time           2.75055 0.19647    14 139.10  3.4069 9.495e-05
***
## Sex:Time            0.58400 0.29200     2  30.24  5.0655   0.01267
*
## Treatment:Time      0.26233 0.13117     2  26.99  2.2758   0.12208
## Sex:Treatment:Time  0.13666 0.06833     2  27.04  1.1855   0.32097
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
FullModel <- update(FullModel, ~ . -Sex:Treatment:Time)
```

```
## fixed-effect model matrix is rank deficient so dropping 4 columns /
coefficients
```

```
## boundary (singular) fit: see ?isSingular
```

```
## Warning: Model failed to converge with 5 negative eigenvalues: -2.8
e-02 -1.4e-01
## -1.9e-01 -2.3e+01 -2.6e+02
```

```
anova(FullModel, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                      Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## AvgTHI              0.05425 0.05425     1 168.67  0.9403 0.3335788
## YesterdayAvgTHI     0.06650 0.06650     1 158.21  1.1527 0.2846278
## YesterdayHighTHI    0.02982 0.02982     1 141.56  0.5168 0.4734034
## YesterdayLowTHI     0.04918 0.04918     1 152.56  0.8523 0.3573528
## AvgSurfaceTempTotal 1.30564 1.30564     1 414.95 22.6293 2.716e-06
***
## AvgHideTempTotal    0.04634 0.04634     1 418.07  0.8032 0.3706511
## Date                0.62603 0.20868     3  62.32  3.6162 0.0178988
*
```

```
## Sex                 0.02032 0.02032     1  17.72  0.3522 0.5603452
## Treatment           0.16827 0.16827     1  15.04  2.9164 0.1082399
## Time                2.11430 1.05715     2  50.07 18.2899 1.089e-06
***
## Date:Sex            0.30157 0.04308     7  61.07  0.7458 0.6342335
## Date:Treatment      0.38213 0.05459     7  69.36  0.9454 0.4777814
## Sex:Treatment       0.23569 0.23569     1  14.90  4.0850 0.0616164
.
## Date:Time           2.71032 0.19359    14 138.67  3.3531 0.0001182
***
## Sex:Time            0.56456 0.28228     2  30.27  4.8912 0.0144325
*
## Treatment:Time      0.21037 0.10518     2  29.56  1.8229 0.1792567
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
FullModel <- update(FullModel, ~ . -Date:Sex)
```

```
## fixed-effect model matrix is rank deficient so dropping 4 columns /
coefficients
```

```
## boundary (singular) fit: see ?isSingular
```

```
## Warning: Model failed to converge with 4 negative eigenvalues: -4.0
e+00 -7.1e+01
## -2.5e+02 -7.2e+02
```

```
anova(FullModel, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                       Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## AvgTHI               0.00190 0.00190     1 235.79  0.0312  0.860011
## YesterdayAvgTHI      0.00887 0.00887     1 232.43  0.1459  0.702874
## YesterdayHighTHI     0.02122 0.02122     1 253.26  0.3488  0.555319
## YesterdayLowTHI      0.00824 0.00824     1 235.43  0.1354  0.713220
## AvgSurfaceTempTotal 1.23943 1.23943     1 427.72 20.3760 8.236e-06
***
## AvgHideTempTotal     0.03512 0.03512     1 431.77  0.5774  0.447743
## Date                 0.93539 0.31180     3  67.96  5.1255  0.002958
**
## Sex                  0.01733 0.01733     1  14.66  0.2850  0.601473
## Treatment            0.17361 0.17361     1  15.03  2.8541  0.111766
## Time                 2.23719 1.11859     2  50.09 18.3569 1.046e-06
***
## Date:Treatment       0.40596 0.05799     7  69.43  0.9527  0.472439
## Sex:Treatment        0.22834 0.22834     1  14.90  3.7540  0.071879
.
```

```
## Date:Time           2.88540 0.20610    14 139.27  3.3860  0.000103
***
## Sex:Time            0.51688 0.25844     2  29.81  4.2471  0.023828
*
## Treatment:Time      0.22277 0.11138     2  29.57  1.8310  0.177963
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
FullModel <- update(FullModel, ~ . -Date:Treatment)
```

```
## fixed-effect model matrix is rank deficient so dropping 4 columns /
coefficients
```

```
## boundary (singular) fit: see ?isSingular
```

```
## Warning: Model failed to converge with 2 negative eigenvalues: -2.4
e+00 -1.2e+02
```

```
anova(FullModel, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                       Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## AvgTHI              0.00506 0.00506     1 198.03  0.0846 0.7714081
## YesterdayAvgTHI     0.01615 0.01615     1 195.81  0.2704 0.6036397
## YesterdayHighTHI    0.02551 0.02551     1 212.74  0.4271 0.5141270
## YesterdayLowTHI     0.01477 0.01477     1 197.79  0.2473 0.6195566
## AvgSurfaceTempTotal 1.30178 1.30178     1 435.44 21.7966 4.043e-06
***
## AvgHideTempTotal    0.03640 0.03640     1 436.76  0.6095 0.4353842
## Date                0.91952 0.30651     3  67.93  5.1316 0.0029382
**
## Sex                 0.01456 0.01456     1  14.67  0.2438 0.6287790
## Treatment           0.26086 0.26086     1  13.57  4.3677 0.0559691
.
## Time                2.21546 1.10773     2  50.01 18.5149 9.601e-07
***
## Sex:Treatment       0.25142 0.25142     1  14.04  4.2097 0.0593356
.
## Date:Time           2.78493 0.19892    14 139.00  3.3282 0.0001301
***
## Sex:Time            0.52898 0.26449     2  29.82  4.4270 0.0207242
*
## Treatment:Time      0.21821 0.10910     2  29.38  1.8267 0.1787582
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
FullModel <- update(FullModel, ~ . -Treatment:Time)
```

```
## fixed-effect model matrix is rank deficient so dropping 4 columns /
coefficients

## boundary (singular) fit: see ?isSingular

## Warning: Model failed to converge with 3 negative eigenvalues: -9.8
e-02 -4.0e+01
## -6.2e+01

anova(FullModel, ddf = "Kenward-Roger")

## Type III Analysis of Variance Table with Kenward-Roger's method
##                       Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## AvgTHI               0.00482 0.00482     1 197.91  0.0811  0.776118
## YesterdayAvgTHI      0.01576 0.01576     1 195.71  0.2654  0.607028
## YesterdayHighTHI     0.02576 0.02576     1 212.64  0.4337  0.510896
## YesterdayLowTHI      0.01454 0.01454     1 197.69  0.2449  0.621262
## AvgSurfaceTempTotal 1.29948 1.29948     1 436.10 21.8775 3.882e-06
***
## AvgHideTempTotal     0.03318 0.03318     1 439.61  0.5586  0.455224
## Date                 0.91545 0.30515     3  67.92  5.1369  0.002921
**
## Sex                  0.01490 0.01490     1  14.67  0.2509  0.623900
## Treatment            0.27662 0.27662     1  13.51  4.6570  0.049454
*
## Time                 2.17544 1.08772     2  49.91 18.2806 1.106e-06
***
## Sex:Treatment        0.25057 0.25057     1  14.05  4.2184  0.059093
.
## Date:Time            2.82867 0.20205    14 139.07  3.3993 9.794e-05
***
## Sex:Time             0.51542 0.25771     2  29.85  4.3371  0.022206
*
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

FullModel <- update(FullModel, ~ . -AvgTHI)

## fixed-effect model matrix is rank deficient so dropping 3 columns /
coefficients

## boundary (singular) fit: see ?isSingular

## Warning: Model failed to converge with 4 negative eigenvalues: -4.1
e-04 -2.0e-02
## -4.5e-02 -5.3e-01

anova(FullModel, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                       Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## YesterdayAvgTHI      0.01816 0.01816     1 209.52  0.2932 0.5887273
## YesterdayHighTHI     0.03799 0.03799     1 224.56  0.6135 0.4342970
## YesterdayLowTHI      0.01855 0.01855     1 217.29  0.2995 0.5847304
## AvgSurfaceTempTotal 1.35296 1.35296     1 435.91 21.8519 3.933e-06
***
## AvgHideTempTotal     0.03481 0.03481     1 439.69  0.5622 0.4537843
## Date                 0.97732 0.24433     4  78.90  3.9433 0.0057120
**
## Sex                  0.01585 0.01585     1  14.67  0.2561 0.6203627
## Treatment            0.28787 0.28787     1  13.51  4.6494 0.0496163
*
## Time                 2.27651 1.13825     2  49.95 18.3520 1.059e-06
***
## Sex:Treatment        0.26094 0.26094     1  14.04  4.2144 0.0591996
.
## Date:Time            2.93404 0.20957    14 139.04  3.3825 0.0001048
***
## Sex:Time             0.54038 0.27019     2  29.84  4.3623 0.0217794
*
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

FullModel <- update(FullModel, ~ . -Sex)

## fixed-effect model matrix is rank deficient so dropping 3 columns /
coefficients

## boundary (singular) fit: see ?isSingular

## Warning: Model failed to converge with 3 negative eigenvalues: -5.1
e-02 -8.2e-02
## -1.9e-01

anova(FullModel, ddf = "Kenward-Roger")

## Type III Analysis of Variance Table with Kenward-Roger's method
##                       Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## YesterdayAvgTHI      0.01727 0.01727     1 209.37  0.2961 0.5868888
## YesterdayHighTHI     0.03602 0.03602     1 224.50  0.6177 0.4327368
## YesterdayLowTHI      0.01774 0.01774     1 217.18  0.3042 0.5818511
## AvgSurfaceTempTotal 1.28011 1.28011     1 436.24 21.9508 3.743e-06
***
## AvgHideTempTotal     0.03271 0.03271     1 440.70  0.5609 0.4542918
## Date                 0.91899 0.22975     4  78.89  3.9366 0.0057695
**
```

```
## Treatment           0.44798 0.44798     1  13.47  7.6818 0.0154407
*
## Time                2.11890 1.05945     2  49.87 18.1354 1.206e-06
***
## Treatment:Sex       0.28331 0.14166     2  18.30  2.3435 0.1241308
## Date:Time           2.75899 0.19707    14 138.93  3.3769 0.0001072
***
## Time:Sex            0.50580 0.25290     2  29.86  4.3352 0.0222361
*
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
FullModel <- update(FullModel, ~ . -YesterdayAvgTHI)
```

```
## fixed-effect model matrix is rank deficient so dropping 2 columns /
coefficients
```

```
## boundary (singular) fit: see ?isSingular
```

```
## Warning: Model failed to converge with 5 negative eigenvalues: -1.8
e+00 -8.9e+00
## -3.0e+01 -6.8e+01 -1.1e+02
```

```
anova(FullModel, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                       Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## YesterdayHighTHI    0.00386 0.00386     1 211.74  0.0636 0.8011838
## YesterdayLowTHI     0.01870 0.01870     1 215.55  0.3082 0.5793328
## AvgSurfaceTempTotal 1.32250 1.32250     1 435.58 21.7999 4.036e-06
***
## AvgHideTempTotal    0.03468 0.03468     1 438.95  0.5717 0.4499740
## Date                0.96350 0.19270     5  78.10  3.1736 0.0116436
*
## Treatment           0.46207 0.46207     1  13.47  7.6167 0.0157936
*
## Time                2.25274 1.12637     2  50.00 18.5345 9.497e-07
***
## Treatment:Sex       0.29307 0.14654     2  18.29  2.3302 0.1254608
## Date:Time           2.88116 0.20580    14 139.20  3.3900 0.0001015
***
## Time:Sex            0.53386 0.26693     2  29.82  4.3985 0.0211849
*
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
FullModel <- update(FullModel, ~ . -YesterdayHighTHI)
```

```
## fixed-effect model matrix is rank deficient so dropping 1 column /
coefficient

## boundary (singular) fit: see ?isSingular

## Warning: Model failed to converge with 3 negative eigenvalues: -2.9
e-01 -3.8e-01
## -8.8e+01

anova(FullModel, ddf = "Kenward-Roger")

## Type III Analysis of Variance Table with Kenward-Roger's method
##                       Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## YesterdayLowTHI     0.02024 0.02024     1 216.70  0.3327 0.5646622
## AvgSurfaceTempTotal 1.32728 1.32728     1 435.54 21.8193 3.997e-06
***
## AvgHideTempTotal    0.03476 0.03476     1 438.93  0.5714 0.4501126
## Date                1.19046 0.19841     6  77.70  3.2584 0.0065515
**
## Treatment           0.46303 0.46303     1  13.47  7.6118 0.0158210
*
## Time                2.25683 1.12842     2  50.00 18.5178 9.591e-07
***
## Treatment:Sex       0.29373 0.14687     2  18.29  2.3292 0.1255697
## Date:Time           2.88782 0.20627    14 139.17  3.3886 0.0001021
***
## Time:Sex            0.53563 0.26781     2  29.83  4.4011 0.0211405
*
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

FullModel <- update(FullModel, ~ . -YesterdayLowTHI)

## boundary (singular) fit: see ?isSingular

## Warning: Model failed to converge with 4 negative eigenvalues: -8.5
e-02 -8.7e-02
## -1.2e-01 -1.6e-01

anova(FullModel, ddf = "Kenward-Roger")

## Type III Analysis of Variance Table with Kenward-Roger's method
##                       Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## AvgSurfaceTempTotal 1.30465 1.30465     1 435.89 21.8506 3.935e-06
***
## AvgHideTempTotal    0.03405 0.03405     1 439.99  0.5702 0.4505816
## Date                1.19627 0.17090     7  80.59  2.8584 0.0102454
*
```

```
## Treatment           0.45703 0.45703     1  13.47  7.6544 0.0155881
*
## Time                2.19239 1.09620     2  49.96 18.3274 1.073e-06
***
## Treatment:Sex       0.28881 0.14440     2  18.29  2.3332 0.1251541
## Date:Time           2.80589 0.20042    14 139.02  3.3543 0.0001172
***
## Time:Sex            0.52542 0.26271     2  29.84  4.3985 0.0211802
*
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
FullModel <- update(FullModel, ~ . -AvgHideTempTotal)
```

```
## boundary (singular) fit: see ?isSingular
```

```
anova(FullModel, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                       Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## AvgSurfaceTempTotal 1.64187 1.64187     1 342.41 27.6610 2.552e-07
***
## Date                1.16954 0.16708     7  77.85  2.8113 0.0115604
*
## Treatment           0.42835 0.42835     1  13.26  7.2165 0.0184122
*
## Time                2.04973 1.02487     2  45.92 17.2297 2.615e-06
***
## Treatment:Sex       0.27273 0.13636     2  18.30  2.2163 0.1374361
## Date:Time           2.78060 0.19861    14 137.45  3.3439 0.0001242
***
## Time:Sex            0.45852 0.22926     2  29.04  3.8610 0.0325942
*
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
FullModel <- update(FullModel, ~ . -Treatment:Sex)
```

```
## boundary (singular) fit: see ?isSingular
```

```
## Warning: Model failed to converge with 2 negative eigenvalues: -4.1
e-02 -3.0e-01
```

```
anova(FullModel, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                       Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## AvgSurfaceTempTotal 1.57088 1.57088     1 339.42 25.1473 8.576e-07
```

```
***
## Date                1.22369 0.17481     7  77.86  2.7952   0.01197
*
## Treatment           0.14719 0.14719     1  14.74  2.3564   0.14596
## Time                1.81948 0.90974     2  36.73 14.5490 2.221e-05
***
## Date:Time           3.13192 0.22371    14 138.28  3.5795 4.818e-05
***
## Time:Sex            0.45383 0.15128     3  29.77  2.3537   0.09208
.
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
FinalModel <- lmer(PercentOMBTotal ~ (1|Block) + AvgSurfaceTempTotal +
Date + Treatment + Time + Date:Time + Time:Sex, data = FullData)
```

```
anova(FinalModel, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                     Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## AvgSurfaceTempTotal 18.392 18.3924     1 478.95 38.8215 1.018e-09 *
**
## Date                18.345  2.6208     7 478.41  5.5314 3.895e-06 *
**
## Treatment            0.716  0.7159     1 468.11  1.5111  0.219585
## Time                26.910 13.4552     2 471.14 28.4003 2.262e-12 *
**
## Date:Time           38.136  2.7240    14 468.63  5.7496 2.157e-10 *
**
## Time:Sex             7.925  2.6417     3  68.83  5.4764  0.001961 *
*
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
EMTreatment <- emmeans(FinalModel, pairwise ~ Treatment)
```

```
## NOTE: A nesting structure was detected in the fitted model:
##     Sex %in% Time
```

```
EMTreatment
```

```
## $emmeans
##  Treatment emmean     SE   df lower.CL upper.CL
##  1          0.400 0.0686 24.4    0.259    0.542
##  2          0.475 0.0684 23.9    0.334    0.616
##
## Results are averaged over the levels of: Date, Sex, Time
## Degrees-of-freedom method: kenward-roger
```

```
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate     SE  df t.ratio p.value
##  1 - 2     -0.0749 0.0609 468 -1.229  0.2196
##
## Results are averaged over the levels of: Date, Sex, Time
## Degrees-of-freedom method: kenward-roger
```

```
EMSexB <- emmeans(FinalModel, pairwise ~ Sex|Treatment)
```

```
## NOTE: A nesting structure was detected in the fitted model:
##     Sex %in% Time
```

```
EMSexB
```

```
## $emmeans
## Treatment = 1:
##  Sex Time emmean     SE    df lower.CL upper.CL
##  Hfr A    0.3382 0.1417  69.4   0.0555    0.621
##  Str A    0.4451 0.1241 102.3   0.1990    0.691
##  Hfr B    0.0221 0.1243  42.8  -0.2286    0.273
##  Str B    0.1604 0.0990  46.2  -0.0389    0.360
##  Hfr C    0.9364 0.1214  41.3   0.6913    1.182
##  Str C    0.4984 0.0999  48.2   0.2975    0.699
##
## Treatment = 2:
##  Sex Time emmean     SE    df lower.CL upper.CL
##  Hfr A    0.4131 0.1411  67.4   0.1314    0.695
##  Str A    0.5200 0.1230 100.6   0.2760    0.764
##  Hfr B    0.0970 0.1249  42.9  -0.1548    0.349
##  Str B    0.2353 0.0988  46.5   0.0365    0.434
##  Hfr C    1.0113 0.1218  41.2   0.7654    1.257
##  Str C    0.5733 0.1002  49.6   0.3719    0.775
##
## Results are averaged over the levels of: Date
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = 1:
##  contrast      estimate     SE    df t.ratio p.value
##  Hfr,A - Str,A  -0.1069 0.1563  42.5 -0.684  0.9976
##  Hfr,A - Hfr,B   0.3161 0.1552 473.4  2.037  0.4745
##  Hfr,A - Str,B   0.1778 0.1712  59.6  1.039  0.9708
##  Hfr,A - Hfr,C  -0.5982 0.1466 472.7 -4.079  0.0015
##  Hfr,A - Str,C  -0.1602 0.1751  65.3 -0.915  0.9861
```

```
##  Str,A - Hfr,B   0.4230 0.1805  71.4  2.343  0.2977
##  Str,A - Str,B   0.2847 0.1307 473.1  2.178  0.3817
##  Str,A - Hfr,C  -0.4914 0.1716  62.5 -2.864  0.1031
##  Str,A - Str,C  -0.0533 0.1366 473.7 -0.390  1.0000
##  Hfr,B - Str,B  -0.1383 0.1507  37.0 -0.918  0.9850
##  Hfr,B - Hfr,C  -0.9143 0.1241 468.4 -7.368  <.0001
##  Hfr,B - Str,C  -0.4763 0.1503  37.0 -3.168  0.0583
##  Str,B - Hfr,C  -0.7760 0.1496  38.0 -5.186  0.0002
##  Str,B - Str,C  -0.3380 0.0991 468.1 -3.411  0.0171
##  Hfr,C - Str,C   0.4380 0.1494  38.1  2.933  0.0988
##
## Treatment = 2:
##  contrast      estimate     SE    df t.ratio p.value
##  Hfr,A - Str,A  -0.1069 0.1563  42.5 -0.684  0.9976
##  Hfr,A - Hfr,B   0.3161 0.1552 473.4  2.037  0.4745
##  Hfr,A - Str,B   0.1778 0.1712  59.6  1.039  0.9708
##  Hfr,A - Hfr,C  -0.5982 0.1466 472.7 -4.079  0.0015
##  Hfr,A - Str,C  -0.1602 0.1751  65.3 -0.915  0.9861
##  Str,A - Hfr,B   0.4230 0.1805  71.4  2.343  0.2977
##  Str,A - Str,B   0.2847 0.1307 473.1  2.178  0.3817
##  Str,A - Hfr,C  -0.4914 0.1716  62.5 -2.864  0.1031
##  Str,A - Str,C  -0.0533 0.1366 473.7 -0.390  1.0000
##  Hfr,B - Str,B  -0.1383 0.1507  37.0 -0.918  0.9850
##  Hfr,B - Hfr,C  -0.9143 0.1241 468.4 -7.368  <.0001
##  Hfr,B - Str,C  -0.4763 0.1503  37.0 -3.168  0.0583
##  Str,B - Hfr,C  -0.7760 0.1496  38.0 -5.186  0.0002
##  Str,B - Str,C  -0.3380 0.0991 468.1 -3.411  0.0171
##  Hfr,C - Str,C   0.4380 0.1494  38.1  2.933  0.0988
##
## Results are averaged over some or all of the levels of: Date
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 8.262 es
timates

EMSexC <- emmeans(FinalModel, pairwise ~ Sex|Treatment|Time)

## NOTE: A nesting structure was detected in the fitted model:
##     Sex %in% Time

EMSexC

## $emmeans
## Treatment = 1, Time = A:
##  Sex emmean     SE    df lower.CL upper.CL
##  Hfr 0.3382 0.1417  69.4   0.0555    0.621
##  Str 0.4451 0.1241 102.3   0.1990    0.691
##
```

```
## Treatment = 1, Time = B:
##  Sex emmean     SE    df lower.CL upper.CL
##  Hfr 0.0221 0.1243  42.8  -0.2286    0.273
##  Str 0.1604 0.0990  46.2  -0.0389    0.360
##
## Treatment = 1, Time = C:
##  Sex emmean     SE    df lower.CL upper.CL
##  Hfr 0.9364 0.1214  41.3   0.6913    1.182
##  Str 0.4984 0.0999  48.2   0.2975    0.699
##
## Treatment = 2, Time = A:
##  Sex emmean     SE    df lower.CL upper.CL
##  Hfr 0.4131 0.1411  67.4   0.1314    0.695
##  Str 0.5200 0.1230 100.6   0.2760    0.764
##
## Treatment = 2, Time = B:
##  Sex emmean     SE    df lower.CL upper.CL
##  Hfr 0.0970 0.1249  42.9  -0.1548    0.349
##  Str 0.2353 0.0988  46.5   0.0365    0.434
##
## Treatment = 2, Time = C:
##  Sex emmean     SE    df lower.CL upper.CL
##  Hfr 1.0113 0.1218  41.2   0.7654    1.257
##  Str 0.5733 0.1002  49.6   0.3719    0.775
##
## Results are averaged over the levels of: Date
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = 1, Time = A:
##  contrast  estimate    SE   df t.ratio p.value
##  Hfr - Str   -0.107 0.156 42.5 -0.684  0.4978
##
## Treatment = 1, Time = B:
##  contrast  estimate    SE   df t.ratio p.value
##  Hfr - Str   -0.138 0.151 37.0 -0.918  0.3648
##
## Treatment = 1, Time = C:
##  contrast  estimate    SE   df t.ratio p.value
##  Hfr - Str    0.438 0.149 38.1  2.933  0.0057
##
## Treatment = 2, Time = A:
##  contrast  estimate    SE   df t.ratio p.value
##  Hfr - Str   -0.107 0.156 42.5 -0.684  0.4978
##
```

```
## Treatment = 2, Time = B:
##  contrast  estimate    SE   df t.ratio p.value
##  Hfr - Str   -0.138 0.151 37.0 -0.918  0.3648
##
## Treatment = 2, Time = C:
##  contrast  estimate    SE   df t.ratio p.value
##  Hfr - Str    0.438 0.149 38.1  2.933  0.0057
##
## Results are averaged over some or all of the levels of: Date
## Degrees-of-freedom method: kenward-roger
```

```
EMSexD <- emmeans(FinalModel, pairwise ~ Treatment|Time)
```

```
## NOTE: A nesting structure was detected in the fitted model:
##     Sex %in% Time
```

```
EMSexD
```

```
## $emmeans
## Time = A:
##  Treatment emmean     SE    df lower.CL upper.CL
##  1         0.3916 0.1079 124.7  0.17815    0.605
##  2         0.4665 0.1068 120.3  0.25503    0.678
##
## Time = B:
##  Treatment emmean     SE    df lower.CL upper.CL
##  1         0.0912 0.0833  51.4 -0.07606    0.258
##  2         0.1661 0.0836  51.7 -0.00172    0.334
##
## Time = C:
##  Treatment emmean     SE    df lower.CL upper.CL
##  1         0.7174 0.0823  49.6  0.55197    0.883
##  2         0.7923 0.0829  50.4  0.62592    0.959
##
## Results are averaged over the levels of: Date, Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Time = A:
##  contrast estimate     SE  df t.ratio p.value
##  1 - 2     -0.0749 0.0609 468 -1.229  0.2196
##
## Time = B:
##  contrast estimate     SE  df t.ratio p.value
##  1 - 2     -0.0749 0.0609 468 -1.229  0.2196
##
```

```
## Time = C:
##  contrast estimate     SE  df t.ratio p.value
##  1 - 2     -0.0749 0.0609 468 -1.229  0.2196
##
## Results are averaged over the levels of: Date, Sex
## Degrees-of-freedom method: kenward-roger
```

```
EMSexE <- emmeans(FinalModel, pairwise ~ Time|Treatment)
```

```
## NOTE: A nesting structure was detected in the fitted model:
##     Sex %in% Time
```

```
EMSexE
```

```
## $emmeans
## Treatment = 1:
##  Time emmean     SE    df lower.CL upper.CL
##  A    0.3916 0.1079 124.7  0.17815    0.605
##  B    0.0912 0.0833  51.4 -0.07606    0.258
##  C    0.7174 0.0823  49.6  0.55197    0.883
##
## Treatment = 2:
##  Time emmean     SE    df lower.CL upper.CL
##  A    0.4665 0.1068 120.3  0.25503    0.678
##  B    0.1661 0.0836  51.7 -0.00172    0.334
##  C    0.7923 0.0829  50.4  0.62592    0.959
##
## Results are averaged over the levels of: Date, Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Treatment = 1:
##  contrast estimate     SE  df t.ratio p.value
##  A - B       0.300 0.1182 475  2.541  0.0305
##  A - C      -0.326 0.1157 475 -2.816  0.0140
##  B - C      -0.626 0.0794 468 -7.891  <.0001
##
## Treatment = 2:
##  contrast estimate     SE  df t.ratio p.value
##  A - B       0.300 0.1182 475  2.541  0.0305
##  A - C      -0.326 0.1157 475 -2.816  0.0140
##  B - C      -0.626 0.0794 468 -7.891  <.0001
##
## Results are averaged over the levels of: Date, Sex
## Degrees-of-freedom method: kenward-roger
```

```
## P value adjustment: tukey method for comparing a family of 3 estima
tes
```

```
EMSex <- emmeans(FinalModel, pairwise ~ Sex|Time)
```

```
## NOTE: A nesting structure was detected in the fitted model:
##     Sex %in% Time
```

```
EMSex
```

```
## $emmeans
## Time = A:
##  Sex emmean     SE   df lower.CL upper.CL
##  Hfr 0.3756 0.1381 62.6  0.09961    0.652
##  Str 0.4825 0.1197 91.0  0.24474    0.720
##
## Time = B:
##  Sex emmean     SE   df lower.CL upper.CL
##  Hfr 0.0595 0.1208 38.0 -0.18496    0.304
##  Str 0.1978 0.0941 38.3  0.00738    0.388
##
## Time = C:
##  Sex emmean     SE   df lower.CL upper.CL
##  Hfr 0.9739 0.1177 36.4  0.73521    1.213
##  Str 0.5358 0.0953 40.5  0.34325    0.728
##
## Results are averaged over the levels of: Date, Treatment
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Time = A:
##  contrast  estimate    SE   df t.ratio p.value
##  Hfr - Str   -0.107 0.156 42.5 -0.684  0.4978
##
## Time = B:
##  contrast  estimate    SE   df t.ratio p.value
##  Hfr - Str   -0.138 0.151 37.0 -0.918  0.3648
##
## Time = C:
##  contrast  estimate    SE   df t.ratio p.value
##  Hfr - Str    0.438 0.149 38.1  2.933  0.0057
##
## Results are averaged over some or all of the levels of: Date, Treat
ment
## Degrees-of-freedom method: kenward-roger
```

```
EMTime <- emmeans(FinalModel, pairwise ~ Time)

## NOTE: A nesting structure was detected in the fitted model:
##     Sex %in% Time

## NOTE: Results may be misleading due to involvement in interactions

EMTime

## $emmeans
##  Time emmean     SE    df lower.CL upper.CL
##  A     0.429 0.1029 106.4   0.2250    0.633
##  B     0.129 0.0777  39.2  -0.0285    0.286
##  C     0.755 0.0768  37.8   0.5994    0.910
##
## Results are averaged over the levels of: Date, Treatment, Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate     SE  df t.ratio p.value
##  A - B       0.300 0.1182 475  2.541  0.0305
##  A - C      -0.326 0.1157 475 -2.816  0.0140
##  B - C      -0.626 0.0794 468 -7.891  <.0001
##
## Results are averaged over the levels of: Date, Treatment, Sex
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estima
tes

EMTime2 <- emmeans(FinalModel, pairwise ~ Time|Date)

## NOTE: A nesting structure was detected in the fitted model:
##     Sex %in% Time

EMTime2

## $emmeans
## Date = 6/21/2019:
##  Time  emmean    SE  df lower.CL upper.CL
##  A     0.6984 0.223 436  0.25984    1.137
##  B     0.3751 0.199 408 -0.01602    0.766
##  C     0.5592 0.195 401  0.17669    0.942
##
## Date = 6/24/2019:
##  Time  emmean    SE  df lower.CL upper.CL
##  A     0.6655 0.207 422  0.25868    1.072
##  B     0.4570 0.193 401  0.07733    0.837
```

```
##  C     0.3084 0.186 389 -0.05824    0.675
##
## Date = 7/17/2019:
##  Time  emmean    SE  df lower.CL upper.CL
##  A     0.1887 0.136 245 -0.07859    0.456
##  B     0.0734 0.154 309 -0.23018    0.377
##  C     0.7225 0.161 331  0.40506    1.040
##
## Date = 7/3/2019:
##  Time  emmean    SE  df lower.CL upper.CL
##  A     0.2853 0.187 389 -0.08279    0.653
##  B    -0.0216 0.142 269 -0.30205    0.259
##  C     1.6652 0.139 264  1.39079    1.940
##
## Date = 8/14/2019:
##  Time  emmean    SE  df lower.CL upper.CL
##  A     0.3701 0.146 283  0.08184    0.658
##  B    -0.0907 0.143 270 -0.37138    0.190
##  C     0.3640 0.142 269  0.08388    0.644
##
## Date = 8/21/2019:
##  Time  emmean    SE  df lower.CL upper.CL
##  A     0.2245 0.189 385 -0.14690    0.596
##  B    -0.1903 0.188 391 -0.55919    0.179
##  C     0.5976 0.182 378  0.23886    0.956
##
## Date = 9/18/2019:
##  Time  emmean    SE  df lower.CL upper.CL
##  A     0.4894 0.202 417  0.09333    0.886
##  B     0.0418 0.184 383 -0.31913    0.403
##  C     0.2854 0.186 390 -0.07970    0.650
##
## Date = 9/4/2019:
##  Time  emmean    SE  df lower.CL upper.CL
##  A     0.5107 0.197 410  0.12423    0.897
##  B     0.3847 0.191 394  0.00957    0.760
##  C     1.5365 0.191 395  1.16003    1.913
##
## Results are averaged over the levels of: Treatment, Sex
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## Date = 6/21/2019:
##  contrast estimate    SE  df t.ratio p.value
##  A - B     0.32334 0.256 469  1.262  0.4174
```

```
##  A - C     0.13917 0.253 469  0.550  0.8466
##  B - C    -0.18417 0.247 468 -0.745  0.7366
##
## Date = 6/24/2019:
##  contrast estimate    SE  df t.ratio p.value
##  A - B     0.20847 0.246 468  0.846  0.6747
##  A - C     0.35716 0.251 469  1.424  0.3293
##  B - C     0.14869 0.245 468  0.606  0.8168
##
## Date = 7/17/2019:
##  contrast estimate    SE  df t.ratio p.value
##  A - B     0.11533 0.201 471  0.575  0.8337
##  A - C    -0.53378 0.207 472 -2.573  0.0280
##  B - C    -0.64910 0.174 468 -3.737  0.0006
##
## Date = 7/3/2019:
##  contrast estimate    SE  df t.ratio p.value
##  A - B     0.30683 0.232 474  1.322  0.3834
##  A - C    -1.37994 0.229 474 -6.030  <.0001
##  B - C    -1.68677 0.174 469 -9.721  <.0001
##
## Date = 8/14/2019:
##  contrast estimate    SE  df t.ratio p.value
##  A - B     0.46087 0.207 472  2.226  0.0679
##  A - C     0.00616 0.207 472  0.030  0.9995
##  B - C    -0.45471 0.173 468 -2.625  0.0242
##
## Date = 8/21/2019:
##  contrast estimate    SE  df t.ratio p.value
##  A - B     0.41479 0.262 470  1.585  0.2529
##  A - C    -0.37312 0.251 469 -1.486  0.2986
##  B - C    -0.78790 0.247 468 -3.194  0.0043
##
## Date = 9/18/2019:
##  contrast estimate    SE  df t.ratio p.value
##  A - B     0.44759 0.258 469  1.734  0.1935
##  A - C     0.20404 0.252 469  0.808  0.6981
##  B - C    -0.24355 0.247 468 -0.984  0.5873
##
## Date = 9/4/2019:
##  contrast estimate    SE  df t.ratio p.value
##  A - B     0.12598 0.278 471  0.453  0.8933
##  A - C    -1.02581 0.279 471 -3.672  0.0008
##  B - C    -1.15179 0.244 468 -4.717  <.0001
##
## Results are averaged over the levels of: Treatment, Sex
```

```
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estima
tes

EMTime3 <- emmeans(FinalModel, pairwise ~ Date|Time)

## NOTE: A nesting structure was detected in the fitted model:
##     Sex %in% Time

EMTime3

## $emmeans
## Time = A:
##  Date       emmean    SE  df lower.CL upper.CL
##  6/21/2019  0.6984 0.223 436  0.25984    1.137
##  6/24/2019  0.6655 0.207 422  0.25868    1.072
##  7/17/2019  0.1887 0.136 245 -0.07859    0.456
##  7/3/2019   0.2853 0.187 389 -0.08279    0.653
##  8/14/2019  0.3701 0.146 283  0.08184    0.658
##  8/21/2019  0.2245 0.189 385 -0.14690    0.596
##  9/18/2019  0.4894 0.202 417  0.09333    0.886
##  9/4/2019   0.5107 0.197 410  0.12423    0.897
##
## Time = B:
##  Date       emmean    SE  df lower.CL upper.CL
##  6/21/2019  0.3751 0.199 408 -0.01602    0.766
##  6/24/2019  0.4570 0.193 401  0.07733    0.837
##  7/17/2019  0.0734 0.154 309 -0.23018    0.377
##  7/3/2019  -0.0216 0.142 269 -0.30205    0.259
##  8/14/2019 -0.0907 0.143 270 -0.37138    0.190
##  8/21/2019 -0.1903 0.188 391 -0.55919    0.179
##  9/18/2019  0.0418 0.184 383 -0.31913    0.403
##  9/4/2019   0.3847 0.191 394  0.00957    0.760
##
## Time = C:
##  Date       emmean    SE  df lower.CL upper.CL
##  6/21/2019  0.5592 0.195 401  0.17669    0.942
##  6/24/2019  0.3084 0.186 389 -0.05824    0.675
##  7/17/2019  0.7225 0.161 331  0.40506    1.040
##  7/3/2019   1.6652 0.139 264  1.39079    1.940
##  8/14/2019  0.3640 0.142 269  0.08388    0.644
##  8/21/2019  0.5976 0.182 378  0.23886    0.956
##  9/18/2019  0.2854 0.186 390 -0.07970    0.650
##  9/4/2019   1.5365 0.191 395  1.16003    1.913
##
## Results are averaged over the levels of: Treatment, Sex
## Degrees-of-freedom method: kenward-roger
```

```
## Confidence level used: 0.95
##
## $contrasts
## Time = A:
##  contrast              estimate    SE  df t.ratio p.value
##  6/21/2019 - 6/24/2019  0.03288 0.257 480  0.128  1.0000
##  6/21/2019 - 7/17/2019  0.50971 0.239 475  2.134  0.3944
##  6/21/2019 - 7/3/2019   0.41313 0.261 474  1.583  0.7606
##  6/21/2019 - 8/14/2019  0.32825 0.225 474  1.458  0.8290
##  6/21/2019 - 8/21/2019  0.47391 0.258 473  1.839  0.5936
##  6/21/2019 - 9/18/2019  0.20895 0.251 478  0.832  0.9912
##  6/21/2019 - 9/4/2019   0.18770 0.259 478  0.725  0.9962
##  6/24/2019 - 7/17/2019  0.47683 0.229 474  2.078  0.4306
##  6/24/2019 - 7/3/2019   0.38025 0.259 477  1.466  0.8252
##  6/24/2019 - 8/14/2019  0.29537 0.219 473  1.348  0.8799
##  6/24/2019 - 8/21/2019  0.44103 0.254 477  1.734  0.6652
##  6/24/2019 - 9/18/2019  0.17607 0.250 473  0.705  0.9968
##  6/24/2019 - 9/4/2019   0.15482 0.246 472  0.629  0.9985
##  7/17/2019 - 7/3/2019  -0.09658 0.215 473 -0.450  0.9998
##  7/17/2019 - 8/14/2019 -0.18146 0.179 469 -1.013  0.9724
##  7/17/2019 - 8/21/2019 -0.03580 0.216 475 -0.166  1.0000
##  7/17/2019 - 9/18/2019 -0.30076 0.223 473 -1.346  0.8805
##  7/17/2019 - 9/4/2019  -0.32200 0.222 473 -1.449  0.8339
##  7/3/2019 - 8/14/2019  -0.08488 0.216 474 -0.393  0.9999
##  7/3/2019 - 8/21/2019   0.06078 0.247 476  0.246  1.0000
##  7/3/2019 - 9/18/2019  -0.20418 0.252 477 -0.810  0.9925
##  7/3/2019 - 9/4/2019   -0.22543 0.254 476 -0.886  0.9872
##  8/14/2019 - 8/21/2019  0.14566 0.214 474  0.681  0.9975
##  8/14/2019 - 9/18/2019 -0.11930 0.216 473 -0.553  0.9993
##  8/14/2019 - 9/4/2019  -0.14054 0.216 473 -0.651  0.9981
##  8/21/2019 - 9/18/2019 -0.26496 0.249 475 -1.063  0.9639
##  8/21/2019 - 9/4/2019  -0.28620 0.252 478 -1.138  0.9482
##  9/18/2019 - 9/4/2019  -0.02125 0.251 476 -0.085  1.0000
##
## Time = B:
##  contrast              estimate    SE  df t.ratio p.value
##  6/21/2019 - 6/24/2019 -0.08199 0.255 480 -0.321  1.0000
##  6/21/2019 - 7/17/2019  0.30169 0.261 476  1.156  0.9438
##  6/21/2019 - 7/3/2019   0.39662 0.247 475  1.607  0.7460
##  6/21/2019 - 8/14/2019  0.46578 0.247 475  1.886  0.5610
##  6/21/2019 - 8/21/2019  0.56535 0.272 473  2.075  0.4325
##  6/21/2019 - 9/18/2019  0.33320 0.256 479  1.301  0.8982
##  6/21/2019 - 9/4/2019  -0.00965 0.284 479 -0.034  1.0000
##  6/24/2019 - 7/17/2019  0.38368 0.259 475  1.484  0.8157
##  6/24/2019 - 7/3/2019   0.47861 0.245 475  1.957  0.5126
##  6/24/2019 - 8/14/2019  0.54777 0.245 475  2.238  0.3308
```

```
##  6/24/2019 - 8/21/2019  0.64734 0.272 477  2.376  0.2555
##  6/24/2019 - 9/18/2019  0.41519 0.255 474  1.626  0.7347
##  6/24/2019 - 9/4/2019   0.07234 0.275 475  0.263  1.0000
##  7/17/2019 - 7/3/2019   0.09493 0.174 468  0.545  0.9994
##  7/17/2019 - 8/14/2019  0.16409 0.174 468  0.943  0.9817
##  7/17/2019 - 8/21/2019  0.26366 0.215 475  1.224  0.9247
##  7/17/2019 - 9/18/2019  0.03151 0.230 473  0.137  1.0000
##  7/17/2019 - 9/4/2019  -0.31135 0.215 473 -1.447  0.8346
##  7/3/2019 - 8/14/2019   0.06916 0.172 468  0.402  0.9999
##  7/3/2019 - 8/21/2019   0.16873 0.213 474  0.793  0.9935
##  7/3/2019 - 9/18/2019  -0.06342 0.221 473 -0.287  1.0000
##  7/3/2019 - 9/4/2019   -0.40628 0.214 473 -1.896  0.5541
##  8/14/2019 - 8/21/2019  0.09957 0.213 474  0.468  0.9998
##  8/14/2019 - 9/18/2019 -0.13258 0.221 473 -0.599  0.9989
##  8/14/2019 - 9/4/2019  -0.47543 0.214 473 -2.219  0.3420
##  8/21/2019 - 9/18/2019 -0.23215 0.252 474 -0.920  0.9840
##  8/21/2019 - 9/4/2019  -0.57501 0.250 479 -2.298  0.2965
##  9/18/2019 - 9/4/2019  -0.34286 0.258 476 -1.326  0.8885
##
## Time = C:
##  contrast              estimate    SE  df t.ratio p.value
##  6/21/2019 - 6/24/2019  0.25087 0.254 480  0.988  0.9760
##  6/21/2019 - 7/17/2019 -0.16324 0.265 476 -0.615  0.9987
##  6/21/2019 - 7/3/2019  -1.10598 0.239 475 -4.629  0.0001
##  6/21/2019 - 8/14/2019  0.19524 0.243 475  0.804  0.9929
##  6/21/2019 - 8/21/2019 -0.03838 0.256 473 -0.150  1.0000
##  6/21/2019 - 9/18/2019  0.27382 0.248 479  1.103  0.9560
##  6/21/2019 - 9/4/2019  -0.97727 0.282 479 -3.461  0.0135
##  6/24/2019 - 7/17/2019 -0.41411 0.253 475 -1.639  0.7265
##  6/24/2019 - 7/3/2019  -1.35685 0.231 475 -5.874  <.0001
##  6/24/2019 - 8/14/2019 -0.05563 0.234 474 -0.238  1.0000
##  6/24/2019 - 8/21/2019 -0.28925 0.254 477 -1.137  0.9483
##  6/24/2019 - 9/18/2019  0.02295 0.249 473  0.092  1.0000
##  6/24/2019 - 9/4/2019  -1.22814 0.266 474 -4.626  0.0001
##  7/17/2019 - 7/3/2019  -0.94274 0.179 469 -5.272  <.0001
##  7/17/2019 - 8/14/2019  0.35848 0.177 469  2.029  0.4636
##  7/17/2019 - 8/21/2019  0.12486 0.228 476  0.547  0.9994
##  7/17/2019 - 9/18/2019  0.43706 0.245 473  1.784  0.6313
##  7/17/2019 - 9/4/2019  -0.81403 0.216 472 -3.761  0.0047
##  7/3/2019 - 8/14/2019   1.30122 0.172 469  7.549  <.0001
##  7/3/2019 - 8/21/2019   1.06760 0.216 475  4.952  <.0001
##  7/3/2019 - 9/18/2019   1.37980 0.225 474  6.127  <.0001
##  7/3/2019 - 9/4/2019    0.12871 0.215 473  0.599  0.9989
##  8/14/2019 - 8/21/2019 -0.23362 0.217 475 -1.077  0.9614
##  8/14/2019 - 9/18/2019  0.07859 0.228 473  0.345  1.0000
##  8/14/2019 - 9/4/2019  -1.17251 0.214 473 -5.471  <.0001
```

```
##  8/21/2019 - 9/18/2019  0.31220 0.250 475  1.250  0.9164
##  8/21/2019 - 9/4/2019  -0.93889 0.255 480 -3.682  0.0062
##  9/18/2019 - 9/4/2019  -1.25110 0.266 476 -4.710  0.0001
##
## Results are averaged over the levels of: Treatment, Sex
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 8 estima
tes
```

```
EMDate <- emmeans(FinalModel, pairwise ~ Date)
```

```
## NOTE: A nesting structure was detected in the fitted model:
##     Sex %in% Time
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
EMDate
```

```
## $emmeans
##  Date      emmean     SE    df lower.CL upper.CL
##  6/21/2019  0.544 0.1457 251.2   0.2573    0.831
##  6/24/2019  0.477 0.1337 211.5   0.2134    0.741
##  7/17/2019  0.328 0.1007 100.2   0.1284    0.528
##  7/3/2019   0.643 0.0989  95.2   0.4467    0.839
##  8/14/2019  0.214 0.0885  64.5   0.0377    0.391
##  8/21/2019  0.211 0.1155 138.0  -0.0179    0.439
##  9/18/2019  0.272 0.1224 169.8   0.0306    0.514
##  9/4/2019   0.811 0.1155 141.5   0.5823    1.039
##
## Results are averaged over the levels of: Treatment, Sex, Time
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast              estimate    SE  df t.ratio p.value
##  6/21/2019 - 6/24/2019  0.06725 0.155 474  0.435  0.9999
##  6/21/2019 - 7/17/2019  0.21605 0.186 480  1.161  0.9424
##  6/21/2019 - 7/3/2019  -0.09874 0.169 479 -0.583  0.9991
##  6/21/2019 - 8/14/2019  0.32976 0.162 480  2.037  0.4579
##  6/21/2019 - 8/21/2019  0.33363 0.169 478  1.969  0.5043
##  6/21/2019 - 9/18/2019  0.27199 0.155 480  1.753  0.6520
##  6/21/2019 - 9/4/2019  -0.26641 0.182 482 -1.464  0.8261
##  6/24/2019 - 7/17/2019  0.14880 0.173 479  0.858  0.9894
##  6/24/2019 - 7/3/2019  -0.16600 0.161 481 -1.033  0.9692
##  6/24/2019 - 8/14/2019  0.26250 0.151 479  1.734  0.6649
##  6/24/2019 - 8/21/2019  0.26637 0.164 482  1.619  0.7385
##  6/24/2019 - 9/18/2019  0.20474 0.149 479  1.378  0.8670
```

```
##  6/24/2019 - 9/4/2019  -0.33366 0.166 479 -2.013  0.4744
##  7/17/2019 - 7/3/2019  -0.31480 0.112 471 -2.807  0.0958
##  7/17/2019 - 8/14/2019  0.11370 0.106 470  1.074  0.9619
##  7/17/2019 - 8/21/2019  0.11757 0.135 482  0.874  0.9882
##  7/17/2019 - 9/18/2019  0.05594 0.154 478  0.363  1.0000
##  7/17/2019 - 9/4/2019  -0.48246 0.131 478 -3.693  0.0060
##  7/3/2019 - 8/14/2019   0.42850 0.108 471  3.957  0.0022
##  7/3/2019 - 8/21/2019   0.43237 0.133 481  3.246  0.0273
##  7/3/2019 - 9/18/2019   0.37074 0.145 480  2.551  0.1771
##  7/3/2019 - 9/4/2019   -0.16766 0.133 480 -1.258  0.9137
##  8/14/2019 - 8/21/2019  0.00387 0.126 481  0.031  1.0000
##  8/14/2019 - 9/18/2019 -0.05776 0.137 479 -0.422  0.9999
##  8/14/2019 - 9/4/2019  -0.59616 0.126 479 -4.744  0.0001
##  8/21/2019 - 9/18/2019 -0.06163 0.152 481 -0.406  0.9999
##  8/21/2019 - 9/4/2019  -0.60004 0.150 478 -3.990  0.0019
##  9/18/2019 - 9/4/2019  -0.53840 0.158 482 -3.412  0.0160
##
## Results are averaged over the levels of: Treatment, Sex, Time
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 8 estima
tes
```

```
EMSurfTotal <- emmeans(FinalModel, pairwise ~ AvgSurfaceTempTotal)
```

```
## NOTE: A nesting structure was detected in the fitted model:
##     Sex %in% Time
```

```
EMSurfTotal
```

```
## $emmeans
##  AvgSurfaceTempTotal emmean     SE   df lower.CL upper.CL
##                 36.7  0.438 0.0614 15.6    0.307    0.568
##
## Results are averaged over the levels of: Date, Treatment, Sex, Time
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast  estimate SE df z.ratio p.value
##  (nothing)   nonEst NA NA NA      NA
##
## Results are averaged over the levels of: Date, Treatment, Sex, Time
## Degrees-of-freedom method: kenward-roger
```

```
summary(FinalModel, ddf = "Kenward-Roger")
```

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger's method
[
## lmerModLmerTest]
## Formula: PercentOMBTotal ~ (1 | Block) + AvgSurfaceTempTotal + Date
+
##     Treatment + Time + Date:Time + Time:Sex
##    Data: FullData
##
## REML criterion at convergence: 1122.3
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.5702 -0.4631 -0.0834  0.2869  5.9083
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  Block    (Intercept) 0.03813  0.1953
##  Residual             0.47377  0.6883
## Number of obs: 511, groups:  Block, 16
##
## Fixed effects:
##                       Estimate Std. Error         df t value Pr(>|t
|)
## (Intercept)          -0.911569   0.254178 323.923176  -3.586 0.0003
87 ***
## AvgSurfaceTempTotal   0.041423   0.006648 478.951123   6.231 1.02e-
09 ***
## Date6/24/2019        -0.032881   0.257342 479.975794  -0.128 0.8983
82
## Date7/17/2019        -0.509709   0.238824 475.143184  -2.134 0.0333
34 *
## Date7/3/2019         -0.413130   0.261023 474.204292  -1.583 0.1141
48
## Date8/14/2019        -0.328249   0.225068 474.175056  -1.458 0.1453
82
## Date8/21/2019        -0.473909   0.257682 472.766815  -1.839 0.0665
24 .
## Date9/18/2019        -0.208951   0.251245 478.498535  -0.832 0.4060
15
## Date9/4/2019         -0.187704   0.259014 478.246443  -0.725 0.4689
97
## Treatment2            0.074920   0.060946 468.109040   1.229 0.2195
85
## TimeB                -0.339056   0.287053 468.798312  -1.181 0.2381
37
## TimeC                 0.133288   0.282284 468.834020   0.472 0.6370
```

```
21
## Date6/24/2019:TimeB   0.114872   0.354095 468.055851   0.324 0.7457
74
## Date7/17/2019:TimeB   0.208016   0.305429 468.292652   0.681 0.4961
69
## Date7/3/2019:TimeB    0.016509   0.326496 470.375115   0.051 0.9596
94
## Date8/14/2019:TimeB  -0.137532   0.307567 468.634990  -0.447 0.6549
66
## Date8/21/2019:TimeB  -0.091444   0.349078 468.155619  -0.262 0.7934
68
## Date9/18/2019:TimeB  -0.124253   0.345571 468.026368  -0.360 0.7193
39
## Date9/4/2019:TimeB    0.197359   0.362921 468.631395   0.544 0.5868
32
## Date6/24/2019:TimeC  -0.217990   0.351363 468.096495  -0.620 0.5352
88
## Date7/17/2019:TimeC   0.672949   0.304924 468.593363   2.207 0.0278
03 *
## Date7/3/2019:TimeC    1.519110   0.323125 470.627175   4.701 3.40e-
06 ***
## Date8/14/2019:TimeC   0.133009   0.304644 468.743642   0.437 0.6625
99
## Date8/21/2019:TimeC   0.512287   0.344201 468.076450   1.488 0.1373
35
## Date9/18/2019:TimeC  -0.064874   0.342312 468.072285  -0.190 0.8497
69
## Date9/4/2019:TimeC    1.164976   0.361069 468.707652   3.226 0.0013
41 **
## TimeA:SexStr          0.106870   0.156300  42.500432   0.684 0.4978
44
## TimeB:SexStr          0.138299   0.150713  37.010209   0.918 0.3647
52
## TimeC:SexStr         -0.438045   0.149373  38.109811  -2.933 0.0056
61 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

##
## Correlation matrix not shown by default, as p = 29 > 12.
## Use print(x, correlation=TRUE)  or
##     vcov(x)        if you need it

cor.test(FullData$PercentOMBTotal, FullData$AvgHideTempTotal)
```

```
##
##  Pearson's product-moment correlation
##
## data:  FullData$PercentOMBTotal and FullData$AvgHideTempTotal
## t = 11.131, df = 509, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.3698976 0.5096251
## sample estimates:
##       cor
## 0.4424426
```

```
cor.test(FullData$PercentOMBTotal, FullData$AvgSurfaceTempTotal)
```

```
##
##  Pearson's product-moment correlation
##
## data:  FullData$PercentOMBTotal and FullData$AvgSurfaceTempTotal
## t = 11.721, df = 509, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.3898756 0.5267035
## sample estimates:
##       cor
## 0.4610254
```

```
cor.test(FullData$PercentOMBTotal, FullData$YesterdayAvgTHI)
```

```
##
##  Pearson's product-moment correlation
##
## data:  FullData$PercentOMBTotal and FullData$YesterdayAvgTHI
## t = 4.1038, df = 510, p-value = 4.732e-05
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.0935845 0.2613967
## sample estimates:
##       cor
## 0.1787906
```

```
cor.test(FullData$PercentOMBTotal, FullData$YesterdayLowTHI)
```

```
##
##  Pearson's product-moment correlation
##
## data:  FullData$PercentOMBTotal and FullData$YesterdayLowTHI
## t = 4.0089, df = 510, p-value = 7.012e-05
```

```
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.08948272 0.25753870
## sample estimates:
##       cor
## 0.1747834
```

```
cor.test(FullData$PercentOMBTotal, FullData$YesterdayHighTHI)
```

```
##
##  Pearson's product-moment correlation
##
## data:  FullData$PercentOMBTotal and FullData$YesterdayHighTHI
## t = 2.7264, df = 510, p-value = 0.006624
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.03354844 0.20438914
## sample estimates:
##      cor
## 0.119856
```

```
cor.test(FullData$PercentOMBTotal, FullData$AvgTHI)
```

```
##
##  Pearson's product-moment correlation
##
## data:  FullData$PercentOMBTotal and FullData$AvgTHI
## t = 6.7411, df = 510, p-value = 4.265e-11
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.2044408 0.3636715
## sample estimates:
##       cor
## 0.2860295
```

```
cor.test(FullData$PercentOMBTotal, FullData$HighTHI)
```

```
##
##  Pearson's product-moment correlation
##
## data:  FullData$PercentOMBTotal and FullData$HighTHI
## t = 6.5199, df = 510, p-value = 1.695e-10
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.1954172 0.3554874
## sample estimates:
```

```
##       cor
## 0.277376
```

```
cor.test(FullData$PercentOMBTotal, FullData$LowTHI)
```

```
##
##  Pearson's product-moment correlation
##
## data:  FullData$PercentOMBTotal and FullData$LowTHI
## t = 5.5785, df = 510, p-value = 3.941e-08
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.1564083 0.3198235
## sample estimates:
##       cor
## 0.2398139
```

```
par(mfrow = c(2,2))
plot(FinalModel)
```