# EXPERIMENTS TOWARDS A GREATER UNDERSTANDING OF THE LIVER ABSCESS COMPLEX IN FED BEEF

By

RYAN TRAVIS HERRICK

B.S., California Polytechnic State University at San Luis Obispo, 2011

M.Sc., University of Illinois at Urbana-Champaign, 2014

A Dissertation

Submitted in Partial Fulfilment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

Department of Agricultural Sciences

College of Agriculture and Natural Sciences

WEST TEXAS A&M UNIVERSITY

Canyon, TX

January 2018

## ABSTRACT

Four trials (national liver audit, a multiple response feedlot questionnaire, association of liver abscesses to carcass trim and quality, and association of blood chemistry variables and liver abscess outcome) were conducted to examine national liver abscess incidence, determine conditions at the feedyard that may affect these outcomes, quantify trim losses and carcass characteristics associated with liver abscesses, and identify blood chemistry variables that may be associated with liver abscess incidence in fed Holstein steers. The first trial involved observational liver audits that occurred at 7 fed beef (n = 130,845 livers evaluated) and 4 cull cow (n = 30,646 livers evaluated) processing facilities. Processing facilities were selected to target the greatest frequency of Holsteins harvested per region and were audited for one week. At each processing facility, 30 (10 A-, 10 A, 10 A+) intact liver abscess samples were collected and cultured for *Fusobacterium necrophorum*, *Trueperella pyogenes*, and *Salmonella enterica*. Average liver abscess incidence was 20.3% for cattle harvested at fed beef processing facilities; processors in the Pacific Northwest had the greatest ($P < 0.01$) abscess incidence rate (33.8%) whereas those in the Northeast had the fewest liver abscesses (10.0%). Average liver abscess incidence was 17.6% for cull beef processing facilities. Within cattle type, fed Holsteins had greater ($P < 0.01$) abscess incidence rates (25.0%) than fed beef steers (18.2%) or heifers (19.1%). Cull dairy cows, cull bulls, and cull range cows had total abscess incidence rates (19.3%, 19.8%, and 16.7%, respectively)

similar to fed steers and fed heifers. *Fusobacterium necrophorum* subsp. *necrophorum* was present in 79.9% of samples collected from fed beef processors and 76.9% of samples from cull beef processors, whereas *Trueperella pyogenes* was present in 14.8 of samples from fed beef processors and 8.8% of samples from cull beef processors. *Salmonella enterica* was present in 27.5% of abscess samples collected from fed beef processors and 16.5% of samples from cull cow processors. *Fusobacterium necrophorum*, regardless of subspecies, was present at every processing facility; whereas *S. enterica* tended to occur in processing facilities in warm and dry climates and *T. pyogenes* tended to occur in processing facilities in colder and wetter climates.

In order to associate conditions at the feedyard with abscess outcomes observed at the processing facility, the second trial utilized survey data that were assimilated to corresponding individual lots of fed cattle. Thirteen nutritionists participated in the survey representing 43,255 animals, in 321 lots, from 32 individual feedlots throughout the major cattle feeding areas of the U.S. The survey contained 57 questions divided into information categories that included: general (n = 13); health (n = 6); source and background (n = 7); growth promoting technologies (n = 3); diet (n = 10); feeding management (n = 6); cattle management (n = 8); and liver abscess control technologies (n = 4). Cattle type (beef or dairy; Adj. $R^2 = 0.13$), diet dry matter percentage (Adj. $R^2 = 0.11$), G:F (Adj. $R^2 = 0.11$), mortality (Adj. $R^2 = 0.07$), and tylosin supplementation (yes or no; Adj. $R^2 = 0.06$) all yielded univariate equations capable of predicting total abscess percentage. Using the maximum likelihood method in the CALIS Procedure of SAS, the full model to predict total liver abscess incidence was: total liver abscess percentage = 113.5 – (217.39 × G:F) – (18.48 × cattle type [1 = native beef; 0 = Holstein]) – (46.35 ×

Diet DM, %) – (14.39 × tylosin supplementation [1 = yes; 0 = no]), and accounted for 34% of the variation to predict total liver abscess incidence.

In the third trial, fed Holsteins were tracked through two commercial processing facilities, one in the High Plains region (n = 1,073) and one in the Central Plains region (n = 1,070). Liver abscesses were visually assessed and scored according to a modified scoring system based on the Elanco Liver Check Service; simultaneously, lungs were manually palpated to assess degree of consolidation and fibrin tag formation and its association to liver abnormality. Finally, carcass trim was weighed from carcasses moved off-line for zero tolerance trimming. Carcass and viscera values were assigned using USDA market reports and adjusted based on viscera condemnations along with premiums and discounts for quality and yield outcomes. Cattle exhibited liver abnormality rates of A- = 3.73%, A = 7.28%, A+ = 7.56%, A+Adhesion (A+AD) = 17.50%, A+Open (A+OP) = 4.39%, A+Adhesion/Open (A+AD/OP) = 3.92%, and Contamination = 6.16%, with 49.46% of all livers being edible. Hot carcass weight was reduced ($P < 0.01$; -25.1 kg, -6.6%) in carcasses that had an A+AD/OP liver abscess as compared to carcasses with edible livers. Carcasses with A+AD or A+OP liver scores had increased ($P < 0.01$) carcass trim (3.92 and 3.39 kg, respectively), when compared to carcasses with edible livers (0.38 kg). A greater degree ($P < 0.001$) of lung consolidation was observed in carcasses with A+AD and A+AD/OP liver scores than carcasses with edible livers. Livers with abscesses yielded an average loss to the beef processor of $3.25 per condemned liver ($3.89/edible liver vs $0.64/condemned liver). Given the 44.38% incidence of liver abnormalities, and the 25.81% incidence of open and adhered abscesses observed, fed beef processors lose an estimated $21.8 million annually in

condemned viscera due to liver abnormalities in fed Holsteins. Although not different ($P$ = 0.27), carcasses with A+AD liver outcomes were worth 4.9% less (-$73.93/carcass) than their edible counterparts, and carcasses with A+OP livers were worth 4.6% less (-$69.65/carcass) than carcasses with edible livers.

For the final trial, blood samples for complete blood count (CBC) and sera were collected from fed Holsteins (n = 153) approximately 30 s after exsanguination; liver abscesses were visually assessed after evisceration. Greater ($P$ = 0.03) hot carcass weights were observed from carcasses with minor (387.1 kg) abscesses, which were 5.41% heavier than carcasses with major abscesses (366.2 kg) but not different ($P$ > 0.10) from carcasses with no liver abnormality (381.8 kg). Of the CBC variables analyzed, platelet counts were increased ($P$ = 0.02) whereas hemoglobin and hematocrit values ($P$ < 0.01) were decreased in carcasses with major abscesses (214 K/uL, 12.9 g/dL, 41.0%) when compared to carcasses without abscesses (137 K/uL, 13.8 g/dL, 43.4%). Serum analysis indicated that carcasses with major abscesses had increased ($P$ < 0.01) globulin (5.8 g/dL) concomitant with decreased ($P \leq 0.03$) sodium (141 mmol/L), albumin (2.8 g/dL), alanine aminotransferase (19 u/L) and aspartate aminotransferase (89 u/L) when compared to carcasses with no liver abscess (5.2 g/dL; 142 mmol/L; 3.0 g/dL; 21 u/L; 97 u/L, respectively). Logistic regressions equations using CBC variables (platelets and hemoglobin) or serum variables (aspartate aminotransferase and total protein), yielded equations that were able to explain 72% (c-statistic = 0.72) and 69% (c-statistic = 0.69) of the area under the curve, respectively, for predicting major liver abscesses.

In conclusion, total visceral losses ($/animal) did not differ by region ($P$ = 0.40) or cattle type ($P$ = 0.85), conservative estimates indicate that liver abscesses and other

liver abnormalities cost the beef industry approximately $60 million annually in viscera losses. While predictive equations revealed that dietary factors can contribute to, and likely exacerbated liver abscess incidence, additional factors, beyond dietary measures, contributed to the multifactorial disease. Models generated were unable to explain more than 40% of the variation involved in predicting either total or major abscess incidence, indicating that liver abscesses are likely a multifactorial disease influenced beyond dietary factors alone. Holsteins are an important segment of the beef industry and are disproportionally affected by liver abscesses, when compared to traditional (non-Holstein) beef. Unless the liver abscess issue is addressed by the fed beef industry, Holsteins will continue to incur greater financial risk to both cattle feeders and processors. Finally, cattle with major liver abscesses undergo metabolic differentiation which can be detected in whole blood and serum at slaughter and identifying blood parameters that could be used to diagnose liver abscesses, during the live phase, may have useful implications for cattle welfare and feedlot management.

**ACKNOWLEDGEMENTS**

I would like to express my sincere gratitude to Dr. Ty Lawrence, my advisor, for taking a chance on a kid from California and for the constant mentorship he has provided throughout my time of study. I would also like to express my appreciation to the members of my committee: Drs. Casey Maxwell, Trent McEvers, T.G. Nagaraja, Bridget Guerrero, and Tim Steffens. Your tutelage and guidance have been invaluable to me and without you this dissertation would not have been possible.

I would also like to extend my greatest thanks to the staff of the Beef Carcass Research Center. Countless hours of work and assistance were provided by the graduate and undergraduate students including: Tyler Wells, Fidel Burciaga, Jessica Sperber, Forest Francis, Taylor Buckley, Avery Jones, Coy Mercer, Oscar Rodriguez, Andreah Meador, Erin Watt, Ryan Bone, Olga Rodriguez, Adrian Lunsford, Adam Lunsford, Quent Roach, Lee-Anne Walter, Angela Schmitz, Daniel Guadian, Taylor Loyd, Ashley Davis, Jordan Doetker, Kaitlin Doughty, Becca Grimes, and John Mitchell.

I would like to express sincere appreciation to my friends, Shelby Roberts, Hunter Galloway, Tyler Harper, Carson Rogers, Trenton Jones, and Ryan Dahl, for providing assistance, and words of encouragement over the last three and a half years. To my family, you have educated me in agriculture, determination, integrity, and persistence; blessed me with a passion for learning and encouraged my desire to pursue a career in agriculture. I cannot thank you enough for all that you have done for me. Last, but

certainly not least, I would like to thank Kaylee. You have been my rock, my sounding board, and my greatest supporter. This dissertation is for you.

## TABLE OF CONTENTS

## LIST OF TABLES

### Chapter 3



### Chapter 4



### Chapter 5

**Chapter 6**

## LIST OF FIGURES

# CHAPTER 1:

# INTRODUCTION

Liver abscesses can form in all types of cattle, including range cattle, at any time in their life cycle. While the formation of an abscess can be detrimental to the performance of any animal, liver abscesses have the greatest economic impact on grain-fed cattle. The current dogma for the pathogenesis of liver abscesses is that abscesses form as a result of high starch, or low roughage, intensive feeding programs. A high starch or low roughage diet can lead to rumenitis from lactic acidosis (Brent, 1976). Rumenitis allows for the creation of microscopic pores in the wall of the rumen from which bacteria can escape the rumen and become sequestered in the liver; with the major pathogens of concern believed to be *Fusobacterium necrophorum*, and *Trueperella pyogenes* (Nagaraja and Chengappa, 1998). Once the bacteria are sequestered in the liver they can form a puss filled sac resulting in an abscess.

After an abscess forms within the liver, metabolism and performance of the animal will be shifted due to damaged hepatocytes, and overall metabolic efficiency can be greatly diminished. Cattle with liver abscesses have been reported having a 14% reduction in ADG and up to a 13% reduction in G:F (Brink et al., 1990). Reductions in ADG and G:F due to liver abscesses can result in significant reductions (up to 3.6%) in HCW (Davis et al., 2007), and are associated with a decrease in overall carcass quality

(Brown and Lawrence, 2010). Carcasses with severe liver abscesses (A+ or greater) have been reported as having decreased marbling scores concomitant with decreased *Longissimus* muscle area (Montgomery, 1985; Brown and Lawrence, 2010). Decreases in performance and decreases in carcass quality as a consequence of liver abscesses, results in cattle that are less efficient and carcasses that are less valuable than non-abscessed cattle.

Liver abscess incidence rates can vary greatly in specific groups of cattle, from as little as 1% to as high as 90%; however, incidence rates of liver abscesses generally range from 12-30% (Brink et al., 1990; Brown and Lawrence, 2010). Because liver abscesses are generally attributed to feeding practice, diet is thought to be a major factor involved with incidence. Typically, incidence and severity of abscesses increase as roughage inclusion in the diet decreases (Gill et al., 1979; Zinn and Plascencia, 1996). However, other studies reported no differences in liver abscess incidence in diets with 0% roughage vs. 3 to 15% roughage supplied throughout the finishing phase (Kreikemeier et al.,1990; Stock et al., 1990). The variability in outcome related to roughage inclusion suggests that apart from diet, feeding management may have an effect on liver abscess incidence rates.

Management of liver abscesses has mostly been limited to oral administration of feed grade antibiotics. According to the U.S. Feed Additive Compendium, six antibiotics are approved for the prevention of liver abscesses in cattle: bacitracin methylene disalicylate, chlortetracycline, neomycin sulfate in conjunction with oxytetracycline, oxytetracycline, tylosin, and virginiamycin (Lundeen, 2013). However, the new FDA Veterinary Feed Directive (VFD) will require these drugs to have veterinarian prescriptions before use (US CFR. 2015. Title 21, Parts 514 and 558), which may deter

some feedlots from using these products to prevent liver abscesses. Current public perceptions of feed grade antibiotics in conjunction with increased FDA regulations will likely necessitate shifts in cattle management practices to reduce liver abscess incidence while concurrently decreasing antibiotic usage. These management shifts will require effort by all sectors of the beef industry; however most of the burden will fall upon feedlots, since the feedlot is the final staging ground for most slaughtered cattle in the U.S. and the greatest impact can be felt there. Regardless of why the change is made, management decisions made by beef feeders, and influenced by beef packers, should be aided by correlations and predictive models based upon conditions occurring at the feedlot along with outcomes seen at the processing facility in order to provide the industry with the best decision-making tools as possible.

# CHAPTER 2:

# REVIEW OF THE LITERATURE

## 2.1 ETIOLOGIC AGENTS

Liver abscesses are poly-microbial infections, which are sequestered in the liver, and if able to putrefy, are walled off by scar tissue, eventually forming an abscess (Amachawadi and Nagaraja, 2016). Because liver abscesses are caused by bacteria, the bacterial flora of liver abscesses, both aerobic and anaerobic, has been investigated extensively (Lechtenberg et al., 1988; Nagaraja et al., 1996a; Tan et al., 1996); most studies conclude that *Fusobacterium necrophorum* is the primary etiologic agent. This organism is also the primary pathogen of concern involved in footrot, foot abscesses in cattle, and necrotic laryngitis (Tan et al., 1996). Cultured liver abscesses have been reported as having an incidence of *F. necrophorum* ranging from 81-100% (Nagaraja and Chengappa, 1998). *Fusobacterium necrophorum* has been isolated as a single pathogen from an abscess (Lechtenberg et al., 1988), but often it has been associated with other anaerobic and facultative bacteria (Scanlan and Hathcock, 1983). Other bacteria isolated from liver abscesses include, *Trueperella pyogenes*, previously named *Actinomyces pyogenes* (Lechtenberg et al., 1988), *Clostridium* spp., *Pasteurella* spp. (Simon and Stovell, 1971), *Staphylococcus* spp. (Lechtenberg et al., 1988), *Streptococcus* spp. (Simon and Stovell, 1971; Lechtenberg et al., 1988), and *Salmonella enterica*

(Amachawadi and Nagaraja, 2015). Typically, *T. pyogenes* is the second most frequently isolated pathogen from liver abscesses (Lechtenberg et al., 1988; Nagaraja and Chengappa, 1998), and evidence suggests a pathogenic synergy exists between *F. necrophorum* and *T. pyogenes* (Takeuchi et al., 1983).

### *2.1.1 Fusobacterium necrophorum*

*Fusobacterium necrophorum* is an anaerobic, Gram-negative, nonmotile, nonsporulating, rod-shaped bacterium (Langworth, 1977). Typically, this species does not ferment carbohydrates, rather its major energy substrate is lactic acid, which it ferments to produce acetate, butyrate, and propionate (Lechtenberg et al., 1988). *Fusobacterium necrophorum* has historically been classified into 4 biotypes/biovars: A, B, AB, and C (Langworth, 1977). Biotypes A and B, are the most common isolated from liver abscesses, and have been classified as subspecies *necrophorum* (biotype A) and subspecies *funduliforme* (biotype B; Shinjo et al., 1991). Biotype AB is generally isolated from the foot lesions of cattle and sheep (Emery et al., 1985), and its features are midway those of biotypes A and B (Berg and Scanlan, 1982). Biotype C has a structure similar to biotypes A and B, but is non-pathogenic in mice and does not produce similar virulence factors, and has therefore been reclassified as a new species, *Fusobacterium pseudonecrophorum* (Shinjo et al., 1990). Although *F. necrophorum* is a causative agent for liver abscesses, footrot, and calf diphtheria, literature does not exist that correlates the incidence of one disease to another (i.e. increased incidence of liver abscesses with increased incidence of footrot). The fact that these diseases are all caused by the same organism is mentioned, but no further associations have been made. In regards to liver abscesses, biotypes A and B are often the organisms of concern because biotypes A and

B are often isolated from liver abscesses due to the toxins and other virulence factors produced by these biotypes that facilitate their survival and promote abscess formation.

Several toxins have been identified as virulence factors in the pathogenesis of *F. necrophorum* infections that aid in the bacterium's survival within the host (Tan et al., 1996; Nagaraja and Chengappa, 1998). Components of the virulence factors include: endotoxic lipopolysaccharide, hemolysin, leukotoxin, and a number of extracellular enzymes including proteases (Tan et al., 1994a; Tan et al., 1996). Of these virulence factors, endotoxic lipopolysaccharide and leukotoxin are believed to be the major virulence factors involved in fusobacterial infection (Tan et al., 1996). The role of leukotoxin on the potency of *F. necrophorum* infection has been demonstrated through correlations between toxin production and abscess formation in laboratory animals (Emery et al., 1986). It is the difference in production of these virulence factors that attributes to why biotypes A and B are most commonly isolated from liver abscesses. Additionally, subspecies *necrophorum* has been reported to produce more leukotoxin than subspecies *funduliforme* (Tan et al., 1992), which could explain why subspecies *necrophorum* is isolated from 71 to 95% of liver abscesses while subspecies *funduliforme* is isolated at a rate of 5 to 29% (Lechtenberg et al., 1988).

*Fusobacterium necrophorum* is a normal resident of the gastrointestinal tract of mammals (Langworth, 1977), and has been isolated from the ruminal contents of cattle fed various diets (Berg and Scalan, 1982; Tan et al., 1994a). Generally, the concentration of *F. necrophorum* in the rumen is in the range of $10^5$ to $10^6$/g of rumen contents, but this concentration can be affected by diet (Nagaraja and Chengappa, 1998). Tan et al. (1994c) reported that the concentration of *F. necrophorum* increased when diet was

changed from roughage (7 x $10^5$/g) to high-grain (3 – 7 x $10^6$/g). Unlike most bacteria in the rumen, *F. necrophorum* uses lactate as its major energy substrate and not sugar; therefore, the increase in concentration due to dietary changes is most likely a result of increased lactate production. This means that as the pH of the rumen decreases, concentrations of the bacterial agent that causes liver abscesses will increase at the same time the rumen's structural integrity is decreasing.

### *2.1.2 Trueperella pyogenes*

*Trueperella pyogenes* is the second most common bacterium isolated from liver abscesses. *Trueperella pyogenes* is a Gram-positive, rod-shaped, and facultatively-anaerobic organism (Amachawadi and Nagaraja, 2016). This organism is often cultured from mucus membranes and the upper respiratory and digestive tracts of animals (Biberstein, 1990). Additionally, *T. pyogenes*, when present, is more commonly cultured from the rumen wall than rumen contents (Narayanan et al., 1998), which would help explain how *T. pyogenes* ends up in liver abscesses. Aiding in the co-existence of *T. pyogenes* and *F. necrophorum* within liver abscesses is a nutritional and pathogenic synergy that exists between the two species (Tadepalli et al., 2009). *Trueperella pyogenes* utilizes oxygen to create anaerobic conditions, which creates a more favorable environment for *F. necrophorum*. Furthermore, the waste product of *T. pyogenes* is lactic acid, which is the primary energy substrate of *F. necrophorum.*

Occasionally, *T. pyogenes* is the only organism isolated from liver abscesses (Nagaraja and Lechtenberg, 2007). When steers were inoculated intraportally with *T. pyogenes* in a pure culture, liver abscesses did not develop (Lechtenberg et al., 1993). However, when *T. pyogenes* was administered with *F. necrophorum*, liver abscesses

formed; indicating that it is possibly the leukotoxin provided by *F. necrophorum* that allows *T. pyogenes* to survive in the liver and aid in abscess formation. The primary virulence factor of *T. pyogenes* is a hemolysin that is cytotoxic to polymorphonuclear leukocytes (Billington et al., 1997). Along with hemolysin, other virulence factors of *T. pyogenes* include proteases, DNases, and extracellular matrix binding protein (Jost and Billington, 2005). All of these factors aid in the adherence, colonization and pathogenicity of *T. pyogenes.*

### *2.1.3 Salmonella enterica*

Anaerobic *Salmonella enterica* has also been isolated from liver abscess samples collected from Holstein steers, with all isolates belonging to the Lubbock serotype (Amachawadi and Nagaraja, 2015). *Salmonella enterica* has been isolated from human liver abscesses (Qu et al., 2013), but this was the first reported isolate from beef liver abscesses. Until recently, *Salmonella* Lubbock was the predominant serotype isolated from beef liver abscess samples (Amachawadi and Nagaraja, 2015). However, when liver abscesses were collected from cattle that originated from 22 feedlots in the Central Plains, Desert Southwest, and High Plains regions that were slaughtered in 6 abattoirs throughout Arizona, California, Colorado, and Kansas, researchers isolated 5 unique serotypes of *Salmonella* (Lubbock, Agona, Cerro, Give, and Muenster; Amachawadi, 2017). The greater diversity observed in the later experiment is likely a result of greater diversity in the origins of the cattle tested. The initial experiment that isolated *S. enterica* from beef liver abscess samples only sampled cattle from the same source, while the later experiment had multiple sources which spanned several geographic regions. However, at this time it is not known if *S. enterica* is one of the etiologic agents of liver abscesses or if

it entered after the abscess was initiated by *F. necrophorum*. *Salmonella* present in the gut could cross the intestinal lumen, enter portal circulation, and become sequestered in the liver to initiate infection. Entry though the gut epithelium could be enabled through inflammation or ruminal acidosis. *Salmonella* has been isolated in the lymph nodes of beef cattle at the time of slaughter (Arthur et al., 2008), indicating the possibility of it being systemic to some beef cattle.

*Salmonella* possess several virulence factors that aid in their survival. While most bacterium actively attempt to avoid internalization/phagocytosis by leukoctyes, *Salmonella* can actively infect phagocytic and non-phagocytic cells (Ibarra and Steele-Mortimer, 2009). Once internalized within a target cell, *Salmonella* survive within a modified phagosome (*Salmonella* – containing vacuole; Ibarra and Steele-Mortimer, 2009). This *Salmonella* – containing vacuole survives destruction by interaction with the cell's endocytic pathway, allowing the vacuole to avoid destruction by lysozymes while allowing the vacuole to intercept nutrients from the cell and direct them towards *Salmonella* growth.

Along with its internalization survival mechanisms, *Salmonella* have several other virulence factors that make it a formidable pathogen. A primary defensive mechanism of most host cells is the production of reactive oxygen species. To circumvent destruction by reactive oxygen species, Salmonella uses a superoxide dismutase to protect itself from free radicals (Pacello et al., 2008). Superoxide dismutase is an enzyme that neutralizes free radicals by disproportionation of the superoxide into either molecular oxygen or hydrogen peroxide (McCord et al., 1969). The conversion of superoxide into hydrogen

peroxide and molecular oxygen reduces the reactivity of superoxide, thereby decreasing the damage these free radicals can inflict upon *Salmonella*.

Within a eukaryotic host, iron is the most limiting nutrient to most bacterial cells (Schaible and Kaufmann, 2004), and *Salmonella* is no exception (Ibarra and Steele-Mortimer, 2009). Free iron within the body acts as a free radical that can catalyze the oxidation of lipids, therefore free iron is limited and is often bound to iron-binding proteins. To combat the iron limitations within the host, *Salmonella* produces siderophores (iron chelating proteins) to increase iron sequestration within the host (Müller et al., 2009). Iron-binding proteins produced by bacteria compete with the host's iron-binding proteins to sequester free iron within the cells, which can increase the survivability of *Salmonella*. This mechanism is also utilized in *Salmonella* vaccines. Iron restricted *Salmonella* are utilized in some vaccines, in order to prevent the growth of *Salmonella* in the host (Woodward et al., 2002), which if unchecked could result in serious infection and disease

*Salmonella* are facultative intracellular pathogens that are able to quickly adapt and survive in diverse environments. Therefore, it is possible that *Salmonella* is a causative agent or contributing factor in this disease, but further research is needed to determine the precise role *Salmonella* plays in the liver abscess complex.

### *2.1.4 Klebsiella pneumoniae*

Another pathogen that may contribute to the liver abscess complex is *Klebsiella pneumoniae*. *Klebsiella pneumoniae* is a Gram-negative, facultative anaerobe that has been isolated from the liver abscesses of beef cattle (Amachawadi and Nagaraja, 2016). Currently, no data exists to suggest *Klebsiella pneumoniae* is an etiologic agent involved

in the formation of liver abscesses in beef cattle, but it has been shown to result in pyogenic liver abscesses in humans. Pyogenic liver abscess is typically a polymicrobial disease resulting from intra-abdominal or biliary tract infection (Wang et al., 1998). Prior to the 1980s, the primary pathogen of concern was *Escherichia coli*; however, during the past few decades *Klebsiella pneumoniae* has emerged as the predominant causative agent behind pyogenic liver abscesses in Asia (Jiang and Liu, 2013). *Klebsiella* spp. are ubiquitous in nature, but its primary habitats are in the environment (surface water, sewage, soil, and plants) and mucosal surfaces of mammals (Podschun and Ullmann, 1998).

Vital to the survival and virulence of *Klebsiella* is the production of capsular acidic polysaccharides (Podschun and Ullmann, 1998). These capsular acidic polysaccharides coat the outside of *Klebsiella* in multiple layers, preventing the bacterium from being phagocytized by leukocytes or from being killed by bacterial serum factors (Podschun and Ullmann, 1998). Furthermore, injection of large doses of these capsular polysaccharides into mice has been shown to cause some immunological paralysis (Batshon et al., 1963). When mice were injected with *Klebsiella* capsular polysaccharides, a dose-dependent decrease in the production of antibodies to the specific capsular antigen occurred; meaning that as concentration of capsular polysaccharide increased, antibody response decreased. While it is unknown at this time if *Klebsiella* infection could cause a liver abscess in beef cattle, its ability to survive in anaerobic and aerobic environments as well as its unique virulence factor could potentially be a contributing factor to the beef liver abscess complex and warrants further research.

## 2.2 PATHOGENESIS

The pathogenesis of liver abscesses has been researched since the 1940's when Smith (1944) observed a relationship between ulcerative lesions of the rumen and liver abscesses in feedlot cattle. The relationship between rumen ulcers and liver abscesses was again noted by Jensen et al. (1954a, b) when they reported strong correlations between liver abscesses and rumen pathology; coining the phrase "rumenitis – liver abscess complex". While Smith (1944) and Jensen et al (1954 a, b) reported strong associations between rumen pathology and liver abscess formation, Weiser et al. (1966) described no correlation between incidence of rumen lesions and liver abscesses. Since then the pathogenesis of liver abscesses has been extensively researched, and still no definite pathogenic mechanism exists (Nagaraja and Chengappa, 1998; Nagaraja and Lechtenberg, 2007). However, it is well accepted in the literature that ruminal lesions as a result of acidotic events are the predisposing factor for liver abscesses (Jensen et al., 1954b; Nagaraja and Chengappa, 1998; Nagaraja and Lechtenberg, 2007).

Rumenitis, as a result of acidosis, is typically associated with a sudden change to high-energy diets or other dietary insults such as change in feeding patterns, periods of fasting, and feeding little roughage (Elam, 1976). Damage to the rumen may be further intensified by foreign objects in the feed, sharp feed particles, or hair (Jensen et al, 1954c; Amachawadi and Nagaraja, 2016). A rumen damaged by acidity or by some foreign object becomes susceptible to invasion and colonization by *F. necrophorum*. After colonization, it is thought that *F. necrophorum* can then enter portal blood supply or cause an abscess in the rumen wall which can then shed emboli into circulation (Nagaraja

and Chengappa, 1998). Bacteria are filtered out of the blood supply by the liver where they can congregate and possibly lead to abscess formation.

The virulence factors of *F. necrophorum* are vital in its infiltration of the rumen and eventual colonization and infection of the liver. Proteases secreted by *F. necrophorum* aid in the degradation of the rumen wall, further increasing rumen permeability. Once in the liver *F. necrophorum* has to overcome an environment inhospitable to bacteria and other foreign microorganisms. The liver is a highly vascularized organ with a steady supply of leukocytes to phagocytize any foreign organisms. Therefore, in the liver, *F. necrophorum* has to overcome a high oxygen environment and phagocytes in order to survive and proliferate prior to abscess formation.

Aiding in its survival are *F. necrophorum's* own virulence factors and, if present, *T. pyogenes*. The primary virulence factor facilitating survival in the liver is leukotoxin, which may protect *F. necrophorum* from phagocytosis by leukocytes (Tan et al., 1996). Furthermore, the destruction of phagocytes releases oxygen metabolites, like hydrogen peroxide, that will further damage local tissues (Babior, 1984). *Fusobacterium necrophorum* is also capable of damaging erythrocytes (through hemolysin production), further decreasing oxygen transport and availability. Additionally, *T. pyogenes* aids in the survival of *F. necrophorum* by utilizing oxygen to create more favorable anaerobic conditions while also producing lactic acid, which is a major energy substrate of *F. necrophorum* (Tadepalli et al., 2009). All of these factors may contribute to the development of an anaerobic micro environment within the liver, conducive to the growth of anaerobic bacteria and ultimately the development of a liver abscess.

Although the pathogenesis is the same, not all abscesses are the same size or severity. Modulations in microbial virulence, environment, immune status and a host of other factors can contribute to more severe liver abscesses. Not all abscesses are created equally, liver abscesses are often classified by severity; because size and severity can affect overall economic impact, marketability, and performance characteristics of fed cattle. Liver abscesses are traditionally classified using either the Elanco Liver Check Service or a modified scoring system based on the criteria outlined by Elanco (✓ = no abscesses, A- = 1 or 2 small abscesses, A = 2 to 4 small active abscesses, A+ = 1 or more large active abscesses, A+ Adhesion (A+AD) = liver adhered to GI tract, A+ Open (A+OP) = open liver abscess, A+Adhesion/Open (A+AD/OP) = liver adhered to GI tract with an open abscess; adapted from Brown & Lawrence, 2010).

### *2.2.1 Portal vein circulation*

The pathogenesis of liver abscesses is believed to begin with ruminal acidosis resulting from high energy feeds and the escape of *F. necrophorum* through the rumen wall to the portal vein and then the liver (Smith, 1944; Nagaraja and Lechtenberg, 2007). However, it is possible that other routes of ingress may affect incidence. *Fusobacterium necrophorum* is ubiquitous to the gastrointestinal tract of ruminants (Langworth, 1977; Nagaraja et al., 2005), and tears or cuts in the epithelial lining of the gastrointestinal tract may provide a route for this bacterium to enter portal circulation. Therefore, while the rumen is the most likely route of *F. necrophorum* ingress into the ruminant, it is possible for *F. necrophorum* to infect the animal at any point through the gastrointestinal tract.

Essentially all blood from the abdominal viscera drains into the portal vein where it is filtered by the liver (Pasquini et al., 1997). The vast capillary network surrounding

the lower gastrointestinal tract that is vital for absorption of nutrients from the small intestine (Madara, 2011) also provides multiple routes for *F. necrophorum* to enter portal circulation. Upstream of the rumen, there is another capillary network which feeds oxygenated blood to the tongue and esophagus that eventually drains into the jugular vein (Pasquini et al., 1997). In the circulatory system, blood flows throughout the body where it can be filtered for impurities by the liver. Because the circulatory system is not a closed system, if *F. necrophorum* is able to enter circulation at any point throughout the gastrointestinal tract, eventually it will pass through or be sequestered in the liver where it may develop into a liver abscess.

## 2.3 DIAGNOSIS

Cattle that develop liver abscesses do not display any outward clinical signs, and abscesses are typically only detected at the time of slaughter (Nagaraja and Lechtenberg, 2007). This presents several challenges for producers, because liver abscesses will negatively impact carcass characteristics and their presence goes undetected until it is too late to be changed by either diet or medication. Because liver abscesses pose a serious financial risk to the beef cattle industry, several techniques have been tested to attempt to diagnose liver abscesses at the feedlot. One technology that has been tested in feedlot cattle is ultrasonography.

Ultrasonography (ultrasound) works by measuring rebounding high frequency sound waves that bounce back after coming into contact with various tissues (Houghton and Turlington, 1992). As density of the tissue changes, (i.e. bone vs. muscle) the reflectance of the sound waves change which can then be interpreted by the transducer

into an image that is projected onto a screen, thus allowing for a non-invasive view of the interior of an animal (Houghton and Turlington, 1992). Although this technology has been adopted in some feedlots, it has primarily been utilized to gauge finish, marbling and ribeye area; however, several research trials have used this technology to evaluate presence and development of liver abscesses (Lechtenberg and Nagaraja, 1991; Saginala et al., 1997; Braun, 2009).

When cattle were experimentally induced with *F. necrophorum* to test the efficacy of a possible vaccine, ultrasound was used to verify that all animals were abscess free at d 0 (Saginala et al., 1997). Although ultrasound was utilized for initial diagnosis, final determination of abscess status was performed via necropsy. Additionally, when ultrasonography was used to diagnose liver abscesses in Holstein cows, of the 5,519 animals evaluated only 18 (0.3%) were clinically diagnosed with liver abscesses (Dore et al., 2007). Dore et al. (2007) noted that their liver abscess prevalence was low compared to the 23.4% prevalence previously reported in culled Holstein cattle (Nagaraja et al., 1996b), and attributed the low discovery of abscesses via ultrasound to limitations of the technology.

Although helpful in imaging some internal tissues, one of the major limitations of using ultrasonography for diagnosis of liver abscesses is that the entire liver cannot be evaluated by ultrasound examination (Braun et al., 1995). Only one side of the liver is visible with ultrasonography and liver abscesses are not contained to only one side of the liver, abscesses can develop and appear anywhere within and upon the liver. Along with not being able to fully visualize the liver, other limitations of ultrasonography for the diagnosis of liver abscesses include lack of rumen fill causing the liver to lose contact

with body wall, lung tissue that may overshadow liver due to age, diet, and orientation in the chute, and excessive internal fat may distort proper image (Nagaraja and Lechtenberg, 2007). These limitations are why the use of ultrasound to diagnose and or monitor liver abscesses is rarely utilized outside of research settings or veterinary practices.

Because ultrasonography in the feedlot is expensive, has limitations, and is often impractical for normal feedlot processing schedules, other metrics have been evaluated to attempt to diagnose liver abscesses prior to slaughter. Several research trials have evaluated blood metabolites to determine if they are impacted by the presence of a liver abscesses. When cattle with diagnosed liver abscesses had their blood analyzed for complete blood counts and other serum biochemistry metabolites, the authors concluded that findings were characteristic of chronic inflammation (Dore et al., 2007). In cattle with experimentally induced liver abscesses, total leukocyte count increased post intra-portal *F. necrophorum* challenge, whereas serum albumin decreased; which the authors attributed to hepatic dysfunction (Lechtenberg and Nagaraja, 1991). These research trials have demonstrated that biomarkers in the blood are indicative of a liver abscesses; however little has been done with this information to develop a feedlot applicable device or test that can rapidly and accurately test for liver abscesses.

Although previous literature has expanded the knowledge of what changes occur in the blood of an abscessed animal, the data reported have not been adapted or applied in a manner to screen for or diagnose liver abscesses at the feedlot. Therefore, recent research was conducted to determine if analysis of blood metabolites could be used to diagnose liver abscesses in feedlot cattle. Macdonald et al. (2017) followed 29 beef bulls throughout the finishing phase and sampled blood from the cattle at 9 time-points prior to

slaughter and at slaughter hoping that cattle would develop abscesses and that blood metabolites analyzed would be indicative of liver abscess development. Even though the sample size was small (only 9 of the 29 animals developed liver abscesses) and data presented was averaged over the 56-day sampling period prior to slaughter, animals with liver abscesses had elevated plasma cortisol and aspartate aminotransferase concentrations concomitant with decreased albumin and cholesterol concentrations (Macdonald et al., 2017). Changes in these parameters indicate that liver abscesses impacted liver function while also acting as stressors to the animal. Although this article indicated that it may be possible to screen for liver abscesses during cattle processing at the feedlot, additional research is needed before this practice is used to make diagnoses or management decisions.

## 2.4 PREVENTION AND CONTROL

Historically, the prevention and control of liver abscesses has been achieved through the use of antimicrobial feed additives, vaccines, and nutritional management.

### *2.4.1 Antimicrobial feed additives*

The rumen is a fermentation vat which comprises an ecosystem that is conducive to microbial growth. Unfortunately, liver abscess are a microbial issue and the rumen provides a suitable environment in which the bacteria that cause liver abscesses can reside and propagate. This means that beef cattle can be constantly inoculated with the bacteria that cause liver abscesses because their physiology is dependent on a symbiotic relationship with microorganisms, and the animal is unable to select which microbes populate its rumen. Therefore, although other methods exist to prevent and control for

liver abscesses, the feedlot industry has depended primarily on the use of antimicrobial feed additives to minimize the incidence of liver abscesses in fed beef.

Because liver abscesses are caused by bacteria, the antimicrobial sensitivity of *F. necrophorum* has been extensively researched (Nagaraja and Chengappa, 1998). It has been reported that *F. necrophorum* is susceptible to macrolides, penicillins, and tetracyclines, while being resistant to aminoglycosides and ionophores (Baba et al., 1989; Lechtenberg and Nagaraja, 1989). Furthermore, the susceptibility and resistance of the two primary *F. necrophorum* subspecies to antibiotics are not different from each other (Lechtenberg and Nagaraja, 1989). Therefore, any antimicrobial feed additive that is effective in suppressing either subspecies will be effective against the other.

According to the Feed Additive Compendium, six antibiotics (bacitracin methylene disalicylate, chlortetracycline, neomycin sulfate in combination with oxytetracycline, oxytetracycline, tylosin, and virginiamycin) are approved for use in feed to reduce the incidence of liver abscesses in fed beef (Lundeen, 2013). These antibiotics vary in their ability to reduce the incidence of liver abscesses as well as in their ability to inhibit *F. necrophorum* and *T. pyogenes* (Nagaraja and Chengappa, 1998). Of the antimicrobial feed additives approved for reduction in incidence of liver abscesses, tylosin is the most effective and most commonly used in the feedlot (Amachawadi and Nagaraja, 2016).

Tylosin phosphate is a macrolide antibiotic that is primarily effective against Gram-positive bacteria but has been proven to be effective against the Gram-negative *F. necrophorum* (Berg and Scanlan, 1982; Lechtenberg and Nagaraja, 1989). Macrolide is a class of antibiotics characterized by a large macrolytic lactone ring (Tenson et al., 2003).

Macrolides inhibit microbial growth by causing peptidyl-tRNAs to dissociate from the ribosome (Tenson et al., 2003). This dissociation results in an inability of the ribosome to synthesize new proteins or elongate existing proteins within the bacterium, halting growth and causing eventual death due to lack of functional proteins. It has been hypothesized that tylosin may have some antimicrobial effects outside of the rumen (Nagaraja and Chengappa, 1998). This theory was based upon previous research that demonstrated that tylosin can be absorbed from the gut and be detected in the serum (Gingerich et al., 1977). Although it has been demonstrated that tylosin could affect microbial populations in the liver (Gingerich et al., 1977), the predominant theory is that tylosin mainly functions in the rumen (Nagaraja and Chengappa, 1998). Supplementing diets with tylosin has been reported to decrease ruminal *F. necrophorum* concentrations by 80 to 90%, when cattle were fed an 85% concentrate diet (Nagaraja et al., 1999).

Because tylosin is effective at minimizing the proliferation of *F. necrophorum* in the rumen, dietary supplementation has been shown to markedly reduce liver abscess incidence in finishing cattle. A cursory review of the literature yields results demonstrating that feeding tylosin reduces liver abscess incidence by 40 to 70% (Brink et al., 1990; Nagaraja and Chengappa, 1998; Nagaraja and Lechtenberg, 2007; Reinhardt and Hubbert, 2015). By reducing *F. necrophorum* concentrations in the rumen, tylosin decreases the opportunity for *F. necrophorum* to bind to an acidosis compromised rumen wall, which could provide the pathway for *F. necrophorum* to leave the rumen, enter portal circulation, and eventually result in a liver abscess. Even though tylosin is capable of significantly reducing liver abscesses, cattle fed tylosin still exhibit 12 to 18% liver abscesses incidence (Reinhardt and Hubbert, 2015). Cattle lacking anaphylactic

treatment for liver abscesses had a reported liver abscess incidence in excess of 40% (Brown and Lawrence, 2010). The ability of tylosin to significantly reduce liver abscess incidence by up to 70% in some incidences is why it has become the primary antimicrobial feed additive used in North American cattle feeding to combat liver abscesses.

Although tylosin phosphate is the most effective and most commonly used antibiotic to control for liver abscesses in feedlot cattle, other feed grade antibiotics are utilized to reduce liver abscess incidence. Tetracycline antibiotics (chlortetracycline, neomycin sulfate in combination with oxytetracycline, and oxytetracycline) have also been approved for control of liver abscesses in finished cattle (Lundeen, 2013). Tetracycline antibiotics are classified by a fused linear tetracyclic backbone that can have a variety of attached functional groups (Chopra and Roberts, 2001). This class of antibiotics have demonstrated efficacy against a wide array of microorganisms including gram-negative and gram-positive bacteria, mycoplasmas, and protozoan parasites (Chopra and Roberts, 2001). Tetracyclines inhibit microbial growth via disruption of the target organism's mechanism of protein synthesis. Tetracyclines prevent the association of aminoacyl-tRNA with the bacterial ribosome (Schnappinger and Hillen, 1996, Chopra and Roberts, 2001). Disruption of protein synthesis inhibits the microorganism ability to divide, grow, and function; resulting in eventual death of the microorganism.

Though the mode of action of tetracycline antibiotics is similar to that of the macrolide class of antibiotics; in practice, tylosin has proved to be more efficacious than tetracyclines. When chlortetracycline was fed at 70 mg/animal/d, liver abscess prevalence was shown to be reduced by 21% when compared to negative control cattle

(Brown et al., 1975). However, in the same trial when tylosin was fed at 75 mg/animal/d, liver abscess prevalence was reduced by 67% when compared to the same negative control (Brown et al., 1975). Increased reductions in liver abscesses demonstrates tylosin's greater efficacy at reducing liver abscesses over chlortetracycline at that particular dosage.

Although tylosin is effective in reducing liver abscesses and is commonly utilized by cattle feeders, increased pressure by consumers and the Food and Drug Administration to utilize fewer feed grade antibiotics (US CFR. 2015. Title 21, Parts 514 and 558) has led to some researching alternatives to feed grade antibiotics for the control of liver abscesses in the feedlot.

### *2.4.2 Vaccine*

Despite the fact that antimicrobial compounds are the primary agent used to combat liver abscesses, these compounds are unable to eliminate the problem. Liver abscesses are the result of a bacterial infection, and for many years, the pathogenicity and virulence factors of *F. necrophorum* have been extensively researched and cataloged (Tan et al., 1996; Nagaraja and Chengappa, 1998; Nagaraja and Lechtenberg, 2007). This research has allowed for the development of vaccines to control for incidences of liver abscesses. Two vaccines have been developed to control for liver abscesses, Fusoguard (Elanco Animal Health, Greenfield, IN) and Centurion (Merck Animal Health, Madison, NJ). Fusoguard is a *F. necrophorum* bacterin, a suspension of killed or attenuated bacteria, approved for the control of liver abscesses and foot rot in cattle (Amachawadi and Nagaraja, 2016). Centurion is no longer commercially available, but it was a combination of the leukotoxoid from *F. necrophorum* and a bacterin of *T. pyogenes*

(Amachawadi and Nagaraja, 2016). Although both vaccines have been approved by USDA, industry has been slow to adopt these products even though vaccination can alleviate some of the public health concerns surrounding the use of medically-important antimicrobial feed additives.

In a randomized and blinded field trial, vaccinating cattle with Fusoguard reduced incidence of A and A+ liver abscesses from 9.5% to 2.8% in cattle with a low total incidence (< 10%) of liver abscesses, but was not deemed effective by the authors in cattle with a high (30%) incidence of liver abscesses (reduced incidence from 30.0% to 24.3%; Checkley et al., 2005). Fox et al. (2009) conducted a study to test the efficacy of these two vaccines in natural-fed feedlot cattle. Cattle were randomly assigned to 1 of 3 treatments: control (no vaccination) or vaccination with either Fusoguard or Centurion. All cattle were fed a finishing diet consisting of 73% steam flaked corn and 13% roughage (as-fed basis). Cattle were harvested after 238 days on feed and livers were evaluated using the Elanco Liver Scoring System. Incidence of liver abscesses was 56% and incidence of A and A+ abscesses was 39%. Vaccine treatment did not affect incidence of total liver abscesses or A and A+ abscess incidence rates.

Recent research in development of additional *F. necrophorum* vaccines have focused on the outer membrane proteins of the bacterium, which are responsible for *F. necrophorum's* ability to bind to bovine cells (Kumar et al., 2013). Adhesion of bacterial cells to those of the host organism is a vital step in the establishment of infection and disease pathogenesis of many Gram-negative bacteria (Bavington and Page, 2005), and is a major constituent of the theory of the liver abscess complex. The adhesion of *F. necrophorum* to the rumen wall is a pivotal to the development of a rumen wall abscess

and the eventual leakage of bacteria into portal blood. Adding to this challenge, the outer membrane of subsp. *necrophorum* is different from that of subsp. *funduliforme* (Kumar et al., 2014); meaning that a vaccination that targets the outer membrane would be subspecies specific, which could decrease its overall efficacy. Antibodies to an outer membrane protein of *F. necrophorum* have been found in cattle with liver abscesses (Kumar et al., 2015), while a similar outer membrane protein (FomA) has been found in humans with *F. nucleatum* infections (Han et al., 2005). In a mouse model, a vaccine containing the FomA protein was reported as being able to prevent an oral infection (Liu et al., 2010); however additional research is needed to determine if the targeting of *F. necrophorum* binding protein is an efficacious method to control for liver abscesses in feedlot cattle. The challenges associated with vaccines to control or reduce liver abscess incidence, have led most feedlot managers to use antimicrobial feed additives in conjunction with nutritional management to control for liver abscesses.

### *2.4.3 Nutritional management*

In conjunction with administration of antimicrobial compounds in the feed, proper nutrition and bunk management is well accepted in the literature as a vital component for minimizing rumen imbalance and the eventual development of liver abscesses. Acidosis is typically classified as either acute or sub-acute, also referred to as clinical and sub-clinical, respectively (González et al., 2012). Acute acidosis is an overt illness exhibited by cattle that have consumed readily fermentable carbohydrates in a quantity that is life threatening (Nagaraja and Titgemeyer, 2007), whereas sub-acute acidosis is typically asymptomatic; although feed intake and performance may be diminished (Owens et al., 1998). Ruminal fluid pH below 5.6 is typically the designator of sub-acute acidosis, and

once pH is below 5.0 it is considered to be acute acidosis (Owens et al., 1998; Nagaraja and Titgemeyer, 2007). Liver abscesses are theorized to be a secondary product of acidosis and therefore methods to reduce liver abscesses center on decreasing the incidence and duration of acidosis in feedlot cattle.

In order to minimize the risk of acidosis, some experts have recommended that cattle be gradually adapted to high-grain feedlot diets. Feedlot diets are energy intensive and often contain high concentrations of readily fermentable carbohydrates (González et al., 2012). Starch is rapidly fermented to lactate by lactic-acid producing bacteria in the rumen and an un-adjusted rumen microbiome will be unable to adapt to increasing lactate concentrations, resulting in the death of lactate-fermenting bacteria and eventual pH decline (Nagaraja and Titgemeyer, 2007). Gradually increasing starch concentration in the diet allows lactate-fermenting bacteria to adjust to fluctuations in pH and reduce the incidence of acidosis. Others have recommended that cattle feeders increase the roughage concentration in the diet to reduce incidence of acidosis (Brent, 1976; Nagaraja and Chengappa, 1998).

Roughage feeds are high in fiber, which are slower to ferment by rumen microbes, leading to decreased rate of pH decline (Allen, 1997). Increased fermentation time means that rate of production of lactic acid will be decreased, resulting in a gradual rumen pH decline. Furthermore, greater concentrations of roughage in the diet at an increased particle size promotes a decreased consumption rate and increased mastication time, which further promotes saliva production (González et al., 2012). Saliva contains bicarbonate and phosphate, which can buffer ruminal pH (Bailey and Balch, 1961). Decreased rate of consumption in conjunction with increased saliva production is why

large particle roughage is recommended for inclusion in feedlot diets to decrease the incidence of acidosis. However, an increase in forage in a feedlot ration isn't without some tradeoffs. Increasing roughage in the diet usually comes at the cost of decreasing corn and other concentrates, resulting in a diet that is less energy dense. A diet that contains less starch will not be fermented in the rumen as rapidly as a corn and concentrate dense ration (González et al., 2012), resulting in diminished animal efficiency. Therefore, producers must balance the diet to maximize animal and fermentative efficiency while at the same time formulate a ration that is less acidogenic.

Along with gradual adjustment to high concentrate diets and increased dietary roughage inclusion, bunk management can be essential in decreasing the incidence of acidosis in the feedlot. Gradually increasing the number of times cattle are fed per day may reduce the incidence of acidosis in the feedlot by decreasing the fluctuations in acid production associated with large meals while further increasing saliva production through increased mastication (Soto-Navarro et al., 2000). Providing feed *ad libitum* or in a "slick-bunk" program may also affect incidence of acidosis. *Ad libitum* feeding allows animals to consume feed without restrictions but can be wasteful, whereas "slick-bunk" programs reduce feed wastage but may promote consumption of large meals through the day which can cause greater variations in ruminal pH (González et al., 2012). Finally, consistency of feeding can further affect rate of acidosis in the feedlot. In experimental models, acidotic conditions have been achieved by delaying and withholding feed from cattle for extended periods of time, so that when feed is offered cattle will over consume and enter an acidotic state (Nagaraja and Titgemeyer, 2007). However, these results have not always been replicated. When González et al. (2009) delayed feeding; intake was

decreased in some cattle because cortisol had been released, due to stressful conditions, resulting in decreased eating rate and size of first meal. Regardless of feeding program, the consensus of current literature is that cattle should be fed near the same times every day to not disrupt their feeding and fermentation equilibrium.

### *2.4.4 Feed ingredient alternatives*

Apart from nutritional management, research has been conducted to evaluate the effect that alternative feed ingredients have upon liver abscess incidence. Chief among these alternative ingredients are probiotics and essential oils. Probiotics are live microorganisms that are fed to beef, with the goal to modify the digestive flora and fauna in a positive way (Chaucheyras-Durand and Durand, 2009). The thought process behind this methodology is that through inundation of the rumen with "desired" microorganisms, they will become a larger percentage of the rumen microbiome, resulting in a beneficial shift towards improved performance or decreased acidosis. The most common probiotics administered to feedlot cattle are *Megasphaera elsdenii* and *Saccharomyces cerevisiae* (Chaucheyras-Durand and Durand, 2009).

*Megasphaera elsdenii* is a gram-negative bacterium that is the primary ruminal microorganism involved in the fermentation of lactic acid to pyruvate (Counotte et al., 1981; Nagaraja and Titgemeyer, 2007). It has been estimated that *M. elsdenii* ferments 60 – 80% of the DL-lactate produced in the rumen (Counotte et al., 1981). The ability of *M. elsdenii* to ferment most of the lactate present in the rumen is vital for pH regulation. If lactate is able to accumulate in the rumen, pH will decline towards more acidic conditions, and may eventually lead to the development of acidosis. It is for this reason that some administer *M. elsdenii* to potentially reduce the incidence of liver abscesses in

feedlot cattle. *Megasphaera elsdenii* does not inhibit the growth of *F. necrophorum* (Chaucheyras-Durand and Durand, 2009); however, a reduction in lactate concentration, along with an overall increased pH, may decrease the incidence of acidosis and ultimately reduce liver abscess incidence.

Along with *M. elsdenii*, *Saccharomyces cerevisiae* is often administered as a probiotic to feedlot cattle. *Saccharomyces cerevisiae* is a live yeast culture which has been reported to improve growth and performance of calves (Lesmeister et al., 2004). Hip-height and average daily gain was improved in calves that received 2% yeast culture in the diet over calves that received no yeast culture (Lesmeister et al., 2004); however, calves were euthanized at only 6 wks of age, and therefore not fed to slaughter weight. It has been postulated that live yeast cultures are capable of stabilizing rumen pH and reducing the incidence of acidosis (Chaucheyras-Durand et al., 2008); however, the mode of action is not understood and the ability of live yeast culture to affect pH has not been easily duplicated (Kung et al., 1997). Along with *Saccharomyces cerevisiae* and other probiotics, essential oils have been included in feedlot diets to combat liver abscesses.

Essential oils are volatile and aromatic plant-based compounds which have some antibiotic properties due to their unique structure and wide variety of sidechains that can inhibit the growth of certain microorganisms (Nazzaro et al., 2013). In low concentrations, essential oils have been shown to disrupt microbial enzyme activity, and in high concentrations, essential oils have shown the capability to denature proteins (Tiwari et al., 2009). Generally, essential oils have been shown to be more effective against Gram-positive bacteria than Gram-negative bacteria, because the outer membrane

of Gram-positive bacteria is more conducive for allowing hydrophobic compounds to permeate the membrane and act upon the cell wall and cytoplasm (Nazzaro et al., 2013).

The greater complexity of the membrane of Gram-negative bacteria allows for greater resistance to essential oils. Gram-negative bacteria have a peptidoglycan layer, bound to an outer membrane, coupled to lipopolysaccharide matrix; the increased complexity of the membrane structure along with the phospholipid bi-layer of the outer membrane greatly inhibits the ability of hydrophobic compounds to cross this membrane (Nazzaro et al., 2013). Although Gram-negative bacteria are more resistant to essential oils than Gram-positive bacteria, Gram-negative bacteria can still be affected by essential oils due to the wide array of reactive side-chains compounded to these molecules.

Because essential oils have some antibiotic properties, their efficacy against *F. necrophorum* has been evaluated *in vitro* and *in vivo*. Elwaakeel et al. (2013) evaluated the effect of 5 essential oils (eugenol, vanillin, thymol, guaicol, and limonene) on the growth of *F. necrophorum in vitro* and observed that limonene (20 or 100 µg/mL), and thymol (100 µg/mL) inhibited *F. necrophorum* growth. However, when these two essential oils were included in a proprietary mixture with the other five essential oils evaluated, growth of *F. necrophorum* was not inhibited (Elwaakeel et al., 2013). The authors attributed this lack of affect to the decreased concentrations of limonene and thymol in the mixture as compared to the individual application. The remaining essential oils tested had no effect on *F. necrophorum*. The lack of effect by some of the essential oils is likely due to the inability of some essential oils to cross the outer membrane of *F. necrophorum* and interact with the proteins inside the cell. However, this *in vitro* experiment demonstrated that some essential oils are capable of inhibiting the growth of

*F. necrophorum*. However as demonstrated by the lack of effect by the proprietary mixture, there is a minimum inhibitory concentration needed for even the efficacious essential oil to elicit an effect. Because some essential oils were shown to inhibit *F. necrophorum* growth in the lab, the efficacy of these compounds *in vivo* has been evaluated.

When feedlot steers were fed an essential oil mixture containing: thymol, eugenol, vanillin, guaiacol, and limonene (targeted at 1.0 g/steer daily), liver abscess incidence tended to be reduced when compared with control (Control = 27.2%; Essential Oil Mixture = 16.6%; Meyer et al, 2009). However, liver abscess incidence was still increased when compared to cattle fed monensin and tylosin, without the experimental essential oil mixture (6.5% vs 21.6% liver abscess incidence, respectively). Essential oils may have some antibiotic capabilities; however, their efficacy has not yet been shown to match a true antibiotic compound. Furthermore, although essential oils have demonstrated bacterial inhibition in a petri dish, once they are introduced to the ruminal environment; their effect may be diminished due to volume and bacterial load of the rumen.

The rumen can hold a volume in excess of 100 L (Adams and Kartchner, 1984) and feedlot cattle fed a grain-based diet can have concentrations of *F. necrophorum* in excess of $10^7$/g within the rumen (Nagaraja and Titgemeyer, 2007). Therefore, a feedlot animal can have more than 1 trillion *F. necrophorum* bacteria within the rumen. Furthermore, due to the volume of the rumen, 1 g of product is diluted to 0.00001 g/mL once administered. As observed with the *in vitro* experiment, reduced concentrations of essential oils, even those proven to be affective against *F. necrophorum*, were no longer

efficacious at reducing liver abscesses when concentration was reduced below a certain level. In an environment as large and diverse as the rumen, 1 g of essential oils, administered daily, is not enough to enact significant change. Regardless of the product, and the overall efficacy, all these measures: antibiotic, vaccine, probiotic, essential oil, are administered to cattle with the single purpose of reducing the incidence of liver abscesses in feedlot cattle and to ultimately diminish the economic losses incurred to the industry due to this particular ailment.

## 2.5 INCIDENCE

Incidence of liver abscesses in a lot of cattle can vary greatly, ranging from as low as 0% to in excess of 90%; however in the last decade, incidence has typically hovered around 10-20% (Brown and Lawrence, 2010; Amachawadi and Nagaraja, 2016). Documentation of liver abscess incidence has been a pivotal component of the National Beef Quality Audit (NBQA), since its inception in 1991. The NBQAs primarily documented all offal and by-product condemnations, but percentage of livers condemned due to abscesses are documented within the overall liver condemnation summary. Total liver condemnation experienced a spike in the 2000 audit, however overall liver condemnation rates have changed little since the initial audit (Eastwood et al., 2017). In the latest audit, total abscess incidence was 17.8%, which was increased over all previous audits (Eastwood et al., 2017); however, this increase may be due to the increase in total number of Holsteins audited. Although vital for understanding national liver abscess incidence, the NBQA does not segregate liver abscess incidence by cattle type. When cattle are segregated by type (native vs. Holstein) it is revealed that incidence and

severity is greater in fed Holsteins. In the same time period as the previous publications, incidence of liver abscesses in fed Holsteins averaged 28.3% whereas native beef steers and heifers averaged 16.0% and 13.9%, respectively (Amachawadi and Nagaraja, 2016).

### *2.5.1 Liver abscess incidence in fed Holsteins*

Increased incidence of liver abscesses in Holsteins may have contributed to increased overall abscess incidence reported by Eastwood et al. (2017). Fed Holsteins have become a sizeable proportion of total slaughter cattle (20.4% of total; Eastwood et al., 2017), and their increased abscess incidence over native beef likely increases total abscess incidence, nationally. Along with having a greater overall incidence of liver abscesses, fed Holsteins have a greater proportion of severe abscesses, with upwards of 50% of abscesses being A+ or greater or adhered to the diaphragm and other visceral organs (Amachawadi and Nagaraja, 2016). Because fed Holsteins have become a greater proportion of total slaughter cattle in recent years, and their abscess incidence is increased over non-Holstein fed beef, some have speculated as to why incidence is increased in fed Holsteins.

One of the more common hypotheses as to why incidence is increased in Holsteins over traditional beef is cumulative days on finishing ration (Nagaraja and Chengappa, 1998; Amachawadi and Nagaraja, 2016). Holsteins not destined for the dairy are introduced to energy intensive diets at an early age, which translates to greater days on feed when compared to non-Holstein feedlot cattle. It is not uncommon for Holsteins to be on feed for 300 to 400 d prior to slaughter (Duff and McMurphy, 2007) whereas traditional beef are on feed closer to 120 to 150 days (Vogel and Parrott, 1994). As days on feed increases, the probability for an acidotic event to occur increases,

resulting in a subsequent increase in the probability of an abscess developing. Along with being on feed for longer periods of time, Holsteins typically consume more than their native beef counterparts. Holstein steers can consume up to 12% more feed (on a dry matter basis) than traditional beef cattle, at the same weight (Hicks et al., 1990), due to the increased maintenance energy demand needed to support their larger frame and greater proportion of gut and organ tissue. Furthermore, increased feed consumption increases the total fermentable substrate in the rumen, allowing for prolonged ruminal fermentation and a potential for greater decreases in rumen pH (Owens et al., 1998). Increasing the energy density of the diet may alleviate some of the issues associated with increased intake. A feedlot steer's energy intake is linearly related to total diet energy density to about 1.90 Mcal/kg NEm (Plegge et al., 1984; Zinn and Plascencia, 1996). Therefore, increasing the energy density of a feedlot diet for Holstein steers should reduce total intake; however, increased energy density may still result in rapid fermentation and subsequent pH decline depending on the energy source provided. Along with variations in intake and energy requirements, Holsteins have also been documented as having different mannerisms and behavior when compared to traditional feedlot cattle.

Holsteins in a feedlot setting have been described as having behavior different from traditional feedlot cattle, and these mannerisms unique to Holsteins may further contribute to increased abscess incidence. Eng (2005) discussed characteristics that make Holsteins unique from traditional beef: 1) Holsteins can become bored in confinement, and therefore are more likely to groom and play with their feed, 2) pens containing Holsteins are often wetter due to greater water intake and subsequent urination, and 3)

Holsteins have greater incidences of metabolic disease and overall death loss. While these factors may not immediately correlate to increased liver abscesses, liver abscesses appear to be multifactorial diseases, and these factors could have additive effects upon liver abscesses apart from increased intake and days on feed.

It has been primarily theorized that increased grooming and hair intake further contributes to increased abscess incidence in fed Holsteins (Amachawadi and Nagaraja, 2016). When sheep were fed diets containing ground up cattle hair, liver abscess incidence was increased over sheep fed diets that did not contain beef hair (Fell et al., 1972). Hair may be able to penetrate the rumen wall, allowing for multiple routes of entry for *F. necrophorum* to enter portal blood supply, resulting in increased abscess incidence. It has also been postulated that the bacterial flora of Holsteins is different from that of traditional beef, and the species present in the gastrointestinal tract of fed Holsteins are more virulent than native beef (Amachawadi and Nagaraja, 2016). However, no published data exits that compares the gastrointestinal tract microbiome of Holsteins to native beef.

### *2.5.2 Liver abscess incidence in dairy cattle*

Although fed Holsteins receive most consideration when discussing liver abscess incidence, dairy cattle are also capable of developing liver abscesses. Dairy cattle are fed diets not as energy intensive as a feedlot diet, but are still capable of producing an acidotic event in the rumen. Furthermore, the transition from a forage intensive dry cow diet to an energy intensive lactation diet can produce acidotic conditions in the rumen. Dairy cows are prone to subacute acidosis in the first month of lactation due to increased VFA production as they transition from the high forage diet of the dry cow period to the

high energy diet of the lactation period (Plaizer et al., 2008). Although liver abscesses are rarely diagnosed in cows at the dairy (Dore et al., 2007), they have been documented at slaughter (Rezac et al., 2014) and may be artifacts that contributed to why the animal was culled.

When Hadley et al. (2006) analyzed dairy culling trends by herd size and region, they reported that the primary reason cattle were culled from small herds (<300 head) was injury; although injury remain the principal culling factor in larger herds, additional culling factors (i.e. disease, mastitis, low production) increased as herd size increased. Furthermore, Rezac et al. (2014) documented abscess incidence in cull dairy cows from the Great Lakes region and reported that 32% of culled cows observed had liver abscesses; and within the population of animals with liver abscesses, 58% had abscess severities of A+ or greater. Increased severity of liver abscesses in dairy cows is likely due to multiple ration changes annually concomitant with a lack of available antimicrobial products to reduce liver abscesses (i.e. tylosin phosphate), which is not approved in dairy cattle. Although data is lacking in literature, liver abscesses could affect milk production, due to decreased metabolic efficiency of the liver, which would consequently be an artifact of dairy cattle culled for decreased milk production, further contributing to increased incidence of liver abscesses in culled dairy cattle.

### *2.5.3 Liver abscess incidence in range cows*

Although infrequently discussed in literature, range cows not fed a concentrate diet prior to slaughter can also develop liver abscesses. The National Non-Fed Beef Quality Audits have reported liver condemnation rates in cull beef as 30.8% (1994) and 24.1% (1999), with 27.3% of liver condemnations in the 1999 audit being from liver

abscesses (e.g. 6.6% liver abscess incidence; Roeber et al., 2001). The 2007 National Market Cow and Bull Beef Quality Audit reported 14% liver abscess incidence in cull beef (Hale et al., 2007). Because range cows are not supplemented with an energy intensive diet similar to dairy cows or feedlot cattle, it is intriguing that cull cows still develop liver abscesses at incidences similar to fed beef.

It is possible that cull range cows develop liver abscesses because *F. necrophorum* is capable of entering portal circulation via routes other than rumen wall abscesses caused by acidotic events. *Fusobacterium necrophorum* can survive in the soil of pastures and has been isolated from the oral cavity, gastrointestinal tract, and genitourinary tract of normal, healthy animals (Langworth, 1977). Additionally, it has been reported that liver abscesses can be formed as a result of traumatic reticuloperotinitis ("hardware disease"; Nagaraja and Smith, 2000). Because *F. necrophorum* is ubiquitous to the gastrointestinal tract of ruminants, tears or cuts in the epithelial lining of the gastrointestinal tract can provide a route for this bacterium to enter portal circulation. Range cows are more likely to interact with debris and harsh feedstuffs that can cause trauma to the lining of the mouth or esophagus, which could explain why cattle not fed a concentrate based ration have liver abscess incidences similar to feedlot and dairy cattle. Furthermore, range cows are not supplemented with tylosin phosphate and are often components of a low input system which has little overhead to keep costs down due to low margins.

Regardless of cattle type, liver abscesses can incur significant financial losses to producers and packers, which is why a wide variety of products and procedures have been evaluated and marketed to reduce liver abscess incidence.

## 2.6 ECONOMIC IMPACT

Liver abscesses pose a significant economic detriment to the producer, the packer, and ultimately the consumer, because increased production costs will be passed on. The greatest economic impact of liver abscesses is reduced animal performance and diminished carcass yield (Nagaraja et al., 1996b). Multiple research trials have documented that cattle with liver abscesses exhibit: decreased feed intake, decreased weight gain, decreased feeding efficiency, and overall decreased carcass weights. However, decreases in performance due to liver abscesses have not always been noted. The effect of abscesses on cattle performance has ranged from no effect (Smith, 1944; Harman et al., 1989), to reductions up to 11% in daily gain and an overall decreased feed efficiency over 9% (Brink et al., 1990).

Changes in performance metrics by abscess severity have also been noted. Typically, smaller abscesses (A- to A liver scores) have minimal effect on rate of gain, feed efficiency, or carcass weight, whereas large active abscesses (A+ or greater) can significantly reduce feed intake (up to 13.8%), overall weight gain (up to 11.4%), feed efficiency (up to 29.5%), and final carcass weight (up to 4.6%; Nagaraja et al., 1996b). When Brink et al. (1990) summarized data from 12 research trials that evaluated the association of abscess severity to growth characteristics; they reported a 5.1% reduction in intake, 9.4% reduction in average daily gain, a 9.7% increase in feed-to-gain, and 1.5% decrease in dressed carcass yield. Severe liver abscesses, abscesses which are open or adhered to the diaphragm, can require additional trimming due to carcass contamination or through modification of standard evisceration procedures to ensure that adhered abscesses are fully removed from the carcass.

Montgomery (1985) evaluated 1447 commercially fed cattle from feedlots throughout the Texas Panhandle to document the effect liver abscess severity has on carcass grading outcomes and trim. As observed when documenting feeding performance, major liver abscesses negatively impacted carcass trim and quality. Cattle with normal livers had increased dressed carcass yield over cattle with major liver abscesses (63.3% vs. 61.7%; Montgomery, 1985). Furthermore, cattle with severe liver abscesses had increased carcass trim over cattle with normal livers (0.4538% of carcass weight, vs. 0.0214% of carcass weight, respectively; Montgomery, 1985). Increased carcass trim along with decreased growth performance, resulted in reduced hot carcass weights (Montgomery, 1985). Severe abscesses can have diaphragm and flank tissue attached to the liver, resulting in subsequent condemnation of those tissues by USDA inspectors, whereas ruptured abscesses may result in disruption of the flow of the carcasses through the slaughter floor and may even facilitate removal from the production line (Montgomery, 1985; Nagaraja et al., 1996b). When a carcass is removed from the production line due to open abscess, all tissues contaminated by puss must be trimmed from the carcass, resulting in a lighter and less valuable carcass.

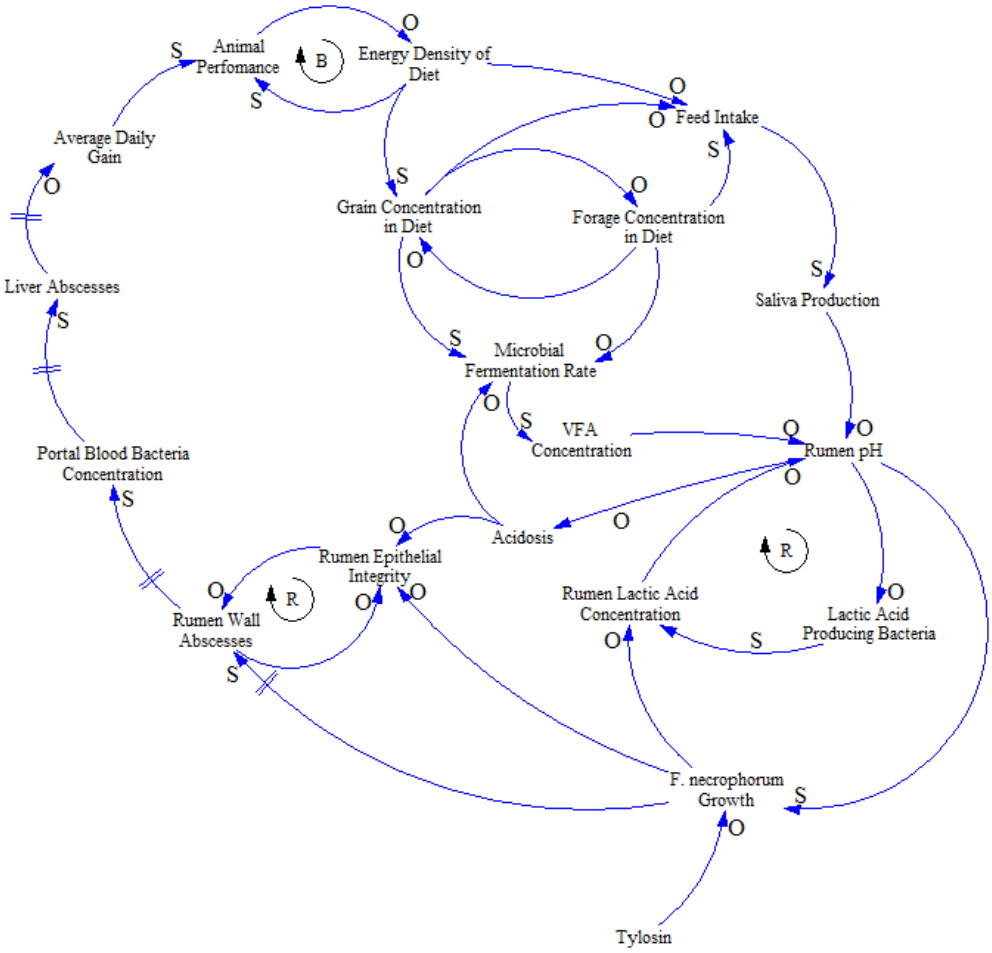
Brown and Lawrence (2010) reported results similar to Montgomery (1985). Cattle with severe liver abscesses had decreased carcass weights, longissimus muscle area, and 12$^{th}$ rib subcutaneous fat when compared to carcasses with normal livers (Brown and Lawrence, 2010). These decreases resulted in carcasses that were worth 3.8% ($38.26) less than carcasses with no liver abscesses (Brown and Lawrence, 2010). Minor reductions in dressed carcass yield, and increased carcass trimming can result in significant reductions in carcass weight which ultimately decreases carcass value.

Regardless of severity, liver abscesses result in the liver being condemned and could potentially result in condemnation of the entire viscera. Severe adhesions and open abscesses may cause the entire gastrointestinal tract to be condemned, which creates a significant financial loss to the beef processor. Abscesses are the primary reason for liver condemnation at slaughter (Eastwood et al., 2017). Because livers are a valuable offal commodity, their condemnation and subsequent rendering is a loss to the processor. Although viscera and offal value is not included in mandatory price reporting (US CFR. 2008. Title 7, Part 59), some packers report offal values (USDA, NW_LS441), which can be used to estimate the value of a liver and the gastrointestinal tract. When Brown and Lawrence (2010) associated liver abscesses to viscera loses, they calculated that liver abscesses resulted in losses up to $15.8 million annually due to condemned livers, and $7 million annually from to condemned gastrointestinal tracts due to open and adhered abscesses.

Liver abscesses present significant financial losses to producers, packers, and consumers alike. Liver abscesses decrease live animal performance, resulting in lighter, less-valuable carcasses, which ultimately increase costs to the consumer. Because liver abscess incidence and severity is increased in Holsteins, their risk to the industry is magnified beyond the normal production risk associated with feeding native beef. Furthermore, calf-fed Holsteins have become a greater proportion of the fed beef population as beef-type cattle numbers have become more limited. Although the liver abscess complex has been researched since the 1940s, the pathogenic mechanism is yet to be definitively proven. Because underlying mechanism of this disease is still unverified, it is possible that the liver abscess complex is a multifactorial disease.

Current research into the liver abscess complex primarily focuses on cattle feeding and solely attributes liver abscesses to acidotic events in the rumen. Furthermore, contemporary data do not exist that quantify trim losses and lost economic opportunity from liver abscesses in Holstein steers. Additionally, despite decades of research, there are still many questions left unanswered regarding the pathogenesis and causative factors of liver abscesses. The rumenitis/acidosis liver abscess complex remains theoretical after more than 60 years. Potential point sources of causative bacteria may include the small and large intestine. Therefore, we designed a series of experiments to expand our knowledge of the rumenitis/acidosis liver abscess complex and to better understand the effect this disease has upon calf-fed Holsteins. We hypothesize that the rumenitis/acidosis liver abscess complex is a multifactorial disease, which can be exacerbated by feeding practices, but is primarily driven by as-of-yet unknown circumstances. Our objective is to expand the current knowledge of the rumenitis/acidosis liver abscess complex using: audits, surveys, carcass tracking and quality data, and analysis of blood parameters, from animals with and without abscesses at slaughter.

**Figure 1. Causal Loop Diagram of the Liver Abscess Complex**



## 2.7 CAUSAL LOOP DIAGRAM DISCUSSION

The attached figure (Figure 1) is a causal loop diagram. A causal loop diagram, is a model/diagram that can aid in the visualization of a system and highlight how variables can interact with each other (Kim, 1992). The diagram is composed of "variables" and "connectors/arrows". The variables are the items listed, and connectors represent the relation between the two variables being connected (denoted by a directional arrow). If the arrow is labeled with an "S", then that indicates a similar relationship (i.e. if one variable increases, the other variable eventually increases), and if the arrow is labeled with an "O", then that indicates an opposite relationship (i.e. if one variable increases, the other variable eventually decreases; Richardson, 1986; Kim, 1992). Within these connections there can also be delays, identified by double parallel lines (Kim, 1992). These delays signify that while the two variables are connected, the effect from one variable may take time to have an observable effect on the connected variable, which can further complicate the system and the ways to respond to the problem.

Also within the causal loop are reinforcing and balancing loops. A reinforcing loop, denoted as an "R" with a circle around it, is a cycle where the change in any of the variables flows throughout the loop and returns to the original variable, strengthening the initial action (Richardson, 1986; Kim, 1992). Furthermore, within the reinforcing loop, if no outside force acts upon it the system will be stuck in a brutal cycle of chain reactions that may ultimately result in the downfall of the overall system. On the other hand, a balancing loop is when variables in the system interact with each other to result in an effect that was opposite to the original change (Richardson, 1986; Kim, 1992). These

two loops within the overarching causal loop allow one to better understand the consequences of certain actions and to better understand why potential fixes may have failed.

The attached causal loop diagram (Figure 1) is meant to be an illustrative representation of the liver abscess complex as outlined in the above literature review. The intricacies and nuances surrounding each individual factor was covered in detail in the body of the literature review, while this diagram is meant to show provide an overview of the complex from a wider perspective. This causal loop illustrates how different factors are connected to each other and allows for one to visualize how changes in one variable can impact another. Instead of focusing on an issue and narrowing the focus down to the minutia, the causal loop diagram is meant to expand the scope of the focus and provide a less detailed, more encompassing view of a system/issue.

In the causal loop diagram (Figure 1) describing the mechanisms of the liver abscess complex, the primary focal point is "Rumen Epithelial Integrity". Although rumen epithelial integrity is not the most interconnected or impactful variable in this model, it is by far the most crucial independent variable. The proliferation of *F. necrophorum* or the decrease in ruminal pH are the drivers, may be the more interconnected or immediately visible contributors to liver abscesses, but they both attack on the same fundamental point, rumen epithelial integrity. If the acidotic event or *F. necrophorum* are contained within the rumen, then the rest of the liver abscess system will fall apart because the causative agent of liver abscesses will have been unable to leave the rumen and enter portal blood supply, where it could eventually form a liver abscess.

While “Rumen Epithelial Integrity” is the focal point of this diagram it is not the primary leverage point. To affect rumen epithelial integrity, one must back up a few steps and apply leverage to the energy density of the diet. Although other variables are in closer proximity to “Rumen Epithelial Integrity”, the energy density of the diet is one of the few variables in this model that can be directly influenced by cattle feeders. Energy density of the diet is the leverage point of this model because if leverage is applied properly to the energy density, the entire outlook of this described liver abscess complex can shift. The manipulation of energy density in the diet begins the cascade that eventually results in the formation of liver abscesses.

To reduce the energy density of the diet, high-energy grains must be removed, and forage must be substituted, since forages inherently are less energy dense than corn, wheat, or barley, the primary grains fed to cattle. Increasing the forage concentration of the diet will increase overall intake by the animal, which in-turn will increase saliva production. Saliva contains natural buffers, like bicarbonate and urea, which can increase the pH of the rumen. An increased ruminal pH decreases the potential for an acidotic event, along with keeping the growth of lactic acid producing bacteria in check, which eventually benefits rumen epithelial integrity. Adding further to this system is the grain concentration of the diet.

Grains are often more readily fermentable than forage sources, which directly contributes to the fermentation rate of the microbes in the rumen. As the fermentation rate of the rumen microbes increases, so too does the VFA concentration of the rumen; since the microbes convert starch and fiber to the VFAs: acetate, propionate, and butyrate. Since VFAs are acidic molecules, they further contribute to the decrease in

rumen pH. If the pH of the rumen becomes acidotic, water from the surrounding tissues will traverse the epithelial lining of the rumen to attempt to buffer the pH. However, the passage of water through the epithelium can result in tissue damage and microscopic tearing, which can provide an optimal environment for *F. necrophorum* to attach to. Once *F. necrophorum* attaches to the rumen wall it can create a rumen wall abscess which may eventually leach *F. necrophorum* from the rumen into the portal blood supply. Since the circulatory system is all connected, *F. necrophorum* in the blood will eventually end up in the liver where it can form an abscess. If the abscess becomes severe enough it can impact the performance and gain of the animal, since the active capacity of the liver is diminished due to an abscess, which may prompt a feedlot nutritionist to increase the energy density of the diet to improve performance. Further propagating this system towards liver abscess development, since increasing the grain concentration of the diet will increase the energy density of the diet, which further fuels the system towards the development of a liver abscess.

Within this system, leverage can be applied to several points to mitigate the development of liver abscesses. The primary leverage point in this system is the energy density of the diet. Force should be applied to this lever in a manner that keeps rumen pH above acidotic levels. This can be achieved through increasing forage concentrations in the diet and decreasing grain concentrations in the diet. Forage decreases the surface area of the diet, resulting in increased fermentation times, reduced rates of volatile fatty acid (VFA) production and a reduced likelihood of an acidic pH. Furthermore, increasing forage concentration in the diet, increases feed intake, subsequently stimulating the salivary response. Saliva contains sodium bicarbonate, which has a basic pH and can

buffer an acidic rumen. The outcome from this "pulling of the lever" will decrease acidotic events, which will allow for increased rumen epithelial integrity and a decrease in the incidence of rumen wall abscesses. While increasing rumen pH will provide for a more favorable environment for *F. necrophorum* growth, an uncompromised rumen epithelium will make adhesion and formation of rumen wall abscess difficult. If rumen wall abscess formation is reduced, the inflow of *F. necrophorum* into portal circulation will be reduced as well; resulting in a decrease in liver abscesses.

The archetype/template described in this causal loop is the "Fixes that Backfire" archetype (Wolstenholme, 2003), meaning that by trying to fix/improve animal performance by increasing the energy density of the diet, the ultimate result will be the opposite of what is desired (i.e. decreased performance due to a liver abscess). Decreased animal performance prompts for an increase in the energy density of the diet to fix the observed problem. Increasing the energy density allows for faster fermentation rates and can temporarily improve animal performance. However, that quick solution has unintended consequences and it ultimately makes the original symptoms even worse.

Adding to the complexity of this problem are delays (identified with parallel lines on the arrows between nodes). Liver abscess formation is not immediate and takes time to proceed to the point of negatively affecting average daily gain. Therefore, by the time the problem presents itself it is not always clear why performance is decreasing. Because we don't know what's causing the decrease in performance, we return to our previous fix; increasing the energy density of the diet, and the cycle begins anew. The fix only addresses the symptom of liver abscesses and not the underlying problem because the reinforcing loop is longer term and not always obvious. The only way to break this cycle

is to address the underlying problem and give up the fix that works only on the symptom. In this case that means decreasing the overall energy density of the diet. However, because the liver abscess complex is a multifaceted system with many components changes to any one area may not result in the desired effect and liver abscesses could possibly still develop. This causal loop diagram aids in the visualization of the problem and highlights how the multiple variables are interconnected but is not an all-encompassing diagram that can immediately solve this problem. This disease has many factors, which serve to increase the complexity and the challenges when trying to reduce liver abscess incidence. Therefore, a visual interpretation of the disease may help some view the problem in a way never considered before or allow others to better understand the outcomes of their management decisions when dealing with liver abscesses in a commercial beef herd.

## 2.7 LITERATURE CITED

Adams, D.C. and R. J. Kartchner. 1984. Effect of level of forage intake on rumen ammonia, pH, liquid volume and liquid dilution rate in beef cattle. J. Anim. Sci. 58:708-713. doi:10.2527/jas1984.583708x.

Allen, M.S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J. Dairy Sci. 80:1447-1462. doi:10.3168/jds.S0022-0302(97)76074-0.

Amachawadi, R. G., and T. G. Nagaraja. 2015. First report of anaerobic isolation of *Salmonella enterica* from liver abscesses of feedlot cattle. J. Clin. Microbiol. 53:3100-3101. doi: 10.1128/JCM.01111-15

Amachawadi, R. G., and T. G. Nagaraja. 2016. Liver abscesses in cattle: A review of incidence in Holsteins and of bacteriology and vaccine approaches to control in feedlot cattle. J. Anim. Sci. 94:1620-1632. doi:10.2527/jas.2015-0261.

Amachawadi, R. G.,T. J. Purvis, B. V. Lubbers, J. W. Homm, C. L. Maxwell, and T. G. Nagaraja. 2017. Bacterial flora of liver abscesses in crossbred beef cattle and Holstein steers fed finishing diets with or without tylosin. J. Anim. Sci. 95:3425-3434. doi:10.2527/jas2016.1198.

Arthur, T. M., D. M. Brichta-Harhay, J. M. Bosilevac, M. N. Guerini, N. Kalchayanand, J. E. Wells, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2008. Prevalence and characterization of *Salmonella* in bovine lymph nodes potentially destined for use in ground beef. J. Food Prot. 71:1685-1688. doi:10.4315/0362-028X-71.8.1685.

Baba, E., T. Fukata, A. Arakawa, H. Ikawa, and M. Takeda. 1989. Antibiotic susceptibility of *Fusobacterium necrophorum* from bovine hepatic abscesses. Br. Vet. J. 145:195-197. doi:10.1016/0007-1935(89)90105-X.

Babior, B. M. 1984. The respiratory burst of phagocytes. J. Clin. Invest. 73:599-601. doi:10.1172/JCI111249.

Bailey, C. B. and C. C. Balch. 1961. Saliva secretion and its relation to feeding in cattle. 2. The composition and rate of secretion of mixed saliva in the cow during rest. Brit. J. Nutrition, 15:383-402.

Berg, J. N., and C. M Scanlan. 1982. Studies of *Fusobacterium necrophorum* from bovine hepatic abscesses: biotypes, quantitation, virulence, and antibiotic susceptibility. Am. J. Vet. Res. 43:1580-1586.

Batshon, B. A., H. Baer, and M. F. Shaffer. 1963. Immunologic paralysis produced in mice by *Klebsiella pneumoniae* type 2 polysaccharide. J. Immunol. 90:121-126.

Bavington, C., and C. Page. 2005. Stopping bacterial adhesion: A novel approach to treating infections. Respiration. 72:335–344. doi:10.1159/000086243.

Biberstein, E. L. 1990. Corynebacteria; *Actinomyces pyogenes*; Rhodococcus equi. In: E. L. Biberstein and Y. C. Zee, editors, Review of veterinary microbiology. Blackwell Scientific, Boston, MA. p. 165.

Billington, S. J., B. H. Jost, W. A. Cuevas, K. R. Bright, and J. G. Songer. 1997. The *Arcanobacterium (Actinomyces) pyogenes* hemolysin, pyolysin, is a novel member of the thiol-activated cytolysin family. J. Bacteriol. 179:6100-6106. doi:10.1128/jb.179.19.6100-6106.1997.

Brent, B. E. 1976. Relationship of acidosis to other feedlot ailments. J. Anim. Sci. 43:930-935. doi:10.2527/jas1976.434930x.

Braun, U., N. Pusterla, and K. Wild. 1995. Ultrasonographic findings in 11 cows with a hepatic abscess. Vet. Rec. 137:284-290. doi: 10.1136/vr.137.12.284.

Braun, U. 2009. Ultrasonography of the liver in cattle. Vet. Clin. North Am. Food Anim. Pract. 25:591-609. Doi:10.1016/j.cvfa.2009.07.003.

Brink, D. R., S. R. Lowry, R. A. Stock, and J. C. Parrott. 1990. Severity of liver abscesses and efficiency of feed utilization of feedlot cattle. J. Anim. Sci. 68:1201-1207. doi:10.2527/1990.6851201x.

Brown, H., R. F. Bing, H. P. Grueter, J. W. McAskill, C. O. Cooley, and R. P. Rathmacher. 1975. Tylosin and chlortetracycline for the prevention of liver abscesses, improved weight gains and feed efficiency in feedlot cattle. J. Anim. Sci. 40:207-213. doi:10.2527/jas1975.402207x.

Brown, T. R., and T. E. Lawrence. 2010. Association of liver abnormalities with carcass grading performance and value. J. Anim. Sci. 88:4037-4043. doi:10.2527/jas.2010-3219.

Chaucheyras-Durand, F., N. D. Walker, and A. Bach. 2008. Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. Anim. Feed Sci. Technol. 145: 5-26. doi:10.1016/j.anifeedsci.2007.04.019.

Chaucheyras-Durand, F., and H. Durand. 2009. Probiotics in animal nutrition and health. Benef Microbes. 1:3-9. doi:10.3920/BM2008.1002.

Checkley, S. L., E. D. Janzen, J. R. Campbell, and J. J. McKinnon. 2005. Efficacy of vaccination against *Fusobacterium necrophorum* infection for control of liver abscesses and footrot in feedlot cattle in western Canada. Can. Vet. J. 46:1002–1007.

Chopra, I. and M. Roberts. 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol. Mol. Biol. Rev. 65:232-260. doi: 10.1128/MMBR.65.2.232-260.2001.

Counotte, G. H. M., R. A. Prins, and R. H. A. M. Janssen. 1981. Role of *Megasphaera elsdenii* in the fermentation of DL-[2-13C] lactate in the rumen of dairy cattle. Appl. Environ. Microbiol. 42:649-655.

Davis, M. S., W. C. Koers, K. J. Vander Pol, and O. A. Turgeon Jr. 2007. Liver abscess score and carcass characteristics of feedlot cattle. J. Anim. Sci. 85:126-127.

Doré, E., G. Fecteau, P. Hélie, and D. Francoz. 2007. Liver abscesses in Holstein dairy cattle: 18 cases (1992–2003). J. Vet. Intern. Med. 21:853-856.

Duff, G. C., and C. P. McMurphy. 2007. Feeding Holstein steers from start to finish. Vet. Clin. North Am. Food Anim. Pract. 23:281–297. doi:10.1016/j.cvfa.2007.04.003.

Eastwood, L. C., C. A. Boykin, M. K. Harris, A. N. Arnold, D. S. Hale, C. R. Kerth, D. B. Griffin, J. W. Savell, K. E. Belk, D. R. Woerner, J. D. Hasty, R. J. Delmore, J. N. Martin, T. E. Lawrence, T. J. McEvers, D. L. VanOverbeke, G. G. Mafi, M. M. Pfeiffer, T. B. Schmidt, R. J. Maddock, D. D. Johnson, C. C. Carr, J. M. Scheffler, T. D. Pringle, and A. M. Stelzleni. 2017. National Beef Quality Audit-2016: Transportation, mobility, and harvest-floor assessments of targeted characteristics that affect quality and value of cattle, carcasses, and by-products1. Trans. Anim. Sci. 1:229-238. doi:10.2527/tas2017.0029.

Elwakeel, E. A., R. G. Amachawadi, A. M. Nour, M. E. A. Nasser, T. G. Nagaraja, and E. C. Titgemeyer. 2013. In vitro degradation of lysine by ruminal fluid-based fermentations and by *Fusobacterium necrophorum*. J. Dairy Sci. 96:495-505. doi:10.3168/jds.2012-5810.

Emery, D. L., J. A. Vaughan, B. L. Clark, J. H. Dufty, and D. J. Stewart. 1985. Cultural characteristics and virulence of strains of *Fusobacterium necrophorum* isolated from

the feet of cattle and sheep. Aust. Vet. J. 62:43-46. doi:10.1111/j.1751-0813.1985.tb14231.x.

Emery, D. L., J. A. Vaughan, B. L. Clark, and D. J. Stewart. 1986. Virulence determinants of *Fusobacterium necrophorum* and their prophylactic potential in animals. In Footrot in ruminants. Proceedings of a workshop, Melbourne 1985. (pp. 267-274). CSIRO Division of Animal Health.

Eng, K. S. 2005. Dairy beef production past, present, and future In: Proceedings from managing and marketing quality Holstein steers. Univ. of Minnesota Dairy Extension, Rochester, MN, http://www.extension.umn.edu/agriculture/dairy/beef/#nutrition. (Accessed 10 November, 2017.)

Fell, B.F., M. Kay, E.R. Ørskov, and R. Boyne. 1972. The role of ingested animal hairs and plant spicules in the pathogenesis of rumenitis. Res. Vet. Sci. 13:30–36.

Fox, J. T., D. U. Thomson, N. N. Lindberg, and K. Barling. 2009. A comparison of two vaccines to reduce liver abscesses in natural-fed beef cattle. Bovine Practitioner. 43:168-174.

González, Correa, L.A., L. B. Ferret, A. Manteca, X. Ruíz-de-la-Torre, and J. L. Calsamiglia. 2009. Intake, water consumption, ruminal fermenattion, and stress response of beef heifers fed alter different lengths of delays in the daily feed delivery time. J. Anim. Sci. 87, 2709–2718. doi:10.2527/jas.2008-1709.

González, L.A., X. Manteca, S. Calsamiglia, K. S. Schwartzkopf-Genswein, and A. Ferret. 2012. Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). Anim. Feed Sci. Technol. 172:66-79. doi:10.1016/j.anifeedsci.2011.12.009.

Gill, D. R., F. N. Owens, R. W. Fent, and R. K. Fulton. 1979. Thiopeptin and roughage level for feedlot steers. J. Anim. Sci. 49:1145-1150. doi:10.2527/jas1979.4951145x.

Gingerich, D. A., J. D. Baggot, and J. J. Kowalski. 1977. Tylosin antimicrobial activity and pharmacokinetics in cows. Can. Vet. J. 18:96-100.

Hadley, G. L., C. A. Wolf, and S. B. Harsh. 2006. Dairy cattle culling patterns, explanations, and implications. J. Dairy Sci. 89:2286-2296. doi:10.3168/jds.S0022-0302(06)72300-1

Hale, D., J. Savell, R. Delmore, D. Johnson, T. Pringle, W. Henning, R. Maddock, T. Lawrence, and J. Nicholson. 2007. National market cow and bull beef quality audit-2007: A survey of producer-related defects. Final Report. National Cattlemen's Beef Association, Centennial, CO.

Han, Y. W., A. Ikegami, C. Rajanna, H. I. Kawsar, Y. Zhou, M. Li, H. T. Sojar, R. J. Genco, H. K. Kuramitsu, and C. X. Deng. 2005. Identification and characterization of a novel adhesin unique to oral fusobacteria. J. Bacteriol. 187:5330–5340. doi: 10.1128/JB.187.15.5330-5340.2005.

Harman, B. R., M. H. Brinkman, M. P. Hoffman, and H. L. Self. 1989. Factors affecting in-transit shrink and liver abscesses in fed steers. J. Anim. Sci. 67:311-317. doi:10.2527/jas1989.672311x.

Hicks, R. B., Owens, F. N., Gill, D. R., Oltjen, J. W., and R. P. Lake. 1990. Daily dry matter intake by feedlot cattle: influence of breed and gender. J. Anim. Sci. 68:245-253.

Houghton, P. L. and L. M. Turlington. 1992. Application of ultrasound for feeding and finishing animals: a review. J. Anim. Sci. 70:930-941. doi:10.2527/1992.703930x.

Ibarra, J. A., and O. Steele-Mortimer. 2009. *Salmonella*–the ultimate insider. *Salmonella* virulence factors that modulate intracellular survival. Cell. Microbiol. 11:1579-1586. doi:10.1111/j.1462-5822.2009.01368.x.

Jensen, R., W. E. Connell, and A. W. Deem. 1954a. Rumenitis and its relation to rate of change of ration and the proportion of concentrate in the ration of cattle. Am. J. Vet. Res. 15:425-428.

Jensen, R., H. M. Deane, L. J. Cooper, V. A. Miller, and W. R. Graham. 1954b. The rumenitis-liver abscess complex in beef cattle. Am. J. Vet. Res. 15:202-216.

Jensen, R., J. C. Flint, and L. A. Griner. 1954c. Experimental hepatic necrobacillosis in beef cattle. Am. J. Vet. Res. 15:5-14.

Jiang, W., and Y. Liu. 2013. *Klebsiella pneumoniae* liver abscess. Asian Pac. J. Life Sci. 7:273-282.

Jost, B. H., and S. J. Billington. 2005. *Arcanobacterium pyogenes*: molecular pathogenesis of an animal opportunist. Antonie van Leeuwenhoek. 88:87-102. doi: 10.1007/s10482-005-2316-5.

Kim, D. H. 1992. Guidelines for Drawing Causal Loop Diagrams. Systems Thinker. 3: 5-7.

Kreikemeier, K. K., D. L. Harmon, R. T. Brandt, T. G. Nagaraja, and R. C. Cochran. 1990. Steam-rolled wheat diets for finishing cattle: effects of dietary roughage and feed intake on finishing steer performance and ruminal metabolism. J. Anim. Sci. 68:2130-2141. doi:10.2527/1990.6872130x.

Kung, L., E. M. Kreck, R. S. Tung, A. O. Hession, A. C. Sheperd, M. A. Cohen, H. E. Swain, and J. A. Z. Leedle. 1997. Effects of a Live Yeast Culture and Enzymes on In

Vitro Ruminal Fermentation and Milk Production of Dairy Cows1. J. Dairy Sci. 80:2045-2051. doi:10.3168/jds.S0022-0302(97)76149-6.

Kumar, A., E. Gart, T. G. Nagaraja, and S. Narayanan. 2013. Adhesion of *Fusobacterium necrophorum* to bovine endothelial cells is mediated by outer membrane proteins. Vet. Microbiol. 162:813–818.

Kumar, A., G. Peterson, T. G. Nagaraja, and S. Narayanan. 2014. Outer membrane proteins of *Fusobacterium necrophorum* subsp. *necrophorum* and subsp. *funduliforme*. J. Basic Microbiol. 54:812–817.

Kumar, A., S. Menon, T. G. Nagaraja, and S. Narayanan. 2015. Identification of an outer membrane protein of *Fusobacterium necrophorum* subsp. *necrophorum* that binds with high affinity to bovine endothelial cells. Vet. Microbiol. 176:196–201.

Langworth, B. F. 1977. *Fusobacterium necrophorum*: its characteristics and role as an animal pathogen. Bacteriol. Rev. 41:373-390.

Lechtenberg, K. F., T. G. Nagaraja, H. W. Leipold, and M. M. Chengappa. 1988. Bacteriologic and histologic studies of hepatic abscesses in cattle. Am. J. Vet. Res. 49:58-62.

Lechtenberg, K. L. and T. G. Nagaraja. 1989. Antimicrobial sensitivity of *Fusobacterium necrophorum* isolates from bovine hepatic abscesses. J. Anim. Sci. 67:544.

Lechtenberg, K.F. and T. G. Nagaraja. 1991. Hepatic ultrasonography and blood changes in cattle with experimentally induced hepatic abscesses. Am. J. Vet. Res. 52:803-809.

Lechtenberg, K. F., T. G. Nagaraja, and J. C. Parrot. 1993. The role of *Actinomyces pyogenes* in liver abscess formation. Scientific update on Rumensin/Tylan for the professional feedlot consultant. Greenfield (IN): Elanco Animal Health, E1-6.

Lesmeister, K. E., A. J. Heinrichs, and M. T. Gabler. 2004. Effects of supplemental yeast (*Saccharomyces cerevisiae*) culture on rumen development, growth characteristics, and blood parameters in neonatal dairy calves. J. Dairy Sci. 87:1832-1839. doi:10.3168/jds.S0022-0302(04)73340-8.

Liu, P. F., W. Shi, W. Zhu, J. W. Smith, S. L. Hsieh, R. L. Gallo, and C. M. Huang. 2010. Vaccination targeting surface FomA of *Fusobacterium nucleatum* against bacterial co-aggregation: Implication for treatment of periodontal infection and halitosis. Vaccine 28:3496–3505. doi:10.1016/j.vaccine.2010.02.047.

Lundeen, T. (Ed.) 2013. Feed additive compendium, The Penton, Inc., Minneapolis, MN.

Macdonald, A.G., S. L. Bourgon, R. Palme, S. P. Miller, and Y. R. Montanholi. 2017. Evaluation of blood metabolites reflects presence or absence of liver abscesses in beef cattle. Vet. Rec. Open. 4:1-7. doi: 10.1136/vetreco-2016-000170.

Madara, J. L. 2011. Functional Morphology of Epithelium of the Small Intestine. Compr. Physiol. 83–120. doi: 10.1002/cphy.cp060403.

McCord, J. M., and I. Fridovich. 1969. Superoxide dismutase an enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244:6049-6055.

Meyer, N. F., G. E. Erickson, T. J. Klopfenstein, M. A. Greenquist, M. K. Luebbe, P. Williams, and M. A. Engstrom. 2009. Effect of essential oils, tylosin, and monensin on finishing steer performance, carcass characteristics, liver abscesses, ruminal fermentation, and digestibility. J. Anim. Sci. 87:2346-2354. doi:10.2527/jas.2008-1493.

Montgomery, T. H. 1985. The influence of liver abscesses upon beef carcass yields. Special technical bulletin. West Texas State University.

Müller, S. I., M. Valdebenito, and K. Hantke. 2009. Salmochelin, the long-overlooked catecholate siderophore of *Salmonella*. Biometals. 22:691-695. doi: 10.1007/s10534-009-9217-4.

Nagaraja, T. G., and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: A review. J. Anim. Sci. 76:287-298. doi:10.2527/1998.761287x.

Nagaraja, T. G., S. B. Laudert, and J. C. Parrott. 1996a. Liver abscesses in feedlot cattle. Part I. Causes, pathogenesis, pathology, and diagnosis. Compend. Cont. Educ. Prac. Vet. 18:S230–S241.

Nagaraja, T. G., S. B. Laudert, J. C. Parrot. 1996b. Liver abscesses in feedlot cattle, part II: incidence, economic importance and prevention. Compend. Cont. Educ. Prac. Vet. 18:S264–S273

Nagaraja, T. G., and K. F. Lechtenberg. 2007. Liver abscesses in feedlot cattle. Vet. Clin. North. Am. Food. Anim. Pract. 23:351-369. doi:10.1016/j.cvfa.2007.05.002.

Nagaraja, T. G., S. K. Narayanan, G. C. Stewart, and M. M. Chengappa. 2005. *Fusobacterium necrophorum* infections in animals: pathogenesis and pathogenic mechanisms. Anaerobe, 11:239-246. doi: 10.1016/j.anaerobe.2005.01.007.

Nagaraja, T., and R. Smith. 2000. Liver abscesses in beef cattle: Potential for dairy monitoring? Page 65–68 in Proc. 33rd Annu. Conf. Am. Assoc. Bovine Pract., Rapid City, SD. American Association of Bovine Practitioners, Auburn, AL.

Nagaraja, T.G., Y. Sun, N. Wallace, K. E. Kemp, and J. C. Parrott. 1999. Effects of tylosin on concentrations of *Fusobacterium necrophorum* and fermentation products in the rumen of cattle fed a high-concentrate diet. Am. J. Vet. Res. 60:1061-1065.

Nagaraja, T. G. and E. C. Titgemeyer. 2007. Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook 1, 2. J. Dairy Sci. 90:E17-E38. doi:10.3168/jds.2006-478.

Narayanan, S., T. G. Nagaraja, N. Wallace, J. Staats, M. M. Chengappa, and R. D. Oberst. 1998. Biochemical and ribotypic comparison of *Actinomyces pyogenes* and *A. pyogenes*-like organisms from liver abscesses, ruminal wall, and ruminal contents of cattle. Am. J. Vet. Res. 59:271-276.

Nazzaro, F., F. Fratianni, L. De Martino, R. Coppola. and V. De Feo. 2013. Effect of essential oils on pathogenic bacteria. Pharmaceuticals. 6:1451-1474. doi: 10.3390/ph6121451.

Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1998. Acidosis in cattle: a review. J. Anim. Sci. 76:275-286. doi:10.2527/1998.761275x

Pacello, F., P. Ceci, S. Ammendola, P. Pasquali, E. Chiancone, and A. Battistoni. 2008. Periplasmic Cu, Zn superoxide dismutase and cytoplasmic Dps concur in protecting *Salmonella enterica* serovar *Typhimurium* from extracellular reactive oxygen species. Biochim. Biophys. Acta. 1780:226-232. doi:10.1016/j.bbagen.2007.12.001.

Pashine, A., N. M. Valiante, and J. B. Ulmer. 2005. Targeting the innate immune response with improved vaccine adjuvants. Nat. Med. 11:S63-S68. doi:10.1038/nm1210.

Pasquini, C., T. Spurgeon, and S. Pasquini. 1997. Anatomy of Domestic Animals: Systemic and Regional Approach. 11th ed. Sudz Pub. Pilot Point, TX.

Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. Vet. J. 176:21–31. doi: 10.1016/j.tvjl.2007.12.016.

Plegge, S. D., R. D. Goodrich, S. A. Hanson, and M. A. Kirick. 1984. Predicting dry matter intake of feedlot cattle. Proc. Minnesota. Nutr. Conf. p 56

Podschun, R., and U. Ullmann. 1998. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin. Microbiol. Rev, 11:589-603.

Qu, F., Z. Fan, E. Cui, W. Zhang, C. Bao, S. Chen, Y. Mao, and D. Zhou. 2013. First report of liver abscess caused by *Salmonella enterica* serovar Dublin. J. Clin. Microbiol. 51:3140- 3142. doi: 10.1128/JCM.01034-13.

Reinhardt, C.D. and Hubbert, M.E., 2015. Control of liver abscesses in feedlot cattle: A review1. Prof. Anim. Sci. . 31:101-108. doi:10.15232/pas.2014-01364.

Rezac, D. J., D. U. Thomson, M. G. Siemens, F. L. Prouty, C. D. Reinhardt, and S. J. Bartle. 2014a. A survey of gross pathologic conditions in cull cows at slaughter in the Great Lakes region of the United States. J. Dairy Sci. 97:4227-4235. doi:10.3168/jds.2013-7636.

Richardson, G. P. 1986. Problems with causal-loop diagrams. System dynamics review. 2:158-170.

Roeber, Deborah L., P. D. Mies, C. D. Smith, K. E. Belk, T. G. Field, J. D. Tatum, J. A. Scanga, and G. C. Smith. 2001.National market cow and bull beef quality audit-1999: a survey of producer-related defects in market cows and bulls. J. Anim. Sci. 79:658-665. doi:10.2527/2001.793658x.

Saginala, S., T. G. Nagaraja, K. F. Lechtenberg, M. M. Chengappa, K. E. Kemp, and P. M. Hine. 1997. Effect of *Fusobacterium necrophorum* leukotoxoid vaccine on susceptibility to experimentally induced liver abscesses in cattle. J. Anim. Sci. 75:1160-1166. doi:10.2527/1997.7541160x.

Scanlan, C. M., and T. L. Hathcock. 1983. Bovine rumenitis-liver abscess complex: a bacteriological review. Cornell Vet. 73:288-297.

Schaible, U. E., and S. H. Kaufmann. 2004. Iron and microbial infection. Nat. Rev. Microbiol. 2:946-953. doi:10.1038/nrmicro1046.

Schnappinger, D. and W. Hillen. 1996. Tetracyclines: antibiotic action, uptake, and resistance mechanisms. Arch. Microbiol. 165:359-369.

Shinjo, T., T. Fujisawa, and T. Mitsuoka. 1991. Proposal of two subspecies of *Fusobacterium necrophorum* (Flügge) Moore and Holdeman: *Fusobacterium necrophorum* subsp. *necrophorum* subsp. nov., nom. rev.(ex Flügge 1886), and *Fusobacterium necrophorum* subsp. *funduliforme* subsp. nov., nom. rev.(ex Hallé 1898). Int. J. Syst. Evol. Microbiol. 41:395-397. doi: 10.1099/00207713-41-3-395.

Shinjo, T., K. Hiraiwa, and S. Miyazato. 1990. Recognition of biovar C of *Fusobacterium necrophorum* (Flügge) Moore and Holdeman as *Fusobacterium pseudonecrophorum* sp. nov., nom. rev.(ex Prévot 1940). Int. J. Syst. Evol. Microbiol. 40:71-73. doi:10.1099/00207713-40-1-71.

Simon, P. C., and P. L. Stovell. 1971. Isolation of *Sphaerophorus necrophorus* from bovine hepatic abscesses in British Columbia. Can. J. Comp. Med. 35:103-106.

Smith, H. A. 1944. Ulcerative lesions of the bovine rumen and their possible relation to hepatic abscesses. Am. J. Vet. Res, 5, 234-242.

Soto-Navarro, S.A., G. C. Duff, C. R. Krehbiel, M. L. Galyean, K. J. Malcom-Callis, 2000. Influence of feed intake fluctuation, feeding frequency, time of feeding and rate gain on performance by limit-fed steers. Prof. Anim. Sci. 16:13–20. doi:10.15232/S1080-7446(15)31655-7.

Stock, R. A., M. H. Sindt, J. C. Parrott, and F. K. Goedeken. 1990. Effects of grain type, roughage level and monensin level on finishing cattle performance. J. Anim. Sci. 68:3441-3455. doi:10.2527/1990.68103441x.

Tadepalli, S., S. K. Narayanan, G. C. Stewart, M. M. Chengappa, and T. G. Nagaraja. 2009. *Fusobacterium necrophorum*: a ruminal bacterium that invades liver to cause abscesses in cattle. Anaerobe. 15:36-43. doi: 10.1016/j.anaerobe.2008.05.005.

Takeuchi, S., Y. Nakajima, and K. Hashimoto. 1983. Pathogenic synergism of *Fusobacterium necrophorum* and other bacteria in formation of liver abscess in BALB/c mice. Jpn. J. Vet. Sci. 45:775−781.

Tan, Z. L., T. G. Nagaraja, and M. M. Chengappa. 1994a. Biochemical and biological characterization of ruminal Fusobacterium necrophorum. FEMS microbiology letters, 120: 81-86.

Tan, Z. L., T. G. Nagaraja, and M. M. Chengappa. 1994b. Selective enumeration of *Fusobacterium necrophorum* from the bovine rumen. Appl. Environ. Microbiol. 60:1387-1389.

Tan, Z. L., T. G. Nagaraja, and M. M. Chengappa. 1996. *Fusobacterium necrophorum* infections: virulence factors, pathogenic mechanism and control measures. Vet. Res. Commun. 20:113-140.

Tenson, T., M. Lovmar. and M. Ehrenberg, 2003. The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. J. Mol. Biol. 330:1005-1014. doi:10.1016/S0022-2836(03)00662-4.

Tiwari, B. K., V. P. Valdramidis, C. P. O'Donnell, K. Muthukumarappan, P. Bourke, and P. J. Cullen. 2009. Application of natural antimicrobials for food preservation. J. Agric. Food Chem. 57:5987-6000. doi:10.1021/jf900668n.

US CFR (US Code of Federal Regulations). 2008. Title 7, part 59. Livestock Mandatory Reporting.

US CFR (US Code of Federal Regulations). 2015. Title 21, parts 514 and 558. Veterinary feed directive; Final Rule.

USDA. 2017. NW_LS441, By-Product Drop Value (Steer). Accessed Jan. 27, 2017. https://www.ams.usda.gov/mnreports/nw_ls441.txt

Vogel, G. J., and J. C. Parrott. 1994. Mortality survey in feed yards: The incidence of death from digestive, respiratory and other causes in feed yards on the Great Plains. Compend. Contin. Educ. Pract. Vet. 16:227–234.

Wang, J.H., Y. C. Liu, S. S. Lee, M. Y. Yen, Y. S. Chen, J. H. Wang, S. R. Wann, and H. H. Lin. 1998. Primary liver abscess due to *Klebsiella pneumoniae* in Taiwan. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 26:1434-1438.

Wieser, M. F., T. R. Preston, A. Macdearmid, and A. C. Rowland. 1966. Intensive beef production. The effect of chlortetracycline on growth, feed utilisation and incidence of liver abscesses in barley beef cattle. Anim. Prod. 8:411-423.

Woodward, M. J., G. Gettinby, M. F. Breslin, J. D. Corkish, and S. Houghton. 2002. The efficacy of Salenvac, a *Salmonella enterica* subsp. Enterica serotype Enteritidis iron-restricted bacterin vaccine, in laying chickens. Avian Pathol. 31:383-392. doi: 10.1080/03079450220141660.

Wolstenholme, E. F. 2003. Towards the definition and use of a core set of archetypal structures in system dynamics. System Dynamics Review. 19:7-26.

Zinn, R. A., and A. Plascencia. 1996. Effects of forage level on the comparative feeding value of supplemental fat in growing-finishing diets for feedlot cattle. J. Anim. Sci. 74:1194-1201. doi:10.2527/1996.7461194x.

# CHAPTER 3:

# EXPLORATORY OBSERVATIONAL QUANTIFICATION OF LIVER ABSCESS INCIDENCE, SPECIFIC TO REGION AND CATTLE TYPE, AND THEIR ASSOCIATIONS TO VISCERA VALUE AND BACTERIAL FLORA

## 3.1 ABSTRACT

Liver abscesses in finished cattle have been a topic of interest since the 1940's; since that time research has been conducted to better understand their incidence, causes, and importance. Our objective was to quantify incidence and economic impact of liver abscesses and identify predominant bacterial species specific to: severity of abscesses, geographical region, and cattle type. Observational liver audits occurred at 7 fed beef (n = 130,845 livers) and 4 cull cow (n = 30,646 livers) processing facilities. At each processing facility, 30 (10 A-, 10 A, 10 A+) intact liver abscess samples were collected and cultured for *Fusobacterium necrophorum*, *Trueperella pyogenes*, and *Salmonella enterica*. Outcome frequency and economic data were analyzed using the GENMOD procedure of SAS v9.4 (SAS Inst. Inc., Cary, NC), with fixed effects of region, cattle type or liver score. Average liver abscess incidence was 20.3% for cattle harvested at fed beef processing facilities; processors in the Pacific Northwest had the greatest ($P < 0.01$) abscess incidence rate (33.8%) whereas those in the Northeast had the fewest liver abscesses (10.0%). Average liver abscess incidence was 17.6% for cull beef processing

facilities. Within cattle type, fed Holsteins had greater ($P < 0.01$) abscess incidence rates (25.0%) than fed beef steers (18.2%) or heifers (19.1%). Cull dairy cows, cull bulls, and cull range cows had total abscess incidence rates (19.3%, 19.8%, and 16.7%, respectively) similar to fed steers and fed heifers. *Fusobacterium necrophorum* subsp. *necrophorum* was present in 79.9% of samples collected from fed beef processors and 76.9% of samples from cull beef processors whereas *Trueperella pyogenes* was present in 14.8% of samples from fed beef processors and 8.8% of samples from cull beef processors. *Salmonella enterica* was present in 27.5% of abscess samples collected from fed beef processors and 16.5% of samples from cull cow processors. *Fusobacterium necrophorum*, regardless of subspecies, was present at every processing facility; whereas *S. enterica* tended to occur in processing facilities in warm and dry climates and *T. pyogenes* tended to occur in processing facilities in colder and wetter climates. Total visceral losses ($/animal) did not differ by region ($P = 0.40$) or cattle type ($P = 0.85$), yet conservative estimates indicate that liver abscesses and other liver abnormalities cost the beef industry approximately $60 million annually in viscera losses.

**Keywords**: bacterial flora; beef; Holstein; liver abscesses; viscera value

## 3.2 INTRODUCTION

Liver abscesses in fed beef have been observed and documented since Smith (1940) discussed incidence of liver abscesses and their contribution to reduced viscera yields. Of cattle harvested in 1940, 5.3% had liver abscesses; since then reported incidence has more than tripled (17.8%; Eastwood et al., 2017). Liver abscess incidence of beef-type cattle by lot may range from 0% to upwards of 90% (Brown and Lawrence, 2010), but has averaged 15% in non-Holstein beef for the last decade; while in the same period, fed Holsteins averaged 30% (Amachawadi and Nagaraja, 2016). Liver abscesses are typically poly-microbial infections that are believed to be ancillary to rumen acidosis (Nagaraja and Chengappa, 1998). Multiple species of bacteria have been cultured from liver abscesses, with the predominant flora being *Fusobacterium necrophorum*, *Trueperella pyogenes*, and *Salmonella enterica* (Lechtenberg et al., 1988; Nagaraja and Chengappa, 1998; Amachawadi and Nagaraja, 2016). However, there has been no study reported on the relationship of bacterial flora to severity of liver abscesses.

Although the pathogens and mechanisms of the liver abscess complex are similar, regional differences in liver abscess incidence have been noted. Feedlot cattle harvested at processing facilities in Texas and Kansas were reported as having a 20.6% liver abscess incidence (Rezac et al., 2014b), while cull dairy cows slaughtered in the Great Lakes region had a 32.1% liver abscess incidence (Rezac et al., 2014a). Additionally, feedlot cattle in western Canada were reported as having a 30% incidence of A and A+ classified liver abscesses (Checkley et al., 2005). In order to better understand the liver abscess complex, a comprehensive audit was needed to better understand liver abscesses incidence by geographical region and cattle type. Therefore, our objective was to

quantify incidence and economic impact of liver abscesses and identify predominant bacterial species specific to: severity of abscesses, geographical region and cattle type.

## 3.3 MATERIALS AND METHODS

Animal care and use committee approval was not required for this study because data were recorded on carcasses, and no live animals were used for sample and data collection.

### *3.3.1 Liver audit*

Observational liver audits occurred at 7 fed beef and 4 cull cow processing facilities. Processing facilities were selected to target the greatest frequency of Holsteins harvested per region and were audited for one week of production from October 2015 through March 2016. Fed beef processors evaluated were located in the following regions: Central Plains (n = 25,813), Desert Southwest (n = 10,119), High Plains (n = 27,034), Northeast (n = 7,958), North Plains (n = 50,671), and Pacific Northwest (n = 9,250). Cull beef processors evaluated were located in the following regions: Great Lakes (n = 7,680), High Plains (n = 9,654), Northeast (n = 7,791), and West Coast (n = 5,521). For all regions, except for the North Plains (facilities = 2), only one processing facility by type (cull or fed) was audited. Names and locations of processing facilities were kept anonymous to ensure that liver abnormality and bacterial prevalence outcomes were not equated to an individual processor.

Within each processing facility, liver abscesses were visually assessed and scored according to a modified scoring system based on the Elanco Liver Check Service (✓ = no abscesses, A- = 1 or 2 small abscesses, A = 2 to 4 small active abscesses, A+ = multiple

small abscesses or 1 or more large active abscesses, A+ Adhesion = liver adhered to GI tract, and A+ Open = open liver abscess). Other recorded liver abnormalities included: cirrhosis, flukes, telangiectasis, carotenosis, sawdust, torn, and if a liver was condemned for contamination (adapted from Brown & Lawrence, 2010). Liver abscesses and other abnormalities were documented by individual carcass and matched with processor lot, sex, cattle type, and any additional information available. Liver abscess and other abnormalities are reported by processing facility (fed beef or cull beef) and by cattle type. Within each processing facility, various cattle types were processed; therefore, data were reorganized to clarify incidence of liver abscesses and other abnormalities regardless of region or processing facility.

### *3.3.2 Visceral loss*

Visceral loss was estimated from the USDA Agricultural Marketing Service by-product drop value (steer) report (NW_LS441; USDA, October 2015 through March 2016, Friday report of every week) and weekly USDA by-product drop value (cow) report (NW_LS444; USDA, Oct 2015 through March 2016). Value of lost viscera was estimated using the unrealized revenue from condemned liver or condemned liver and gut mass (hearts, reg, bone out; tripe, scalded edible, bleached; tripe, honeycomb bleached; lungs, inedible; melts) when an open abscesses occurred. Using those reports, the average edible fed beef liver was valued at $6.44 and every edible cull beef liver was valued at $2.23. Similarly, an edible fed beef gastrointestinal (GI) was valued at $14.84 and an edible cull beef GI mass was valued at $16.14. When a liver or GI mass is condemned by USDA, the product goes to rendering and is salvaged as inedible meat and bone meal. Regardless of whether the GI mass is passed or condemned by USDA

inspectors, fat deposits from around the viscera are salvaged as inedible tallow. These values were not included in viscera loss calculations because tallow is salvaged regardless of condemnation.

Meat and bone meal salvage values were estimated using the same reports and time periods. A rendered fed beef liver was valued at $0.39 whereas a rendered liver from cull beef was valued at $0.28. Value of rendered GI mass was estimated to be $0.72 for fed beef and $0.83 for cull beef. To estimate lost value per animal, the value of rendered product was subtracted from the value of edible product to establish a non-region specific baseline loss for fed beef viscera and cull beef viscera. Once adjusted, liver values were then multiplied by total abscessed liver rate, total contamination rate, and total other abnormality rate for each cull and fed beef processor. Also, GI mass value was multiplied by the open abscess rate, because GI mass is condemned when an open abscess is present. Values were summed to quantify total liver and GI mass value (i.e $6.05 x liver loss (abscess+contamination+other) + $14.12 x open abscesses). Then total, value was divided by the total number of animals evaluated per processing facility to generate viscera losses per animal, by region and type.

Once viscera loss per animal was generated, by region and type, calculations were scaled to a national level. National slaughter numbers were generated using the USDA estimated daily Livestock Slaughter under Federal Inspection report (SJ_LS710; USDA, Jan. 2017). Estimated daily livestock slaughter is approximately 113,000 animals/day, 80% (90,400) of that number are fed beef and 20% (22,600) are cull beef. Using a 5.5 day work week, 286 slaughter days in the U.S. were estimated, resulting in approximately

25,854,400 fed beef and 6,463,600 cull beef are slaughtered per year. Data are reported in dollars per animal.

### *3.3.3 Bacterial sampling and analysis*

At each processing facility, 30 (10 A-, 10 A, 10 A+) intact liver abscess samples were collected. Due to lab schedules and sampling constraints presented by processors, samples were not collected on the same day and were not able to be collected in a matter that would balance cattle type by region. Therefore, samples were collected on a "first-come, first-sampled" basis at the processing facility; consequently, only region-specific differences in bacterial prevalence was statistically analyzed. Intact liver abscesses were excised from the liver to preserve the anaerobic environment inside the abscess. Samples were individually identified in plastic bags, placed in coolers packed with ice, and shipped overnight to Kansas State University to determine incidence of *Fusobacterium necrophorum*, *Trueperella pyogenes*, and *Salmonella enterica*. The procedures described by Amachawadi et al. (2017) were used for isolation and identification of the three bacterial species.

The abscess was opened by searing the surface of the abscess with a hot spatula and incising the capsule with a sterile scalpel. Once opened, inside wall of the abscess was swabbed and streaked onto three plates of blood agar (Remel, Lenexa, KS), and with a different swab, two plates of Hektoen-Enteric (HE) agar (Beckton and Dickson, Sparks, MD; for isolation of *Salmonella*). One blood agar plate and one HE plate were incubated anaerobically inside an Anaerobic Glove Box (Thermo Fisher Scientific Inc., Waltham, MA) and, another set of blood agar and HE plates were incubated aerobically. The blood agar plate for *Trueperella pyogenes* isolation was incubated in a 5% $CO_2$ incubator. In

addition to direct plating on HE plate, samples for *Salmonella* determination were also subjected to an enrichment step, first in tetrathionate broth (Beckton and Dickson; at 37 ºC for 24 h), and then in Rappaport-Vassiliadis broth (Beckton and Dickson; at 37 ºC for 24 h) before plating onto HE agar and incubated at 37 ºC for 24 h. Colonies presumed to be *Salmonella* were tested by agglutination with *Salmonella* polyvalent O antiserum (Beckton and Dickson) for genus confirmation. If positive, the colony was then subjected to B, C1, C2, D1, D2, and E antisera for serogroup identification.

Presumptive *F. necrophorum* colonies were cultured in Brain Heart Infusion broth that had been pre-reduced with 0.05% cysteine HCl and anaerobically sterilized (BHI-S). Purity of possible *F. necrophorum* isolates were verified, microscopic morphology was determined, and species confirmation was with the RapId-ANA II test kit (Thermo Fisher Scientific Inc.). *Fusobacterium necrophorum* isolates were subspeciated (*necrophorum* or *fundiliforme*) based on sedimentation characteristics in BIH-S broth and phosphate test as described by Tan et al. (1994). Likely *T. pyogenes* colonies, identified as pin-point colonies with a narrow encompassing zone of beta hemolysis, were inoculated into tryptic soy broth (Beckton Dickson) and re-plated on blood agar plates to confirm purity of the isolate. The species confirmation of *Salmonella* and *T. pyogenes* was by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF; Bruker Daltonics Inc., Billerica, MA) mass spectrometry (Veterinary Diagnostic Lab, Kansas State University, Manhattan, KS).

### *3.3.4 Statistical analysis*

This experiment was a non-randomized observational study with carcass or liver as the experimental unit. Outcome frequency and economic data were analyzed using the

GENMOD procedure of SAS v9.4 (SAS Inst. Inc., Cary, NC), with the fixed effects of region, cattle type or liver score. Least square means were generated and separated using the PDIFF option with a Tukey-Kramer adjustment for multiple comparisons. Significance was declared at $P \leq 0.05$.

## 3.4 RESULTS AND DISCUSSION

### *3.4.1 Liver abscess and other abnormality incidence by region and cattle type*

Average liver abscess incidence was 20.3% (Table 3.1) for cattle harvested at fed beef processing facilities, which is consistent with the 2016 National Beef Quality Audit (NBQA) report (Eastwood et al., 2017). Within fed beef processing facilities, High Plains and Northeast regions had the greatest ($P < 0.01$) edible liver incidence rates (76.9 and 72.9%, respectively) whereas the Pacific Northwest region had the lowest edible liver incidence rate (46.8%). Furthermore, the greatest ($P < 0.01$) total abscess incidence rate for a fed beef processor occurred in the Pacific Northwest (33.8%) whereas the Northeast region had the fewest liver abscesses (10.0%). Cattle in the Northeast are typically fed a silage based ration, which may have contributed to reduced abscess incidence rates, since a "traditional" feedlot diet is grain corn or concentrate based and not silage based. Silage has a larger particle size than most concentrate feeds, and larger particle size promotes increased feeding durations and decreased rate of intake (Allen, 1996; Addah et al., 2016); leading to a slower rate of fermentation and a potential decrease in the incidence of acidosis.

Grain type has been shown to affect incidence of liver abscesses (Hale, 1985) and in the U.S. Pacific Northwest and Western Canada regions, wheat and barley are

commonly produced and incorporated into feedlot rations (Nelson et al., 2000; Beauchemin and Koening, 2005). These grains are rapidly fermented in the rumen, which allows for greater variations in ruminal pH and the subsequent development of acidosis, rumenitis and liver abscesses (Nagaraja and Chengappa, 1998). Cattle in this region are more likely to encounter diets containing these ingredients which may explain the increased liver abscess incidence observed at the processor in the Pacific Northwest region. Cattle from the Pacific Northwest region also had the greatest ($P < 0.01$) incidence of other abnormalities of the fed beef facilities (10.2% vs. 2.8%, respectively), due to increased incidence of liver flukes, which contributed to the decreased rate of edible livers. The lifecycle of liver flukes is dependent on its snail intermediate host; therefore, the distribution of the parasite is limited to geographical areas (Gulf Coast and Pacific Northwest) where annual rainfall is high and pastures are poorly drained (Kaplan, 2001).

Average liver abscess incidence was 17.6% for cull beef processing facilities (Table 3.1). Within cull beef processing facilities, cattle from the Great Lakes region had the greatest ($P < 0.01$) incidence of edible livers (76.4%) whereas cattle from the High Plains and Northeast regions had the lowest edible liver incidence rate (56.7 and 57.8%, respectively). Cull animals from the High Plains region had the greatest ($P < 0.01$) total abscess incidence rate (22.6%) whereas the Northeast region had the fewest total abscesses (13.7%). It is possible that dairy herd size may have affected observed liver abscess incidence by region. The High Plains region is becoming a major dairy producing region, where milk production increased by 6.2 billion pounds from 1994 to 2006 and the average herd size in the region is greater than 500 head (MacDonald et al.,

2007). In the same time period, milk production in the Great Lakes remained steady and the average herd size in the region remained less than 100 head (MacDonald et al., 2007). When Hadley et al. (2006) analyzed dairy culling trends by herd size and region, they found that the primary reason cattle were culled from small herds (<300 head) was injury; although injury remains the principal culling factor in larger herds, additional culling factors (i.e. disease, mastitis, production) increased as herd size increased. Although data is lacking in literature, liver abscesses could affect milk production, due to decreased metabolic efficiency of the liver, which would consequently be an artifact of dairy cattle culled for decreased milk production.

When data were segregated by cattle type (Table 3.2), Holsteins had greater ($P <$ 0.01) abscess incidence rates (25.0%) than fed beef steers (18.2%) or heifers (19.1%). Reasons for greater liver abscess incidence in Holsteins are not known, but the predominant theory is based upon increased days on a high-energy diet (Amachawadi and Nagaraja, 2016). Each additional day an animal consumes a high concentrate diet increases the risk to develop an abscess; Holsteins are on feed for longer periods of time than conventional fed beef animals (300 to 400 DOF for Holsteins vs. 120 to 150 DOF in non-Holstein beef; Vogel and Parrott, 1994; Duff and McMurphy, 2007). Additionally, Holstein steers have a greater daily DMI (on average, up to 12% greater) than beef breeds at similar weights (Hicks et al., 1990), which is attributed to an increased maintenance energy demand due to a greater proportion of GI and organ tissue. Furthermore, increased DMI increases the fermentable substrate in the rumen, allowing for prolonged ruminal fermentation and greater decreases in rumen pH (Owens et al., 1998).

It is also likely that management of Holstein calves might affect rumen health and total abscess incidence. Holstein calves are commonly removed from their dams immediately after birth and are bottle-fed as they are transitioned to concentrate diets, with some calves being completely weaned by 4 weeks of age (Franklin et al., 2003). Although introduction of easily fermentable carbohydrates along with early ruminal muscular development due to GI fill allow for early weaning and ruminal development of Holstein calves, few precautions are taken to mitigate the development of acidosis in calves (Kristensen et al., 2007). Calves that ingested corn-based starter diets had subsequent decreased ruminal pH, with measurements nearing a pH of 5.5 (Anderson et al., 1987) which has been considered by some to be at risk for subacute acidosis (Nordlund et al., 2004).

Generally, chronic acidosis is considered when ruminal pH decreases to 5.6, while acute acidosis is when ruminal pH is below 5.2 (Owens et al., 1998). Calves with measured ruminal pH near 5.5 are not nearing acute acidotic conditions, but the amount of time subjected to suboptimal ruminal pH may have unknown long-term effects. However, what is still unknown is how long a rumen must be subjected to suboptimal pH before detrimental damage is inflicted upon the rumen microbial community, resulting in subsequent decreases in digestion and rumen function (Nagaraja and Titgemeyer, 2007). Like feedlot cattle, calves may experience acidosis, and damage to the epithelial lining of the rumen may promote the development of rumen wall abscesses (Nagaraja and Chengappa, 1998). Feedlot cattle undergo these conditions in the later stages of their lives whereas Holstein calves undergo them from earlier on, in some instances, because they spend less time on milk and are transitioned to an energy intensive diet before most

non-Holstein beef. The increased potential for acidotic events throughout the life of a fed Holstein may further contribute to the increased incidence of liver abscesses exhibited.

Cull beef had abscess incidence rates (18.8%) similar ($P = 0.99$) to fed beef (20.0%; Table 3.2). Cull dairy cows and cull bulls exhibited total abscess incidence rates (19.3% and 19.8%, respectively) similar ($P > 0.10$) to fed steers and heifers (18.2% and 19.1%, respectively). Because the conditions animals were subjected to prior to slaughter were unattained, it is possible that some cull beef could have been fed an energy intensive diet prior to slaughter. Within cull beef, cull range cows had an abscess incidence rate (16.7%) similar ($P = 0.40$) to cull dairy cows (19.8%), which was unexpected because cull range cows are not fed diets similar to dairy cows or fed beef animals, unless fed prior to slaughter. National Non-Fed Beef Quality Audits have reported liver condemnation rates in cull beef as 30.8% (1994) and 24.1% (1999), with 27.3% of liver condemnations in the 1999 audit being from liver abscesses (e.g. 6.6% liver abscess incidence; Roeber et al., 2001). The 2007 National Market Cow and Bull Beef Quality Audit reported 14% liver abscess incidence in cull beef (Hale et al., 2007), while a more recent survey reported 32% incidence of liver abscesses in cull beef; however 87% of that population were Holstein cows (Rezac et al., 2014a).

It is possible that cull range cows develop liver abscesses from *F. necrophorum* entering portal circulation via routes other than through rumen wall abscesses caused by acidotic events. *Fusobacterium necrophorum* is able to survive in the soil of pastures and has been isolated from the oral cavity, gastrointestinal tract, and genitourinary tract of normal, healthy animals (Langworth, 1977). Furthermore, it has been reported that liver abscesses may be formed as a result of traumatic reticuloperotinitis ("hardware disease";

Nagaraja and Smith, 2000). Because *F. necrophorum* is ubiquitous to the gastrointestinal tract of ruminants, tears or cuts in the epithelial lining of the GIT may provide a route for this bacterium to enter portal circulation. Range cows are more likely to interact with debris and harsh feedstuffs that may cause trauma to the lining of the mouth or esophagus, which could explain why cattle not fed a concentrate-based ration have liver abscess incidences similar to feedlot and dairy cattle.

### *3.4.2 Bacterial incidence by region and liver score*

*Fusobacterium necrophorum* subsp. *necrophorum* was present in 79.9% of abscesses from fed beef processing facilities and in 76.9% of abscesses from cull beef plants (Table 3.3). These data are consistent with incidence rates reported by Nagaraja and Chengappa (1998). Fed beef processing facilities in the Central Plains and High Plains regions had the greatest ($P < 0.05$) incidence of *F. necrophorum* subsp. *necrophorum* (88.0 and 88.5%, respectively) in liver abscesses when compared to other geographic regions. *Fusobacterium necrophorum* subsp. *funduliforme* was isolated from 24.3% of abscess samples taken from fed beef processors, and 17.6% of abscess samples collected at cull beef facilities. Liver abscesses cultured from the Pacific Northwest region had the greatest ($P < 0.05$) incidence (44.0%) of *F. necrophorum* subsp. *funduliforme,* whereas abscesses cultured from cull beef processors in the High Plains (8.3%) and West Coast (8.7%) regions had the lowest incidence. Of the two subspecies isolated, *F. necrophorum* subsp. *necrophorum* is the more virulent strain of *F. necrophorum* due to its increased production of leukotoxin (Nagaraja and Chengappa, 1998). Leukotoxin protects the organism against phagocytosis, and in addition, the resulting lysis of leukocytes (white blood cells) releases products that are cytolytic to

hepatic parenchymal cells contributing to the accumulation of purulent and necrotic material in the liver (Tan et al., 1996), which forms the basis of a liver abscess.

*Trueperella pyogenes* was present in 14.8% of abscesses from fed beef processing facilities and in 8.8% of abscesses from cull beef processors (Table 3.3). However, *T. pyogenes* was not isolated from liver abscesses collected from every processor. *Trueperella pyogenes* was not detected in liver abscesses from fed beef facilities in: Central Plains, Desert Southwest, and High Plains regions or at cull beef facilities in the West Coast region. In contrast, *T. pyogenes* was present in 60% of Pacific Northwest samples. The Central Plains, Desert Southwest, High Plains, and West Coast regions average annual rainfall less than 50.8 cm and can reach temperatures in excess of 37°C in the summer, whereas the Pacific Northwest and Northeast regions can average 119 cm of rainfall and average max temperatures of 26°C in summer (U.S. Climate Data). *Trueperella pyogenes* was primarily isolated from samples collected in geographical regions with colder climates and increased rainfall. Regardless of average temperature and rainfall, it is surprising that *T. pyogenes* was not isolated from several processing facilities.

*Salmonella enterica* was present in 27.5% of abscess samples collected from fed beef processors and 16.5% of samples from cull cow processors (Table 3.3). Similarly to *T. pyogenes*, *S. enterica* was not present in liver abscesses from every processing facility. *Salmonella enterica* was not isolated in samples collected from fed beef processors in North Plains – A, North Plains – B, and Pacific Northwest or cull beef processors in High Plains, Northeast, and West Coast. *Salmonella enterica* was recently discovered in beef liver abscesses (Amachawadi and Nagaraja, 2015) and at this time it is not known if *S.*

*enterica* is an etiologic agent or whether it enters after an abscess is initiated by *F. necrophorum*. *Salmonella enterica* has also been isolated from lymph nodes of slaughtered beef cattle (Gragg et al., 2013). When swine were orally inoculated with *S. enterica*, samples collected from lymphatic tissue and synovial fluid were positive for *Salmonella* (Broadway et al., 2015), suggesting that *Salmonella* is able to migrate from the gastrointestinal tract to musculoskeletal lymph nodes. Furthermore, *S. enterica* has been isolated from retail ground beef (Zhao et al., 2002); however, it is possible that samples were cross contaminated at the retail level prior to packaging. Isolation of *S. enterica* from beef liver and lymphatic tissue indicates that *Salmonella* may present future food safety issues in a variety of beef products.

Although the three major bacterial species were isolated in the study, not all species were present within the same liver abscess sample (Table 3.4). *Fusobacterium necrophorum*, regardless of subspecies, and *T. pyogenes* were present together in 14.3% of abscesses from fed beef facilities and 8.8% of samples collected from cull cow processing plants. However, these bacteria were not isolated together in samples cultured from the Central Plains, Desert Southwest, High Plains (fed beef), or West Coast regions, because no *T. pyogenes* was isolated from samples collected in these geographical regions. Of all the geographic regions, the Pacific Northwest had the greatest ($P < 0.01$) incidence (56.0%) of both *F. necrophorum* and *T. pyogenes* within the same liver abscess.

The Pacific Northwest also had the greatest total abscess incidence out of all the geographical regions, which may result from the increased incidence of *F. necrophorum* and *T. pyogenes*, together, within liver abscesses. A pathogenic synergy exists between

*F. necrophorum* and *T. pyogenes* (Tadepalli et al., 2009). *Trueperella pyogenes* utilizes oxygen to create anaerobic conditions, which creates a favorable environment for *F. necrophorum*; whereas the waste product of *T. pyogenes* is lactic acid, which is the primary energy substrate of *F. necrophorum*. Furthermore, it has been demonstrated that mice inoculated with a sub-infective dose of *F. necrophorum* developed liver abscesses after they were inoculated with *T. pyogenes* (Lechtenberg et al., 1993). These two pathogens aid each other in survival within the host and ultimately result in increased liver abscess incidence.

*Fusobacterium necrophorum* and *S. enterica* were present together in 23.8% of abscess samples from fed beef plants and 16.5% of samples collected from cull cow processing plants. Within fed beef processing facilities, cattle from the North Plains (A or B) and the Pacific Northwest regions did not exhibit both bacterial species within the same abscess, because samples cultured from these regions did not contain *S. enterica*. Within cull beef processing facilities, samples collected from cattle in the High Plains, Northeast, and West Coast regions, also did not have viable *F. necrophorum* and *S. enterica* within the same abscess sample. When these bacterial species did occur within the same abscess sample, samples from the High Plains fed beef processor and the Great Lakes cull beef processor had high frequencies together (76.7% and 68.2%, respectively), due to the high incidence of *S. enterica* that was present in both of those regions. It is possible that pathogenic synergy exists between *F. necrophorum* and *S. enterica*, because both have potent virulence factors and are capable of surviving in various environments; however, *S. enterica* was fairly recently discovered in beef liver abscesses and the interactions that exist between these two bacterial species are yet to be fully defined.

Finally, *T. pyogenes* and *S. enterica* were present together in 0.5% of abscess samples from fed beef processing facilities and 2.2% of samples from cull cow processing facilities. The combination of these two pathogens within the same abscess was infrequent; with occurrence only in samples from 2 regions (Northeast fed beef and Great Lakes cull beef facilities). Of the over 300 abscess samples cultured, only three abscesses had both *T. pyogenes* and *S. enterica* within the same liver abscess sample. Additionally, those three samples were the only abscesses cultures that had all three bacterial species cultured for present.

Data exists on *Salmonella* in beef liver abscesses (Amachawadi and Nagaraja, 2015; Amachawadi and Nagaraja, 2016; Amachawadi et al., 2017) however its ability to interact with *T. pyogenes* in these conditions is limited. Recent research has reported the incidence of *T. pyogenes* and *S. enterica* within the same liver abscess sample, with crossbred cattle having an incidence of 15.4% and fed Holsteins having an incidence of 5.8%; which was less than the mixed infection incidence of *F. necrophorum* and *S. enterica* or *F. necrophorum* and *T. pyogenes* (Amachawadi et al., 2017). Additional research is still needed to better understand the relationship between *T. pyogenes* and *S. enterica* within beef liver abscesses; however, these data suggest that there might be some competitive inhibition between these two bacteria, which may explain their rare incidence together with liver abscesses.

When segregated by abscess severity (Table 3.5), A+ abscesses had the greatest ($P < 0.01$) incidence rate for *F. necrophorum* subsp. *necrophorum* (100.0%) and *S. enterica* (27.7%), compared to A (62.2% and 23.3%, respectively) and A- (71.8% and 20.5%, respectively) abscesses. *Trueperella pyogenes* incidence increased ($P < 0.01$) as

abscess size increased from A- to A, but incidence did not differ ($P > 0.05$) between A and A+ abscesses (9.0, 13.3 and 13.9%, respectively). Generally, as abscess severity score increased, bacteriological incidence increased.

Once *S. enterica* was isolated, serogroup identification was determined for each isolate and frequency of each was segregated by liver score (Table 3.5). Of the 8 unique serotypes isolated, the most common serotypes observed were: Anatum (20 isolates), Lubbock (16 isolates), and Montevideo (18 isolates). Until recently, *Salmonella* Lubbock was the predominant serotype isolated from beef liver abscess samples (Amachawadi and Nagaraja, 2015). However, when liver abscesses were collected from cattle that originated from 22 feedlots in the Central Plains, Desert Southwest, and High Plains regions that were slaughtered in 6 abattoirs throughout Arizona, California, Colorado, and Kansas, researchers isolated 5 unique serotypes of *Salmonella* (Lubbock, Agona, Cerro, Give, and Muenster; Amachawadi, 2017). The greater diversity of *Salmonella* serotypes observed in this experiment is likely due to the increased diversity of geographical regions sampled. Although not from liver abscess samples, fecal samples from multiple feedlots have yielded results similar to what was observed in this experiment. Fecal samples from feedlot and range cattle have yielded other serotypes of *Salmonella*, with Anatum and Montevideo being the most common in feedlot cattle (Fedorka-Cray et al., 1998), whereas Oranienburg and Cerro were the most prevalent serotypes cultured from range cows (Dargatz et al., 2000). *Salmonella* are facultatively anaerobic pathogens that can rapidly adapt to new environments and are known to be rather virulent in anaerobic conditions (Amachawadi and Nagaraja, 2015). The presence of various serotypes of *Salmonella* in the feces of feedlot and range beef indicates that

these pathogens are present in the gastrointestinal tract and if one serotype is able to pass through the epithelial lining of the gut it is unsurprising that others are able to enter portal circulation as well.

### *3.4.3 Bacterial incidence by cattle type*

Incidence of bacterial species was also segregated by cattle type (Table 3.6). Fed beef, regardless of region and individual type, had an 81.3% incidence of *F. necrophorum* subsp. *necrophorum*, 25.9% incidence of *F. necrophorum* subsp. *funduliforme*, 14.5% incidence of *T. pyogenes*, and 29.5% incidence of *S. enterica*. Cull cattle, regardless of region and individual type, had 73.7% incidence of *F. necrophorum* subsp. *necrophorum*, 10.5 % incidence of *F. necrophorum* subsp. *funduliforme*, 6.6% incidence of *T. pyogenes*, and 10.5% incidence of *S. enterica*. It is interesting to note that greater than 40% of fed Holstein samples contained *S. enterica*, and other than fed cattle from mixed lots and bulls, all cattle types had samples positive for all bacterial species selectively cultured. It is likely that bulls and cattle from mixed lots would have had samples positive for *F. necrophorum* subsp. *funduliforme*, *T. pyogenes*, or *S. enterica*, but those groups had low sample numbers and were not collected throughout all geographical regions.

### *3.4.4 Estimated visceral losses due to liver abscesses and other abnormalities*

No differences ($P = 0.48$; Table 3.1) in total visceral losses ($/animal) were noted by region or cattle type ($P = 0.86$; Table 3.2). Fed beef losses were estimated at $2.05/animal due to liver abscess and other abnormalities, whereas cull beef losses were estimated at $1.05/animal. Total viscera losses are comprised of: losses due to liver abscess ($1.46/animal, fed beef; $0.60/animal, cull beef), losses due to liver

contamination ($0.43/animal, fed beef; $0.23/animal, cull beef), and losses due to other abnormalities ($0.16/animal, fed beef; $0.22/animal, cull beef). Therefore, using calculated values for national, annual fed and cull beef slaughter cattle, viscera losses for fed beef are estimated at $53.1 million ($37.7 million due to liver abscesses, $11.1 million due to contamination, and $4.3 million due to other abnormalities) annually and $6.8 million ($3.9 million due to liver abscesses, $1.5 million due to contamination, and $1.4 million due to other abnormalities) for cull beef. Based on conservative estimates liver abscesses and other abnormalities cost beef processors approximately $60 million annually in viscera losses.

## 3.5 IMPLICATIONS AND FUTURE RESEARCH

Liver abscesses in feedlot cattle significantly impact the beef industry, not only from the loss of a condemned liver and often all viscera, but also through reduced animal performance, diminished carcass yield, and decreased processor efficiency. Generally, as size of the abscess increased, bacteriological incidence increased. However, *T. pyogenes* and *S. enterica* were rarely present together within the same abscess. Using conservative estimates, viscera losses were approximated at $60 million annually. Because the incidence and severity of abscesses are increased in Holsteins, their potential to negatively impact the industry is magnified.

These data will be used in conjunction with survey data, focusing on diet and management practices, to better understand the relationships between cattle feeding inputs and liver abscess outcomes. Furthermore, these data will allow generation of

predictive models by input condition for liver abscess outcomes, thus providing the beef industry knowledge to manage risk and the incidence of liver abscesses in their herd.

## 3.6 LITERATURE CITED

Addah, W., J. Baah, and T. A. McAllister. 2016. Effect of silage chop length on feed intake and feeding behaviour of finishing feedlot steers. Acta Agriculturae Scandinavica, Section A—Animal Science. 66: 106-114. doi: 10.1080/09064702.2016.1267789.

Allen, M. S. 1996. Physical Constraints on Voluntary Intake of Forages by Ruminants1. J. Anim. Sci. 74:3063-3075. doi:10.2527/1996.74123063x.

Amachawadi, R. G., and T. G. Nagaraja. 2015. First report of anaerobic isolation of Salmonella enterica from liver abscesses of feedlot cattle. J. Clin. Microbiol. 53:3100-3101. doi:10.1128/JCM.01111-15.

Amachawadi, R. G., and T. G. Nagaraja. 2016. Liver abscesses in cattle: A review of incidence in Holsteins and of bacteriology and vaccine approaches to control in feedlot cattle. J. Anim. Sci. 94:1620-1632. doi:10.2527/jas2015-0261.

Amachawadi, R. G.,T. J. Purvis, B. V. Lubbers, J. W. Homm, C. L. Maxwell, and T. G. Nagaraja. 2017. Bacterial flora of liver abscesses in crossbred beef cattle and Holstein steers fed finishing diets with or without tylosin. J. Anim. Sci. 95:3425-3434. doi:10.2527/jas2016.1198.

Anderson, K. L., T. G. Nagaraja, and J. L. Morrill. 1987. Ruminal metabolic development in calves weaned conventionally or early. J. Dairy Sci. 70:1000–1005.

Beauchemin, K A., and K. M. Koenig. 2005. Feedlot cattle diets based on barley or corn supplemented with dry corn gluten feed evaluated using the NRC and CNCPS beef models. Can. J. Anim. Sci. 85: 365-375. doi:10.4141/A04-060.

Broadway, P. R., J. A. Carroll, J. C. Brooks, J. R. Donaldson, N. C. Sanchez, T. B. Schmidt, T. R. Brown, and T R. Callaway. 2015. Salmonella prevalence of lymph nodes and synovial fluid of orally inoculated swine. Agric. Food Anal. Bacteriol. 5:6-14.

Brown, T. R., and T. E. Lawrence. 2010. Association of liver abnormalities with carcass grading performance and value. J. Anim. Sci. 88:4037-4043. doi: 10.2527/jas.2010-3219.

Checkley, S. L., E. D. Janzen, J. R. Campbell, and J. J. McKinnon. 2005. Efficacy of vaccination against Fusobacterium necrophorum infection for control of liver abscesses and footrot in feedlot cattle in western Canada. Can. Vet. J. 46:1002–1007.

Dargatz, D. A., P. J. Fedorka-Cray, S. R. Ladely, and K. E. Ferris. 2000. Survey of Salmonella serotypes shed in feces of beef cows and their antimicrobial susceptibility patterns. J. Food Prot. 63:1648-1653. doi:10.4315/0362-028X-63.12.1648.

Duff, G. C., and C. P. McMurphy. 2007. Feeding Holstein steers from start to finish. Vet. Clin. North Am. Food Anim. Pract. 23:281–297. doi:10.1016/j.cvfa.2007.04.003.

Eastwood, L. C., C. A. Boykin, M. K. Harris, A. N. Arnold, D. S. Hale, C. R. Kerth, D. B. Griffin, J. W. Savell, K. E. Belk, D. R. Woerner, J. D. Hasty, R. J. Delmore, J. N. Martin, T. E. Lawrence, T. J. McEvers, D. L. VanOverbeke, G. G. Mafi, M. M. Pfeiffer, T. B. Schmidt, R. J. Maddock, D. D. Johnson, C. C. Carr, J. M. Scheffler, T. D. Pringle, and A. M. Stelzleni. 2017. National Beef Quality Audit-2016: Transportation, mobility, and harvest-floor assessments of targeted characteristics that affect quality and value of cattle, carcasses, and by-products. Trans. Anim. Sci. 1:229-238. doi:10.2527/tas2017.0029.

Fedorka-Cray, P. J., D. A. Dargatz, L. A. Thomas, and J. T. Gray. 1998. Survey of Salmonella Serotypes in Feedlot Cattle. J. Food Prot. 61:525-530. doi:10.4315/0362-028X-61.5.525.

Franklin, S. T., D. M. Amaral-Phillips, J. A. Jackson, and A. A. Campbell. 2003. Health and performance of Holstein calves that suckled or were hand-fed colostrum and were fed one of three physical forms of starter. J. Dairy Sci. 86:2145-2153. doi:10.3168/jds.S0022-0302(03)73804-1.

Gragg, S. E., G. H. Loneragan, M M Brashears, T. M. Arthur, J. M. Bosilevac, N. Kalchayanand, R. Wang, J. W. Schmidt, J. C. Brooks, S. D. Shackelford, T. L. Wheeler, T. R. Brown, T. S. Edrington, and D. M. Brichta-Harhay. 2013. Cross-sectional study examining Salmonella enterica carriage in subiliac lymph nodes of cull and feedlot cattle at harvest. Foodborne Pathog. Dis. 10:368-374. doi: 10.1089/fpd.2012.1275.

Hadley, G. L., C. A. Wolf, and S. B. Harsh. 2006. Dairy cattle culling patterns, explanations, and implications. J. Dairy Sci. 89:2286-2296. doi:10.3168/jds.S0022-0302(06)72300-1.

Hale, D., J. Savell, R. Delmore, D. Johnson, T. Pringle, W. Henning, R. Maddock, T. Lawrence, and J. Nicholson. 2007. National market cow and bull beef quality audit-2007: A survey of producer-related defects. Final Report. National Cattlemen's Beef Association, Centennial, CO.

Hale, W. H. 1985. Liver abscesses and founder. Anim. Nutr. Health. Sept: 12−20.

Hicks, R. B., Owens, F. N., Gill, D. R., Oltjen, J. W., and R. P. Lake. 1990. Daily dry matter intake by feedlot cattle: influence of breed and gender. J. Anim. Sci. 68:245-253.

Kaplan, R. M. 2001. Fasciola hepatica: a review of the economic impact in cattle and considerations for control. Vet. Ther. 2:40-50.

Kristensen, N. B., J. Sehested, S. K. Jensen, and M. Vestergaard. 2007. Effect of milk allowance on concentrate intake, ruminal environment, and ruminal development in milk-fed Holstein calves. J. Dairy Sci.. 90:4346-4355. doi:10.3168/jds.2006-885.

Langworth, B. F. 1977. Fusobacterium necrophorum: its characteristics and role as an animal pathogen. Bacteriol. Rev. 41:373-390.

Lechtenberg, K. F., T. G. Nagaraja, H. W. Leipold, and M. M. Chengappa. 1988. Bacteriologic and histologic studies of hepatic abscesses in cattle. Am. J. Vet. Res. 49: 58-62.

Lechtenberg, K. F., T. G. Nagaraja, and J. C. Parrot. 1993. The role of Actinomyces pyogenes in liver abscess formation. Scientific update on Rumensin/Tylan for the professional feedlot consultant. Greenfield, IN: Elanco Animal Health, E1-6.

MacDonald, J. M., W. D. McBride, E. O'Donoghue, R. F. Nehring, C. Sandretto, and R. Mosheim. 2007. Profits, costs, and the changing structure of dairy farming. USDA Economic Research Report Number 47.

Nagaraja, T. G., and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: a review. J. Anim. Sci. 76:287-298. doi:10.2527/1998.761287x.

Nagaraja, T., and R. Smith. 2000. Liver abscesses in beef cattle: Potential for dairy monitoring? Page 65–68 in Proc. 33rd Annu. Conf. Am. Assoc. Bovine Pract., Rapid City, SD. American Association of Bovine Practitioners, Auburn, AL.

Nagaraja, T. G. and E. C. Titgemeyer. 2007. Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook 1, 2. J. Dairy Sci. 90:E17-E38. doi:10.3168/jds.2006-478.

Nelson, M. L., J. R. Busboom, J. D. Cronrath, L. Falen, and A. Blankenbaker. 2000. Effects of graded levels of potato by-products in barley-and corn-based beef feedlot diets: I. Feedlot performance, carcass traits, meat composition, and appearance. J. Anim. Sci. 78:1829-1836. doi:10.2527/2000.7871829x.

Nordlund, K. V., N. B. Cook, and G. R. Oetzel. 2004. Investigation strategies for laminitis problem herds. J. Dairy Sci. 87:E27–E35. doi:10.3168/jds.S0022-0302(04)70058-2.

Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1998. Acidosis in cattle: a review. J. Anim. Sci. 76:275-286. doi:10.2527/1998.761275x

Rezac, D. J., D. U. Thomson, M. G. Siemens, F. L. Prouty, C. D. Reinhardt, and S. J. Bartle. 2014a. A survey of gross pathologic conditions in cull cows at slaughter in the Great Lakes region of the United States. J. Dairy Sci. 97:4227-4235. doi:10.3168/jds.2013-7636.

Rezac, D. J., D. U. Thomson, S. J. Bartle, J. B. Osterstock, F. I. Prouty, and C. D. Reinhardt. 2014b. Prevalence, severity, and relationships of lung lesions, liver abnormalities, and rumen health scores measured at slaughter in beef cattle. J. Anim. Sci. 92:2595-2602. doi:10.2527/jas.2013-7222

Roeber, D. L., P. D. Mies, C. D. Smith, K. E. Belk, T. G. Field, J. D. Tatum, J. A. Scanga, and G. C. Smith. 2001.National market cow and bull beef quality audit-1999: a survey of producer-related defects in market cows and bulls. J. Anim. Sci. 79:658-665. doi:10.2527/2001.793658x.

Smith, H. R. 1940. Beef liver condemnations. Proceedings of the American Society of Animal Nutrition, 1940:272-276.

Tadepalli, S., S. K. Narayanan, G. C. Stewart, M. M. Chengappa, and T. G. Nagaraja. 2009. Fusobacterium necrophorum: a ruminal bacterium that invades liver to cause abscesses in cattle. Anaerobe. 15:36-43. doi:10.1016/j.anaerobe.2008.05.005.

Tan, Z. L., Nagaraja, T. G., and M. M. Chengappa, 1994. Biochemical and biological characterization of ruminal Fusobacterium necrophorum. FEMS microbiology letters. 120:81-86.

Tan, Z. L., T. G. Nagaraja, and M. M. Chengappa. 1996. Fusobacterium necrophorum infections: virulence factors, pathogenic mechanism and control measures. Vet. Res. Commun. 20:113-140.

USDA. Oct. 2015 – Mar. 2016. NW_LS441, By-Product Drop Value (Steer). Accessed Jan. 27, 2017. https://www.ams.usda.gov/mnreports/nw_ls441.txt

USDA. Oct. 2015 – Mar. 2016. NW_LS444, Weekly USDA By-Product Drop Value (Cow). Accessed Jan. 27, 2017. https://www.ams.usda.gov/mnreports/nw_ls444.txt

USDA. 2017. SJ_LS710, Estimated daily Livestock Slaughter under Federal Inspection. Accessed Jan. 27, 2017. https://www.ams.usda.gov/mnreports/sj_lj710.txt

Vogel, G. J., and J. C. Parrott. 1994. Mortality survey in feed yards: The incidence of death from digestive, respiratory and other causes in feed yards on the Great Plains. Compend. Contin. Educ. Pract. Vet. 16:227–234.

Zhao, T., M. P. Doyle, P. J. Fedorka-Cray, P. Zhao, and S. Ladely. 2002. Occurrence of Salmonella enterica serotype Typhimurium DT104A in retail ground beef. J. Food Prot. 65:403-407. doi:10.4315/0362-028X-65.2.403

Table 3.1. Liver abscess incidence and estimated processor visceral loss per animal by region.

| | | Liver Score, % | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Region** | **n** | **Edible** | **Total Abscess[1]** | **A-** | **A** | **A+** | **A+ Adhesion** | **A+ Open** | **A+Adhesion/ Open** | **Contamination** | **Other Abnormality[2]** | **Viscera Loss ($/animal)[3]** |
| **Fed Beef Processors** | **130,845** | **69.2** | **20.3** | **8.2** | **2.5** | **2.9** | **5.0** | **1.2** | **0.5** | **7.5** | **3.0** | **2.11** |
| Central Plains | 25,813 | 70.0$^{bc}$ | 25.5$^{b}$ | 10.5$^{ab}$ | 2.9 | 3.6 | 7.7$^{abc}$ | 0.4 | 0.4 | 3.4$^{d}$ | 1.1$^{f}$ | 1.93 |
| Desert Southwest | 10,119 | 66.8$^{cd}$ | 24.3$^{b}$ | 7.9$^{bc}$ | 3.0 | 2.5 | 8.4$^{abc}$ | 1.7 | 0.9 | 7.6$^{bcd}$ | 1.3$^{f}$ | 2.38 |
| High Plains | 27,034 | 76.9$^{a}$ | 15.5$^{d}$ | 6.0$^{bcd}$ | 1.8 | 2.1 | 4.7$^{bcd}$ | 0.6 | 0.2 | 5.6$^{cd}$ | 2.0$^{ef}$ | 1.51 |
| Northeast | 7,958 | 72.9$^{ab}$ | 10.0$^{e}$ | 4.1$^{cd}$ | 1.7 | 1.6 | 1.7$^{d}$ | 0.7 | 0.2 | 11.2$^{ab}$ | 5.9$^{cde}$ | 1.80 |
| North Plains - A | 26,485 | 71.8$^{b}$ | 16.0$^{d}$ | 6.8$^{bcd}$ | 2.5 | 2.1 | 4.0$^{cd}$ | 0.4 | 0.3 | 8.6$^{bc}$ | 3.6$^{def}$ | 1.80 |
| North Plains - B | 24,186 | 65.1$^{d}$ | 21.4$^{bc}$ | 8.9$^{b}$ | 3.4 | 4.4 | 1.9$^{d}$ | 2.3 | 0.5 | 10.8$^{b}$ | 2.7$^{ef}$ | 2.51 |
| Pacific Northwest | 9,250 | 46.8$^{f}$ | 33.8$^{a}$ | 14.0$^{a}$ | 1.7 | 2.6 | 8.5$^{abc}$ | 4.6 | 2.3 | 9.2$^{bc}$ | 10.2$^{abc}$ | 4.20 |
| **Cull Beef Processors** | **30,646** | **63.0** | **17.6** | **5.0** | **1.9** | **1.9** | **7.1** | **1.1** | **0.6** | **10.0** | **9.4** | **0.97** |
| High Plains | 9,654 | 56.7$^{e}$ | 22.6$^{b}$ | 6.3$^{bcd}$ | 2.3 | 3.2 | 8.7$^{ab}$ | 1.5 | 0.6 | 8.1$^{bc}$ | 12.5$^{ab}$ | 1.17 |
| Great Lakes | 7,680 | 76.4$^{a}$ | 14.9$^{d}$ | 6.6$^{bcd}$ | 1.2 | 1.3 | 4.1$^{cd}$ | 1.3 | 0.4 | 5.9$^{cd}$ | 2.8$^{ef}$ | 0.72 |
| Northeast | 7,791 | 57.8$^{e}$ | 13.7$^{de}$ | 3.7$^{cd}$ | 2.3 | 1.5 | 5.6$^{bcd}$ | 0.4 | 0.2 | 15.5$^{a}$ | 13.0$^{a}$ | 0.92 |
| West Coast | 5,521 | 63.0$^{d}$ | 17.8$^{cd}$ | 2.3$^{d}$ | 1.6 | 1.1 | 10.7$^{a}$ | 0.9 | 1.2 | 11.2$^{ab}$ | 8.0$^{bcd}$ | 1.05 |
| ***P* - value** | | **< 0.01** | **< 0.01** | **< 0.01** | **0.92** | **0.37** | **< 0.01** | **0.11** | **0.94** | **< 0.01** | **< 0.01** | **0.48** |

$^{a,b,c,d,e,f}$Means within column lacking common superscripts differ ($P < 0.05$).

[1]Total Abscess is a sum of: A-, A, A+, A+Adhesion, A+Open, and A+Adhesion/Open.

[2]Other Abnormality includes: cirrhosis, flukes, telangiectasis, carotenosis, sawdust and torn.

[3]Viscera Loss includes unrealized revenue from condemnation of liver and gut mass (presence of open abscess).

Table 3.2. Liver abscess incidence and estimated processor visceral loss per animal by cattle type.

| | | Liver Score, % | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Type** | **n** | **Edible** | **Total Abscess[1]** | **A-** | **A** | **A+** | **A+ Adhesion** | **A+ Open** | **A+Adhesion /Open** | **Contamination** | **Other Abnormality[2]** | **Viscera Loss ($/animal)[3]** |
| **Fed Beef** | **132,796** | **70.1** | **20.0** | **8.1** | **2.5** | **2.9** | **4.8** | **1.2** | **0.5** | **7.1** | **2.7** | **2.05** |
| Steer | 64,449 | 72.5$^{a}$ | 18.2$^{bc}$ | 7.7$^{ab}$ | 2.4 | 2.7 | 4.0$^{cd}$ | 1.0 | 0.4 | 6.8$^{b}$ | 2.5$^{c}$ | 1.85 |
| Heifer | 33,240 | 70.3$^{ab}$ | 19.1$^{bc}$ | 8.2$^{a}$ | 2.6 | 2.9 | 3.7$^{d}$ | 1.3 | 0.4 | 7.2$^{b}$ | 3.4$^{c}$ | 2.04 |
| Holstein[4] | 26,169 | 65.0$^{cd}$ | 25.0$^{a}$ | 8.7$^{a}$ | 2.4 | 3.0 | 8.1$^{abc}$ | 1.8 | 1.0 | 7.9$^{b}$ | 2.1$^{c}$ | 2.52 |
| Mixed Lot | 8,938 | 67.3$^{bc}$ | 21.7$^{ab}$ | 8.8$^{a}$ | 2.9 | 3.1 | 5.3$^{bcd}$ | 1.0 | 0.6 | 7.0$^{b}$ | 4.0$^{c}$ | 2.20 |
| **Cull Beef** | **28,695** | **58.1** | **18.8** | **5.2** | **2.0** | **1.9** | **8.1** | **0.7** | **0.8** | **11.8** | **11.3** | **1.05** |
| Bull | 984 | 61.5$^{de}$ | 19.3$^{bc}$ | 3.9$^{b}$ | 2.0 | 2.1 | 10.4$^{a}$ | 0.3 | 0.6 | 10.2$^{ab}$ | 9.0$^{b}$ | 0.89 |
| Dairy Cow | 18,306 | 57.6$^{e}$ | 19.8$^{bc}$ | 5.1$^{ab}$ | 2.0 | 1.7 | 9.3$^{ab}$ | 1.0 | 0.7 | 12.4$^{a}$ | 10.1$^{ab}$ | 1.09 |
| Range Cow | 9,405 | 58.8$^{e}$ | 16.7$^{c}$ | 5.7$^{ab}$ | 2.1 | 2.3 | 5.5$^{bcd}$ | 0.3 | 1.0 | 10.8$^{ab}$ | 13.7$^{a}$ | 1.00 |
| ***P* - value** | | **< 0.01** | **< 0.01** | **< 0.01** | **0.99** | **0.95** | **< 0.01** | **0.94** | **0.99** | **< 0.01** | **< 0.01** | **0.86** |

$^{a,b,c,d,e,f}$Means within column lacking common superscripts differ ($P < 0.05$).

[1]Total Abscess is a sum of: A-, A, A+, A+Adhesion, A+Open, and A+Adhesion/Open

[2]Other Abnormality includes: cirrhosis, flukes, telangiectasis, carotenosis, sawdust and torn

[3]Viscera Loss includes unrealized revenue from condemnation of liver and gut mass (presence of open abscess).

[4]Holstein includes: Holstein steers (n = 23,364), Holstein heifers (n = 1,294), and mixed Holsteins (n = 1,511).

Table 3.3. Incidence of bacteria cultured from liver abscesses by region and processor type.

| | | Incidence, % | | | |
|---|---|---|---|---|---|
| | | *Fusobacterium necrophorum* | | | |
| **Region** | **n** | **Subsp. *necrophorum*** | **Subsp. *funduliforme*** | ***Trueperella pyogenes*** | ***Salmonella enterica*** |
| **Fed Beef Processors** | **189** | **79.9** | **24.3** | **14.8** | **27.5** |
| Central Plains | 25 | 88.0$^{a}$ | 24.0$^{bc}$ | 0.0$^{e}$ | 52.0$^{c}$ |
| Desert Southwest | 26 | 76.9$^{cd}$ | 15.4$^{d}$ | 0.0$^{e}$ | 26.9$^{e}$ |
| High Plains | 26 | 88.5$^{a}$ | 15.4$^{d}$ | 0.0$^{e}$ | 84.6$^{a}$ |
| Northeast | 28 | 75.0$^{d}$ | 21.4$^{c}$ | 10.7$^{c}$ | 35.7$^{d}$ |
| North Plains - A | 30 | 80.0$^{bc}$ | 23.3$^{bc}$ | 23.3$^{b}$ | 0.0$^{f}$ |
| North Plains - B | 29 | 75.9$^{cd}$ | 27.6$^{b}$ | 10.3$^{c}$ | 0.0$^{f}$ |
| Pacific Northwest | 25 | 76.0$^{cd}$ | 44.0$^{a}$ | 60.0$^{a}$ | 0.0$^{f}$ |
| **Cull Beef Processors** | **91** | **76.9** | **17.6** | **8.8** | **16.5** |
| High Plains | 24 | 79.2$^{bcd}$ | 8.3$^{e}$ | 8.3$^{cd}$ | 0.0$^{f}$ |
| Great Lakes | 22 | 81.8$^{b}$ | 27.3$^{b}$ | 22.7$^{b}$ | 68.2$^{b}$ |
| Northeast | 22 | 77.3$^{bcd}$ | 27.3$^{b}$ | 4.5$^{de}$ | 0.0$^{f}$ |
| West Coast | 23 | 69.6$^{e}$ | 8.7$^{e}$ | 0.0$^{e}$ | 0.0$^{f}$ |
| ***P* - value** | | **< 0.01** | **< 0.01** | **< 0.01** | **< 0.01** |

$^{a,b,c,d,e,f}$Means within column lacking common superscripts differ ($P < 0.05$).

Table 3.4. Incidence of bacteria cultured in combination from liver abscesses by region and processor type.

| | | Incidence, % | | |
|---|---|---|---|---|
| **Region** | **n** | ***F. necrophorum* and *T. pyogenes*** | ***F. necrophorum* and *S. enterica*** | ***T. pyogenes* and *S. enterica*** |
| **Fed Beef Processors** | **189** | **14.3** | **23.8** | **0.5** |
| Central Plains | 25 | 0.0[e] | 44.0[c] | 0.0[b] |
| Desert Southwest | 26 | 0.0[e] | 23.1[e] | 0.0[b] |
| High Plains | 26 | 0.0[e] | 76.7[a] | 0.0[b] |
| Northeast | 28 | 10.7[c] | 28.6[d] | 3.6[b] |
| North Plains - A | 30 | 23.3[b] | 0.0[f] | 0.0[b] |
| North Plains - B | 29 | 10.3[c] | 0.0[f] | 0.0[b] |
| Pacific Northwest | 25 | 56.0[a] | 0.0[f] | 0.0[b] |
| **Cull Beef Processors** | **91** | **8.8** | **16.5** | **2.2** |
| High Plains | 24 | 8.3[cd] | 0.0[f] | 0.0[b] |
| Great Lakes | 22 | 22.7[b] | 68.2[b] | 9.1[a] |
| Northeast | 22 | 4.6[de] | 0.0[f] | 0.0[b] |
| West Coast | 23 | 0.0[e] | 0.0[f] | 0.0[b] |
| ***P* - value** | | **< 0.01** | **< 0.01** | **< 0.01** |

[a,b,c,d,e,f]Means within column lacking common superscripts differ ($P < 0.05$).

Table 3.5. *Fusobacterium necrophorum, Trueperella pyogenes, and Salmonella enterica* and serotype incidence by liver score.

| | Liver Score | | | | | | |
|---|---|---|---|---|---|---|---|
| | A- (n = 78) | | A (n = 90) | | A+ (n = 101) | | |
| **Bacterial species** | **No. Isolated** | **Percent** | **No. Isolated** | **Percent** | **No. Isolated** | **Percent** | ***P*- value** |
| *F. necrophorum* | | | | | | | |
| Subsp. *necrophorum* | 56 | 71.8[b] | 56 | 62.2[c] | 101 | 100.0[a] | < **0.01** |
| Subsp. *funduliforme* | 8 | 10.3[b] | 22 | 24.4[a] | 28 | 27.7[a] | < **0.01** |
| *Trueperella pyogenes* | 7 | 9.0[b] | 12 | 13.3[a] | 14 | 13.9[a] | < **0.01** |
| *Salmonella enterica* | 16 | 20.5[b] | 21 | 23.3[b] | 28 | 27.7[a] | < **0.01** |
| ***Salmonella* serotype** | **No. Isolated** | **Percent** | **No. Isolated** | **Percent** | **No. Isolated** | **Percent** | **Total** |
| Anatum | 6 | 9.0 | 5 | 7.5 | 9 | 13.4 | 20 |
| Give | 1 | 1.5 | 0 | 0.0 | 2 | 3.0 | 3 |
| Kentucky | 0 | 0.0 | 1 | 1.5 | 4 | 6.0 | 5 |
| Lubbock | 3 | 4.5 | 8 | 11.9 | 5 | 7.5 | 16 |
| Mbandaka | 1 | 1.5 | 0 | 0.0 | 1 | 1.5 | 2 |
| Montevideo | 5 | 7.5 | 7 | 10.4 | 6 | 9.0 | 18 |
| Reading | 0 | 0.0 | 0 | 0.0 | 2 | 3.0 | 2 |
| Schwarzengrund | 0 | 0.0 | 1 | 1.5 | 0 | 0.0 | 1 |

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

Table 3.6. Incidence of bacteria cultured from liver abscesses by cattle type.

| | | Incidence, % | | | |
|---|---|---|---|---|---|
| | | *Fusobacterium necrophorum* | | | |
| **Type** | **n** | **Subsp. *necrophorum*** | **Subsp. *funduliforme*** | ***Trueperella pyogenes*** | ***Salmonella enterica*** |
| **Fed Beef** | **193** | **81.3** | **25.9** | **14.5** | **29.5** |
| Steer | 87 | 79.3 | 26.4 | 10.3 | 33.3 |
| Heifer | 55 | 76.4 | 29.1 | 27.3 | 14.5 |
| Holstein[1] | 42 | 88.1 | 26.2 | 9.5 | 47.6 |
| Mixed Lot | 9 | 100.0 | 0.0 | 0.0 | 0.0 |
| **Cull Beef** | **76** | **73.7** | **10.5** | **6.6** | **10.5** |
| Bull | 5 | 60.0 | 0.0 | 0.0 | 0.0 |
| Dairy Cow | 44 | 70.5 | 9.1 | 4.5 | 13.6 |
| Range Cow | 27 | 81.5 | 14.8 | 11.1 | 7.4 |

[1]Holstein includes: Holstein steers (n = 41) and Holstein heifers (n = 1).

# CHAPTER 4:

# ASSOCIATION OF CATTLE NUTRITIONAL AND MANAGEMENT CONDITIONS TO LIVER ABSCESS INCIDENCE

## 4.1 ABSTRACT

The pathogenesis of liver abscesses is believed to begin with ruminal acidosis resulting from high energy feeds and the escape of *Fusobacterium necrophorum* through the rumen wall to the portal vein and then the liver. However, we hypothesized that liver abscesses in feedlot cattle are multifactorial and our objective was to determine which factors, management, nutritional, or otherwise are associated with the development of liver abscesses in fed beef. To achieve this objective, survey data were assimilated corresponding to individual lots of fed cattle. Thirteen nutritionists participated in the survey representing 43,255 animals, in 321 lots, from 32 individual feedlots throughout the major cattle feeding areas of the U.S. The survey contained 57 questions divided into information categories that included: general (n = 13); health (n = 6); source and background (n = 7); growth promoting technologies (n = 3); diet (n = 10); feeding management (n = 6); cattle management (n = 8); and liver abscess control technologies (n = 4). Data were analyzed using the REG, CORR, and CALIS procedures of SAS v9.4 (SAS Inst. Inc., Cary, NC). Cattle type (beef or dairy; Adj. $R^2 = 0.13$), diet dry matter percentage (Adj. $R^2 = 0.11$), G:F (Adj. $R^2 = 0.11$), mortality (Adj. $R^2 = 0.07$), and tylosin

supplementation (yes or no; Adj. $R^2$ = 0.06) all yielded univariate equations capable of predicting total abscess percentage. Using the maximum likelihood method in the CALIS Procedure of SAS, the full model to predict total abscess incidence was: total abscess percentage = 113.5 – (217.39 × G:F) – (18.48 × cattle type [1 = native beef; 0 = Holstein]) – (46.35 × Diet DM, %) – (14.39 × tylosin supplementation [1 = yes; 0 = no]), and accounted for 34% of the variation to predict total liver abscess incidence. Predictive equations reveal that while dietary factors can contribute to and likely exacerbate liver abscess incidence, additional factors, beyond dietary measures, contribute to this multifactorial disease. Models generated were unable to explain more than 40% of the variation involved in predicting either total or major abscess incidence, indicating that liver abscesses are likely a multifactorial disease that are influenced beyond dietary factors alone.

**Keywords**: feedlot survey; Holstein; liver abscess

## 4.2 INTRODUCTION

Liver abscesses in beef animals are not a recent problem; Smith (1940) reported their detriment, and their prevalence has not diminished in the 75 years since (Eastwood et al., 2017). The dogma of liver abscess pathogenesis has changed little since Smith (1944) reported a relationship between ulcerative lesions in the rumen and abscesses in the liver of feedlot cattle. Since then, the pathogenesis has expanded to include ruminal acidosis resulting from high energy concentrate feeds (Brent, 1976) and the development of rumen wall abscesses caused by *Fusobacterium necrophorum* (Nagaraja and Lechtenberg, 2007). Studies that have evaluated effects of management practices on acidosis and rumen health (Elam, 1976; Galyean and Rivera, 2003) have continued to link their results back to diet and dry-matter intake.

Liver abscesses are polymicrobial bacterial infections (Nagaraja and Lechtenberg, 2007; Amachawadi and Nagaraja, 2016), and while the major route of bacterial ingress into portal circulation is believed to be the rumen (Smith, 1944; Nagaraja and Lechtenberg, 2007), it is likely that other routes of ingress may affect incidence. Essentially all blood from the abdominal viscera drains into the portal vein where it is filtered by the liver (Pasquini et al., 1997). Although the rumen is believed to be the primary route of entry for bacteria to enter portal blood, any tear or hole in the gastrointestinal tract can allow bacteria to enter portal blood supply and be sequestered in the liver. Bovine respiratory disease has long been accepted as a multifactorial disease (Bowland and Shewen, 2000), and it may be appropriate to also consider liver abscesses as multifactorial disorders. Therefore, we hypothesized that liver abscesses in feedlot

cattle are multifactorial and our objective was to quantify factors, nutritional or otherwise, that contribute to the development of liver abscesses in fed beef.

## 4.3 MATERIALS AND METHODS

Animal care and use committee approval was not obtained for this study because data were recorded on carcasses, and no live animals were used for data collection. West Texas A&M University Institutional Review Board approval was obtained (IRB Permit Number 01-11-15) prior to distribution of the survey.

### *4.3.1 Survey participants*

Participants in this experiment were selected based on the criteria of whether or not cattle under their management were evaluated in our national liver audit. Feedlot managers and nutritionists managing those cattle were contacted and requested to participate in the survey. Thirteen persons returned surveys representing 43,255 head of cattle, across 321 lots, from 32 individual feedlots throughout the major cattle feeding areas (e.g. Central Plains, High Plains, North Plains, and Southwest).

### *4.3.2 Data collection*

Potential participants were initially contacted via phone; those that agreed to take part in this experiment were sent an email describing the process and were required to sign and return an informed consent form prior to being granted access to the survey questionnaire. All participants received a guarantee of anonymity. Survey data were collected via three options: a Web-based survey tool (https://www.qualtrics.com/academic-solutions/west-texas-am-university/), paper hard copy of the survey, or a Microsoft Excel (Microsoft, Redmond, WA) spreadsheet. Data

generated from questionnaires were matched with the liver audit data generated for each individual lot of cattle.

Survey data gathered from participants were matched to individual liver scores gathered for each lot of cattle that a survey was completed for. Liver abscesses were visually assessed and scored according to a modified scoring system based on the Elanco Liver Check Service (✓ = no abscesses, A- = 1 or 2 small abscesses, A = 2 to 4 small active abscesses, A+ = multiple small abscesses or 1 or more large active abscesses, A+ Adhesion = liver adhered to GI tract, and A+ Open = open liver abscess). Other recorded liver abnormalities included: cirrhosis, flukes, telangiectasis, carotenosis, sawdust, torn, and if a liver was condemned for contamination (adapted from Brown & Lawrence, 2010). Liver abscesses and other abnormalities were documented by individual carcass and matched with processor lot, sex, cattle type, and any additional information available.

### *4.3.3 Survey questions*

The survey contained 57 questions divided into several categories including: general information (n = 13); health information (n = 6); source and backgrounding information (n = 7); growth promoting technologies (n = 3); general diet information (n = 3); roughage source and levels (n = 4); co-product sources and levels (n = 3); feeding management (n = 6); cattle management (n = 8); and liver abscess control technologies (n = 4). Survey questions were formatted to attempt to account for multiple points of variation that could lead to the development of liver abscesses. Liver abscesses are believed to be a result of ruminal acidosis, thus multiple questions were focused upon nutrition. Questions were also developed to attempt to determine animal management, or the effect that feeding regimen, pen space, bunk space, and bunk reads have on abscess

incidence. Finally, additional questions were formatted in an attempt to determine if backgrounding, weaning, transitional period, health status, or breed contributed to liver abscess incidence beyond traditional nutritional concerns. Because we hypothesized that liver abscesses are multifactorial, we generated a myriad of questions covering a wide variety of topics in an attempt to determine if liver abscesses are truly a multifactorial disorder.

### *4.3.4 Statistical analysis*

Data were analyzed using SAS v9.4 (SAS Inst. Inc., Cary, NC). Prior to generation of predictive models, univariate analysis using the REG procedure of SAS was conducted for each variable to determine which variables of interest should be tested for inclusion in prediction equations. Because of several variables were auto-correlated to each other, these variables were combined into their own classification variables. Implant status, ractopamine supplementation, and ionophore supplementation were combined to create the new variable "technology" and tetracycline supplementation and metaphylaxis were combined to create the new category "other antibiotic". If a cattle feeder is going to implement growth promoting technologies it is likely that they will implement multiple technologies, which was the case in this population. If a feeder supplemented ractopamine, in almost every circumstance they also administered an implant and added ionophores in their diet, which led to a high degree of auto-correlation.

Variables that yielded univariate regression equations with Type-I error less than 0.05, and Adjusted $R^2$ (Adj. $R^2$) values greater than 0.05, were considered for multivariate regression analysis. Correlations between variables were tested using the CORR procedure of SAS and items that possessed multicollinearity were denoted and

removed from multiple regression equations. Multiple variable prediction equations were developed using the stepwise selection in the REG procedure of SAS and analyzed for prediction of total abscess incidence and major abscess (A+ liver score or greater) incidence. Variables were excluded from the equation at the $P > 0.10$. Selection criterion for best fit regression models included Adj. $R^2$, root mean square error (RMSE), Mallows Cp statistic (Cp; Mallows, 1973), variance inflation factor (VIF), tolerance (TOL), and condition index.

Because survey responses had missing data, traditional model selection using the REG procedure had unique challenges and limitations. If any cell had missing data, that entire lot of cattle was removed from analysis by SAS, regardless if other variables in that lot included a survey response. Initially, candidate equations were generated using the $R^2$ selection method in the REG procedure. Once best 1- p candidate parameter equations for the prediction of total and major liver abscess percentage were populated using the $R^2$ selection method, equations were reselected using the stepwise selection method of SAS to generate best-fit equations. Equations were reselected using the stepwise selection method of SAS because the $R^2$ selection method does not account for multicollinearity or variance inflation factors for each individual variable added to the equation. Finally, the maximum likelihood method using the CALIS procedure of SAS (Allison, 2012) was used to fill in missing data to allow all lots of cattle to be analyzed via regression. Since the CALIS procedure must be specifically coded for which variables are in the model and will not remove variable if they are insignificant, models were first generated using the stepwise selection method in the REG procedure of SAS and then ran using the CALIS

procedure of SAS to account for incomplete survey responses. Model fit statistics included are: Adjusted $R^2$, and Aiaike information criterion (AIC).

## 4.4 RESULTS AND DISCUSSION

### *4.4.1 Sample population*

Descriptive statistics of the sample population (Table 4.1) indicated that total liver abscess outcomes are similar to those described by Eastwood et al. (2017) in the 2016 National Beef Quality Audit (NBQA). Our sample population was represented by 21.4% total liver abscess incidence, with 10.7% incidence of minor liver abscesses (sum of A- and A liver abscesses) and 10.8% incidence of major liver abscesses (sum of A+ or greater liver abscesses). For cattle that had no survey response, total liver abscess incidence was 18.7%, minor liver abscess incidence was 8.6%, and major abscess incidence was 10.1%. Total, minor, and major liver abscess incidence was not different ($P \geq 0.06$) between the survey population and the population without survey responses. The 2016 NBQA similarly reported that 11.8% of livers were condemned for having a minor abscess and 6.0% of livers were condemned for having major abscesses (Eastwood et al., 2017). Incidence of flukes, contamination and cirrhosis in the sample population were also similar to those reported in the 2016 NBQA (Eastwood et al., 2017).

Descriptive statistics of the sample population represented by survey response variables with continuous data outcomes are reported in Table 4.2. Mean latitude and longitude of feedlots for survey recipients is in Southeastern Colorado, which is not surprising because most feedlot cattle in the U.S. are located in High Plains region. Mean elevation and rainfall is also consistent with this region at 931.0 m and 48.5 cm,

respectively. Average morbidity of audited cattle was 7.9% and mean mortality was 2.0%. Morbidity and mortality can vary greatly due to disease outbreaks, weather anomalies, and other unforeseen circumstances; however, mean morbidity and mortality of surveyed cattle falls within the ranges described by Kelly and Janzen (1986), who reported morbidity (0 – 69%) and mortality (0 – 15%) rates in feedlot cattle across North America. Additionally, Smith (1998) reported that feedlot morbidity averages between 5 to 11% of cattle received. More recently, the National Animal Health Monitoring System (NAHMS) reported that mortality for all feedlot cattle was 1.6% (NAHMS, 2013), which is less than what was reported by survey participants; however, the most recent NAHMS report uses data from 2011.

In regard to the study population's performance and diet composition (Table 4.2), average weight when cattle were placed on feed was 65.8% greater than those reported by Snowder et al. (2006), who evaluated 18,112 calves prior to and throughout the feeding period. Similarly, ADG was 40.0% greater for survey cattle, but total DOF (196.4 vs 205, respectively) was not different from those reported by Snowder et al. (2006). Increased feedlot in-weight and ADG in the current group of cattle is likely due to the overall genetic improvements observed in the beef industry from year to year (Miller, 2010). Additionally, average total diet NEg (1.5 Mcal/kg) was similar to values reported by Vasconcelos and Galyean (2007) when they surveyed feedlot nutritionists for their diet recommendations. Primary grain source in respondent's diets was corn; whereas roughage source tended to vary based on region, with alfalfa hay and corn silage being the most common sources. Additionally, the average grain inclusion rate was 72.7%; however, there was also a wide range in average grain inclusion, ranging from 41

to 95%. This value was highly dependent upon available co-products and their energy density.

On average, 9.2% of the diet was forage/roughage on a dry matter basis; however, for this population of cattle, forage inclusion ranged from 6.0 to 18.0%. These values are consistent with what was reported by Vasconcelos and Galyean (2007). Mean total diet eNDF was calculated at 8.6% based on survey responses of diet ingredients and inclusion level. Meaning that on average 8.6% of the diet was eNDF. This value was calculated by multiplying an ingredients NDF% by its eNDF% to generate the eNDF of a feedstuff as a percentage of the feed, which was then multiplied by the components inclusion percentage to generate the total percentage of the diet that was eNDF.

Survey participants also answered questions concerning general cattle management (Table 4.2). On average cattle were fed 2.8 times/d and mean daily bunk reads was 2.9. Multiple feedings throughout the day is thought to decrease the incidence of acidosis because multiple meals have been shown to stabilize rumen pH, and multiple meals may help mitigate the risks associated with a single feeding system (Gonzalez et al., 2012). If animals are only fed once a day, any fluctuations in delivery or timing could affect intake and result in decreased ruminal pH; whereas in a multiple feeding system, if one meal is mistimed the other meals in the day can help to restore the rumen to homeostasis. For survey respondents, average diet chop length was 6.1 cm; however, diet chop length was highly viable, and ranged from 1.0 to 12.7 cm. Eating rate of ruminants is partially regulated by particle length, with longer particles requiring more mastication and rumination (Gonzalez et al., 2012). Mastication promotes saliva production, which contains bicarbonate and phosphate, both of which are vital in

buffering rumen pH (Bailey and Balch, 1961). Mean pen area allotted per animal was 16 $m^2$, and on average each animal was allocated 24.1 cm of linear bunk space.

Finally, some survey response questions were nominal in nature, and were included in subsequent univariate and multivariate analyses. Of cattle with survey responses, 87.5% were "native" beef type, and 12.5% were Holstein. These values are different than those reported in the latest NBQA (79.6% native vs 20.4% Holstein; Eastwood et al., 2017); however, survey responses did not include all cattle audited. Almost all cattle (92.2%) received an implant during the feeding period whereas only 82.6% were supplemented with ractopamine hydrochloride. Along with growth promoting feed additives, 96.5% of survey cattle were fed an ionophore throughout the feeding period. Few cattle were fed tetracycline (12.1%) with even less receiving fusoguard (3.7%), with the primary product to manage liver abscesses being tylosin phosphate (96.5%). Finally, 12.1% of cattle received metaphylaxis upon entry into the feedlot during processing. These variables were utilized in the development of univariate and multivariate models to predict total and major liver abscess prevalence in feedlot cattle.

### *4.4.2 Univariate analysis of survey data*

Because the REG procedure of SAS is unable to simultaneously utilize 93 individual variables for a predictive model, univariate analysis was performed on each variable to determine likely candidates for a multivariate predictive equation. When data were analyzed in univariate models, grain inclusion percentage on a dry matter basis had the greatest Adj. $R^2$ in a univariate model (Adj. $R^2 = 0.17$) to predict total abscess percentage (Table 4.3). However, these results are misleading when not viewed in

context with the rest of the variables. The feedyard that had an average grain inclusion rate of 41% administered potato waste as a starch source and had a high incidence of liver abscesses while the feedlot that had an average grain inclusion percentage of 95% had a low liver abscess incidence. Additionally, the NEg for both rations were similar at 69 Mcal NEg/45.35kg and 68 Mcal NEg/45.35kg, respectively. Further highlighting how important dietary management and nutrient digestibility are in the development of liver abscesses. Therefore, for this population of cattle, if viewed out of context, it appears that increasing grain inclusion percentage in the diet decreases liver abscess incidence. Because of those reasons, grain inclusion percentage was removed from multivariate analysis.

Cattle type (beef or dairy; Adj. $R^2 = 0.13$), diet dry matter percentage (Adj. $R^2 = 0.11$), G:F (Adj. $R^2 = 0.11$), technology (Adj. $R^2 = 0.11$), mortality (Adj. $R^2 = 0.07$), other antibiotics (Adj. $R^2 = 0.07$), and tylosin supplementation (yes or no; Adj. $R^2 = 0.06$) all yielded significant univariate equations capable of predicting total abscess percentage (Table 4.3). Total diet eNDF was able to account for 3.0% of the variation when predicting total abscess incidence (Adj. $R^2 = 0.03$), whereas forage inclusion percentage and NEg were unable to yield a significant equation to predict total liver abscess incidence.

Similarly, when used in univariate equations to predict major abscess prevalence percentage (Table 4.3), cattle type (beef or dairy; Adj. $R^2 = 0.14$), technology (Adj. $R^2 = 0.11$), diet dry matter percentage (Adj. $R^2 = 0.06$), G:F (Adj. $R^2 = 0.06$), tylosin supplementation (yes or no; Adj. $R^2 = 0.06$), and other antibiotics (Adj. $R^2 = 0.07$) yielded univariate equations that were able to explain more variation in the data than

other variables queried in the survey. Total diet eNDF, diet NEg, and forage inclusion percentage were unable to yield a significant equation to predict major liver abscess incidence.

It is unsurprising that dietary factors and components represented so much of the variation in models to predict either total or major liver abscess percentage. Wetter starches, like high moisture corn and potato waste, are rapidly fermentable in the rumen and have been shown to reduce mean ruminal pH over diets containing dry ground corn (Krause et al., 2002). Furthermore, the acidogenic effect of a diet increases as the proportion of starch in the diet increases (Gonzalez et al., 2012), which is why some recommend increasing forage concentration of the diet to decrease acidosis and eventual liver abscess incidence (Nagaraja and Lechtenberg, 2007). However, for this population of cattle, total diet eNDF and forage inclusion percentage of the diet were non-predictive of major abscess incidence and forage inclusion was non-predictive of total abscess incidence, in univariate models (Table 4.3).

Average forage inclusion percentage for this population of cattle was 9.2%, and abscess incidence for cattle that received that forage inclusion rate ranged from 0-100%. Highlighting that liver abscesses can develop in feedlot cattle, regardless of dietary forage inclusion percentage. While we are not suggesting that forage inclusion doesn't affect abscess incidence, it is more likely that feeding management can mitigate the risks associated with a highly fermentable diet. Unfortunately, this questionnaire was unable to quantify fluctuations in feeding management and while mean times fed per day was generated; regularity of feeding or subsequent fluctuations was not obtained. Increasing dietary effective fiber has been shown to increase fermentation time in the rumen,

allowing for more gradual pH decline and a decrease in the incidence of acidosis (Krause and Oetzel, 2006). However, liver abscesses appear to be a multifactorial condition, affected by items beyond nutrition alone, the fact that some of these non-nutritional variables yielded significant univariate models lends credence to that hypothesis.

Although some feed ingredients had the greatest individual effect on liver abscess incidence, other compounds were able to explain some of the variability surrounding liver abscess incidence. It was unsurprising that supplementation of tylosin phosphate yielded a significant ($P < 0.01$) univariate model, because tylosin phosphate is labeled for the treatment and control of liver abscesses in feedlot cattle (Feed Additive Compendium, 2013). Along with tylosin supplementation, the usage of certain technologies (ionophores and anabolic implants) produced a univariate equation capable to predict liver abscess incidence ($P < 0.01$). While ionophores are not labeled for liver abscess reduction, it is possible that their ability to increase rumen pH (Nagaraja et al., 1982) could contribute to reduced acidosis and an eventual reduction in liver abscesses. Also, it has been previously demonstrated that anabolic implants are immunomodulatory (Burton et al., 1993), and an immune system augmented by anabolic implants may provide for decreased abscess incidence and severity. Although, because ionophores, ractopamine, and implants are often administered in conjunction with tylosin phosphate, the univariate equation generated may be auto-correlated with tylosin administration. If a cattle feeder is going to implement one of these technologies, it is not unlikely that they will utilize all of them in their cattle feeding operation.

It was expected that cattle type affected liver abscess incidence, because Holsteins have often been reported as having an increased liver abscess incidence (Amachawadi

and Nagaraja, 2016). One hypothesis as to why Holsteins have a greater incidence is the increased days on feed that Holsteins are subjected to (Amachawadi and Nagaraja, 2016). It is theorized that the longer an animal is on feed, the greater the chance they will have to experience acidosis and eventually develop a liver abscess. However, with this population of cattle, days on feed was unable to explain any of the variation surrounding total abscess incidence in a univariate model, and was only capable of explaining 2.0% of the variation when used to model major abscess incidence. The longer an animal is on an energy-intensive feedlot ration, the greater the likelihood of an acidotic event and subsequent liver abscess development (Nagaraja and Chengappa, 1998); however, that does not appear to be a major factor in predicting liver abscess incidence in this population of cattle. Therefore, it is likely that the unique rearing and management conditions young Holsteins are subjected to affects liver abscess incidence beyond the effects that diet and days on feed contribute to this malady (Franklin et al., 2003; Kristensen et al., 2007).

### *4.4.3 Multivariate analysis of survey data*

When all variables with significant univariate results were analyzed via multivariate analysis, less than half (129 out of 321) of all lots were included in the model, due to mussing data. When missing data were filled using the maximum likelihood method using the CALIS procedure of SAS (Allison, 2012), all lots of cattle were included in the model and the same parameters that yielded the greatest Adj. $R^2$ when data were missing, continued to yield equations with the greatest Adj. $R^2$ when missing data were filled. These variables (diet dry matter percentage; cattle type [1 = native beef; 0 = Holstein]; gain to feed; morbidity; tylosin; technology; other antibiotics)

were then used to generate multivariate models using the stepwise selection method which could then account for multicollinearity and identify individual parameter *P*-values to determine if their inclusion in the model was significant and appropriate to calculate using the maximum likelihood method.

Using the maximum likelihood method to compute missing data as described by Allison (2012), a best-fit model was generated to predict major abscess incidence and includes: intercept, coefficients, Adj. $R^2$, and Aiaike information criterion (AIC; Table 4.4). Cattle type (native or Holstein) was the best single predictor of total liver abscess incidence, accounting for 13% of the total variation. The addition of G:F into the equation accounted for another 13%, increasing the Adj. $R^2$ to 0.26, making Cattle type and G:F the best two-variable model for predicting total liver abscess incidence. The Adj. $R^2$ was further increased by including Diet DM, % (0.26 to 0.32). Finally, including Tylosin supplementation (yes or no) into the model resulted in an equation that accounted for 34% of the total variation. Therefore, the full model to predict total abscess incidence was: total abscess percentage = 113.5 – (217.39 × G:F) – (18.48 × cattle type [1 = native beef; 0 = Holstein]) – (46.35 × Diet DM, %) – (14.39 × tylosin supplementation [1 = yes; 0 = no]).

Using the same methodology for predicting total abscess incidence, a best-fit model was generated to predict major abscess incidence and include: intercept, coefficients, Adj. $R^2$, and AIC (Table 4.5). Cattle type (native or Holstein) was the best single predictor of major liver abscess incidence, accounting for 14% of the total variation. The addition of G:F into the equation accounted for another 8%, increasing the Adj. $R^2$ to 0.22, making Cattle type and G:F the best two-variable model for predicting

major liver abscess incidence. The Adj. $R^2$ was further increased by including Diet DM, % (0.22 to 0.24). Finally, including Tylosin supplementation (yes or no) into the model resulted in an equation that accounted for 28% of the total variation. Therefore, the model to predict major abscess incidence is: major abscess percentage = 66.15 – (118.72 × G:F) – (13.73 × cattle type [1 = native beef; 0 = Holstein]) – (20.53 × Diet DM, %) – (12.93 × tylosin supplementation [1 = yes; 0 = no]).

While neither of these models were able to account for more than 40% of the variation regarding total or major liver abscess incidence, each of the factors used in the analysis represent variables that are objective while also being non-invasive and were observed to be significant predictor variables ($P < 0.05$). However, it is important to note that these equations have not been validated and are only applicable to the population evaluated. The inability of these models, which were developed using questions that ranged beyond nutrition and management, to explain more than 40% of the variation involved in predicting either total or major abscess incidence, is indicative that liver abscesses are a multifactorial disease that are influenced beyond dietary factors alone. Unfortunately, models generated were unable to explain more than half of the variation involved in predicting liver abscesses. However, the inability of dietary factors to explain more of the variation does add some credence to our hypothesis that liver abscess are multifactorial disorders; nevertheless, additional research is needed in order to quantify the extemporaneous factors that may contribute to liver abscess incidence.

While unquantified by the survey questionnaire and model, cattle type (native beef or Holstein), did have a significant impact on the prediction of total and major abscess incidence. Holsteins are often cited as having increased abscess incidence

(Amachawadi and Nagaraja, 2016); however, the reason for the increased incidence is poorly understood. One possible reason why Holsteins have increased abscess incidence is *Mycobacterium avium* subsp. *paratuberculosis*. *Mycobacterium avium* subsp. *paratuberculosis* is the causative agent of Johne's disease, a chronic infectious disease of the gastrointestinal tract (Ott et al., 1999), which has been estimated to infect at least 22% of all U.S. dairy herds (Wells et al., 1998). *Mycobacterium avium* subsp. *paratuberculosis* is believed to be capable of permeating the intestinal mucosa and has been detected in blood, lymph, muscle and liver samples from slaughter cattle (Zarei et al., 2017). If *Mycobacterium avium* subsp. *paratuberculosis* is capable of creating a breach in the intestinal mucosa, it is possible that *F.necrophorum* may be able to leave the gastrointestinal tract through these same opening and enter portal circulation. Johne's disease is prevalent in dairy herds and animals are often infected at a young age (Wells et al., 1998). While non-definitive, this infection model may provide some explanation as to why liver abscess incidence in increased in fed Holsteins, beyond days on feed or dietary management.

Traditional methods to diagnose liver abscesses have required ultrasound examination (Lechtenberg and Nagaraja, 1991), or blood analysis (Lechtenberg and Nagaraja, 1991; Doré et al., 2007; Macdonald et al., 2017), to detect liver abscesses prior to slaughter. However, these methods require individual animal analysis and are often time intensive and potentially costly. Being able to predict liver abscess incidence prior to slaughter with non-invasive methods may allow for better management decisions by cattle feeders, which could lead to fewer or less severe liver abscesses at slaughter. The inability of the measured factors to generate predictive equations able to explain more

than 40% of the variation surrounding liver abscesses is likely an indication of how multifactorial this disease is. Predictive equations generated show that while dietary factors can contribute and likely exacerbate liver abscess incidence, additional factors contribute to the disease. Viewing liver abscesses as a multifactorial disease will allow future researchers to break away from the traditional dogma and allow for new schools of thought on the matter.

## 4.5 LITERATURE CITED

Allison, P. D. 2012. Handling missing data by maximum likelihood. In SAS global forum (Vol. 23). Haverford, PA, USA: Statistical Horizons.

Amachawadi, R. G., and T. G. Nagaraja. 2016. Liver abscesses in cattle: A review of incidence in Holsteins and of bacteriology and vaccine approaches to control in feedlot cattle. J. Anim. Sci. 94:1620-1632. doi:10.2527/jas2015-0261.

Bailey, C. B., and C. C. Balch. 1961. Saliva secretion and its relation to feeding in cattle. 1. The composition and rate of secretion of parotid saliva in a small steer. Br. J. Nutr. 15:371-382.

Bowland, S.L. and P. E. Shewen. 2000. Bovine respiratory disease: commercial vaccines currently available in Canada. Can. Vet. J. 41:33-48.

Brent, B. E. 1976. Relationship of acidosis to other feedlot ailments. J. Anim. Sci. 43:930-935.

Burton, J. L., T. H. Elsasser, T. S. Rumsey, J. K. Lunney, and B. W. McBride. 1993. Immune responses of growing beef steers treated with estrogen/progesterone implants or insulin injections. Domest. Anim. Endocrinol. 10:31-44. doi:10.1016/0739-7240(93)90006-W.

Doré, E., G. Fecteau, P. Hélie, and D. Francoz. 2007. Liver abscesses in Holstein dairy cattle: 18 cases (1992–2003). J. Vet. Intern. Med. 21:853-856.

Eastwood, L. C., C. A. Boykin, M. K. Harris, A. N. Arnold, D. S. Hale, C. R. Kerth, D. B. Griffin, J. W. Savell, K. E. Belk, D. R. Woerner, J. D. Hasty, R. J. Delmore, J. N. Martin, T. E. Lawrence, T. J. McEvers, D. L. VanOverbeke, G. G. Mafi, M. M. Pfeiffer, T. B. Schmidt, R. J. Maddock, D. D. Johnson, C. C. Carr, J. M. Scheffler, T. D. Pringle, and A. M. Stelzleni. 2017. National Beef Quality Audit-2016: Transportation, mobility, and harvest-floor assessments of targeted characteristics that affect quality and value of cattle, carcasses, and by-products1. Translational Animal Science. 1:229-238. doi:10.2527/tas2017.0029.

Elam, C. J. 1976. Acidosis in feedlot cattle: Practical observations. J. Anim. Sci. 43:898−901.

Feed Additive Compendium. 2013. Feed additive compendium. Book. Miller Publishing Co., Minnetonka, Minn.

Franklin, S. T., D. M. Amaral-Phillips, J. A. Jackson, and A. A. Campbell. 2003. Health and performance of Holstein calves that suckled or were hand-fed colostrum and were fed one of three physical forms of starter. J. Dairy Sci. 86:2145-2153. doi:10.3168/jds.S0022-0302(03)73804-1.

Galyean, M. L., and J. D. Rivera. 2003. Nutritionally related disorders affecting feedlot cattle. Can. J. Anim. Sci. 83:13-20.

González, L.A., X. Manteca, S. Calsamiglia, K. S. Schwartzkopf-Genswein, and A. Ferret, 2012. Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). Anim. Feed Sci. Technol. 172:66-79. doi:10.1016/j.anifeedsci.2011.12.009.

Kelly, A.P. and E. D. Janzen. 1986. A review of morbidity and mortality rates and disease occurrence in North American feedlot cattle. Can. Vet. J. 27:496- 500.

Krause, K. M., D. K. Combs, and K. A. Beauchemin. 2002. Effects of forage particle size and grain fermentability in midlactation cows. II. Ruminal pH and chewing activity. J. Dairy Sci. 85:1947–1957.

Krause, K.M. and G. R. Oetzel. 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Anim. Feed Sci. Technol. 126:215-236. doi:10.1016/j.anifeedsci.2005.08.004.

Kristensen, N. B., J. Sehested, S. K. Jensen, and M. Vestergaard. 2007. Effect of milk allowance on concentrate intake, ruminal environment, and ruminal development in milk-fed Holstein calves. J. Dairy Sci.. 90:4346-4355. doi:10.3168/jds.2006-885.

Lechtenberg, K.F., and T. G. Nagaraja. 1991. Hepatic ultrasonography and blood changes in cattle with experimentally induced hepatic abscesses. Am. J. Vet. Res. 52:803-809.

Macdonald, A.G., S. L. Bourgon, R. Palme, S. P. Miller, and Y. R. Montanholi. 2017. Evaluation of blood metabolites reflects presence or absence of liver abscesses in beef cattle. Vet. Rec. Open. 4:1-8. doi:10.1136/vetreco-2016-000170.

Mallows, C. 1973. Some comments on Cp. Technometrics. 15:661-675.

Miller, S. 2010. Genetic improvement of beef cattle through opportunities in genomics. R. Bras. Zootec. 39:247-255. doi:10.1590/S1516-35982010001300027.

Nagaraja, T.G., T. B. Avery, E. F. Bartley, S. Roof, and A. Dayton. 1982. Effect of lasalocid, monensin or thiopeptin on lactic acidosis in cattle. J. Anim. Sci. 54:649-658. doi:10.2527/jas1982.543649x.

Nagaraja, T. G., and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: a review. J. Anim. Sci. 76:287-298.

Nagaraja, T.G. and K. F. Lechtenberg. 2007. Liver abscesses in feedlot cattle. Vet. Clin. N. Am-Food. A. 23:351-369. Doi:10.1016/j.cvfa.2007.05.002.

National Animal Health Monitoring System. Feedlot 2011: Part I: Management practices on U.S. feedlots with a capacity of 1,000 or more head. USDA APHIS National Animal Health Monitoring System, 2013. https://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_dr_PartIV.pdf

Ott, S. L., S. J. Wells, and B. A. Wagner. 1999. Herd-level economic losses associated with Johne's disease on US dairy operations. Prev. Vet. Med. 40:179-192. doi:10.1016/S0167-5877(99)00037-9.

Pasquini, C., T. Spurgeon, and S. Pasquini. 1997. Anatomy of Domestic Animals: Systemic and Regional Approach. 11th ed. Sudz Pub. Pilot Point, TX.

Smith, H. R. 1940. Beef liver condemnations. Proceedings of the American Society of Animal Nutrition, 1940:272-276.

Smith, H. A. 1944. Ulcerative lesions of the bovine rumen and their possible relation to hepatic abscesses. Am. J. Vet. Res. 5:234-242.

Smith, R.A., 1998. Impact of disease on feedlot performance: a review. J. Anim. Sci. 76:272-274. doi:10.2527/1998.761272x.

Snowder, G. D., L. D. Van Vleck, L. V. Cundiff, and G. L. Bennett. 2006. Bovine respiratory disease in feedlot cattle: environmental, genetic, and economic factors. J. Anim. Sci. 84: 1999-2008. doi:10.2527/jas.2006-046.

Tan, Z. L., T. G. Nagaraja, and M. M. Chengappa. 1996. Fusobacterium necrophorum infections: virulence factors, pathogenic mechanism and control measures. Vet. Res. Commun. 20:113-140.

Vasconcelos, J. T. and M. L. Galyean. 2007. Nutritional recommendations of feedlot consulting nutritionists: The 2007 Texas Tech University survey. J. Anim. Sci. 85: 2772-2781. doi:10.2527/jas.2007-0261.

Vestal, M. K., J. L. Lusk, E. A. DeVuyst, and J. R. Kropp. 2013. The value of genetic information to livestock buyers: a combined revealed, stated preference approach. Agric. Econ. 44:337-347. doi: 10.1111/agec.12016.

Wells, S.J., S. L. Ott, and A. H. Seitzinger. 1998. Key health issues for dairy cattle—new and old. J. Dairy. Sci. 81:3029-3035. doi: 10.3168/jds.S0022-0302(98)75867-9.

Zarei, M., M. Ghorbanpour, S. Tajbakhsh, and N. Mosavari. 2017. Comparison of rapid diagnostic tests to detect Mycobacterium avium subsp. paratuberculosis disseminated infection in bovine liver. Trop Anim. Health Prod. 49:1195-1200. doi:10.1007/s11250-017-1317-5.

Tale 4.1. Descriptive statistics of viscera outcomes for the sample population.

| Item | n[1] | Mean | Std. Dev | Min | Max |
|---|---|---|---|---|---|
| Edible liver, % | 321 | 70.7 | 16.1 | 0.0 | 94.0 |
| Contaminated liver, % | 321 | 5.5 | 3.6 | 0.0 | 25.0 |
| Total liver abscess[2], % | 321 | 21.4 | 15.0 | 0.0 | 100.0 |
| Minor liver abscess[3], % | 321 | 10.7 | 6.8 | 0.0 | 43.0 |
| A- liver abscess, % | 321 | 8.4 | 5.9 | 0.0 | 40.0 |
| A liver abscess, % | 321 | 2.3 | 2.3 | 0.0 | 11.0 |
| Major liver abscess[4], % | 321 | 10.8 | 11.0 | 0.0 | 100.0 |
| A+ liver abscess, % | 321 | 3.0 | 3.2 | 0.0 | 22.0 |
| A+Adhesion liver abscess, % | 321 | 6.0 | 8.5 | 0.0 | 100.0 |
| A+Open liver abscess, % | 321 | 1.3 | 2.9 | 0.0 | 27.0 |
| A+Adhesion/Open liver abscess, % | 321 | 0.5 | 1.2 | 0.0 | 9.0 |
| Cirrhosis liver, % | 321 | 0.1 | 0.5 | 0.0 | 4.0 |
| Liver flukes, % | 321 | 1.8 | 5.1 | 0.0 | 61.0 |
| Telangiectasis liver, % | 321 | 0.5 | 0.9 | 0.0 | 5.0 |

[1]n indicates individual feedyard lots, representing 43,255 total cattle.

[2]Total liver abscess = sum of all liver abscess scores.

[3]Minor liver abscess = sum of A- and A liver abscess scores.

[4]Major liver abscess = sum of A+, A+Adhesion, A+Open, and A+Adhesion/Open liver abscess scores.

Tale 4.2. Descriptive statistics of continuous survey response variables for the sample population.

| Item | n | Mean | Std. Dev | Min | Max |
|---|---|---|---|---|---|
| Latitude | 321 | 37.1 | 3.0 | 32.6 | 46.1 |
| Longitude | 321 | -102.9 | 4.8 | -118.9 | -95.1 |
| Elevation, m | 321 | 931.0 | 295.6 | 75.0 | 1425.9 |
| Annual rainfall, cm | 317 | 48.5 | 14.2 | 8.4 | 70.9 |
| Morbidity, % | 151 | 7.9 | 8.5 | 0.0 | 55.0 |
| Mortality, % | 263 | 2.0 | 2.6 | 0.0 | 21.0 |
| In BW, kg | 272 | 340.5 | 207.5 | 94.1 | 619.6 |
| Out BW, kg | 267 | 614.8 | 49.9 | 412.8 | 725.7 |
| Total gain, kg | 267 | 275.2 | 84.8 | 57.2 | 525.7 |
| DMI, kg | 271 | 9.1 | 1.2 | 6.2 | 13.1 |
| ADG, kg | 273 | 1.4 | 0.2 | 0.7 | 2.0 |
| DOF | 272 | 196.4 | 69.6 | 50.0 | 385.0 |
| G:F | 271 | 0.15 | 0.02 | 0.08 | 0.21 |
| Diet NEg, Mcal/45.35 kg | 294 | 68.6 | 2.7 | 62.0 | 74.5 |
| Diet dry matter, % | 308 | 63.3 | 6.5 | 43.0 | 74.0 |
| Grain inclusion, % | 290 | 72.7 | 15.0 | 41.0 | 95.0 |
| Forage inclusion, % | 308 | 9.2 | 1.4 | 6.0 | 18.0 |
| Forage absolute eNDF, %[1] | 285 | 9.9 | 4.0 | 4.0 | 22.0 |
| Forage actual eNDF, %[1] | 286 | 5.5 | 2.2 | 0.0 | 13.0 |
| Total diet eNDF, %[1] | 281 | 8.6 | 2.4 | 6.0 | 17.0 |
| Forage chop length, cm | 301 | 6.1 | 3.6 | 1.0 | 12.7 |
| Time fed/d | 321 | 2.8 | 0.4 | 1.0 | 3.0 |
| Bunk reads/d | 321 | 2.9 | 0.8 | 1.0 | 4.0 |
| Pen density, $m^2$ | 240 | 16.0 | 3.6 | 11.1 | 46.5 |
| Linear bunk space, cm | 264 | 24.1 | 4.6 | 17.8 | 50.8 |

[1]eNDF values are represented as a percent of pure item within total mass of the variable. (i.e. 9.9% of the forage supplied is pure eNDF).

Table 4.3. Univariate models to predict major and total liver abscess incidence.

| Item | Major abscess, % Adj. $R^2$ | Effect | Total abscess, % Adj. $R^2$ | Effect |
|---|---|---|---|---|
| Grain inclusion, % | 0.12 | - | 0.17 | - |
| Cattle-type[1] | 0.14 | - | 0.13 | - |
| Latitude | 0.05 | + | 0.12 | + |
| Diet dry matter, % | 0.06 | - | 0.11 | - |
| G:F | 0.06 | - | 0.11 | - |
| Technology[2,4] | 0.11 | - | 0.11 | - |
| Longitude | 0.06 | - | 0.08 | - |
| Mortality, % | 0.04 | + | 0.07 | + |
| Tylosin[2] | 0.06 | - | 0.06 | - |
| Other antibiotic[2,5] | 0.05 | + | 0.07 | + |
| Annual rainfall, cm | 0.02 | - | 0.03 | - |
| Total diet eNDF, % | 0.00 | . | 0.03 | - |
| Out BW, kg | 0.02 | + | 0.03 | + |
| Bunk reads/d | 0.01 | - | 0.02 | - |
| Sex[3] | 0.00 | . | 0.00 | . |
| DMI, kg | 0.00 | . | 0.00 | . |
| DOF | 0.02 | + | 0.00 | . |
| Forage inclusion, % | 0.00 | . | 0.00 | . |
| Diet NEg, Mcal/45.35 kg | 0.00 | . | 0.00 | . |
| Chop length, cm | 0.00 | . | 0.00 | . |
| Times fed/day | 0.00 | . | 0.00 | . |
| Pen density, $m^2$ | 0.00 | . | 0.00 | . |
| Linear bunk space, cm | 0.00 | . | 0.00 | . |

[1]Cattle type: 1 = native beef; 0 = Holstein.
[2]Nominal outcome: 1 = yes; 0 = no.
[3]Sex: 1 = steer; 0 = heifer.
[4]Technology is a combination of implant status, ractopamine supplementation, and ionophore supplementation.
[5]Other antibiotic is a combination of metaphalyxis and tetracycline supplementation.

Table 4.4. Adjusted R2, and Akaike Information Criterion of best fit models containing 1 …..n variables for equations to predict total liver abscess incidence using the maximum likelihood method to calculate missing data.

| **Total Liver Abscess Incidence[1]** | Adj. $R^2$ | AIC[2] | *P*-value |
|---|---|---|---|
| Variable(s) in model | | | |
| Cattle type[3] | 0.13 | 2806.6 | <0.001 |
| Cattle type; G:F | 0.26 | 1356.3 | <0.001 |
| Cattle type; G:F; Diet DM, % | 0.32 | 499.8 | <0.001 |
| **Cattle type; G:F; Diet DM, %; Tylosin supplementation[4]** | **0.34** | **267.2** | **<0.001** |

*Bold denotes best fit model for prediction total liver abscess incidence (%).

[1]Total liver abscess incidence = sum of all liver abscess outcomes (%) as described by Brown and Lawrence (2010).

[2]AIC = Akaike Information Criterion (smaller is

[3]Cattle type: 1 = native beef; 0 = Holstein.

[4]Tylosin supplementation: 1 = yes; 0 = no.

Maximum likelihood method used as described by Allison (2012).

Table 4.5. Adjusted $R^2$, and Akaike Information Criterion of best fit models containing 1 …..n variables for equations to predict major liver abscess incidence using the maximum likelihood method to calculate missing data.

| **Major Liver Abscess Incidence[1]** | Adj. $R^2$ | AIC[2] | *P*-value |
|---|---|---|---|
| Variable(s) in model | | | |
| Cattle type[3] | 0.14 | 2605.0 | <0.001 |
| Cattle type; G:F | 0.22 | 1172.8 | <0.001 |
| Cattle type; G:F; Diet DM, % | 0.24 | 329.6 | <0.001 |
| **Cattle type; G:F; Diet DM, %; Tylosin supplementation[4]** | **0.28** | **91.8** | **<0.001** |

*Bold denotes best fit model for prediction total liver abscess incidence (%).

[1]Major liver abscess incidence = sum of A+, A+AD, A+OP, and A+AD/OP liver abscess outcomes (%) as described by Brown and Lawrence (2010).

[2]AIC = Akaike Information Criterion (smaller is better).

[3]Cattle type: 1 = native beef; 0 = Holstein.

[4]Tylosin supplementation: 1 = yes; 0 = no.

Maximum likelihood method used as described by Allison (2012).

# CHAPTER 5:

# ASSOCIATION OF LIVER ABSCESS PRESENCE AND SEVERITY WITH TRIM LOSS, HARVEST YIELD, CARCASS GRADING PERFORMANCE, LUNG LESIONS, AND VALUE OF FED HOLSTEINS

## 5.1 ABSTRACT

Fed Holsteins were tracked through two commercial processing facilities, one in the High Plains region (n = 1,073) and one in the Central Plains region (n = 1,070) from fall of 2016 through spring of 2017. Our objective was to evaluate the effect of liver abscesses on carcass characteristics and value of calf-fed Holstein carcasses. Liver abscesses were visually assessed and scored according to a modified scoring system based on the Elanco Liver Check Service; simultaneously, lungs were manually palpated to assess degree of consolidation and fibrin tag formation, and its association to liver abnormality. Finally, carcass trim was weighed from carcasses moved off-line for zero tolerance trimming. Carcass and viscera values were assigned using USDA market reports and adjusted based on viscera condemnations along with premiums and discounts for quality and yield outcomes. Data were analyzed using the MIXED and GENMOD procedures of SAS v9.4 (SAS Inst. Inc., Cary, NC) with carcass as the experimental unit. Cattle exhibited liver abnormality rates of A- = 3.73%, A = 7.28%, A+ = 7.56%, A+Adhesion (A+AD) = 17.50%, A+Open (A+OP) = 4.39%, A+Adhesion/Open

(A+AD/OP) = 3.92%, and Contamination = 6.16%, with 49.46% of livers being edible. Hot carcass weight was reduced ($P < 0.01$; -25.1 kg, -6.6%) in carcasses that had an A+AD/OP liver abscess as compared to carcasses with edible livers. Carcasses with A+AD or A+OP liver scores had increased ($P < 0.01$) carcass trim (3.92 and 3.39 kg, respectively), when compared to carcasses with edible livers (0.38 kg). A greater degree ($P < 0.001$) of lung consolidation was observed in carcasses with A+AD and A+AD/OP liver scores than carcasses with edible livers. Livers with abscesses yielded an average loss to the beef processor of $3.25 per condemned liver ($3.89/edible liver vs $0.64/condemned liver). Given the 44.38% incidence of liver abnormalities, and the 25.81% incidence of open and adhered abscesses observed, fed beef processors lose an estimated $21.8 million annually in condemned viscera due to liver abnormalities in fed Holsteins. Although not different ($P = 0.27$), carcasses with A+AD liver outcomes were worth 4.9% less (-$73.93/carcass) than their edible counterparts, and carcasses with A+OP livers were worth 4.6% less (-$69.65/carcass) than carcasses with edible livers. Unless the liver abscess issue is addressed by the fed beef industry, Holsteins will continue to incur great financial risk to the beef industry.

**Keywords**: carcass quality; carcass trim; Holstein; liver abscess

## 5.2 INTRODUCTION

Liver abscesses in cattle are commonly thought to result from aggressive feeding programs in which readily available dietary starches are rapidly fermented by rumen microbes, resulting in increased acid concentrations, lower ruminal pH, and eventual ruminal acidosis (Brent, 1976). Decreases in ruminal pH cause damage to the epithelial lining of the rumen, allowing *Fusobacterium necrophorum* to implant itself in the rumen wall and form a rumen wall lesion (Lechtenberg et al., 1988). Once implanted, *F. necrophorum* is believed to be able to pass through the rumen wall, enter portal circulation and eventually be sequestered in the liver, where it can proliferate and form a liver abscess due to accumulation of necrotic and purulent material (Lechtenberg et al., 1988; Nagaraja and Chengappa, 1998).

Liver abscesses are associated with reduced growth performance (Brink et al., 1990), diminished viscera yield and value (Smith, 1940; Brown and Lawrence, 2010), increased carcass trim losses (Montgomery, 1985), and decreased carcass quality (Montgomery, 1985; Brown and Lawrence, 2010). Although liver abscesses may impact the value of any carcass, these outcomes disproportionally affect fed Holsteins because the incidence and severity of abscesses are increased when compared to "native" beef-type cattle (Montgomery, 1985; Amachawadi and Nagaraja, 2016). Calf-fed Holsteins have become a significant proportion of the national slaughter market, with the latest National Beef Quality Audit (NBQA) reporting 20.4% of cattle at harvest were Holstein (Eastwood et al, 2017). Data on the effect liver abscesses have on carcass value has not been established in calf-fed Holsteins. Therefore, the objective of this research was to

evaluate the effect of liver abscesses on carcass characteristics and value of calf-fed Holstein carcasses.

## 5.3 MATERIALS AND METHODS

Animal care and use committee approval was not required for this study, because no live animals were used for data collection.

### *5.3.1 Data collection*

Fed Holsteins were tracked through two commercial processing facilities, one in the High Plains region (n = 1,073), and one in the Central Plains region (n = 1,070), from the fall of 2016 through the spring of 2017. In order to match measured carcass parameters with individual animals, individual ear tags and or lot tags were recorded for each animal on pre-numbered sheets which matched a unique harvest order number. Poly-vinyl tags (6.65 × 6.98 cm, Label Logic Inc., Elkhart, IN) matching the harvest order number were assigned when ear tags were recorded and then pinned (magnetic shroud pin, Koch Supplies, Kansas City, MO) to the carcasses for positive identification throughout the data collection procedure.

Liver abscesses were visually assessed and scored by trained university personnel, according to a modified scoring system based on the Elanco Liver Check Service (✓ = no abscesses, A- = 1 or 2 small abscesses, A = 2 to 4 small active abscesses, A+ = multiple small abscesses or 1 or more large active abscesses, A+ Adhesion (A+AD) = liver adhered to GI tract, A+ Open (A+OP) = open liver abscess, A+Adhesion/Open (A+AD/OP) = liver adhered to GI tract with an open abscess). Other liver abnormalities recorded if present included: telangiectasis, flukes, contamination (liver condemned for

contamination), cirrhosis or any other abnormality (adapted from Brown & Lawrence, 2010). Six carcasses exhibited livers with flukes and thirteen carcasses exhibited livers with telangiectasis; these carcasses were removed from the study due to low frequency and because the focus of this study was on the effect that liver abscesses have on carcass trim and carcass value. Liver abscesses were also classified by severity in order to consolidate outcomes by overall abscess severity: None (liver did not possess abscess at slaughter), Minor (combination of A and A- liver abscesses), and Major (combination of all other liver abscess outcomes; A+ or greater).

Lungs were visually inspected and palpated for lung health evaluation. Dorsal and ventral surfaces were visually evaluated for consolidated lung tissue. Cranial, caudal, and accessory lobes of each lung were manually palpated to detect presence of fibrin tags. Absence of lung tissue was noted to assess presence and severity of adhesion to the thoracic cavity. Lung score outcomes were: N = normal, no lesions observed; M or E = presence of minor (M) or extensive (E) interlobular adhesions; 1 = consolidation of lung lobes, presence of mycoplasma-like lesions, a portion of lung missing, or a combination of these affecting >5% to 15% of lung tissue; 2 = pleural adhesions, a portion of lung missing, or a combination of these affecting >15% to 50% of lung tissue; 3 = pleural adhesions, a portion of lung missing, or a combination of these affecting >50% of lung tissue; C = lung was condemned for contamination; X = lung did not deflate at death. Other than normal and extent of fibrin tags or consolidation, scores were not mutually exclusive and therefore a lung could be scored with a combination of outcomes (i.e. M, 1, C; adapted from Tennant et al., 2014)

When a carcass was moved off-line and placed in the "railout" bay, all trim removed from the carcass was weighed and recorded along with harvest order number to match data with the other carcass parameters measured at the processing facility. Carcasses were moved off-line if contamination was present on the carcasses in quantities exceeding what normal on-line production procedures could handle for zero tolerance trimming. Trim was weighed in plastic totes (Toteall 2000 molded poly totes, Koch Supplies, Kansas City, MO) on a platform scale (Model UFM-B60, Summit Measurements, South Deerfield, MA) prior to the carcass being placed back on the production line. The processor carcass ID number was recorded for all carcasses tagged with a harvest order number in order to match lot-based ear tags with harvest floor parameters measured. Additional data collected included carcass grading information provided by the processor. Grade data was obtained from video image analysis (VIA) camera systems (E+V VGB 2000, Technology GmbH & Co. KG) and matched to individual processor carcass ID for further analysis. Data obtained included: marbling score, calculated yield grade, HCW, 12$^{th}$-rib subcutaneous fat depth, LM area, and percentage KPH (estimated at 2.5%; USDA, 2016).

### *5.3.2 Carcass valuation*

Each carcass was assigned a base value using prices generated from every Friday report of the USDA Agricultural Marketing Service weekly beef carcass price equivalent index value report (USDA, 2016; NW_LS410), for the 2016 calendar year, to develop a yearly average carcass base value. Carcass premiums (USDA Prime or Premium Choice quality grades; yield grades 1 or 2) and discounts (USDA Select or Standard quality grades; yield grades 4 or 5; dark cutter; dairy type) were calculated using combined

averages from every Friday report of the USDA Agricultural Marketing Service weekly slaughter cattle premium and discount report (USDA, 2016; LM_CT155) for the 2016 calendar year, to generate average yearly premiums and discounts to be applied to carcasses.

### *5.3.3 Viscera valuation*

Viscera value was calculated based on the value of a liver and other visceral organs reported in the USDA Agricultural Marketing Service by-product drop value (steer) report (USDA, Jan 2016 through Dec 2016; NW_LS441), using the Friday report of every week. Values obtained included unrealized revenue from condemnation of liver or liver and gut mass (hearts, reg, bone out; tripe, scalded edible, bleached; tripe, honeycomb bleached; lungs, inedible; melts), when open abscess was present; as well as price of inedible tallow and meat and bone meal, to account for recovered revenue from rendered product. Harvest yields from May et al., (2016) were used to calculate mass of meat and bone meal and tallow generated from condemned viscera organs for value calculations.

Using those reports, the average edible beef liver was valued at $3.89, while a condemned liver that was rendered into meat and bone meal was valued at $0.64. Similarly, an edible beef GI mass was valued at $14.35 and a rendered GI mass was valued at $6.11. When a liver or GI mass is condemned by USDA the product goes to rendering and is salvaged as inedible meat and bone meal. Regardless of whether the GI mass is passed or condemned by USDA inspectors, fat deposits from around the viscera are salvaged as inedible tallow. Calculated values from that product were not included in viscera loss calculations because tallow is salvaged regardless of condemnation.

To estimate total value lost, the value of rendered product was subtracted from the value of edible product. Once adjusted, liver values were then multiplied by total abscessed liver rate, and total contamination rate. Also, GI mass value was multiplied by the open abscess rate (both A+Open and A+Adhesion/Open), because GI mass is condemned when an open abscess is present. Lastly, values were summed to quantify total liver and GI mass value (i.e $3.25 x liver loss (abscess+contamination+other) + ($8.24 x open abscesses). Once viscera loss per liver score was generated, calculations were scaled to a national level. Using the USDA 2016 Livestock Slaughter Summary and the Holstein incidence at slaughter noted by the 2016 NBQA (Eastwood et al., 2017), approximately 6.1 million fed Holsteins are slaughtered in the United States each year. Viscera value lost per liver score was then multiplied by total number of Holsteins slaughtered to generate approximate losses to the industry due to liver abscesses in fed Holsteins.

### *5.3.4 Statistical analysis*

For all analyses in this experiment, individual carcass served as the experimental unit (n = 2,143). Comparisons of carcass traits and value variables across liver abnormality outcomes and abscess classes were analyzed using the MIXED procedure of SAS v9.4 (SAS Inst. Inc., Cary, NC), with the fixed effects of liver score or abscess class and the random effects of processor, date, and source. Processor (n = 2), date (n = 9), and source (n = 15) were used as random effects to account for variations in processing plant personnel, cattle marketing strategies, and varying feeding management practices, respectively. Single degree of freedom contrast statements were generated using the ESTIMATE option to test for differences in carcass characteristics and value between

carcasses with edible (normal) livers and carcasses with livers of each abscess classification. Percentage of caresses that were choice or better, percentage of carcasses railed out, and liver and lung associations were analyzed using the GENMOD procedure of SAS v9.4 with the fixed effect of liver score or abscess classification. Least square means were generated using the LSMEANS option and separated using the PDIFF option with a Tukey-Kramer adjustment for multiple comparisons to determine differences in carcass characteristics and carcass value parameters, along with differences in lung health associations, across liver score and abscess classes. Significance was declared at $P \leq 0.05$ and tendencies were declared at $P \leq 0.10$.

## 5.4 RESULTS AND DISCUSSION

### *5.4.1 Liver abnormality incidence*

Cattle in this experiment exhibited liver abnormality rates of A- = 3.73%, A = 7.28%, A+ = 7.56%, A+AD = 17.50%, A+OP = 4.39%, A+AD/OP = 3.92%, Contamination = 6.16%, with 49.46% of livers being edible (Table 5.1). Total abscess incidence rate was 44.38% with 11.01% incidence of minor abscesses, and 33.36% major abscesses (Table 5.2). Total liver abscess incidence was more than double the 17.8% liver abscess incidence reported in the 2016 NBQA, an audit that was comprised of both beef breed and Holstein animals (Eastwood et al., 2017). Although liver abscess incidence in this experiment was greater than those observed in the National Beef Quality Audit, an increased liver abscess incidence in Holsteins is not a new phenomenon. Amachawadi and Nagaraja (2016) reported a 10-year average of liver abscess incidences in Holstein steers of 28.3% and from 2010-2014 liver abscess incidences ranged from 30-

50%. Dietary regimen for the study population is not known, but we presume that cattle were fed antimicrobials to control for liver abscesses and were reared in a traditional calf-fed Holstein production system (i.e. more days on feed with an energy-intensive diet).

### *5.4.2 Carcass characteristics*

Hot carcass weight was reduced ($P = 0.04$; -25.1kg, -6.6%) in carcasses that had an A+AD/OP liver abscess as compared to carcasses with edible livers (Table 5.1). Carcasses with livers scored A+AD were 17.3 kg lighter (-4.6%; $P = 0.27$) than carcasses with edible livers, and carcasses with A+OP liver outcomes were 15.5 kg lighter (-4.1%; $P = 0.74$) than their edible liver counterparts. These values were not different likely due to varying ending live weights and marketing strategies for these cattle; however, a 15.5 to 17.3 kg reduction in hot carcass weight is financially important to cattle producers. Similar effects were observed when hot carcass weight was delineated by abscess class (Table 5.2). Although hot carcass weight did not differ ($P = 0.23$) by abscess class, carcasses with major liver abscesses had carcasses that were 13.6 kg (-3.6%) lighter than their counterparts with no liver abscess.

Parallel to decreased carcass weights, carcasses with A+AD or A+OP livers had increased ($P < 0.001$) carcass trim relative to carcasses with edible livers (3.92, 3.39, and 0.38 kg, respectively), which further contributed to decreased carcass weights (Table 5.1). When separated by abscess class (Table 5.2), carcasses with liver outcomes in the major classification had greater carcass trim than carcasses with minor liver abscesses or carcasses with no liver abscess at slaughter (1.40 and 0.39 kg, respectively; $P < 0.001$). Montgomery (1985) reported similar findings in Holsteins; observing that as abscesses progressed from minor to major, trim attributable to abscesses increased. Although

Montgomery's (1985) observation follows the same trends observed in this experiment, average trim removed in this experiment was more than double what Montgomery reported in the 1980's.

Concomitant with increased carcass trim, carcasses with more severe liver abscess outcomes were more often removed from the production line for additional trimming than carcasses with edible livers or less severe abscess outcomes (Tables 5.1, and 5.2). Carcasses with A+AD/OP (22.9%), A+OP (21.3%) and contamination (9.1%) liver outcomes were railed-out at increased ($P < 0.001$) rates when compared to carcasses with edible (3.6%) livers. Similarly, carcasses with major liver abscesses were railed-out at increased ($P < 0.001$) rates than carcasses with no liver abnormality at slaughter (9.7 and 4.2%, respectively). When a liver is adhered to the diaphragm or when an abscess ruptures, beef processing personnel must trim the contaminated area. This additional trimming is not often possible on a moving production line and requires the removal of the carcass from the production line. Once there, the carcasses will be trimmed of all adhering contaminates, resulting in increased carcass trim and decreased hot carcass weights. In addition, regardless of abscess classification, 5.9% of all carcasses in this study were removed from the production line for additional trimming.

Carcasses with A+AD and A+OP livers also had less ($P < 0.01$) $12^{th}$ rib subcutaneous fat than carcasses with edible livers (-6.8% and -6.8%, respectively; Table 5.1). Moreover, carcasses with A+AD and A+OP livers had less ($P < 0.01$) LM area than their counterparts with edible livers (-2.8% and -4.4%, respectively). These changes in $12^{th}$ rib subcutaneous fat and muscling were not reflected in the calculated yield grade, which was not affected ($P = 0.13$) by liver abscess outcome. Carcasses with liver

abnormalities in the major classification had 3.9% less ($P = 0.01$) 12$^{th}$ rib subcutaneous fat compared to carcasses with no liver abscess, coupled with a 2.3% decrease ($P < 0.01$) in LM area compared to carcasses with no liver abscess at slaughter (Table 5.2). These differences resulted in a trend ($P = 0.06$) for a 1.8% decrease in calculated carcass yield grade for carcasses with major liver abscesses compared to carcasses with no liver abscess at slaughter.

Marbling score was decreased ($P = 0.03$) in carcasses with A+AD and A+AD/OP liver abnormalities when compared against carcasses with edible livers (Small$^{59}$, Small$^{55}$, and Small$^{78}$, respectively; Table 5.1). These results are similar to those reported by Brown and Lawrence (2010), who reported that carcasses with A+AD liver abscesses had decreased marbling scores when compared to carcasses with edible livers. When marbling score was segregated by percentage Choice or better (Small$^{00}$ or greater), carcasses with A+ liver scores had a greater proportion ($P < 0.001$) of carcasses that graded Choice or better than carcasses with edible livers (79.6% and 72.9%, respectively). Carcasses with A-, A+OP, A+AD/OP and contaminated livers had decreased ($P < 0.001$) proportions of carcasses that graded Choice or better than carcasses with edible livers (72.9%). When analyzed by abscess class (Table 5.2), carcasses with major liver abscesses had less ($P < 0.01$) marbling than carcasses with no liver abscess (Small$^{62}$ vs. Small$^{77}$) but were not different ($P > 0.05$) from carcasses with minor liver abscesses (Small$^{75}$). Although differences in marbling scores were statistically significant, these differences were not enough to change the quality grade of these cattle. No difference ($P = 0.95$) was observed in the proportion of carcasses that graded Choice or better when segregated by abscess class

Average quality marketing group for all carcasses, regardless of abscess classification, was Low Choice (Small$^{00}$ to Small$^{90}$). Slight numerical differences in marbling score and calculated yield grade did not affect overall premiums and discounts for these metrics when individual liver scores were separated ($P \geq 0.11$; Table 5.3). However, when liver scores were grouped by abscess classification, there was a trend ($P = 0.09$) for a larger quality grade discount for carcasses with livers classified as major compared to none and minor (-2.87, $/45.35 kg and -2.27, $/45.35 kg, respectively; Table 5.6).

### *5.4.3 Association of liver abnormalities and lung health*

During the course of this experiment lung abnormalities were also recorded (Table 5.3, Table 5.4) to quantify associations between liver abnormalities and lung health. A greater degree ($P < 0.001$) of lung consolidation was observed in carcasses with A+AD (32.53% with consolidation score 2, and 19.73% with consolidation score 3) and A+AD/OP (26.19% with consolidation score 2, and 14.29% with consolidation score 3) liver scores than carcasses with normal livers (13.98% with consolidation score 2, and 5.67% with consolidation score 3). Severe liver abscesses are capable of adhering to the diaphragm, which in turn can become adhered to the lung; likely contributing to increased lung consolidation and more severe abscess scores. Along with increased lung consolidation, carcasses with A+AD livers had more ($P < 0.001$) extensive fibrin tag formation than carcasses with edible livers (25.33% and 17.37%, respectively). Additionally, carcasses that had A+OP or A+AD/OP liver abscesses had greater rates ($P < 0.001$) of pluck condemnation than carcasses with edible livers or carcasses without

open abscesses. This result was not unexpected as an open abscess typically results in all viscera being condemned due to concerns of contamination by USDA inspectors.

These associations were further highlighted when segregated by abscess class (Table 5.4). Carcasses with major abscesses had greater ($P < 0.001$) rates of lung consolidation (25.98% with consolidation score 2 and 14.61% with consolidation score 3) than carcasses with no liver abscess (14.36% with consolidation score 2 and 5.96% with consolidation score 3) or carcasses with minor liver abscesses (19.49% with consolidation score 2 and 4.66% with consolidation score 3). These results indicate that strong associations exist between overall liver and lung health. When segregated by abscess class, no difference ($P = 0.31$) in the incidence of extensive fibrin tag formation was detected, whereas carcasses with no liver abscess and minor abnormalities had increased ($P < 0.001$) incidence of minor fibrin tag formation compared to carcasses with major liver abnormalities (27.54%, 26.27%, and 14.33%, respectively).

A major liver abscess is the result of a severe bacterial infection, which results in a localized immune response (Garcia et al., 1974). Concomitantly, the immune system could be fighting an infection in the lung, and ultimately the whole animal may suffer from trying to fight off multiple bacterial infections simultaneously. Associations between liver and lung health further help to explain the numeric decreases in hot carcass weight observed in carcasses with major liver abscesses. When an animal is fighting an infection, energy that could have been utilized for growth will be redirected towards immunity (Waggoner at al., 2009), because living is more important than growth at that point in the animal's life.

### *5.4.4 Economic analysis*

The incidence of liver abscesses observed in this experiment represents a significant economic loss to the beef feeding and processing industries. Livers with abnormalities yield an inedible liver with an average loss to the beef processor of $3.25 per condemned liver ($3.89/edible liver vs $0.64/condemned liver; USDA NW LS441, 2016). When a liver is condemned, it is rendered and sold as meat and bone meal, which drastically reduces its value. Using the USDA 2016 Livestock Slaughter Summary (USDA, 2017) and the Holstein incidence at slaughter noted by the 2016 National Beef Quality Audit (Eastwood et al., 2017), approximately 6.1 million fed Holsteins are slaughtered in the United States each year. Holsteins that are fed in feedlots and commercially slaughtered are a by-product of the dairy industry and given that Americans consume approximately 112.7 kg of dairy products per capita annually (USDA, 2016; Economic Research Service, Food Availability (Per Capita) Data System), fed Holsteins will continue to exist in the fed beef market.

Given the 44.38% incidence of liver abnormalities observed in this trial, the direct economic loss due to liver abscesses in fed Holsteins is estimated near $8.8 million/year to beef processors. Additionally, liver abscess scores of A+AD, A+OP, and A+AD/OP pose an even greater economic loss due to the loss of the liver, the gastrointestinal tract, and occasionally the pluck (heart, trachea, and lungs). Liver abscesses inducing viscera condemnations resulted in an average loss to the processor of $8.24 per incident ($14.35/edible viscera vs. $6.11/condemned viscera; USDA, 2016; NW_LS441), or an approximate annual loss of $13 million, given the 25.81% incidence of A+AD, A+OP, and A+AD/OP liver abscess outcomes. These values are similar to the $15.8 million for

the loss of livers due to abnormalities and the $7 million lost for condemned gastrointestinal tracts reported by Brown and Lawrence (2010); however, that experiment was focused at the entire fed beef population and not specific to fed Holsteins like the current experiment. Furthermore, these values are conservative because USDA offal value reports use non-Holstein beef for their carcass basis calculations, and visceral organs from fed Holsteins are typically heavier than those in non-Holstein beef (May et al., 2016).

Segregating outcomes by liver abscess score (Table 5.5) and abscess class (Table 5.6), further highlights the detrimental effect that certain liver abscesses may have on gross carcass and viscera value. As previously mentioned, no differences ($P > 0.10$) were observed in carcass grading premiums or discounts when segregated by liver score (Table 5.5). Gross carcass value was reduced ($P = 0.03$; $110.99/carcass -7.3%) when carcasses had A+AD/OP liver outcomes when compared to carcasses with edible livers. Although not different ($P = 0.27$), carcasses with A+AD liver outcomes were worth 4.9% less (-$73.93) than carcasses with edible livers, and carcasses with A+OP livers were worth 4.6% less (-$69.65) than carcasses with edible livers. These values are greater, than those reported by Brown and Lawrence (2010), who reported that carcasses with A+OP livers were 2.9% less valuable than carcasses with edible livers, and carcasses with A+AD livers were valued 3.9% less than carcasses with edible livers. All carcasses with liver abnormalities had reduced ($P < 0.001$) gross offal value when compared to carcasses with edible livers. Carcasses with edible livers had the most valuable offal at $17.98 per carcass whereas carcasses with A+AD/OP ($6.86/carcass; -61.8%), A+OP

($6.94/carcass; -61.4%), and A+AD ($7.76/carcass; -56.8%) livers were the least valuable.

Similar results were observed when carcasses were segregated by abscess classification (Table 5.6). Carcasses with abscesses in the major classification tended ($P = 0.08$) to have increased total carcass discounts ($-6.28/45.35 kg) and an overall decreased ($P = 0.09$) market value ($180.48/45.35 kg), when compared to carcasses with no liver abscess at slaughter ($-5.56/45.35 kg and $181.20/45.35 kg, respectively) and those with minor abnormalities ($-5.35/45.35 kg and $181.41/45.35 kg, respectively). This tendency was not reflected in gross carcass value. Although carcasses with major liver abnormalities were valued at $58.54/carcass (-3.9%) less than carcasses with no liver abscess, this difference was not significant ($P = 0.20$).

When a severe abscess is adhered to adjacent tissue it makes viscera removal difficult and increases the risk of gastrointestinal tract rupture, which in turn, increases carcass trim and results in a carcass that has reduced hot carcass weight and subsequent value. Additionally, when an abscess ruptures during viscera removal, more viscera is condemned by USDA inspectors, resulting in increases in rendered product, while simultaneously increasing the likelihood that purulent material contaminates the carcass, which must be trimmed from the carcass. Total gross viscera value was decreased ($P < 0.001$) in carcasses with major liver abnormalities ($9.05/carcass; -48.3%) and minor liver abnormalities ($14.58/carcass; -16.8%) when compared to carcasses with no liver abnormalities ($17.52/carcass; Table 5.6). Since many components of the GI mass are further processed for sale as edible product, open abscesses that exude puss or are adhered to the GI tract, render these products inedible. The condemnation of these

products results in a loss to the packer because they must now be rendered or disposed of instead of sent to a retail store for consumption.

The observed liver and lung health associations, in conjunction with increased carcass trim, may explain why carcasses with major liver abnormalities weighed less at the hot scale and were ultimately valued less than their counterparts lacking any liver abnormality. Additionally, carcasses with major liver abscesses had overall decreased GI mass value and increased rates of carcasses removed from the production line for trimming, resulting in a loss to the entire production system that negatively impacts both the packer and the producer. Fed Holsteins do not appear to be leaving the beef industry anytime soon, even though Holsteins have increased rates of severe liver abscesses compared to their non-Holstein equivalents. Unless the liver abscess issue is addressed by the fed beef industry, Holsteins will continue to incur greater financial risk to both cattle feeders and processors.

## 5.5 LITERATURE CITED

Amachawadi, R. G., and T. G. Nagaraja. 2016. Liver abscesses in cattle: A review of incidence in Holsteins and of bacteriology and vaccine approaches to control in feedlot cattle. J. Anim. Sci. 94:1620-1632. doi:10.2527/jas2015-0261.

Brent, B.E., 1976. Relationship of acidosis to other feedlot ailments. J. Anim. Sci. 43:930-935.

Brink, D. R., S. R. Lowry, R. A. Stock, and J. C. Parrott. 1990. Severity of liver abscesses and efficiency of feed utilization of feedlot cattle. J. Anim. Sci. 68:1201-1207.

Brown, T. R., and T. E. Lawrence. 2010. Association of liver abnormalities with carcass grading performance and value. J. Anim. Sci. 88:4037-4043. doi: 10.2527/jas.2010-3219.

Eastwood, L. C., C. A. Boykin, M. K. Harris, A. N. Arnold, D. S. Hale, C. R. Kerth, D. B. Griffin, J. W. Savell, K. E. Belk, D. R. Woerner, J. D. Hasty, R. J. Delmore, J. N. Martin, T. E. Lawrence, T. J. McEvers, D. L. VanOverbeke, G. G. Mafi, M. M. Pfeiffer, T. B. Schmidt, R. J. Maddock, D. D. Johnson, C. C. Carr, J. M. Scheffler, T. D. Pringle, and A. M. Stelzleni. 2017. National Beef Quality Audit-2016: Transportation, mobility, and harvest-floor assessments of targeted characteristics that affect quality and value of cattle, carcasses, and by-products1. Translational Animal Science. 1:229-238. doi:10.2527/tas2017.0029.

Garcia, M.M., D. C. Alexander, K. A. McKay, W. J. Dorward, and S. E. Magwood. 1974. Results of a preliminary trial with Sphaerophorus necrophorus toxoids to control liver abscesses in feedlot cattle. Canadian Journal of Comparative Medicine. 38:222-226.

Lechtenberg, K. F., T. G. Nagaraja, H. W. Leipold, and M. M. Chengappa. 1988. Bacteriologic and histologic studies of hepatic abscesses in cattle. Am. J. Vet. Res. 49:58-62.

May, N.D., T. J. McEvers, L. J. Walter, J. A. Reed, J. P. Hutcheson, and T. E. Lawrence. 2016. Byproduct yields of serially harvested calf-fed Holstein steers fed zilpaterol hydrochloride. J. Anim. Sci. 94:4006-4015. doi:10.2527/jas2016-0486.

Montgomery, T. H. 1985. The influence of liver abscesses upon beef carcass yields. Special technical bulletin. West Texas State University.

Nagaraja, T. G., and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: a review. J. Anim. Sci. 76:287-298.

Smith, H. R. 1940. Beef liver condemnations. Proceedings of the American Society of Animal Nutrition, 1940:272-276.

Tennant, T.C., S. E. Ives, L. B. Harper, D. G. Renter, and T. E. Lawrence. 2014. Comparison of tulathromycin and tilmicosin on the prevalence and severity of bovine respiratory disease in feedlot cattle in association with feedlot performance, carcass characteristics, and economic factors. J. Anim. Sci. 92:5203-5213. doi: 10.2527/jas.2014-7814.

USDA. 2016. Economic Research Service, Food Availability (Per Capita) Data System. Accessed 28 Aug, 2017. https://www.ers.usda.gov/data-products/food-availability-per-capita-data-system/

USDA. 2016. Official United States standards for grades of carcass beef. Accessed Jun. 12, 2017. https://www.ams.usda.gov/sites/default/files/media/Carcass%20Beef%20Standard.pdf.

USDA. 2016. LM_CT155, National Weekly Direct Slaughter Cattle - Premiums And Discounts. Accessed 28 Aug, 2017. https://www.ams.usda.gov/mnreports/lm_ct155.txt

USDA. 2016. NW_LS410, USDA Beef Carcass Price Equivalent Index Value. Accessed 28 Aug, 2017. https://www.ams.usda.gov/mnreports/nw_ls410.txt

USDA. 2016. NW_LS441, By-Product Drop Value (Steer). Accessed Jan. 27, 2017. https://www.ams.usda.gov/mnreports/nw_ls441.txt

USDA. 2017. Livestock Slaughter 2016 Summary (April 2017). USDA, National Agricultural Statistics Service http://usda.mannlib.cornell.edu/usda/current/LiveSlauSu/LiveSlauSu-04-19-2017.pdf

Waggoner, J.W., C. A. Löest, J. L. Turner, C. P. Mathis, and D. M. Hallford. 2009. Effects of dietary protein and bacterial lipopolysaccharide infusion on nitrogen metabolism and hormonal responses of growing beef steers. J. Anim. Sci. 87:3656-3668. doi:10.2527/jas.2009-2011.

Table 5.1. Beef carcass yield and quality attributes of carcasses with normal livers and carcasses that had livers with abnormalities.

| | Liver Score[1] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Item** | **Edible (n = 1,060)** | **A- (n = 80)** | **A (n = 156)** | **A+ (n = 162)** | **A+AD (n = 375)** | **A+OP (n = 94)** | **A+AD/OP (n = 84)** | **Contamination (n = 132)** | **Pooled SEM** | ***P*-value** |
| Trim weight, kg | 0.38 | 0.60 | 0.23 | 0.36 | 0.72* | 3.92* | 3.39* | 0.95 | 0.382 | < 0.001 |
| Hot carcass weight, kg | 379.9 | 373.4 | 377.6 | 381.0 | 362.6 | 364.4 | 354.8* | 377.9 | 11.304 | 0.04 |
| 12th-rib subcutaneous fat depth, cm | 0.79 | 0.80 | 0.79 | 0.80 | 0.74* | 0.74* | 0.77 | 0.77 | 0.033 | 0.01 |
| LM area, $cm^2$ | 84.26 | 83.16 | 83.10 | 84.19 | 81.93* | 80.52* | 81.81* | 83.16 | 4.006 | < 0.001 |
| USDA calculated yield grade | 2.77 | 2.79 | 2.82 | 2.80 | 2.68 | 2.77 | 2.71 | 2.79 | 0.077 | 0.13 |
| Marbling score[2] | 478 | 482 | 471 | 469 | 459* | 465 | 455* | 473 | 31.592 | 0.03 |
| Choice or better[3], % | 72.9 | 64.6* | 76.3 | 79.6* | 70.8 | 63.8* | 67.5* | 65.4* | - | < 0.001 |
| Carcasses "railed out"[4], % | 3.6 | 5.0 | 1.9 | 4.3 | 6.2 | 21.3* | 22.9* | 9.1* | - | < 0.001 |

*Designates means of carcasses with liver abnormality differ ($P < 0.05$) from carcasses with edible livers.

[1]A− = 1 or 2 small abscesses or inactive scars; A = 2 to 4 small active abscesses; A+ = multiple small abscesses or 1 or more large active abscesses; A+AD = liver adhered to part of the gastrointestinal tract or diaphragm or both; A+OP = ruptured abscesses; A+AD/OP = liver adhered to part of the gastrointestinal tract or diaphragm or both and ruptured abscess.

[2]Scores: 300 to 390 = Slight; 400 to 490 = Small; 500 to 590 = Modest.

[3]Choice or better = percentage of cattle within liver score that had at least a marbling score of 400 ($Small^{00}$).

[4]Rail out indicates that carcass was removed from the production line for additional trimming.

Table 5.2. Beef carcass yield and quality attributes of carcasses by abscess class.

| Item | Abscess Class[1] None (n = 1,192) | Minor (n = 236) | Major (n = 715) | Pooled SEM | *P*-value |
|---|---|---|---|---|---|
| Trim weight, kg | 0.42[b] | 0.35[b] | 1.40[a] | 0.451 | < 0.001 |
| Hot carcass weight, kg | 379.7 | 376.3 | 366.1 | 10.893 | 0.23 |
| 12th-rib subcutaneous fat depth, cm | 0.79[a] | 0.79[a] | 0.76[b] | 0.030 | 0.01 |
| LM area, $cm^2$ | 84.13[a] | 83.10[ab] | 82.26[b] | 3.916 | < 0.001 |
| USDA calculated yield grade | 2.78 | 2.81 | 2.73 | 0.072 | 0.06 |
| Marbling score[2] | 477[a] | 475[ab] | 462[b] | 31.355 | < 0.01 |
| Choice or better[3], % | 72.1 | 72.3 | 71.5 | - | 0.95 |
| Carcasses "railed out"[4], % | 4.2[b] | 3.0[b] | 9.7[a] | - | < 0.001 |

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

[1]None = liver did not possess abscess at slaughter; Minor = combination of A and A- liver abscesses ( A− = 1 or 2 small abscesses or inactive scars; A = 2 to 4 small active abscesses); Major = combination of all other liver abscess outcomes (A+ = multiple small abscesses or 1 or more large active abscesses; A+AD = liver adhered to part of the gastrointestinal tract or diaphragm or both; A+OP = ruptured abscesses, A+AD/OP = liver adhered to part of the gastrointestinal tract or diaphragm or both and ruptured abscess).

[2]Scores: 300 to 390 = Slight; 400 to 490 = Small; 500 to 590 = Modest.

[3]Choice or better = percentage of cattle within liver score that had at least a marbling score of 400 ($Small^0$).

[4]Rail out indicates that carcass was removed from the production line for additional trimming.

Table 5.3. Association of lung score outcomes to liver abnormalities segregated by liver score.

| | | Consolidation[1], % | | | | Fibrin Tags[2], % | | | Contamination[3], % | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Liver score[4]** | **n** | **1** | **2** | **3** | **None** | **Minor** | **Extensive** | **None** | **No** | **Yes** |
| Edible | 1,059 | 19.83$^{a}$ | 13.98$^{c}$ | 5.67$^{cd}$ | 60.53$^{bc}$ | 29.37$^{a}$ | 17.37$^{bc}$ | 53.26$^{d}$ | 87.54$^{a}$ | 12.46$^{g}$ |
| Contamination | 132 | 11.36$^{b}$ | 17.42$^{c}$ | 8.33$^{c}$ | 62.88$^{b}$ | 12.88$^{c}$ | 12.12$^{d}$ | 75.00$^{b}$ | 46.97$^{e}$ | 53.03$^{c}$ |
| A- | 80 | 10.00$^{b}$ | 25.00$^{b}$ | 3.75$^{d}$ | 61.25$^{b}$ | 28.75$^{ab}$ | 15.00$^{cd}$ | 56.25$^{cd}$ | 75.00$^{bc}$ | 25.00$^{ef}$ |
| A | 156 | 17.31$^{a}$ | 16.67$^{c}$ | 5.13$^{cd}$ | 60.90$^{b}$ | 25.00$^{b}$ | 20.51$^{b}$ | 54.49$^{d}$ | 76.28$^{b}$ | 23.72$^{f}$ |
| A+ | 160 | 18.13$^{a}$ | 17.50$^{c}$ | 8.13$^{c}$ | 56.25$^{cd}$ | 25.63$^{ab}$ | 19.38$^{b}$ | 55.00$^{cd}$ | 71.25$^{c}$ | 28.75$^{e}$ |
| A+AD | 375 | 8.80$^{b}$ | 32.53$^{a}$ | 19.73$^{a}$ | 38.93$^{e}$ | 15.47$^{c}$ | 25.33$^{a}$ | 59.20$^{c}$ | 54.93$^{d}$ | 45.07$^{d}$ |
| A+OP | 93 | 9.68$^{b}$ | 13.98$^{c}$ | 5.38$^{cd}$ | 70.97$^{a}$ | 3.23$^{d}$ | 3.23$^{e}$ | 93.55$^{a}$ | 12.90$^{f}$ | 87.10$^{b}$ |
| A+AD/OP | 84 | 7.14$^{b}$ | 26.19$^{b}$ | 14.29$^{b}$ | 52.38$^{d}$ | 0.00$^{d}$ | 4.76$^{e}$ | 95.24$^{a}$ | 4.76$^{g}$ | 95.24$^{a}$ |
| ***P* - value** | | **< 0.001** | **< 0.001** | **< 0.001** | **< 0.001** | **< 0.001** | **< 0.001** | **< 0.001** | **< 0.001** | **< 0.001** |

$^{a,b,c,d,e,f,g}$Means within column lacking common superscripts differ ($P < 0.05$).

[1]Consolidation: 1 = consolidation of lung lobes, presence of mycoplasma-like lesions, a portion of lung missing, or a combination of these affecting >5% to 15% of lung tissue; 2 = pleural adhesions, a portion of lung missing, or a combination of these affecting >15% to 50% of lung tissue; 3 = pleural adhesions, a portion of lung missing, or a combination of these affecting >50% of lung tissue.

[2]Fibrin Tags: M or E = presence of minor (M) or extensive (E) fibrin tag formation or interlobular adhesions between lobes.

[3]Contamination: Yes = lung was condemned for contamination.

[4]A− = 1 or 2 small abscesses or inactive scars; A = 2 to 4 small active abscesses; A+ = multiple small abscesses or 1 or more large active abscesses; A+AD = liver adhered to part of the gastrointestinal tract or diaphragm or both; A+OP = ruptured abscesses; A+AD/OP = liver adhered to part of the gastrointestinal tract or diaphragm or both and ruptured abscess.

Table 5.4. Association of lung score outcomes to liver abnormalities segregated by abscess classification.

| | | Consolidation[1], % | | | | Fibrin Tags[2], % | | | Contamination[3], % | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Abscess class[4]** | **n** | **1** | **2** | **3** | **None** | **Minor** | **Extensive** | **None** | **No** | **Yes** |
| None | 1,192 | 18.89[a] | 14.36[c] | 5.96[b] | 60.79[a] | 27.54[a] | 16.79 | 55.67[b] | 83.04[a] | 16.96[c] |
| Minor | 236 | 14.83[b] | 19.49[b] | 4.66[b] | 61.02[a] | 26.27[a] | 18.64 | 55.08[b] | 75.85[b] | 24.15[b] |
| Major | 715 | 10.81[c] | 25.98[a] | 14.61[a] | 48.60[b] | 14.33[b] | 18.68 | 66.99[a] | 47.19[c] | 52.81[a] |
| ***P* - value** | | **< 0.001** | **< 0.001** | **< 0.001** | **< 0.001** | **< 0.001** | **0.31** | **< 0.001** | **< 0.001** | **< 0.001** |

[a,b,c]Means within column lacking common superscripts differ ($P < 0.05$).

[1]Consolidation: 1 = consolidation of lung lobes, presence of mycoplasma-like lesions, a portion of lung missing, or a combination of these affecting >5% to 15% of lung tissue; 2 = pleural adhesions, a portion of lung missing, or a combination of these affecting >15% to 50% of lung tissue; 3 = pleural adhesions, a portion of lung missing, or a combination of these affecting >50% of lung tissue.
[2]Fibrin Tags: M or E = presence of minor (M) or extensive (E) fibrin tag formation or interlobular adhesions between lobes.
[3]Contamination: Yes = lung was condemned for contamination.
[4]None = liver did not possess abscess at slaughter; Minor = combination of A and A- liver abscesses ( A− = 1 or 2 small abscesses or inactive scars; A = 2 to 4 small active abscesses); Major = combination of all other liver abscess outcomes (A+ = multiple small abscesses or 1 or more large active abscesses; A+AD = liver adhered to part of the gastrointestinal tract or diaphragm or both, A+OP = ruptured abscesses, A+AD/OP = liver adhered to part of the gastrointestinal tract or diaphragm or both and ruptured abscess).

Table 5.5. Least squares means of discounts or premiums for quality and yield attributes for carcasses with edible livers and those with liver abnormalities[1].

| | Liver Score[2] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Item** | **Edible (n = 1,060)** | **A- (n = 80)** | **A (n = 156)** | **A+ (n = 162)** | **A+AD (n = 375)** | **A+OP (n = 94)** | **A+AD/OP (n = 84)** | **Contamination (n = 132)** | **Pooled SEM** | ***P*-value** |
| Hot carcass weight premiums and discounts, $/45.35 kg | -0.73 | -0.46 | -0.79 | -0.84 | -0.76 | -1.21 | -0.69 | -1.00 | 0.244 | 0.45 |
| Quality grade premiums and discounts, $/45.35 kg | -2.22 | -2.27 | -2.32 | -1.90 | -3.19 | -3.18 | -3.10 | -2.52 | 1.520 | 0.11 |
| Yield grade premiums and discounts, $/45.35 kg | 0.95 | 1.12 | 0.77 | 1.03 | 1.23 | 0.97 | 0.87 | 1.05 | 0.235 | 0.67 |
| Other discounts[3], $/45.35 kg | -3.64 | -3.19 | -3.45 | -3.75 | -3.76 | -3.58 | -4.22 | -3.34 | 0.401 | 0.72 |
| Sum of premiums and discounts, $/45.35 kg | -5.55 | -4.70 | -5.70 | -5.39 | -6.38 | -6.90 | -6.99 | -5.68 | 1.754 | 0.21 |
| Market price[4], $/45.35 kg | 181.22 | 182.06 | 181.06 | 181.37 | 180.38 | 179.86 | 179.77 | 181.08 | 1.755 | 0.20 |
| Gross carcass value, $ | 1,517.36 | 1,499.09 | 1,508.46 | 1,523.33 | 1,443.43 | 1,447.71 | 1,406.37* | 1,510.60 | 31.212 | 0.03 |
| Gross offal value, $ | 17.98 | 14.55* | 14.53* | 14.41* | 7.76* | 6.94* | 6.86* | 14.03* | 0.085 | < 0.001 |

*Designates means of carcasses with liver abnormality differ ($P < 0.05$) from carcasses with edible livers.

[1]Carcass basis, premiums and discounts, and offal value were generated using:
USDA. LM_CT155, National Weekly Direct Slaughter Cattle - Premiums and Discounts; USDA. NW_LS410; USDA Beef Carcass Price Equivalent Index Value; USDA. 2016. NW_LS441, By-Product Drop Value (Steer).

[2]A− = 1 or 2 small abscesses or inactive scars; A = 2 to 4 small active abscesses; A+ = multiple small abscesses or 1 or more large active abscesses; A+AD = liver adhered to part of the gastrointestinal tract or diaphragm or both; A+OP = ruptured abscesses; A+AD/OP = liver adhered to part of the gastrointestinal tract or diaphragm or both and ruptured abscess.

[3]Summation of discounts from dairy type, and dark cutter.

[4]Includes base price and all premiums and discounts.

Table 5.6. Least squares means of discounts or premiums for quality and yield attributes for carcasses with different abscess classifications[1].

| | Abscess class[2] | | | | |
|---|---|---|---|---|---|
| **Item** | **None (n = 1,192)** | **Minor (n = 236)** | **Major (n = 715)** | **Pooled SEM** | ***P*-value** |
| Hot carcass weight premiums and discounts, $/45.35 kg | -0.76 | -0.67 | -0.83 | 0.236 | 0.56 |
| Quality grade premiums and discounts, $/45.35 kg | -2.25 | -2.29 | -2.87 | 1.508 | 0.09 |
| Yield grade premiums and discounts, $/45.35 kg | 0.96 | 0.88 | 1.11 | 0.206 | 0.44 |
| Other discounts[3], $/45.35 kg | -3.60 | -3.36 | -3.78 | 0.358 | 0.32 |
| Sum of premiums and discounts, $/45.35 kg | -5.56 | -5.35 | -6.28 | 1.720 | 0.08 |
| Market price[4], $/45.35 kg | 181.20 | 181.41 | 180.48 | 1.720 | 0.09 |
| Gross carcass value, $ | 1,516.46 | 1,505.52 | 1,457.92 | 29.139 | 0.20 |
| Gross offal value, $ | 17.52[a] | 14.58[b] | 9.05[c] | 0.258 | < 0.001 |

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

[1]Carcass basis, premiums and discounts, and offal value were generated using:
USDA. LM_CT155, National Weekly Direct Slaughter Cattle - Premiums and Discounts; USDA. NW_LS410; USDA Beef Carcass Price Equivalent Index Value; USDA. 2016. NW_LS441, By-Product Drop Value (Steer).
[2]None = liver did not possess abscess at slaughter; Minor = combination of A and A- liver abscesses ( A− = 1 or 2 small abscesses or inactive scars, A = 1 to 2 large abscesses or multiple small abscesses); Major = combination of all other liver abscess outcomes (A+ = multiple small abscesses or 1 or more large active abscesses, A+AD = liver adhered to part of the gastrointestinal tract or diaphragm or both, A+OP = ruptured abscesses, A+AD/OP = liver adhered to part of the gastrointestinal tract or diaphragm or both and ruptured abscess).
[3]Summation of discounts from dairy type, and dark cutter.
[4]Includes base price and all premiums and discounts.

# CHAPTER 6:

# ASSESSMENT OF BLOOD METABOLITES IN FED HOLSTEIN STEERS WITH AND WITHOUT LIVER ABSCESSES

## 6.1 ABSTRACT

Liver abscesses in feedlot cattle are known to reduce growth performance of the animal and yield of the carcass and viscera. Cattle with liver abscesses do not display observable clinical signs that we know of, and abscesses are only detected upon slaughter. Considering that, methods to detect liver abscesses prior to slaughter have been evaluated. However, these procedures are often time intensive and have yielded inconsistent results. The objective of this research was to compare whole blood and serum analyses from cattle with and without liver abscesses to quantify differences associated with the presence and severity of liver abscesses in fed beef animals. Blood samples for complete blood count (CBC) and sera were collected from fed Holsteins (n = 153) approximately 30 s after exsanguination; liver abscesses were visually assessed after evisceration. Data were analyzed using mixed model ANOVA and logistic regression procedures of SAS v9.4 (SAS Inst. Inc., Cary, NC) with carcass serving as the experimental unit. Of the CBC variables analyzed, platelet counts were increased ($P$ = 0.02) and hemoglobin and hematocrit values ($P < 0.01$) were decreased in carcasses with major abscesses (214 K/uL, 12.9 g/dL, 41.0%, respectively) than in carcasses without

abscesses (137 K/uL, 13.8 g/dL, 43.4%, respectively). Serum analysis indicated that carcasses with major abscesses had increased ($P < 0.01$) globulin (5.8 g/dL) concomitant with decreased ($P \leq 0.03$) sodium (141 mmol/L), albumin (2.8 g/dL), alanine aminotransferase (19 u/L) and aspartate aminotransferase (89 u/L) when compared to carcasses with no liver abscess (5.2 g/dL; 142 mmol/L; 3.0 g/dL; 21 u/L; 97 u/L, respectively). Logistic regression equations using CBC variables (platelets and hemoglobin) or serum variables (aspartate aminotransferase and total protein), yielded equations that were able to explain 72% (c-statistic = 0.72) and 69% (c-statistic = 0.69) of the area under the curve, respectively, for predicting major liver abscesses. These results indicate that cattle with major liver abscesses undergo metabolic differentiation which can be detected in whole blood and serum. Identifying blood parameters that could be used to diagnose liver abscesses have useful significant implications for cattle welfare and feedlot management.

**Keywords**: complete blood count; Holstein; liver abscess; serum chemistry

## 6.2 INTRODUCTION

Liver abscesses in feedlot cattle are not a recent phenomenon. In the 1940's, Smith (1944) observed that a relationship existed between ulcerative lesions in the rumen and abscesses in the livers of feedlot cattle. Although the pathogenesis remains theoretical, the literature suggests that ruminal lesions due to acidotic events are the primary predisposing factor for liver abscesses because they allow an entry point into the hepatic portal vein for the causative agent, *F. necrophorum* (Jensen et al., 1954; Nagaraja and Chengappa, 1998; Nagaraja and Lechtenberg, 2007). Cattle with liver abscesses do not display clinical signs, and abscesses are only detected at slaughter (Nagaraja and Lechtenberg, 2007). Because liver abscesses pose a financial liability to the beef feeding and processing industries, technologies to diagnose and detect liver abscesses prior to slaughter have been evaluated.

Ultrasound of bovine liver has been used to identify liver abscesses in fed beef with some success (Lechtenberg and Nagaraja, 1991), however this technology is time intensive, and suffers from poor accessibility and visibility of the entire liver (Braun, 2009). When blood of finished bulls was sampled at exsanguination, Macdonald et al. (2017) reported decreased concentrations of thyroxine, albumin, cholesterol, and alkaline phosphatase from carcasses with liver abscesses when compared to those without abscesses. Furthermore, dairy cattle with liver abscesses diagnosed via ultrasound, had increased serum globulin (Doré et al., 2007); in Holstein steers with experimentally-induced liver abscesses, cattle with liver abscesses were reported to have hepatic dysfunction as a result of their decreased serum albumin concentrations (Lechtenberg and Nagaraja, 1991).

The objective of this research was to compare complete blood count and serum chemistry variables from fed Holstein steers with and without liver abscesses to quantify changes in blood to better understand the liver abscess complex in finished cattle, and explore the efficacy of blood variables for diagnosis of liver abscesses.

## 6.3 MATERIALS AND METHODS

Animal care and use committee approval was not obtained for this study because data were recorded on carcasses, and no live animals were used for data collection.

### *6.3.1 Carcass identification and liver scoring*

Fed Holsteins steers (n = 153) were tracked through a commercial processing facility, in the High Plains region in the summer of 2017. In order to match measured carcass parameters with individual animals, individual ear tags and/or lot tags were manually recorded for each animal on pre-numbered sheets, which matched a unique harvest sequence number. Blood was collected approximately 30 s post-exsanguination in pre-labeled collection tubes (BD Vacutainer serum separator tube, 10mL, reference number 367985; BD Vacutainer K2 EDTA tube, 4 mL, reference number 367844) that matched the harvest sequence number. Prior to collection, the rubber cap was removed from the blood tube, and the open tube was held under the carcasses on the exsanguination rail at the processing facility. After collection, the cap was replaced on the blood tube, and filled tubes were placed in a cold pack lined cooler, being careful to ensure that blood tubes did not come into direct contact with the cold packs. Poly-vinyl tags (6.65 × 6.98 cm, Label Logic Inc., Elkhart, IN) matching the harvest sequence number assigned when ear tags were recorded, were pinned (magnetic shroud pin, Koch

Supplies, Kansas City, MO) to the carcasses so they could be positively identified during the harvest process.

Liver abscesses were visually assessed and scored according to a modified scoring system based on the Elanco Liver Check Service (✓ = no abscesses, A- = 1 or 2 small abscesses, A = 2 to 4 small active abscesses, A+ = 1 or more large active abscesses, A+ Adhesion (A+AD) = liver adhered to GI tract, A+ Open (A+OP) = open liver abscess, A+Adhesion/Open (A+AD/OP) = liver adhered to GI tract with an open abscess; adapted from Brown & Lawrence, 2010). Processor carcass number was recorded for all carcasses tagged with a slaughter sequence number to match blood and liver score outcomes with grade data provided by the processor. Grade data were obtained from the Video Image Analysis (VIA) camera system (E+V VGB 2000, Technology GmbH & Co. KG) and matched to individual carcasses via processor carcass number for further analysis. Data included marbling score, calculated yield grade, HCW, $12^{th}$-rib subcutaneous fat depth, LM area, and percentage KPH, estimated at 2.5% (USDA, 2016).

### *6.3.2 Whole blood analysis and serum chemistry*

Because liver status was not known prior to evisceration, blood was collected from a larger population of 494 carcasses. Although all carcasses were tracked throughout the processing facility, only carcasses with noted liver abscesses containing suspected purulent material on the gut table were selected for further analysis, within their respective abscess classification. Carcasses with and without liver abscesses were further separated into abscess classes {None (n = 51) = no abscess was present at slaughter; Minor (n = 51) = carcass had either A- or A liver abscess score; Major (n = 51) = liver had a scored abscess of A+, A+AD, A+OP, or A+AD/OP} based on the previous

works of Brown and Lawrence (2010) in order to increase the power of this analysis. Using data from Macdonald et al. (2017), we conducted a power analysis, with $\beta = 0.80$, and determined that at least 46 carcasses per abscess classification were needed to detect a difference, which was rounded up to 50 per group. After the final collection period, there were 51 carcasses per abscess classification, and instead of trying to decide which samples should be removed, all were left in for analysis.

Immediately post-collection, blood was chilled in coolers lined with frozen cold packs prior to transport back to the West Texas A&M University – Animal Health Laboratory. Approximately 6 h post-collection, samples collected into 4 mL evacuated tubes containing 7.2 mg EDTA, were used for determination of complete blood count (CBC) using an automated hemocytometer (ProCyte Dx Hematology Analyzer; IDEXX Laboratories, Inc., Westbrook, ME). Secondary samples stored in 10 mL serum separator tubes were centrifuged at 1780 x *g* (Beckman Coulter Inc., model Allegra™ 6R centrifuge, Brea, CA) set to 20 °C for 20 min. After serum was separated from whole blood, serum was pipetted out of the tube using a 3 mL disposable pipet (Karter Scientific, Lake Charles, LA) and stored in duplicate in 1.7 mL micro-centrifuge vials (VWR International, Radnor, PA). Serum samples were analyzed using Preventative Care Profile Plus rotors in an Abaxis VetScan VS2 chemistry analyzer (Abaxis Inc., Union City, CA). Serum chemistry variables analyzed were: albumin, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, blood urea nitrogen, calcium, chloride, creatinine, globulin, glucose, potassium, sodium, total bilirubin, and total protein.

Because blood was collected from these cattle at slaughter, post exsanguination, reference ranges (if available) were provided for each variable to validate the results and methodology. Reference ranges were populated using The Merck Veterinary Manual, (1998), since this commonly used by veterinarians for reference ranges and general health discussion. However, the population used for these reference ranges is poorly defined and is not specified with regard to age, type (steer, bull, heifer, cow), or health status of animals. Furthermore, reference ranges reported were generated using means for each variable within this population ± the 95% confidence interval of that variable. Therefore, the range may not constitute a "normal" or "healthy" range, but simply, commonly seen numbers.

Although cattle in this experiment were shipped as a lot to the processor, the processor harvested Holsteins in groups of approximately 30, due to the excessive rate of open abscesses that led to additional trimming and reduced operating efficiency. Therefore, cattle were noted by harvest group and analyzed samples were balanced within harvest group with the least frequent liver abscess class within kill group setting the number to be analyzed within group (i.e. if three minor abscesses occurred within a kill group only three livers with no abnormality and three with major abnormality were selected). After the minimum number of samples per group was selected, remaining samples were selected on a "first-come, first-sampled" basis to balance outcome within kill group.

### *6.3.3 Statistical analysis*

Data were analyzed as a completely randomized design; individual carcass served as the experimental unit. Data were analyzed using the MIXED procedure of SAS v9.4

(SAS Inst. Inc., Cary, NC) with the fixed effect of liver score and the random effects of source, date, and harvest group. Cattle originated from 3 unique sources and were harvested over 3 kill dates. Significance was declared at $P \leq 0.05$. When main effects were significant, means were separated using the PDIFF option of SAS, and data are presented as least squares means. Logistic regression was conducted using the LOGISTIC procedure. Major abscess was classified as an event "1" while no abscess was classified as a non-event "0". Minor abscesses were removed from logistic regression analysis because differences in this experiment were primarily noted between major and no liver abscesses. Serum predictor variables included AST, and total protein, whereas platelets and hemoglobin were used for CBC variables. Predictive variables were selected based on outcomes observed in the MIXED analysis because they had some of the most variability and the widest ranges of the variables analyzed and because MIXED procedure analysis revealed them to be statistically different from each other when analyzed by abscess class. Regression equations are better predictors of an outcome if they data used to formulate the encompasses a wide range of outcomes for a given variable.

## 6.4 RESULTS AND DISCUSSION

### *6.4.1 Whole blood parameters*

Prior to conducting this experiment, we hypothesized that an animal with a major liver abscess would have different CBC results than an animal that was not challenged with a bacterial infection in the liver. Typically, white blood cell numbers increase under acute bacterial infections (The Merck Veterinary Manual, 1998). In general, this was not

the case with these cattle, because there were few detectable differences in most of the complete blood count variables observed (Table 6.1). No difference ($P \geq 0.21$) was observed in total white blood cell concentration or in differentials (neutrophils, lymphocytes, monocytes, eosinophils) and percentages across abscess classification. Although not different ($P \geq 0.78$) across abscess classifications, mean and percentage neutrophil concentration were greater than the reference ranges provided in The Merck Veterinary Manual (1998). Of the animals with major abscesses, minor abscesses, and no liver abscesses, 64.7%, 56.8%, and 58.8%, respectively, had neutrophil concentrations that exceeded the high reference range (The Merck Veterinary Manual, 1998). These values are likely above the reference ranges cited because cattle are subjected to various stressors prior to slaughter, and stress has been shown to increase neutrophil concentrations (Buckham-Sporer et al., 2007).

When an animal undergoes stress, glucocorticoids (i.e. cortisol) are released into circulation and result in a downregulation of L-selectin (Weber et al., 2004). L-selectin is an adhesion molecule whose primary function is to slow-down and promote the capture of leukocytes which are rapidly flowing past target endothelial tissue (Tedder et al., 1995). Downregulation of L-selectin in times of stress results in increased circulating leukocytes, and eventual neutrophilia (Buckham-Sporer et al., 2007). Cattle that arrive at the processing facility have most likely incurred stress via removal from their pen, withholding of feed, transportation to slaughter, and the eventual systemic stresses of stunning and exsanguination. Because blood samples were collected after cattle had likely experienced all of these stressors, it is not surprising that neutrophil concentrations observed were outside The Merck Veterinary Manual (1998) references ranges.

Red blood cell concentration, and red cell distribution width did not differ ($P \geq 0.15$) between abscess classes (Table 6.1). Carcasses with major abscesses had decreased ($P < 0.01$) hemoglobin (12.9 g/dL) and hematocrit (41.0%) when compared to carcasses with minor liver abscesses (13.8 g/dL; 44.0%, respectively) or no liver abscess (13.8 g/dL; 43.4%). Because these values are used to calculate mean corpuscular hemoglobin concentration (MCHC = hemoglobin ÷ hematocrit; The Merck Veterinary Manual, 1998) and the difference between the values was similar within treatment, the ratio of those values was not different ($P = 0.22$) across treatments, nor were they outside of The Merck Veterinary Manual (1998) reference ranges. Platelets were 36.0% increased ($P = 0.02$) in carcasses with major abscesses (214 K/uL) when compared to carcasses with no liver abscess (137 K/uL), and values were not outside of The Merck Veterinary Manual (1998) reference ranges. Platelets are involved in tissue repair and coagulation, as well as facilitating recruitment of leukocytes (Golebiewska and Poole, 2015). The virulence factors produced by *Fusobacterium necrophorum* include endotoxic lipopolysaccharide, hemolysin (protein that lyses red blood cells), leukotoxin (protein that causes destruction and necrosis of white blood cells), and extracellular enzymes including proteases (exogenous enzymes that denature host proteins; Tan et al., 1994; Tan et al., 1996). All of those virulence factors can cause cellular damage, which may explain why carcasses with major abscesses have increased platelet counts.

### *6.4.2 Serum chemistry*

Serum samples analyzed by abscess classification yielded results similar to those observed in the CBC analysis (Table 6.2). Serum globulin was increased ($P < 0.01$) by 10.3% in cattle with major liver abscesses (5.8 g/dL) over cattle with minor liver

abscesses (5.2 g/dL) and cattle with no liver abscess (5.2g/dL). Nielsen et al. (1978) previously reported that cattle experimentally infected with *Trypanosoma congolense* had greater globulin concentrations than control cattle. Immunoglobulins are proteins produced by the immune system; their production is stimulated by infections. Therefore, cattle that are challenged with a bacterial infection of the liver would be expected to have increased serum globulin concentrations. Serum albumin was decreased ($P < 0.01$) in carcasses with major abscesses (2.8 g/dL) when compared to cattle with minor (3.1 g/dL; +10.7%) or no liver abscess (3.0 g/dL; +7.1%). Serum albumin is primarily produced in the liver (Peters, 1962), and a liver compromised by a major abscess would likely have a diminished ability to synthesize this protein. Total serum protein was also increased ($P < 0.01$) in cattle with major liver abscesses (8.6 g/dL) over cattle with minor liver abscesses (8.3 g/dL; -3.5%) and cattle with no liver abnormality (8.2 g/dL; -4.7%). Serum protein is inclusive of serum albumin and serum globulin; thus, it is not surprising that total serum protein is increased in cattle with major abscesses, because they also exhibited greater globulin concentrations than carcasses in the other abscesses classifications.

In human medicine, the albumin-to-globulin (AGR) ratio is sometimes used as an additional non-specific diagnostic tool to indicate immunological changes in a patient (Azab et al., 2013). Therefore, in order to add to the discussion on serum changes in animals with and without liver abscesses, the ratio of albumin to globulin was calculated for each animal (Table 6.2). Cattle with a major liver abscess had a decreased ($P < 0.01$; AGR= 0.5) when compared to cattle with minor liver abscesses (AGR = 0.6) or those with no liver abscess (AGR = 0.6). Discussion of this ratio is limited in animal science literature; however, previous studies have reported a numerically decreased AGR in

healthy versus scouring calves (0.8 vs. 1.0, respectively; Nakamura et al., 2017), while dairy cows with subclinical endometritis were reported as having a numerically decreased AGR than their healthy counterparts (0.8 vs. 0.9, respectively; Green et al., 2011). Ratios observed in this experiment were generally less than those observed in previous literature; however, this is due to the elevated globulin concentrations observed in this population of cattle.

Serum glucose was not different ($P = 0.12$) across abscess classifications (Table 6.2). Although not different, mean serum glucose for each abscess class was greater than the upper Merck Veterinary Manual (1998) reference range. One possible explanation for the increased serum glucose concentrations is stress. Shaw and Tume (1992) reported that cattle subjected to the stress of transport and slaughter had increased plasma glucose concentrations (100.8 and 108 mg/dL, respectively), which were also beyond The Merck Veterinary Manual reference ranges; however, these values were still less than what was observed in the current experiment. Additionally, cattle fed high starch feedlot diets have been reported as having increased serum glucose than when the same cattle were fed a predominately forage based diet (Berzins and Manns, 1979). When a diet is predominately starch, some of the starch escapes the rumen, where it can enter the lower gastrointestinal tract (Wheeler and Noller, 1977). There the starch will be hydrolyzed to glucose and absorbed like in a monogastric animal potentially leading to hyperglycemic conditions.

Serum glucose concentrations observed in this experiment differ from those of Macdonald et al. (2017), who reported that carcasses with abscesses had deceased serum glucose concentrations at slaughter. The liver is the primary site of the Cori cycle, which

is vital to gluconeogenesis (Nocek, 1997), and if a liver is impaired due to abscesses one might expect gluconeogenesis would be decreased. However, cattle observed in this experiment had been taken off feed prior to slaughter and were in lairage for several hours prior to slaughter, which could explain why no difference was observed. Galyean et al., (1981) reported that cattle that had been fasted and transported had increased serum glucose concentrations when compared to cattle that has just been fasted and tended to have increased serum glucose when compared to control cattle (cattle not fasted or transported). Furthermore, because cattle in this experiment were slaughtered in small groups with ample chain-time in between, lairage time was likely increased over what was observed by Macdonald et al. (2017).

Blood urea nitrogen was increased ($P < 0.01$) in cattle with minor liver abscesses (13 mg/dL) when compared to carcasses with major liver abscesses (12 mg/dL; -7.7%) or carcasses with no liver abscess (12 mg/dL; -7.7%), and values were not outside of The Merck Veterinary Manual (1998) reference ranges. Blood urea nitrogen is a byproduct of protein catabolism (Eisemann et al., 1989), and an animal in the initial stages of liver abscess development, prior to the abscess being sequestered by scar tissue, could exhibit increased protein catabolism due to the degradative virulence factors of *F. necorophorum*. No difference was observed in serum calcium ($P = 0.32$), chloride ($P = 0.76$), or potassium ($P = 0.22$) across abscess class (Table 6.2). However, serum sodium was decreased ($P = 0.02$) in carcasses with major abscesses (141 mmol/L) when compared to carcasses with minor or no liver abscesses (142 mmol/L). Sodium is tightly regulated within the body and decreased serum sodium in humans is associated with cirrhosis and an increased risk of death (Waikar et al., 2011). Although the decrease is

slight, decreased sodium could be indicative of liver damage in cattle with severe liver abscesses; though, values observed were not outside of The Merck Veterinary Manual (1998) reference ranges.

### *6.4.3 Serum liver enzymes*

Along with serum ions and proteins, enzymes that are primarily produced in the liver were also analyzed (Table 6.2). Alanine aminotransferase (ALT) was decreased ($P < 0.01$) in carcasses that had major liver abscesses (19 u/L) versus cattle with minor liver abscesses (22 u/L; +15.8%) and cattle with no liver abnormality (21 u/L; +10.5%). Alanine aminotransferase, an enzyme that catalyzes the two parts of the alanine cycle, is chiefly produced in the liver, but can be synthesized in the kidney, and is used as a marker of hepatic disease in human medicine (Sherman, 1991). Humans diagnosed with multiple pyogenic liver abscesses were reported as having numerically decreased ALT concentrations when compared to patients diagnosed with a single liver abscess (Seeto and Rockey, 1996). However, all patients with liver abscesses had ALT concentrations above the normal range for humans.

Aspartate aminotransferase (AST) was decreased ($P = 0.03$) in carcasses with major abscesses (89 u/L) when compared to carcasses with no liver abscess (97 u/L; +9.0%). Aspartate aminotransferase is a transaminase enzyme that facilitates the reversible transferal of an amino group between aspartate and glutamate (Karmen et al., 1955), which is an important step in either amino acid degradation or biosynthesis. Decreased concentrations of AST will diminish an animal's ability to metabolize or synthesize amino acids which can negatively impact the growth and performance of that animal.

Alkaline phosphatase (ALP) is an enzyme, found in most mammalian tissues, that dephosphorylates compounds and functions best under alkaline conditions (Coleman, 1992). No difference ($P = 0.22$) in ALP was observed across the varying abscess classifications. This is contrary to what was reported by Macdonald et al. (2017), who observed decreased serum ALP concentrations in carcasses with liver abscesses. These differing results could be an artifact of the differing sex classifications used in both trials (bulls vs. steer) or due to the lack of cattle with abscesses observed by Macdonald et al. (2017) because only nine bulls had liver abscesses at slaughter. Mean ALT, AST, and ALP concentrations did not exceed the reference ranges reported in The Merck Veterinary Manual (1998), and all were numerically decreased in carcasses with major liver abscesses when compared against carcasses with minor or no liver abscesses. The numerical decrease is not surprising, because if functional hepatocytes are diminished due to large or multiple abscesses, the quantity of enzymes produced may also be diminished.

Liver abscesses can develop in all ages and types of fed beef, and unfortunately for veterinarians and feedlot personnel, liver abscesses do not exhibit any discernable clinical signs (Nagaraja and Lechtenberg, 2007). Furthermore, because liver abscesses do not present any known clinical pathogenic signs, we must look to human medicine to better understand potential symptoms and clinical signs. Humans diagnosed with pyogenic liver abscesses have exhibited symptoms of fever (79%), chills (60%), abdominal pain (55%), nausea (37%), vomiting (30%), weight loss (28%), chest pain (21%), cough (21%), diarrhea (20%) and abdominal distension (5%; Seeto and Rockey, 1996). Human patients reported in Seeto and Rockey (1996) also exhibited signs of

increased core temperature > 38.5 °C (39%), tender right upper quadrant (43%), hepatomegaly (enlarged liver, 28%), jaundice (22%), and abdominal distention (13%). Humans with pyogenic liver abscesses typically exhibit non-specific signs of liver abscess, but their ability to express discomfort to medical professionals and the ability to use computerized tomography and magnetic resonance imaging, allows for liver abscess diagnosis in humans. These technologies are not cost effective or practical for feedlot cattle; however rapid CBC and serum analyzers are available and can potentially be used chute-side. Additional research is needed to determine if CBC and serum differences observed in this experiment are present during the development of a liver abscess. Further quantification of these variables and their associations to liver abscess development may allow for identification of liver abscesses prior to slaughter.

### *6.4.4 Logistic regression of serum and CBC from cattle with and without major liver abscesses*

Within both serum and CBC variables, logistic regression equations were generated to predict the probability of a major liver abscess. There is no direct equivalent to the coefficient of determination ($R^2$) in logistic regression; however, the area under the receiver operating characteristic curve (c–statistic) has been used as a measure of the discriminatory power of the logistic regression equation (Hosmer and Lemeshow, 2000). The c-statistic can range from 0.5 (the model is no better than chance at predicting an outcome) to 1.0 (the model is perfectly accurate at prediction).

Using CBC variables of hemoglobin and platelets, univariate (Figures 6.1 and 6.2) and multivariate logistic equations were generated. In a univariate model to estimate the probability of a major abscess from blood hemoglobin, the equation, exp(4.2299 –

(0.3207 × hemoglobin, g/dL))/(1+exp(4.2299 – (0.3207 × hemoglobin, g/dL))) was generated, with a c-statistic of 0.64. When platelets were used in a univariate model, the equation to estimate the probability of a major abscess equaled, exp(-0.9669 + 0.00558 × platelets, K/uL)/(1+exp(-0.9669 + 0.00558 × platelets, K/uL)), with a c-statistic of 0.67. Although both models were significant and yielded predictive equations better than chance, when combined in a multivariate analysis the predictive power of the model increased (c-statistic = 0.72). Using this analysis, the probability of a major abscesses estimated as: exp(3.0682 + (0.00538 × platelets, K/uL) – (0.3032 × hemoglobin, g/dL)))/(1+exp(((0.00538 × platelets, K/uL) – (0.3032 × hemoglobin, g/dL)))). Although both univariate equations are predictive of major liver abscesses, their predictive power is increased when combined.

When serum variables were analyzed using logistic regression, AST and total protein yielded univariate (Figures 6.3 and 6.4) and multivariate equations capable of predicting liver abscesses. In the univariate model, knowledge of blood AST estimates the probability of a major liver abscess as: exp(1.9560 + -0.0216 × AST, u/L)/(1+exp(1.9560 + -0.0216 × AST, u/L)), with a c-statistic of 0.65. When total protein was entered into the univariate model, the probability of a major abscess equaled: exp(-7.0668+0.8453 × total protein, g/dL)/(1+exp(-7.0668+0.8453 × total protein, g/dL), with a c-statistic of 0.64. Similar to the CBC variables, the combined serum variables had greater ability to predict major liver abscesses (c-statistic = 0.69) when calculated via multiple logistic regression: (exp(-4.9698 - (0.0201 × AST, u/L) + (0.08131 × total protein, g/dL)))/(1+exp(((-0.0201 × AST, u/L) + (0.08131 × total protein, g/dL)))).

Because blood was collected from cattle at slaughter, these equations are unknown in their ability to predict major liver abscesses in cattle throughout the finishing period. However, these equations demonstrate that parameters quantified in a complete blood count and serum analysis are capable of predicting the outcome of a major liver abscess. Therefore, these variables and equations should serve as the basis for further research using live animals throughout the feeding period in order to develop equations that are capable of predicting major liver abscesses in cattle prior to slaughter.

### *6.4.5 Carcass characteristics*

Aside from blood collection and liver evaluation, carcass grading data (Table 6.3) was collected on all cattle to quantify differences in CBC and serum in context with carcass quality metrics. Greatest ($P$ = 0.03) hot carcass weights were observed in carcasses with minor abscesses (387.1 kg), which were 5.4% heavier than carcasses with major abscesses (366.2 kg) and not different from carcasses with no liver abscess (381.8 kg). These findings were not unexpected. Brown and Lawrence (2010) reported that carcasses with "A" liver scores had numerically greater, but similar, hot carcass weights to cattle with normal livers. Liver abscesses are a potential outcome of acidotic events, which in turn are caused by decreased rumen pH as a result of the increased fermentation rates associated with concentrate-based feedlot rations (Nagaraja and Chengappa, 1998; Nagaraja and Lechtenberg, 2007). Cattle are fed grain-based, energy dense diets in order to maximize growth performance and increase intramuscular fat deposition (Dinius and Cross, 1978); with increased marbling leading to greater carcass value (USDA, 2017; LM_CT155). Liver abscesses are a consequence of this system, and while a major abscess is detrimental to carcass weight or quality, a carcass with a minor abscess may be

heavier than a carcass with an edible liver (Montgomery, 1985; Brown and Lawrence, 2010). A minor liver abscess likely indicates that an animal was consuming enough feed to realize its full genetic potential for growth in the feedlot; however, the line between enough and too much is unknown and the development of a major liver abscess can be costly to feeders and processors alike.

Subcutaneous fat depth measured between the $12^{th}$ and $13^{th}$ rib was decreased ($P = 0.02$) in carcasses with major abscesses (0.71 cm) when compared to carcasses with minor abscesses (0.84 cm; +18.3%) but was not different ($P > 0.10$) from carcasses with no liver abscesses (0.76 cm). Additionally, there was a tendency ($P = 0.09$) for carcasses with minor liver abscesses to have greater LM area (88.1 $cm^2$) than carcasses with major liver abscesses (84.2 $cm^2$). Although these individual metrics were different, they did not result in a difference ($P = 0.41$) in calculated yield grade among abscess classifications (None = 2.65, Minor = 2.71, Major = 2.58). There was also no difference ($P = 0.66$) in marbling score across abscess class, with the least square mean from all abscess classes culminating in a $Small^{43}$ to $Small^{59}$ marbling score and a low Choice quality grade. Liver abscess incidence of beef-type cattle by lot can range from 0% to upwards of 90% but has averaged 15% in non-Holstein beef for the last decade, while in the same period, fed Holsteins averaged 30% liver abscess incidence (Amachawadi and Nagaraja, 2016). Liver abscesses result in decreased carcass yields (Montgomery, 1985) and have been reported to greatly decrease total carcass value (Brown and Lawrence, 2010).

Methods that have been developed to diagnose liver abscesses prior to slaughter are time intensive and often produce inconsistent results (Braun, 2009). Use of ultrasound to diagnose liver abscesses in fed beef has been successfully implemented

(Lechtenberg and Nagaraja, 1991). However, this technology is not without its limitations. When ultrasonography was used to diagnose liver abscesses in Holstein dairy cows, of the 5,519 animals evaluated only 18 (0.3%) were clinically diagnosed with liver abscesses (Doré et al., 2007). In that trial, diagnosis was confirmed with laparotomy in 10 cases, and via ultrasound in the other 8. Only the side of the liver closest to the body wall is visible with ultrasound (Braun et al., 1995), and because liver abscesses are not confined to only one side of the liver, there is a high rate of false negative diagnoses with this technology. Beside the issue of poor sensitivity, other cited limitations of ultrasound for the diagnosis of liver abscesses include lack of rumen fill causing liver to lose contact with body wall, lung tissue that may overshadow liver due to age, diet, and orientation in the chute and excessive internal fat that may distort proper image (Nagaraja and Lechtenberg, 2007).

Limitations with this technology have led others to research alternative methods to diagnose liver abscesses prior to slaughter. Alternatives evaluated are primarily blood based and have included complete blood cell counts as well as analysis of serum metabolites. Cattle with liver abscesses were reported to have serum biochemistry and complete blood cell counts comparable to findings in cattle with chronic active inflammation (Doré et al., 2007). Conversely, cattle with liver abscesses have been reported as having decreased serum albumin concentrations, which may be symptomatic of liver damage (Lechtenberg and Nagaraja, 1991).

The liver is a vital organ which demands copious amount of energy to function, and whose outputs reflect variations in metabolic status of an animal (Johnson et al., 1990), leading to the hypothesis that markers of liver functionality may correlate with

changes in performance metrics of fed beef as well as being predictive of liver abscesses (Macdonald et al., 2017). Although differences in CBC and serum variables across abscess classifications were observed in this experiment, changes observed in steers at slaughter may not be predictive in live animals during the finishing period. Additional research is needed in order to determine if the blood-based variables tested in this experiment are capable of predicting or diagnosing liver abscesses in feedlot cattle with different days on feed.

## 6.5 LITERATURE CITED

Amachawadi, R. G., and T. G. Nagaraja. 2016. Liver abscesses in cattle: A review of incidence in Holsteins and of bacteriology and vaccine approaches to control in feedlot cattle. J. Anim. Sci. 94:1620-1632. doi:10.2527/jas2015-0261.

Azab, B., S. Kedia, N. Shah, S. Vonfrolio, W. Lu, A. Naboush, F. Mohammed, and S. W. Bloom. 2013. The value of the pretreatment albumin/globulin ratio in predicting the long-term survival in colorectal cancer. Int. J. Colorectal Dis. 28:1629-1636. doi:10.1007/s00384-013-1748-z.

Berzins, R., and J. G. Manns. 1979. How concentrate feeding affects glucoregulatory hormones in ruminants: implications in bovine ketosis. J. Dairy. Sci. 62:1739-1745.

Boyd, J. W. 1984. The interpretation of serum biochemistry test results in domestic animals. Vet. Clin. Pathol. 13:7-14.

Braun, U., N. Pusterla, and K. Wild. 1995. Ultrasonographic findings in 11 cows with a hepatic abscess. Vet. Rec. 137:284-290. doi: 10.1136/vr.137.12.284.

Braun, U., 2009. Ultrasonography of the liver in cattle. Vet. Clin. N. Am.-Food. A. 25:591-609.

Brown, T. R., and T. E. Lawrence. 2010. Association of liver abnormalities with carcass grading performance and value. J. Anim. Sci. 88:4037-4043. doi: 10.2527/jas.2010-3219.

Buckham-Sporer, K.R., J. L. Burton, B. Earley, and M. A. Crowe. 2007. Transportation stress in young bulls alters expression of neutrophil genes important for the regulation of apoptosis, tissue remodeling, margination, and anti-bacterial function. Vet Immunol Immunop. 118:19-29. doi: 10.1016/j.vetimm.2007.04.002.

Coleman, J.E., 1992. Structure and mechanism of alkaline phosphatase. Annu. Rev. Bioph. Biom. 21:441-483.

Dinius, D.A., and H. R. Cross. 1978. Feedlot performance, carcass characteristics and meat palatability of steers fed concentrate for short periods. J. Anim. Sci. 47:1109-1113.

Doré, E., G. Fecteau, P. Hélie, and D. Francoz. 2007. Liver abscesses in Holstein dairy cattle: 18 cases (1992–2003). J. Vet. Intern. Med. 21:853-856.

Eisemann, J. H., A. C. Hammond, T. S. Rumsey, and D. E. Bauman. 1989. Nitrogen and protein metabolism and metabolites in plasma and urine of beef steers treated with somatotropin. J. Anim. Sci. 67:105-115.

Galyean, M. L., R. W. Lee, and M. E. Hubbert. 1981. Influence of fasting and transit on ruminal and blood metabolites in beef steers. J. Anim. Sci. 53:7-18. doi:10.2527/jas1981.5317.

Golebiewska, E.M., and A. W. Poole. 2015. Platelet secretion: From haemostasis to wound healing and beyond. Blood Rev. 29:153-162. doi:10.1016/j.blre.2014.10.003

Green, M.P., A. M. Ledgard, S. E. Beaumont, M. C. Berg, K. P. McNatty, A. J. Peterson, and P/ J. Back. 2011. Long-term alteration of follicular steroid concentrations in relation to subclinical endometritis in postpartum dairy cows. J. Anim. Sci. 89:3551-3560. doi:10.2527/jas.2011-3958.

Hosmer, D. W., and S. Lemeshow. 2000. Applied Logistic Regression. 2nd ed. John Wiley and Sons, Inc., New York, NY.

Jensen, R., H. M. Deane, L. J. Cooper, V. A. Miller, and W. R. Graham. 1954. The rumenitis-liver abscess complex in beef cattle. Am. J. Vet. Res. 15: 202-216.

Johnson, D.E., K. A. Johnson, and R. L. Baldwin. 1990. Changes in liver and gastrointestinal tract energy demands in response to physiological workload in ruminants. J. Nutr. 120:649-655.

Karmen, A., F. Wróblewski, and J. S. LaDue. 1955. Transaminase activity in human blood. J. Clin. Invest. 34:126-133.

Lechtenberg, K.F., and T. G. Nagaraja. 1991. Hepatic ultrasonography and blood changes in cattle with experimentally induced hepatic abscesses. Am. J. Vet. Res. 52:803-809.

Macdonald, A.G., S. L. Bourgon, R. Palme, S. P. Miller, and Y. R. Montanholi. 2017. Evaluation of blood metabolites reflects presence or absence of liver abscesses in beef cattle. Vet. Rec. Open. 4:1-8. doi:10.1136/vetreco-2016-000170.

Montgomery, T. H. 1985. The influence of liver abscesses upon beef carcass yields. Special technical bulletin. West Texas State University.

Nagaraja, T. G., and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: A review. J. Anim. Sci.76:287-298.

Nagaraja, T. G., and K. F. Lechtenberg. 2007. Liver abscesses in feedlot cattle. Vet. Clin. N. Am.-Food. A. 23:351-369. doi: 10.1016/j.cvfa.2007.05.002

Nakamura, S.I., Y. H. Kim, K. Takashima, A. Kimura, K. Nagai, T. Ichijo, and S. Sato. 2017. Composition of the microbiota in forestomach fluids and feces of Japanese Black calves with white scours. J. Anim. Sci. 95:3949-3960. doi:10.2527/jas2017.1431.

Nielsen, K., J. Sheppard, W. Holmes, and I. Tizard. 1978. Experimental bovine trypanosomiasis. Changes in the catabolism of serum immunoglobulins and complement components in infected cattle. Immunology. 35:811-816.

Nocek, J. E., 1997. Bovine acidosis: Implications on laminitis. J. Dairy Sci. 80:1005-1028.

Peters, T., 1962. The biosynthesis of rat serum albumin I. Properties of rat albumin and its occurrence in liver cell fractions. J. Biol. Chem. 237:1181-1185.

Seeto, R.K. and D. C. Rockey. 1996. Pyogenic liver abscess changes in etiology, management, and outcome. Medicine. 75:99-113.

Shaw, F.D. and R. K. Tume. 1992. The assessment of pre-slaughter and slaughter treatments of livestock by measurement of plasma constituents—a review of recent work. Meat Sci. 32:311-329.

Sherman, K.E. 1991. Alanine aminotransferase in clinical practice: a review. Arch. Intern. Med, 151:260-265.

Smith, H. A. 1944. Ulcerative lesions of the bovine rumen and their possible relation to hepatic abscesses. Am. J. Vet. Res. 5:234-242.

Tan, Z. L., T. G. Nagaraja, and M. M. Chengappa. 1994. Biochemical and biological characterization of ruminal Fusobacterium necrophorum. FEMS microbiology letters. 120:81-86.

Tan, Z. L., T. G. Nagaraja, and M. M. Chengappa. 1996. Fusobacterium necrophorum infections: virulence factors, pathogenic mechanism and control measures. Vet. Res. Commun. 20:113-140.

Tedder, T.F., D. A. Steeber, A. N. Chen, and P. Engel. 1995. The selectins: vascular adhesion molecules. The FASEB Journal. 9:866-873.

The Merck Veterinary Manual, 8th edition. 1998. National Publishing, Inc., Philadelphia, Pennsylvania

USDA. 2016. Official United States standards for grades of carcass beef. Accessed Jun. 12,2017.https://www.ams.usda.gov/sites/default/files/media/Carcass%20Beef%20Standard.pdf.

USDA. 2017. LM_CT155, National Weekly Direct Slaughter Cattle - Premiums And Discounts. Accessed 10 Oct, 2017.https://www.ams.usda.gov/mnreports/lm_ct155.txt

Waikar, S.S., G. C. Curhan, and S. M. Brunelli. 2011. Mortality associated with low serum sodium concentration in maintenance hemodialysis. Am. J. Med. 124:77-84. doi: 10.1016/j.amjmed.2010.07.029.

Weber, P.S., T. Toelboell, L. C. Chang, J. D. Tirrell, P. M. Saama, G. W. Smith, and J. L. Burton. 2004. Mechanisms of glucocorticoid-induced down-regulation of neutrophil L-selectin in cattle: evidence for effects at the gene-expression level and primarily on blood neutrophils. J. Leukocyte Biol. 75:815-827.

Wheeler, W.E., and C. H. Noller. 1977. Gastrointestinal tract pH and starch in feces of ruminants. J. Anim. Sci. 44:131-135.

Table 6.1. Complete blood count variables by abscess class.

| Item | Abscess class[1] None (n = 51) | Minor (n = 51) | Major (n = 51) | SEM | *P*-value | Reference Range[2] |
|---|---|---|---|---|---|---|
| White blood cells, K/uL | 9.10 | 9.19 | 9.51 | 0.460 | 0.75 | **4.0 - 12.0** |
| Neutrophils, K/uL | 4.70 | 4.82 | 5.09 | 0.357 | 0.78 | **0.6 - 4.0** |
| Lymphocytes, K/uL | 3.45 | 3.32 | 3.32 | 0.288 | 0.88 | **2.5 - 7.5** |
| Neutrophil : Lymphocyte | 1.65 | 1.70 | 1.76 | 0.152 | 0.81 | **.** |
| Monocytes, K/uL | 0.71 | 0.77 | 0.86 | 0.077 | 0.21 | **0.025 - 0.85** |
| Eosinophils, K/uL | 0.23 | 0.26 | 0.23 | 0.064 | 0.63 | **0.0 - 2.4** |
| Neutrophils, % | 51.5 | 51.1 | 53.1 | 2.412 | 0.94 | **15 - 45** |
| Lymphocytes, % | 37.9 | 37.2 | 35.5 | 2.653 | 0.69 | **45 - 75** |
| Monocytes, % | 8.0 | 8.6 | 8.9 | 0.558 | 0.29 | **2 - 7** |
| Eosinophils, % | 2.6 | 3.0 | 2.5 | 0.643 | 0.51 | **2 - 20** |
| Red blood cells, M/uL | 8.95 | 8.92 | 8.62 | 0.217 | 0.15 | **5 - 10** |
| Hemoglobin, g/dL | 13.8[a] | 13.8[a] | 12.9[b] | 0.480 | < 0.01 | **8 - 15** |
| Hematocrit, % | 43.4[a] | 44.0[a] | 41.0[b] | 1.574 | < 0.01 | **24 - 46** |
| Mean corpuscular volume, fL | 48.8 | 49.6 | 47.9 | 1.000 | 0.07 | **40 - 60** |
| Mean corpuscular hemoglobin, pg | 15.5[ab] | 15.6[a] | 15.1[b] | 0.261 | 0.05 | **11 - 17** |
| Mean corpuscular hemoglobin concentration, g/dL | 31.7 | 31.4 | 31.5 | 0.003 | 0.22 | **30 - 36** |
| Red cell distribution width, fL | 36.4 | 36.7 | 36.1 | 0.775 | 0.42 | **.** |
| Platelets, K/uL | 137[b] | 143[ab] | 214[a] | 16.357 | 0.02 | **100 - 800** |

[a,b]Means within row lacking common superscripts differ ($P < 0.05$).

[1]Abscess class: None - indicates liver possessed no abscesses at slaughter; Minor - indicates liver possessed up to 4 small active abscesses at time of slaughter; Major - indicates that liver possessed at least one large active abscess at time of slaughter.

[2]Reference ranges listed were generated from values reported in The Merck Veterinary Manual, 8th Edition. Merck & Co., Inc. 1998.

Table 6.2. Serum metabolites, proteins, and ions by abscess classification.

| Item | Abscess class[1] None (n = 51) | Minor (n = 51) | Major (n = 51) | SEM | *P*-value | Reference Range[2] |
|---|---|---|---|---|---|---|
| Albumin, g/dL | 3.0[a] | 3.1[a] | 2.8[b] | 0.093 | < 0.01 | **2.8 - 3.9** |
| Blood urea nitrogen, mg/dL | 12[b] | 13[a] | 12[b] | 0.511 | < 0.01 | **7.8 - 24.6** |
| Creatinine, mg/dL | 1.4 | 1.4 | 1.4 | 0.033 | 0.29 | **0.6 - 1.8** |
| Globulin, g/dL | 5.2[b] | 5.2[b] | 5.8[a] | 0.127 | < 0.01 | **2.9 - 4.9** |
| Glucose, mg/dL | 127 | 116 | 121 | 14.037 | 0.12 | **42.1 - 74.5** |
| Albumin:Globulin | 0.6[a] | 0.6[a] | 0.5[b] | 0.030 | < 0.01 | **0.6-1.3**[*] |
| **Enzymes** | | | | | | |
| Alanine aminotransferase, u/L | 21[a] | 22[a] | 19[b] | 0.569 | < 0.01 | **6.9 - 35.3** |
| Alkaline phosphatase, u/L | 142 | 148 | 138 | 10.843 | 0.22 | **17.5 - 152.7** |
| Aspartate aminotransferase, u/L | 97[a] | 95[ab] | 89[b] | 6.596 | 0.03 | **45.3 - 110.2** |
| **Ions** | | | | | | |
| Calcium, mg/dL | 10.6 | 10.6 | 10.5 | 0.099 | 0.32 | **8.4 - 11.0** |
| Chloride, mmol/L | 97 | 97 | 97 | 0.404 | 0.76 | **95.7 - 108.6** |
| Potassium, mmol/L | 7.4 | 7.6 | 7.4 | 0.155 | 0.22 | **4.0 - 5.8** |
| Sodium, mmol/L | 142[a] | 142[a] | 141[b] | 0.611 | 0.02 | **134.5 - 148.1** |
| Total bilirubin, mg/dL | 0.4 | 0.4 | 0.4 | 0.044 | 0.79 | **0.00 - 0.8** |
| Total protein, g/dL | 8.2[b] | 8.3[b] | 8.6[a] | 0.113 | 0.01 | **6.2 - 8.2** |

[a,b]Means within row lacking common superscripts differ ($P < 0.05$).

[1]Abscess class: None - indicates liver possessed no abscesses at slaughter; Minor - indicates liver possessed up to 4 small active abscesses at time of slaughter; Major - indicates that liver possessed at least one large active abscess at time of slaughter.

[2]Refrence ranges listed were generated from values reported in The Merck Veterinary Manual, 8th Edition. Merck & Co., Inc. 1998.

[*]Reference range for Albumin:Globulin generated from values reported by Boyd, 1984.

Table 6.3. Carcass characteristics by abscess classification.

| | Abscess class[1] | | | | |
|---|---|---|---|---|---|
| **Item** | **None (n = 51)** | **Minor (n = 51)** | **Major (n = 51)** | **SEM** | ***P*-value** |
| Hot carcass weight, kg | 381.8[ab] | 387.1[a] | 366.2[b] | 5.876 | 0.03 |
| 12[th] rib fat thickness, cm | 0.76[ab] | 0.84[a] | 0.71[b] | 0.084 | 0.02 |
| *Longissimus* muscle area, cm[2] | 86.6 | 88.1 | 84.2 | 3.316 | 0.09 |
| Calculated yield grade | 2.65 | 2.71 | 2.58 | 0.224 | 0.41 |
| Marbling score[2] | 445 | 459 | 443 | 14.582 | 0.66 |

[a,b]Means within row lacking common superscripts differ ($P < 0.05$).

[1]Abscess class: None - indicates liver possessed no abscesses at slaughter; Minor - indicates liver possessed up to 4 small active abscesses at time of slaughter; Major - indicates that liver possessed at least one large active abscess at time of slaughter.

[2]Scores: 300 to 390 = Slight; 400 to 490 = Small; 500 to 590 = Modest.

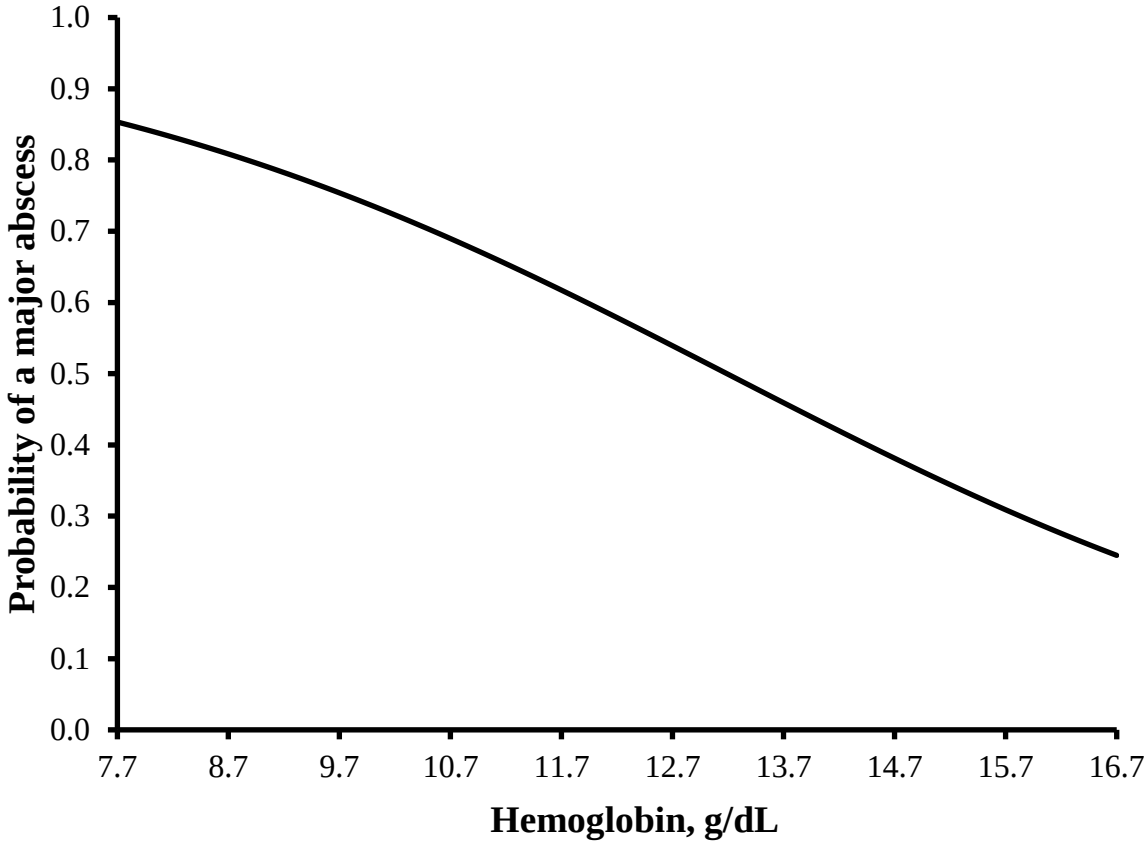



**Figure 6.1.** Probability of an animal having a major abscess using hemoglobin as a predictor.

exp(4.2299 - 0.3207 × hemoglobin, g/dL)/(1+exp(4.2299 - 0.3207 × hemoglobin, g/dL))

c-stastic = 0.64

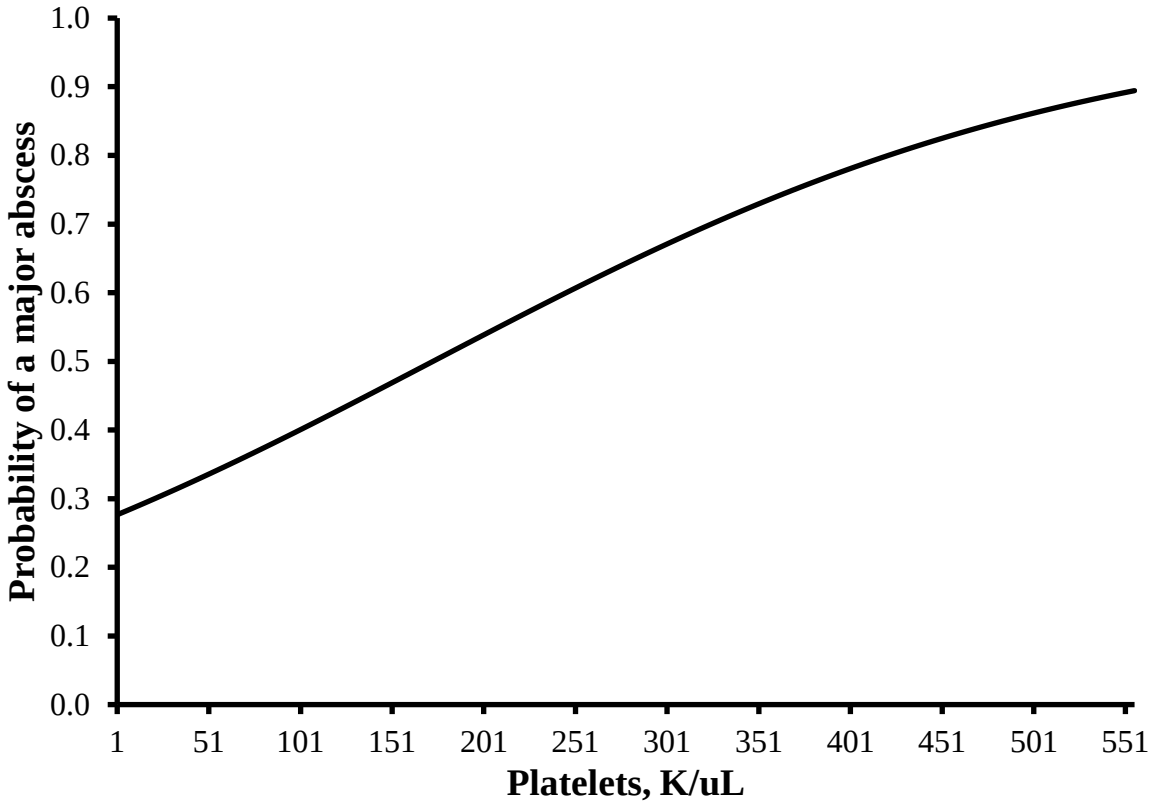



**Figure 6.2**. Probability of an animal having a major abscess using platelets as a predictor.

exp(-0.9669 + 0.00558 × platelets, K/uL)/(1+exp(-0.9669 + 0.00558 × platelets, K/uL))

c-stastic = 0.67

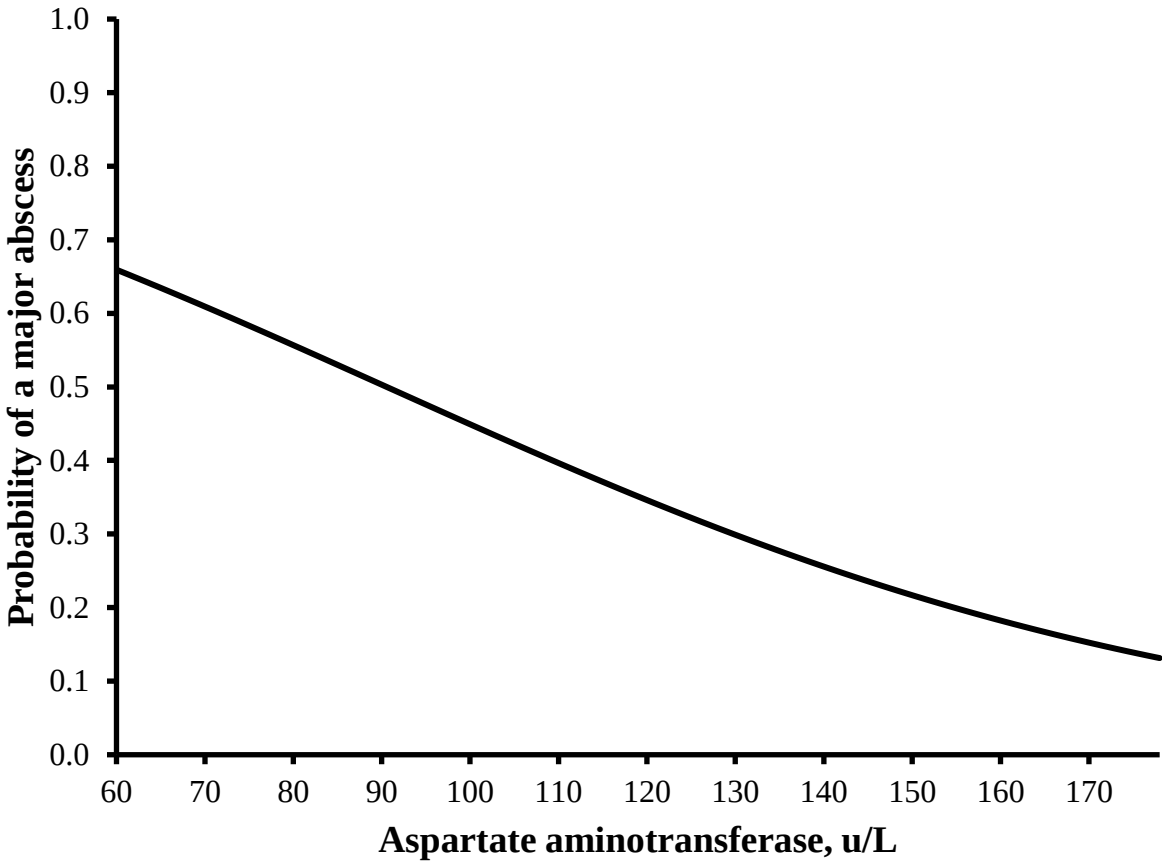



**Figure 6.3.** Probability of an animal having a major abscess using aspartate aminotransferase as a predictor.

exp(1.9560 + -0.0216 × AST, u/L)/(1+exp(1.9560 + -0.0216 × AST, u/L))

c-stastic = 0.65

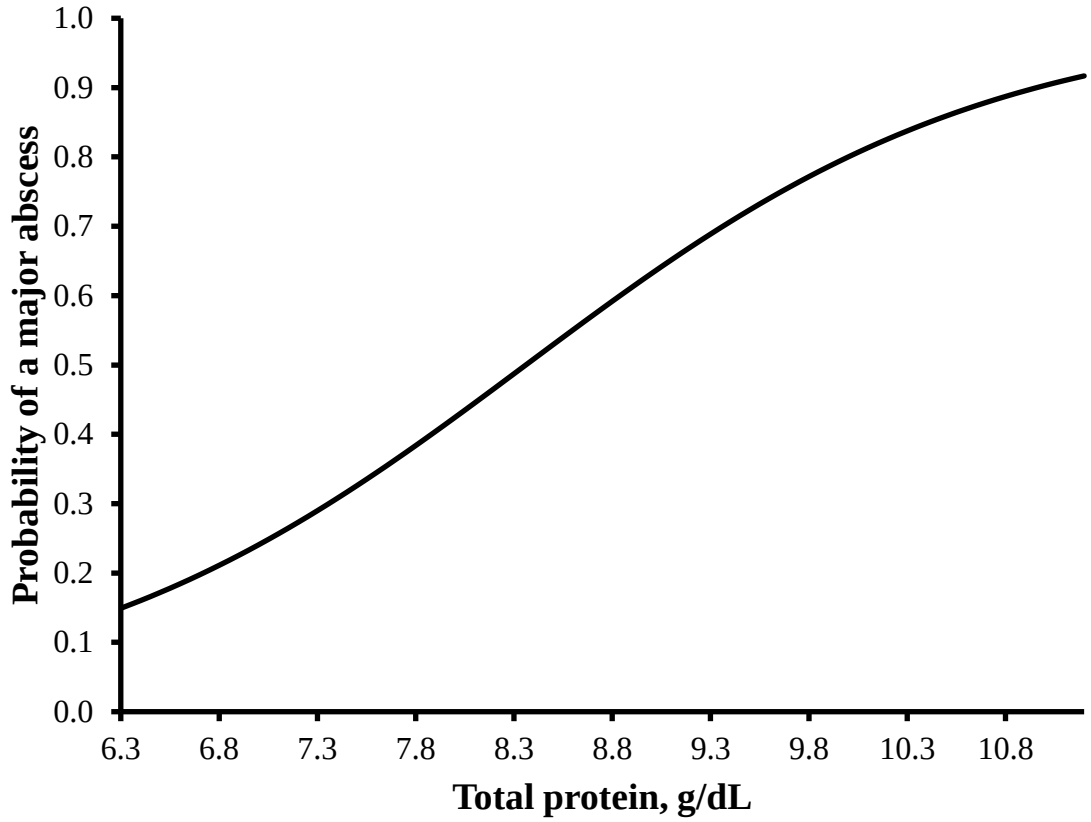



**Figure 6.4.** Probability of an animal having a major abscess using serum total protein as a predictor.

exp(-7.0668+0.8453 × total protein, g/dL)/(1+exp(-7.0668+0.8453 × total protein, g/dL))

c-stastic = 0.64