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DISSERTATION

ACUTE INTERSTITIAL PNEUMONIA, BOVINE RESPIRATORY DISEASE COMPLEX AND POTENTIAL PNEUMOTOXICITY IN FEEDLOT CATTLE

Submitted by

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In partial fulfillment of the requirements

for the degree of Doctor of Philosophy

Colorado State University

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY GUY HEATON LONERAGAN ENTITLED ACUTE INTERSTITIAL PNEUMONIA, BOVINE RESPIRATORY DISEASE COMPLEX AND POTENTIAL PNEUMOTOXICITY IN FEEDLOT CATTLE BE ACCEPTED AS FULFILLING IN PART THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

ACUTE INTERSTITIAL PNEUMONIA, BOVINE RESPIRATORY DISEASE COMPLEX AND POTENTIAL PNEUMOTOXICITY IN FEEDLOT CATTLE

The broad aims of these studies were to describe trends in mortalities, and evaluate a potential role of 3-methylindole (3MI) or its pneumotoxic metabolite, 3-methyleneindolenine (3MEIN), in acute interstitial pneumonia (AIP) and bovine respiratory disease complex (BRD) in feedlot cattle.

A retrospective cohort study was performed using data collected by the USDA's National Animal Health Monitoring System to evaluate trends in mortalities in feedlot cattle. The study population included approximately 21.8 million cattle placed in 121 feedlots from 1994 through 1999. Frequency counts of mortalities were modeled using Poisson regression. The mortality ratio was 12.6 deaths per 1,000 placements. The risk of feedlot mortalities increased from 1994 through 1999 because of increases in the proportion of animals that died from respiratory disorders.

Prospective case-control studies were performed to evaluate potential causes of AIP. Lung and blood samples were collected from animals from 14 feedlots located in CO, NE, KS and TX. Histological diagnoses were categorized as AIP cases, bronchopneumonia (BP) cases, and controls. Acute interstitial pneumonia cases had

been at the feedlot for an average of 127.2 days, which was longer than BP cases (98.6 days) and controls (84.0 days). The presence of a viral respiratory pathogen was not associated with histological category. Bovine respiratory syncytial virus was detected in 8.3% of AIP cases and 24.0% of controls. Histological category was associated with isolation of an aerobic bacteria and mycoplasma. Bronchopneumonia cases were at greater risk for aerobic bacteria and mycoplasma isolation compared to AIP cases and controls. Lung 3MEIN-adduct concentrations were greater in AIP ($P < 0.01$) and BP ($P < 0.01$) cases compared to controls. Blood 3MEIN-adduct concentrations were greater in AIP-affected animals than controls ($P < 0.01$) or animals with BP ($P = 0.02$). The odds of an AIP case being a heifer was 3.1 times greater than the odds of an AIP case being a steer.

Time-dependant patterns and magnitudes of plasma 3-methylindole and blood 3MEIN-adduct concentrations in feedlot cattle were described using 64 single-source yearling steers in a completely randomized study design with repeated measures. Blood samples were collected from all steers approximately 4 times per week for 3 weeks, 3 times per week for the following 5 weeks, then weekly for an additional 10 weeks. All plasma samples were analyzed for 3MI concentrations. Blood samples collected during the first 8 weeks from 32 animals were analyzed for 3MEIN-adduct concentration. Blood 3MEIN-adduct concentrations peaked during the period of greatest risk of the BRD complex. Conversely, plasma 3MI concentrations decreased during the same period.

The effect of dietary inclusion of aspirin and vitamin E on plasma 3MI concentration, blood and lung 3MEIN-adduct concentration, occurrence of grossly

identifiable lung lesions at harvest, and animal performance was evaluated. Two trials were conducted concurrently using steers (316 kg) obtained from a single source. Sixty-four animals were used in trial 1, and 192 animals were used in trial 2. A 2x2 factorial treatment design was used in each trial and treatment factors were aspirin (0 or 3 g PO daily), and supplemental vitamin E (0 or 1,500 iu PO daily). Animals from trial 1 were harvested on day 59. 3-methylindole concentration was measured in plasma and 3MEIN-adduct concentration was determined in blood and lung tissue. Trial 2 animals were weighed every 28 days and harvested on day 138. Vitamin E was associated with an increased concentration of plasma 3MI ($P = 0.08$). Without supplemental vitamin E, aspirin was associated with increased blood 3MEIN-adduct concentration ($P = 0.01$). Of trial 1 animals, 57.1 % had grossly identifiable lesions at harvest although none were observed to be sick. The presence of lung lesions was not associated with adverse effects on carcass characteristics, 3MI and 3MEIN concentrations, or treatment. Nor was treatment associated with improvements or adverse effects in body weight, mean daily weight gain, dry matter intake, or feed conversion in trial 2.

An increasing proportion of placements died from respiratory tract disorders from 1994 through 1999. Increased pulmonary generation of the pneumotoxin 3MEIN, may be important in the pathogenesis of AIP. Additionally, 3MEIN-adduct concentrations were elevated during the period temporally associated with greatest risk for BRD.

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My Ph.D. training has been wholly satisfying and immensely rewarding. At many levels, it is difficult to neatly compartmentalize my graduate training into distinct masters and Ph.D. programs. However, some understanding of my Ph.D. research commitments is needed so as to identify those people who warrant acknowledgment. The primary research efforts include:

1. Execution of a collaborative agreement with the Centers for Epidemiology and Animal Health (USDA:APHIS:VS:CEAH). My chief responsibility was the National Animal Health Monitoring System's Feedlot '99 study. This was a nationwide survey of feedlot producers that included the specific testing of biological samples and the use of data acquisition tools such as questionnaires,
2. Field research of potential microbial and toxicological causes of acute interstitial pneumonia of feedlot cattle, and
3. Patterns of plasma 3-methylindole and blood 3-methyleneindolenine concentrations in feedlot cattle with specific relevance to bovine respiratory disease complex.

Thus, research activities were broad in scope and only possible through extensive teamwork which involved many, many diligent and hardworking personnel.

Of those at CEAH, I would like to specifically thank Bruce Wagner. He was integral in the success of the Feedlot '99 study and always provided me much needed help relating to study design and data analysis for non-Feedlot '99 related activities.

Field trials were conducted at many feedlots throughout the U.S. and would not have been possible without the collaborative efforts of Drs. Leon Mills, Bruce Hoffman, and Del Miles. Numerous feedlot personnel were also instrumental in the successful completion of research; one such person who deserves special thanks is Joel Chisum.

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No phraseology can adequately acknowledge the integral role that my graduate committee played in my higher education. Members of the committee included Drs. Frank Garry, David Dargatz, Paul Morley, and Daniel Gould. The latter two were co-advisors. Dr. Dargatz was my supervisor while working at CEAH. This period was one of the most valuable experiences of the entire Ph.D. program. His clinical reasoning, patience and work-ethic were exemplary and provide an excellent model for which all should strive.

The Integrated Livestock Management program (ILM) was at the center of my graduate training. Thus, no acknowledgment is complete without recognizing the unflagging leadership provided by Dr. Garry. Oftentimes, his efforts go unrecognized or without gratitude. Yet, he appreciates that successful ILM graduate programs must focus on producer-related issues, and that research should provide beneficial outcomes for producers. I am extremely grateful to efforts Dr. Garry has imparted. Special thanks also must go to Dr. Gould, with whom I have closely worked for the past five and a half years. His friendship and advice have been at the core of my academic development. I would like to acknowledge Dr. Morley for his advisement.

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A.M.D.G.

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CHAPTER 1

The purpose of this chapter is to provide the reader with an overview of the format of this dissertation. There are 3 related subject areas described in the body of this work that, at their core, pertain to respiratory disease in feedlot cattle. The subject areas are feedlot mortalities, acute interstitial pneumonia (AIP), and bovine respiratory disease (BRD) complex. A relationship with a rumen generated-toxin is also evaluated in the latter two subject areas.

Chapter 2 is a review of the pertinent literature relating to the specific topic areas of the research described herein. There are 5 sections covered and include:

I. Bovine respiratory disease complex.

II. Acute interstitial pneumonia.

III. 3-methylindole metabolism.

IV. Potential role of 3-methylindole and 3-methyleneindolenine in respiratory disease.

V. Research hypotheses and objectives.

The bulk of the dissertation, chapters 3 through 7, are stand alone manuscripts. As such, there is a certain degree of repetition from chapter to chapter.

Chapter 3 describes trends in mortalities in U.S. feedlots for the period of 1994 through 1999. The data were collected through the Sentinel Feedlot Monitoring Program, which is administered by United States Department of Agriculture's National Animal Health Monitoring System (NAHMS) personnel. The data are offered voluntarily and constitute a substantial proportion of the U.S. fed cattle inventory. A retrospective cohort study was used to evaluate the data. The reader should use care when making generalizations to the wider feedlot industry as the data do not represent a random sample of feedlots.

Chapters 4 and 5 report results from case-control studies performed to investigate proposed causes of AIP in feedlot cattle. Chapter 4 deals with microbial pathogens whereas chapter 5 evaluates an association with 3-methyleneindolenine, a toxic metabolite of 3-methylindole.

The final subject area is covered in chapters 6 and 7. The first of these is a descriptive report that provides an explorative evaluation of the patterns and magnitudes of plasma 3-methylindole and blood 3-methyleneindolenine concentrations in feedlot cattle. The research described in chapter 7, an intervention study, is a natural progression from these results.

Overall conclusions are provided in chapter 8. These are presented in bullet format.

Chapter 2.

Section I. BOVINE RESPIRATORY DISEASE COMPLEX

Overview

By far the most important animal health concern for the North American feedlot industry is bovine respiratory disease complex (BRD).[1,2] This disorder is typically attributed to bacterial bronchopneumonia and is the principal cause of morbidity and mortality in feedlot cattle.[1,3,4] Consequently, BRD imparts a substantial economic burden on feedlots.[5]

Rarely if ever does BRD result from an uncomplicated infection with a single viral or bacterial species. Rather, feedlot-associated BRD is the sequella of a complex relationship between stressors, animal susceptibility, and respiratory pathogens.[6] Viral agents and stressors decrease an animal's innate and acquired pulmonary defense mechanisms, thereby facilitating multiplication of bacteria in the lower respiratory tract.

Estimates of morbidity in the literature vary widely, and it is often unclear how they were calculated. Estimates vary from approximately 5 to 95%.[7,8] A nation-wide survey

conducted by United States Department of Agriculture's National Animal Health Monitoring System (NAHMS) personnel identified that 14.4% of all cattle that entered feedlots developed BRD (Table 2.1.).[1] Nationally, 1.4% of all feedlot placements died before reaching a desirable harvest weight.[4] Vogel and Parrott reported that approximately two-thirds of feedlot mortalities are attributable to respiratory disease.[9] Therefore, a greater proportion of cattle died from BRD than all other disorders combined.[10]

Substantial time and money is afforded to prevention and control of BRD, and identification and treatment of BRD-affected animals. Despite our increased understanding of the pathogenesis of BRD, the use of vaccines and efficacious antimicrobials, the proportions of animals that do not survive to a desirable harvest weight because of BRD appears to be increasing.[10]

Economic burden of BRD

The exact cost of BRD is unknown and estimates are difficult to calculate. Other authors have reported values vary between $458 to $624 million annually.[5,11] Griffin estimated that investments associated with efforts to prevent, control, and effects of BRD account for 7% of all productions costs; this may represent billions of dollars.[12]

Feedlot personnel reported that they spent an average of $12.59 in 1999 to treat BRD-affected animals.[1] This estimate included the cost of pharmaceuticals and other

Table 2.1. Percentage of cattle placed during the year ending June 30, 1999 that developed the following disease conditions.[a] Estimates include those animals identified with the disorders and treated, identified with the disorders and not treated, or died without treatment.

	Feedlot capacity					
	1,000 to 7,999		8,000 or more		All feedlots	
Disease condition	Percent	SE	Percent	SE	Percent	SE
Bovine respiratory disease	8.7	0.7	15.5	4.7	14.4	4.0
Acute interstitial pneumonia	2.9	0.4	3.1	0.4	3.1	0.3
Digestive problems (excluding non-eaters)	1.1	0.1	2.0	0.3	1.9	0.3
Buller steer syndrome	1.4	0.2	2.3	0.4	2.2	0.3
Lameness	1.3	0.2	2.0	0.9	1.9	0.8
Diseases of the central nervous system.	0.3	0 1	0.4	0.1	0.4	0.1

[a] Table adapted from: NAHMS. Part III: Health management and biosecurity in U.S. feedlots, 1999. #N336.1200

expendables (such as syringes and needles), but did not include labor or veterinary service expenditures. Of the animals identified as BRD-affected by feedlot managers (i.e. 14.4% of placements),[1] it seems reasonable that 95% were treated for their disease. During the calendar year of 1999, approximately 25.2 million animals were placed in feedlots.[13,14] Therefore, BRD resulted an estimated $45.7 million in treatment costs alone.

Feedlots spent $157.1 million to purchase animals that ultimately died as due to BRD based on assumptions of an average arrival weight of 318.2 kg (700 lbs), a purchase price of $2.09 / kg ($95 per cwt), and that two-thirds of mortalities resulted from BRD.[9] These animals consumed an average of 405 kg of feed based on a mean of 45 days from arrival until death and a daily dry matter intake of 9 kg.[15] Although feed costs can vary widely, a reasonable cost of the diet prepared and delivered to the pen is $29.60 / 100 kg ($65 / cwt).[16] Based on these assumptions, animals that ultimately died from BRD consumed $2.8 million in feed costs annually in 1999.

Further costs are difficult to quantify but are likely extensive and include interest, associated labor expenses, veterinary services, opportunity losses and losses from those animals sold prior to a desirable harvest weight because of BRD that was refractive to available therapeutic regimens.

Investigators have recently discovered that a substantial proportion of animals who were never observed to be suffering from BRD had pulmonary lesions at harvest.[17,18] In one

study, 67.2% of non-treated animals had evidence of either resolved or active bronchopneumonia at harvest.[17] Animals with lesions gained an average of 0.07 to 0.2 kg less per day than animals without lesions. Therefore, the greatest financial cost of BRD likely results from decreased performance in animals that are apparently healthy but who actually have performance-affecting pulmonary lesions that affect rates of gain.

Pathogens

Bovine respiratory disease is a classic example of a condition that results from a complex, multifactorial etiology.[6,19] The disease is widely accepted to result from a complex interaction of stressors, animal susceptibility and infectious respiratory pathogens.[6] Viral pathogens are often thought to be primary invaders that compromise the animal's innate and acquired pulmonary defense mechanisms.[20-22] Subsequently, bacterial colonization of the animal's lower respiratory may occur.[6] Bovine respiratory disease complex then develops and its clinical manifestations are a function of the dynamic host-pathogen interaction.

Despite extensive research, the precise role of each infectious pathogen in the pathogenesis of feedlot BRD is unclear. This is primarily because BRD is generally accepted to result from an interrelationship of pathogens with stressors and animal susceptibility.[6,23] This complex relationship includes many unknown factors that make experimental reproduction of the exact disease process unlikely. However, most of the understanding of the role of infectious pathogens has developed from interpretation of

case reports, controlled experiments, vaccine efficacy studies, and seroepidemiological surveys.

Viral pathogens

There have been numerous viral agents implicated in BRD of feedlot cattle including bovine herpesvirus type 1 (BHV1),[24] which is the etiological agent associated with infectious bovine rhinotracheitis (IBR), bovine respiratory syncytial virus (BRSV),[25] parainfluenza virus type 3 (PI3), and bovine viral diarrhea virus (BVDV).[20] Recently, there has been increasing interest in bovine coronavirus (BCV) and BRD.[26,27] By themselves, these pathogens do not typically induce clinically significant disease in healthy, non-stressed animals.[28] However, in the feedlot environment, infections with these virus are capable of leading to BRD.[19]

Bovine herpesvirus type 1

Bovine herpesvirus type 1 is an alpha herpesvirus has been recognized as an important pathogen in feedlot BRD since 1957.[24] The classical disease associated with BHV1 infection is commonly referred to as infectious bovine rhinotracheitis (IBR), or red nose; lesions are characterized by fibrinonecrotic inflammation of the nasal and tracheal mucosa.[24,29] Tracheas from severely affected animals are often described as having a "sewer pipe" appearance. Virulent strains of BHV1 have resulted in IBR outbreaks with substantial mortality.[30] However, classically described IBR is rarely reported -now, presumably as a consequence of industry-wide vaccination against BHV1 or a decrease in

its virulence.[31] However, BHV1 is still generally believed to be an important organism in the complex pathogenesis of BRD.[6,32]

Exposure to BHV1 is common,[33] and as with other alpha herpesviruses BHV1 may become latent in paravertebral ganglia.[19] Stresses, such as those associated with induction into the feedlot environment, may result in recrudescence and viral shedding.[19] This can potentially result in exposure of immunologically naive animals to BHV1.

Martin and others reported that seroconversion to BHV1 at arrival was uncommon in feedlot cattle.[34] However, others have found the opposite may be true indicating that pre-arrival exposure to BHV1 varies substantially.[33] Initial antibody titers to BHV1 have been associated with increased risk of treatment for respiratory disease.[34] Booker and coworkers, found that the odds of treatment were lower in those animals that seroconverted to BHV1 G-IV glycoprotein.[32] In their study, the odds of treatment in animals that seroconverted to BHV1, but not to BHV1 G-IV glycoprotein, did not differ from those animals that did not seroconvert.[32]

Of cattle placed on feed during the year ending June 30, 1999, 96.9% of cattle were vaccinated against BHV1.[31] Perino and Hunsaker performed a comprehensive review of vaccine efficacy field trials.[35] Only 3 papers pertaining to BHV1 withstood their selection criteria (inclusion of a valid control group, randomization of treatments, blinding of evaluators of a subjective outcome, adequate statistical power, and use of relevant

outcome variables). One of the papers, showed a dramatic beneficial effect of vaccination against BHV1.[36] However, this manuscript was published in 1958 and its relevance to modern feedlot production is questionable.[36]

Bovine respiratory syncytial virus

Bovine respiratory syncytial virus, a pneumovirus, is closely related to the respiratory syncytial virus of humans (HRSV).[37] Indeed, BRSV infections of cattle have been used as models for human infections with HRSV.[37] Bovine respiratory syncytial virus replicates in ciliated and non-ciliated cells of the large and small airways as well as epithelial cells of the alveolus.[38] Numerous BRSV-associated outbreaks of respiratory disease have been documented and it is clear that virulent strains can result in life-threatening disease, particularly in newly weaned calves.[20,38] Characteristic lesions include edema, emphysema, syncytia formation, and necrotizing bronchiolitis.[38]

Seroepidemiological surveys have revealed that approximately three quarters of all cattle entering feedlots have antibodies to BRSV, presumably resulting from either natural exposure or vaccination.[21] Disease in feedlot cattle directly attributed to BRSV is rarely reported in the literature. This may be a consequence of wide-spread vaccine usage as 70.9% of cattle placed in feedlots were vaccinated against BRSV.[31]

Seroconversion to BRSV was associated with increased risk of treatment for BRD.[34] Bingham and others found that animals vaccinated with a modified live BRSV vaccine

were less likely to be treated for BRD than unvaccinated controls.[39] Beneficial effects of vaccine administration varied with the type of animal.[35] Vaccination against BRSV decreased morbidity in younger animals whereas limited or no beneficial effect was detected in older animals.[35,40]

Parainfluenza virus type 3

Parainfluenza virus type 3 is a paramyxovirus that is considered a pathogen of feedlot cattle. Griffin reported that PI3 can, on rare occasions, cause a severe respiratory disease of cattle.[30] However, most infections only result a very mild disease that is of little clinical concern.[6] Parainfluenza virus type 3 infects many types of cells of the respiratory tract,[33,34] which may result in impaired mucociliary clearance and phagocytosis of foreign material.[41,42]

Arrival titers against PI3 were negatively associated with the odds of treatment for BRD.[43] There have been few field trials of vaccine efficacy where the effect on desirable feedlot outcomes were reported.[35] Of the cattle places in U.S. feedlots, 73.5% were vaccinated against PI3.[31]

Bovine viral diarrhea virus

Although not generally considered a primary respiratory pathogen, this pestivirus is considered important in the pathogenesis of BRD in feedlot cattle.[44,45] Many U.S. cattle have been exposed to BVDV and animals infected *in utero* with a non-cytopathic strain

may be immunotolerant to the virus and become persistently infected (PI).[46,47] These PI animals serve as reservoir for exposure of immunologically naive animals.[48] The mechanism of BVDV involvement in BRD of feedlot cattle is unclear. It is possible that BVDV increases the likelihood for BRD development by infecting alveolar macrophages and adversely affects the animal's immune competency.[49,50] There appears to be considerable synergy between BVDV and other respiratory pathogens.[45] Diseases associated with experimental challenge with BHV1, BRSV, and *M. haemolytica* were more severe in animals with concurrent BVDV infections.[51-54]

Based on serological surveys, antibodies to BVDV in feedlot cattle are relatively common.[48] Initial titers to BVDV were associated with decreased odds of treatment.[32,43] No studies of the effect of vaccination against BVDV on BRD in feedlot cattle have been published in peer review journals.[35]

Bovine coronavirus

For years BCV has been implicated in winter dysentery of dairy cattle.[55-59] Recently there has been increasing interest in the possibility that BCV may contribute to the pathogenesis of BRD in feedlot cattle.[8,26,27,60-62]

Exposure to BCV is almost ubiquitous in cattle.[27,60] Increased antibody titers at feedlot arrival were associated with decreased likelihood for treatment of BRD and improved weight gains during the first 28 days on feed.[60] Animals that shed virus at processing and

seroconverted were 1.6 (OR, 1.6; 95% CI, 0.8 to 3.4) times more likely to be treated for BRD than other animals.[26] Additionally, animals that shed BCV were greater than twice as likely to have grossly identifiable pulmonary lesions evident at harvest.[26] In a documented outbreak, BCV was isolated from approximately of 75% of BRD-affected animals just prior to development of disease.[8] Storz and coworkers concluded that BCV may have a causative role in outbreaks of BRD in feedlot cattle.

Bacterial pathogens

Mannheimia haemolytica

Mannheimia haemolytica (formerly *Pasteurella haemolytica*) is a Gram negative, non-spore forming, facultatively anaerobic bacterium.[63] *Mannheimia haemolytica* is an intracellular survivor and multiplier. Its virulence factors include but are not limited to proteases, fimbria and leukotoxin.[63] Like all Gram negative bacteria, endotoxin is liberated from the cell wall during bacterial degradation.[63]

It is a common inhabitant of the nasal mucosa, sinuses and tonsils of cattle.[64] In clinically healthy animals, serotype 2 is the predominant form of *M. haemolytica* isolated from nasal mucosa.[64,65] During times of stress (such as transportation or a viral infection), the pathogenic *M. haemolytica* biotype A serotype 1 (*M. haemolytica* A1) can colonize nasal mucosa and tonsilar tissue.[64-67] This may give rise to colonization of the lower respiratory tract and subsequent fibrinopurulent bronchopneumonia.[6]

Initial antibody titers to *M. haemolytica* and seroconversion to leukotoxin were associated with increased risk of treatment for BRD.[43] This was supported by a more recent investigation where the odds of treatment were 2.8 times greater (OR, 2.8; 95% CI, 1.4 to 5.6) in animals that seroconverted to leukotoxin compared to other animals.[32]

Studies evaluating the efficacy of leukotoxin toxoids and *M. haemolytica* bacterins have provided mixed results. Several of these field trials have yielded beneficial results indicating that some degree of protection was afforded to vaccinates.[68-70] In 1999, 27.5% of cattle were vaccinated against *M. haemolytica* or its leukotoxin.[31]

Pasteurella multocida

Pasteurella multocida is a Gram negative bacterium that is frequently cultured from pulmonary tissues of BRD-affected animals.[6] This bacterium induces fibrinopurulent bronchopneumonia that is less severe than by *M. haemolytica*-induced disease.[6] Griffin suggested that *P. multocida* may be an important pathogen of young animals.[6] Therefore, *P. multocida* may pose increased animal health concerns for feedlots in which calves comprise a substantial proportion of placements.

Haemophilus somnus

Haemophilus somnus is often included in the posse of pathogens associated with BRD of feedlot cattle.[71] Although this Gram negative, intracellular pathogen is commonly associated with morbidity and mortality in some areas,[72] rarely does it result in classical

bronchopneumonia.[71] Haemophilosis may be characterized as a syndrome resulting from the hematogenous spread of this bacterium.[71] Lesions included in the haemophilosis syndrome include thrombotic meningoencephalitis (TME), polyarthritis, myocardial infarcts, fibrinopurulent pleuritis, and occasionally bronchopneumonia.[73] Haemophilosis may be responsible for a greater proportion of all mortalities than BRD in some regions.[72,74,75] This may be a consequence of climatic or animal factors.

In one study, most animals that succumbed to fatal pasteurellosis (or mannheimiosis) died within 30 days of arrival, whereas haemophilosis-affected animals typically died between 30 and 60 days on feed.[76] Although some epidemiological differences between haemophilosis and BRD exist, clinical manifestations of haemophilosis are often similar to or indistinguishable from *Mannheimia*- or *Pasteurella*-induced bronchopneumonia.[32,72] Because of the potential for misclassification of etiology, some have suggested that the term undifferentiated fever (UF) is superior to BRD when a clinical diagnosis is based on:

1. An elevated rectal temperature,
2. A lack of clinical signs referable to a non-respiratory system.[32,72]

Thus, UF would encompass both haemophilosis and BRD. Use of the term UF may be more appropriate than BRD in situations where haemophilosis accounts for a substantial proportion of total morbidity or mortality.

Higher arrival antibody titers and seroconversion to *H. somnus* were associated with decreased risk of treatment for UF.[32 66,69] The incidence of haemophilosis has been

reported in one study to be increasing.[75,77]

Stressors

Stressors are believed to be integrally involved in the pathogenesis of BRD, but are complex and not wholly understood. This is partly because stress in animals can only be vaguely defined and no reliable measure of stress or the effect of stress on the immune system exists.[78] As such, stress and stressors are ambiguous terms that include physical and psychological conditions that are unpleasant to the animal.[79,80] Further, different animals will respond to similar stressors in very dissimilar ways indicating that an experience that one animal finds stressful, another animal may not.[81,82] Importantly, many stressors are temporally associated with exposure of naive animals to pathogens as cattle move from pastures to feedlots. Hence, it is often impossible to distinguish between the effect of stress and exposure to novel pathogens. Potential stressors to feedlot cattle can include transportation, passage through salebarns, commingling, weaning, and animal handling.

Animals that are crowded on trucks could potentially be exposed to quantities of pathogenic organisms that overwhelm apparently normal pulmonary defense mechanisms. But probably the most important effect of transportation is increased animal susceptibility so that exposure to lower quantities of pathogenic organisms can result in disease.[79,83] Transportation resulted in increased serum ACTH, adrenaline and cortisol concentrations.[79,83-88] Maximum concentrations of these stress-markers were achieved

within 30 to 60 minutes of initiation of transportation.[86] Presumably such biochemical alterations may affect immunocompetence.[83,89] Extended transportation also results in increased heart rate and dehydration (commonly referred to as transit shrink).[80,90] Often, calves are weaned immediately prior to loading on to trucks which likely contributes to stress. Transportation has been associated with colonization of tonsilar tissue with *M. haemolytica* A1 and BRD.[64,66] However, the exact role of transportation in BRD is not known and Ribble and coworkers have questioned its importance.[90] The distance calves were transported was not associated with BRD mortality in their study.[90]

Only 25.5% of cattle that entered U.S. feedlots were procured directly from ranches, or born on operations owned by feedlots during the year ending June 30, 1999.[4] Presumably a significant proportion of the remaining 74.5% of cattle placements were purchased from salebarn sources. Auction-derived animals have greater morbidity than ranch-origin cattle.[3] Unfortunately, it not uncommon for cattle to pass through several salebarns prior to their arrival at feedlots.

Most cattle are processed soon after arrival at feedlots.[4,91] Procedures performed on the newly arrived cattle include vaccination against respiratory diseases.[31,92] Processing invariably involves animal handling and restraint that may further compound stress.[79,81,82,93] In one study, processing was associated with increased mortality and the benefits of processing have been questioned by others.[94-96] This finding does not imply that vaccination of cattle against respiratory disease is not of value, but the results should

provoke meaningful discussion concerning optimal processing techniques that impose minimal stress.[97]

Pathogenesis of Bovine Respiratory Disease Complex

Rarely does a single viral or bacterial species result in clinically important disease in healthy animals. Even virulent strains of virus derived from naturally-occurring disease outbreaks have resulted in little or no apparent disease in attempts to reproduce disease experimentally.[28,98]

Innate and acquired pulmonary defense mechanisms must be overcome for an event of BRD to occur. This could possibly happen by overwhelming the respiratory clearance mechanisms with a single pathogenic species such as *M haemolytica*.[6] However, the majority of BRD events result from a more complex interrelationship of factors.[6]

As a consequence of modern feedlot managerial practices, cattle are likely to be exposed to most or all respiratory pathogens as they pass through auction markets, or soon after their arrival at feedlots.[8,27,32-34,43,60,66,67,74,99] Naivety of the immune system or stress-induced increases in animal susceptibility and possibly increase the likelihood for BRD in response to exposure to respiratory pathogens.

Stressors associated with recent weaning, passage through auction markets, transportation, animal handling and commingling presumably result in increased

susceptibility to infection.[6,100] Therefore, stressed animals would more likely develop BRD in response to a pathogen challenge compared to similar yet unstressed animals.[3] Stress may also result in recrudescence of a latent BHV1 infection.[30]

Viral pathogens infect, replicate within, and injure ciliated cells, alveolar epithelial cells, and alveolar macrophages.[41,42,101] Lymphocytes may also be adversely affected by BVDV infections.[50] As a consequence, mucociliary clearance, and phagocytosis and killing of bacterial pathogens, such as *M. haemolytica* A1, is likely impaired. Bacterial colonization of the lower respiratory tract may ensue. The animal mounts a profound inflammatory response which includes increased production of arachidonic acid metabolites, cytokine secretion, platelet activation and aggregation, fibrinogen accumulation and activation, margination of neutrophils into the alveolar spaces, and vascular congestion and coagulation.[102-108]

The cranioventral aspect of the lung is primarily affected indicating inhalation of pathogens as opposed to hematogenous spread. Gross lesions include congestion, consolidation (hepatization), interlobular fibrin accumulation, and adhesions between parietal and parenchymal pleural surfaces.[23,109] The area of affected lung generally increases caudodorsally as disease progresses.[29]

Clinical manifestations of *M. haemolytica*-induced BRD are a result of the interaction of the host's response and the viral/bacterial infection. Early manifestations may include

excessive lacrimation, serous nasal discharge, increased respiration rate, shallow respiration, inappetence, and signs of depression.[6] A soft cough may also be occasionally present. As the disease progresses, clinical manifestations become more pronounced and death may be an outcome of disease.

Epidemiology

Most BRD events that are manifested as clinically apparent disease occur soon after arrival at the feedlot.[3] This is because stressors and exposure to respiratory pathogens are temporally associated with the movement of cattle from ranches of origin (likely including passage through auction markets) to the feedlot, and induction into the feedlot environment.[3] Hence, BRD is commonly referred to as *shipping fever*.

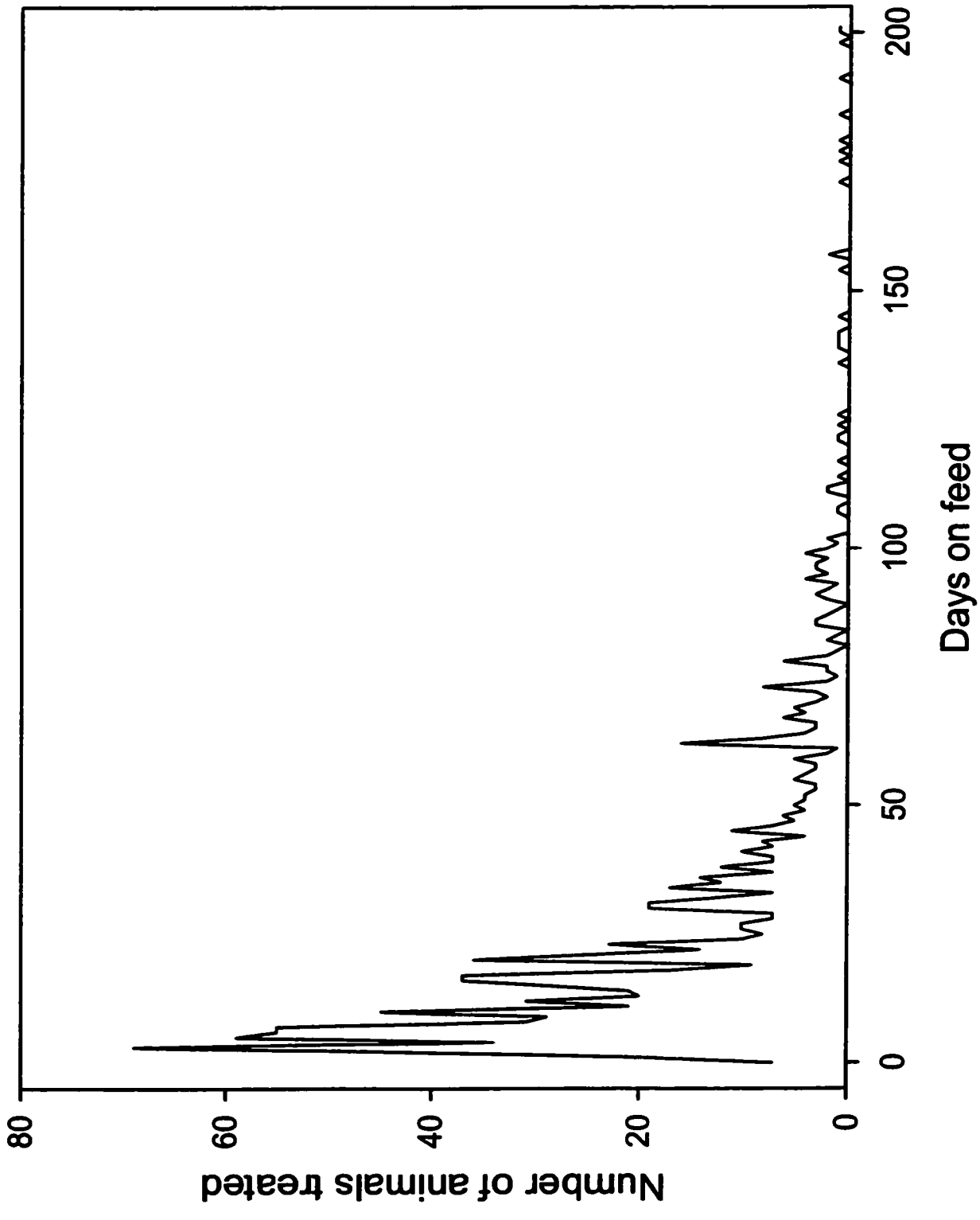
Estimates of morbidity and mortality have been widely published.[1,10,7,61,74,111,112] However, substantial difficulty exists in interpreting these results. It is frequently unclear what criteria were used for an event to be included in the numerator and denominator. Sometimes there is no definition of what constitutes a case, or if the event of interest is an initial episode or a relapse of disease (the latter shortcoming is not a problem for estimates of mortality). Hence, the use of the term morbidity is somewhat imprecise. Treatment rate (or risk) may be a more accurate definition of what is being reported as morbidity in the literature.[3] However, variation in the definition of treatment may vary.

Published treatment rates have varied between 5 and 95%.[7,61,74,111,112] However, the vast majority of reports that included data on large numbers of cattle suggest that treatment rates typically vary between 10 and 25%.[1,3] A recent NAHMS study indicated that 14.4% of placements were affected with respiratory disease.[1] This estimate included animals that were treated, and those that did not receive treatment such as those that died before an antimicrobial could be administered. These data were collected via questionnaires administered to feedlot personnel. Data were not collect to ascertain how diagnoses were made so the potential for misclassification exists.

Estimates of mortality risks for respiratory disease are often confusing because the criteria for including animals in the denominator often varies.[3] Most reports present the number of deaths as a proportion of number of animals placed within a given time period.[4,7,9,10,91] These estimates may not be accurate when the time period is relatively short, such as one month. For example, some animals that died during December (numerator eligibility) may not have been among those animals placed in December (denominator eligibility). Hence, animals may be included in the numerator even though they are not included in the denominator. The use of longer time periods would likely to be more accurate estimators of risk. Other estimates have used average inventory as the denominator.[113] Published mortality risks attributable to respiratory disease vary from 0.25 to 2.7%.[3,7,9,10,74,111,114,115]

Most BRD events occur in cattle soon after they arrive at feedlots.[3,71,74,113] Data collected as part of the NAHMS feedlot '99 study support these claims.[116] A total of 20,236 cattle were monitored from arrival to shipment during this study. Seven percent of monitored animals were treated for an initial event of respiratory disease.[116] The mean duration from arrival to initial treatment for respiratory disease was 27.1 (SEM ± 1.1) days.[117] Sixty-seven and 86.1% of initial treatments for respiratory disease occurred with in the first 28 and 56 days on feed, respectively. The mean days from arrival to initial treatment was 11.1 (± 0.4) and 17.3 (± 0.6) for those animals treated within the first 28 and 56 days of arrival, respectively. Hence, the data are right skewed (Figure 2.1.). These data compare favorably to other published reports.[3]

Figure 2.1. Frequency count of bovine respiratory disease complex (BRD) events by days on feed. Data derived from NAHMS Feedlot '99 study.



Section II. ACUTE INTERSTITIAL PNEUMONIA

Overview

An important, yet enigmatic disease of feedlot cattle is acute interstitial pneumonia (AIP). This disease is frequently mentioned in the literature, but little is known about its etiology. It is not uncommon for authors to report contradictory findings. For example, some authors have suggested that the disease events occur with equal frequency during all stages of finishing,[118,119] whereas others report that the disease is clustered towards the latter part of the feeding period.[120]

Considerable confusion exists with regard to AIP. Many causal associations have been proposed including dust,[119] BRSV,[121] increased production of a pneumotoxic metabolite of 3-methylindole (3MI),[120] bacterial pneumonia,[118] hypersensitivity,[100] molds,[122] alterations in digestive function,[123] and other rumen-generated pneumotoxins.[124]

Because considerable misconception surrounds this disease, case definitions are unclear in many reports of AIP. Therefore, accurate estimates of morbidity and mortality rates are difficult to obtain and even harder to interpret. One report indicated that 3.1% of all placements were affected with AIP.[1] Published data indicate the proportional mortality

attributable to AIP is between 5 to 10%.[119,125] However, during the hottest months of the year, AIP may be the single leading cause of mortality in some feedlots.[123,126]

Acute Interstitial Pneumonia

Acute interstitial pneumonia describes specific histological lesions resulting from injury at the level of the alveolar wall.[127] The term is, however, nonspecific with regard to etiology. Lesions may result from a number of etiologies that occasion damage to the alveolar wall including 3-methylindole,[128] 4-ipomeanol,[129] Perilla ketones,[130,131] unidentified compounds from cruciferous crops,[132] and BRSV.[38] The human equivalent of the disease, known as acute respiratory distress syndrome (ARDS), has been associated with increased concentrations of proinflammatory cytokines.[133]

Acute interstitial pneumonia has been known by many different names depending on the production setting where the disease occurred.[73] *Acute interstitial pneumonia* encompasses diseases better known by their common name and include acute bovine pulmonary edema and emphysema (ABPE),[134] fog fever, dust pneumonia,[100] allergic pneumonia,[122] cow asthma, ARDS, panters, pneumoconiosis, and pulmonary adenomatosis.[119,135] Further confusion ensued because AIP has been referred to as atypical interstitial pneumonia.[119,121] This terminology developed because interstitial pneumonias were believed to be chronic in nature and characterized solely by a proliferative response involving the alveolar walls.[73] However, it was discovered that AIP involves an acute intra-alveolar exudative phase that precede the more *typical*

cellular proliferation. Because the disease did not fit the accepted understanding of interstitial pneumonia pathogenesis, it was designated atypical interstitial pneumonia. However, it was felt that the word *atypical* created considerable confusion, so it has been suggested that the term be abandoned[73] in preference for acute interstitial pneumonia.

Pathology of Acute Interstitial Pneumonia

Gross Pathologic Findings

At postmortem examination, lungs fail to collapse and possess a resilient or rubbery texture.[119] The dorsocaudal aspect of the lungs are most severely affected but lesions may involve the entire lung. If bronchopneumonia is also present, the cranioventral lung may be consolidated. In cases of uncomplicated AIP the pleural surface is edematous and free from fibrin deposition and there is minimal pleural effusion.

Affected lobules are independently movable and their color varies from dark red or purple, to pinkish gray;[119] they are often interspersed with over inflated yet normal-appearing lobules. This gives the lung a distinctive checkerboard appearance (Figure 2.2.).

Edema and emphysema are evident when cut surfaces of lung are inspected. Also, cut surfaces often ooze clear exudate (Figure 2.3.).[119,136] Because AIP-affected lungs are grossly enlarged and have lost compliance, costal impressions may also be observed (Figure 2.2.). Other postmortem findings sometimes noted include elevated ruminal fluid

Figure 2.2. Surface of acute interstitial pneumonia-affected lung. The pattern of affected and unaffected lobules produce a checkerboard appearance. Costal impressions are evident caudally (arrows).

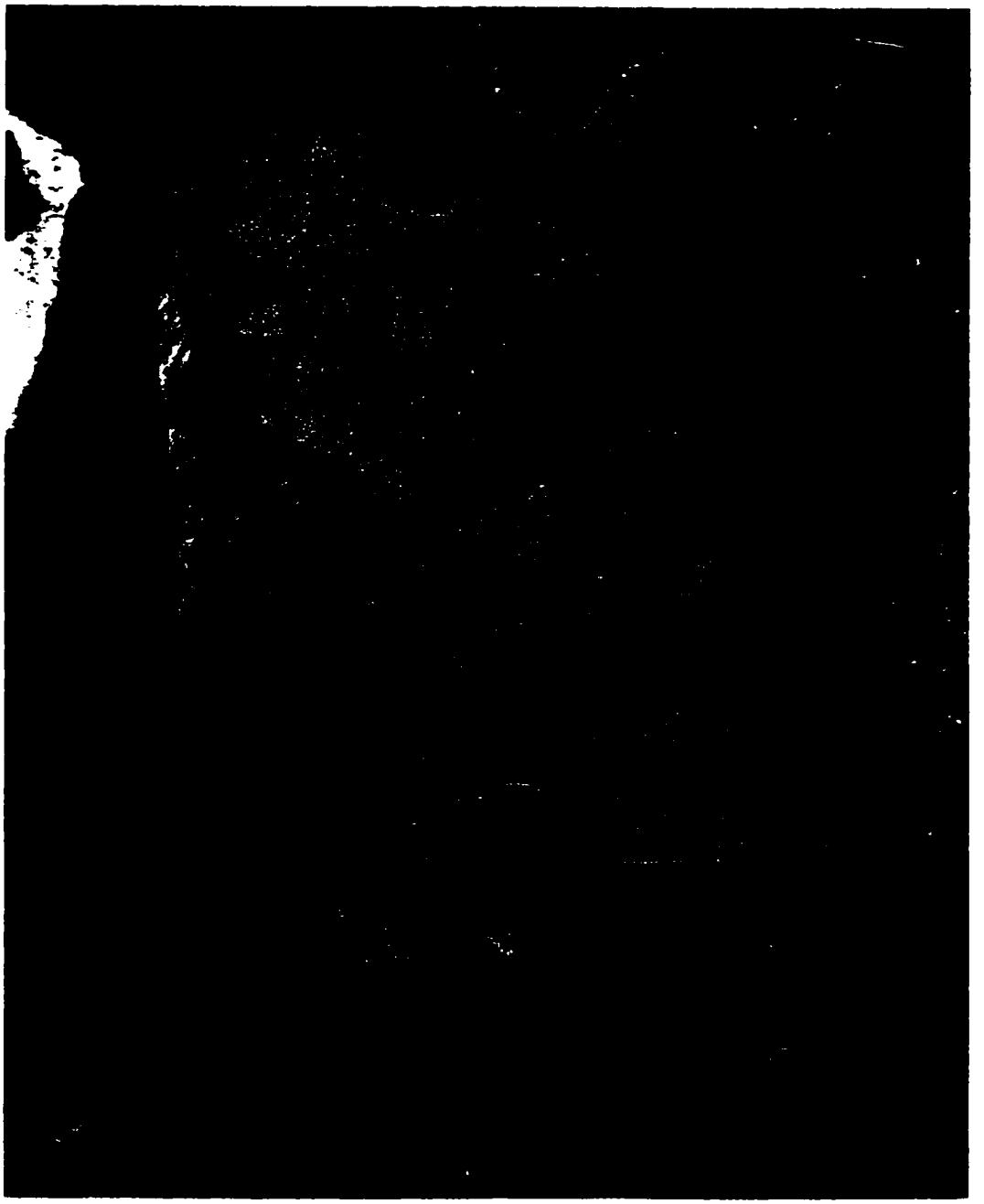


Figure 2.3. Cut surface of acute interstitial pneumonia-affected lung. Note prominent interlobular edema and increased opacity of pleural surface.

pH and occasionally *corpa hemorrhagica* are evident, which indicates recent ovulation.[123,126,137]

Histological Characteristics

A definitive diagnosis requires histological evaluation of lung tissue because AIP can be grossly confused with other types of pneumonia. Histological evaluation of AIP-affected tissues reveals fibrin accumulation and hyaline membrane formation in alveolar spaces, alveolar epithelial hyperplasia and congestion and edema.[138]

Lesions may be categorized as either:

1. Acute (exudative) phase. In the acute phase, alveolar edema and hyaline membranes (compacted fibrin) are evident (Figure 2.4.). Basophilic chromatin-like strands may be present in alveolar spaces and terminal bronchioles.[119,121] Hemorrhage into alveolar spaces and interlobular lymphatics may be observed.[119] Alveolar walls are thickened with edema fluid, and the interlobular space is edematous.[113,115]
2. Proliferative phase. Cuboidal alveolar type II cells proliferate in response to destruction of type I cells (Figure 2.4.). This can result in a glandular appearance and likely led to the early description of AIP as pulmonary adenomatosis.[135] The alveolar wall may be enlarged with edema fluid; mononuclear, and sometimes neutrophilic infiltrate may be present, and, when extensive, likely represents inflammation of several days' duration.[119,121,127]

Figure 2.4. Photomicrographs of (a) the acute phase of AIP (20X magnification) with intra-alveolar exudation, and hyaline membrane formation, and (b) the proliferative phase of AIP (20X magnification) with cuboidal type II alveolar epithelial cells present.

Figure 2.4.a

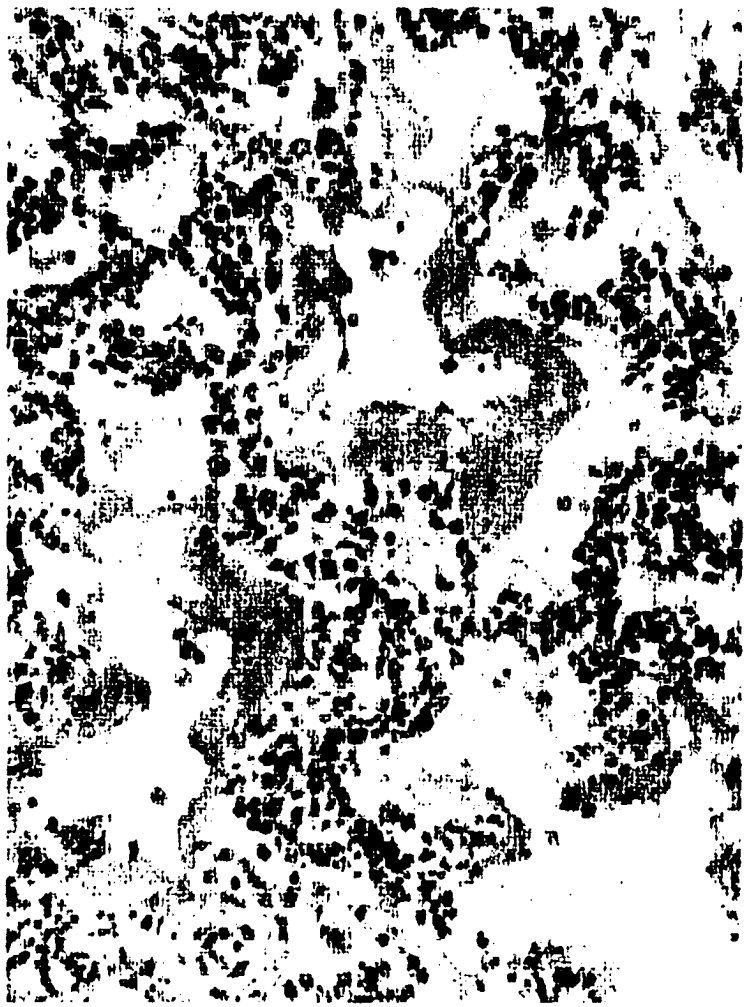
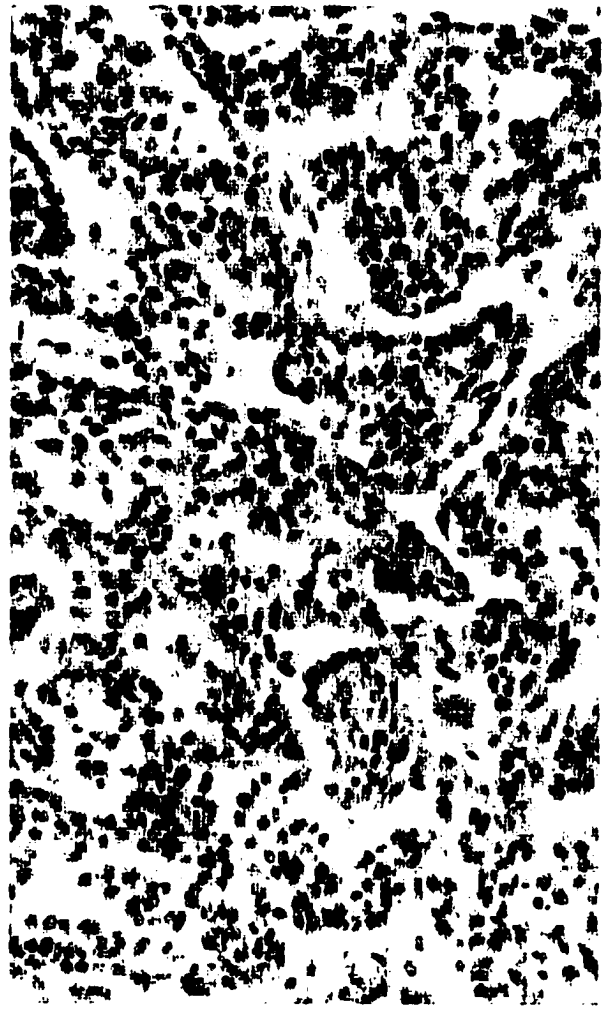


Figure 2.4.b.



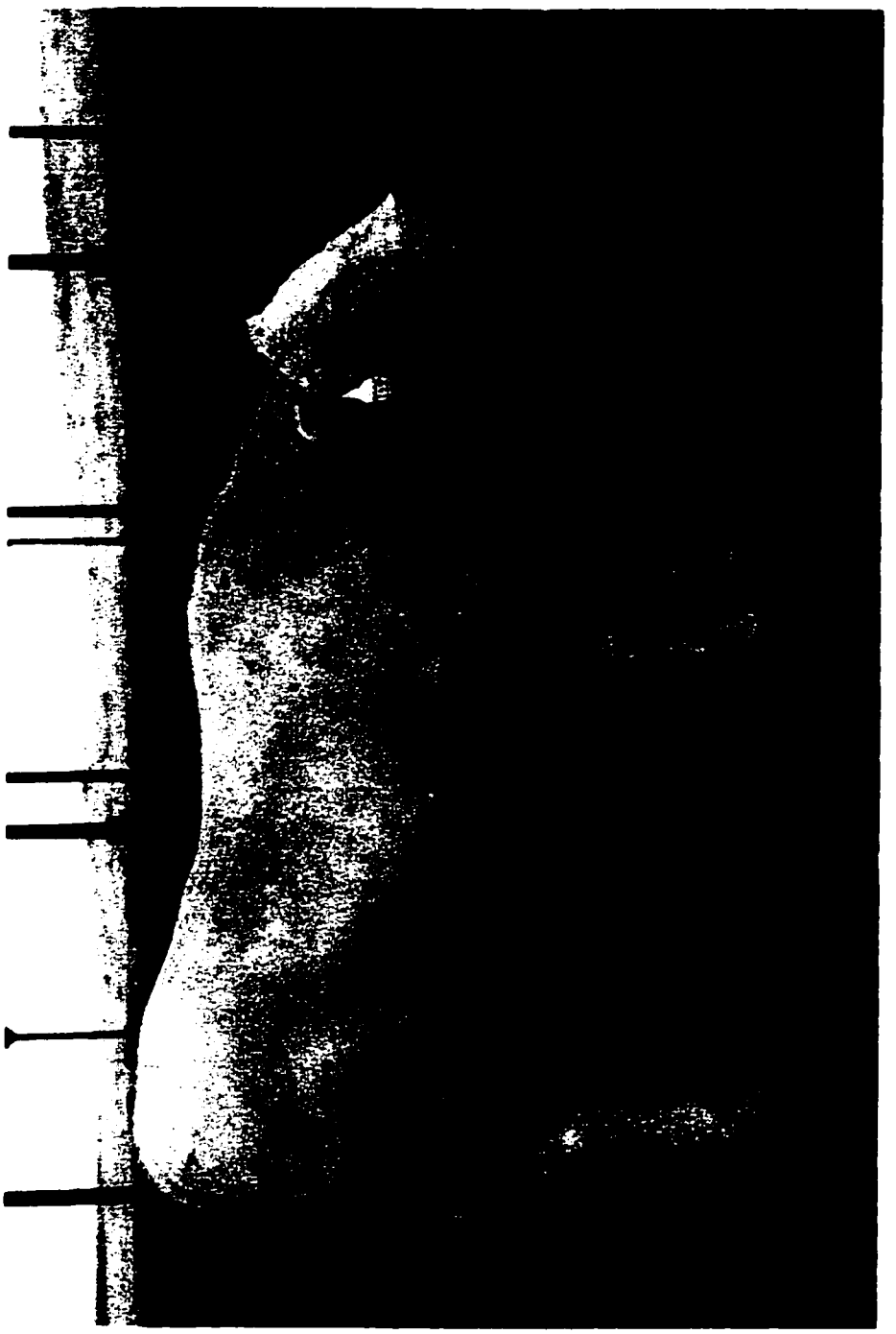
Other histological findings often include bronchiolitis obliterans,[119,121] indicating chronic bronchiolar inflammation. It is unclear if this chronic inflammation is an incidental finding or associated with the pathogenesis of AIP.

Clinical Manifestations of Acute Interstitial Pneumonia

Because of the acute nature of the disease, many AIP-affected animals are found dead in their *home pens* even though they were never observed to be sick. Animals clinically affected with acute interstitial pneumonia display acute respiratory distress.[120]

Early clinical signs include abdominal exertion during respiration that may audibly manifest as a grunt. Affected animals adopt a sway-back posture and extend their neck. Open-mouth breathing often develops as the disease progresses (Figure 2.5.). Frothy fluid may drain from the mouth or nares especially when the head is lowered. Unless complicated by bronchopneumonia, AIP-affected animals do not appear obtunded and are afebrile.[73] In advanced stages of the disease, animals can demonstrate a lethal exercise intolerance.

Figure 2.5. Animal displaying clinical manifestations of acute interstitial pneumonia. Note that the animal has adequate gastrointestinal *fill*, has adopted a sway-back appearance, and is open-mouth breathing.



Proposed Causes of Feedlot-Associated Acute Interstitial Pneumonia

Infectious agents

Bovine respiratory syncytial virus

Bovine respiratory syncytial virus has frequently been implicated as a potential etiological agent of feedlot AIP.[25,121,139,140] The similarity of clinical signs, gross and microscopic pathology of AIP and BRSV infections presumably contributed to the hypothesis that BRSV infections lead to AIP. The vast majority of BRSV infections in feedlot cattle are mild and potentially inconsequential. Occasionally, however, BRSV can result in an acute and severe dyspnea that may lead to death.[141] Histological lesions include proliferation of type II cells, and emphysema.[141-143] Bronchiolitis obliterans may be observed in both AIP-affected animals and subsequent to a BRSV infection.[119,142] However, the severe alveolar edema, and hyaline membranes characteristic of AIP are not typically observed with BRSV.[141-143] Further, the severe disease associated with BRSV infections is seen more often in young naive animals whereas AIP is clustered towards the latter stages of feeding.[120] Animals infected with BRSV can be markedly febrile, whereas AIP-affected animals are typically afebrile.

An association between feedlot AIP and BRSV was identified in one study.[121] Eleven of 15 AIP-affected lungs were positive for BRSV via immunohistochemistry whereas only 5 of 18 lungs without AIP were positive for BRSV ($P = 0.01$). No association was detected with other microbial pathogens that were identified in lung samples used in their study (BHV1, *Mannheimia haemolytica*, *Pasteurella multocida*, mycoplasmas, and

Arcanobacterium pyogenes).[121] Recent reports have not found an association of AIP with BRSV. Bovine respiratory syncytial virus was not detected in any of 31 AIP-affected animals in one study using immunohistochemistry.[120] In another study, only 2 of 28 (7%) AIP-affected lungs were positive for BRSV via immunohistochemistry.[144] These authors concluded that AIP likely developed independently of BRSV.

Concurrent bronchopneumonia

Hjerpe documented findings from necropsies performed on feedlot cattle.[125] Of 149 animals with postmortem lesions of AIP, 144 (97%) also had cranioventral consolidation (evidence of concurrent bronchopneumonia). Bronchopneumonia was evident in a significantly greater number of AIP cases than in animals that died of non-respiratory tract-related disorders. It is unclear if the observed bronchopneumonia developed before or concurrently with AIP. Cattle with AIP were more likely to have been treated previously for BRD than cattle that died from other disorders.[125] Hence, it may be possible that prior bronchopneumonia may contribute to the pathogenesis of AIP.

Sorden and coworkers reported that 32 and 75% of 28 AIP-affected lungs had gross and histological evidence of bronchopneumonia, respectively.[144] Chronic bronchitis or bronchiolitis was present in 43% of AIP-affected lungs and bronchiolitis obliterans was present in 89% of cases.

A contribution of bronchopneumonia in the pathogenesis of feedlot-associated AIP seems

plausible because the human form of AIP, ARDS, may develop as a consequence of bacterial pneumonia.[145,146] Proinflammatory cytokines, in particular tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β), are closely associated with the development of ARDS. People at risk for ARDS who subsequently developed the disease had higher concentrations of TNF-α and IL-1β in bronchoalveolar lavage (BAL) fluid than those people who did not develop ARDS.[133] Further, greater concentrations of TNF-α and IL-1β in plasma and BAL fluid at the outset of ARDS was associated with increased case fatality rates.[147]

Tumor necrosis factor alpha and IL-1β are liberated in response to many stimuli, including phagocytosis of bacteria,[148] exposure to lipopolysaccharide[148] or leukotoxin,[149] and viral infection.[150] Both cytokines induce a variety of effects that may lead to damage of alveolar capillaries and epithelial cells, resulting in alveolar hemorrhage, edema, and hyaline membrane formation.[151-153]

It is possible that proinflammatory cytokines may contribute to the pathogenesis of feedlot-associated AIP. Calves experimentally infected with *M. haemolytica* resulted in increased concentrations of TNF-α and IL-1β lavage fluid.[154] Increases in serum TNF-α has also been reported in response to *M. haemolytica* infection.[155,156] Administration of TNF-α leads to lesions indistinguishable from AIP.[157]

A further mechanism by which Gram negative bacterial infections may contribute to the

pathogenesis of AIP is via liberation of endotoxin. Administration of endotoxin to calves resulted in increased serum concentrations of TNF-α.[158] Calves administered endotoxin intravenously developed varying degrees of respiratory distress.[158-160]

It is possible, or even probable, that bacterial infections contribute to the pathogenesis of AIP because bacterial infections of the respiratory tract and liver are common in feedlot cattle.[17,18,161,162] In a recent investigation, animals with experimentally-induced BRD had greater TNF-α, IL-1β, and interleukin 8 (IL-8) in lung parenchyma and BAL fluid than controls.[105]

Non-Infectious Causes

3-Methylindole

There has recently been interest in the role that 3MI may play in feedlot-associated AIP because increased ruminal production,[163] or experimental administration of 3MI can lead to AIP.[98,128] If pulmonary metabolism of 3MI is prevented by specific enzyme inhibitors, however, acute lung injury does not develop.[164,165 166] Although several metabolites of 3MI have been identified,[167,168] 3-methyleneindolenine (3MEIN) is thought to be the major metabolite produced in lung tissue and responsible for cellular injury that gives rise to AIP.[169,170] Free radicals may also contribute to 3MI-induced pulmonary disease.[171,172]

Only one study has evaluated the association of feedlot AIP and 3MEIN.[120] Investigators discovered that animals clinically affected with AIP had greater plasma concentrations of

3MEIN compared to controls. However, they did not detect a difference in lung 3MEIN between AIP-affected animals and animals that had died from other disorders.

Hypersensitivity

Acute interstitial pneumonia has frequently been thought of as a hypersensitivity disease.[23,119,173] This hypothesis likely developed because histological similarities exist between AIP and pulmonary type III hypersensitivity lesions in humans.[173]

Although it has never been documented, hypersensitivity reactions to *Micropolysporum faeni* spores have been proposed to play a role in feedlot AIP.[119] Inhalation of spores of *M. faeni* or other antigens can, however, result in extrinsic allergic alveolitis (bovine farmer's lung). This disease includes a combination of type III and type IV hypersensitivity, as well as other immune-mediated mechanisms.[174-176] Acute cases are characterized by moderate to severe dyspnea, tachypnea, and coughing that may be confused with AIP.[176]

However, there are significant differences in the two conditions:

1. Lungs of animals with extrinsic allergic alveolitis often appear almost normal with only mild anomalies.[174,176] Lungs are firm in cases of extended duration.
2. Alveolar edema and hemorrhage may be present which shares similarities with AIP.[175,176] However, granulomas are uniformly seen and are not seen in AIP cases. Hyaline membranes are rarely if ever observed in cases of extrinsic allergic

alveolitis.

Type I hypersensitivity due to milk allergy in dairy cattle can cause acute severe dyspnea.[177] Lesions include hyaline membrane formation, alveolar hemorrhage, pronounced pulmonary edema and congestion.[177] These are all characteristic of AIP. However, type I hypersensitivity is rapidly fatal or the disease resolves within hours.[174] Feedlot-associated AIP is often fatal within a day yet a substantial proportion of AIP-affected animals have proliferation of alveolar type II epithelial cells.[119,120] Therefore, a substantial proportion of animals survive for a period of days before they ultimately succumb to AIP.

The literature is devoid of studies that evaluated an association of AIP with hypersensitivity. Acute interstitial pneumonia is unlikely related to extrinsic allergic alveolitis. Although AIP is possibly related to type I hypersensitivity, it is unlikely that the majority of feedlot AIP result from anaphylactic events.

Alterations in rumen function

Because AIP events are acute and occur in animals adapted to high-concentrate diets, an association of AIP with elevated ruminal fluid pH was unexpected.[137] In another study, AIP was associated with decreased cellulolytic bacterial numbers which was possibly the result of a period of acidosis.[120] Although these findings seem paradoxical, it is possible that the events are related. A low-grade episode of ruminal acidosis could result in death

of cellulolytic bacteria and ruminal stasis. The remaining carbohydrates would be consumed by microbial fermentation. After microbial exhaustion of readily fermentable carbohydrates, the pH would rise possibly through systemic absorption of acids (such as lactate) and the buffering effect of saliva.[178]

Ammonia was measured in the ruminal gas cap of AIP-affected and other animals at postmortem.[124] All (n = 9) AIP-affected animals had detectable ammonia in the ruminal gas cap, while none of the controls (n = 4) had detectable ammonia. In a follow up study, animals were clinically categorized to be suffering from BRD or AIP; ruminal gas cap ammonia concentrations were measured antemortem.[124] Detectable ammonia was evident in 2 of 3 animals suffering AIP and only 1 of 15 animals with bronchopneumonia. Even though these studies were performed on relatively few animals, it provided additional evidence that AIP was associated with an alterations in ruminal metabolism.

Epidemiology

There are marked inconsistencies in the literature with regard to the epidemiology of feedlot-associated AIP. Hjerpe,[125] and Jensen and others,[119] reported that AIP occurred at equal frequencies during all stages of the finishing process. Jensen and coworkers found that 30% (n = 27) occurred during the first 45 days on feed, 17% (n = 15) between 46 and 90 days on feed, 30% (n = 27) between 91 and 140 days on feed, and 22% (n = 19) occurred in animals with more than 141 days on feed.[119]

Unfortunately, the numbers of cattle at risk during these time periods were not reported by Jensen and coworkers. Thirty seven percent occurred with 90 days or fewer on feed and 63% occurred with more than 90 days on feed. In general, the number of animals at risk of AIP would have decreased as days since arrival increased because animals would have been removed from the population at risk by harvest or illness-related death. It is possible, therefore, the risk of AIP-related death was greater for animals with more than 90 days on feed in Jensen and coworker's study. Thus, the disease may not have occurred at equal frequencies as documented.

Ayroud et al. proposed that AIP events are clustered towards the end of the feeding period.[120] Acute interstitial pneumonia cases had a mean days on feed of 114, which was on average 24 days prior to harvest.

Hjerpe suggested that AIP events occurred independent of time of year.[125] However, Ayroud et al., and Jensen et al. found that animals were more likely to be diagnosed with AIP during the summer and fall months.[119,120] In contrast, Sorden and other reported that 86% (n = 24) of their 28 AIP cases occurred between November 1 and April 1.[144] Unpublished data indicated that the majority of events occurred during the hottest months of the year.[123,126]

Even though steers constituted 11% of the at risk population in one study, all AIP-positive lungs came from heifers.[120] This may have indicated an association with sex. In

another study, however, 15 of 28 were steers whereas only 3 were heifers (sex was not specified in 10 of 28 cases).[144] The steer to heifer ratio in the at-risk population was not provided in their publication.

Estimates of morbidity and mortality are difficult to obtain. Acute interstitial pneumonia has been estimated to be responsible for 5 to 15% of all mortalities.[119,123,125,126]

Section III. 3-METHYLINDOLE METABOLISM

Overview

3-methylindole is a lipophilic compound produced in the gastrointestinal tract of mammals.[172,179,180] Ruminants are very sensitive to sudden increases in 3MI generation. Adverse effects of 3MI exposure are also observed in horses, rats, rabbits and mice.[181-183]

The role of 3MI in bovine respiratory disease was discovered fortuitously. During experiments designed to evaluate tryptophan (TRP) metabolism, 5 of 8 animals died from acute pulmonary injury.[184] The tryptophan-induced lung pathology was similar to cases of naturally-occurring, pasture-associated AIP (ABPE, fog fever). The unexpected finding of this study ultimately led to the discovery of excessive ruminal generation of 3MI as the cause of pasture-associated AIP.[128,163,185]

3-Methylindole Metabolism

Ruminal Generation of 3-Methylindole

Cattle challenged with D,L-TRP (0.5-0.6 g/kg body weight) developed clinical manifestations and pulmonary lesions consistent with pasture-associated AIP.[186] Cattle were subsequently challenged with oral, intravenous, or intraperitoneal TRP, but only oral

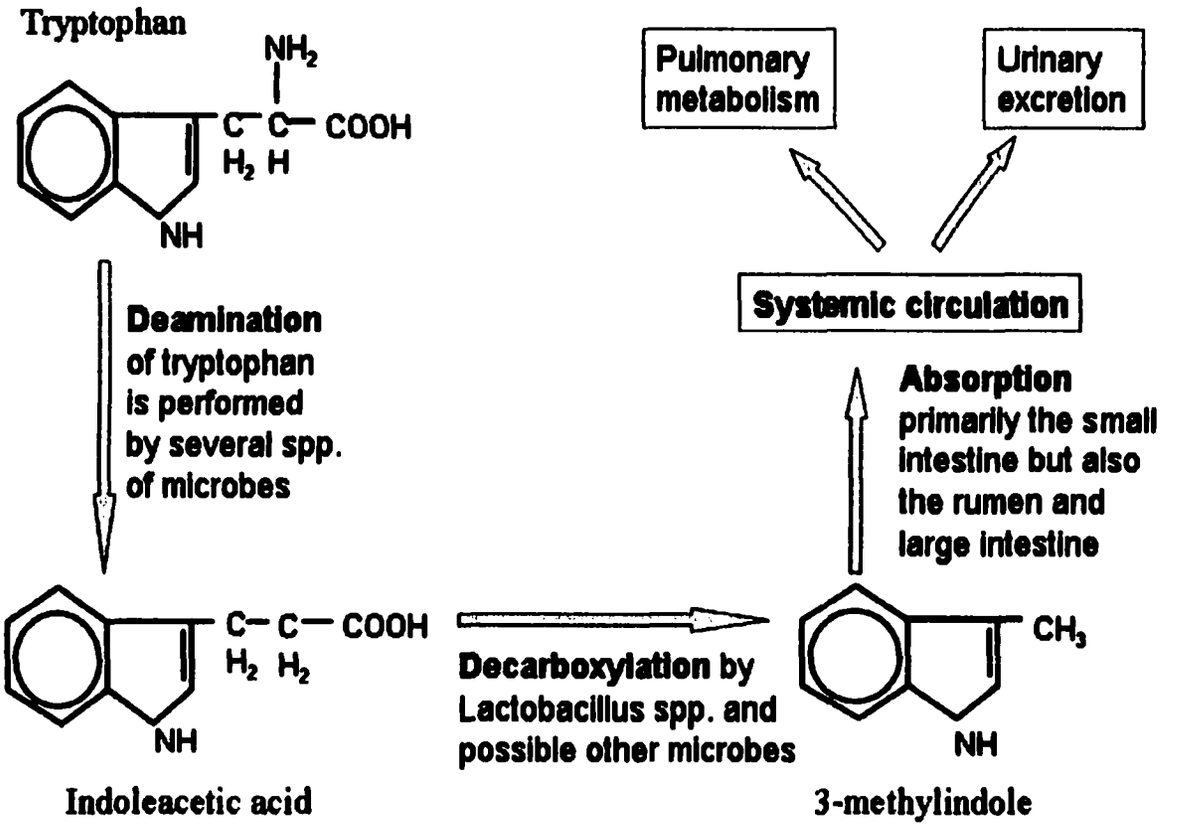
administration resulted in AIP.[187] Therefore, ruminal metabolism of TRP was determined to be critical in the pathogenesis of this acute pulmonary injury. Further research was performed to compare oral administration of D- and L-TRP; only L-TRP resulted in pulmonary injury.[188]

Aerobic incubation of L-TRP in ruminal fluid revealed 3MI as a metabolite of TRP.[188] Ruminal conversion of TRP to 3MI is a 2 step process initially involving deammination of TRP to form indoleacetic acid (IAA). This first step can be performed by a variety of ruminal microbial species.[189,190] *Lactobacillus* spp. then decarboxylate IAA to 3MI (Figure 2.6.).[189,190] It seems possible that there are other ruminal microbes capable of catalyzing IAA to 3MI.[186]

Carlson and coworkers then administered 3MI orally and intravenously to cattle and goats. Both routes resulted in clinical manifestations and pulmonary injury identical to the disease seen in pastured cattle.[188] Therefore, ruminal metabolism of TRP to 3MI was hypothesized to result in naturally-occurring ABPE.[163 191]

In studies performed by Yokoyama et al., 3-methylindole was detected in the ruminal fluid within 6 hours of TRP administration (0.35 g/kg body weight).[163] Maximum plasma 3MI concentrations occurred 12 to 24 hours following administrations and were 3.0 to 9.0 μg/ml. Clinical manifestations of AIP developed in 3 of 5 animals. Their findings indicated that cumulative (peak concentration and duration) exposure to 3MI may be

Figure 2.6. Schematic representation of ruminal fermentation of tryptophan to form 3-methylindole.



predictive of the severity of pulmonary injury.[163] Although the magnitude of and time to maximum plasma 3MI concentrations vary slightly depending on route and dose of challenge, other studies have reproduced similar patterns in plasma 3MI concentrations to those of Yokoyama and coworkers.[128,185] Authors have concluded that plasma and ruminal 3MI concentrations are correlated.[192,193 194]

Rumen and plasma 3MI concentrations were measured in animals exposed to natural conditions that were believed conducive for the development of pasture-associated AIP.[194,195] Animals that developed respiratory distress were exposed to ruminal 3MI concentrations of greater than 2 μg/ml for an average of 42 hours whereas those animals that remained clinically normal were exposed to these concentrations for only 14 hours on average.[189] This provided additional evidence indicating that cumulative exposure to 3MI is important in the pathogenesis of AIP.

Fate of Ruminal 3-Methylindole

3-methylindole is a lipophilic compound that is rapidly absorbed across the intestinal wall and into the systemic circulation.[163,196] It is then widely disseminated throughout the body.

Absorbed 3MI may be metabolized and excreted in the urine as processed 3MI-glutathione adducts (mercapturates).[197,198] Metabolism of 3MI has been demonstrated in lung, liver, testicle and kidney.[179,199-202] Ruminants, particularly cattle, are very sensitive

to 3MI challenges possibly because they possess and abundant capacity to bioactivate 3MI in lung tissue.[172,179]

Pulmonary Metabolism of 3-methylindole

3-methylindole displays species, organ, and cellular variation in toxicity.[172,179,180,203,204] Goats, cattle and sheep are the species most susceptible to increased production of 3MI.[179] The lungs are the primary organ affected in ruminants and most cellular injury has been observed in alveolar type I epithelial and Clara cells.[205,206] This is because 3MI is metabolized by enzymes associated with smooth endoplasmic reticulum (SER) which is in relatively high concentrations in these cells.[205,207] Metabolism of 3MI has also been detected in alveolar macrophages.[208] The enzymes responsible for bioactivation of 3MI to reactive intermediates include specific families of cytochrome P450 enzymes and prostaglandin H synthetase (PHS).[201,202,209,210]

Evidence for 3MI bioactivation by P450 enzymes is extensive.[166,168-170,209-215] Investigators pretreated goats with either a cytochrome P450 enzyme inhibitor, piperonyl butoxide, or an inducer, phenobarbital;[209] a third untreated group of animals served as controls. All animals were challenged with 3MI. Those animals that received piperonyl butoxide were spared from 3MI-induced disease, whereas those that received phenobarbital developed a more severe disease than controls. Bioactivation of 3MI by Clara cells was reduced 94% by the cytochrome P450 inhibitor, 1-aminobenzotriazole *in vitro*.[208] However, bioactivation in alveolar macrophages was only decreased by 24%. In another study,

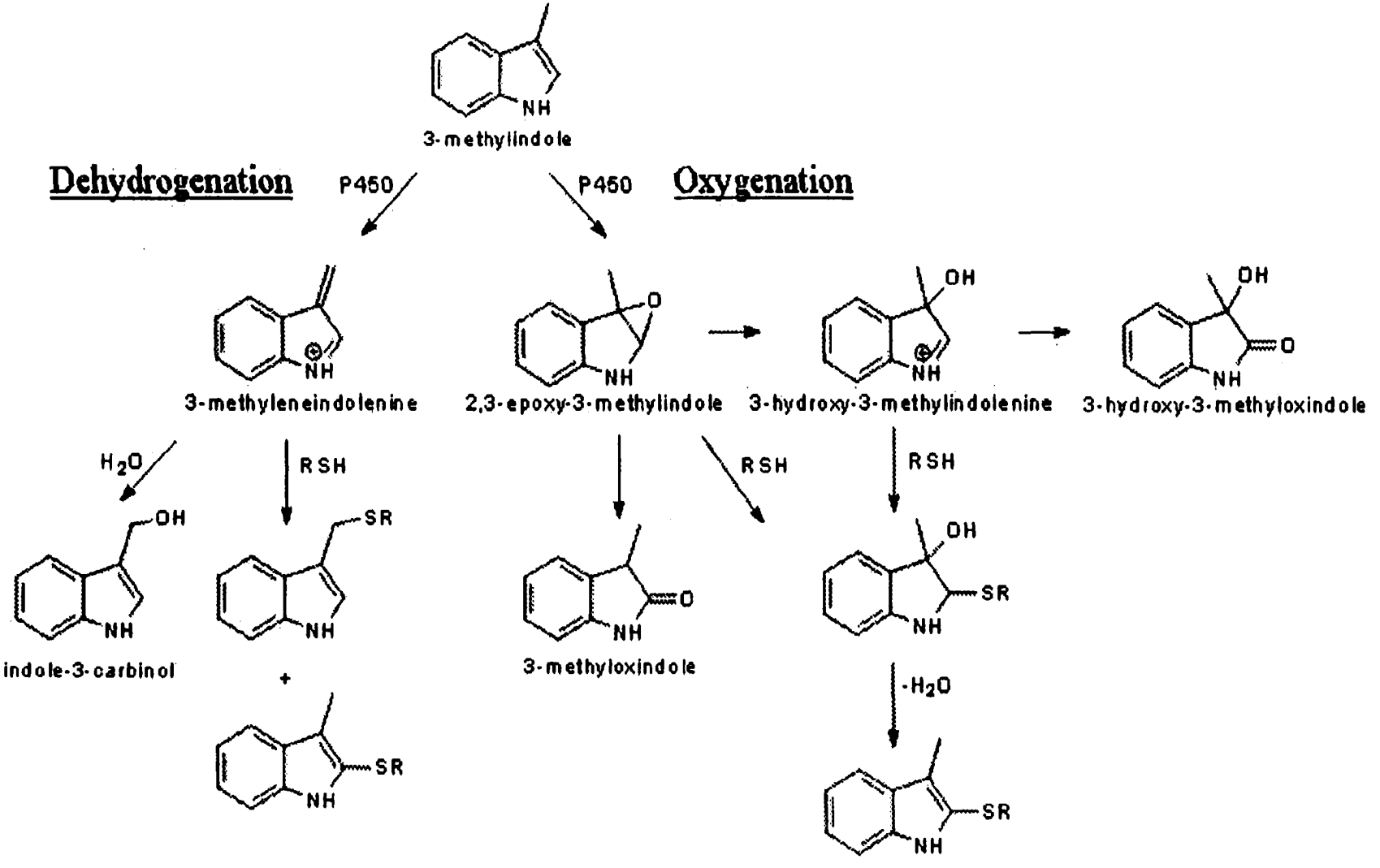
cytochrome P450 bioactivation of 3-methylindole and resultant covalent binding, an indicator of 3MI toxicity,[169,216] were decreased 100% by the addition of 0.1 mM 1-aminobenzotriazole *in vitro*.[217]

Incubation of 3MI and specific families of cytochrome P450 enzymes[212] result in the generation of various electrophilic metabolites of 3MI (Figure 2.7.). These include a reactive methylene imine, 3-methyleneindolenine (3MEIN),[169,214] 2,3-epoxy-3-methylindole and 3-methyloxindole,[168] and 3- hydroxy-3-methylindolenine.[167,168,218] These electrophiles are produced by dehydrogenation, epoxation, and oxidation. 3-methyleineindolenine is produced in the greatest quantities in lung microsomes, forms the majority of the cellular adducts that have been detected, and adduct formation is proportional to cellular injury. For these reasons, it has been proposed that 3MEIN is primarily responsible for 3MI-induced pulmonary injury in ruminants.[214,215]

Literature relating to other pathways through which 3MI may be bioactivated is relatively sparse. Bray and Emmerson hypothesized that the PHS metabolic pathway acts synergistically with cytochrome P450 enzymes to induce pulmonary injury.[172] Investigators demonstrated increased oxygen usage by PHS when incubated with 3MI *in vitro*.[202] These researchers used the microsomal fraction derived from ram seminal vesicles because they contain abundant PHS but are devoid of cytochrome P450 activity. An electrophilic, free radical metabolite of 3MI was detected using a horseradish peroxidase model of PHS.[202] Incubation of goat microsomes *in vitro* with 3MI resulted in

Figure 2.7. Schematic depiction of the proposed metabolism of 3M1 by cytochrome P450 enzymes.[a]

[a] Figure adapted from: Skordos et al. *Chem. Res. Toxicol.* 1998;11:741-749



increased metabolic activity of PHS.[201] The authors speculated that 3MI toxicity results from co-oxidation of 3MI by cytochrome P450 enzymes and PHS.[201,202]

Pretreatment of goats with non-steroidal anti-inflammatory drugs (NSAID) such as aspirin and indomethacin resulted in reduced severity in the clinical manifestations and pathology of 3MI-induced disease compared to controls.[164] This disease sparing effect of NSAID was only evident if the test article was administered prior to the 3MI challenge. Therefore, the effect of NSAID is likely explained through inhibition of PHS activity and not solely post insult inhibition of prostanoid formation.[164,219]

Mechanism of 3MI-induced Pulmonary Damage

The mode of action of 3MI is complex and not fully understood. It is clear, however, that 3MI requires bioactivation in Clara and type I alveolar epithelial cells to induce cellular derangements.[164,208,217] Evidence suggests that covalent binding of electrophilic 3MI metabolites to cellular macromolecules occurs[220-223] and is proportional to cellular dysfunction and injury.[169,179,216] Adducts that have been identified and extensively studied are specific for cytochrome P450 enzymes.[169,170,197,210,212,215,224,225]

It has been proposed that the primary toxic metabolite of 3MI is 3MEIN. This electrophilic compound covalently binds to cellular macromolecules resulting in profound oxidative damage.[169,197,216] Antibodies to 3MEIN adducts have been developed[204] and used to estimate concentrations of 3MEIN in tissues obtained from animals with

naturally-occurring respiratory disease.[120]

Spin-trapping techniques have also demonstrated the production of N- and C-centered free radicals when goat lung microsomes were incubated with 3MI *in vitro*.[171,226] These radical species were also evident *in vivo*.[171] It is unclear if these are products of cytochrome P450 enzymes or PHS, or if they are intermediates in the formation of 3MEIN or other electrophiles. Bray and Emmerson speculated that the N-centered free radical undergoes a structural rearrangement to form a reactive electrophilic metabolite.[172] However, this has not been confirmed.

The preponderance of data indicate that cytochrome P450 bioactivation is required for 3MI-induced pulmonary disease.[208,209,217] Evidence also exists that implicates PHS metabolism of 3MI as a pathways for cellular injury.[164,201,202] It is possible that these two enzyme systems act synergistically,[172] but this proposition has been questioned.[227]

Section IV. POTENTIAL ROLE OF 3-METHYLINDOLE AND 3-METHYLENEINDOLENINE IN RESPIRATORY DISEASE

Bovine Respiratory Disease Complex

3-methylindole is produced normally in the rumen of all animals.[190] Small fluctuations in ruminal generation of 3MI may result in increased quantities presented to cytochrome P450 enzymes and PHS in lung tissue. Metabolism in type I alveolar epithelial cells, Clara cells and alveolar macrophages may result in damage to structural barriers that prevent microbial invasion and impaired phagocytosis of bacterial pathogens.[172,208] Therefore, at concentrations below those required to induce AIP, small increases in circulating 3MI concentrations may increase the likelihood for BRD. Further, viral infections that would normally be inconsequential, may lead to BRD when they are associated with small increases in 3MI generation.

Castleman and coworkers evaluated an interaction between 3MI and BRSV.[28] Calves were challenged with 3MI (0.25 g/kg body weight) 3 days prior to intratracheal inoculation with BRSV. The authors failed to demonstrate synergy between 3MI and BRSV. However, the BRSV strain used underwent minimal replication within alveolar tissue. This indicated that the strain of BRSV was probably not particularly virulent.[28]

A 2x2 factorial design was used in a subsequent study.[98] Treatment factors were 3MI challenge (0.1 g/kg of body weight) and BRSV inoculation (aerosolization and intratracheal). Twenty calves were randomly assigned to 1 of 4 treatments.

The most acute and severe disease was evident in animals exposed to a combined challenge of BRSV and 3MI.[98] Lungs from these animals also had the greatest displacement volume and histopathology. Thus, Bingham and coworkers demonstrated that 3MI increased the severity of BRSV-associated disease.

Eight of 10 animals that were challenged with 3MI developed clinical manifestations of AIP so severe they either died or were euthanized prior to the completion of study (day 7). Further research is required to evaluate potential synergy with lower 3MI challenges that would more closely mimic naturally-occurring challenges.

Blood samples were collected from 256 animals on day 0 (the day they arrived at the feedlot) and day 3 in a BRSV vaccine efficacy field trial.[39] Cattle were housed in a feedlot-type environment for the duration of the study. Serum 3MI concentrations ranged from undetectable (0.0) to 9.0 μg/ml. Maximum serum 3MI concentration from day 0 or 3 was associated with a decreased overall rate of gain. An association of maximum serum 3MI concentrations and BRD or lung lesions identifiable at harvest was not detected. Immunity against BRSV did not modify the effect of 3MI on BRD

development or mean daily weight gain (MDG). Hence, a synergy between 3MI and BRSV was not observed.

A second field study was conducted by Bingham and coworkers and included 244 beef cattle.[11] Half of the animals were treated with 31.2 g of aspirin on arrival. 3-methylindole concentrations were determined in serum samples collected on days 0, 3, and 6, and in ruminal fluid collected on day 3. Serum and ruminal fluid 3MI concentrations ranged from 0.3 to 3.5, and 1.2 to 12.5 μg/ml, respectively. The investigators did not detect an effect of 3MI on MDG or risk of BRD treatment.[11] However, increased 3MI concentrations were associated with increased likelihood of pulmonary lesions detectable at harvest.

It is unclear why an association of 3MI with MDG or BRD treatment was not detected in both studies.[11,39] This may have occurred because:

1. The time period from sample collection to serum harvest may have resulted in loss of volatile 3MI. Presumably the rate of 3MI loss would have been greater in samples with the greatest 3MI concentrations. Plasma harvested soon after collection may have prevented this potential pitfall.
2. 3-methylindole needs to be metabolized in order to induce respiratory disease. The metabolic capacity of lung tissue may be more important than the actual 3MI concentrations. In this case, measurement of 3MEIN-adduct concentration may have provided a better estimate of cellular injury in the lung.

3. The cattle in these experiments had rates of gain approximately half those observed in more typical feedlot cattle.[15]
4. Differences in serum 3MI concentrations between study populations may explain disparities between study outcomes.
5. Serum collected on either day 0 or 3 may not provide 3MI concentrations that are representative of the entire feeding period. Therefore, associations of serum 3MI concentration (collected on day 0 or 3) with other parameters may not have been detected.

These factors may have resulted in estimates that were biased towards the null.

Their results do suggest, however, that increases in 3MI generation are associated with detrimental effects on lung health and MDG.[11,39] Studies have demonstrated that animals with lung lesions at harvest gained weight more slowly than animals without lung lesions.[17,18] Therefore, if increases in 3MI generation occur in feedlot cattle, they could increase the severity of viral infections, contribute to BRD development, increase antimicrobial usage, decrease rates of gain, and produce adverse effects on carcass quality.[18]

A large proportion of cattle enter feedlots after passage through auction markets.[4,10,91,228] These animals undergo a period of feed restriction and after arrival, are provided with high quality diets containing abundant quantities of protein and readily fermentable

starch. *Lactobacillus* spp. propagate under these conditions.[189] This would likely result in an increase in ruminal 3MI generation if TRP is available.[163,190,229] However, most feedlot cattle are fed an ionophore,[4,91] which has been shown to inhibit 3MI generation *in vitro* and *in vivo*.[192,230,231] No research has investigated the patterns of 3MI or 3MEIN production in cattle fed typical feedlot diets.

Because NSAID provided protection against 3MI-induced respiratory disease,[164,172,219] and because free radicals are produced during pulmonary metabolism of 3MI,[171,172,202,226,232] the use of efficacious quantities of aspirin and vitamin E in feedlot cattle may be beneficial if feedlot cattle are exposed to increases in 3MI or 3MEIN production.

Acute Interstitial Pneumonia

3-methylindole exposure has occasionally been proposed as an etiology of feedlot AIP because of its association with pasture-associated AIP.[100,132,144,233] There have been no studies that have evaluated an association of 3MI with AIP in U.S. feedlots.

There is, however, evidence generated by Canadian researchers that 3MI contributes to the pathogenesis of feedlot AIP.[120] These researchers identified 38 cattle displaying clinical manifestations of AIP. All animals were taken to nearby abattoirs for emergency slaughter. Blood, lung, ruminal fluid, and urine were harvested. Acute interstitial pneumonia was confirmed in 31 of these animals. Blood samples were also collected from 17 clinically healthy pen-mates. Therefore, 24 animals served as controls when

comparing blood indices, and 7 served as controls for all other comparisons. 3-methyleneindolenine absorbence per μg protein (a proxy for concentration) was determined for plasma, and homogenate and microsomal fractions of lung tissue.[204] Concentrations of 3MI mercapturate were determined in urine specimens by HPLC techniques.[234]

Plasma concentrations of 3MEIN were significantly higher in AIP cases than in animals with other respiratory tract disorders (Table 2.2.). It seems likely that a significant portion of blood 3MEIN-adducts were derived from bioactivation of 3MI by lung cytochrome P450 enzymes because the lung enzymes are the most efficient at catalyzing this process.[203,212,224] However, a difference in lung 3MEIN concentration was not detected between controls and cases. Urinary 3MI mercapturates were significantly lower in AIP-affected animals compared to controls.

In their study, only 7 animals served as controls and these animals were clinically diagnosed as suffering from AIP. Histological evaluation of lung tissue did not confirm this diagnosis, yet a description of findings from these animals was not included in the report. It is possible the controls used to compare lung 3MEIN were not appropriate. The authors concluded that 3MI contributes to the pathogenesis of feedlot-associated AIP because they identified an association with 3MEIN in plasma.[120]

Table 2.2. 3-methylindole mercapturate concentration (nmol/mL) in urine and 3-methyleneindolenine absorbance (per μg protein) in plasma and lung tissue from acute interstitial pneumonia- (AIP) affected animals and controls.[a]

I	AIP-affected	Non-AIP			
Biological sample	(n = 31)	AIP negative (n = 7)	Healthy pen-mates (n = 17)	SEM	P value
Urine	36.5	133.3	-	21.7	<0.01
Plasma	0.79	0.60	-	0.06	0.02
	0.79	-	0.41	0.03	0.01
Lung - homogenate	1.08	1.18	-	0.08	0.44
Lung - microsomal protein	0.76	0.79	-	0.08	0.79

[a] adapted from: Ayroud et al. *Can Vet J.* 2000;41:547-554.

They also noted that all AIP cases were heifers and fed melengesterol acetate (MGA), a progestin used to suppressant estrus. Popp and others evaluated the effect of MGA on 3MI-induced respiratory disease.[234] Ten sheep were fed high-concentrate diets. Half were pretreated with MGA (0.15 mg daily). All sheep were challenged with 3MI (0.2 g/kg body weight) and serial blood samples were collected.

Those animals that received MGA developed a more acute and severe disease than those animals that did not receive MGA.[234] Melengesterol acetate-treated animals had higher plasma 3MEIN than untreated sheep. The authors proposed that MGA treatment may have induced PHS or cytochrome P450 enzymes in pulmonary tissue. It is possible that MGA induced these enzyme systems. However, their study did not provide evidence that PHS was induced because 3MEIN appears to be a cytochrome P450-specific metabolite.[203,212,224]

Even though there are differences between Canadian and U.S. feedlots, it is possible that feedlot-associated AIP in U.S. cattle is associated with increased 3MEIN production. Further, heifers constitute a substantial proportion of feedlot inventory and most heifers are fed MGA.[4,91] Therefore, an ideal opportunity exists to evaluate an association of 3MEIN and AIP in U.S. feedlots.

Section V. RESEARCH HYPOTHESES AND OBJECTIVES

Global Research Hypothesis

Increased ruminal generation of 3-methylindole and pulmonary conversion to its toxic metabolite, 3-methyleneindolenine, is associated with bovine respiratory disease complex and acute interstitial pneumonia in feedlot cattle.

Bovine Respiratory Disease Complex

Specific Research Hypotheses

1. Plasma 3-methylindole concentrations increase early in the feeding period,
2. Blood 3-methyleneindolenine-adduct concentrations increase early in the feeding period,
3. Aspirin, but not vitamin E, will decrease metabolism of 3-methylindole to 3-methyleneindolenine, and
4. Feeding aspirin and vitamin E will improve performance and decrease the occurrence of respiratory disease in feedlot cattle.

Research Objectives

1. Monitor patterns of plasma 3-methylindole concentrations in cattle over a feeding period,
2. Monitor patterns of blood 3-methyleneindolenine-adduct concentrations in cattle over a feeding period,
3. Measure blood and lung concentrations of 3-methyleneindolenine-adduct and plasma 3-methylindole in cattle fed aspirin and vitamin E.
4. Measure the effect of feeding aspirin and vitamin E on rates of gain, feed conversion, carcass characteristics, and prevalence of lung lesions in feedlot cattle.

Acute Interstitial Pneumonia

Specific Research Hypotheses

1. Feedlot-associated acute interstitial pneumonia is associated with increased lung 3-methyleneindolenine-adduct concentrations,
2. Feedlot-associated acute interstitial pneumonia is associated with increased blood 3-methyleneindolenine-adduct concentrations, and
3. Feedlot-associated acute interstitial pneumonia is not associated with bovine respiratory syncytial virus or other common infectious respiratory pathogens.

Research Objectives

1. Compare lung 3-methyleneindolenine-adduct concentrations in feedlot cattle that died from acute interstitial pneumonia with animals that died from other causes,
2. Compare blood 3-methyleneindolenine-adduct concentrations in feedlot cattle suffering from acute interstitial pneumonia with clinically healthy animals, and
3. Evaluate possible associations of feedlot-associated acute interstitial pneumonia with viral and bacterial respiratory pathogens, and *Mycoplasma* spp. isolated from lung tissue.

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CHAPTER 3

Trends in cattle mortalities in U.S. feedlots.

Summary

The objectives of this study were to evaluate 1) trends in feedlot cattle mortality over time, 2) monthly proportional cattle mortality by primary body system affected, and 3) risk of mortality by type of animal placed in feedlots. A retrospective cohort study was used. The study population included approximately 21.8 million cattle placed in 121 U.S. feedlots from 1994 through 1999. Yearly and monthly mortality ratios were calculated. Frequency counts of mortalities were modeled using Poisson regression with repeated measures methodologies. Relative risk for mortalities over time, and by animal-type were estimated. Averaged over time, the mortality ratio was 12.6 deaths per 1,000 placements. The risk of feedlot mortalities increased from 1994 to 1999. This was because of an increase in the proportion of feedlot placements died from disorders of the respiratory system. There did not appear to be a change in the proportion of animals dying from digestive and other disorders. Animals of dairy breeds were at increased risk of all mortality etiologies compared to beef steers. During 1994-1996, a similar percentage of beef heifers and steers developed fatal respiratory disease. However, during 1997-1999, the percentage of beef heifers that developed fatal respiratory disease was 63% greater

than the percentage of beef steers that developed fatal respiratory disease. Beef heifers were less likely to die from digestive disorders than beef steers. Direct economic losses associated with feedlot mortalities include animal purchase price, consumed feed, processing/medicine costs, and disposal costs. Because respiratory mortality represents only a small proportion of respiratory-associated morbidity, the increased rates of respiratory mortality reported here may also reflect other adverse production effects in surviving animals.

Introduction

Cattle deaths result in significant losses for U.S. feedlots.[1] Economic losses include, but are not limited to, the purchase price of the animal, feed consumed from arrival at the feedlot to time of death, processing and medicine costs incurred, disposal costs, any labor associated with animal disposal, and interest on invested money.

Most feedlot-associated mortality results from bovine respiratory disease (BRD) complex, and at postmortem, lesions often include fibrinous bronchopneumonia.[2] Bovine respiratory disease is a consequence of complex interactions of stressors, host immunity, pathogenicity of agents, and environmental factors that alter the probability of pathogen exposure. Factors that may influence overall animal health in feedlot cattle include arrival weight (which provides a crude estimate of an animal's age and maturity), distance transported to the feedlot, water and feed restriction during transportation, commingling, and even the experience of the feedlot personnel.[3-5] These factors can directly and

indirectly affect the proportion of feedlot placements that do not survive to harvest.

Careful monitoring of causes of death may be useful to elucidate broad trends in animal health within and between feedlots. These trends may include increasing or decreasing mortality rates, or changes in proportional mortality ratios. The sentinel feedlot monitoring program was developed under the umbrella of the United States Department of Agriculture's National Animal Health Monitoring System (NAHMS) to monitor cattle in feedlots and serves as a *benchmarking* tool for participating feedlot managers and veterinary consultants. This program has been collecting placement and mortality data since March, 1993. Feedlots supply data to NAHMS voluntarily and confidentially through their veterinary consultants. This is therefore an ideal source of data to monitor trends in cattle mortalities. The objectives of this study were to evaluate 1) trends in feedlot cattle mortality over time, 2) monthly proportional mortality of cattle by primary body system affected, and 3) risk of mortality by type of animal placed by those feedlots participating in the NAHMS sentinel feedlot monitoring program.

Criteria for Selection of Cases

Data regarding feedlot cattle were submitted to the NAHMS sentinel feedlot monitoring program through veterinary consultants. The confidentiality of the participating feedlots were maintained by their consulting veterinarians who were located in Texas (n = 5), Nebraska (n = 2), Kansas (n = 2), and Idaho (n = 1). All submitted data were eligible for inclusion in the study. Data were included in the study and subjected to further analysis

based on the following broad criteria 1) data were relevant to the time-period of January 1994 through December 1999, and 2) complete placement and mortality data had been provided by the consultant.

Procedures

Data acquisition

Participating veterinary consultants assigned numeric codes for each of their participating feedlots. Completed data entry forms were identified with the feedlot code and submitted monthly by veterinary consultants to ensure confidentiality of participating feedlots. Feedlots submitted month-end data including total placements, cattle inventory, and the number of deaths attributable to respiratory, digestive and other disorders for the preceding month. Feedlot personnel, in consultation with their veterinarian (with or without ancillary diagnostic tests), attributed each mortality into 1 of 3 etiological categories. These categories were death attributable to the respiratory system, digestive system, or any *other* cause. Some feedlots in this study also reported the number of placements and mortalities that were beef steers, beef heifers or dairy animals. Submitted data were entered into an electronic database;[a] monthly summary reports were generated and returned to the veterinary consultants for distribution to the participating feedlot managers.

Data analysis

Data submitted from January 1994 through December 1999 were classified as having been derived from feedlots that supplied at least 10 months of data each year from January 1994 through 1999 (regular contributors), and *Other* feedlots (feedlots that irregularly contributed data, feedlots that entered the program after January 1994, and feedlots that left the program). Yearly estimates of mortality risk for the regular contributors and *Other* feedlots were calculated. Overall and etiology specific producer-attributed mortality frequency counts for regular contributors and *Other* feedlots were compared using Cochran-Mantel-Haenszel statistics.[6] The relative risk (RR) of mortality of regular contributors relative to *Other* feedlots and 95% confidence limits were estimated while controlling for year.[7]

Data were further analyzed at two levels: 1) data from all participating feedlots, and 2) data submitted that were classified as beef steers, beef heifers, and dairy breeds (Group-Subset). Group-Subset feedlots (those that provided Group-subset data) were a subset of all feedlots included in the study that elected to provide more detailed information about placement and mortality counts. Yearly cattle placements and deaths for each feedlot were summed for all feedlots and when possible by animal-type, and attributed etiology of death.

Data analysis: All feedlots

Yearly mortality and placement counts for all participating feedlots were aggregated across months within each year. Month-specific mortality and placement counts were also aggregated across years. Ratios of etiology specific mortality counts to placement counts (mortality ratio) were then calculated for each year. Mortality ratios were calculated for each month across years. Animals that died in a certain month may or may not have been placed within that month, but this monthly mortality ratio provided the best estimator of risk that was available from the data. It was more likely that animals dying within a given year were placed within that year so the yearly mortality ratio was a better estimator of risk.

Relative risks of producer attributed mortality etiology for each year compared to 1994 were estimated using Poisson regression techniques[a]. Frequency counts of total mortalities and for each etiology were dependant variables and year was the independent variable. Yearly placements within each feedlot were considered a cohort of cattle. Within each feedlot, first order autoregressive matrices were used to model the covariance structure of cohorts within feedlots over time.[7] The offset used in the marginal models was the log of placements for each cohort. Parameter estimates were generated using generalized estimator equations.[7] There was evidence that dependence in the outcome variables within feedlots (over-dispersion) was present in the data. To adjust for this over-dispersion, the exponential dispersion parameter was scaled to the Pearson's chi-square statistic divided by the model degrees of freedom, thereby adjusting standard

errors of the estimates.[7]

Data analysis: Group-Subset

Group-Subset yearly mortality risks for each etiology by animal type were calculated and examined graphically. An association of mortality risk by animal-type was evaluated using Poisson regression as described above. Frequency counts for producer attributed mortality etiology were the dependent variables of interest and animal-type was included in the model as the independent variable. Based on preliminary graphical evaluation of respiratory mortality risk for animal-type, the risk of respiratory mortality for beef heifers relative to beef steers was further analyzed in two time-periods; these being 1994 through 1996, and 1997 through 1999.

Results

One hundred and thirty-eight feedlots supplied at least one month's data related to placements and mortalities for the period of January, 1994 through December, 1999. This resulted in 5,139 feedlot-month data records. Of which, 792 were not included in the study because of incomplete placement or mortality counts. Hence, 4,347 feedlot-month records submitted from 121 feedlots were included in the study. These provided information on 21,753,082 placements. The number of participating feedlots and total placements monitored through the sentinel feedlot monitoring program has increased over this period (Table 3.1.).

Table 3.1. Frequency count of veterinary consultants, eligible feedlots, enrolled feedlots, and number of placements from enrolled feedlots in the NAHMS sentinel feedlot monitoring program by year.

	Number of participants and placements			
Year	Veterinarians	Eligible Feedlots	Enrolled Feedlots	Total Placements
1994	6	56	56	2,889,150
1995	9	78	71	3,636,107
1996	9	78	69	3,583,161
1997	9	77	64	3,486,750
1998	9	97	81	3,815,659
1999	10	108	94	4,342,255
Total	10	138	121	21,753,082

Averaged over time, the ratio of total mortalities to placements was 12.6 per 1,000 placements (Table 3.2.). Deaths attributed to respiratory, digestive, and other disorders accounted for 7.2, 2.9, and 2.5 animals per 1,000 placements, respectively. Respiratory disorders were the most common attributed cause of death and resulted in 57.1% of total mortalities averaged over time. Statistical analyses indicated that mortality risks were significantly associated with regularity of data submission (regular contributor versus *Other* feedlots; $P < 0.01$). Of the 121 enrolled feedlots, 24 were classified as regular contributors as they supplied at least 10 months of data each year from 1994 through 1999. However, the effect associated with regularity of data submission, albeit statistically significant, was deemed biologically negligible as placements in the regular contributors were only 4% less likely to die than *Other* feedlots' placements (RR, 0.959; 95% CL, 0.955, 0.962). Because this effect was deemed clinically insignificant, no further analysis was performed stratifying the data by regularity of data submission.

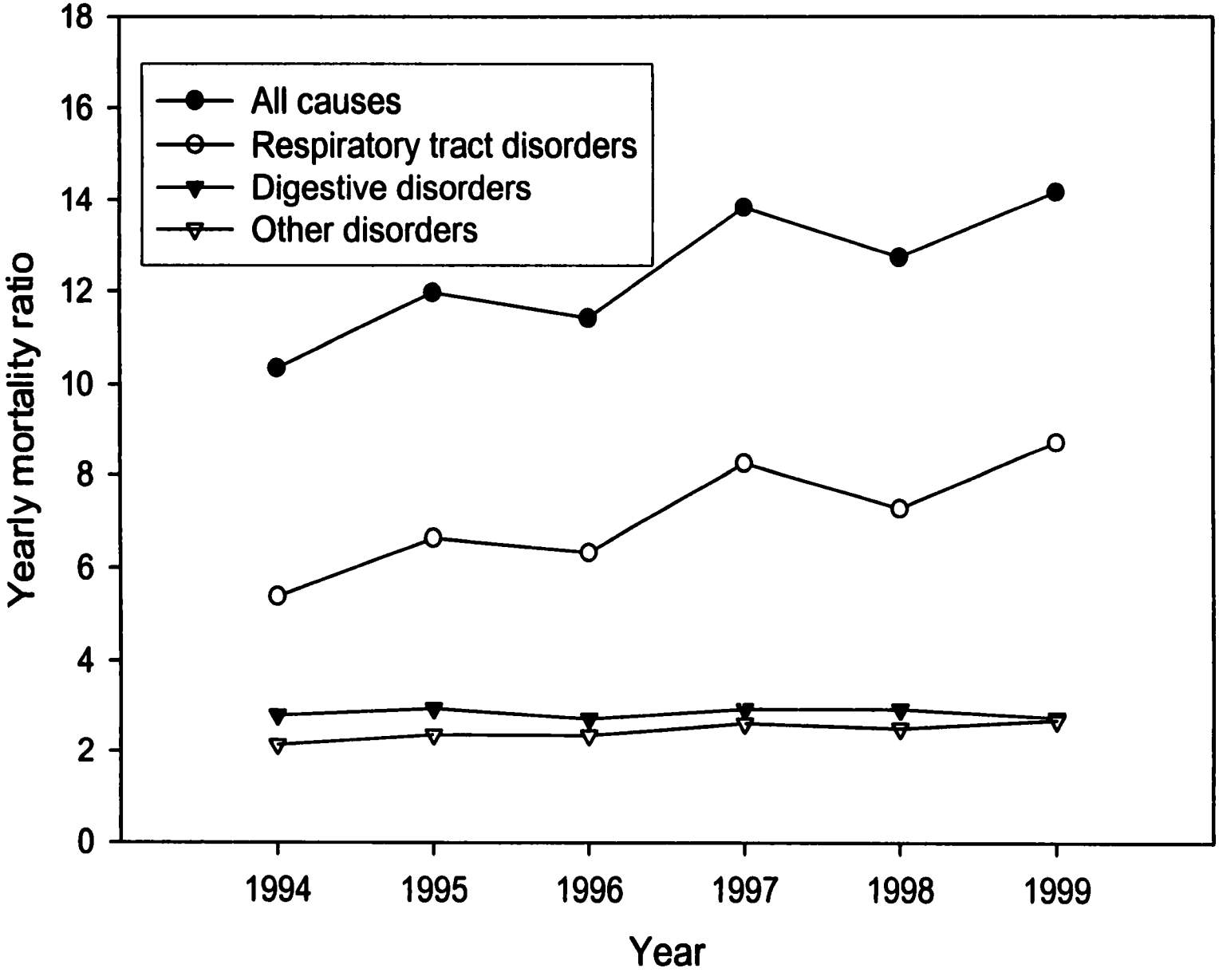
All feedlots

The yearly mortality ratio increased over time (Figure 3.1.) and as a result, cattle placed in feedlot during the years subsequent to 1994 were at greater risk of death than those placed in 1994 ($P = 0.09$). The number of placements that died during 1994 was 10.3 per 1,000 placements whereas 14.2 animals per 1,000 placements died in 1999. Overall, there was a 37.9% increase in the yearly mortality ratio from 1994 to 1999.

Table 3.2. Yearly mortality ratios and etiology-specific mortality ratios for all participating feedlots.

	Yearly mortality ratio per 1,000 placements			
Year	All etiologies	Respiratory etiologies	Digestive etiologies	*Other* etiologies
1994	10.3	5.4	2.8	2.1
1995	12.0	6.6	3.0	2.4
1996	11.4	6.3	2.7	2.4
1997	13.8	8.2	3.0	2.6
1998	12.7	7.2	3.0	2.5
1999	14.2	8.7	2.8	2.7
Average	12.6	7.2	2.9	2.5

Figure 3.1. Yearly mortality ratios for deaths attributed to respiratory, digestive and other etiologies during the period of 1994 through 1999. Estimates are expressed as deaths per 1,000 placements. Data were use relating 21,753,082 cattle placed in 121 feedlots.



The major reason for the increase in the total mortality risk over time was an increased mortality risk of animals succumbing to fatal respiratory disease (Figure 3.1.). The relative risk of respiratory mortality compared to 1994 was significantly greater than 1.0 for the years 1995 (RR, 1.16; 95% CL, 1.01, 1.33), 1997 (RR, 1.35; 95% CL, 1.02, 1.79), and 1999 (RR, 1.46; 95% CL, 1.13, 1.88; Table 3.3.). Cattle placed on-feed during 1999 were at a 46% increased risk of respiratory death compared to 1994-placements. Although not statistically significant, there appears to be evidence supporting variation in the risk of death attributable to digestive disorders relative to 1994 ($P = 0.11$). All RR estimates are less than 1.0 and varied from 0.92 to 0.65 implying that post-1994 placements were less likely to be diagnosed at postmortem with a digestive disorder compared to 1994. The data did not support a conclusion of significant variation in risk of death due to *other* mortality etiologies relative to 1994 ($P = 0.52$).

When data were aggregated across years by month, there appeared to be two distinct peaks in number of cattle placed in feedlots. The first occurred during spring whereas the second, and larger peak, occurred during the late summer and fall months (Figure 3.2.).

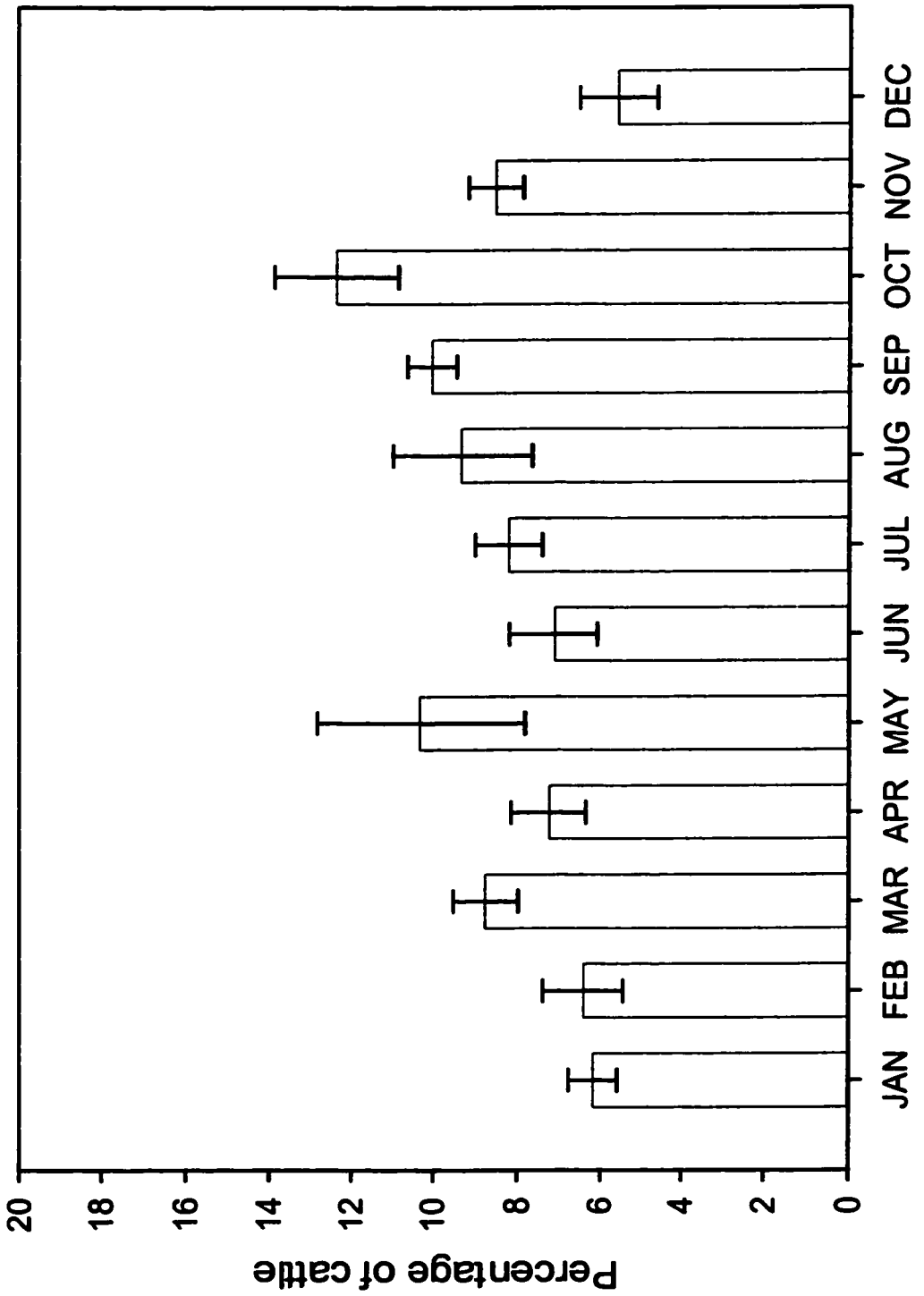
From August through November, one-third of the year, 40.1% of all feedlot cattle were placed by the participating feedlots. October is a disproportionately high-placement month and accounted for 12.3% of yearly placements whereas December placements accounted for only 5.5%. Numerically, December accounted for the lowest proportion of yearly placements. These data generally agree with other published reports regarding the

Table 3.3. Relative risk (and 95 % confidence limits) of mortality attributable to respiratory, digestive, and other causes compared to 1994.

	Cause of death		
Year	Respiratory	Digestive	Other
1994	1	1	1
1995	1.16* (1.01, 1.33)	0.91 (0.72, 1.16)	1.03 (0.89, 1.19)
1996	0.87 (0.54, 1.41)	0.69 (0.40, 1.17)	0.83 (0.54, 1.25)
1997	1.35* (1.02, 1.79)	0.92 (0.69, 1.23)	0.95 (0.70, 1.29)
1998	0.92 (0.48, 1.77)	0.65 (0.30, 1.39)	0.81 (0.50, 1.31)
1999	1.46* (1.13, 1.88)	0.75 (0.49, 1.14)	1.02 (0.76, 1.37)
Overall *P* value	< 0.01	0.11	0.52

* estimates are significantly different from 1 at the 0.05 level.

Figure 3.2. Monthly placements as a percentage of total annual placements.



placement profile of the entire U.S. feedlot industry.[8-15]

The monthly respiratory mortality ratio was greatest from November through January (Figure 3.3.). During December, the ratio of respiratory mortalities to monthly placements was 17.3 animals per 1,000 placements compared to 3.5 animals per 1,000 placements in May. Although there does not appear to be as distinct patterns in digestive and *other* mortality ratios, the monthly mortality ratios for both these categories were also numerically greatest for December and January (Figure 3.3.).

Group-Subset (data categorized by animal-type)

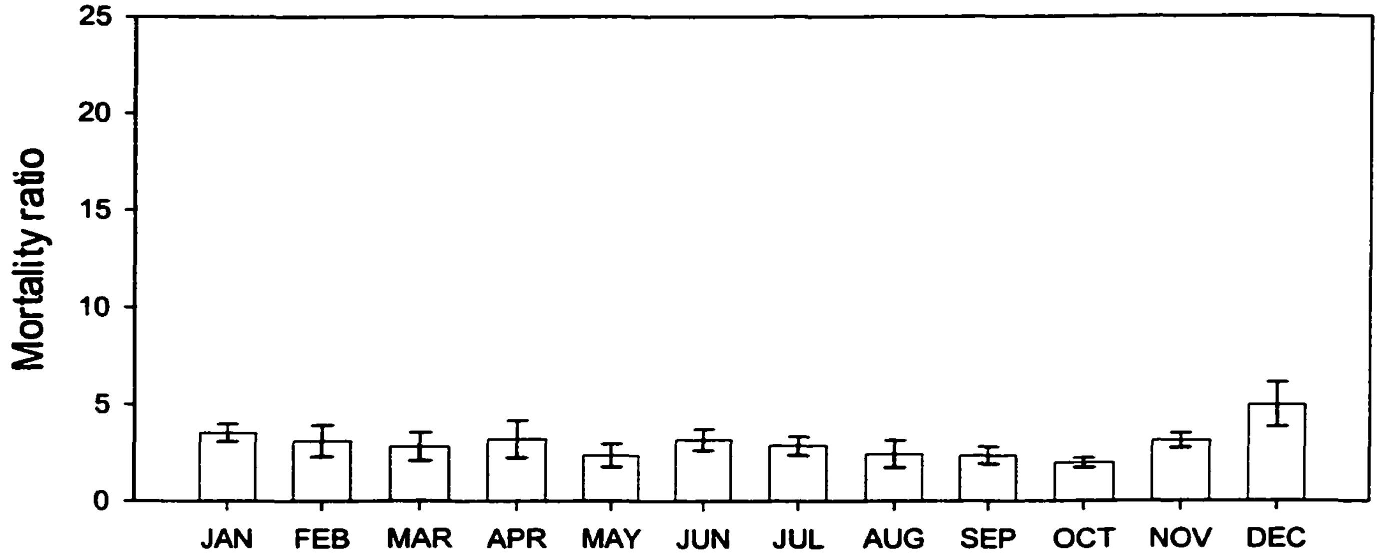
There were 83 feedlots that provided at least one month's mortality and placement data categorized by type of animal (beef steer, beef heifer and dairy breeds). These data included 5,057,441 beef steers, 2,649,219 beef heifers, and 101,471 cattle of dairy breed influence. There were only 2 feedlots that placed only one type of animal such as beef steer. Both feedlots only supplied 2 months of data and reported that only heifers were placed during these time periods. In contrast to data from *all feedlots*, the differences in yearly mortality ratios and yearly respiratory mortality ratios relative to 1994 were not detected ($P > 0.20$). Placements categorized as dairy breeds had an increased relative risk of mortality attributable to respiratory (RR, 1.99; 95% CL, 1.23, 3.21), digestive (RR, 4.18; 95% CL, 2.30, 7.58) and other (RR, 2.52; 95% CL, 1.92, 3.30) disorders compared to beef steers (Table 3.4.). Beef heifer placements had a 28% increased risk of death due to respiratory causes compared to beef steers ($P = 0.09$). Death loss due to digestive

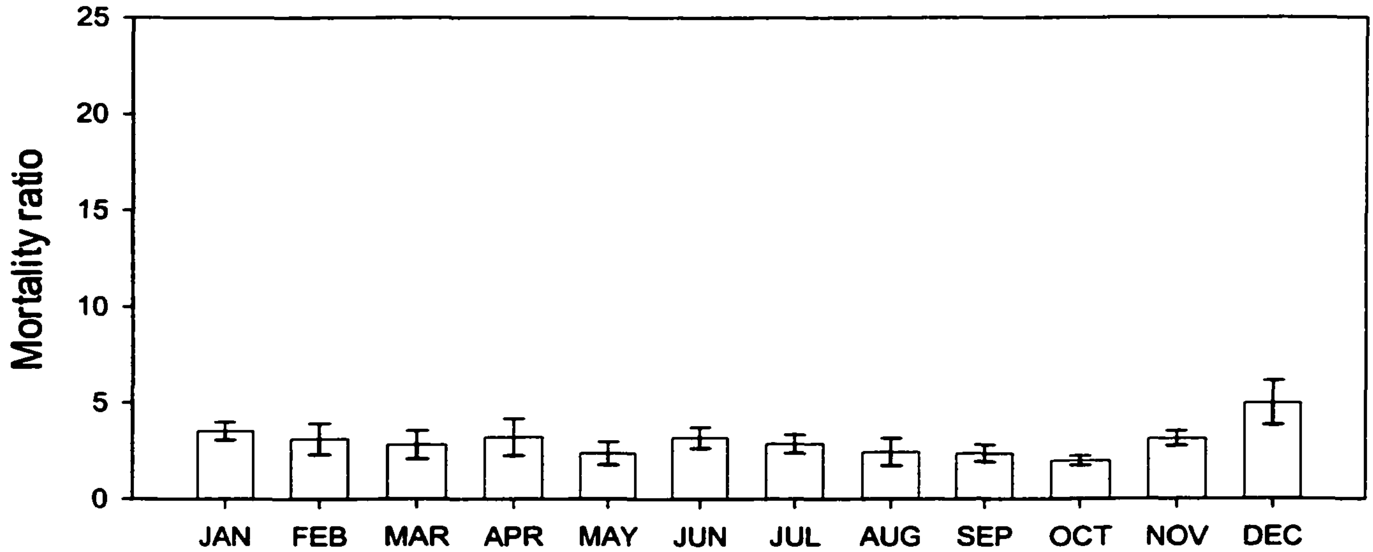
Table 3.4. Relative risks (RR), 95 % confidence limits (CL), and associated *P* values of deaths due to respiratory, digestive and other disorders for beef heifers and dairy cattle compared to beef steer placements.

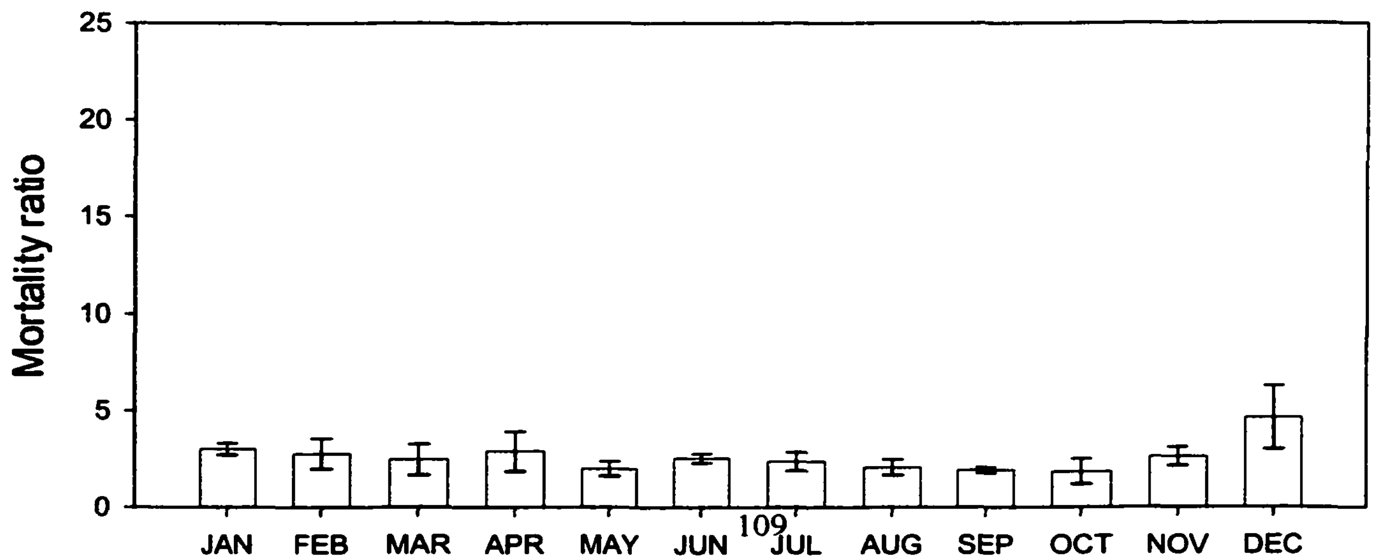
	Beef Heifer placements		Dairy placements		Beef steer placements
Mortality category	RR (95 % CL)	*P* Value	RR (95 % CL)	*P* Value	RR
Respiratory	1.28 (0.96, 1.71)	0.09	1.99 (1.23, 3.21)	< 0.01	1
Digestive	0.77 (0.66, 0.91)	< 0.01	4.18 (2.30, 7.58)	< 0.01	1
Other	1.35 (1.14, 1.60)	< 0.01	2.52 (1.92, 3.30)	< 0.01	1
Respiratory 1994 - 1996	0.98 (0.64, 1.51)	0.94	-	-	1
Respiratory 1997 - 1999	1.63 (1.36, 1.96)	< 0.01	-	-	1
Digestive 1994 - 1996	0.66 (0.54, 0.80)	< 0.01	-	-	1
Digestive 1997 - 1999	0.90 (0.75, 1.09)	0.29	-	-	1

- = analysis not performed.

Figure 3.3. Monthly mortality ratios for deaths attributed to respiratory, digestive and *other* etiologies. Monthly mortality ratios are expressed as deaths per 1,000 placements. Error bars represent the maximum and minimum monthly estimates for the period of 1994, through 1999.







disorders was varied by animal type. Whereas dairy breeds were more likely ($P < 0.01$) to die from digestive disorders than beef steers, beef heifers were 23% less likely ($P < 0.01$) to die from digestive disorders than beef steers (RR, 0.77; 95% CL, 0.66, 0.91). Graphically, there appears to be little difference between the percentage of beef heifers and beef steers that died from respiratory causes from 1994 through 1996 (Figure 3.4.). However, the proportion of placed heifers that succumbed to respiratory diseases increased after 1996. When analyzed in two time-periods, there was no evidence ($P = 0.94$) that beef heifers were at increased risk of respiratory mortality from 1994 through 1996 compared to beef steers . However, the risk of respiratory mortality for beef heifers was 63% greater (RR, 1.63; 95% CL, 1.36, 1.96; $P < 0.01$) than beef steers from 1997 through 1999.

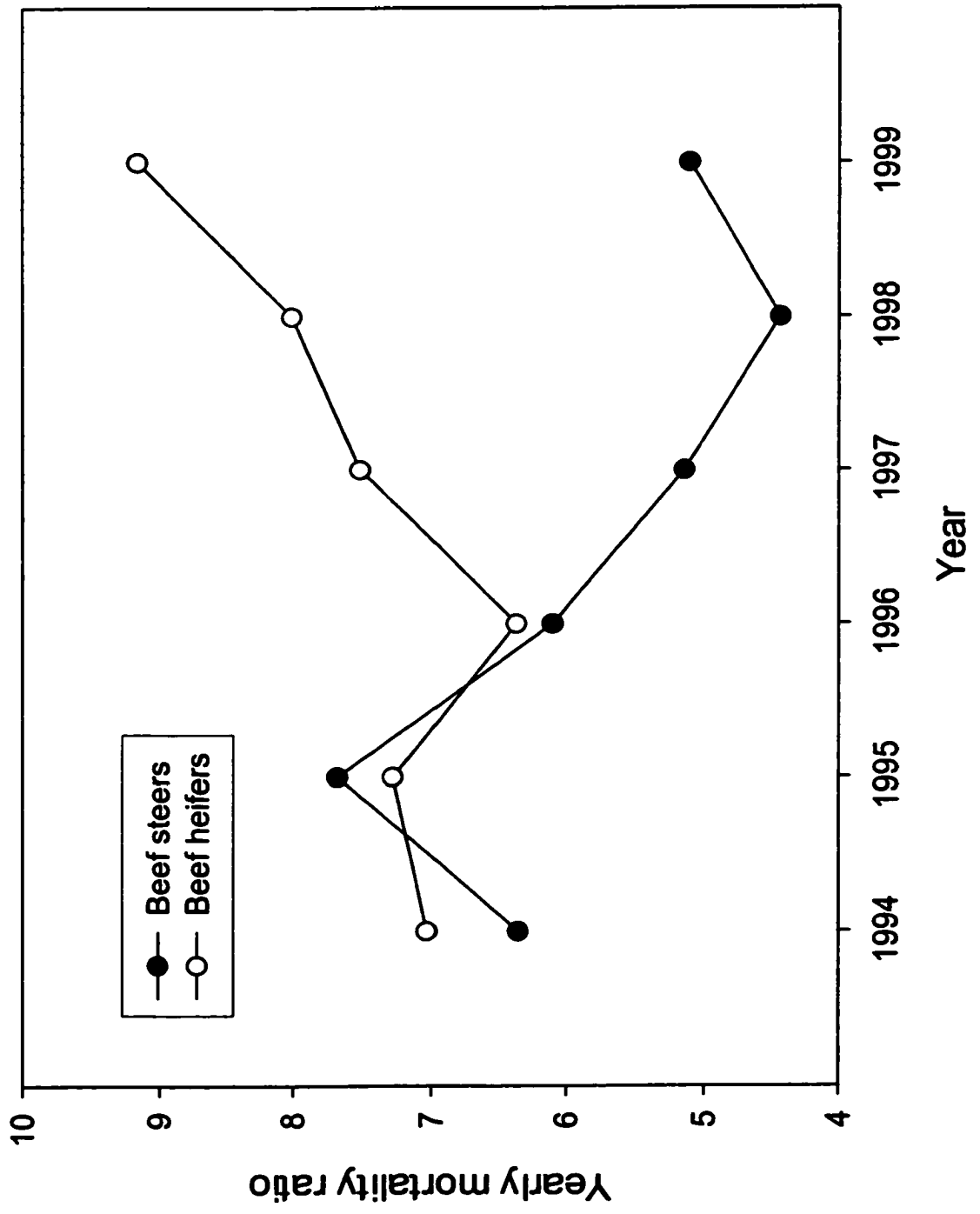
Discussion

The yearly mortality ratio increased over the period of the study and was primarily related to an increased proportion of placements dying from respiratory disorders. The relative risk estimators for respiratory deaths increased over time relative to 1994 ($P < 0.01$). Although the variation in risk of deaths due to digestive causes was not significant over time ($P = 0.11$), there was some evidence of a decrease in the mortality attributable to digestive disorders relative to 1994 because all RR estimates were numerically less than one.

Figure 3.4. Yearly mortality ratios for beef steers and beef heifers for the period of 1994 through 1999. Estimates are expressed as deaths per 1,000 placements. Data relating to 5,057,441 beef steers and 2,649,219 beef heifers from 83 feedlots were used.

The NAHMS sentinel feedlot monitoring program is a voluntary program. This provided some strengths and, potentially, weakness in the present study. The data are not necessarily a representative sample of all U.S. feedlots. The best estimator of risk from these data was the ratio of deaths to placements. However, it should be noted that animals dying were not necessarily placed at the feedlot during the same month or even the same year that they died. It is likely that some of the animals that succumbed to fatal respiratory disease did so in the months subsequent to the month in which they were placed. Hence, month-specific mortality ratios may not be accurate estimates of risk. If a substantial percentage of mortalities occurred subsequent to the month of placement, the mortality ratio for those months with fewer average placements would have been an over estimate of the true risk; conversely, the mortality ratio for those months with greater than the average number of placements would have been an under estimate of the true mortality risk. Estimators of risk would have been improved if animals were included in the denominator if they were indeed at risk. The accuracy of month-specific estimates of relative risk likely varies with etiology. This is because the majority of respiratory deaths occur soon after arrival where as digestive tract-related deaths occur later during the feeding period. It was more likely that deaths occurred during the same year that cattle were placed. Therefore, the ratio of yearly mortalities to placements is a more accurate estimate of risk.

Because many disease conditions present with similar clinical manifestations, categorization of cause of death based on antemortem manifestations can lead to



misclassification.[16] A thorough postmortem examination is more likely to correctly identify the affected body system.[17] The respiratory mortality category included deaths due to bronchopneumonia, acute interstitial pneumonia, respiratory-associated haemophylosis deaths, and any other disease condition with primary manifestations associated with the respiratory system. Digestive mortalities included ruminal tympany (typically frothy bloat), rumen lactic acidosis, and enterotoxemia. Mortality etiologies that were categorized as *other* (other than those attributable to respiratory or digestive disorders) included diseases of the central nervous system, musculoskeletal system, urogenital system, other miscellaneous causes, and undetermined disorders.

Nation-wide surveys of the feedlot industry indicate that the percentage of dead animals that were examined postmortem increased slightly from 45.9 (SE = 2.5) in 1994 to 53.9 (SE = 2.3) in 1999.[18,19] As such, a further weakness of the present study was that it is unlikely that postmortem examinations were performed on all animals that died at the participating feedlots. Thus, the potential for misclassification of diseases exists and would have been greatest among animals that did not receive a postmortem examination.

Newly arrived placements are at greatest risk of developing BRD (shipping fever) and the majority of respiratory-associated deaths occurs soon after arrival.[2] Conversely, the majority of digestive deaths occurs at later stages in the feeding period. This may have affected the accuracy of monthly etiologic-specific mortality ratios. Jensen and others reported that 72% of shipping fever deaths occurred in the first 45 days, whereas Pierson

and others found that 87.5% of bloat-associated deaths occurred after the first 45 days after arrival.[20,21] Vogel and Parrott reported that the average number of days from arrival to death attributable to respiratory and digestive disorders were 48.6 and 93.2, respectively.[b] If an increased proportion of placements in the present study died early in the feeding period from respiratory disease, then a smaller proportion of placements survived to the stage of feeding when digestive deaths are more likely to occur. Data presented in this report suggested that the risk of respiratory mortality increased over time (Figure 3.1.). Time from feedlot arrival to death was not collected in the present study, but it is expected that the majority of respiratory deaths occurred early in the feeding period.

All factors being equal, one would expect that as the proportion of placements dying from respiratory disease increased, proportionally fewer placements would have had an opportunity to die from digestive disorders. Group-Subset data did not support this expectation. The increased respiratory mortality risk of heifers compared to steers (Group-Subset) was only apparent after 1996 and was without an accompanying decrease in risk of digestive mortality for heifers. Over the course of the study period, heifers were less likely ($P < 0.01$) to die from digestive disorders than steers (Table 3.4.).

The reason for the increase in the respiratory mortality risk over time is unclear from these data. Many factors could have contributed to the increase over time such as a change in the source of cattle (e.g. groups purchased directly from a ranch compared to

assembled through the salebarn system), change in pre-arrival animal health management, increased transportation stressors, commingling of animals, changing environmental conditions, and changes in dietary management of new arrivals (such as micronutrient supplementation or the concentration of non-structural carbohydrates in receiving diets). These factors, either singularly or in combination, are generally expected to compromise immune function. The effect of transportation distance on occurrence of fatal fibrinous pneumonia has been questioned.[22] Other factors that may have contributed to increased risk of respiratory death include placement of a greater proportion of light-weight animals (body-weight may serve as a crude proxy for an animal's age and maturity), changes in treatment protocols, decreases in the ratio of animal health employees to feedlot inventory, or changes in disease awareness, skill levels and experience of feedlot personnel.

Interestingly, the disproportionate increase in respiratory death loss of beef heifers compared to beef steers from 1996 through 1999 coincides with a reduction in the U.S. cattle herd inventory.[14,23] It is likely a greater number of heifers were culled from herds at a younger age compared to periods of herd expansion when more heifers would be kept for breeding purposes. It is possible that herd contraction contributed to the increase in heifers that developed fatal respiratory disease by supplying a greater number of light-weight heifers to the feedlot industry. This explanation is supported by information regarding feedlot placements; heifers as a proportion of total beef animal placed in feedlots increased from 34.1% (SE = 0.9) in 1994 to 41.4% (SE = 1.0) in 1999.[18,19]

However, during the same period the proportion of total feedlot placements that were less than 600 pounds at arrival only changed from 27.0 to 28.2%.[8-15] The animal-type distribution within arrival weight categories are not available.

Morbidity data for participating feedlots were not collected as part of the sentinel feedlot monitoring program. However, respiratory morbidity and mortalities are associated.[24] Because increasing proportion of placements are succumbing to fatal respiratory disease, it is likely that increased proportions of those animals surviving to harvest experienced an episode or episodes of non-fatal bovine respiratory disease whilst at the feedlot; this assumes that the case fatality rate for BRD did not change significantly during the study period. Others have demonstrated that the presence of pulmonary lesions at harvest was associated with significant decreases in feedlot gains of 0.07 to 0.20 kg per animal-day and was also associated with decreased carcass quality.[25,26] Ultimately, the trend for increased respiratory mortalities reported herein may therefore indicate that decreased rates of gain and feed efficiency occurred in cattle placed in participating feedlots. So economic losses of fatal respiratory disease likely extend beyond those losses directly attributable to animals that failed to survive to harvest.

Because dairy breeds of cattle are generally placed in feedlots at lighter weights and have lower average daily gains than beef animals, their stay at the feedlot is typically longer than beef animals. The increased risk of all mortalities in dairy cattle compared to beef steers may in part be due to increased time at the feedlot for dairy cattle. Therefore, dairy

breeds of cattle would have a greater opportunity to develop fatal disease than beef animals. It was not possible to control for time at-risk in the analyses as *days-on-feed* data are not supplied as part of the sentinel feedlot monitoring program. It was also not possible to evaluate differences in mortality risks between breeds of cattle (such as Simmental and Angus) for those animals classified as beef breeds.

Data reported here suggest that the risk of death is associated with gender for beef breeds of cattle. Except for differences such as type of implant, and inclusion of melengesterol acetate (MGA) in the ration, there is little variation in day to day management of beef heifers and beef steers in feedlots. Heifers generally have lower dry matter intakes and average daily gains compared to steers. Thus, they may be less likely to develop fatal rumen lactic acidosis or frothy bloat than steers. The reason for increased risk of respiratory death associated with heifers is unclear.

Other causes of mortality include diseases that are either sex specific or display a predilection for sex such as dystocia, metritis, urolithiasis (water belly), and the buller steer syndrome.[27] Therefore, comparison of differences in relative risks between beef heifers and steers for other causes of mortality is of limited value.

Benchmarking is an important practice that feedlot managers and veterinary consultants can use to evaluate well defined parameters of their feedlot compared to a population of other feedlots. The sentinel feedlot monitoring program provides timely reports to

veterinary consultants to aid the identification of strengths and weaknesses in their animal health programs, and changes in mortality patterns of their clientele's feedlots. Although random sampling was not used to select the participating feedlots, the results presented herein may serve as benchmarks and comparisons for use by nutritionists, veterinarians, researchers and managers working in the wider feedlot industry.

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ENDNOTES

[a] The SAS System release 8.00, SAS Institute Inc., Cary, NC.

[b] Gary L. Vogel and Cal Parrott. Mortality survey in feedlot : the incidence of digestive, respiratory and other death losses. In: Scientific update on Rumensin/Tylan for the professional veterinary consultant. August 25, 1993, Amarillo, TX.

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CHAPTER 4

Acute interstitial pneumonia in feedlot cattle I. Involvement of microbial respiratory pathogens.

Summary

The objectives of this research were to evaluate possible associations of feedlot-associated acute interstitial pneumonia (AIP) with viral and bacterial respiratory pathogens, and *Mycoplasma* spp. isolated from lung tissue. A prospective case-control study was performed through collaboration with 14 feedlots located in Colorado, Nebraska, Kansas and Texas. Lung samples were collected from 186 animals during routine postmortem examination for histology, microbiology and toxicology evaluation. Histological diagnoses were categorized as AIP cases, bronchopneumonia (BP) cases, controls and *other*. Acute interstitial pneumonia cases had been at the feedlot for an average of 127.2, which was longer than BP cases (98.6 days) and controls (84.0 days). The presence of a viral respiratory pathogen (bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus, bovine herpesvirus 1, or parainfluenza virus 3) was not associated with histological category. Bovine respiratory syncytial virus was detected in 8.3% of AIP cases and 24.0% of controls. Histological category was associated with isolation of an aerobic bacterial agent and *Mycoplasma* spp. Bronchopneumonia cases

were at greater risk for aerobic and *Mycoplasma* spp. isolation compared to AIP cases and controls. Results suggest that feedlot-associated AIP does not occur as a consequence of BRSV infection. The increased risk of aerobic bacterial identification from AIP cases compared to controls may indicate a causal role, or an opportunistic infection following AIP development.

Introduction

By far the most important cause of morbidity and mortality in U. S. feedlot cattle is the bovine respiratory disease complex (BRD).[1-3] This disease complex is etiologically multifactorial and results from an interaction of stressors, animal susceptibility and infectious respiratory pathogens that ultimately give rise to bacterial bronchopneumonia.[4]

Quite distinct from BRD, a second important respiratory disorder of feedlot cattle is acute interstitial pneumonia (AIP). Acute interstitial pneumonia describes a pattern of lesions that results from injury to Clara and alveolar type I epithelial cells. Characteristic lesions of AIP include hyaline membrane formation and proliferation of alveolar type II epithelial cells.[5] Acute interstitial pneumonia manifests with a sudden onset of respiratory distress and is frequently fatal.[6] The case fatality rate of animals suffering AIP may exceed 60% and the disease appears to be refractive to available therapeutic regimens. The United States Department of Agriculture's National Animal Health Monitoring System (NAHMS) reported that 97.6% of feedlots had at least one case of AIP in the year ending June 30, 1999. Participants in the NAHMS study reported that 3.1% of all cattle placed

in feedlots during the same time-period developed AIP. With the exception of BRD, AIP affected more cattle than any other disorder. Although the criteria used by feedlot personnel to diagnose AIP was not described in the NAHMS study, it is apparent that AIP is perceived to be a substantial animal health concern by the feedlot industry.

More than one causal mechanism may lead to AIP and include bioactivated forms of 3-methylindole (3MI),[7,8] 4-ipomeanol and perilla ketones,[9,10] and infection with bovine respiratory syncytial virus (BRSV).[11] The epidemiology and pathogenesis of AIP in pastured cattle (fog fever, cow asthma or acute bovine pulmonary edema and emphysema) has been well described.[9,10,12] Pasture-associated AIP results from an abrupt dietary change when cattle are moved from dry dormant to lush pasture that gives rise to marked increases in ruminal 3-methylindole (3MI) generation.[7,8,13-15] Feedlot-associated AIP, also known as dust pneumonia or allergic pneumonia, typically affects animals close to market weight during a period of their feedlot production when they are presumably well adapted to high-concentrate diets.[16,17] Although the lesions are histologically identical to ABPE, the etiology of feedlot-AIP remains obscure.

Because BRSV may produce lesions similar to AIP, an infectious etiology for feedlot-associated AIP has often been suggested.[4,18] Collins and others identified bovine respiratory syncytial virus (BRSV) in lung tissue from 11 of 15 animals with AIP and from 5 of 18 animals with other disorders.[18] In their study, AIP was associated with isolation of BRSV ($P = 0.01$). However, other investigators have not found evidence

supporting an association with BRSV.[19,20] Hjerpe reported that a greater proportion of feedlot animals that died from AIP also had gross lesions of bronchopneumonia compared to animals that had died from other non-BRD causes.[17] In another study, 75% of animals, from which AIP was confirmed histologically, also had concurrent suppurative bronchopneumonia.[19] The purpose of this study was to evaluate possible associations of feedlot-associated AIP with viral and bacterial respiratory pathogens, and *Mycoplasma* spp. isolated from lung tissue.

Materials and methods

A prospective case-control study was performed to evaluate associations of lung microbial findings with AIP. Feedlots were enrolled in the study by their consultant veterinarians and sample collection was performed during routine postmortem examinations of animals during the period of May 15 to September 15, 1999.

In accordance with usual feedlot practices, cause of death was assigned by the consulting veterinarians or trained feedlot personnel. Those performing postmortem examinations were instructed on sample collection technique. In all, six samples were taken from the right lung. Tissue samples, each approximately 5 x 5 x 1 cm, included 2 adjacent samples from the dorsal aspect of the caudal lobe for toxicology (n = 1) and histology (n = 1), 3 adjacent samples from the caudal margin of the middle lobe for microbiology (n = 2) and histology (n = 1), and 1 sample from the apex of the middle lobe for histology (n = 1). Tissue was collected in the following order to minimize the possibility of formalin

contamination of tissue samples submitted for microbiological and toxicological evaluation. One sample from the caudal lung lobe and two samples from the base of the middle lobe were collected and each sample was placed in individual air-tight plastic bags and frozen. The remaining three samples were collected and fixed in 10% neutral buffered formalin. At regular intervals, one of the investigators visited participating feedlots or their veterinary consultants and retrieved fixed and frozen samples. Frozen tissue samples were stored at -20 °C (two samples from middle lobe) and -76 °C (sample from caudal lung lobe).

Fixed tissue samples were sliced at 10 mm intervals. A block for light microscopical examination was selected from each section (3 per animal) and embedded in paraffin using routine methods, sectioned at 5 μm and stained with hematoxylin and eosin. Some sections were stained with periodic acid Schiff for protein transudate, and phosphotungstic acid hematoxylin for hyaline membrane. Lung tissue was examined by two investigators using light microscopy and their findings recorded. Criteria for diagnosis of AIP included multifocal or diffuse microscopic lesions of alveolar septal edema with serofibrinous exudation into alveolar spaces or hyaline membrane formation with or without admixed chromatin strands or type II epithelial hyperplasia. Cattle with this pattern of injury present in one or more lung sections were categorized as AIP even if other concurrent disease processes (such as bronchopneumonia) were present. At the time of histological examination of lung tissue, the pathologists were not aware of the microbial findings.

Virus identification was performed using fluorescent antibody detection and virus isolation techniques. Frozen lung was sectioned at 6 μm and stained using a direct methodology with fluorescein conjugated antibodies against bovine herpesvirus 1 (BH-1), BRSV, bovine viral diarrhea virus (BVDV) and parainfluenza virus 3 (PI3). Sections were then illuminated with a xenon epifluorescent lamp and observed for specific viral fluorescence. Virus isolation proceeded as follows. Lung tissue was homogenized in minimal essential media (MEM) at a concentration of approximately 10% lung tissue (weight vol^{-1}). The homogenate was placed onto a confluent bovine turbinate (BT) cell culture monolayer for one hour at 37 °C then washed. Minimal essential media containing antimicrobials and 10% fetal bovine serum (vol vol^{-1}) were added. Cells were observed for viral cytopathic effects for 7 days. Monolayers that exhibited no cytopathic effects were passed onto fresh BT monolayers and further observed for 7 days. Monolayers showing no cytopathic effect were then again passed onto fresh BT monolayers and after 3 days were fixed using room temperature acetone for 10 minutes. Monolayers were then stained by an avidin-biotin immunohistochemical technique using antibodies against non-cytopathic strains of BVDV. If cytopathic effects were observed, viruses were identified utilizing fluorescein conjugated antibodies against BH-1, BRSV, BVDV and parainfluenza virus 3 PI3. Fluorescence was detected as described above. During the study period, laboratory personnel routinely tested fetal calf serum for BVDV.

The remaining lung sample for microbiology was thawed and sectioned using alcohol-sterilized scissors. Interior samples of lung tissue were aseptically obtained. A sterile

swab was placed on the sectioned surface and directly plated onto blood, Columbia and MacConkey agar plates. Blood and MacConkey agar plates were incubated aerobically and the Columbia agar plates were incubated microaerophilically (10% CO_2) for 18-24 hours at 35 °C. Bacterial isolates were identified according to standard Colorado State University Veterinary Diagnostic Laboratory (D-Lab) protocols. *Mycoplasma* spp. were isolated from lung tissue using a second swab inoculated into F-broth. The broth was incubated for 24 hours at 35 °C and 5% CO_2 then filtered (0.45 μm), swabbed on F-agar plates, and incubated for five days at 35 °C and 5% CO_2 for *Mycoplasma* spp. *Mycoplasmas* were speciated as *Mycoplasma bovis* or other spp. using PCR techniques in accordance with D-Lab protocols.

At the time of sample acquisition, information pertaining to feedlot of origin, sex, days from arrival at the feedlot to death, and suspected cause of death were recorded.

Statistical analyses.

Histological findings were categorized into one of four groups: AIP, bronchopneumonia (BP), control, and *other* diagnoses. Descriptive statistics were calculated for all categories whereas only the former three categories were used in formal statistical analyses. Viral detection methods (VI and FA) were interpreted as tests performed in parallel i.e. if either test was positive then the animal was classified positive. Aerobic culture was considered positive if *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*), *Pasteurella multocida*, or *Haemophilus somnus* were isolated. Other

isolates, such as enterobacteria and *Actinomyces pyogenes*, were considered postmortem contaminants or opportunistic invaders and not included in the analyses. Frequency counts by virus, aerobic bacteria and *Mycoplasma* spp. were tabulated and validated.

A commercially available statistical analysis software package was used to generate estimates (and SEM), test statistics, confidence limits and *P* values where applicable.[a] Days from arrival at the feedlot to death was compared between histological categories using analysis of variance techniques. Feedlot was considered a random variable and Satterthwaite approximations for the denominator degrees of freedom were used to test least squares means estimates.[21] Means were compared using Tukey's method of adjustment.[22] For count data, contingency tables were analyzed to test the compatibility of the data with the null hypothesis of no association of histological category (AIP, BP, and control) with BRSV, BVDV, BHV1, PI3, concomitant BRSV and BVDV infection, aerobic bacteria, and *Mycoplasma* isolation. Probability values were calculated from the χ^2 statistic or Fisher's exact test where appropriate. When the data provided evidence of an association (*P* less than or equal to 0.05), logistic regression was used to generate odds ratios for AIP and BP cases compared to controls, and BP compared to AIP cases. Ninety-five percent confidence limits were calculated using partially maximized likelihood functions.[21]

Results

Fourteen feedlots submitted lung samples from at least one animal during the study period. Lung samples from 186 animals were collected in the present study. Three feedlots submitted samples from one animal and three feedlots submitted samples from greater than 20 animals. One feedlot supplied samples from 60 animals. Study feedlots were located in Colorado (n = 4), Kansas (n = 3), Nebraska (n = 4), and Texas (n = 3). These feedlots submitted lung samples from 101, 49, 14 and 22 animals, respectively.

Histological grouping of lungs are presented in Table 4.1 and included AIP (n = 108), bronchopneumonia (n = 50), control (n = 25); congestion and edema, atelectasis and no microscopic anomalies), and other (n = 3). Acute interstitial pneumonia was confirmed in at least 1 animal from all participating feedlots. One feedlot supplied 29 lung samples confirmed with AIP. Control lung samples were submitted from 7 feedlots. Nine feedlots submitted at least 1 animal that was classified to have BP. Two feedlots supplied 1 BP case each and one feedlot supplied 23 BP cases.

Least squares means ± SEM for days from feedlot arrival until time of death (*days on-feed*) were 127.2 ± 8.3, 98.6 ± 9.8, and 84.0 ± 11.8 for AIP cases, BP cases, and controls respectively. The ranges of *days on-feed* were 23 to 249 (AIP cases), 5 to 236 (BP cases), and 3 to 145 (controls). Animals that died from AIP were *on-feed* for a longer period of time than BP cases ($P < 0.01$) and controls ($P < 0.01$). Controls and BP cases did not differ significantly in the days from feedlot arrival to death ($P = 0.40$).

Table 4.1. Frequency counts of sample submission by histological category and by feedlot. Histological categories included acute interstitial pneumonia (AIP) cases, bronchopneumonia (BP) cases, controls or *other* diagnoses. The frequency counts for the *other* category are not shown but are included in the total.

		Histological grouping			
Feedlot	State	AIP	Control	BP	Total
A	Colorado	14	2	5	22
B	Colorado	7	4	2	13
C	Colorado	5	0	1	6
D	Colorado	29	7	23	60
E	Kansas	4	0	2	6
F	Kansas	8	1	3	12
G	Kansas	21	5	5	31
H	Nebraska	1	0	0	1
I	Nebraska	1	0	0	1
J	Nebraska	8	0	3	11
K	Nebraska	1	0	0	1
L	Texas	5	4	5	15
M	Texas	2	0	1	3
N	Texas	2	2	0	4
Total	Four states	108	25	50	186

Bovine respiratory syncytial virus, BVDV, or BHV1 was identified in 30.1% (n = 56) lungs. Parainfluenza was not identified in any animal. Bovine herpesvirus 1 was identified in lung tissue from 2 animals suffering AIP, and 3 animals suffering BP. There was no evidence supporting an association of histological category and the presence of BHV1 (P = 0.33; Table 4.2).

Twenty one animals (11.3%) were positive for BRSV. Similar percentages of animals were positive using fluorescent antibody detection, virus isolation or both techniques (Table 4.3). Bovine respiratory syncytial virus was detected in 24.0% of controls, 8.3% of AIP cases and 12.0% of BP cases. There was evidence for an association of histological category with isolation of BRSV (P = 0.09; Table 4.3).

Bovine viral diarrhea virus was identified in 20.4% (n = 38) of study animals. Similar percentages of animals were positive for BVDV using fluorescent antibody detection, virus isolation or both techniques (Table 4.2). Seven controls (28.0%), 19 AIP cases (17.6%), and 11 BP cases (22.0%) were positive for BVDV. The data did not support a hypothesis that BVDV presence varied with histological category (P = 0.47; Table 4.3).

Seven animals were positive for both BRSV and BVDV, these included 1.9% of animals with AIP, 6.0% of BP cases, and 8.0% of controls. There was no evidence for an association of concurrent BRSV and BVDV infection with histological category (P = 0.18). No animals in the *other* category were positive for both BRSV and BVDV. One

Table 4.2. Percent and frequency counts for identification of bovine respiratory syncytial virus (BRSV), or bovine viral diarrhea virus (BVDV) using virus isolation (VI) and fluorescent antibody (FA) detections techniques. There were seven animals positive for bovine herpesvirus 1 (not shown) and no animals positive for parainfluenza virus 3.

	Technique			
Virus	VI	FA	Percent	Count (n=186)
BRSV	-*	-	88.71	165
BRSV	-	+†	4.30	8
BRSV	+	-	3.23	6
BRSV	+	+	3.76	7
BVDV	-	-	79.57	148
BVDV	-	+	5.38	10
BVDV	+	-	6.99	13
BVDV	+	+	8.06	15

*Negative, †Positive.

Table 4.3. Percent and frequency count of lung samples positive for bovine herpesvirus 1 (BHV1), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus (BVDV), and concomitant BRSV and BVDV infections using either virus isolation or fluorescent antibody detection, aerobic bacteria‡, and *Mycoplasma* spp. *P* values were calculated using χ^2 goodness of fit (BRSV, BVDV, aerobic bacteria and *Mycoplasma* spp.) and Fisher's exact (BHV1, and combined BRSV and BVDV) tests.

	Histological category			
Virus	AIP* percent (n = 108)	BP† percent (n = 50)	Control percent (n = 25)	*P* value
BHV1	1.85 (2)	6.0 (3)	0.0 (0)	0.33
BRSV	8.33 (9)	12.0 (6)	24.0 (6)	0.09
BVDV	17.59 (19)	22.0 (11)	28.0 (7)	0.47
BRSV and BVDV	1.85 (2)	6.0 (2)	8.0 (2)	0.18
Aerobic bacteria	13.89 (15)	36.0 (18)	4.0 (1)	<0.01
Mycoplasma spp.	30.56 (33)	52.0 (26)	16.0 (4)	<0.01

‡*Mannheimia haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus* (enterobacteria and *Actinomyces pyogenes* were not included), *acute interstitial pneumonia, †bronchopneumonia.

animal, a BP case, was positive for both BHV1 and BVDV.

Overall there were 37 aerobic bacterial isolates identified *as Mannheimia haemolytica* (n = 9), *Pasteurella multocida* (n = 26), or *Haemophilus somnus* (n = 2; Table 4.4). These isolates were cultured from 18.28% (n = 34) of animals. There were three animals from which more than one isolate was cultured; *P. multocida* and *H. somnus*, and *M. haemolytica* and *P. multocida* were isolated from one AIP case, and two BP cases, respectively. Isolation of aerobic bacteria varied with histological category ($P < 0.01$, Table 4.3). One control (4.0%), 15 AIP cases (13.9%), and 18 BP cases (36.0%) were culture positive. The odds of an aerobic bacterial isolate being cultured from an AIP case did not differ from the odds of a control having an aerobic isolate (OR, 3.9; 95% CI, 0.73 to 71.7; $P = 0.20$). Bronchopneumonia cases were at greater risk of aerobic bacterial isolation than AIP cases (OR, 3.5; CI, 1.6 to 7.8; $P < 0.01$).

Mycoplasma spp. were cultured from 33.3% of study animals. Isolation of *Mycoplasma* spp. was associated with histological category ($P < 0.01$; Table 4.3). The proportions of controls, AIP cases, and BP cases positive for *Mycoplasma* spp. were 16.0, 30.6 and 50.2%, respectively. There was no evidence that AIP cases were at increased risk of *Mycoplasma* isolation compared to controls (OR, 2.3; 95% CI 0.8 to 8.4; $P = 0.15$) whereas BP cases were at increased risk relative to controls (OR, 5.7; 95% CI 1.8 to 21.7; $P < 0.01$). Risk of a *Mycoplasma* isolation was greater for BP cases than AIP cases (OR, 2.6; 95% CI 1.2 to 4.9; $P = 0.01$).

Table 4.4. **Percent and frequency count of *Mannheimia haemolytica*, *Pasteurella multocida* and *Haemophilus somnus* isolates cultured from lung tissue from acute interstitial pneumonia (AIP) cases, bronchopneumonia (BP) cases, and controls. The *other* category are not shown but included in the total.**

	Histological category			
Microbial isolate	AIP (n = 108)	BP (n = 50)	Control (n = 25)	Total (n = 186)
M. haemolytica	1.85 (2)	14.0 (7)	0.0 (0)	4.84 (9)
P. Multocida	11.11 (12)	26.0 (13)	4.0 (1)	13.98 (26)
H. somnus	1.85 (2)	0.0 (0)	0.0 (0)	1.08 (2)

Discussion

Acute interstitial pneumonia represents a distinct histological lesion pattern that includes edema of the alveolar wall and interlobular spaces, accumulation of protein rich fluid within the alveolar spaces, hyaline membrane formation, and proliferation of alveolar type II epithelial cells.[16] Hemorrhage from pulmonary capillaries may also be present and its presence probably reflects the severity of tissue injury. Grossly, lesions may be diffuse but the caudodorsal aspect of lung is typically the most severely affected.[19] At postmortem examination, lungs fail to collapse, have a rubbery texture, and exude clear edema fluid from cut surfaces. Interlobular edema is often marked and emphysematous bullae may be present. Lobules vary in the extent to which they are affected and often an apparently normal lobule may be adjacent to an affected lobule. When variation is present within an animal, it results in a distinctive *checkerboard* appearance.

Although there has been considerable interest in infectious organisms and feedlot-associated AIP, we found no evidence to support an association of an infection with commonly identified respiratory pathogens and AIP of feedlot cattle. Hjerpe reported that bronchopneumonia was present in a greater proportion of AIP cases than in animals that had died from other causes, and animals with AIP were more likely to have been administered prior therapeutic regimens.[17] In the present study, gross evidence of concurrent bronchopneumonia was not recorded. Although not statistically significant ($P = 0.20$), of the 25 controls, the only aerobe cultured was a single isolate of *Mannheimia*

haemolytica, whereas 13.9% AIP cases were culture positive for at least one aerobic bacterium. Animals suspected to be suffering from AIP in the participating feedlots were treated with antimicrobials in accordance with their routine treatment protocols. Aerobic culture may have been unsuccessful in some AIP cases with bacterial infections of the lower respiratory tract because of residual antimicrobial effects. The majority of the isolates were *P. multocida*, although *M. haemolytica* ($n = 2$) and *H. somnus* ($n = 2$) were occasionally isolated. It is unclear from the present study whether or not bacterial infection of the lower respiratory tract occurred prior to the development of AIP. Because proliferation of alveolar type II epithelial cells was evident in some animals, these animals must have survived after the initial AIP episode for at least 2 to 3 days. Bacterial colonization of the lower respiratory tract would presumably be more likely in animals with severe pulmonary injury than in healthy animals. The susceptibility to bacterial colonization would also likely increase as time at-risk, i.e. duration of pulmonary injury-induced respiratory distress, increased. Although no association was observed in the present study, it is still possible that aerobic bacterial pathogens may have a causal role in development of feedlot-AIP, or simply an opportunistic role. An opportunistic infection could have contributed to the death of some of the animals without involvement in the pathogenesis of AIP.

The etiological factors responsible for feedlot-associated AIP are uncertain. This disease reportedly affects cattle advanced in the finishing period.[16] The data reported here support this finding with the mean *days on-feed* until death for AIP cases being 127.2

days. At this stage cattle are presumably well adapted to their finishing diet and, in contrast to pasture-associated AIP, sudden dietary changes are unlikely. Since root crops are not commonly fed to feedlot cattle,[23] it is doubtful that feedlot-associated AIP results from contamination of the diet with 4-ipomeanol. Further; root crops were not included in the diets of cattle in the present study. If perilla ketones were involved, then those animals with the greatest exposure to purple mint would be at greatest risk for AIP. Purple mint plant could potentially contaminate the roughage component of the diet. The proportion of roughage fed to new arrivals is greater than that fed to established cattle, but AIP typically affects cattle on low-roughage diets i.e. those at lowest risk of purple mint exposure. Perilla ketones are not likely involved in feedlot-AIP. Although sudden increases in dietary tryptophan are unlikely in feedlot diets, associations of feedlot-AIP with increased blood and lung concentrations of protein adducts of the 3MI metabolite, 3-methyleneindolenine (3MEIN), have been demonstrated.[20]

Woolums and others discussed the potential role of a subclinical bacterial infection of the lung or liver.[24,25] It was proposed that a bacterial nidus may result in increased concentrations of pro-inflammatory cytokines, particularly TNF-α and IL-1β, thereby priming the lung for AIP. This hypothesis has been supported by data presented in reports of humans with the acute respiratory distress syndrome (ARDS) which shares many histological lesion characteristics with bovine AIP. Patients at risk of ARDS that subsequently developed ARDS had higher concentrations of TNF-α and IL-1β in bronchoalveolar lavage fluid than those who did not develop ARDS.[26] Tumor necrosis

factor-α and IL-1β were elevated in lung and lavage fluid from calves experimentally infected with *M. haemolytica*.[27] It may be possible that TNF-α and IL-1β prime the lung for an AIP-trigger, such as increased generation of 3MEIN. More research is required to evaluate the association of bacterial infections either of the lung or other organs, particularly the liver, with feedlot-AIP.

Bovine respiratory syncytial virus has been implicated in AIP because BRSV-induced disease can manifest with similar clinical signs and pathology to AIP. Calves experimentally challenged with BRSV developed acute respiratory distress and at postmortem had gross and microscopic lesions similar to AIP.[11] In one study, BRSV was identified in 66.7% animals with feedlot-AIP whereas it was only identified in 44% animals that died from other causes ($P = 0.01$).[18] Data collected by others have not supported an association of AIP with BRSV infection.[19,20] In the present study, we identified an association ($P = 0.09$) of histological category with BRSV infection. However, controls had the greatest proportion of lung tissue positive (24.0%) for BRSV whereas AIP cases had the lowest proportion positive (8.3%). These data indicate that BRSV commonly infects feedlot cattle without inducing lesions identifiable using standard light microscopy techniques. Although only 25 animals served as controls, the prevalence estimate of BRSV infections in animals without observed lung pathology can be adequately estimated from the present study. The prevalence of BVDV infection in similar animals probably cannot be estimated accurately because BVDV infection may be associated with increased risk of death from non-respiratory disorders such as enteric

disease. Bovine respiratory syncytial virus infection would not likely lead to increased risk of death due to non-respiratory disorders. The standard error of the estimate of BRSV infection is 8.5%. Based on the data presented herein, the prevalence estimate and 95% confidence interval of BRSV infection in cattle without pulmonary disease are 24.0%, and 7.3 to 40.7%, respectively.

There are substantial differences in the presentation and signalment of BRSV and feedlot-AIP. These include: 1) calves develop marked pyrexia and are obtunded when BRSV-associated respiratory disease is induced experimentally,[11] and animals suffering AIP are typically not febrile and are alert.[6] In fact, feedlot personnel have often noted that AIP-affected animals can be unusually aggressive. 2) Acute interstitial pneumonia affects feedlot cattle with substantial *days on-feed*, 127.2 days in the present study. Infectious respiratory disease, such as that caused by BRSV, typically causes greatest morbidity in newly arrived cattle.[2] Further, AIP cases had the smallest proportion of animals positive for BRSV in the present study. Therefore, the majority of AIP cases seen in feedlot cattle are not likely to be BRSV-induced.

In situ infection of alveolar macrophages with both BRSV and BVDV resulted in a greater inhibition of specific macrophage functional parameters than either BRSV or BVDV alone.[28] Bovine viral diarrhea virus and BRSV co-infections were identified in 4.0% of animals. There was no evidence that co-infection was associated with histological grouping; however, the number of positive animals was limited. The greatest

proportion of BRSV-BVDV co-infections were identified in controls (8.0%). It is possible that synergy between these two viruses occurs *in vivo*, but was not detected in the present study.

The role of *Mycoplasma* spp. in feedlot-associated BRD is unclear. Bronchopneumonia cases were at greater risk to have a positive *Mycoplasma* culture than AIP cases ($P = 0.01$) or controls ($P < 0.01$). The odds ratios were 2.6 and 5.7, respectively. Fifty two percent of BP cases were culture positive for *Mycoplasma*. It is unclear if these organisms serve a primary or an opportunistic role in the pathogenesis of BP. Sixteen of the 50 BP cases had been at feedlots for greater than or equal to 120 days. Therefore, some of the animals that developed fatal BP were presumably close to marketing. Commonly used antimicrobials in animals close to marketing include those with a short slaughter withdrawal period such as ceftiofur sodium. This cell wall inhibitor would not be effective against *Mycoplasmas*, but this may be inconsequential if *Mycoplasmas* are not involved in BP pathogenesis, do not propagate disease as an opportunistic pathogen, or if effective treatment of aerobic bacteria leads to resolution of *Mycoplasma* infection. More research is required to better understand the ecology of *Mycoplasma* in feedlots, and the role *Mycoplasma* spp. play in BP.

ENDNOTES

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CHAPTER 5

Acute interstitial pneumonia in feedlot cattle II. Association with 3-methyleneindolenine, a toxic metabolite of 3-methylindole.

Summary

The objectives of this study were to compare lung 3-methyleneindolenine- (3MEIN) adduct concentrations in feedlot cattle that died from acute interstitial pneumonia (AIP) to animals that died from other causes, and to compare blood 3MEIN-adduct concentrations in feedlot cattle suffering from AIP to clinically healthy animals. Side-by-side prospective case-control studies were performed through collaboration with 14 feedlots located in Colorado, Nebraska, Kansas and Texas. Lung samples were collected from 186 animals during routine postmortem examination for histology, microbiology and toxicology evaluation. Blood samples were collected from animals with clinical manifestations of AIP and clinically healthy pen-mates. Histological diagnoses were categorized as AIP cases, bronchopneumonia (BP) cases, controls and *other* 3-Methyleneindolenine-adduct concentration (absorbence per μg protein) was determined in lung and blood tissue using an ELISA technique. Lung 3MEIN-adduct concentrations were greater in AIP ($P < 0.01$) and BP ($P < 0.01$) cases than controls. Absorbence per μg of protein did not differ between BP cases and AIP cases ($P = 0.39$). Blood 3MEIN-

adduct concentrations were greater in animals suffering from AIP than clinically healthy animals ($P < 0.01$) or animals with BP ($P = 0.02$). The odds of an AIP case being a heifer was 3.1 times greater than the odds of an AIP case being a steer. Increased pulmonary production of 3MEIN may be an important etiological factor in feedlot-associated AIP.

Introduction

Acute interstitial pneumonia (AIP) is a relatively sporadic bovine disease that may occur as isolated events or in outbreaks affecting a large proportion of a group at risk.[1,2] In pastured cattle, the disorder is known as acute bovine pulmonary edema and emphysema (ABPE), fog fever or cow asthma.[3] This malady transpires when cattle are moved from dry dormant pasture to lush pastures containing a high concentration of tryptophan (TRP).[4] Anaerobic ruminal fermentation of large amounts of TRP leads to a surge in 3-methylindole (3MI) generation. 3-methylindole is readily absorbed across the ruminal and intestinal wall, and disseminated throughout the body.[5,6] Bioactivation of 3MI by Clara cells leads to profound cellular injury in Clara and type I alveolar epithelial cells and ultimately AIP.[7,8] The putative compound responsible for effecting injury is the electrophilic metabolite of 3MI, 3-methyleneindolenine (3MEIN), which forms stable adducts with cellular macromolecules.[9-11] Other causes of AIP include bioactivated forms of 3-substituted furans (4-ipomeanol and perilla ketones),[12,13] and bovine respiratory syncytial virus (BRSV) infection.[14]

In contrast to the well described condition affecting pastured cattle, the etiology of

feedlot-associated AIP is uncertain. Feedlot-AIP is also known as dust pneumonia, allergic pneumonia, or pulmonary adenomatosis. Bovine respiratory syncytial virus is often causally implicated and in one study, investigators found a significantly greater proportion of BRSV infections in lung tissue from animals that died from AIP compared to animals that died from other conditions.[15] However, further studies have failed to identify an association between AIP and BRSV.[16,17] Concurrent bronchopneumonia has also been implicated with feedlot-AIP. One hundred and forty-four of 149 animals with AIP also had evidence of bronchopneumonia[18] and Sorden and others identified bronchopneumonia in 21 of 28 animals that succumbed to AIP.[17]

Recently there has been interest in the role of 3MI in feedlot-associated AIP. Cattle in western Canadian feedlots with AIP had significantly greater plasma 3MEIN and lower urinary concentrations of excreted 3MI metabolites (mercapturates) concentrations than animals without AIP.[16] However, significant differences between lung 3MEIN-adduct concentrations of AIP cases and controls were not observed in their study. The objectives of the present study were to compare lung 3MEIN-adduct concentrations in feedlot cattle that died from AIP to animals that died from other causes, and to compare blood 3MEIN-adduct concentrations in feedlot cattle suffering from AIP to clinically healthy animals.

Materials and methods.

Side-by-side prospective case-control studies were performed to evaluate and compare lung and blood 3MEIN-adduct concentrations from animals with AIP to comparison

groups. Feedlots were enrolled in the study by their consultant veterinarians and sample collection was completed during the period of May 15 to September 15, 1999.

In accordance with usual feedlot practices, mortality etiology was assigned by the consulting veterinarians or feedlot personnel. Those performing postmortem examinations were instructed on sample collection technique. In all, six samples were taken from the right lung. Tissue samples, each approximately 5 x 5 x 1 cm, included 2 adjacent samples from the dorsal aspect of the caudal lobe for 3MEIN analysis (n = 1) and histology (n = 1), 3 adjacent samples from the caudal margin of the middle lobe for microbiology (n = 2) and histology (n = 1), and 1 sample from the apex of the middle lobe for histology (n = 1). Tissue was collected in the following order to minimize the possibility of formalin contamination of tissue samples submitted for microbiological and toxicological evaluation. One sample from the caudal lung lobe and two samples from the base of the middle lobe were collected and each sample was placed in individual air-tight plastic bags and frozen. The remaining three samples were collected and fixed in 10% neutral buffered formalin.

Lung samples were collected from animals suspected to have died from AIP. Additionally, lung samples were collected from animals with apparently healthy lung tissue. Controls were selected from this group.

Where possible, whole-blood samples were collected from animals suspected to be

suffering from AIP. On the same day, blood was collected from either 1 or 2 clinically healthy animals from the home-pen of the animal suspected to be suffering from AIP. These animals served as controls. Approximately 9 ml of blood were collected via jugular venipuncture into an evacuated heparinized tube.[a] Blood was gently agitated then divided into two aliquots by pouring blood into 4.2-mL freezer-safe containers[b] and frozen. If the animal died, lung samples were collected as described above and histologically evaluated. At regular intervals, one of the investigators traveled to participating feedlots or their veterinary consultants and retrieved fixed lung and frozen lung and blood samples. Frozen tissues were stored at -20 °C (2 samples from middle lobe) and -76 °C (sample from caudal lung lobe and blood for 3MEIN analysis).

Frozen tissue samples for 3MEIN analysis were shipped to the Department of Pharmacology and Toxicology, University of Utah. Samples were analyzed for 3MEIN-adduct concentration by a enzyme-linked immunosorbent assay (ELISA) technique as previously described.[19] Briefly, tissue samples were thawed on ice and homogenized. Homogenized lung tissue was centrifuged at 105,000 x g and the supernate (cytosol fraction) harvested. Analyses were normalized for protein content of the sample. Samples containing 10 mg protein were homogenized and suspended in 1 M phosphate-buffered saline (PBS) at a concentration of 0.2 mg protein mL^{-1} PBS and 50-μL aliquots (10 μg protein) of each sample were dispensed into six replicate wells on standard 96-well polystyrene plates. The plates were gently agitated on an orbital shaker overnight to allow the protein to bind to the bottom of the wells. Primary polyclonal antibodies to

thioether adducts of 3MEIN were added to each well at a dilution of 1:100,000. Following incubation for 1 h at room temperature, the plate was washed with 0.1% (vol vol^{-1}) Tween 20 in PBS (PBS+T). Secondary antibody (donkey anti-sheep IgG coupled with horseradish peroxidase) was added to the wells at a 1:1000 dilution and incubated for 1 h at room temperature. Developer (0.05 M phosphate-citrate buffer containing 0.4 mg mL^{-1} *o*-phenylenediamine dihydrochloride and 0.3 mg mL^{-1} sodium perborate) was added to each well. Color was developed for 20 min and absorbance per μg protein at 450 nm was recorded by a microplate reader.[c] Absorbance per μg protein are arbitrary units that are directly proportional to primary antibody binding to tissue proteins.

Fixed tissue samples were sliced at 10 mm intervals. A block for light microscopical examination was selected from each lung sample and embedded in paraffin using routine methods, sectioned at 5 μm and stained with hematoxylin and eosin. Some samples were stained with periodic acid Schiff for protein transudate, and phosphotungstic acid hematoxylin for hyaline membrane. Stained lung tissue was examined using light microscopy and the findings were recorded. Criteria for diagnosis of AIP included multifocal or diffuse microscopic lesions of alveolar septal edema with serofibrinous exudation into alveolar spaces of hyaline membrane formation with or without admixed chromatin strands or type II epithelial hyperplasia. Cattle with this pattern of injury present in one or more lung section were categorized as AIP even if other concurrent disease processes (such as bronchopneumonia) were present. At the time of histological examination of lung tissue, the pathologists were not aware of the 3MEIN absorbance per

μg protein values.

Virus isolation, fluorescent antibody detection for specific viral pathogens, aerobic culture, and Mycoplasma culture was performed on the remaining frozen lung samples. The methods used were described in Chapter 3 of this dissertation.

At the time of sample acquisition, information on feedlot of origin, sex, days from arrival at the feedlot to death, and suspected cause of death were recorded.

Statistical analyses.

Lungs were categorized as AIP cases, bronchopneumonia (BP) cases, controls, and *other* disorders based on histological findings. Only the former 3 categories were used in formal statistical analyses of lung cytosol 3MEIN absorbance per μg protein. Animals from which blood samples were collected, were grouped into diagnosis categories that consisted of AIP (lesions confirmed histologically), AIP-suspects (AIP was suspected clinically but lung samples were not available for histological confirmation), BP cases (BP confirmed histologically) and controls (samples from clinically healthy pen-mates). A commercially available statistical analysis software package was used to generate estimates (and SEM), test statistics, confidence limits and *P* values where applicable.[d] Lung and blood 3MEIN absorbance per μg protein values were dependant variables of interest. Independent variables were histological category for lung tissue (AIP, BP, and control), or diagnostic category for blood (AIP, AIP-suspect, BP and control). Feedlot

was considered a random variable. A restricted maximum likelihood method was used to generate least squares means and a Satterthwaite procedure was used to approximate the denominator degrees of freedom for the estimates[20] and estimates were compared using Tukey's method of adjustment. The correlation of blood and lung 3MEIN-adduct concentrations was evaluated in animals from which both blood and lung samples were collected.

Where sex was recorded, lung samples from the AIP and control groups were categorized as being derived either from a heifer or steer. The odds ratios and 95% confidence interval (95% CI) of an AIP case being a heifer compared to a control being a heifer were calculated for all feedlots. The relative risk and 95% CI of an AIP cases being a heifer relative to a steer were calculated for the single feedlot that supplied the greatest number of AIP cases.

Results

Fourteen feedlots submitted lung samples from at least 1 animal during the study period. Lungs samples were collected from 186 animals. Three feedlots submitted samples from 1 animal and 3 feedlots submitted samples from greater than 20 animals. The feedlot that submitted the greatest number of samples supplied lung tissue from 60 animals. Four feedlots in Colorado, 3 in Kansas, 4 in Nebraska, and 3 in Texas were enrolled in the study. These feedlots submitted lung samples from 101, 49, 14 and 22 animals for lung 3MEIN analysis, respectively.

Lung 3MEIN-adduct concentration was determined in 108 AIP cases, 50 BP (bronchopneumonia) cases, 24 controls (congestion and edema, atelectasis and no abnormalities identified) and 3 animals with *other* disorders. The distribution of histological groupings for study feedlots is presented in Table 4.1. In brief, AIP was confirmed in at least 1 animal from all participating feedlots. One feedlot supplied 29 lung samples confirmed with AIP. Controls were submitted from 7 feedlots. Nine feedlots submitted at least 1 animal that was classified as a BP case. Two feedlots supplied 1 BP case each and 1 feedlot supplied 23 BP cases.

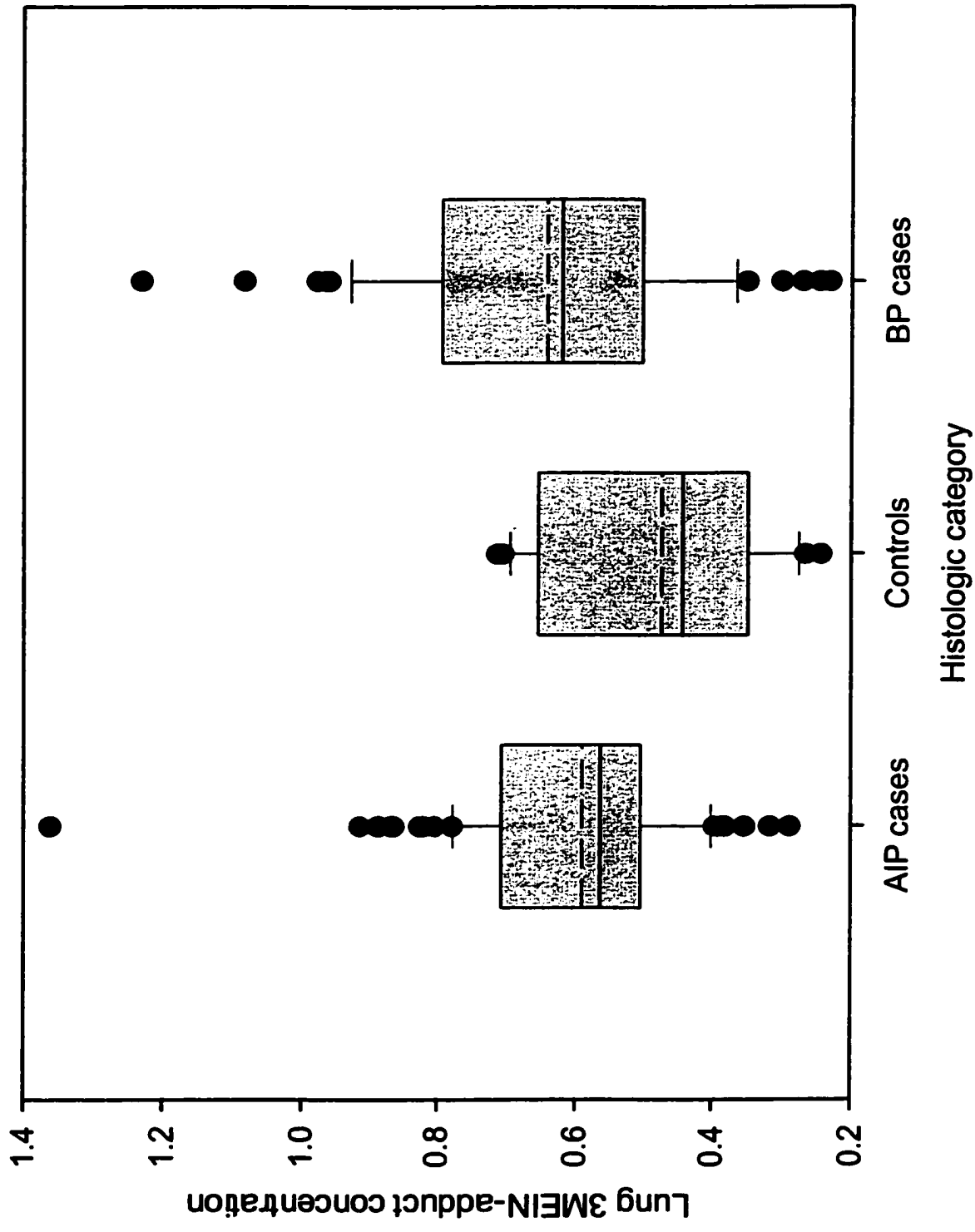
Lung 3MEIN-adduct concentrations are graphically presented in Figure 5.1. There was significant variation in lung 3MEIN-adduct concentrations between histological categories ($P < 0.01$). Least squares means ± SEM for lung 3MEIN-adduct concentrations for AIP cases, controls and BP cases were 0.58 ± 0.04, 0.45 ± 0.05 and 0.62 ± 0.04, respectively. Acute interstitial pneumonia cases had greater lung 3MEIN than did controls ($P < 0.01$; Table 5.1). Bronchopneumonia cases had greater 3MEIN-adduct concentration than controls ($P < 0.01$) but did not differ significantly from AIP cases ($P = 0.39$).

Blood samples were collected from 69 animals. Thirteen were confirmed to have AIP and 3 with BP. Twelve animals were suspected to have AIP but lung samples were not available for histological confirmation (these animals may have died without having postmortem samples collected or survived the disease event). A total of 41 healthy pen-

Table 5.1. Least squares means (and 95 % confidence limits) for lung 3-methyleneindolenine- (3MEIN) adduct concentration for acute interstitial pneumonia (AIP) cases, bronchopneumonia (BP) cases, and controls. Estimates with differing superscripts are significantly differently (at $P < 0.05$).

	Histological grouping		
	AIP cases	Controls	BP cases
Number of samples	108	24	50
3MEIN concentration	0.58^{a} (0.41, 0.75)	0.46^{b} (0.33, 0.58)	0.62^{a} (0.48, 0.76)

Figure 5.1. Box plot of lung 3-methyleneindolenine- (3MEIN) adduct concentration for acute interstitial pneumonia (AIP, n = 108) cases, controls (n = 24) and bronchopneumonia (BP; n = 38) cases. Means are represented by hatched lines. Whiskers represent 10 and 90 percentiles and solid dots are measurements beyond these percentiles.



mates of the AIP or AIP-suspects served as controls. Animals confirmed with AIP had higher blood 3MEIN-adduct concentrations than clinically healthy pen-mates ($P < 0.01$) and animals with BP ($P = 0.02$; Figure 5.2). Blood 3MEIN-adduct concentrations in clinically healthy animals did not differ from animals with BP ($P = 0.74$). Least squares means ± SEM for 3MEIN-adduct concentrations for AIP cases, BP cases and controls were 0.55 ± 0.03, 0.40 ± 0.05 and 0.45 ± 0.02, respectively (Table 5.2). The data did not support a difference between animals confirmed to have had AIP and those suspected, but not confirmed, to have AIP ($P = 0.94$).

The mean blood and lung 3MEIN-adduct concentrations for the 13 AIP cases from which whole blood samples were collected in addition to lung samples were 0 558 and 0.556, respectively The data did not support a correlation between lung and blood 3MEIN-adduct concentrations (r = 0.22, $P = 0.48$; Figure 5.3).

Of the 108 AIP cases, animal sex was recorded for 99 animals and included 68 heifers and 31 steers. Controls lung samples were collected from 10 heifers and 14 steers. The odds of an AIP case identified at postmortem being a heifer was 3.1 times greater than the odds of a control being a heifer (95% CI 1.9 to 4.9). Acute interstitial pneumonia cases included 24 heifers and 5 steers from the feedlot that supplied the greatest number of AIP cases ($n = 29$). There were 29,776 steers and 29,426 heifers *on-feed* for at least part of the study period within this feedlot and as such, represent the population at risk. The risk of an AIP case being a heifer was 4.9 times greater (95% CI 1.9 to 12.7) than the risk of

Table 5.2. Least squares means (and 95 % confidence limits) for blood 3-methyleneindolenine- (3MEIN) adduct concentration for acute interstitial pneumonia (AIP) cases confirmed histologically, controls, bronchopneumonia (BP) cases, and animals suspected to be suffering from AIP but not confirmed histologically. Estimates with differing superscripts are significantly differently (at $P < 0.05$).

	Histological grouping			
	AIP cases	Controls	BP cases	AIP suspects
Number of samples	13	41	3	12
3MEIN concentration	0.55^{a} (0.50, 0.60)	0.45^{b} (0.40, 0.49)	0.40^{bc} (0.30, 0.50)	0.53^{ac} (0.47, 0.59)

Figure 5.2. Box plot of blood 3-methyleneindolenine- (3MEIN) adduct concentrations for acute interstitial pneumonia (AIP, n = 13) cases, controls (n = 41), bronchopneumonia cases (n = 3) and animals suspected to be suffering from AIP but not confirmed (AIP suspects; n = 12). Means are represented by hatched lines. Whiskers represent 10 and 90 percentiles and solid dots are measurements beyond these percentiles.

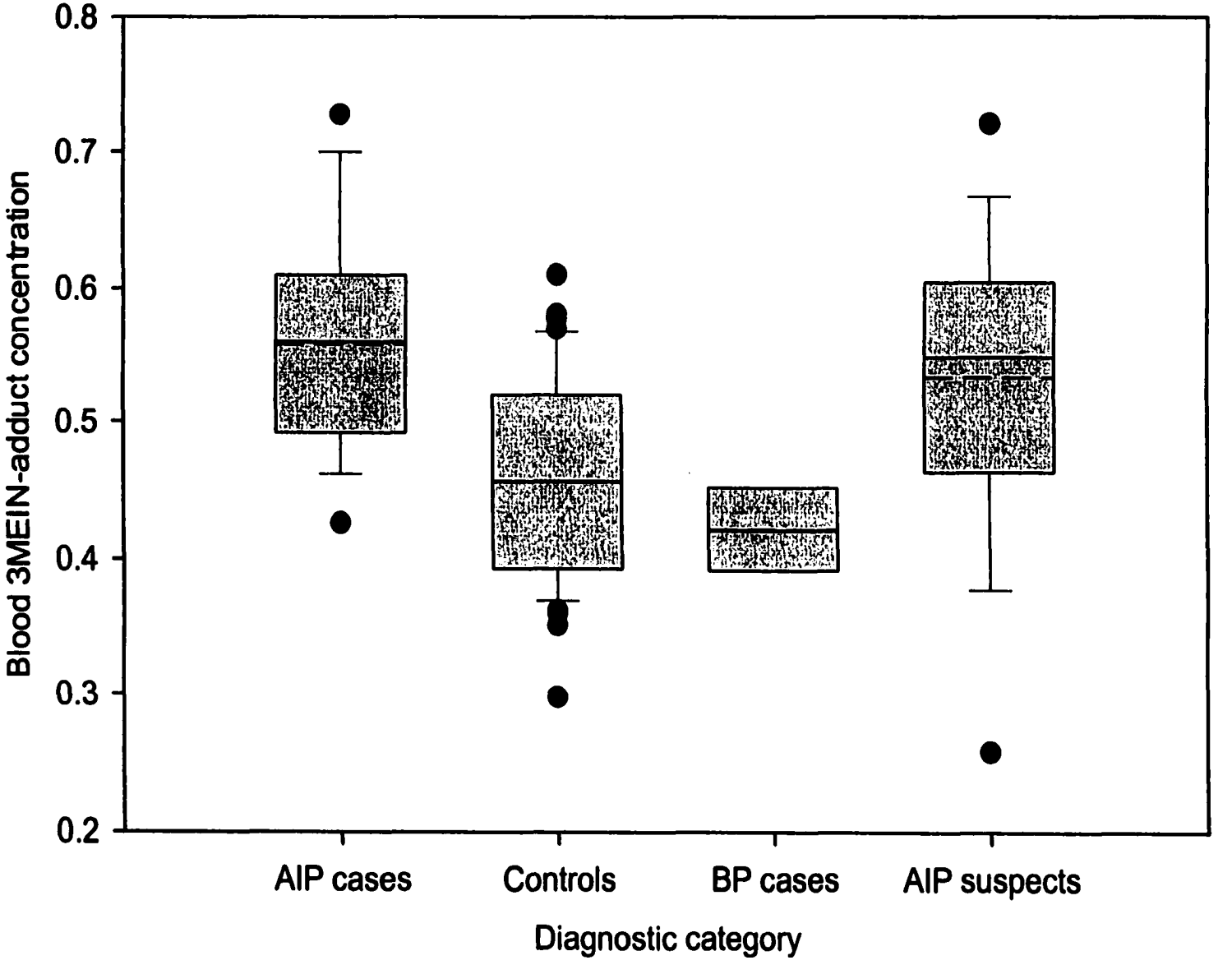
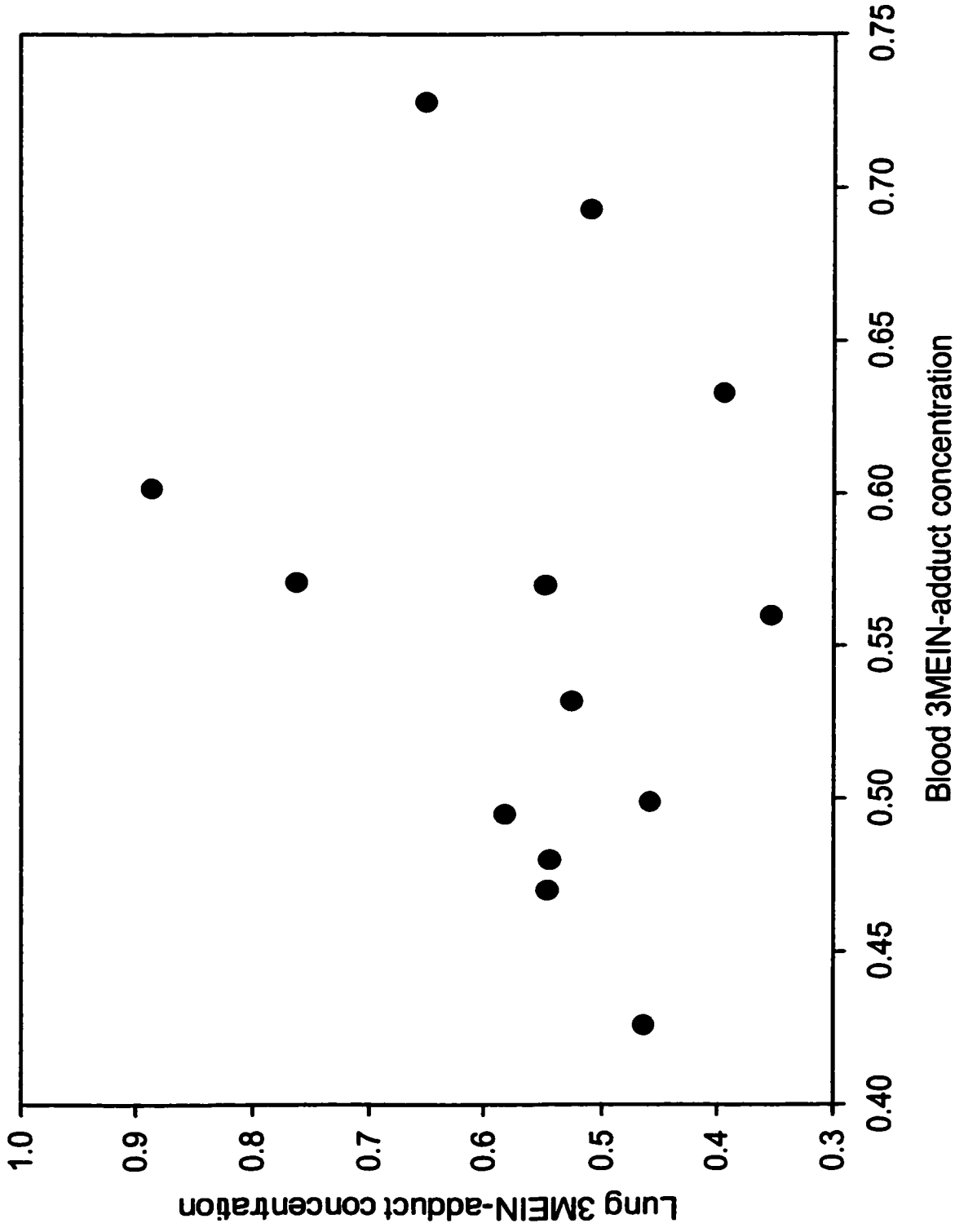


Figure 5.3. Scatter plot of blood and lung 3-methyleneindolenine- (3MEIN) adduct concentrations from acute interstitial pneumonia-affected animals (n = 13) from which both blood and lung samples were collected.



an AIP case being a steer.

Discussion

Acute interstitial pneumonia is a frustrating disease for feedlot personnel. A nation-wide feedlot survey reported that 3.1% of all cattle placed in feedlots developed AIP.[21] Although sporadic, this condition primarily occurs during the hottest months of the year and typically affects cattle close to marketing.[16,22] In one study, 80% of AIP cases occurred in cattle within 24 days of harvest.[16] As such, animals that succumb to AIP have incurred considerable direct and indirect costs. Many of the affected animals are apparently healthy and observed to be eating only a matter of hours prior to the onset of disease. Animals develop acute respiratory distress, adopt a sway-back posture, exhibit a respiratory grunt, and often have a profuse frothy discharge from the mouth and nares.[2,3] Many affected animals die in their home-pen, while being moved to treatment facilities, or within a few days after administration of a treatment regimen. When recorded, affected animals are typically afebrile.[2] The rumen of AIP-affected cattle are typically full at postmortem indicating an acute pathogenesis. A chronic disease process would result in a period of decreased feed intake or anorexia and consequentially, a rumen that is not full.

We demonstrated that 3MEIN adducts were present in lung tissue of all cattle regardless of histological category. However, animals with AIP had greater lung 3MEIN-adduct concentration than animals with apparently normal pulmonary tissue, and greater blood

3MEIN-adduct concentrations than clinically healthy pen-mates. It is unclear if the majority of the blood 3MEIN-adducts originated in hepatic or pulmonary tissue. It seems likely, however, that a significant portion of blood 3MEIN-adducts were derived from bioactivation of 3MI by lung P450 enzymes because the lung enzymes are the most efficient at catalyzing this process.[8,11,23] Hence, AIP is associated with increased pulmonary metabolism of 3MI to 3MEIN. Increased 3MEIN-adduct concentrations may have occurred secondary to altered rumen metabolism, or induction of P450 and prostaglandin H synthetase (PHS) enzymes that bioactivate 3MI.

3-Methylindole is a product of normal anaerobic ruminal fermentation of TRP and is readily absorbed across the rumen wall and disseminated hematogenously.[5,6] Pasture-associated AIP occurs when animals are abruptly exposed to a diet high in TRP concentration,[1,4] which gives rise to an abnormal surge in rumen-microbial generation of 3MI. Bioactivation of 3MI chiefly occurs by specific families of P450 enzymes and to some extent by PHS.[7,8,23] Inhibition of P450 function prevented cellular injury following 3MI challenge whereas inhibition of PHS was only partially protective.[24,25] Hence both enzymes may be required for 3MI-induced pulmonary disease. In ruminants, alveolar type I epithelial and Clara cells are the target cells for 3MI-induced injury; Clara cells contain high concentration of both P450 and PHS.[9,26] The electrophile 3MEIN is one of several metabolites of 3MI that have been identified.[27] It is proposed that 3MEIN is responsible for 3MI-induced AIP and occasions injury through covalent binding to cellular macromolecules.[9,11] Metabolism of 3MI also produces free radicals that may

contribute to cellular injury.[28]

The results presented herein are in partial agreement with the only other study in which an association between feedlot-AIP and 3MEIN was evaluated.[16] Those investigators found that animals with AIP had greater plasma 3MEIN-adduct concentrations than animals that did not have AIP. Their controls consisted of clinically healthy animals as well as animals suspected to be suffering from AIP. They did not find evidence to support altered lung 3MEIN-adduct concentration in AIP cases compared to reference animals. Blood samples from our study were analyzed in a similar manner to the plasma collected in that study. Lung 3MEIN optical densities reported herein are for the cytosol fraction whereas 3MEIN-adduct concentration was determined in total lung homogenate and mitochondrial fraction in their study. Differences in lung fractions analyzed, and number and type of controls may explain the disparities between the results of these studies.

It is unlikely that increased pulmonary 3MEIN production was the sole cause of AIP in the present study because lung 3MEIN-adduct concentrations did not significantly differ between AIP and BP cases ($P < 0.39$). However, one may postulate that a high concentration of 3MEIN adducts is a necessary, yet not in itself sufficient, cause of feedlot-associated AIP. If this is so, then other factors that increase the susceptibility of the lung to 3MEIN must be present. At least 1 of these unknown contributing factors must not have been present in BP cases. Calves experimentally challenged with both 3MI and BRSV developed more severe disease than those exposed to 3MI alone.[29] This

suggests that an infection with BRSV, and potentially other respiratory pathogens, lowered the 3MI challenge required to induce AIP. Data presented elsewhere found no association of BRSV and AIP, in fact only 8.3% of AIP cases were positive for BRSV whereas 24.0% of controls were positive. Lung 3MEIN concentrations in AIP-affected animals with BRSV infections were similar to those from BRSV-negative animals (P = 0.23). The means ± SEM were 0.65 ± 0.05 and 0.58 ± 0.02, respectively. Hence, we did not observe a synergistic relationship between BRSV and 3MEIN. If an aerobic bacterial infection increases the risk of AIP as has been proposed,[30] then we would have expected BP cases to also have AIP because they had similar 3MEIN-adduct concentration to AIP cases. It is unclear what role, if any, concurrent aerobic bacterial infection has in the pathogenesis of feedlot-associated AIP based on the data presented here and elsewhere.

Animals with AIP had been at the feedlot for an average of 127.2 days and presumably had been exposed to their finishing diet for weeks and in many cases months. Typically, feedlot personnel ensure cattle receive a consistent finishing diet and control variation in dry matter intake. Therefore, increased 3MEIN-adduct concentration in animals suffering from feedlot-AIP in the present study was not associated with abrupt dietary changes in contrast to pasture-associated AIP. However, others have reported that ruminal fluid pH in animals with typical postmortem lesions of AIP was elevated compared to the animals that had died from other maladies.[31] Ayroud and others found that the concentration of cellulolytic bacteria in the rumen was less than that of controls.[16] Results from these studies indicate that AIP and by association, increased pulmonary 3MEIN production,

occur concurrently with altered ruminal metabolism. Rumen metabolic parameters were not evaluated in the present study.

The odds of an AIP case being a heifer was 3.1 times greater than odds of an AIP case being a steer. Within the feedlot that supplied that largest number of AIP cases, the relative risk estimate was 4.9. As AIP is a relatively rare event, the OR is a reasonable approximation for relative risk and this appears to be so because 3.1 may be considered a relatively good approximation for 4.9.[32] In another investigation, all AIP cases (n = 31) were heifers, although these cases were derived from a population of approximately 87.5% heifers.[16] These data indicate feedlot heifers are at a substantially greater risk of succumbing to AIP than steers. Day-to-day management of steers and heifers is similar except for types of implant administered and addition of melengestrol acetate (MGA)[c] to the diet. Popp and others demonstrated that sheep consuming MGA developed greater plasma concentrations of 3MEIN adducts, developed AIP more acutely, and displayed more severe clinical signs than controls.[33] The authors speculated that inclusion of MGA in the diet induced PHS or P450 in lung tissue and this may have been responsible for the increased 3MEIN-adduct concentration. 3-Methyleneindolenine has not been identified as a 3MI metabolite of PHS but is a metabolite of P450.[9,10] Therefore, MGA induced-P450 enzymes may explain the over representation of AIP seen in heifers. Melengestrol acetate was included in the diet of all female cattle in the present study and all cattle were implanted with growth promotants. Thus, the use of MGA or implants could not be evaluated as possible risk factors for AIP. Further research is required to evaluate factors

that may result in over-representation of AIP in heifers.

Animals with BP had similar concentration of lung 3MEIN to AIP cases. Increased production of 3MEIN may have resulted in cellular injury that, although insufficient to induce AIP without the presence of other factors, predisposed the lower respiratory tract to bacterial colonization. Alternatively, the pathogenesis of BP may have led to an increase in 3MEIN production. Increased pulmonary interleukin-1β and TNF-α have been demonstrated in calves experimentally challenged with *Mannheimia haemolytica*,[34] Pro-inflammatory cytokines and interferons increased the expression of some P450s in the liver.[35] It is possible that specific pulmonary P450 enzymes are induced in response to cytokine exposure so the potential for post-inflammation increases in pulmonary 3MEIN production exists. Only 3 blood samples were collected from animals histologically classified as BP cases. However, these 3 animals had significantly lower blood 3MEIN absorbance per μg protein than AIP cases ($P = 0.02$). This may indicate that BP cases experienced either increased retention of 3MEIN in lung tissue rather than increased production, or increased pulmonary production of 3MEIN without increases in other organs. More research is required to evaluate a possible role of 3MEIN in the pathogenesis of BP.

ENDNOTES

a. Vacutainer®, Becton Dickinson and Company, Franklin Lakes, NJ, 07417.

b. Sterilized Freestanding Cryule (4 ml), Wheaton Scientific Products, Millville, NJ.

c. Molecular Devices Corporation, Sunnyvale, CA.

d. The SAS System for Windows release 8.00, SAS Institute Inc., Cary, NC.

e. Pharmacia and Upjohn, Kalamazoo, MI.

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CHAPTER 6

Time-dependant changes in plasma 3-methylindole and blood 3-methyleneindolenine-adduct concentrations in feedlot cattle.

Summary

The objectives of this study were to describe time-dependant patterns and magnitudes of plasma 3methylindole (3MI) and blood 3-methyleneindolenine- (3MEIN) adduct concentrations in feedlot cattle. The study population included 64 single-source yearling steers in a completely randomized study design with repeated measures. The steers were assigned to 2 groups of 32 animals each. Groups of cattle were managed slightly differently for the first 8 weeks of sample collection. Blood samples from Group 1 were collected before the morning diet was delivered whereas samples from Group 2 were collected 2 to 3 hours after the diet was delivered. Blood samples were collected from all steers approximately 4 times per week for 3 weeks, 3 times per week for the following 5 weeks, then weekly for an additional 10 weeks. All plasma samples from Groups 1 and 2 were analyzed for 3MI concentrations. Blood samples collected during the first 8 weeks from Group 2 were analyzed for 3MEIN-adduct concentration. Mean blood 3MEIN-adduct concentration increased to a maximum value on day 33 (1.05 units per μg protein) then decreased to a minimum on day 54 (0.30 units per μg protein). Plasma 3MI

concentrations initially decreased and remained low until after day 54. There were significant differences in 3MI concentrations between groups over time. Animals in Group 2 gained weight faster than Group 1, which may reflect differences in dry matter consumption that also may have affected plasma 3MI concentrations. Blood 3MEIN-adduct or plasma 3MI concentrations were not associated with deleterious effects on live weight gains. Blood 3MEIN-adduct concentrations peaked during the period of greatest risk of the bovine respiratory disease (BRD) complex. Conversely, plasma 3MI concentrations decreased during the same period. Animal to animal variation in metabolic capacity to convert 3MI to 3MEIN may be of more importance than differences in plasma 3MI concentration.

Introduction

Bovine respiratory disease (BRD) complex is the leading cause of morbidity and mortality in feedlot cattle.[1-3] The majority of BRD events occur soon after cattle arrive at feedlots and accordingly, the disease complex is commonly referred to as shipping fever. It is well accepted that BRD results from an interaction of host susceptibility, stressors, and infectious pathogens.[4,5] Traditionally, viral pathogens are thought to be primary invaders of the respiratory tract which may give rise to bacterial colonization of the lower respiratory and consequentially, bronchopneumonia.

Rumen-generated 3-methylindole (3MI) is a toxicant that may contribute to BRD of feedlot cattle even though toxins have not generally been considered important in the

pathogenesis. 3-methylindole is produced in the rumen via microbial fermentation of tryptophan.[6] Following production, 3MI is readily absorbed across the ruminal and intestinal walls then hematogenously disseminated throughout the body.[7-9] Excessive ruminal production of 3MI is associated with development of severe pulmonary injury known as acute interstitial pneumonia (AIP).[10,11] This form of pulmonary injury has also been referred to as atypical interstitial pneumonia, fog fever, acute bovine pulmonary edema and emphysema, and cow asthma.[12] It is possible that at concentrations below those required to induce AIP, 3MI through its reactive metabolites, may injure pulmonary tissues or host defenses and thereby potentiate the development of BRD complex.

Experimentally-induced respiratory disease was most severe when calves were challenged with both 3MI and bovine respiratory syncytial virus (BRSV) compared to animals challenged with either 3MI or BRSV alone.[13] The synergy observed between 3MI and BRSV may also occur with other respiratory pathogens of importance to the pathogenesis of feedlot-associated BRD. Data from a field study demonstrated that feedlot animals with greater concentrations of serum 3MI were more likely to be treated for BRD than animals with lower serum 3MI.[14]

It is unlikely that 3MI directly contributes to the pathogenesis of BRD because it must be bioactivated in the lung to induce pulmonary injury.[10,11,15] Two enzyme systems have been shown to metabolize 3MI producing intermediates that are thought to be involved in the pathogenesis of 3MI-related pulmonary disease; cytochrome P450 enzymes[16-20] and

prostaglandin H synthetase (PHS).[21,22] Inhibition of both enzyme systems has been shown to provide protection from 3MI-induced injury using both *in vivo* and *in vitro* experimental models.[21,22] The presence and functionality of both enzymes may be required for 3MI to effect cellular toxicity seen in naturally-occurring disease and these enzymes are abundant in ruminant pulmonary Clara cells.[10,15,18,23]

The proximate metabolite from P450 enzyme metabolism which is believed responsible for a majority of 3MI-induced pulmonary disease via this pathway is 3-methyleneindolenine (3MEIN).[16,24,25] This electrophile covalently binds to cellular macromolecules resulting in profound cellular dysfunction.[25] Other 3MI-metabolites have also been identified and these electrophiles may also contribute to cellular injury.[26,27] Feedlot animals that died from bronchopneumonia had greater lung 3MEIN-adduct concentration than animals without lung disease.[28] It is possible that if 3MI and 3MEIN contribute to the pathogenesis of BRD, production should be increased during times when cattle are at greatest risk of BRD. However, variation in 3MI or 3MEIN concentrations in feedlot cattle during times of greatest risk of respiratory disease have not been reported. This research was undertaken to describe the time-dependant patterns and magnitudes of plasma 3MI and blood 3MEIN concentrations in feedlot cattle.

Materials and Methods

The study protocol was reviewed and approved by the Colorado State University Animal Care and Use Committee. Sixty-four single-source, crossbred yearling steers (318 kg)

were used for this research and managed as 2 equally sized groups. Blood samples from Group 1 were collected during times they would normally be fed. Group 2 animals were allowed to eat for 2 to 3 hours prior to sample collection. Blood samples were collected approximately 4 times per week for 3 weeks, 3 times per week for the following 5 weeks, then weekly for the remainder of the research. All plasma samples were analyzed for 3MI concentration. 3-methyleneindolenine-adduct concentrations from Group 2 animals were determined in whole blood samples collected during the first 8 weeks of sample collection. All animals were monitored daily by trained feedlot personnel for manifestations of illness.

Animals

Animals used in this project were procured from a single-source in western Oklahoma. Upon arrival, the steers were moved to a receiving pen and provided with long-stem grass hay and *ad libitum* access to water. Within 24 hours after arrival at the feedlot, cattle were moved through a cattle handling facility, vaccinated with modified-live bovine herpesvirus 1, parainfluenza 3 virus, bovine viral diarrhea virus and BRSV[a]. Cattle were also administered *Clostridium perfringens* C and D toxoids[b], doramectin (10 mg/ml)[c] at 2 ml per 100kg body-weight, and implanted with a growth promotant containing 120 mg trenbalone acetate and 24 mg estradial[d].

The following day (day -34) the steers were weighed, provided with unique identification ear-tags, and metaphylactically administered long-acting oxytetracycline (200 mg/ml)[c]

subcutaneously at a dose of 10 ml per 100 kg body-weight. Metaphylaxis was used to reduce the likelihood of cattle developing BRD.[29] Animals were assigned to 1 of 8 pens so that each pen would contain 8 animals. Allocation to pens was based on body weights so that the mean body weight of each pen was similar. The steers were reweighed on day -33 and moved to their allocated pens. Steers were reweighed on day -1, then at approximately two-week intervals (days 13, 28, 42, 54, 68, 82 and 97) and twice at the completion of the research (days 117 and 118). The average of the 2 initial and 2 final weights were used as the arrival and shipment weights, respectively.

Animals were transported to a commercial abattoir[f] on day 118. Carcass characteristics including hot carcass weight, liver abscess score, marbling units, longissimus muscle cross section area and fat thickness over the 12th rib, kidney-pelvic-heart fat score, and USDA quality and yield grades were recorded. Final weights were adjusted to reflect transportation "shrink" by multiplying them by 0.96. Dressing percentage was calculated by dividing adjusted final weight into hot carcass weight.

Feeding

Study animals selected for the research had been consuming a diet that consisted primarily of mature pasture for at least a month prior to their arrival at the feedlot. After the steers were moved from the receiving pen to their allocated pens, the were fed a straw-based diet to mimic the intake of cattle consuming mature pasture. Crude protein concentration of the straw diet was 6.1% on a dry matter basis. A mineral block[g]

designed for range cattle was included in the bunk of each pen during the pasture simulation phase. This straw-based diet was fed for 32 days to allow for thorough adaptation. On day -1, cattle were only fed in the morning and then fed again during the afternoon of day 0. On this day, the first of 4 "step-up" diets (Table 6.1.) was fed. The period of feed restriction from day -1 to day 0 was used to simulate a feed restriction associated with typical transportation of cattle from the ranch of origin or sale barn to a feedlot. After this, steers were fed twice daily and provided subsequent step-up diets on the afternoon of days 5, 8, and 12. The finishing diet (Table 6.1.) was first delivered to the cattle on the afternoon of day 15. The weight of each feed delivery was recorded and adjusted to reflect dry matter content. Dry matter delivery was further adjusted for feed refusal to provide a more accurate estimate of dry matter consumed by each pen.

Sample collection

Animals were brought to the animal handling facility for blood collection purposes in 2 groups. Group 1 consisted of pens 1 through 4 and Group 2 consisted of pens 5 through 8. For each sample day, pens were brought to the animal handling facility in a randomized order within each Group. Blood samples were collected on days -2, -1, 1, and 2, most days that the diet was changed, and each day subsequent to a diet change (days 5, 6, 9, 12, 13, 15 and 16). Because of severe inclement weather, samples were not collected on day 8 which was the day the 3rd step-up diet was initially delivered to the cattle. Starting on day 19, blood samples were collected 3 times per week for 5 weeks, then weekly for the remainder of the research, a further 10 weeks. During days -2

Table 6.1. Percent of dietary dry matter by commodity included in each of the 4 step-up and finishing diets. Dry matter content as a percent of as-fed is also included.

	Diet				
Commodity	1st step-up	2nd step-up	3rd step-up	4th step-up	Finishing
Steam flaked corn	34.715	45.972	52.571	65.359	69.141
Alfalfa hay	31.371	24.030	11.541	8.128	-
Corn silage	27.756	22.678	27.229	17.260	20.943
CCDS*	3.979	4.064	3.903	4.124	4.003
Soybean meal	1.798	2.547	2.831	1.924	1.634
Salt (NaCl)	0.173	0.176	0.169	0.179	0.174
Limestone	0.171	0.413	0.697	0.920	1.106
Soy oil	0.008	0.014	0.026	0.036	0.044
Fat	-	-	0.677	1.430	2.083
Urea	-	-	0.320	0.607	0.844
Monensin[h]	0.007	0.007	0.007	0.007	0.014
Tylosin[i]	-	0.001	0.002	0.003	0.004
Trace minerals[j]	0.015	0.015	0.014	0.015	0.014
Vitamin E[k]	0.006	0.006	0.006	0.006	0.006
Vitamin A[l]	0.001	0.001	0.001	0.001	0.001
Dry matter content	69.01	70.48	67.70	71.52	69.43

* Condensed corn distiller's solubles.

through 54 (intensive sampling phase), Group 1 animals were moved to the animal handing facility immediately prior to the morning feed delivery whereas Group 2 animals were removed from their pens approximately 2 to 3 hours after the morning feed delivery. Following the intensive sampling phase, both Groups were removed from their pens at the same time for blood collection purposes.

Sample processing and analysis

Blood samples were collected by jugular venipuncture into 2 evacuated, 10-ml blood collection tubes[m]; one of which contained potassium EDTA and the other sodium heparin. Blood samples were kept on ice and processed immediately after the cattle were returned to their pens. Whole-blood containing potassium EDTA was centrifuged at 3200 rpm for 20 minutes at 4° C using a refrigerated centrifuge[n]. Aliquots of plasma were harvested, snap-frozen in liquid nitrogen, then stored at -20° C. Aliquots of heparinized whole-blood samples were frozen and stored at -20° C.

Plasma 3MI concentrations were determined by use of a microplate methods adapted from procedures as described.[14,30] Samples were extracted with absolute ethanol and then centrifuged. The supernatant was harvested and mixed with a solution containing 4-dimethylaminobenzaldehyde. A reaction product is formed when 3MI is present. Absorbance was determined spectrophotometrically, and the concentration of 3MI was calculated by comparing the results to a standard curve. 3-methylindole concentrations were determined for all samples obtained from all animals at all time points (n = 2204)

Whole-blood samples were analyzed for 3MEIN absorbance per μg protein using methods described elsewhere.[24] Briefly, prepared samples of known protein content were placed into wells of a standard 96-well polystyrene plate. Primary polyclonal antibodies to thioether adducts of 3MEIN were added to each well. Following incubation, a second antibody (donkey anti-sheep IgG coupled with horseradish peroxidase) was added to the wells. Developer was added following a second incubation and color was measured spectrophotometrically. Absorbance per μg protein are arbitrary units directly proportional to primary antibody binding to tissue proteins.

3-methyleneindolenine-adduct concentrations were measured per μg protein in blood samples collected during the intensive sampling phase (day -2 through 54) from Group 2 animals (n = 864 samples). These samples were selected because of limited fiscal resources, most respiratory disease events typically occur during the first 8 weeks that cattle are at feedlots, and Group 2 animals were apparently less affected by handling than Group 1 animals.

Statistical Analyses.

Individual and pen-level mean daily weight gains (MDG_i and MDG_p, respectively) were calculated for the pasture simulation phase and subsequent 2 weekly periods. Mean daily dry matter intake for cattle in a pen (DMI) was calculated for each 2-week period using daily dry matter consumed per pen of cattle divided by the number of animal-days for the period. Feed efficiency ratios (FE) were estimated for each weigh-period by dividing

DMI into MDG_p.

Statistical analyses were performed using commercially available software[o]. Period 1 was considered day -33 to day -1, then periods 2 through 9 represented approximately 2-week intervals between the times that animal weights were recorded; periods 2, 3, 4, 5, 6, 7, 8, and 9 were comprised of 14, 15, 14, 12, 14, 14, 15, and 21 days, respectively. Study animals were considered the experimental unit for the analysis of live weights, MDG_i, carcass characteristics, plasma 3MI and blood 3MEIN concentrations. Pens were considered experimental unit for the analysis of DMI and FE. Time-period was considered a classification variable for the analysis of MDG_i, DMI and FE. Trial day was treated as a continuous variable when constructing 3MI and 3MEIN models. First order auto-regressive matrices were used to model the covariance structure within experimental units over time.[31] Carcass characteristics measured on a continuous scale were analyzed as a one-way analysis of variance with Group as the effect of interest. Categorical carcass characteristics (USDA quality and yield grades) were analyzed using a Chi-square goodness-of-fit test. Arrival weight was included as a covariate when analyzing MDG_i, DMI, continuous carcass characteristics, 3MI and 3MEIN if its P value was < 0.10.

The effect of maximum 3MI and 3MEIN concentration on day 1, 2, 5, 6 or 9, on MDG_i was evaluated. Area under the curve[p] (AUC) for 3MI and 3MEIN was calculated for each inter-sampling time-period. Animal- and pen-level AUC for each weigh-period was calculated and the effect of plasma 3MI and blood 3MEIN AUC on MDG_i and MDG_p

was evaluated. Arrival weight and pen-level DMI were included in the models as covariates if their P values were < 0.10.

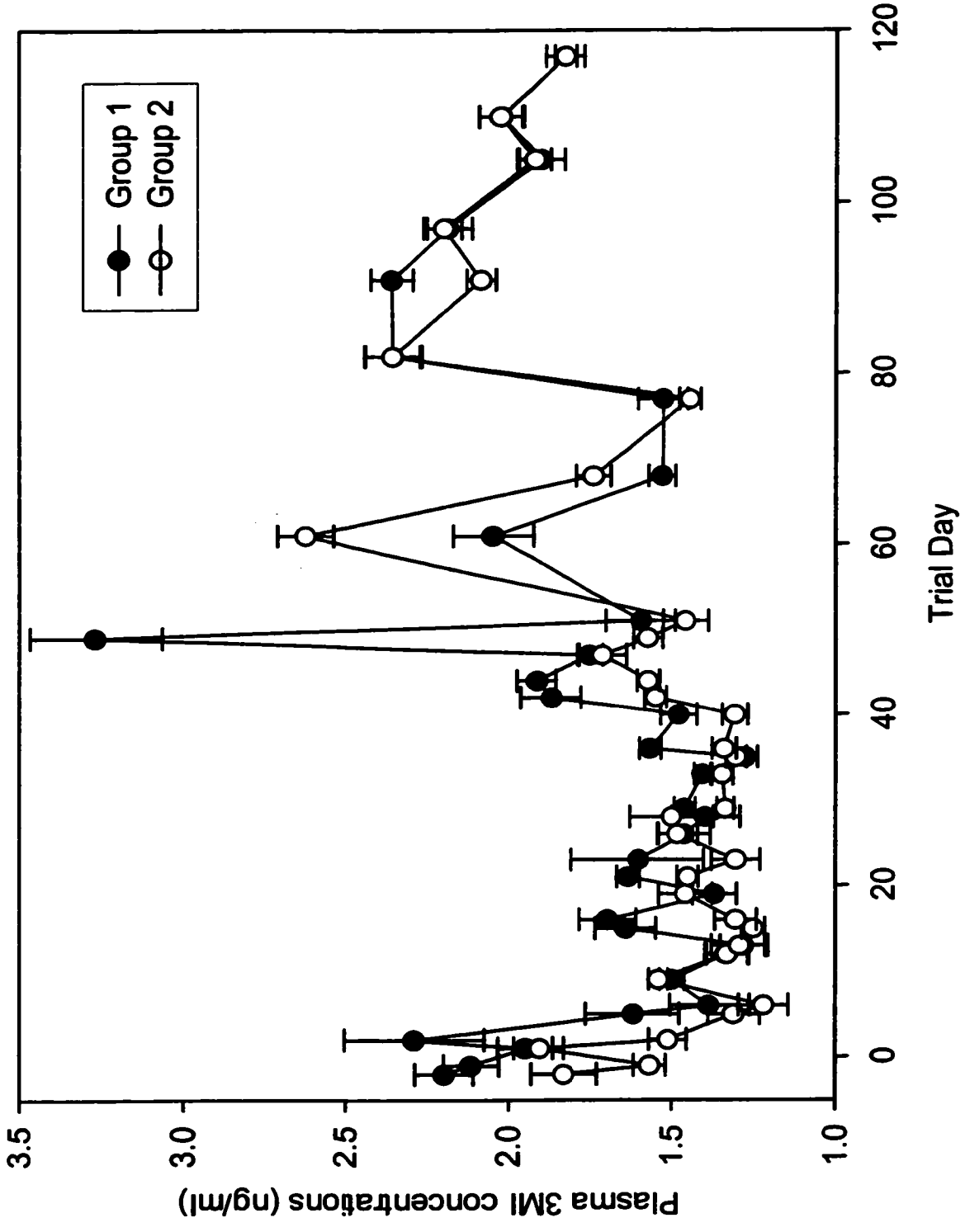
Results

No animals developed manifestations of illness or died during the study period.

Averaged across all time points, plasma 3MI concentration for Groups 1 and 2 differed ($P < 0.01$) and their means ± SEM were 1.60 ± 0.04 and 1.77 ± 0.04 ng/ml. The maximum, minimum and median 3MI concentrations were 7.57, 0.12, 1.58 ng/ml, respectively. A statistical interaction between sample day and Group was identified ($P < 0.01$) in the final model of plasma 3MI concentrations indicating the difference in 3MI concentrations between Groups varied significantly over time. Plasma 3MI concentrations on the day of a diet change did not differ ($P = 0.60$) from plasma 3MI concentrations on the day after the diet change. Averaged across the diet changes, means were 1.55 ± 0.08 and 1.53 ± 0.08 ng/ml, respectively. Plasma 3MI concentrations were lower during days -2 to 54 compared to the phase when samples were collected weekly ($P < 0.01$; Figure 6.1); mean 3MI concentrations were 1.58 ± 0.07 and 2.00 ± 0.07 ng/ml, respectively.

Mean 3MEIN-adduct concentration was 0.60 ± 0.01 units per μg blood protein. The maximum, minimum and median 3MEIN-adduct concentrations were 1.05, 0.30 and 0.59 units per μg blood protein, respectively. Blood 3MEIN-adduct concentrations varied

Figure 6.1. Mean plasma 3-methylindole (3MI) concentrations for Groups 1 and 2. Error bars represent standard error of the means.



quadratically with sample day ($P < 0.01$). Concentrations increased after day -1, peaked on day 33 and then decreased to day 54 (Figure 6.2). Mean 3MEIN-adduct concentration was 0.80 ± 0.02 and 0.40 ± 0.01 units per μg blood protein on day 33 and 54, respectively. Blood 3MEIN-adduct concentrations on the day of a diet change was less than ($P < 0.01$) blood 3MEIN-adduct concentrations on the days following a diet change. On the day before and day of a diet change, mean 3MEIN-adduct concentrations were 0.61 ± 0.02 and 0.68 ± 0.02, respectively.

Plasma 3MI concentrations were not strongly predictive of blood 3MEIN-adduct concentration, each 1 ng/ml increase in plasma 3MI concentration was associated with a 0.024 unit decrease in 3MEIN absorbance per μg protein (Figure 6.2; $P = 0.04$). This weak relationship is illustrated in the scatter plot of blood 3MEIN-adduct by plasma 3MI concentration (Figure 6.3).

Mean arrival and day -1 weights of steers were 317.9 ± 2.6 and 368.3 ± 3.1 kg, respectively (Figure 6.4). There was no statistically detectable difference in animal weights between Groups 1 and 2 at arrival ($P = 0.35$) or on day -1 ($P = 0.83$). However, final weight varied between Groups ($P = 0.02$). Mean final weights for Groups 1 and 2 were 564.1 ± 7.5 and 583.5 ± 7.5 kg, respectively. Mean daily weight gains of animals in Groups 1 and 2 did not differ ($P = 0.53$) while receiving the roughage diet (period 1) and overall MDG_i was 1.57 ± 0.05 kg. A statistical interaction between Group and period ($P < 0.01$) was identified in the final model of MDG_i from which period 1 was excluded

Figure 6.2. Mean plasma 3-methylindole (3MI) and blood 3-methyleneindolenine- (3MEIN) adduct concentration for Group 2 during the intensive sampling phase. Error bars represent standard error of the means.

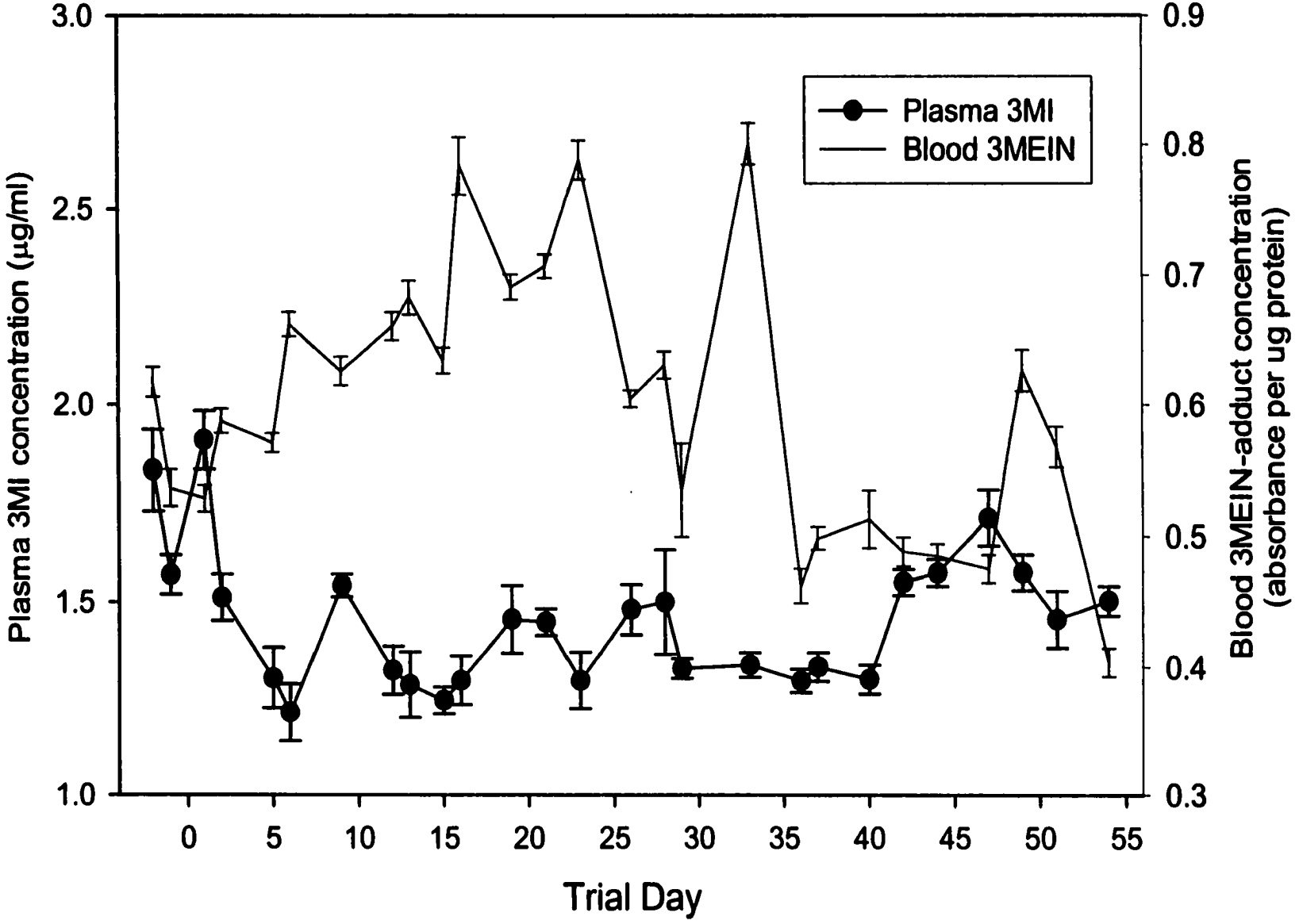


Figure 6.3. A scatter plot of plasma 3-methylindole (3MI) and blood 3-methyleneindolenine- (3MEIN) adduct concentrations. Each ng/ml increase in plasma 3MI concentration was associated with a 0.024 unit decrease in blood 3MEIN-adduct concentration.

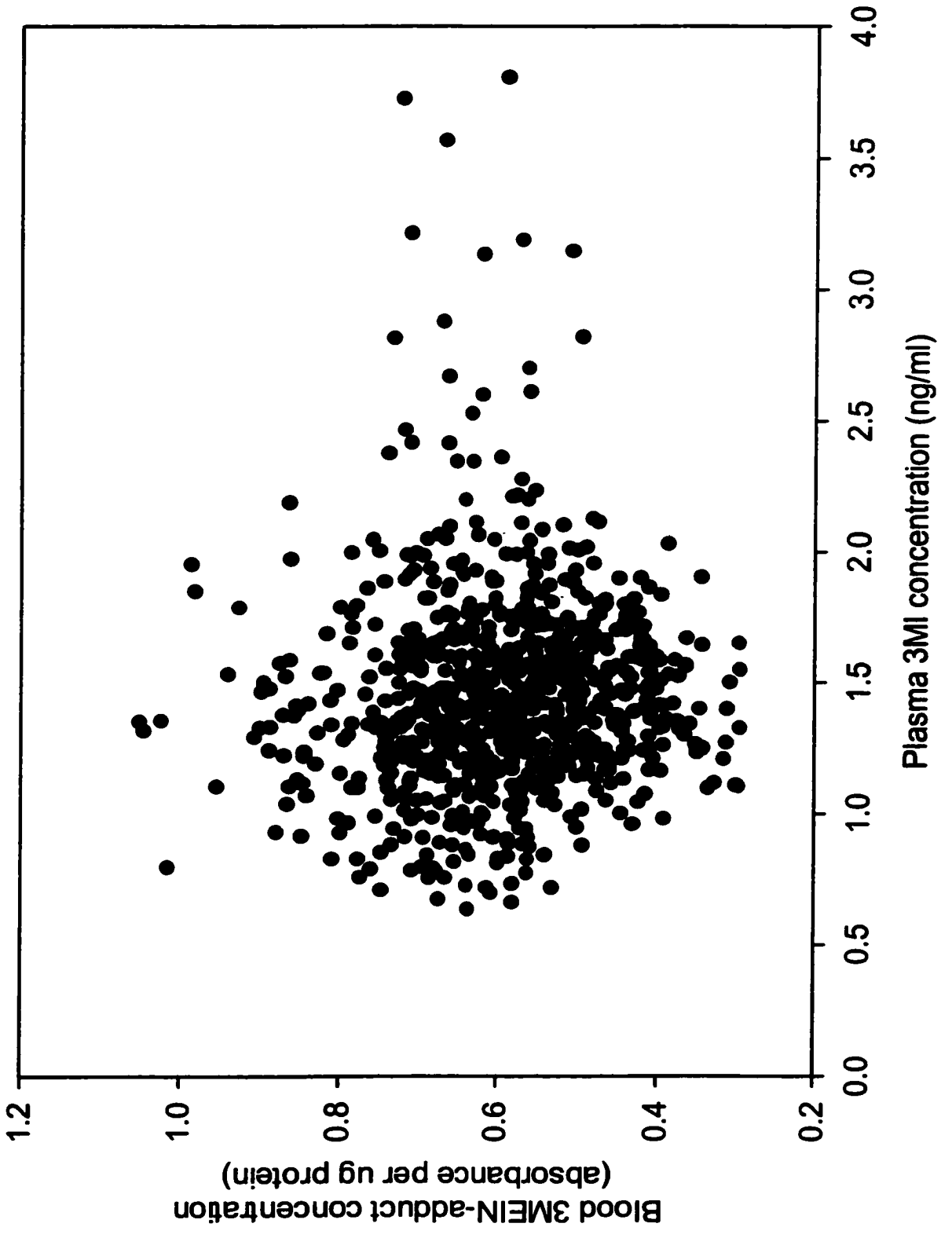
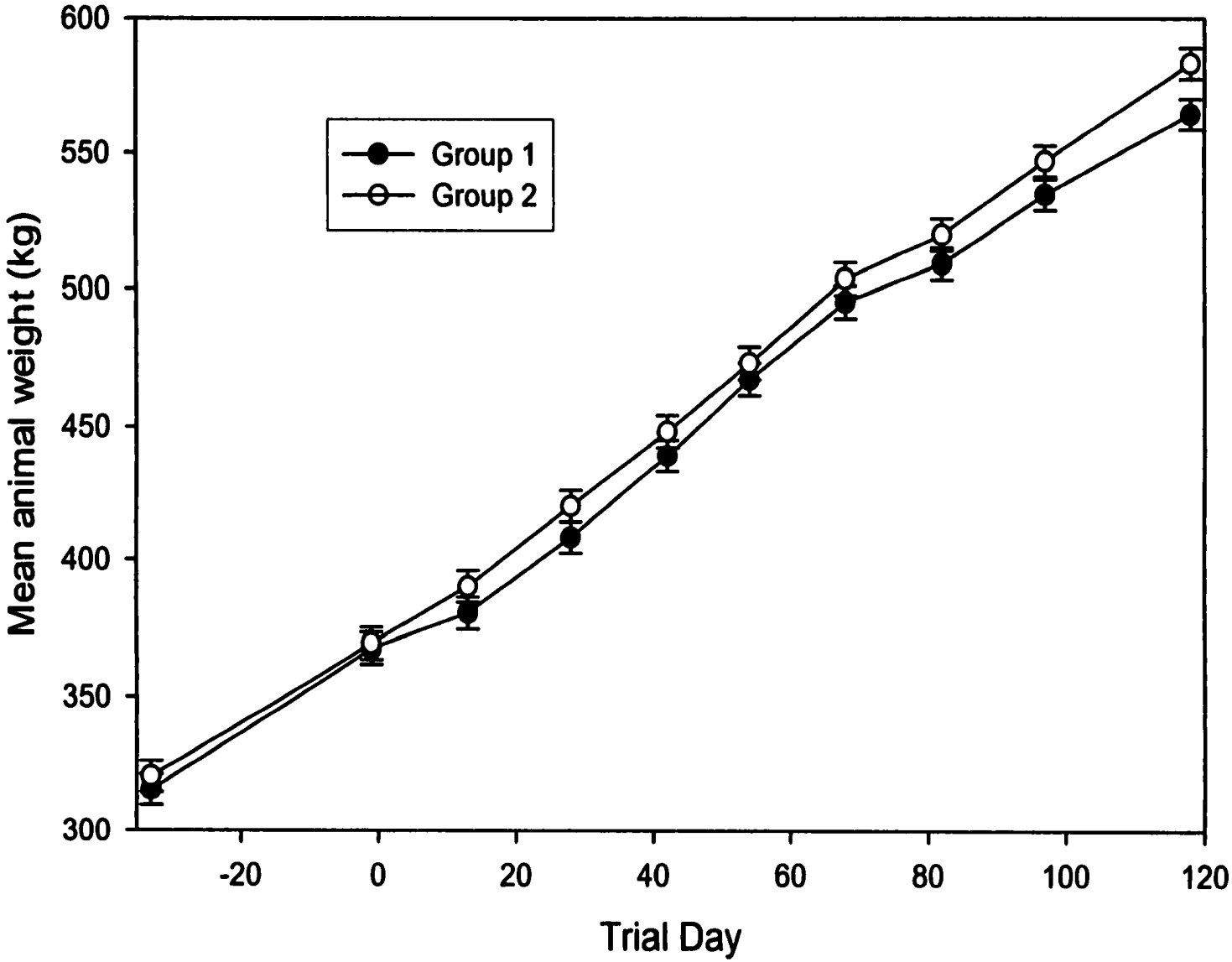


Figure 6.4. Least squares mean live weights of Groups 1 and 2. Error bars represent standard error of the means.

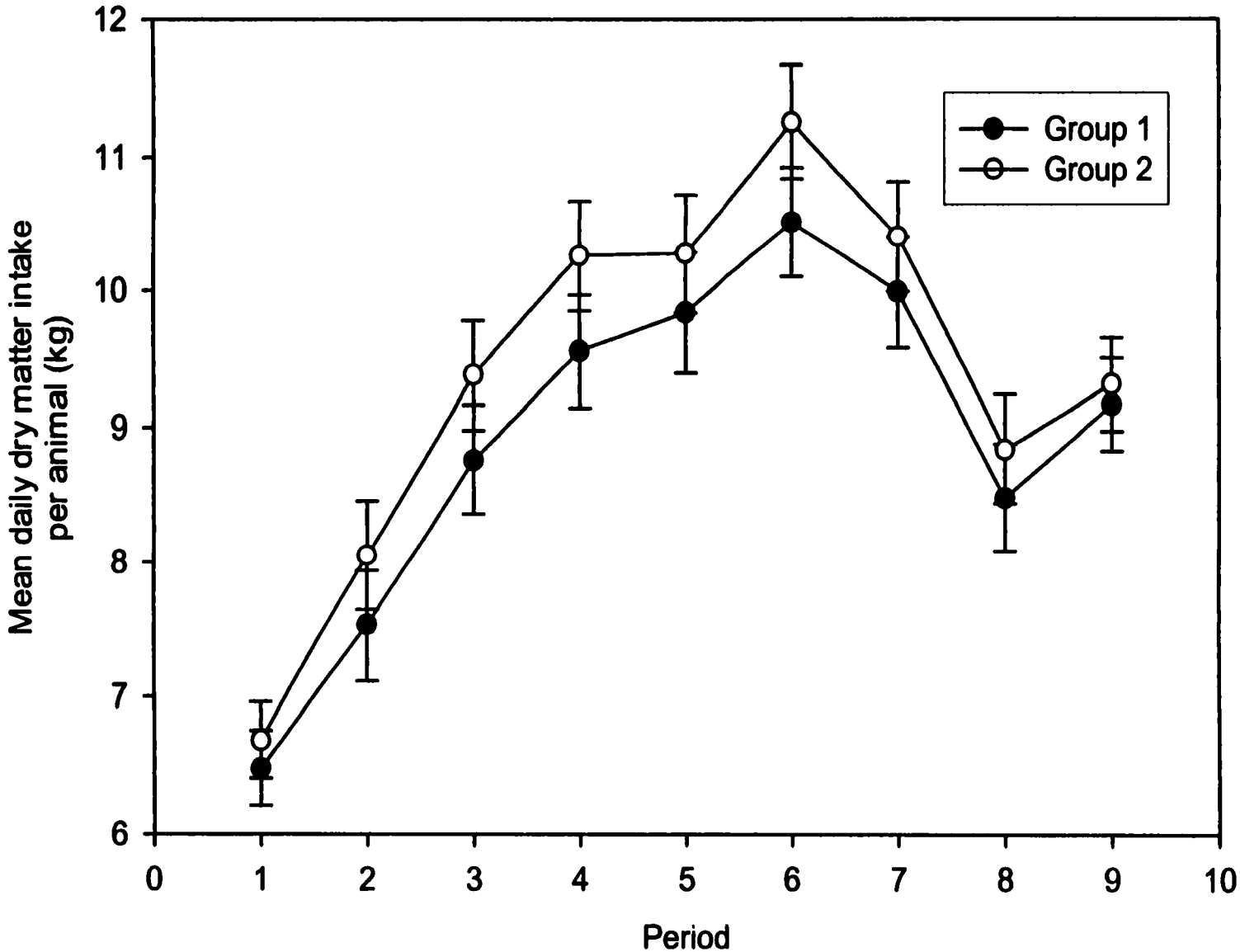


suggesting that differences in MDG_i between Groups 1 and 2 varied over time. During periods 2 ($P < 0.01$) and 9 ($P = 0.04$) Group 2 gained at a greater rate than Group 1. Group 1 gained at a greater rate than Group 2 during period 5 ($P = 0.01$). Averaged over the entire study period, MDG_i for Group 1 and Group 2 varied significantly ($P = 0.02$) and were 1.68 ± 0.04 and 1.82 ± 0.04 kg, respectively.

Maximum blood 3MEIN-adduct concentration from day 1, 2, 5, 6 or 9 was associated with an increase in the overall MDG_i while cattle received feedlot diets ($P < 0.03$). Each 0.1 unit increase in 3MEIN-adduct concentration was accompanied by a 0.52 kg increase in MDG_i. Maximum plasma 3MI concentration from the same period was not associated with a significant difference in MDG_i ($P = 0.92$). Animal-level plasma 3MI ($P = 0.06$) and blood 3MEIN-adduct ($P = 0.03$) AUC were associated with improvements in MDG_i. Each unit increase in plasma 3MI and blood 3MEIN-adduct AUC was associated with a 0.02 and 0.12 kg increase in MDG_i, respectively. There was a significant interaction between period and pen-level blood 3MEIN-adduct AUC ($P < 0.01$). Averaged over time, pen-level 3MEIN AUC was not associated with significant changes in MDG_p ($P = 0.59$). Pen-level plasma 3MI AUC was associated with an increase in MDG_p ($P = 0.04$).

Mean daily DMI of cattle was 6.6 ± 0.2 kg while receiving the roughage-based diet; a difference between Groups was not detected ($P = 0.69$). Dry matter intake varied by period ($P < 0.01$) and by Group (Figure 6.5; $P = 0.01$) in the final model from which period 1 was excluded. Averaged over time, mean daily DMI were 9.2 ± 0.1 and 9.7 ±

Figure 6.5. Least squares mean daily dry matter consumption per animal for Groups 1 and 2. Error bars represent standard error of the means.



0.1 kg for Groups 1 and 2, respectively. A statistical interaction between period and Group was not identified. Dry matter consumption increased during period 6 and was greater than periods 2 ($P < 0.01$), 3 ($P < 0.01$), 4 ($P = 0.02$), 5 ($P = 0.05$), 7 ($P = 0.09$), 8 ($P < 0.01$), and 9 ($P < 0.01$). Feed efficiency did not vary between Groups ($P = 0.33$) for period 1 and was 0.24 ± 0.01. Excluding period 1 from the model, there was no evidence of a statistical interaction between Group and period or an effect of Group ($P = 0.67$) on FE and overall FE was 0.19 ± 0.01. Period was a significant source of variation for FE ($P < 0.01$).

Mean hot carcass weights were 355.0 ± 4.9 for Group 2 and 341.0 ± 4.9 kg for Group 1 ($P = 0.05$). There was no detectable difference between Groups for other carcass characteristics ($P > 0.20$).

Discussion

The vast majority of BRD events are diagnosed early in the feeding period and most occur within 8 weeks of arrival.[3,4,32,33] During this period, blood 3MEIN-adduct concentrations increased with greatest mean values on days 16, 23 and 33, and then declined to their lowest concentration on day 54. This pattern of change in blood 3MEIN-adduct concentrations coincides temporally with typical BRD epidemic curves. Plasma 3MI concentrations were expected to increase early in the feeding period in a similar manner to 3MEIN, however, this was not observed. No cattle developed clinical manifestations of respiratory disease in the present study. An associations between 3MI

or its metabolite, 3MEIN, and the occurrence of BRD was not evaluated in the present study.

Plasma 3MI and blood 3MEIN were negatively correlated. A possible explanation for this unexpected finding is that animals with a high capacity to metabolize 3MI may have converted 3MI to 3MEIN more efficiently than animals with a low metabolic capacity. Therefore, those animals with a high 3MI-metabolic capacity could have lower 3MI and higher 3MEIN than animals with low metabolic capacity.

The primary purpose of this study was to describe the patterns of plasma 3MI and blood 3MEIN-adduct concentration during the period typically associated with the greatest risk of BRD. Hence, blood samples were collected more frequently during the first 54 days "on feed". Sampling frequency was decreased to weekly collections after 54 days because this phase of the feeding period is associated with a lower risk of BRD development. As a consequence of the study design, animals experienced increased handling early in the study period. This may have resulted in lower feed consumption compared to animals under more typical feedlot management practices. Because ruminal 3MI generation, and therefore plasma 3MI concentrations, are largely dependant on tryptophan intake, a decrease in DMI may have resulted in decreased 3MI production during the intensive sampling phase. It is possible that the frequency of handling required for the intensive monitoring resulted in 3MI and 3MEIN-adduct concentrations that are not representative of feedlot cattle under typical management conditions.

Plasma 3MI concentrations initially decreased and remained relatively low until after day 54 (Figure 6.1). This may indicate that the risk period for BRD development was not associated with increases in plasma 3MI concentration. Monensin[h] was included in the diets used in the present study and may have contributed to the reduction in plasma 3MI concentrations by reducing fermentation of tryptophan within the rumen.[34] Serum 3MI concentrations decreased from day 0 to 3 of feedlot arrival in a previous study.[14] However, serum was only analyzed from these two time points in this previous study. Plasma 3MI concentrations increased following the intensive sampling period (Figure 6.1), during the time cattle are believed to be at low risk of infectious respiratory disease development. However, dry matter consumption also increased during period 6 and was greater than all other periods (Figure 6.5). Because of limited fiscal resources, it was not possible to determine 3MEIN-adduct concentration in all blood samples. Blood samples collected during the intensive sampling phase from Group 2 were assayed for 3MEIN as the intensive sampling phase coincided with greatest BRD risk based on previous reports. Additionally, MDG and DMI of Group 2 animals were less affected by sample collection than Group 1 animals. Therefore, Group 2 animals may have provided a better model for blood 3MEIN-adduct concentrations in feedlot cattle under more commonplace production settings.

Concentrations of 3MI measured in this study may represent basal production of 3MI that on average result in no adverse outcome in feedlot cattle. Bioactivation of 3MI to 3MEIN by P450s in lung Clara cells may, however, be of consequence because 3MEIN, a potent

pneumotoxin, peaked during the period of greatest risk of BRD. Therefore, the metabolic capacity of the P450s that bioactivate 3MI may be of more importance as a predictor of the likelihood of disease than the concentration of 3MI in feedlot cattle. A mechanism for increased 3MEIN production in respiratory tissues might be the induction of cytochrome P450 enzymes that catalyze the formation of this putative reactive intermediate in susceptible lung cells. Induction of these enzymes could be associated with drug treatment, exposure to viral pathogens, changes in diet, or other environmental factors.[35] Evaluation of this hypothesis will require further characterization of P450 enzymes in bovine respiratory tissues. In another study, lung 3MEIN-adduct concentrations were greater in animals affected with bronchopneumonia compared to animals without histological evidence of respiratory disease.[28] Increased lung 3MEIN exposure likely propagated injury at the cellular-level in this previous study.

It seems likely that a substantial portion of blood 3MEIN-protein adducts measured in the present study were derived from lung P450 enzymes because these enzymes are thought to be the most efficient at metabolizing 3MI to this specific reactive intermediate compared to P450 enzymes found in other organ systems.[11,16,20,36] If this was so, then animals with greatest blood 3MEIN-adduct concentrations may have undergone some degree of pulmonary compromise without development of respiratory disease that manifested as BRD. Because many events of respiratory injury go undetected,[37,38] it is possible that study animals developed bronchopneumonia but were not identified as sick by feedlot personnel. In one study, 68% of animals that did not receive treatment for

BRD (that is those animals that were not identified as sick) had evidence of bronchopneumonia at harvest.[37] Unfortunately, it was not possible to adequately evaluate the lungs of cattle following harvest in the present study.

Other studies have provided conflicting results on the effect of serum 3MI on MDG_i. In one study, a 1 μg/ml increase in serum 3MI was associated with a 0.02 kg reduction in MDG_i ($P = 0.02$).[14] In another study, no association of serum 3MI and MDG_i was detected.[39] The cattle used in these two studies were not fed typical feedlot diets and their MDG_i were less than half of the MDG_i reported herein. Hence, these studies may not provide inferences that can be appropriately applied to cattle in typical feedlot production settings. We found no association of maximum plasma 3MI on day 0 through 9 with MDG_i ($P = 0.93$). However, maximum blood 3MEIN from the same time-period was associated with improvements in MDG_i ($P = 0.03$). Both plasma 3MI ($P = 0.06$) and blood 3MEIN ($P = 0.03$) AUC were positively associated with increases in MDG_i. It is likely that MDG_i and 3MI (and potentially 3MEIN) are associated with DMI. In our models we attempted to control for DMI intake, however, DMI was measured at the pen-level and plasma 3MI and blood 3MEIN at the animal-level. Hence, it was not possible to appropriately control for animal-level DMI. This may explain why a positive association of 3MI/3MEIN with MDG_i was identified in some models. Alternatively, 3MI and 3MEIN may only be associated with deleterious effects on MDG_i if they induce sufficient cellular damage to result in pulmonary disease. More research is required to evaluate the effect of 3MI and 3MEIN on MDG_i of cattle under more typical management

settings.

The study design resulted in lower DMI and MDG_i for cattle of Group 1 compared to cattle of Group 2. This may have been a consequence of cattle handling procedures. Group 1 also had significantly lower plasma 3MI concentrations compared to Group 2. During the intensive sampling phase, Group 1 was removed from their pen before they received their morning ration, whereas Group 2 was moved 2 to 3 hours after they received their morning ration. The difference in DMI, MDG_i, 3MI between Groups 1 and 2 persisted even after the frequency of sampling was reduced to once a week (when all cattle were removed from their pens prior to the morning feeding) and lasted for the duration of the study. These data suggest that procedures that require handing of feedlot cattle, either for production (such as reimplanting with a growth promotant) or research purposes should be performed so that cattle are in their pens at the time feed is normally delivered to the pen.

ENDNOTES

a. BoviShield 4, Pfizer Animal Health, New York, NY.

b. Fortress CD, Pfizer Animal Health, New York, NY.

c. Dectomax, Pfizer Animal Health, New York, NY.

d. Revalor-S, Hoechst-Roussel Vet, Warren, NJ.

e. Liquamycin LA200®, Pfizer Animal Health, New York, NY.

f. ConAgra Inc., Garden City, KS.

g. Champions Choice Trace Mineral Salt Block, Cargill Inc., Minneapolis, MN.

h. Rumensin 80. Eli Lily and Company, Elanco Animal Health, Indianapolis, IN.

i. Tylan 100. Eli Lily and Company, Elanco Animal Health, Indianapolis, IN.

j. Trace Mineral Mix. Colorado Beef, Lamar, CO.

k. Vitamin E. Roche Vitamins Inc., Parsippany, NJ.

l. Vitamin A. Roche Vitamins Inc., Parsippany, NJ.

m. Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ.

n. Centra CL3R, International Equipment Company, Needham Heights, MA.

o. The SAS System for Windows release 8.0, SAS Institute Inc., Cary, NC.

p. Formula used to calculate AUC: $AUC = (y_x + y_{x+1})/2 * (day_{x+1} - day_x)$; where y = 3MI or 3MEIN concentration, x = sample day, and x+1 = subsequent sample day

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CHAPTER 7

Effect of dietary inclusion of aspirin and vitamin E on plasma 3-methylindole, and blood and lung 3-methyleneindolenine concentrations, lung lesions, and animal performance in feedlot cattle.

Summary

The objectives of this study were to evaluate the effect of dietary inclusion of acetylsalicylic acid (aspirin) and vitamin E on plasma 3MI concentration, blood and lung 3MEIN-adduct concentration, occurrence of grossly identifiable lung lesions at harvest, animal performance, and carcass characteristics in feedlot cattle. Two trials were conducted concurrently using steers (316 kg) obtained from a single source. Sixty-four animals were used in trial 1, and 192 animals were used in trial 2. Within each trial, animals were randomly assigned to each of four treatments. A 2x2 factorial treatment design was used and treatment factors were aspirin (0 or 3 g PO daily), and vitamin E (0 or 1,500 iu PO daily). Treatments were included in the diet of animals. Aspirin, but not vitamin E, was removed approximately 7 days prior to animal harvest to allow for an appropriate drug withdrawal period. Animals from trial 1 were harvested on day 59. Lungs were grossly evaluated. Plasma, blood and lung samples were collected. 3-

methylindole (3MI) concentration was measured in plasma. 3-methyleneindolenine (3MEIN)-adduct concentration was determined in blood and lung tissue. Carcass characteristics were recorded. Trial 2 animals were weighed every 28 days and harvested on day 138. Overall plasma 3MI, blood 3MEIN-adduct, and lung 3MEIN-adduct concentrations were 3.11 ng/mL, 0.51 units per μg protein, and 0.49 units per μg protein, respectively. Vitamin E was associated with an increased concentration of plasma 3MI ($P = 0.08$). When vitamin E was not added to the diet, aspirin was associated with increased blood 3MEIN-adduct concentration ($P = 0.01$). Of trial 1 animals, 57.1% had grossly identifiable lung lesions at harvest although none were observed to be sick during the course of the study. The presence of lung lesions was not associated with treatment, adverse effects on carcass characteristics, or 3MI and 3MEIN concentrations. Treatment was not associated with improvements or adverse effects in body weight, mean daily weight gain, dry matter intake, or feed conversion in Trial 2. The potential disease sparing effects of aspirin and vitamin E could not be adequately evaluated in the present study. Aspirin (3 g daily) and vitamin E (1,500 iu daily) can be safely included in the diet of steers without deleterious effects on animal performance.

Introduction

Sudden increases in ruminal generation of 3-methylindole (3MI), a microbial metabolite of tryptophan, are associated with acute interstitial pneumonia (fog fever, acute bovine pulmonary edema and emphysema).[1,2] 3-methylindole is then absorbed predominantly from the small intestine and disseminated hematogenously.[3] Although the mechanism of

action of 3MI is not fully understood, bioactivation in pulmonary tissue is a critical step in the pathogenesis of 3MI-induced lung injury.[4,5]

Bioactivation of 3MI by cytochrome P450 enzymes in Clara cells, type I alveolar cells, and alveolar macrophages has been extensively studied. Clara cells appear to be the primary site for 3MI bioactivation.[6] The major toxic metabolite of 3MI is believed to be 3-methyleneindolenine (3MEIN) and this electrophile is believed responsible for the majority of 3MI-induced disease.[7,8] Data also exist that suggest 3MI may be bioactivated by prostaglandin H synthetase (PHS).[4,9-11] PHS-dependent free radicals were produced when 3MI was incubated with PHS *in vitro* and during 3MI challenge studies *in vivo*.[12] Free radicals may contribute to the pathogenesis of 3MI-induced disease. It has been proposed that the mechanism of 3MI-induced disease may actually be through co-oxidation by cytochrome P450 enzymes and PHS.[4]

Although 3MI is a potent pneumotoxic precursor and is capable of inducing acute lung injury, it is normally produced at low concentrations in the rumen without apparent adverse effect.[13] Increases in ruminal generation of 3MI that are not sufficient to induce acute interstitial pneumonia may, however, result in increased concentrations of 3MEIN and other electrophiles in lung tissue. Cellular injury and compromises to pulmonary defense mechanisms may be a consequence of these small fluctuations in 3MI generation.

Bingham and co-workers found that increases in serum 3MI concentrations at, or soon

after arrival, were associated with an increased likelihood of treatment for bovine respiratory disease complex (BRD).[14,15] In our previous study, Chapter 6, although increased plasma 3MI concentrations were not detected (Figure 6.1.), blood 3MEIN-adduct concentrations were increased during the period typically associated with the greatest risk of BRD (Figure 6.2.). Therefore, increases in ruminal generation of 3MI or bioactivation in lung tissue may increase the risk of BRD in feedlot cattle.

If this proposition is so, mitigation of 3MI bioactivation or free radical-induced cellular injury may reduce the risk of BRD in feedlot cattle. Acetylsalicylic acid (aspirin), a potent PHS inhibitor,[16] exerted a disease sparing effect during 3MI challenge studies.[17] This effect was only observed if aspirin was administered prior to the 3MI challenge The authors concluded that the protective effect resulted from the prevention of 3MI metabolism by PHS, and not simply from inhibition of prostanoid production.[17] In a different challenge study, 3MI-induced disease was least severe in calves pretreated with aspirin (15.6 g, PO) and vitamin E (1,500 iu, IM) compared to controls, or animals pretreated with either aspirin or vitamin E.[18] However in a field study, no disease sparing effect was detected in cattle that were administered aspirin (31.2 g, PO) at feedlot arrival.[14] The protective effects of aspirin may not have been detected in their study if increases in ruminal 3MI generation occurred after aspirin-induced PHS inhibition had waned. A protective effect of aspirin may have been observed if aspirin was administered to maintain PHS inhibition during periods of increased 3MI bioactivation. Bray and Preston demonstrated that doses of aspirin lower than those provided by Bingham and co-

workers effectively inhibited PHS when provided in the diet for 7 days.[16]

This study was performed to evaluate the effects of dietary inclusion of aspirin and vitamin E on plasma 3MI concentration, blood and lung 3MEIN-adduct concentration, occurrence of grossly identifiable lung lesions at harvest, animal performance, and carcass characteristics in feedlot cattle.

Materials and Methods

The study protocol was reviewed and approved by the Colorado State University Animal Care and Use Committee prior to initiation of the study. Two hundred and fifty-six single-source yearling steers were used in the study. A 2x2 factorial treatment design was used and treatment factors were aspirin (0 or 3 g daily) and vitamin E (0 or 1,500 iu daily). Sixty-four steers were randomly selected at the onset of the research and tissue samples for 3MI and 3MEIN determination were harvested on day 59. The remaining animals, 192, were fed treatment diets until they reached a desirable harvest weight. All animals were monitored daily by trained feedlot personnel for manifestations of illness.

Animals

Upon arrival (day -2), the steers were provided with long-stem grass hay and *ad libitum* access to water. Within 24 hours after arrival at the feedlot, cattle were moved through a cattle handling facility, vaccinated with modified-live bovine herpesvirus 1, parainfluenza 3 virus, bovine viral diarrhea virus and bovine respiratory syncytial virus[a]. Cattle were

also administered *Clostridium perfringens* C and D toxoids[b], doramectin (10 mg/ml)[c] at 2 ml per 100kg body-weight, and implanted with a growth promotant containing 120 mg trenbalone acetate and 24 mg estradial[d].

On the following day (day -1), the steers were again moved through the animal handling facility, weighed, and provided with individually numbered plastic ear tags. Animals were assigned to one of six blocks based on their weights. Blocks 1 through 4 contained 43 animals each while blocks 5 and 6 contained 42 animals each. Eleven animals were randomly selected from each of blocks 1 through 4 and 10 animals were randomly selected from blocks 5 and 6. This procedure was undertaken to provide 2 groups of animals that were to be used in side-by-side trials. Trial 1 was comprised of 64 animals in 2 weight blocks and trial 2 contained 192 animals in 6 weight-blocks. All blocks contained 32 animals. One of 4 treatments was randomly assigned to animals within each weight-block.

The following day (day 0), steers were reweighed and moved to pens so that each pen contained 8 animals (block within treatment). The average of weights recorded on days 1 and 0 was used as the arrival weight. All animals were reweighed on day 26. At harvest, carcass characteristics including hot carcass weight, predicted yield grade, marbling units, longissimus muscle cross section area and fat thickness over the 12th rib, kidney-pelvic-heart fat score, and USDA quality grade were recorded. Final live weights were adjusted to reflect transportation "shrink" by multiplying them by 0.96. Dressing percentage was

calculated by dividing adjusted final weight into hot carcass weight.

Treatments and Feeding

A 2x2 factorial treatment design was used; factors were aspirin (0 or 3 g per day) and supplemental vitamin E (0 or 1500 iu per day). Test articles were included in the diets of the steers, and the four treatments were designated control, aspirin, vitamin E and aspirin-vitamin E. Treatments were added to a mineral supplement, then the supplement was included in the diets. Inclusion rates of supplements varied depending on the diet and expected feed consumption so that the intake of treatments closely resemble target levels.

Cattle were provided with an untreated, ground hay-based diet on day -1. The first of step-up diets (Table 6.1.) was fed on the afternoon of day 0. Following this, steers were fed twice daily and provided subsequent step-up diets on the afternoon of days 3, and 6. The finishing diet was first delivered to the cattle on the afternoon of day 10. The weight of each feed delivery was recorded and adjusted to reflect dry matter content. Dry matter delivery was further adjusted for feed refusal to provide a more accurate estimate of dry matter consumed by each pen.

Trial 1

The purpose of trial 1 was to evaluate the effects of aspirin and vitamin E on the plasma 3MI concentrations, blood and lung 3MEIN concentrations and the occurrence of grossly identifiable lung lesions at harvest. The 64 randomly selected steers were weighed on day

58 and were shipped to a commercial abattoir the following day. Aspirin was removed from the diets on day 51 to allow for an appropriate withdrawal period of 8 days. Vitamin E was not removed from diets. At harvest, blood samples were collected from the cranial vena cava into 2 blood collection tubes[e], one containing sodium heparin and the other potassium EDTA. Blood samples were kept on ice until processed further. Whole-blood containing potassium EDTA was centrifuged at 3200 rpm for 20 minutes at 4° C using a refrigerated centrifuge[f]. Aliquots of plasma were harvested, frozen in liquid nitrogen, then stored at -20° C. Aliquots of heparinized whole-blood samples were frozen and stored at -20° C.

Lungs were removed at harvest and evaluated to identify gross pathology. The type of lesion (bronchopneumonia, plueritis, or interstitial pneumonia) and percent of lung tissue affected was also recorded. A 5 x 5 x 2 cm sample of lung was obtained from the dorsal aspect of the right caudal lobe, frozen on dry ice, and then stored at -20° C.

Plasma 3MI concentrations were determined by use of a microplate method adapted from procedures as described.[19] Whole-blood samples were analyzed for 3MEIN absorbance per μg protein using methods described elsewhere.[7]

Trial 2

The purpose of trial 2 was to evaluate the effect of aspirin and vitamin E on animal performance and carcass characteristics. Animals were weighed on days 26, 54, 82, 110,

137, and 138. The average of the two weights recorded on days 137 and 138 was used as the final weight. Aspirin was removed from the diet on day 130. Animals were shipped to a commercial abattoir on day 138. Carcass characteristics were recorded as described above.

Statistical analyses

Individual and pen-level mean daily weight gain (MDG_i and MDG_p) were calculated for each weigh period in both trials. Mean daily dry matter intake for cattle in a pen (DMI) was calculated for each 2-week period using dry matter consumed daily per pen of cattle, divided by the number of animal-days for the period. Feed efficiency ratios (FE) were estimated for each weigh-period by dividing DMI into pen-level MDG_p.

Statistical analyses were performed using commercially available software[ε]. Treatments and their interaction terms were included in the analysis. The interaction terms were dropped from analytical models if their P value were ≥ 0.15. Study animals were considered the experimental unit for the analysis of live weights, MDG_i, carcass characteristics, blood and lung 3MEIN, and plasma 3MI concentrations. Pens were considered the experimental unit for the analysis of DMI and FE. Time-period was considered a classification variable for the analysis of MDG_i, DMI and FE. First order auto-regressive matrices were used to model the covariance structure within experimental units over time.[20] Lung lesions were classified as present if they were bronchopneumonia or pleuritis. Lungs from one animal with interstitial pneumonia ($n = 1$) were not included

in the analyses. The presence or absence of a lung lesions was analyzed using logistic regression with treatments and their interaction the independent effects of interest. The interaction term was dropped from the model if its P value was ≥ 0.15. Carcass characteristics measured on a continuous scale were analyzed as a two-way analysis of variance. Categorical carcass characteristics (USDA quality and yield grades) were analyzed using a Chi-square goodness-of-fit test. Arrival weight was included as a covariate when analyzing MDG_i, DMI, continuous carcass characteristics, 3MI and 3MEIN if its P value was < 0.15.

Results

Mean arrival weights for cattle of trial 1 and 2 did not differ ($P = 0.98$) and were 316.2 and 316.8 kg, respectively. One animal from trial 2 died as a result of ruminal tympany on day 17. No other animals were observed to be sick in either trial.

Trial 1

Animal performance

At harvest steers weighed 405.7 kg. There was no evidence to support a difference in final weights associated with feeding aspirin ($P = 0.53$), vitamin E ($P = 0.77$) or both (interaction $P = 0.97$). Arrival weight was associated with performance and each 1 kg increase in arrival weight, final weights increased 1.08 kg ($P < 0.01$). Overall MDG_i (± SEM) was 1.55 ± 0.04 kg. An interaction between vitamin E and aspirin was not detected for MDG_i ($P = 0.78$). Feeding aspirin ($P = 0.44$) and vitamin E ($P = 0.88$), were

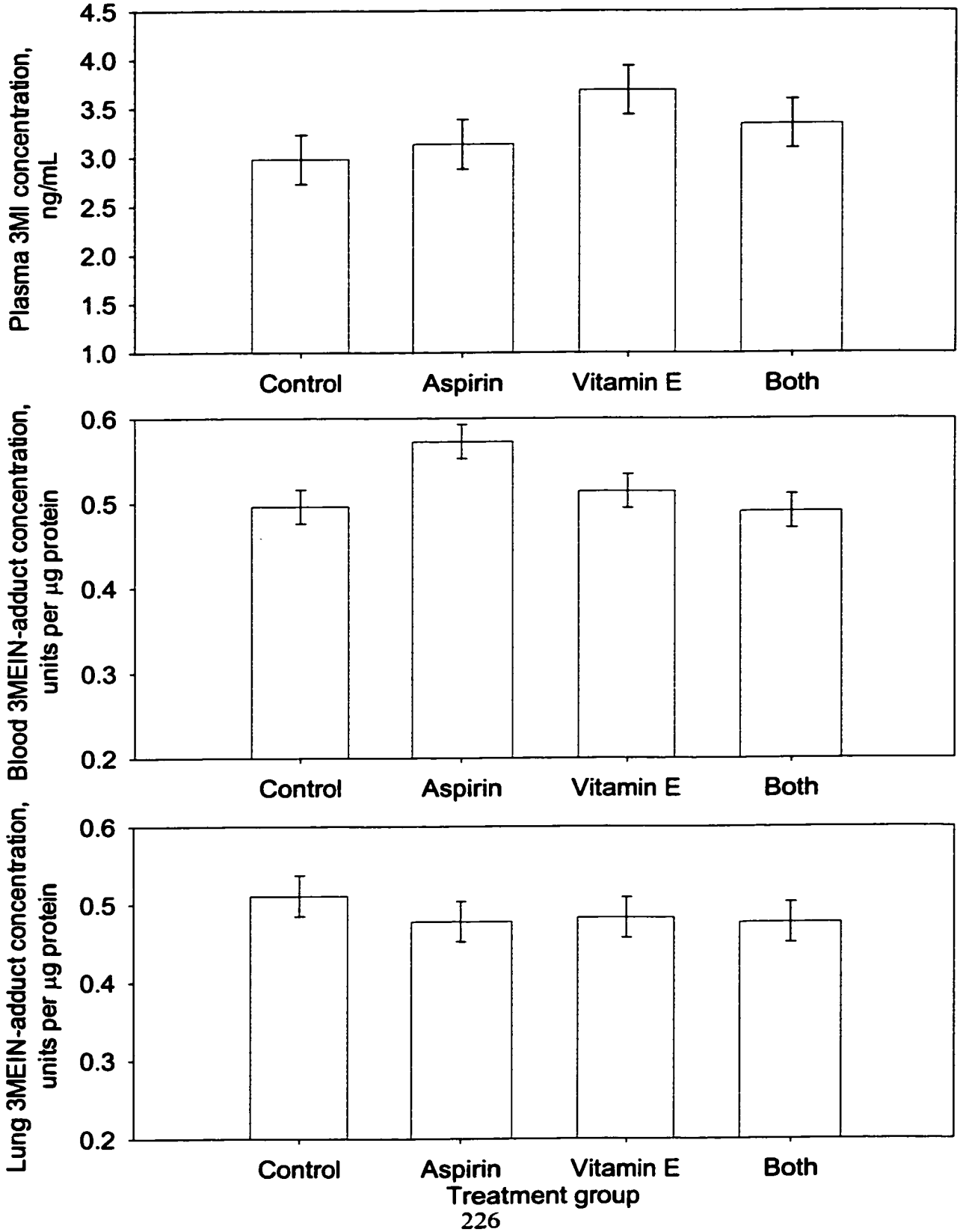
not associated with significant variation in MDG_i.

3MI and 3MEIN concentrations

Feeding vitamin E was associated with increased ($P = 0.08$) plasma 3MI concentrations. Mean plasma 3MI concentrations were 3.51 ± 0.18 and 3.06 ± 0.18 μg/mL for diets with and without supplemental vitamin E, respectively. There was no association between feeding aspirin and plasma 3MI concentration ($P = 0.70$), nor did the effect of vitamin E vary with aspirin usage ($P = 0.33$). A treatment interaction was detected for blood 3MEIN-adduct concentration ($P = 0.01$; Figure 7.1). Without supplemental vitamin E, aspirin was associated with increased blood 3MEIN concentration ($P = 0.01$), however, this effect was not seen if supplemental vitamin E was included in the diet ($P = 0.37$). An interaction ($P = 0.62$), or effect of aspirin ($P = 0.45$) or vitamin E ($P = 0.59$) was not detected for lung 3MEIN-adduct concentrations. Overall blood and lung 3MEIN-adduct concentrations were 0.51 and 0.49 units per μg protein.

An effect of plasma 3MI, or blood or lung 3MEIN-adduct concentration on MDG_i, final weight, carcass weight or dressing percentage was not detected ($P > 0.18$ for all models). Each unit increase in blood ($P = 0.06$) and lung ($P = 0.05$) 3MEIN-adduct concentrations were associated with 0.81 and 0.69 unit increase in predicted yield grade. An effect of plasma 3MI concentration on predicted yield grade was not detected ($P = 0.52$). Each unit increase in blood 3MEIN-adduct concentration was associated with a 207 unit increase in marbling score ($P = 0.11$). An effect of plasma 3MI or lung 3MEIN-adduct

Figure 7.1. Plasma 3-methylindole (3MI) concentrations (a), blood 3-methyleneindolenin- (3MEIN) adduct concentrations (b), and lung 3MEIN-adduct concentrations (c) by treatment group. Error bars represent SEM.



concentration on marbling score was not detected ($P > 0.40$ for both models). Longissimus muscle area, percentage of carcass as KPH fat, and proportion of carcasses grading USDA choice or higher were not affected by plasma 3MI, or blood or lung 3MEIN-adduct concentration ($P > 0.25$ for all models).

Plasma 3MI concentrations were not predictive of blood ($P = 0.78$) or lung ($P = 0.63$) 3MEIN-adduct concentration. A correlation between blood and lung 3MEIN-adduct concentration was not detected ($P = 0.64$).

Lung lesions

Lungs were evaluated from 63 animals and 36 (57.1%) had grossly identifiable lung lesions at harvest. These lesions included interstitial pneumonia ($n = 1$), bronchopneumonia ($n = 21$), pleuritis ($n = 9$) and a combination of bronchopneumonia and pleuritis ($n = 5$). Only three of the lesions identified were considered active. Data from the animal with interstitial pneumonia were excluded from further analyses because it was not deemed representative of either group. Although not statistically significant, animals with lung lesions appeared to be lighter at arrival than animals without lung lesions ($P = 0.23$). Mean arrival weights were 312.8 ± 4.1 kg and 320.2 ± 4.6, respectively. Differences in final weight ($P = 0.58$) or MDG_i ($P = 0.71$) were not detected for those animals with lung lesions compared to animals without lesion.

There was weak evidence that animals with lung lesions had reduced dressing

percentages compared to animals without lung lesions ($P = 0.16$). The dressing percentages were 62.4 ± 1.5 and 63.2 ± 1.5, respectively. Animals with lung lesions tended to have greater marbling scores ($P = 0.13$) and an estimated proportion of their carcass as KPH fat ($P = 0.09$). However, the proportion of carcasses grading USDA select or higher was not associated with the presence of lung lesions ($P = 0.58$). Other carcass characteristics did not differ based on the presence of lung lesions ($P > 0.25$ for all models). The odds of an animal having pulmonary lesions at harvest did not differ with changes observed in plasma 3MI concentration, or blood or lung 3MEIN-adduct concentration ($P > 0.44$ for all models).

There was no evidence for an effect of aspirin ($P = 0.83$), vitamin E ($P = 0.21$), or the interaction of vitamin E and aspirin ($P > 0.45$) on lungs lesions. Whereas 64.5% of animals without supplemental vitamin E had lung lesions, 48.4% of animals that received supplemental vitamin E had lesions. However, this proportional difference was not significant ($P = 0.21$).

Trial 2

Animal performance

There was no evidence for an interaction of aspirin and vitamin E on body weight (P = 0.20) or MDG_i (P = 0.19), nor was an effect of aspirin (P = 0.83 and 0.98, respectively) or vitamin E (P = 0.97 and 0.73, respectively) detected (Figure 7.2). Overall MDG_i was 1.54 ± 0.04 kg. Both body weight and MDG_i varied with time ($P < 0.01$ for both models; Figure 7.3).

An interaction of aspirin and vitamin E was not detected in the models of DMI (P = 0.96) and FE (P = 0.39). Dry matter intake and FE were not influenced by aspirin (P = 0.66 and 0.72, respectively) or vitamin E (P = 0.61 and 0.33, respectively). Overall DMI and FE were 8.16 ± 0.17 kg and 0.19 ± 0.01 kg/kg, respectively. Time was a significant source of variation for DMI and FE ($P < 0.01$ for both models).

Carcass characteristics were recorded, how unique identification of carcasses was lost and analysis of carcass characteristics was not performed.

Discussion

Dietary inclusion of aspirin and supplemental vitamin E did not result in a detectable beneficial or adverse effect on performance parameters recorded in the present study. Bingham and co-workers did not detect an effect on performance of aspirin administered at arrival.[14] Although there is limited data available on long-term dosage of aspirin in

Figure 7.2. Body weights (a), mean daily gains (b), dry matter intake (c), and feed efficiency (d) by treatment group. Error bars represent SEM.

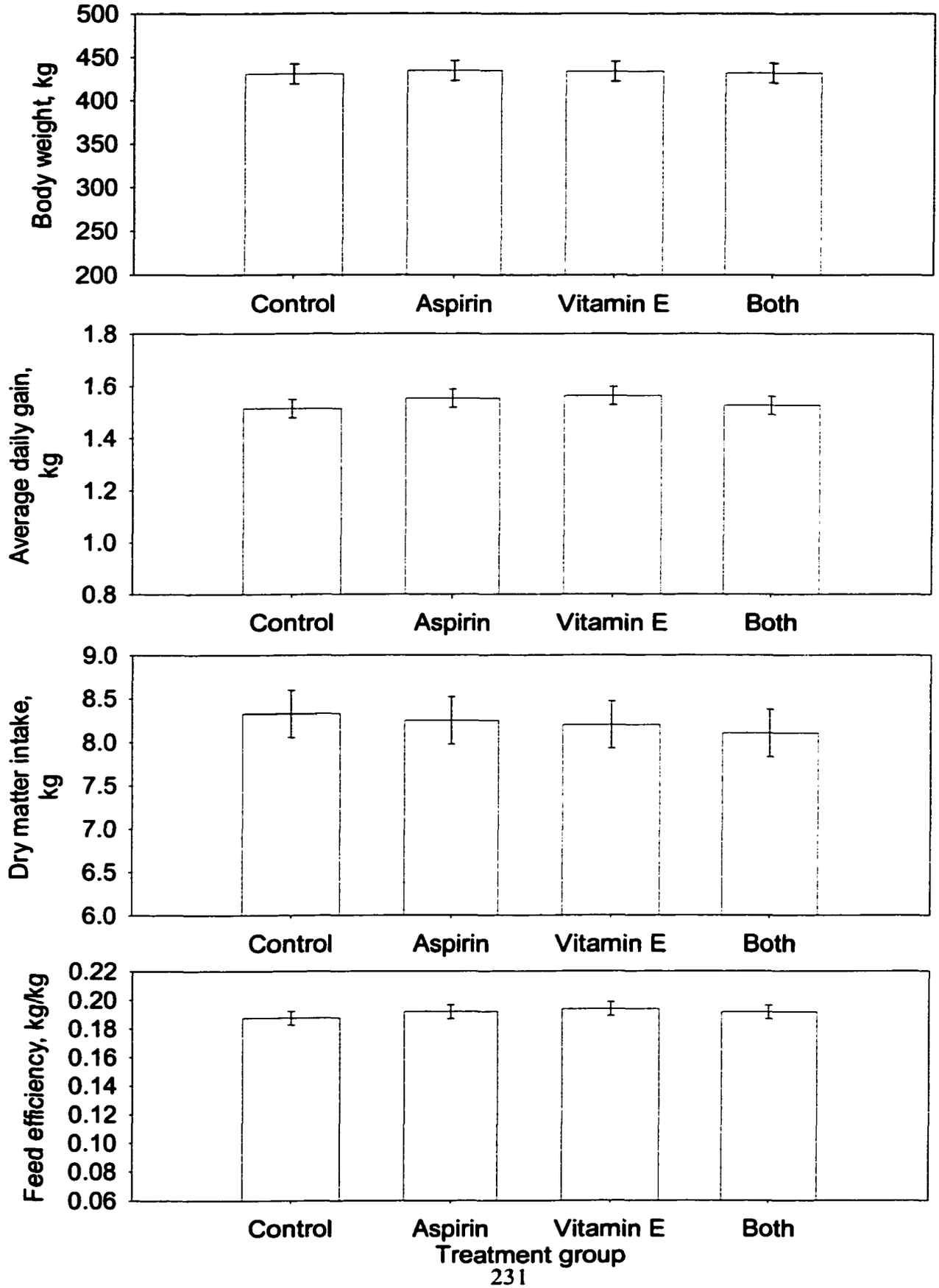
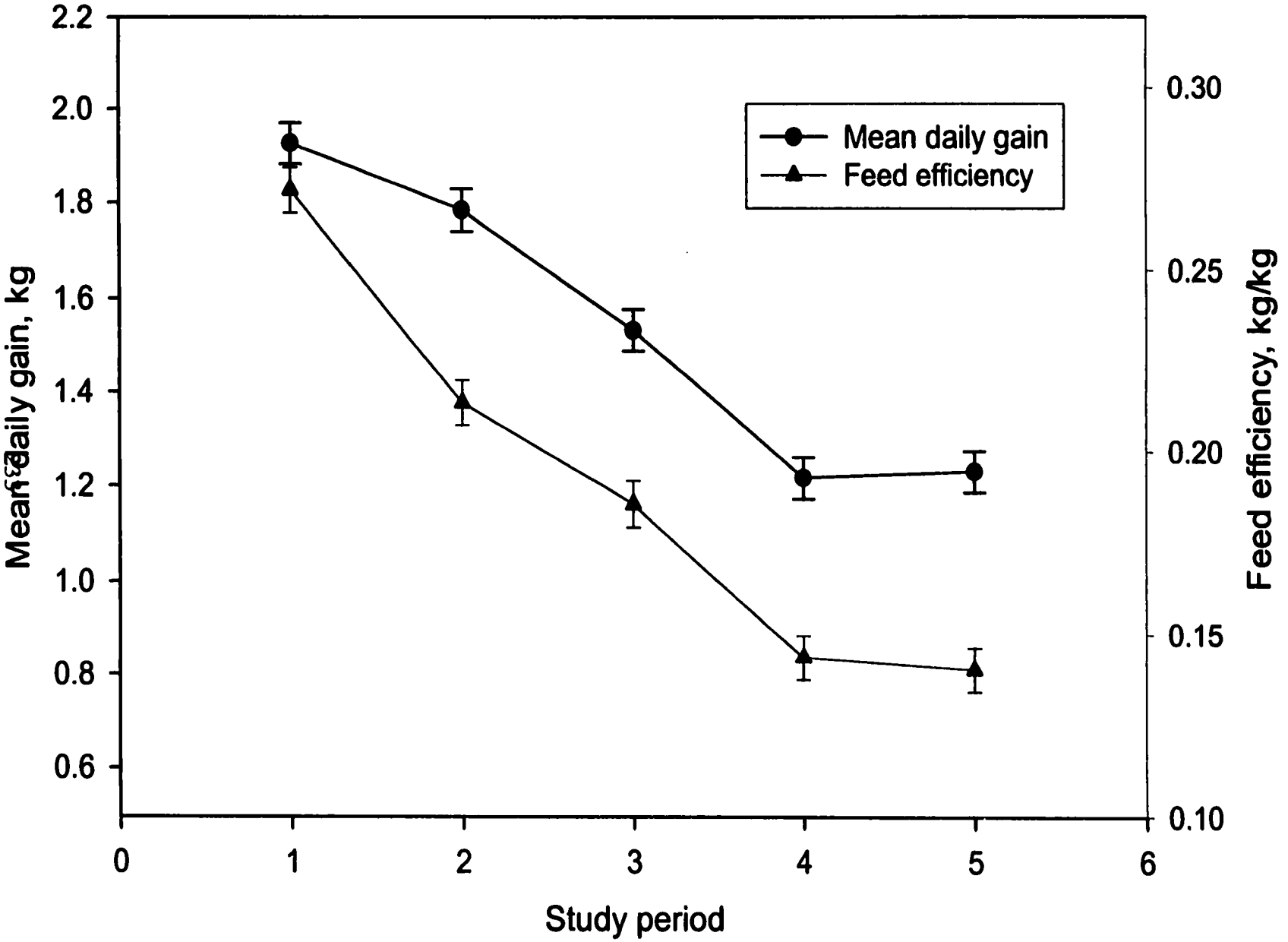


Figure 7.3. Mean daily weight gains and feed efficiency by study period. Error bars represent SEM.



cattle, 3 g per day should have resulted in substantial inhibition of PHS.[16] Ruminant requirements for vitamin E have not been published. However, 1,500 iu of vitamin E daily likely exceeds the animal's requirement,[21] and has provided some degree of protection from a 3MI challenge in a previous study.[18] It is possible that the 3MI concentrations the cattle of this study were exposed to were insufficient to elicit a beneficial effect from aspirin and vitamin E. Alternatively, the daily doses used in this study may have been insufficient to provide protection against naturally-occurring concentrations of 3MI.

Of the animals harvested on day 59 of the trial, 57.1% had lung lesions. Other researchers had reported between one-third and two-thirds of animals had lung lesions regardless of treatment history.[22,23] Interestingly, none of the animals in the present study were identified as sick between feedlot arrival and harvest. The presence of identifiable lung lesions was associated with decreased MDG_i in other studies.[22,23] Further, Gardner and co-workers found that lung lesions were associated with less desirable carcass characteristics.[23] However, no association of pulmonary lesions with MDG_i or deleterious carcass lesions was detected in the present study.

It seems possible that a substantial proportion of the lesions observed may have developed prior to feedlot arrival because the vast majority of the lesions were resolved,

no animals were observed to be sick, and animals with lesions were approximately 8 kg lighter at arrival than animals without lesions. The presence of lesions was not modified by treatment.

There are a number of weaknesses associated with the study. Because a withdrawal period for feed-grade aspirin has not been established, an arbitrary period of approximately 7 days was used. It is possible that the inhibition of PHS had waned by the time that lung and blood samples were harvested. Therefore, if PHS is important for the co-oxidation of 3MI to 3MEIN, no decrease in 3MEIN-adduct concentration would have been observed because the timing of sample collection may have been inappropriate. Inexplicably, aspirin usage was associated with increased 3MEIN-adduct concentrations in blood if no vitamin E was included in the ration. It is also unclear why vitamin E was associated in increased plasma 3MI concentrations.

Another weakness of the study relates to the proposed role that PHS has in the bioactivation of 3MI to 3MEIN. Evidence exists that indicate PHS is involved in 3MI bioactivation.[9,12,17,24] Free radicals were observed when 3MI was incubated with a horseradish peroxidase model of PHS *in vitro*.[9] However, 3MEIN is thought to be cytochrome P450-specific.[25,26] Non-steroidal anti-inflammatory drugs, such as aspirin, do not inhibit cytochrome P450 enzymes. Therefore, aspirin would not be expected to directly affect bioactivation of 3MI to 3MEIN. Aspirin may modulate BRD development via PHS independent of 3MI bioactivation to 3MEIN, or through other mechanisms.

Modulation of BRD development could not be evaluated in the present study because no animals were identified as sick during the study period.

Cattle were housed in pens of 8 animals each in the present study. This small-pen strategy was used because it decreases within pen variance and thereby enhances the ability to detect significant between treatment variation. Thus, the results indicate that doses of aspirin and vitamin E used did not result in beneficial or deleterious effects on production in seemingly healthy animals. However, improvements in performance parameters may only occur when a BRD-sparing effect is present. The use of small pens has the effect of reducing the likelihood for BRD development. Animals used in the present study were from a single-source, and relatively mature. Both these factors are associated with reduced risk of BRD development.[27] Therefore, study design and animal selection may have reduced the likelihood of observing a beneficial effect of aspirin and vitamin E.

It seems likely that a substantial portion of blood 3MEIN-protein adducts measured in the present study were derived from lung P450 enzymes because these enzymes are thought to be the most efficient at metabolizing 3MI to this specific reactive intermediate.[5,6,25,26] However, blood and lung 3MEIN concentrations were not correlated. Further, plasma 3MI concentrations were not predictive of blood of lung 3MEIN-adduct concentration. The reasons for the unexpected findings are unclear.

ENDNOTES

a. BoviShield 4, Pfizer Animal Health, New York, NY.

b. Fortress CD, Pfizer Animal Health, New York, NY.

c. Dectomax, Pfizer Animal Health, New York, NY.

d. Revalor-S, Hoechst-Roussel Vet, Warren, NJ.

e. Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ.

f. Centra CL3R, International Equipment Company, Needham Heights, MA.

g. The SAS System for Windows release 8.2, SAS Institute Inc., Cary, NC.

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CHAPTER 8

Overall conclusions

- The proportion of placements that did not survive to harvest increased during the years of 1994 through 1999. In 1994, there were 10.3 deaths per 1,000 animals placed on feed whereas during 1999 there were 14.2 deaths per 1,000 placements. The majority of this increase was due to an increase risk of death attributable to respiratory disorders relative to 1994 (RR 1.46, CI 1.1 to 1.9). It is uncertain why the risk of respiratory associated death increased so dramatically. Possible explanations include:
  a. A increase in the proportion of animals with treatment-refractive bovine respiratory disease complex (BRD) that were sold prior to a desirable harvest weight. Therefore, a greater proportion of animals with fatal BRD would have died at the feedlot.
  b. A decrease in the quality or quantity of animal health personnel at feedlots. This would have resulted in a reduced ability to visually detected BRD-affected cattle. Therefore, sick cattle would be identified at a more advanced stage of illness. These animals would have been less likely to respond favorably to therapeutic intervention compared with animals identified earlier in the illness.

c. A change in the placement profile of animals entering the feedlot. If the proportion of light-weight placements increased over time then a greater proportion of animals would be expected to succumb to BRD.

d. A change in animal husbandry practices that increased animal stress load such as passage through multiple auction markets prior to feedlot arrival.

- A difference in the risk of death attributed to respiratory disease between heifers and steers was not detected for the years of 1994 through 1996. However, heifers were at increased risk of respiratory-related death relative to steers post 1996. It is unclear why the risk of death varied between heifers and steers.

- Acute interstitial pneumonia (AIP) in feedlot cattle does not appear to be associated with common infectious respiratory pathogens. Although Collins and co-workers identified an association with bovine respiratory syncytial virus (BRSV),[1] our results are consistent with those of others researchers.[2,3] It is possible the ability to culture bacteria was adversely affected due to antemortem antimicrobial therapy. Therefore, an association of AIP with aerobic bacteria may have been present even though it was not detected.

- Bovine respiratory syncytial virus (BRSV) commonly infects feedlot cattle without causing respiratory disease. Twenty-four percent of cattle that did not have histopathological evidence of respiratory tract lesions were positive for

BRSV. Based on these data, the prevalence estimate for seemingly inconsequential BRSV infections in feedlot cattle is 24 %. However, the confidence interval (CI 7.3 to 40.7 %) of this estimate is relatively wide because there were few controls (n = 25).

- Many animals that died as a result of bronchopneumonia did so with a substantial number of days on feed. Sixteen of 50 cattle (32 %) with bronchopneumonia had 120 days on feed or more. At this stage, feedlots personnel typically use antimicrobials with a short withdrawal period such as ceftiofur sodium. However, mycoplasma spp. were cultured from greater than 50 % of bronchopneumonia cases. Cell-wall inhibitors, such as ceftiofur sodium, are not efficacious against these microorganisms. However, this may be inconsequential if mycoplasmas are not involved in bronchopneumonia pathogenesis, do not propagate disease as an opportunistic pathogen, or if effective treatment of aerobic bacteria leads to resolution of mycoplasma infection.

- Acute interstitial pneumonia (AIP) was associated with increased concentrations of lung and blood 3-methyleneindolenine- (3MEIN) adduct compared to controls. It is uncertain if the observed increases in adduct concentration occurred prior to, or in association with disease development. Thus, a causal role for 3MEIN formation in the pathogenesis of acute interstitial pneumonia can, at best, only be pondered. However, 3MEIN is a potent pneumotoxin and increased

concentrations likely propagated pulmonary injury.

It seems likely that the majority of the blood 3MEIN adducts were derived from bioactivation of 3MI by lung P450 enzymes because the lung isoenzymes are the most efficient at catalyzing this process.[4,5]

Therefore, the data indicate that AIP was associated with increased production of 3MEIN and not simply increased retention in pulmonary tissue. This could have occurred secondary to:

a. Increased generation of 3-methylindole (3MI) within the rumen,

b. Decreased urinary excretion of absorbed 3MI, or

c. Increased pulmonary capacity to bioactivate 3MI to 3MEIN.

The former two would have resulted in a greater quantity of substrate, 3MI, being presented to pulmonary cytochrome P450 enzymes.

- Because:

  a. Acute interstitial pneumonia (AIP) cases and controls had considerable overlap in lung 3-methyleneindolenine- (3MEIN) adduct concentrations, and

  b. A difference in lung 3MEIN-adduct concentration was not detected between AIP and bronchopneumonia cases,

it is unlikely that elevated lung 3MEIN production is the sole cause of feedlot-associated AIP. The disease has a complex, multifactorial etiology. The data presented herein do not support an interaction with common infectious pathogens, although an association with aerobic bacteria should not be discounted. Other research indicated that AIP is associated with alterations in digestive function,[2,6] whereas predisposing bronchopneumonia has also been implicated.[3,7] It is possible that AIP is a production-related disease that occurs when specific alterations in rumen function and lung health occur concurrently.

- Heifers are at greater risk of developing acute interstitial pneumonia (AIP) than steers. Based on case-control data from 14 feedlots, the odds of an AIP positive lung coming from a heifer was 3.1 times greater than of an AIP case being a steer. Within a single feedlot, heifers were at 4.9 greater risk of developing AIP relative to steers. The reasons for the association of AIP with sex are uncertain. The use of melengesterol acetate has been implicated,[2,8] however, this was not evaluated in the studies described herein. More research is required to better understand why heifers are predisposed to AIP.

- Timing of cattle handling had deleterious effects on animal performance in the feedlot. Cattle that were moved through a processing facility during the time at which they would normally be fed demonstrated slower live weight gains than animals allowed to consume feed prior handling. Although this management

practice was discontinued after 8 weeks on feed, the adverse performance effects lasted for the duration of the study (a further 10 weeks).

- Plasma 3-methylindole (3MI) decreased and remained low during the period of greatest risk for bovine respiratory disease complex- (BRD) related morbidity and mortality. Concentrations then increased after 8 weeks on feed, which also coincided with the end of intensive animal handling.

  It is possible that the observed decrease in plasma 3MI concentration was design-induced. However, it may indicate that increases in rumen generation of 3MI are rare in feedlot cattle and if involved in the BRD, only explain a very small proportion of all BRD cases.

  Blood 3-methyleneindolenine (3MEIN) increased during the period of greatest risk for the BRD. Further, animals affected with bronchopneumonia had greater concentrations of 3MEIN than controls. Therefore, the capacity to bioactivate 3MI to 3MEIN may be more important in the BRD that actual concentrations of 3MI in the plasma.

- Aspirin and vitamin E at the dosages described did not result in reduced concentrations of plasma 3-methylindole (3MI), or blood or lung 3-methyleneindolenine- (3MEIN) adduct concentrations. Aspirin and vitamin E

were not expected to affect ruminal 3MI generation or its concentration in plasma. However, if 3MI is co-oxidized by cytochrome P450 enzymes and prostaglandin H synthetase (PHS) to 3MEIN, then aspirin, at the dosages described, should have affected blood and lung 3MEIN concentrations. Potential reasons that this was not observed include:

a. Aspirin was removed from the rations approximately 7 days prior to harvest to allow for a withdrawal period. It is possible that the inhibition of PHS had subsided by the time lung and blood samples were harvested. Therefore, if PHS is important in the co-oxidation of 3MI to 3MEIN, then no decrease in 3MEIN-adduct concentration would have been observed because the timing of sample collection was inappropriate.

b. Although evidence exists that PHS is potentially involved in 3MI bioactivation,[9-12] 3MEIN (the putative toxic metabolite of 3MI) is thought to be cytochrome P450-specific.[4,5] Non-steroidal anti-inflammatory drugs, such as aspirin, do not inhibit cytochrome P450 enzymes. Therefore, aspirin would not directly affect bioactivation of 3MI to 3MEIN.

c. Cattle were housed in pens of 8 animals each in the present study. This small-pen strategy is designed to decrease within pen and control within treatment variance and thereby enhances the ability to detect significant between treatment variation. Thus, the results indicate that doses of aspirin and vitamin E used did not result in beneficial or deleterious effects on production in seemingly healthy animals. However, aspirin and

vitamin E-induced improvements in performance parameters may only occur when a BRD-sparing effect is present. The use small pens has the effect of reducing the likelihood for BRD development and as such, the study design may have reduced the likelihood of observing a beneficial effect of aspirin and vitamin E.

- Aspirin and vitamin E did not result in a detectable deleterious effect on animal performance. At 3 g and 1,500 iu per day, respectively, these compounds can be safely included in the diet of feedlot steers.

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