EVALUATING THE IMPACT OF AMMONIA EMISSIONS FROM EQUINE OPERATIONS ON THE ENVIRONMENT AND EQUINE WELFARE

By

JESSIE MCDONNELL WEIR

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIALFULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2016
© 2016 Jessie Mcdonnell Weir
To my Mom and Dad- Thank you for always supporting and pushing me to be my best
ACKNOWLEDGMENTS

First I have to thank my entire family without whom none of this would have been possible. From a young age, my parents and brother have pushed me to further my education and assisted me in pursuing my passions. I only hope I can do the same for my children someday.

A tremendous thank you to my advisor, Dr. Carissa Wickens, for your help, guidance, and faith in the development and completion of all my research projects as well as your dedication to my education and assistance in completing this degree. You have afforded every opportunity you could to allow me to succeed in my PhD as well as life. Without your guidance, I would not be the independent and resourceful scientist that I am today.

I would like to thank Dr. Hong Li for being my co-advisor while at the University of Delaware and staying involved with my research as I moved to the University of Florida. You opened my mind to a whole new world of research which has since become my passion and I cannot fathom this dissertation focusing on anything else. You always challenged me to think outside the box and that will follow me throughout my career.

Dr. Lori Warren deserves more thanks than I could possibly give. You incited this passion for nutrition that I did not realize was there. Thank you for taking a chance on me and teaching me to always think and question. Also, thank you for all the hours spent working on presentations! I truly believe those long hours have given me the confidence to present my work in the best way possible. I would also like to thank Dr. Nicolas DiLorenzo and Dr. Cheryl Mackowiak for being a part of my guidance committee. Your perspective and ideas helped shape me to become a good researcher.
Graduate school would be impossible without the help and support of my peers. I have been fortunate to work with some the best upcoming talent in our field. From volunteering to help on Dr. Sarah White and Jill Bobel’s first research projects, to navigating through my semester with Leigh Ann Skurupey and Dr. Robert Jacobs, we have shared some sorrow and frustration, but even more laughs and smiles that I hope to carry throughout life. Chongyang Lin and Chen Zhang were instrumental in designing and helping me to implement the instruments used to conduct this research. I must give special thanks to both Tayler Hansen and Angie Adkin. You are some of the dearest friends I have and I cannot wait to see where life takes both of you. Tayler you have been in my life since 2007 and I knew right away that we had a special bond. You have always challenged me to be the best scientist I can be, and while I find that frustrating at times, I am so very grateful. Angie Adkin is a special person. You are a rock and always put silly stressors into perspective. I do not know how I would have functioned in grad school without you. I will always remember those long days in the lab fondly.

A special thanks to all the undergraduates who helped on my many research projects, I hope you learned a great deal and had some fun along the way! A very special thanks to Erica Macon for being my lifeline during the digestibility study (still not sure how we made it!). I knew from the first day that you were meant to do great things, and I am so glad you found your passion. I know you will make great contributions to equine nutrition!

I would also like to thank Justin Callaham with the Horse Teaching Unit crew, and Nick Carden with the Equine Science Center crew for all of their help during my
research projects. Without the assistance that I received from the staff of the Horse Teaching Unit, I would not have been able to successfully complete this project.

A huge thanks to Delaware Valley University, Herringswell Stables, and Winbak farm for allowing to take samples from your facilities. All were fantastic experiences and I enjoyed being surrounded by some of the best in the industry.

When I returned to the University of Florida, I was very nervous about what life in Gainesville would be like, as I had spent my early adult years here. I never imagined I would meet my partner in life, Chris Chouinard. You are the most inspirational person I know and I am in awe of you every single day. There is no one I would rather talk to at the end of a long day. I love you and cannot wait to explore life with you!

Most importantly, I would like to send a huge thanks to the horses: Zman, Kip, Cadet, Bucky, Billy, Vinnie, Watson, Dan, Horseradish, Snuffy, Jules, Remy, CJ, Lucy, Blue Ron, Tobias, Jimmy, Cherokee, Shorty, Chile, Hansi, Joe, Ulysses, Quinn, Merlin, Juju, Tootsie, Snickers, Jelly Bean, Up with the Birds, Peace and War, Nucifera, Divine Oath, Dido, Mom’s Bella, Bonita Beach, Turbulent Victoria, Sanabelle, Ms. Virginia, and Zipadeedoodah for being the best research subjects one could hope for. Without the horses, none of this work would have been possible. You will always hold a special place in my heart.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>4</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>10</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>11</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>13</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>16</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1 INTRODUCTORY REMARKS</td>
<td>18</td>
</tr>
<tr>
<td>Impact of Animal Agriculture on the Environment</td>
<td>18</td>
</tr>
<tr>
<td>Formation of Fine Particulate Matter</td>
<td>18</td>
</tr>
<tr>
<td>Eutrophication of Water Systems</td>
<td>20</td>
</tr>
<tr>
<td>Nitrogen Cycle in the Ecosystem</td>
<td>21</td>
</tr>
<tr>
<td>Mineralization</td>
<td>21</td>
</tr>
<tr>
<td>Nitrification</td>
<td>21</td>
</tr>
<tr>
<td>Denitrification</td>
<td>22</td>
</tr>
<tr>
<td>Nitrogen Fixation</td>
<td>22</td>
</tr>
<tr>
<td>Microbial and Plant Uptake of Ammonium</td>
<td>23</td>
</tr>
<tr>
<td>Ammonia Volatilization</td>
<td>23</td>
</tr>
<tr>
<td>Leaching of Nitrate and Nitrite</td>
<td>24</td>
</tr>
<tr>
<td>Ammonia Formation and Volatilization</td>
<td>24</td>
</tr>
<tr>
<td>Quantifying Ammonia Emissions from Animal Facilities</td>
<td>27</td>
</tr>
<tr>
<td>Instrumentation to Measure Aerial Ammonia Concentrations</td>
<td>27</td>
</tr>
<tr>
<td>Detector tubes</td>
<td>28</td>
</tr>
<tr>
<td>Acid scrubbers</td>
<td>28</td>
</tr>
<tr>
<td>Chemiluminescence analyzers</td>
<td>29</td>
</tr>
<tr>
<td>Photoacoustic analyzer</td>
<td>29</td>
</tr>
<tr>
<td>Methods to Estimate Ammonia Emissions</td>
<td>30</td>
</tr>
<tr>
<td>Monitoring Ventilation Rate</td>
<td>32</td>
</tr>
<tr>
<td>Crude Protein in Equine Diets</td>
<td>33</td>
</tr>
<tr>
<td>Pattern of Nitrogen Metabolism</td>
<td>34</td>
</tr>
<tr>
<td>Digestibility of Dietary Protein</td>
<td>35</td>
</tr>
<tr>
<td>Protein Requirements</td>
<td>36</td>
</tr>
<tr>
<td>Dietary Strategies for Reducing Ammonia Loss to the Environment</td>
<td>36</td>
</tr>
<tr>
<td>Environmental Implications of Excreted N from Horses</td>
<td>37</td>
</tr>
<tr>
<td>Impact of Ammonia Emissions on Horse Health</td>
<td>40</td>
</tr>
<tr>
<td>Assessing Impact of Ammonia Emissions on Equine Welfare</td>
<td>43</td>
</tr>
<tr>
<td>Using Aversion Testing to Assess Welfare</td>
<td>43</td>
</tr>
<tr>
<td>Physiological Indicators of Aversion</td>
<td>44</td>
</tr>
</tbody>
</table>
Heart rate and heart rate variability .................................................. 44
Salivary cortisol ............................................................................. 46
Aversion to Higher Ammonia Concentrations in Other Species .......... 46

2 CHARACTERIZING AMMONIA EMISSIONS FROM HORSES FED DIFFERENT CRUDE PROTEIN CONCENTRATIONS .................................................. 52
Introduction ..................................................................................... 52
Material and Methods ..................................................................... 54
Horses ......................................................................................... 54
Dietary Treatments ........................................................................ 55
Experimental Design ...................................................................... 55
Sample Preparation and Analyses .................................................... 56
In Vitro Ammonia Emissions ............................................................ 57
Calculations ................................................................................... 58
Statistical Analysis ......................................................................... 59
Digestibility Study Results ............................................................... 60
Daily Intake and Excretion ............................................................... 60
Apparent Crude Protein Digestibility and Balance ......................... 61
Total Fecal and Urinary Excretion ................................................... 61
Ammonium, Urea-N, Nitrate, and Nitrite Excretion ......................... 62
In Vitro Ammonia Concentrations and Emissions ............................ 63
Discussion ...................................................................................... 64

3 AMMONIA EMISSIONS FROM EQUINE FACILITIES IN THE MID-ATLANTIC REGION ................................................................. 85
Introduction ..................................................................................... 85
Materials and Methods .................................................................. 87
Farm Characteristics ........................................................................ 87
Horses and Management Practices .................................................... 89
Ammonia Concentrations ................................................................. 90
Sample Collection and Analysis ....................................................... 90
Ambient Air .................................................................................... 91
Calculation of Ventilation Rate and Emissions ................................. 91
Statistical Analysis ......................................................................... 92
Results .......................................................................................... 93
Diets and Management Practices ..................................................... 93
Ammonia Concentration ................................................................. 94
Ambient Air and Ventilation Rate ..................................................... 94
Ammonia Emissions ..................................................................... 95
Discussion ...................................................................................... 95

4 DETERMINING THE AVersion OF HORSES TO DIFFERENT AMMONIA CONCENTRATIONS ........................................................................... 112
Introduction ..................................................................................... 112
Materials and Methods ........................................................................................................... 115
Horses .................................................................................................................................... 115
Head Box System .................................................................................................................. 116
Experimental Design ............................................................................................................. 117
Determining Salivary Cortisol .............................................................................................. 118
Monitoring Heart Rate and Heart Rate Variability .............................................................. 119
Behavioral Analysis ............................................................................................................... 119
Statistical Analysis ................................................................................................................ 119
Results ..................................................................................................................................... 120
Acclimation to Head Box System .......................................................................................... 120
Phase 1: Determining Aversion of Horses to Higher NH₃ Concentrations ......................... 121
  Feeding behavior ................................................................................................................. 121
  Heart rate and heart rate variability .................................................................................... 122
  Salivary cortisol .................................................................................................................. 122
Phase 2: Determining Physiological Response to Different NH₃ Concentrations ................ 122
  Feeding behavior ................................................................................................................. 122
  Heart rate and heart rate variability indicators ................................................................. 123
  Salivary cortisol .................................................................................................................. 124
Discussion ............................................................................................................................. 124

5  CONCLUDING REMARKS AND FUTURE DIRECTIONS .................................................. 143
  Overall Conclusions ........................................................................................................... 143
  Future Perspectives ............................................................................................................ 145

LIST OF REFERENCES ......................................................................................................... 147

BIOGRAPHICAL SKETCH ...................................................................................................... 165
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Composition of the three experimental diets and mean daily nutrient intake for each diet.</td>
<td>71</td>
</tr>
<tr>
<td>2-2</td>
<td>Daily excretion and pH in feces and urine.</td>
<td>72</td>
</tr>
<tr>
<td>2-3</td>
<td>Apparent digestibility of dry matter and crude protein and nitrogen balance.</td>
<td>73</td>
</tr>
<tr>
<td>2-4</td>
<td>Concentration of total nitrogen in feces and urine.</td>
<td>74</td>
</tr>
<tr>
<td>2-5</td>
<td>Daily excretion of total nitrogen, ammonium, urea-nitrogen, nitrate and nitrite in feces and urine.</td>
<td>75</td>
</tr>
<tr>
<td>2-6</td>
<td>Least square means of ammonia mean concentration and cumulative emissions of feces.</td>
<td>76</td>
</tr>
<tr>
<td>2-7</td>
<td>Least square means of ammonia mean concentration and cumulative emissions of urine.</td>
<td>77</td>
</tr>
<tr>
<td>3-1</td>
<td>Site descriptions for four equine operations used in study.</td>
<td>101</td>
</tr>
<tr>
<td>3-2</td>
<td>Description of horses from which measurements were taken.</td>
<td>102</td>
</tr>
<tr>
<td>3-3</td>
<td>Description of feeds at the 4 farms.</td>
<td>103</td>
</tr>
<tr>
<td>3-4</td>
<td>Estimation of mean daily dry matter and crude protein intake of horses from the 4 farms.</td>
<td>104</td>
</tr>
<tr>
<td>3-5</td>
<td>Ambient weather data during data collection from the 4 farms.</td>
<td>105</td>
</tr>
<tr>
<td>4-1</td>
<td>Feeding behavior of horses exposed to head box system during initial and washout days.</td>
<td>130</td>
</tr>
<tr>
<td>4-2</td>
<td>Feeding behavior of horses exposed to NH$_3$ using a head box system.</td>
<td>131</td>
</tr>
<tr>
<td>4-3</td>
<td>HR and HRV in horses exposed to NH$_3$ using a head box system.</td>
<td>132</td>
</tr>
<tr>
<td>4-4</td>
<td>Feeding behavior of horses exposed to NH$_3$ using a head box system.</td>
<td>133</td>
</tr>
<tr>
<td>4-5</td>
<td>HR and HRV in horses exposed to NH$_3$ using a head box system.</td>
<td>134</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1-1</td>
<td>Diagram of the nitrogen cycle in nature.</td>
<td>50</td>
</tr>
<tr>
<td>1-2</td>
<td>Conceptual model of ammonia formation and volatilization from manure.</td>
<td>51</td>
</tr>
<tr>
<td>2-1</td>
<td>Schematic of the <em>in vitro</em> emission vessel system.</td>
<td>78</td>
</tr>
<tr>
<td>2-2</td>
<td>Mean (± SE) daily excretion of total nitrogen in feces.</td>
<td>78</td>
</tr>
<tr>
<td>2-3</td>
<td>Mean (± SE) daily excretion of total nitrogen in urine.</td>
<td>79</td>
</tr>
<tr>
<td>2-4</td>
<td>Mean (± SE) daily excretion of ammonium in feces.</td>
<td>79</td>
</tr>
<tr>
<td>2-5</td>
<td>Mean (± SE) daily excretion of ammonium in urine.</td>
<td>80</td>
</tr>
<tr>
<td>2-6</td>
<td>Mean (± SE) daily excretion of urea nitrogen in urine.</td>
<td>80</td>
</tr>
<tr>
<td>2-7</td>
<td>Mean (± SE) daily excretion of nitrate in urine.</td>
<td>81</td>
</tr>
<tr>
<td>2-8</td>
<td>Mean (± SE) daily excretion of nitrite in urine.</td>
<td>81</td>
</tr>
<tr>
<td>2-9</td>
<td>Daily mean ammonia concentration of feces.</td>
<td>82</td>
</tr>
<tr>
<td>2-10</td>
<td>Cumulative ammonia emissions rate of feces.</td>
<td>82</td>
</tr>
<tr>
<td>2-11</td>
<td>Daily mean ammonia concentration of urine.</td>
<td>83</td>
</tr>
<tr>
<td>2-12</td>
<td>Cumulative ammonia emissions rate of urine.</td>
<td>84</td>
</tr>
<tr>
<td>3-1</td>
<td>Outline of Farm A showing the location of the stalls, aisles, and arena.</td>
<td>106</td>
</tr>
<tr>
<td>3-2</td>
<td>Outline of Farm B showing the location of the stalls and aisles.</td>
<td>106</td>
</tr>
<tr>
<td>3-3</td>
<td>Outline of Farm C showing the location of the stalls and aisles.</td>
<td>107</td>
</tr>
<tr>
<td>3-4</td>
<td>Outline of Farm D showing the location of the stalls and aisles.</td>
<td>107</td>
</tr>
<tr>
<td>3-5</td>
<td>Schematics of dynamic NH$_3$ flux chamber system.</td>
<td>108</td>
</tr>
<tr>
<td>3-6</td>
<td>Daily mean NH$_3$ concentrations at the 4 equine operations.</td>
<td>108</td>
</tr>
<tr>
<td>3-7</td>
<td>The relationship between mean NH$_3$ concentrations from dry areas of the stall and percentage of daily crude protein intake (CPI)</td>
<td>109</td>
</tr>
<tr>
<td>3-8</td>
<td>The relationship between mean NH$_3$ concentrations from urine covered areas of the stall and percentage of daily crude protein intake (CPI)</td>
<td>109</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>Amino acid</td>
<td></td>
</tr>
<tr>
<td>AER</td>
<td>Air exchange rates</td>
<td></td>
</tr>
<tr>
<td>ANR</td>
<td>Assimilatory nitrate reduction</td>
<td></td>
</tr>
<tr>
<td>ANS</td>
<td>Autonomic nervous system</td>
<td></td>
</tr>
<tr>
<td>AU</td>
<td>Animal unit</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>Head box 1</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>Head box 2</td>
<td></td>
</tr>
<tr>
<td>BPM</td>
<td>Beats per minute</td>
<td></td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
<td></td>
</tr>
<tr>
<td>CO$_2$</td>
<td>Carbon dioxide</td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>Crude protein</td>
<td></td>
</tr>
<tr>
<td>CPD</td>
<td>Crude protein digestibility</td>
<td></td>
</tr>
<tr>
<td>CPI</td>
<td>Crude protein intake</td>
<td></td>
</tr>
<tr>
<td>Cum ER</td>
<td>Cumulative emissions rate</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>Dry matter</td>
<td></td>
</tr>
<tr>
<td>DMD</td>
<td>Dry matter digestibility</td>
<td></td>
</tr>
<tr>
<td>DMI</td>
<td>Dry matter intake</td>
<td></td>
</tr>
<tr>
<td>DP</td>
<td>Digestible protein</td>
<td></td>
</tr>
<tr>
<td>EBC</td>
<td>Exhaled breath condensate</td>
<td></td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
<td></td>
</tr>
<tr>
<td>EPC</td>
<td>Environmental preference chamber</td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td>Emission rate</td>
<td></td>
</tr>
<tr>
<td>EV</td>
<td>Emission vessel</td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>High frequency band</td>
<td></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>HIGH-CP</td>
<td>150 % NRC requirement for crude protein</td>
<td></td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
<td></td>
</tr>
<tr>
<td>HRV</td>
<td>Heart rate variability</td>
<td></td>
</tr>
<tr>
<td>IAD</td>
<td>Inflammatory airway disease</td>
<td></td>
</tr>
<tr>
<td>IBI</td>
<td>Inter-beat-interval</td>
<td></td>
</tr>
<tr>
<td>IFAS</td>
<td>Institute of Food and Agricultural Sciences</td>
<td></td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
<td></td>
</tr>
<tr>
<td>LF</td>
<td>Low frequency band</td>
<td></td>
</tr>
<tr>
<td>LOW-CP</td>
<td>110 % NRC requirement for crude protein</td>
<td></td>
</tr>
<tr>
<td>LPM</td>
<td>Liters per minute</td>
<td></td>
</tr>
<tr>
<td>L: T</td>
<td>Feeding behavior from box containing lower concentration of NH₃ to total feeding behavior</td>
<td></td>
</tr>
<tr>
<td>Manure</td>
<td>Feces + urine</td>
<td></td>
</tr>
<tr>
<td>MED-CP</td>
<td>130 % NRC requirement for crude protein</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
<td></td>
</tr>
<tr>
<td>N₂</td>
<td>Gaseous nitrogen</td>
<td></td>
</tr>
<tr>
<td>-NH₂</td>
<td>Amine group</td>
<td></td>
</tr>
<tr>
<td>NH₃</td>
<td>Ammonia</td>
<td></td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>Ammonium</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
<td></td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>Nitrite</td>
<td></td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>Nitrate</td>
<td></td>
</tr>
<tr>
<td>NOₓ</td>
<td>Nitrate + nitrite</td>
<td></td>
</tr>
<tr>
<td>NRC</td>
<td>National Research Council</td>
<td></td>
</tr>
</tbody>
</table>
NV  Natural ventilation
P  Phosphorus
PM  Particulate matter
PM$_{2.5}$  Atmospheric fine particulate matter. <2.5 μm
PM$_{10}$  Atmospheric particulate matter. <10 μm
PNS  Parasympathetic nervous system
PPM  Parts per million
PSD  Power spectral density
RH  Relative humidity
RMSSD  Root mean sum of squares difference
RQ  Respiratory quotient
R-R  Beat to beat interval
SDRR  Standard deviation R-R interval
SN  Sinoatrial node
SNS  Sympathetic nervous system
STB  Standardbred
T  Temperature
TB  Thoroughbred
THP  Total heat production
TN  Total nitrogen
VR  Ventilation rate
WB  Warmblood
For the equine industry, concerns about ammonia (NH₃) levels in the barn environment are multifaceted and include issues of animal welfare, animal and human health, and environmental impacts. Ammonia volatilization occurs when excess crude protein (CP) is fed and excreted as urinary nitrogen. Information regarding NH₃ emission from equine facilities is limited, and effects of dietary CP intake on NH₃ emission have not been investigated. In addition, NH₃ concentrations are known to have a negative impact on horse health; however, the impact of NH₃ on horse behavior and welfare is not well understood.

Using both an in vitro system and in the field, this research shows that there is a link between increasing dietary CP intake and NH₃ volatilization. When measuring NH₃ concentrations and calculating the emission rate (ER) in vitro, urinary-N was the main source of NH₃ volatilized from equine manure, primarily due to the high urea-N concentration in the urine. While dietary CP intake did not have an effect on urinary NH₃ concentration, cumulative emissions tended to be higher when horses consumed more CP. When determining NH₃ concentrations and emissions on stall floors from 4 different
equine operations, using a flux chamber system, there was a significant correlation between excess dietary CP intake and increased NH$_3$ volatilization.

Using a novel head box system, the aversion of horses to different NH$_3$ concentrations was tested. Horses were exposed to different NH$_3$ concentrations (0, 25, 50 ppm) and while feeding behavior was not affected by higher NH$_3$ concentrations, when exposed to 50 ppm NH$_3$, there was an increase in salivary cortisol and the low frequency to high frequency (LF: HF) heart rate variability (HRV) ratio. This could potentially indicate an altered physiological response to a negative stimulus. The development of this head box system does show promise as a tool to investigate the effects of air quality on horse behavior and physiology. In summary, this research shows that there is a relationship between dietary CP intake and potential NH$_3$ emissions from equine manure (feces and urine). These studies provide insight into the multifaceted concerns regarding atmospheric NH$_3$ in equine facilities.
CHAPTER 1
INTRODUCTORY REMARKS

Impact of Animal Agriculture on the Environment

There is an increasing awareness of the impact that livestock production systems have on the environment. Environmental concerns can be divided into three categories: accumulation of nutrients in the soil, eutrophication in water systems, and the emission of noxious gases into the air (Jongbloed and Lenis, 1998). When evaluating the impact of emissions from livestock production on air quality, ammonia (NH₃) is by far the greatest concern. Ammonia emitted from animal feeding operations is a major air and water pollutant contributing to eutrophication, soil acidity, and aerosol formation that can impair atmospheric visibility and human health (Hristov et al., 2011). According to the USEPA 2011 Emissions Inventory (USEPA, 2011), an estimated 82 % of total NH₃ emissions is directly related to agriculture, with the majority associated with livestock (waste, 54 %; fertilizer, 27 %). Therefore, it is vital for the sectors of the livestock industry to understand the factors controlling NH₃ emissions and make every effort to reduce emissions from operations.

Formation of Fine Particulate Matter

Atmospheric NH₃ contributes to the formation of fine particulate matter (PM) that is linked to human respiratory issues (Kampa and Castanas, 2008), and its deposition in the environment can lead to the degradation of terrestrial and aquatic ecosystems (Leytem and Dungan, 2014). Ammonia emitted from livestock operations that contributes to atmospheric fine PM (≤ 2.5 µm PM; PM₂.₅) formation is not a well understood process (Hristov et al., 2011). The process of NH₃ formation and volatilization from animal manure is almost instantaneous and begins immediately after
manure (feces and urine) excretion. Once emitted into the atmosphere, NH₃ enters rapidly into simple chemical reactions primarily with sulfur and nitrogen (N) oxides. The dynamics of these reactions, however, are very complex and depend on environmental conditions and concentration of reactants.

Ammonia is present in the troposphere in very low concentrations, and due to its high reactivity, it then reacts with atmospheric acids, such as sulfuric and nitric. These form ammonium sulfate, ammonium bisulfate, or ammonium nitrate which in turn are considered PM₂.₅ (Renard et al., 2004). The World Health Organization (2014) found that these particles contribute to air pollution, which globally is estimated to cause up to 2 million premature deaths annually. Fine PM is considered among the most dangerous air pollutant as, when inhaled, it may reach the peripheral regions of the bronchioles and interfere with gas exchange inside the lungs (World Health Organization, 2014).

Since the livestock industry is considered the greatest contributor to gaseous NH₃ emissions in the United States, it is important to quantify their contribution to PM₂.₅ (Hristov et al., 2011). Assuming that NH₃ contribution to PM₂.₅ is through the formation of ammonium nitrate and ammonium sulfate and that sulfuric acid is rapidly converted to sulfate and is usually not present as free acid in the atmosphere (USEPA, 2004), this contribution can be estimated based on data from the National Air Quality Status and Trends Report (USEPA, 2008). Across different regions and weather conditions, PM₂.₅ attributable to NH₃ emitted from livestock operations averaged 5 to 11 % (Hristov, 2011). Therefore, under certain climatic conditions, the estimated contribution of farm animals to atmospheric PM₂.₅ may be significant in certain areas of the US (Hristov et al., 2011).
Eutrophication of Water Systems

Ammonia emitted from livestock operations can contribute to water pollution, primarily through eutrophication. Eutrophication was once considered a natural biological aging process of aquatic ecosystems where nutrients increase and produce more plants leading to the formation of a pond and finally conversion into a marsh (Wetzel, 1983). However, the term eutrophication is currently used to describe a rapid nutrient enrichment process that takes place in water bodies as a result of anthropogenic activities that cause nutrients to accumulate (Ghaly and Ramakrishnan, 2015). Adverse effects of eutrophication are: (1) increased phytoplankton biomass, (2) a shift in phytoplankton to bloom forming species which are toxic or inedible, (3) increased blooms of gelatinous zooplankton in marine environment, (4) increased biomass of algae, (5) death of coral reefs, (6) decreased transparency of water, (7) depleted oxygen levels, (8) increased incidence of fish kills, and (8) reduced harvest of fish and shellfish (Carpenter et al., 1998).

Atmospheric transport and fate of NH$_3$ depend on meteorological chemical conditions (Wu et al., 2008). Models have predicted an average lifetime atmospheric NH$_3$ of 3 to 4 d and a ratio of wet to dry deposition of about 6 to 7 (Pye et al., 2009). Wu and colleagues (2008) estimated that from 10 to 40 % (summer) and 20 to 50 % (winter) of emitted NH$_3$ can be converted to ammonium near source, and 40 to 100 % downwind. Thus, NH$_3$ emitted from animal operations may impact water quality immediately or at a considerable distance from the emission source (Hristov et al., 2011). Ammonium wet deposition was estimated in the Chesapeake Bay watershed to be 2.6 kg ha$^{-1}$, or about 47 % of the total inorganic N deposition (Grimm and Lynch, 2005).
Nitrogen Cycle in the Ecosystem

The importance of nitrogen in regards to soil fertility has long been recognized and our knowledge concerning the nature, distribution and transformations of nitrogen compounds in soil is extensive (Ghaly and Ramakrishnan, 2015). A schematic diagram adapted from Ghaly and Ramakrishnan (2015) depicting the cycle of nitrogen in nature is shown in Figure 1. The nitrogen cycle includes many biological and non-biological processes, which will be discussed below.

Mineralization

Mineralization (or ammonification) is the term used for the process by which nitrogen in organic compounds is converted by soil microorganisms into NH$_4^+$ (Schimel and Bennett, 2004; Guggenberger, 2005). The soil microflora typically produces NH$_4^+$ from organic compounds when they release more nitrogen from the organic matter on which they are living than they can assimilate into their own protoplasm. This accumulation of NH$_4^+$ in the soil is affected by: (1) the rate of mineralization, (2) uptake of NH$_4^+$ by microbes as a source of nitrogen for growth, (3) uptake by plants as a source of nitrogen for growth, (4) volatilization of NH$_3$, (5) nitrification, (6) loss of nitrate by leaching, and (7) plant uptake of nitrate as a source for growth (Ghaly and Ramakrishnan, 2015). Mineralization is a complicated process, and cannot always simply be explained by saying if there is a low concentration of NH$_4^+$ in the soil then there is a low mineralization.

Nitrification

Nitrification is the oxidation of NH$_4^+$-N to nitrites (NO$_2^-$) and nitrates (NO$_3^-$), and the result of metabolism by chemoautotrophic organisms (Ghaly and Ramakrishnan, 2015). The two groups of organisms that are considered to be the primary nitrifying
bacteria are *Nitrosomonas* Sp. and *Nitrobacter* Sp. *Nitrosomonas* carry out the oxidation of NH$_4^+$ to NO$_2^-$ to obtain energy and *Nitrobacter* oxidizes NO$_2^-$ to NO$_3^-$ for the same reason (Ghaly and Ramakrishnan, 2015). The general oxidative processes involved can be represented by the following equations (Eq. 1-1, 1-2) (Anthonisen et al., 1976):

\[
\text{NH}_4^+ + 1.5\text{O}_2 \xrightarrow{\text{Nitrosomonas}} \text{NO}_2^- + \text{H}_2\text{O} + 2\text{H}^+ + \text{Energy} \quad (1-1)
\]

\[
\text{NO}_2^- + 0.5\text{O}_2 \xrightarrow{\text{Nitrobacter}} \text{NO}_3^- + \text{Energy} \quad (1-2)
\]

**Denitrification**

Nitrate reduction (or denitrification) is a more complex and less understood process than nitrification. There are two processes of nitrate reduction: assimilatory and dissimilatory. Assimilatory nitrate reduction (ANR) is one of the main processes in the nitrogen cycle in which NO$_3^-$ is used as the nitrogen source for growth of new cells. The process includes three steps: (1) NO$_3^-$ uptake, (2) reduction of NO$_3^-$ to NO$_2^-$ and (3) reduction of NO$_2^-$ to NH$_4^+$ (Bonete et al., 2008). Dissimilatory (or respiratory nitrate) reduction is the process in which nitrate serves as the terminal hydrogen acceptor in energy yielding reactions (Ghaly and Ramakrishnan, 2015).

**Nitrogen Fixation**

Although atmospheric nitrogen (N$_2$) is abundant in the atmosphere, it is inert and not a ready form to be used by most organisms. Diazotrophic microorganisms are capable of fixing N by breaking the strong triple bond (Puri et al., 2016). The diazotrophs are available in both soil and in symbiotic association with plants; they use the enzyme nitrogenase to carry out the fixation process. The enzymatic reaction is described as follows (Eq. 1-3):
\[ \text{N}_2 + 4\text{H}_2\text{O} \xrightarrow{\text{Nitrogenase}} 2\text{NH}_4^+ + 2\text{O}_2 \quad (1-3) \]

Nitrogen fixation is inhibited in the presence of high levels of available nitrogen (NH\(_4^+\) or NO\(_3^-\)), and the process is controlled by N:P ratios as phosphorus activates the gene for nitrogen synthesis (Bohlool et al., 1992). The rate of nitrogen fixation is related to the rate of photosynthesis (Ghaly and Ramakrishnan, 2015).

**Microbial and Plant Uptake of Ammonium**

The NH\(_4^+\) uptake across the biological membranes of microbes is generally facilitated by a class of transport proteins called ammonium transporters (Mylona et al., 1995). Most plant species absorb and assimilate NO\(_3^-\), NH\(_4^+\), urea and amino acids as nitrogen sources, but the specificity of these sources vary with different plants (Ghaly and Ramakrishnan, 2015). Both NO\(_3^-\) and NH\(_4^+\) are absorbed by plants in the form of an amino group (-NH\(_2\)) and the availability of both depends on the environmental conditions that affect the production of NH\(_4^+\) and the conversion of NH\(_4^+\) to NO\(_3^-\). However, many plant species show preferences to NO\(_3^-\) over NH\(_4^+\), although thermodynamic analysis suggests that the metabolic energy cost of reducing NO\(_3^-\) to NH\(_2\) is significantly greater (Ghaly and Ramakrishnan, 2015).

**Ammonia Volatilization**

Ammonium exists in two forms: free or unionized form (NH\(_3\)) and ionized form (NH\(_4^+\)). Ammonium is soluble in water while NH\(_3\) is volatile and can easily be removed from water. Volatilization occurs when NH\(_4^+\) ions are present in an alkaline medium and gets dissociated into gaseous NH\(_3\) which then gets released into the atmosphere. That reaction is shown below (Eq. 1-4):

\[ \text{NH}_4^+ + \text{OH}^- \rightarrow \text{NH}_3 + \text{H}_2\text{O} \quad (1-4) \]
Leaching of Nitrate and Nitrite

Leaching refers to the removal of \( \text{NO}_3^- \) or \( \text{NO}_2^- \) from the plant root zone by the movement of water through the soil. Since both are negatively charged, they are found to move freely with water unless soils have a significant anion exchange capacity (Ghaly and Ramakrishnan, 2015). Leaching has a high environmental impact and it reduces the bioavailability to plants.

Ammonia Formation and Volatilization

Urea is the main nitrogenous constituent of urine. Bristow and coworkers (1992) reported that urea N represented from about 60 to 90 \% of all urinary N in cattle. Other significant nitrogenous components were hippuric acid, creatinine, and metabolites of purine bases, such as allantoin, uric acid, xanthine, and hypoxanthine (Hristov et al., 2011). In the urine of high-producing dairy cows, urea represents 60 to 80 \% of total urinary N (Reynal and Broderick, 2005) and proportionally increases as dietary CP level and intake increase (Colmenero and Broderick, 2006). Urea is the main source of \( \text{NH}_3 \) volatilized from cattle manure (Bussink and Oenema, 1998) and these authors indicated that 4 to 41 \% of the urinary N may be volatilized, while N volatilization from feces is considerably less at 1 to 13 \%. In a 1 yr study at two Texas feedlots, Cole and Todd (2009) noted that N volatilization losses ranged from 64 to 124 \% of urinary N excretion, with an average of 79 \%. Urea is not volatile, but once it comes in contact with feces it is rapidly hydrolyzed to \( \text{NH}_3 \) and \( \text{CO}_2 \) by the abundant urease activity in fecal matter. Lee et al. (2009) showed very low \( \text{NH}_4^+ \) concentration in fresh dairy cow manure, but a rapid hydrolysis of urea in urine resulting in a sharp increase in \( \text{NH}_4^+ \) concentration in manure and \( \text{NH}_3 \) volatilization rates. In this study, concentrations of urea in manure decreased from 3.7 to 0.7 mg mL\(^{-1}\) in 24 h, representing an 80 \% loss of urea. Hollmann and
coworkers (2008) estimated that over a 6 h period in a flushing dairy facility, manure is flushed away from the barn floor, the rate of volatilization was 18.1 %/h. However, this estimate would have included some losses of NH₃ from the water used to flush the barn, a factor that may explain the differences in emission rates between these studies. James et al. (1999) observed that essentially all of the urea present in cattle manure was converted to NH₃ and volatilized within 26 h after excretion.

The chemical composition of fresh urine spots have been shown to be different from drier areas of a feedlot pen and that NH₄⁺ concentrations in the pen surface increased 10-fold within 5-min of urine application, then decreased by approximately 50 % over the next 2 h (Cole et al., 2009). They also showed that surface pH increased rapidly, however after 4 d the N concentration and surface pH returned to background levels. These changes in the pen surface chemistry agree well with NH₃ flux data. Using surface isolation flux chambers, NH₃ emissions from urine spots were 10 to 20 times the emissions from dry pen surfaces (Rhoades et al., 2008).

Lee and Hristov (2010) quantified the relative contributions of urinary N and fecal N to NH₃-N volatilization losses from cattle manure. Feces and urine from lactating dairy cows were labeled with ¹⁵N, combined in a 1:1 ratio, and incubated for 10 d in a laboratory-scale closed-chamber system. The proportion of NH₃-N originating from fecal N was negligible in the first 48 h of the incubation and gradually increased to approximately 11 % as mineralization of fecal N progressed. The proportion from urinary N was 94 % at 24 h, decreasing gradually to 87 % over the 10 d incubation. This study shows that urinary N is the principal source of NH₃-N from cattle manure during the initial 10 d of storage.
The complete of hydrolysis of urea to NH$_3$ (or NH$_4^+$) in aqueous environments is catalyzed by the enzyme urease and occurs in two steps (Hristov et al., 2011). In the first step (Eq. 1-5), 1 mole of urea is hydrolyzed into 1 mole of NH$_3$ and 1 mole of unstable carbamic acid. Carbamic acid then spontaneously decomposes into a second mole of NH$_3$ and 1 mole of CO$_2$ (Eq. 1-6). Effectively, a mole of urea produces 2 moles of NH$_3$.

\[
\text{NH}_2(\text{CO})\text{NH}_2 + \text{H}_2\text{O} \rightarrow \text{NH}_3 + \text{NH}_2(\text{CO})\text{OH} \quad (1-5)
\]

\[
\text{NH}_2(\text{CO})\text{OH} \rightarrow \text{NH}_3 + \text{CO}_2 \quad (1-6)
\]

The conversion of organic-N (proteins, amino polysaccharides, and nucleic acids) to NH$_4^+$-N is mediated by enzymes produced by heterotrophic microbes (Hristov et al., 2011). First, extracellular enzymes break down organic-N polymers into monomers. These monomers then enter the microbial cell and are further metabolized by intracellular enzymes into NH$_4^+$. Some of the NH$_4^+$ is assimilated into the microbe as protein and nucleic acids, and the surplus is incorporated into the bulk manure (Barak et al., 1990; Barraclough, 1997).

In aqueous environments, NH$_4^+$-N and NH$_3$-N exist in an equilibrium that is primarily controlled by pH and temperature (Hristov et al., 2011). At a constant temperature, the pH of the solution (or manure, in this case) drives the equilibrium: lower pH favors NH$_4^+$ and hence lowers the potential for NH$_3$. The reverse is also true: increasing the pH shifts the equilibrium towards NH$_3$, thus increasing volatilization. The greatest increase in NH$_3$ release takes place between a pH of 7 and 10, and at a pH of 7 or below volatilization decreases such that at a pH of 4.5 there is essentially no measurable free NH$_3$ (McCarty and Swayer 1978; Hartung and Phillips, 1994; Ndegwa
et al., 2008). Increasing temperature will lead to an increase in dissociation of NH$_4^+$-N to NH$_3$-N, thus increasing volatilization.

Generally, the process of NH$_3$ volatilization involves movement of NH$_3$ to the manure surface and subsequent release of NH$_3$ into the ambient air (Ni 1999). A conceptual model of NH$_3$ formation and volatilization adapted from Hristov et al. (2011) is presented in Figure 1-2.

**Quantifying Ammonia Emissions from Animal Facilities**

Quantifying NH$_3$ or any other gaseous emissions from animal facilities entails two major challenges: 1) measuring NH$_3$ concentration in the air, 2) quantifying NH$_3$ transfer efficiency from surface to atmosphere (Hristov et al., 2011). The complex nature of environmental variables affecting gas production rates and fluxes creates great difficulty for accurate assessment and monitoring of gas emissions. Emissions from soils and manure are well reported as varying both daily and seasonally (Hu et al., 2014). In addition, climatic conditions, such as temperature and precipitation, and porous medium characteristics, including pH, texture, and mineralogy, all act individually and collectively to change emissions spatially and temporally (Amon et al., 2001; Mosier, 1994). In light of these complexities, reliable emission measurements necessitate an integrated whole-system approach applied during real-time agricultural operations (Amon et al., 2001).

**Instrumentation to Measure Aerial Ammonia Concentrations**

There are many techniques and measurement methods available to provide either simple or sophisticated analysis for NH$_3$ concentrations in the air (Phillips et al., 2001). Some of these methods are discussed below.
Detector tubes

These devices provide a simple and convenient way of measuring atmospheric NH$_3$ and have been the main method of previous research to measure NH$_3$ concentrations from horse manure (Williams et al., 2011; Pratt et al., 2000). The NH$_3$ tube has a specified range of ammonia concentration detection of 0.25 to 200 ppm with a standard deviation of ± 10 to 15 % (Parbst et al., 2000). Each tube consists of a scaled glass vial containing a chemical reagent that reacts with NH$_3$. A calibrated 100 ml sample of air is drawn through the tube and if NH$_3$ is present, the reagent in the tube changes color and the length of color change typically indicates the measured concentration (Dräger, 2011). These devices are primarily designed to assess human exposure to NH$_3$ at relatively high concentrations.

Acid scrubbers

Gas washing, or acid scrubbing, is a type of chemical acid absorption that involves actively pulling and bubbling air through an acid solution (boric, sulfuric, hydrochloric, phosphoric), where the NH$_3$ in the air is drawn into solution to form NH$_4^+$ which reacts with the acid and is chemically trapped in the solution (Hristov et al., 2011). The NH$_4^+$ captured can be analyzed in the lab using spectrophotometric techniques. The knowledge of the air flow rate and duration through the acid solution allows for the calculation of the NH$_3$ in the air (Hristov et al., 2011). This method is proven, inexpensive, and accurate for measuring atmospheric NH$_3$ in many situations (Denmead et al., 1974; Beauchamp et al., 1978; Hutchinson et al., 1982; Bussink et al., 1996; Sharpe and Harper 1997; Todd et al., 2006). However, there are some definite drawbacks. The use of acid scrubbers needs long sampling time (hours) to get an average concentration, thus limiting the applicability when finer-scaled measurements
are needed. Also, the acid scrubbers cannot discriminate between NH$_3$ and NH$_4^+$ or N-containing volatile organic compounds that may become entrapped in the acid solution (Sommer and Hutching, 1995).

**Chemiluminescence analyzers**

A chemiluminescence analyzer relies on the high temperature oxidation of NH$_3$ and NO$_x$, and only NO$_x$ converts to NO, which subsequently is converted in the presence of ozone to NO$_2$. When excited NO$_2$ drops to a lower energy state, emitted radiation is proportional to NO concentration, and from that NH$_3$ concentration can be calculated (Hristov et al., 2011). Chemiluminescence instrumentation provides continuous monitoring of NH$_3$ and has been used with micrometeorological methods (Phillips et al., 2004) and closed chambers (Baek et al., 2003).

**Photoacoustic analyzer**

The photoacoustic analyzer uses a measurement system based on the photoacoustic infrared detection method, and is capable of measuring almost any gas that absorbs infrared light (Li and Xin, 2006). Light from an infrared source is reflected off a mirror, passed through a mechanical chopper, which pulsates light, and then through optical filters. This causes the temperature of the gas being monitored to increase which selectively absorbs the light transmitted by the filter. Due to the light pulsating, the gas temperature increases and decreases, causing an equivalent increase and decrease in the pressure of the gas (an acoustic signal) in the closed cell. Two microphones mounted in the cell wall measure this signal, which is proportional to the concentration of the monitored gas in the cell (Li and Xin, 2006).

In the studies included in this dissertation, two separate photoacoustic analyzers were used. A photoacoustic multi-gas analyzer (INNOVA 1412, LumaSense
Technologies, Inc. Santa Clara, CA.) was used to measure the NH$_3$ concentration from horses fed different concentrations of crude protein using an *in-vitro* emission vessel system. A single gas- Chillgard RT analyzer (Mine Safety Appliances Company, Pittsburgh, PA.) was used to measure NH$_3$ concentrations in individual horse stalls using a flux chamber. This Chillgard RT analyzer has been used in several agricultural NH$_3$ emission studies for its advantages over other analyzers, including stability, low cost, and minimal maintenance (Li et al., 2008); however, a drawback is its high detection limit at 1 ppm compared to the detection limit on the INNOVA 1412 being 0.2 ppm. Li and coworkers (2015) found that there was a strong linear relationship between the NH$_3$ concentration readings from the Chillgard RT and the INNOVA under different field conditions. However, the Chillgard RT overestimated NH$_3$ concentrations by an average of 3.2 %, 10 % and 22.9 % with laying-hen houses, stored broiler litter, and broiler houses, respectively (Li et al., 2015).

**Methods to Estimate Ammonia Emissions**

In general, there are two measurement approaches: methods that interfere with gas transport processes (chamber methods) and methods designed to minimize such interferences (micrometeorological methods) (Harper, 2005). Point-scale chamber techniques have some significant limitations (Hu et al., 2014). While these enclosure methods can be versatile in some scenarios, micrometeorological techniques can estimate gas emissions from much broader footprints (Brown et al., 2002). Fundamental assumptions of each method must be weighed in light of each unique measurement scenario, and measurement techniques must be carefully chosen depending on the desired application (Hu et al., 2014).
The basic task of micrometeorological methods that estimate NH₃ emission is to describe the nature of turbulence. Micrometeorological methods have been commonly used to measure multiple gases, including CH₄ and NH₃ from both point and non-point sources (Hatala et al., 2012; Jungbluth et al., 2001; Neftel et al., 2006). These techniques offer advantages in that they are truly in situ and can integrate fluxes over larger areas than chamber techniques (Brown et al., 2002). Generally, micrometeorological methods rely on measurements in and characterization of the atmosphere near the ground (Hristov et al., 2011). These methods are dependent on simplifying assumptions regarding uniformity and homogeneity of airflow (Hu et al., 2014). Also the required equipment is often prohibitively expensive (Flesch et al., 2007).

Chamber methods are commonly used for estimating gas emissions from agricultural and animal feeding operations (McGinn, 2006; Rochette et al., 2005; Spiehs et al., 2011). They are generally less expensive to implement than micrometeorological methods because the required instrumentation is not as complex. These methods are commonly useful for smaller settings (Hu et al., 2014), like a horse stall. The main disadvantage of chamber methods is the artificial, constrained environment they create, which can alter the boundary conditions for natural gas production (Johnson and Johnson, 1995).

Chambers are defined as either steady state (SS), where the gas loop is open and the concentration of the measured gas does not accumulate in the chamber over time, or non-steady state (NSS) where the gas loop is closed and gas accumulates in the chamber (Livingston and Hutchinson, 1995). To prevent gas accumulation in a SS chamber, either air must flow through the chamber or the trapped gas must be removed.
by chemical reaction (McGinn, 2006). Air is circulated through SS chambers at a known, constant rate, and flux rate calculated as function of the enclosed area, flow rate, the inlet and outlet NH₃ concentration, and the molar air volume of air at chamber temperature and pressure (Hristov et al., 2011). Chambers are appropriate for comparing treatments or assessing relative emissions rate, but not for quantifying actual emissions (Meisinger et al., 2001).

**Monitoring Ventilation Rate**

Many of the approaches to measuring NH₃ emission rates require the monitoring of ventilation rates of the barns. Quantifying gaseous emissions from naturally ventilated (NV) barns, however, has additional challenges primarily because of the complexity of air exchange rates (AER) determination (Kiwan et al., 2012). The AER in NV barns are directly dependent on atmospheric conditions (Snell et al., 2003; Ngwabie et al., 2011).

The fundamental approach of determining AER in a NV facility is direct measurement of airflow at the inlets and outlets of the building (hence: direct method) (Wang et al., 2016). Direct measurements of AER in the field, however, are few due to substantial initial investment (Joo et al., 2014). Joo and coworkers (2014) used 16 ultrasonic anemometers (sonics) at select points of the barn openings to measure air velocities at respective air inlets and outlets. They found that the sum of airflows through all openings acting as inlets were the best measure of barn ventilations for that given period.

The AER for NV buildings are also measured using indirect methods such as tracer gas techniques and the CO₂-mass balance. The basic principle of tracer techniques for direct measurement of overall ventilation rate is to release a tracer at a known rate, monitor its concentration at downwind points and hence determine the
airflow necessary to achieve this downwind concentration of the tracer gas (Li and Xin, 2006). The major assumption is that there is complete air mixing, but this may not be the case in practice (Demmers et al., 1998; Joo et al., 2015). The CO$_2$-balance method, based on CO$_2$ production by animals, is one of the most commonly used methods in NV buildings, and research has indicated that the CO$_2$-balance method agreed well with the direct method in mechanically ventilated barns (Blanes and Pedersen, 2005; Li et al., 2005; Xin et al., 2009; Wang et al., 2016). The accuracy of this indirect method, however, depends on CO$_2$, H$_2$O, and heat produced by the housed livestock, which vary with animal weight, productivity, and manure management system (Wang et al., 2016).

The ventilation rate is based on the indoor and outdoor CO$_2$ concentration and can be calculated by (Eq. 1-7):

$$CO_2 = V \times (C_o - C_i) \times 10^{-6}$$

where CO$_2$ is the CO$_2$ production (m$^3$h$^{-1}$), C$_o$ is the outdoor CO$_2$ concentration (ppm), C$_i$ is the indoor CO$_2$ concentration (ppm), and V is the ventilation rate (m$^3$h$^{-1}$) (Li and Xin, 2006).

**Crude Protein in Equine Diets**

It is common in the horse industry to overfeed crude protein (excess of the requirement) (Lawrence et al., 2003; Swinker et al., 2009; Bott et al., 2016). In livestock, successful predictors of N utilization and excretion are primarily based on knowledge of feed protein quality and understanding of the digestive processes. However there is little information regarding this process in horses, and equids are fed a wide variety of forages, cereal grains, and oil seeds that vary in protein quality (Trottier et al., 2016).

Horses have evolved to eat the structural parts of plants. These include the stems and leaves, which contain significant amounts of structural polysaccharides,
including cellulose, hemicellulose, and lignin (Van Soest, 1967). When compared to other grazing animals, equids have a greater feed intake per unit body weight and shorter passage time (Haenlein et al., 1966). Such a digestive strategy has been suggested as being essential for the use of high-fiber herbage and survival on a diet which ruminants of similar body size cannot maintain themselves (Trottier et al., 2016). Herbivore body size is crucial to predicting the fiber to protein ratio with larger animals, like the horse, requiring less protein and tolerating more fiber (Demment and Soest, 1985).

**Pattern of Nitrogen Metabolism**

Dietary protein is digested mainly in the foregut of the horse through enzymatic digestion in the stomach and small intestine. Enzymatic digestion of protein in the stomach occurs via pepsin, and pancreatic proteases secreted into the small intestine continue protein breakdown and enable absorption of amino acids (AA) and dipeptides in the small intestine (NRC, 2007). The ultimate end products of the small intestinal protein digestion processes are the free AAs, which are available for absorption into the enterocytes (Urschel and Lawrence, 2013).

Horses are equipped with a highly specialized gastrointestinal system and have adopted cecal and hindgut digestion, rather than ruminal. In rabbits, hindgut fermenters similar to horses, undigested protein and unabsorbed AAs pass into the large intestine, where cecal peptidase activity is much lower than in the small intestinal segments (Bai 1993). There has been little research in horses about microbial digestion of protein; however, microbial protein digestion in the rumen of cattle has been studied and could provide insight into the processes occurring in the equine large intestine (Urschel and Lawrence, 2013). In ruminants, proteases associated with the bacterial cell wall begin to
degrade proteins into smaller peptide chains, which can then be absorbed by the bacteria, cleaved into AAs, and either used for microbial protein synthesis or further degraded to carbon skeletons and NH$_3$ (Wallace, 1996). Bacterial cells isolated from the cecum of the horse could use partially digested protein as a N source, with small subsets also being able to use NH$_3$ and urea as sole N sources (Maczulak et al., 1985). It is important to note the distinction between the sites of microbial protein synthesis in ruminants versus monogastrics. In ruminants, microbial protein synthesis occurs before gastric and pancreatic secretion and therefore can be digested by the animal in a similar manner as dietary protein in monogastrics. In horses, however, microbial protein synthesis occurs after these secretions and has implications for both the digestion and absorption of these proteins (Urschel and Lawrence, 2013).

**Digestibility of Dietary Protein**

Protein digestibility may be measured at the end of the gastrointestinal tract through fecal sampling, known as apparent total tract digestibility, or at the end of the small intestine prior to the entrance into the cecum through collection of ileal digesta, known as apparent ileal or prececal digestibility (Trottier et al., 2016). Apparent CP (N x 6.25) is calculated from the N output in feces or ileal digesta relative to N intake. The apparent total tract approach is non-invasive and simple, but it does not accurately reflect the end result of dietary CP digestion due to basal and endogenous contribution from microbial protein and secretions in the feces (Urschel and Lawrence, 2013; Trottier et al., 2016). The vast majority of AAs are absorbed prior to the ileum and most postcecal N absorption is likely to be NH$_3$. Ammonia is only available for protein synthesis if it is first incorporated into dispensable AAs following absorption. Thus, prececal protein digestibility may be a more reliable indicator of the amount of protein N
that is absorbed in a form that is readily available for protein synthesis (Urschel and Lawrence, 2013). In mature horses, protein digestibility varies based on the source and the composition of the diet. The NRC (2007) estimated total tract apparent N digestibility to be 79 % and pre-cecal apparent N digestibility to be 51 %. Within forages, apparent total tract CP digestibility varies from 73-83 % for alfalfa, 57-64 % for Coastal Bermudagrass and 67-74 % for grasses (NRC, 2007). Feeding corn, oats, or sorghum in combination with Coastal Bermudagrass resulted in apparent total tract protein digestibility of 88, 82.8 and 84.6 % respectively (Gibbs et al., 1996).

**Protein Requirements**

Endogenous urinary and fecal N have been evaluated in several studies to try to estimate the minimal protein needs of the horse. Nitrogen balance studies in mature horses and ponies have concluded that between 400 and 800 mg DP (digestible protein)/kg BW/d is necessary to achieve N balance in the maintenance horse (NRC, 2007). Applying linear regression to means from studies (N = 12) that measured N intake and N retention resulted in 619 mg DP/kg BW/d. Because N balance tends to underestimate true N loss, due to error as well as loss from hair, skin and sweat, some allowances for retention should be made when determining the requirement (NRC, 2007). Fitting the same data to a broken-line model estimates the requirement to be 1.26 g/kg BW/d, thus for a 500 kg horse, this would equate to 630 g CP/d.

**Dietary Strategies for Reducing Ammonia Loss to the Environment**

In other livestock species, research data indicates that diets fed to animals have profound effects on NH₃ emissions from excreted manure (Hristov et al., 2011; Bougouin et al., 2016). In a meta-analysis conducted with dairy cattle, Bougouin et al. (2016) showed that NH₃ emissions were, in part, influenced by dietary CP content (r =
0.51) and N intake \((r = 0.55)\). Overfeeding of CP and feeding an imbalanced AA supply can lead to excessive urinary N excretion (Hristov et al., 2011). Paul et al. (1998) reported a linear decrease in cattle manure NH\(_3\) losses with decreasing dietary CP concentration. Another study (Chiavegato et al., 2015) showed that decreasing CP content from 13 to 10 % in diets fed to Holstein steers did not affect BW or DMI, but did reduce the NH\(_3\) emissions. In pigs, multiple studies have shown that reducing dietary CP and supplementing with essential AA results in reduced urinary N and NH\(_3\) emissions (Canh et al., 1998; Li et al., 2015).

The quantity and source of dietary N can affect nutrient excretion markedly, because of differences in the site and rate of protein digestion in ruminants (Hristov et al., 2011). Using an in vitro system, Cole et al. (2005) did not find a significant effect of dietary CP source (urea or cottonseed meal) on NH\(_3\) emissions. However, losses from steer feces and urine increased exponentially as dietary CP concentration increased. Another study from the same group noted similar effects when feces and urine were applied to a simulated feedlot surface (Todd et al., 2006). Bierman et al. (1999) fed diets with neutral detergent fiber (NDF) contents of 10 %, 13 %, or 28 % of diet DM to feedlot cattle. Under these conditions, 45 to 57 % of the N fed was volatilized, and the proportion decreased with increasing dietary roughage content. This was most likely due to a partial shift in N excretion from urine to feces (Hristov et al., 2011). Kellemes and coworkers (1979) noted that grain source and concentration in the diet affected NH\(_3\) emissions primarily via their effects on fecal pH.

**Environmental Implications of Excreted N from Horses**

Ammonia emissions (kg/LU, livestock unit 500 –kg live weight) are predicted to be lower in horses than cattle, pigs, poultry, or sheep (Hartung and Phillips, 1994);
however NH$_3$ emitted by horses is no less able to impact the environment on a unit-by-unit basis. According to the American Horse Council (2005), the estimated horse population in the United States is 9.2 million. In 2002, the EPA’s national emissions inventory estimated an NH$_3$ emission factor for horses of 26.9 lb/head/year; therefore, horses produce approximately 123,740 tons of NH$_3$ emissions per year. Similarly, the EPA’s national emissions inventory (2002) estimated an NH$_3$ emission factor for beef cattle of 25.2 lb/head/year.

Multiple factors can influence NH$_3$ volatilization on horse facilities. They include temperature, air velocity, pH, surface area, manure moisture content, and storage time. As discussed in previous sections, high pH and temperature correlate with an increase in NH$_3$ emissions. When collected from soiled stall waste (manure + bedding), horse manure pH typically ranges from 7.7 to 8.2, depending on bedding source (Komar et al., 2012). The pH of horse manure samples collected between 2007 and 2014 ranged from 6.9 to 8.2, which allows for fairly rapid NH$_3$ volatilization (Bott et al., 2016). Only a few studies have been conducted to determine the amount of N and/or NH$_3$ concentration of horse stall waste and none have focused on calculating the emission rate based on environmental factors.

Horse stables are noted to have higher NH$_3$ concentrations than pastures (Bott et al., 2016). Whittaker and coworkers (2009) were interested in determining the exhaled breath condensate (EBC) pH from ponies housed on pasture and in a barn. Measurement of EBC pH provides a simple, highly repeatable and noninvasive method for the longitudinal investigation of changes in airway pH in response to environmental changes. Stabling of horses was found to lead to a significant increase in ambient NH$_3$.
concentrations which was associated with an increase in EBC pH and exhaled NH$_3$ compared to horses housed on pastures.

The majority of previous research conducted to investigate NH$_3$ concentration from horse waste has focused on the impact of bedding type. Garlipp and colleagues (2011) analyzed the influence of horse feces and urine added to different bedding materials on the generation of NH$_3$ from deep litter bedding under standardized laboratory conditions. They found that wheat straw emitted the highest concentration with wood shavings emitting the lowest. Another study compared different bedding types (wheat straw, wood shavings, and wheat straw pellets) used in horse stables on the generation of airborne PM (< 10 µm) and 3 gases, including NH$_3$ (Fleming et al., 2008). The mean gaseous NH$_3$ were found to be 256 ppm for wheat straw, 223.5 ppm for wood shavings, and 86.83 ppm for wheat straw pellets.

In a study conducted in horses fed at approximately 165 % of the recommended protein requirement (Williams et al., 2011), authors showed that elevating protein levels in a horse’s diet increases the NH$_3$ lost to the atmosphere and N levels excreted in manure. More specifically, fecal N and NH$_3$ concentrations were higher (approximately 15 and 100 % higher, respectively) in the high protein fed horses (1042 g CP/d DM) than in the control fed horses (703 g CP/d DM). Nitrogen and NH$_3$ for control horses were 0.242 % and 0.034 %, respectively, whereas N and NH$_3$ were 0.278 % and 0.068 %, respectively, for horses fed high protein diets. When atmospheric NH$_3$ was tested by an 8-hour accumulation via Dräger tube, there was a significantly higher level of NH$_3$ in the air in the stalls of horses fed the high protein diet. The high protein horses averaged 37.8 ppm, whereas the control horses averaged 25.4 ppm. These levels could
potentially be a problem for the horses’ health if left exposed for an extended period of time (Bott et al., 2016).

While these studies have succeeded at determining NH₃ concentrations based upon different variables, none have attempted to characterize NH₃ emissions based on simultaneous consideration of these factors (pH, temperature, dietary CP intake, bedding, and air velocity). Quantifying NH₃ emissions from horse manure, and how diet composition affects N excretion, would provide more meaningful information to assess the potential impact of equine operations on the environment. More importantly, horse-specific information is necessary given that regulatory agencies could potentially use such values as horse emission factors.

**Impact of Ammonia Emissions on Horse Health**

For the equine industry, concerns about NH₃ concentrations in the stable environment are multifaceted and include issues of animal welfare, animal and human health, and environmental impacts. Barn ventilation and the surrounding environment have an important impact on air quality in the barn (Ivester et al., 2014a). Season and stable construction were found to impact particulate concentrations and number when 3 barns were evaluated at a race track (Millerick-May et al., 2011). They found that the barn subjectively judged to have the least natural ventilation had significantly higher PM₁₀ and PM₂.₅ measurements.

While there are recommendations for proper horse stable ventilation (Wathes, 1989; MWPS, 1971) few stables are built to these specifications. Most horse stables employ natural, rather than mechanical, ventilation due to the relatively low density of mature animals within the building (Clarke, 1987; Golden, 2000). By agricultural engineering definition, stables are “cold housing with no supplemental heat and no, or
limited insulation. In recent history, the trend has been to tighten up construction to a more “residential” standard rather than complying with livestock housing ventilation recommendations (Wheeler, 2001). Hence, most modern horse barns are under-ventilated in an attempt to maintain comfortable conditions for handlers and an earnest, but misdirected, attempt to provide thermal comfort for the horse (Sainsbury and Rossdale, 1987). Horses kept indoors can therefore be exposed to ammonia on a regular basis.

Ammonia concentrations have a negative impact on horse health. Chronic and acute respiratory disease is one of the leading causes of wastage in horses used in high performance athletic endeavors and is commonly recognized in pleasure horses as well (Hernandez and Hawkins, 2001). Holcombe et al. (2001) established that stabling is associated with increased airway inflammation and the persistence of upper airway inflammation in young horses. Gerber et al. (2003) found that horses housed in a stable environment while clinically healthy and performing well showed evidence of inflammatory airway disease (IAD). Inflammatory airway disease in horses is a widespread syndrome in which inflammation of the lower airways results in impaired gas exchange and poor performance (Couetil et al., 2007). Exposure to airborne dust and other irritants (such as NH\textsubscript{3}) present in the barn environment appears to play a major role in the pathogenesis of IAD (Ivester et al., 2014b).

Ammonia is the most important gas in the barn air with respect to animal health, particularly of the respiratory tract (Fleming et al., 2009). Various studies have found that ammonia levels are highest near barn and stall floors (Fleming et al., 2009; Pratt et al., 2000). High levels of ammonia have been associated with foal pneumonia.
(McMillian, 1986) and may also contribute to horses being predisposed for chronic obstructive pulmonary disease (Tanner et al., 1998).

Since horses frequently eat off the floor, have their heads down and/or lie down when stabled for long periods of time, they can be exposed to high levels of ammonia. Lawrence et al. (1988) detected ammonia concentrations of 25.3 parts per million (ppm) at the level of the horse’s halter following a 16-day period of stall confinement. There has been evidence to show that horses could be exposed to NH$_3$ concentrations from 25-37 ppm at head level (Williams et al., 2011). As it is common practice to house sport type horses in barns for extended periods of time, these concentrations could be an issue. A study in Japan found that a horse inhaling only 2–17 ppm of NH$_3$ (range of NH$_3$ in an enclosed trailer) over the course of 40 hours in stalls created excessive nasal discharge and slight swelling of the nasal cilia (Katayama et al., 1995). This same study found that another horse inhaling 40–130 ppm (maximum exposure for humans) had a much more severe nasal discharge, swelling and irregular distribution of tracheal epithelium and edema of the submucosa, loss of nasal cilia, and more severe swelling than the horse exposed to 2–17 ppm. This study indicates that inhalation of these concentrations of ammonia is detrimental to the respiratory health of horses. The concentration of ammonia that is harmful to horses, in both the short term and long term, has not been determined. In humans, the short-term exposure limit (15-min) is 35 ppm (ATSDR, 2004) and according to OSHA (2015) the permissible exposure limit for NH$_3$ is 25 ppm.
Assessing Impact of Ammonia Emissions on Equine Welfare

Using Aversion Testing to Assess Welfare

Fraser and coworkers (1997) described animal welfare in terms of feelings, functioning of the body and ability to express natural behaviors. While it cannot be proven that feelings play a role in the causation of behavior, even in humans (Fraser and Duncan, 1998), it is widely suggested that at least some affective states do play a role (Broom, 1998; Fraser and Duncan, 1998) and hence that they have observable effects (Dawkins, 1993). Even if feelings do not have observable consequences, the correlation between reported feelings and behavioral responses to stimuli in humans suggests that behavioral and physiological responses reflect the strength of associated feelings (Kirkden and Pajor, 2006).

The term ‘preference’ denotes a difference between the strength of motivation to obtain or avoid one resource or stimulus and the strength of motivation to obtain or avoid another (Kirken and Pajor, 2006). Aversion tests are designed to assess whether a preference exists or to measure the strength of a motivation of a preference. It is assumed that all negative stimuli contribute to ‘distress’, common motivational state underlying the specific states of fear, pain, anxiety, frustration and boredom (Rushen, 1990). Aversion choice tests are designed to ascertain whether an animal is motivated to avoid a stimulus, or to identify a preference between two different stimuli (Kirken and Pajor, 2006). The stimulus that is chosen fewer times or with less time spent is said to be more aversive (Raj and Gregory, 1991; Jones et al., 1996; Pajor et al., 2003).
Physiological Indicators of Aversion

Heart rate and heart rate variability

Some of the most remarkable, non-invasive, measures of the functioning of the autonomic nervous system (ANS) are indices of heart rate variability (HRV) (von Borell et al., 2007a). Detailed and sophisticated analysis of short-term fluctuations in instantaneous heart period has been widely used to indirectly assess ANS regulation of cardiovascular function (Kautzner, 1995). Analysis of HRV has been used as an indicator of acute and chronic stress (Delaney and Brodie, 2000; Hall et al., 2004).

The sinoatrial node (SN) acts as the primary pulse generator of heart beats and is under the control of the parasympathetic (vagal, PNS) and sympathetic nervous system (SNS) (Akselrod, 1995). Heart rate (HR), at any point in healthy individuals, represents the net interactions between PNS (which reduces HR) and SNS (which increases HR) regulation (Hainsworth, 1995). These separate effects of the ANS cannot simply be added or subtracted, because the two components can work either together or independently. Therefore, mean HR parameters provide information on the net effects of all components inputting into cardiac activity and are of limited use for accurately assessing SNS regulation (Tulppo et al., 1998). Heart rate variability analysis, on the other hand, allows a much more accurate and detailed determination of the functional regulatory characteristics of the ANS (von Borell et al., 2007a). Heart rate variability is characterized by specific fluctuations in the lengths of the inter-beat-intervals (IBI) (Stucke et al., 2015).

There are essentially three approaches for quantifying HRV: time-domain analyses, frequency-domain analysis, and nonlinear analysis methods. Time-domain analysis of HRV reflects various aspects of statistical variability in the IBI data series.
Key measures include the mean IBI, standard deviation of IBI (SDRR) and the root mean sum of squares of differences between successive IBI (RMSSD). The mean IBI (R-R) and SDRR reflect the long term variability of cardiac activity and are influenced by both the SNS and PNS. Measures of variability derived from differences between adjacent IBI, like RMSDD, reflect the high frequency beat-to-beat variations (Stucke et al., 2015). Since the PNS reacts faster than the SNS, high frequency variations represent PNS activity (von Borell et al., 2007a).

Heart rate variability is composed of several oscillations due to the different biological regulation systems involved in HR control (Stucke et al., 2015). In spectral (frequency domain) analysis, characteristics of the oscillating signal, such as frequencies and power, are estimated. A key function in this context is the so-called power spectral density (PSD), which indicates at which frequencies the signal shows strong variations (Stucke et al., 2015). It is widely accepted that the power in the high frequency (HF) band represents vagal (PNS) activity (Akselrod, 1995). The low frequency (LF) band is associated with SNS, or SNS+PNS activity, however the physiological meaning has been debated (Ponikowski et al., 1996; Wagner et al., 1997). The LF/HF ratio is used as an indicator for both SNS tone and cardiac sympathetic-vagal balance (Yamamoto et al., 1991; von Borell et al., 2007a). The recommended frequency band thresholds for interpretation of ANS activity in horses are LF 0.01 ≥ 0.07 Hz and HF 0.07 ≥ 0.6 Hz (Kuwahara et al., 1996).

Stress can result in a shift of the SNS-PNS balance towards the SNS (von Borell et al., 2007a). Different stressors cause a significant decrease in HF power leading to a significant increase in LF/HF ratio (feeding stress test: Bachmann et al., 2003; Nagy et
al., 2009; enforced backward movement Rietmann et al., 2004). It is quite difficult to achieve standardized experimental conditions with horses, hence the control of ANS stimulation to achieve significant changes in HRV remains difficult (Stucke et al., 2015). With horses, there are large inter-individual variations so achieving statistically significant differences is very difficult.

**Salivary cortisol**

The HPA axis is one of the physiological systems almost always activated by stress (Ralph and Tilbrook, 2016). It consists of the hypothalamus of the brain, the anterior pituitary gland, and the cortex of the adrenal glands (Tilbrook, 2007). Once secreted from the anterior pituitary into blood, adrenocorticotropic hormone acts on the adrenal cortex to stimulate synthesis of glucocorticoids, which in all mammals (except rodents), the principle glucocorticoid is cortisol (Ralph and Tilbrook, 2016). Cortisol is synthesized in a diurnal pattern that is governed by environmental factors including light, among other factors including genetics (Turner et al., 2012). Salivary cortisol represents the unbound, that is, biologically active fraction of total plasma cortisol while plasma cortisol is largely bound to carrier proteins (Riad-Fahmy et al., 1983) and has been validated to mirror the concentrations found in plasma cortisol in horses (Van der Kolk et al., 2001; Hughes et al., 2010; Schmidt et al., 2010; Peeters et al., 2011). The measurement of glucocorticoids in animal welfare science is used to identify stress responses, and it is common to associate increased stress with compromised welfare (Ralph and Tilbrook, 2016).

**Aversion to Higher Ammonia Concentrations in Other Species**

Intensively housed livestock are exposed to concentrations of NH₃ that do not arise in normal habitats. The adverse effects of NH₃ exposure on animal health and
productivity is well recognized in pigs (De Boer and Morrison, 1998), poultry (Kristensen and Wathes, 2000), and to some extent in horses (Ivester et al., 2014). However, the impact of NH$_3$ on animal behavior and welfare has not been studied as intensely. These behavioral studies complement physiological investigations by providing an alternative insight into the environmental response of an animal (Wathes et al., 2002).

The majority of studies that have looked at the NH$_3$ aversion of pigs and poultry have used an environmental preference chamber (EPC). Briefly, they consisted of multiple identical compartments kept under positive pressure to control the ventilation rate. The NH$_3$ concentration was maintained independently within each compartment, with all other environment factors kept constant (Jones et al., 2000; Kristensen et al., 2000; Wathes et al., 2002; Green et al. 2008; Sales et al., 2013) Morrison et al. (1993) were the first to study the aversion of pigs and poultry to NH$_3$ in short-term (30 min to 12 h), free choice tests. They found that there was some avoidance at 60 ppm NH$_3$, but not at lower concentrations of NH$_3$.

Another study determined the behavioral preferences of laying hens for different concentrations of ammonia found in commercial poultry houses (Kristensen et al., 2000). Six groups, of six laying hens each, were given the choice of three concentrations of ammonia (0, 25, and 45 ppm) in a preference chamber over a period of 6 days and their location and behavior were recorded every 15 minutes. Hens foraged, preened, and rested significantly more in fresh air than in the ammonia-polluted environments. There was a significant difference between the responses in 0 and 25 ppm, but not between 25 and 45 ppm. This suggests that ammonia may be aversive to hens with a threshold between 0 and 25 ppm.
The behavioral responses of pigs to atmospheric ammonia were tested in a chronic choice test (Jones et al., 1996). The behavior of two pig groups in different concentrations of ammonia gas (0, 10, 20, and 40 ppm) was continuously observed and frequently recorded for 14 days each in a choice test. The location of the pigs was scan sampled every 15 minutes and their behavior at this time was recorded. A significantly greater proportion of their time was spent in the unpolluted compartments (53.4 %) than in the 10 ppm (26.9 %), 20 ppm (7.1 %) or 40 ppm (5.1 %) compartments. Higher concentrations of ammonia were visited less often and once there, the pigs stayed for a comparatively shorter time for approximately 35 minutes. The pigs also chose to rest, feed, and forage more in the unpolluted compartments. Overall more feeding behavior was observed in the fresh air and more food was consumed in these compartments of the chamber.

In pigs, there has been some research to determine if there is an increase in the stress response when exposed to NH$_3$ (Asmar et al. 2001). Von Borell and colleagues (2007) assessed the acute and prolonged effects of 35 and 50 ppm concentrations of atmospheric NH$_3$ on welfare of weaned pigs. Their experiments show that pigs tended to have higher serum cortisol concentrations when exposed to 50 ppm NH$_3$, not only in the acute phase (96 h), but also in the prolonged studies (19 d). Von Borell (2007) also saw increases in absolute counts of white blood cells, lymphocytes, monocytes, and haptoglobin (acute phase protein) when exposed to NH$_3$. Their studies indicate that pigs respond to NH$_3$ with systemic stress response, however there was no effect on animal growth performance.
These studies, along with others, show that there is a preference to fresh air rather than NH$_3$ filled air in other species. However, the use of EPCS are implausible with larger species, like the horse, so alternative approaches to determining the impact of NH$_3$ concentrations on horse behavior and physiology are needed. One such approach is discussed in Chapter 4.

In conclusion, information regarding NH$_3$ emissions from equine facilities is limited. Data in other livestock species have shown how diet and management factors can influence NH$_3$ emissions. Therefore, it is necessary to characterize and quantify NH$_3$ emissions from equine operations, so that we can better understand the contribution of the equine industry to impaired air quality. Therefore, the objectives for the studies included in this dissertation set out to determine: 1) the effects of dietary CP concentrations and bedding type on potential NH$_3$ losses from feces and urine of horses fed an all forage diet; 2) the air emissions from 4 equine operations as affected by house type and feeding practices; 3) if a system can be developed to measure the aversion of horses to different NH$_3$ concentrations; and 4) if horses’ behavioral and physiological responses to acute NH$_3$ exposure can be used as potential indicators of aversion when air NH$_3$ concentrations in barns have become too high.
Figure 1-1. Diagram of the nitrogen cycle in nature (Ghaly and Ramakrishnan, 2015).
Figure 1-2. Conceptual model of ammonia formation and volatilization from manure (Hristov et al., 2011).
CHAPTER 2
CHARACTERIZING AMMONIA EMISSIONS FROM HORSES FED DIFFERENT CRUDE PROTEIN CONCENTRATIONS

Introduction

For the equine industry, concerns about NH$_3$ emissions are multifaceted and include issues of animal welfare, animal and human health, and environmental impacts. In the United States, the largest source of NH$_3$ emissions is animal agriculture (USEPA, 2005). Ammonia is an important pollutant gas that accelerates fine particulate formation in the atmosphere and plays a crucial role in the acidification and the eutrophication of ecosystems (Krupa, 2003). Ammonia is produced as a by-product of the microbial decomposition of the organic nitrogen compounds in manure. Nitrogen occurs as both unabsorbed nutrients in animal feces and either as urea (mammals) or uric acid (poultry) in urine. The term “manure” refers to the combination of feces and urine that is excreted (USEPA, 2002). According to the American Horse Council (2005), the estimated horse population in the United States is 9.2 million. In 2005, the USEPA’s national emissions inventory estimated an NH$_3$ emission factor for horses of 26.9 lb/head/year; therefore, horses produce approximately 123,740 tons of NH$_3$ emissions per year. By 2050, the global emissions of NH$_3$ are expected to double, principally owing to demographic growth, changes in food preferences and the agricultural intensification (Krupa, 2003; Clarisse et al., 2009). Furthermore, large uncertainties remain in the magnitude of NH$_3$ emissions (Reidy et al., 2008). A recent study using satellite monitoring suggests that NH$_3$ emissions have been significantly underestimated, especially in the Northern hemisphere (Clarisse et al., 2009). Therefore, the precise knowledge of influencing factors is greatly needed to determine accurate emission factors (Philippe, 2011).
Ammonia is the most important gas in the barn environment with respect to animal health, particularly of the respiratory tract (Fleming et al., 2009). Various studies have found that ammonia levels are highest near barn and stall floors (Fleming et al., 2009; Pratt et al., 2000). Since horses frequently consume feed from the stall floor, have their heads down and/or lie down when stabled for long periods of time, they can be exposed to high levels of ammonia. High levels of ammonia have been associated with foal pneumonia (McMillian, 1986) and may also predispose horses to inflammatory airway disease or recurrent airway obstruction (Tanner et al., 1998). Lawrence and coworkers (1988) detected ammonia concentrations of 25.3 parts per million (ppm) at the level of the horse’s halter. The concentration of ammonia that is harmful to horses has not been determined; however, in humans, the short-term exposure limit is 35 ppm (ATSDR, 2004).

Feeding protein in excess of the requirement is common in the horse industry (Lawrence et al., 2003; Swinker et al., 2009) and leads to increased urinary N excretion. Urea is the main nitrogenous constituent of equine urine. Urea is not volatile, but once it comes in contact with feces it is rapidly hydrolyzed to NH$_3$ and carbon dioxide by the abundant urease activity in fecal matter (Bussink and Oenema, 1998). Lee et al. (2009) showed, in dairy cows, very low ammonium concentration in fresh manure, but a rapid hydrolysis of urea in urine results in a sharp increase in ammonium concentration in manure and NH$_3$ volatilization rates. Ammonium-N (NH$_4^+$-N) is not volatile, but it is susceptible to volatilization to NH$_3$. In aqueous environments, NH$_4^+$-N and NH$_3$ exist in an equilibrium that is governed by both pH and temperature (Hristov et al., 2011). Lower pH favors NH$_4^+$-N and hence lowers the potential for NH$_3$ volatilization (McCarty and
Sawyer, 1978). The greatest increase in NH$_3$ release takes place between a pH of 7 and 10. At a pH of 7 and below, NH$_3$ volatilization decreases progressively such that at a pH of 4.5 there is essentially no measureable free NH$_3$ (McCarty and Sawyer, 1978; Hartung and Phillips, 1994; Ndegwa et al., 2008). Increasing temperature increases dissociation of NH$_4^+$-N to NH$_3$ and thus enhances NH$_3$ volatilization (Loehr, 1974).

The objective of this study was to determine the effects of dietary CP concentrations and bedding type on potential NH$_3$ losses from feces and urine of horses fed an all forage diet. We hypothesized that feeding horses above the minimum N requirement would result in an increase in NH$_3$ emissions from urine and feces and different bedding material could affect NH$_3$ emissions from urine.

**Material and Methods**

**Horses**

Nine mature (mean, 565 ± 7.4 kg) geldings (3 Thoroughbreds, 6 Quarter Horses) were used to determine the effects of dietary CP concentrations on potential NH$_3$ losses from feces and urine. Horses were housed in individual 3.7 m x 3.7 m box stalls at the Institute of Food and Agricultural Sciences (IFAS) Horse Teaching Unit in Gainesville, FL. Horses also had access to a 3.7 m x 12 m grass-free exercise area attached to each individual stall. Access to the exercise area was permitted for 8 h/d during the diet adaptation phase and limited to two periods of 20 min each during the collection phase. All horses received routine healthcare, including vaccination, anthelmintic treatment and hoof care established in the standard operating procedures for the IFAS Horse Teaching Unit. All animal protocols and procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Florida.
Dietary Treatments

Horses were fed 3 diets that differed in dietary CP concentrations. All forage diets were fed at 110, 130, and 150 % of NRC (2007) requirements for CP for horses at average maintenance. Diets consisted of two warm-season grass hays: bahiagrass (*Paspalum notatum*) and Tifton-85 bermudagrass hay (*Cynodon dactylon*), and ration balancer (Triple Crown Lite, Wayzata, MN). Collectively, the two grass hays were fed in different proportions that resulted in the following diet combinations: 1) 110 % NRC CP (LOW-CP, 10.6 % CP); 2) 130 % NRC CP (MED-CP, 11.4 % CP) and 3) 150 % NRC CP (HIGH-CP, 12 % CP). The goal of this study was to determine the NH₃ losses from horses fed differing concentrations of dietary CP, but it was important to keep the protein quality (AA profile) similar between diets, hence the use of two similar types of hay. Diet composition and mean daily nutrient intake for each diet are provided in Table 2-1. Daily rations were split into three equal sized feedings fed at 0700, 1500, and 2200 h.

Experimental Design

The 3 experimental diets were fed to 9 horses according to a 3 x 3 replicated Latin square design. In each period, 3 horses were randomly assigned to each of the 3 diets. At the conclusion of each period, dietary treatments were switched until all horses received all diets, giving 9 observations for each experimental diet.

Each period consisted of an 11-d dietary adjustment phase, followed by a 3 d total fecal and urine collection. During each 3 d collection phase, horses were fitted with harnesses specifically designed to facilitate collection of voided urine and feces (Stablemaid, PTY, LTD, Melbourne, Australia). The harness permitted the horse to
move about freely within the confines of the stall, as well as allowed the horse to lie
down to rest.

Harnesses were emptied of their contents at 8 h intervals (0700, 1500, and 2200 h). The pH of urine and fecal water (feces were squeezed with cheesecloth to obtain
fecal water) were obtained immediately using a combination electrode. Total urine and
feces voided were weighed, recorded, and a 10 % representative sample for each
collection was stored at 4°C until further analysis. Feed offered at each feeding was
weighed and recorded. In addition, body weights were obtained prior to the start of the
study and at the end of each period and used to adjust dietary intake as needed.

**Sample Preparation and Analyses**

All feedstuffs were analyzed by a commercial laboratory (Equi-Analytical, Ithaca, NY) for nutrient composition prior to the start of the study. Feed samples obtained
during the collection phase of each period were analyzed for DM content by drying in a
60°C forced-air oven for 48 h. During the collection phase, any feed refused was
collected once daily immediately prior to the 0700h feeding. Calculations of daily
nutrient intake were adjusted, where appropriate, to account for feed refusals.

For each horse in each period, fecal and urine samples were thoroughly mixed
and composited into samples that represented each 24 h cycle within a 3-d collection.
For example, the day 1 composite began with the samples obtained at 1600 h on the
first day of the collection phase and ended with the samples obtained at 0700 h on the
second day of the collection phase. Next, each of the three 24 h cycle samples were
combined, thoroughly mixed, pooled by period and stored at -20°C until further analysis.
This resulted in 1 fecal and 1 urine sample per horse per period. Once the composited
period samples were processed and analyzed (as described below), data was represented as one mean for each nutrient or variable of interest per horse per diet (n = 9).

Fecal samples were dried at 60°C in a forced-air oven for 48 h and weight change was recorded. Dried feces were then finely ground to pass a 1 mm screen using a Wiley mill. A sub-sample of ground feces was further oven-dried at 105°C for 24 h for determination of DM content. Total nitrogen (TN) concentration of feces and urine was determined using a carbon-nitrogen analyzer (Vario Max N/CN, Elementar Americas, Inc., Mt. Laurel, NJ). To determine other fecal N components, dried fecal samples were mixed with 50 mL of deionized water to create a 1:20 slurry. The slurry was mixed and centrifuged at 1250 g for 20 min and the supernatant was passed through a 0.45 µm membrane. The NH$_4^+$ content of fecal filtrate and urine was determined by the phenol-hypochlorite method (Weatherburn, 1967). The NOx (nitrate + nitrite) content of urine was determined using a commercial kit (Cayman Chemical, Ann Arbor, MI) and read on a PowerWave XS microplate spectrophotometer (BioTek Instruments, Winooski, VT). Urinary urea-N concentrations were determined colorimetrically using a commercial kit (Sigma Diagnostics, St. Louis, MO).

**In Vitro Ammonia Emissions**

To evaluate in-vitro NH$_3$ concentrations and emissions, fecal and urine samples were composited separately (5 % representative sample), within period, by diet (referred to as pool of horses). This resulted in 1 fecal and 1 urine sample for each diet in each period. Samples were thawed and 500 g of both were placed into a 5-gal (19-liter) emission vessel (EV) with either 250 g wheat straw or 500 g wood shavings to determine NH$_3$ concentrations. Different amounts of bedding were used as the head
space in the EV needed to be similar across all samples. Samples were run in
duplicate, which resulted in each diet per period being in 4 different EVs, 2 mixed with
wheat straw and two mixed with wood shavings. The EV system was in an
environmentally controlled room at the University of Delaware. Each EV, 13 in total, had
a 610 cm$^2$ surface area of each sample, and were evaluated for gaseous emissions
over a 7-d period with a constant air temperature at 20$^\circ$C, and air flow rate at 2.5 L/min
controlled by an air flow meter (Figure 2-1). One EV was empty to serve as a control.
The vessels were operated under positive pressure. Each vessel was equipped with a
small stirring fan located 5 cm below the lid for uniform mixing of the headspace.
Samples of the exhaust air from each of the twelve vessels and the supply air were
sequentially taken and analyzed at 5-min intervals, with the first 3 min for stabilization
and the last 2 min for measurement. This yielded a measurement cycle of 65 min for
each vessel. The NH$_3$ concentration was measured with a photoacoustic multi-gas
analyzer (INNOVA 1412, LumaSense Technologies, Inc. Santa Clara, CA.). The NH$_3$
concentration from the control EV was subtracted from the NH$_3$ concentration of each
EV. Outputs from the multi-gas analyzer were sampled at 1-s interval and logged at 1-
min interval into a measurement and control unit.

Calculations

Ammonia emissions rate (ER) was calculated as a mass of NH$_3$ emitted from the
EV per unit of space, in the following form (Eq. 2-1):

$$ER = \frac{[\text{NH}_3]}{10^5} \times \frac{m_{\text{NH}_3}}{A} \times \frac{V \times 1440 \text{min/day}}{22.414 \text{ L/mol}} \times \frac{273.15 + T, K}{273.15 \text{ K}}$$  \hspace{1cm} (2-1)

where ER is emission rate, mg/m$^2$-d; $m_{\text{NH}_3}$ is the molar mass of ammonia, 17.03 g/mol;
$V$ is the air flow rate of the emission vessels; 2.5 L/min, $T$ is the ambient temperature of
the room containing the system, 20°C and converted to kelvin (K); and A is the surface area of emission vessels, 0.06 m².

Cumulative emission rates were the summation of the dynamic emissions over the 7-d period (Eq. 2-2):

\[
\text{CumuER} = \sum_{i=1}^{7} \text{ER}
\]  

(2-2)

Apparent digestibility of DM and CP (DMD and CPD), plus N balance were calculated according to Eq. 2-3 and 2-4, respectively.

\[
\% \text{ Apparent digestibility} = \left(\frac{\text{intake} - \text{fecal excretion}}{\text{intake}}\right) \times 100
\]  

(2-3)

\[
\text{N balance (g/d)} = \text{N intake} - \text{fecal N excretion} - \text{urinary N excretion} - \text{total NH}_3 \text{ lost}
\]  

(2-4)

To determine amount of NH₃ lost, the difference between the emission of the NH₃ on d 7 and d 1 determined in the EV was used.

**Statistical Analysis**

Data were analyzed using one-way ANOVA as part of the PROC MIXED procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC.). All data, besides NH₃ concentrations and ER, were modeled with horse as a random effect and diet, period, and the period by diet interaction as fixed effects. For NH₃ concentration and ER data, a log transformation was performed, and a mixed model with repeated measures was used. Pool of horses were the random effect with diet, day, bedding, and period included as fixed effects. Day served as the repeated measure with a covariate structure of compound symmetry applied. In all ANOVA, individual treatment means
were compared using a Tukey adjustment of the LSMEANS statement. All data are expressed as LSMEANS ± SE. Differences were considered significant at $P \leq 0.05$ and trends considered at $P \leq 0.1$.

**Digestibility Study Results**

**Daily Intake and Excretion**

Dry matter intake (DMI) differed between diets ($P < .0001$). The highest DMI was the HIGH-CP diet, with MED-CP as the intermediate and LOW-CP as the lowest. As expected, CP intake differed between diets ($P < .0001$). Daily CP intake was lowest in the LOW-CP diet, intermediate in the MED-CP diet, and highest in the HIGH-CP diet (Table 2-1).

On a DM basis, the amount of feces excreted was affected by diet ($P = 0.0025$), where HIGH-CP was higher than LOW-CP ($P = 0.0018$) and tended to be higher than MED-CP ($P = 0.0927$), while LOW-CP and MED-CP were not different ($P = 0.118$) (Table 2-2). Daily excretion of urine was affected by diet ($P = 0.0007$), where the LOW-CP diet and MED-CP diet were not different ($P = 0.4255$), but the HIGH-CP diet was higher than both other diets ($P < 0.0060$) (Table 2-2).

The mean fecal and urinary pH for each diet is shown in Table 2-2. Fecal pH followed a similar pattern to fecal excretion between treatments. There was an overall effect of diet ($P = 0.0040$), where the fecal pH of the LOW-CP and MED-CP were not different ($P = 0.1756$), MED-CP tended to be lower than HIGH-CP ($P = 0.0.0869$), and HIGH-CP was higher than LOW-CP ($P = 0.003$). In contrast, urinary pH was not affected by diet ($P = 0.8921$).
Apparent Crude Protein Digestibility and Balance

Apparent DM digestibility was not affected by diet ($P = 0.4217$) and ranged from 31.94 to 34.66 % (Table 2-3). Crude protein apparent digestibility was affected by diet ($P = 0.0005$), where HIGH-CP was higher than both MED-CP and LOW-CP ($P = 0.0121$ and $P = 0.0004$, respectively), while there was no difference between MED-CP and LOW-CP ($P = 0.1548$) (Table 2-3).

All horses exhibited positive N balance during the study. Nitrogen balance was affected by diet ($P = 0.0114$), and followed a pattern similar to apparent CPD between treatments. The N balance from the HIGH-CP diet was higher than the LOW-CP diet ($P = 0.0110$), and tended to be higher than the MED-CP diet ($P = 0.0554$), while there was no difference between the MED-CP and LOW-CP diets ($P = 0.6481$) (Table 2-3).

Total Fecal and Urinary Excretion

On an as-excreted basis, the concentration of TN in feces was affected by diet ($P = 0.0007$), where LOW-CP had a lower TN concentration than both MED-CP and HIGH-CP ($P < 0.005$), but HIGH-CP and MED-CP were not different ($P = 0.4906$) (Table 2-4). When considering the TN concentration in feces on a DM basis, there was an effect of diet ($P = 0.0406$), however the differences between diets followed a different pattern. The LOW-CP TN concentration was not different from either MED-CP or HIGH-CP ($P = 0.6253$ and $P = 0.1772$, respectively), however the MED-CP diet had a higher concentration than the HIGH-CP diet ($P = 0.0359$) (Table 2-4).

Daily fecal excretion of TN, expressed as either grams per day ($P = 0.0345$; Table 2-5) or as milligrams per kilogram of BW ($P = 0.0506$; Figure 2-2) was affected by diet. On an absolute basis, the LOW-CP diet excreted less fecal TN than both the MED-CP and HIGH-CP diets ($P = 0.0309$ and $P = 0.0181$, respectively), but there was no
difference between HIGH-CP and MED-CP \((P = 0.7761)\) (Table 2-5). When expressed on a BW basis, the HIGH-CP diet tends to excrete more fecal TN than the LOW-CP diet \( (P = 0.0669)\), however there were no differences between the MED-CP and both LOW-CP and HIGH-CP \((P = 0.939 \text{ and } 0.9787)\) (Figure 2-2).

Similar to feces, diet had an effect \( (P = 0.0088)\) on the concentration of TN in urine on an as-excreted basis (Table 2-4). The % of TN excreted in urine from the LOW-CP was significantly lower than both MED-CP and HIGH-CP diets \((P = 0.0214 \text{ and } P = 0.0132, \text{ respectively})\), and there was no difference between the MED-CP and HIGH-CP diets \((P = 0.9613)\) (Table 2-4). Daily urinary excretion of TN, expressed as either grams per day \((P < .0001; \text{ Table 2-5})\) or as milligrams per kilogram of BW \((P = 0.006; \text{ Figure 2-3})\) was also affected by diet. On an absolute and BW basis, daily excretion of TN in urine was greatest with HIGH-CP \((P <.0001)\), intermediate with MED-CP \((P <0.01)\), and lowest with LOW-CP \((P < 0.05)\) (Table 2-5 and Figure 2-3).

When determining how much TN was excreted in urine as a percentage of total urine excreted in manure (feces +urine), there was an overall effect of diet \((P = 0.0062)\). The HIGH-CP diet had a higher percentage of TN excreted from urine compared to the LOW-CP diet \((P = 0.0049)\) and tended to be higher than that excreted from the MED-CP diet \((P = 0.0822)\). There was no effect of diet when comparing LOW-CP and MED-CP diets \((P = 0.2878)\) (Table 2-5).

**Ammonium, Urea-N, Nitrate, and Nitrite Excretion**

Daily excretion of NH\(_4^+\) in feces was not affected by diet when expressed as grams per day \((P = 0.6586)\) or as milligram per kilogram of BW \((P = 0.6237)\) (Table 2-5 and Figure 2-4). The same pattern was seen in urine. Urinary NH\(_4^+\) daily excretion was
not affected by diet as grams per day \((P = 0.7601)\) nor on a BW basis \((P = 0.6694)\)
(Table 2-5 and Figure 2-5).

Daily urea-N excretion followed a similar pattern to daily urinary TN excretion.
There was an overall effect of diet when expressed as grams per day \((P < .0001)\), or as milligram per kilogram of BW \((P < .0001)\) where urinary urea-N excretion was highest on HIGH-CP \((P < .001)\), followed by MED-CP \((P < .001)\), and lowest on LOW-CP \((P < .001)\) (Table 2-5 and Figure 2-6). An important measure of determining the susceptible NH\(_3\) loss is the proportion of total excreted urinary TN that is urea-N. There was an overall effect of diet \((P = 0.0002)\), where HIGH-CP had a higher percentage of urea-N to the total compared to LOW-CP \((P = 0.0001)\) and tended to be higher when compared to MED-CP \((P = 0.0696)\). The proportion of urea-N was higher in the MED-CP diet compared to the LOW-CP diet \((P = 0.0072)\) (Table 2-5).

When expressed on an absolute (mg/d) or on a BW basis, daily excretion of NO\(_3^-\) in urine was affected by diet \((P < 0.0001\text{ and } P < 0.0001,\text{ respectively})\), where HIGH-CP excreted the most \((P < 0.0001)\), followed by MED-CP \((P < 0.0001)\), and the lowest NO\(_3^-\) excreted in urine from LOW-CP \((P = 0.0117)\). When determining the daily excretion of NO\(_2^-\), there was no effect of diet \((P = 0.1922)\) and the excretion was very minimal compared to NO\(_3^-\).

**In Vitro Ammonia Concentrations and Emissions**

Daily mean fecal NH\(_3\) concentrations were not affected by diet nor day in the vessel system \((P = 0.5276\text{ and } P = 0.2468,\text{ respectively})\) (Table 2-6 and Figure 2-9). When considering the cumulative ER, there was an overall effect of diet, day, and diet*day \((P = 0.0049, P < .0001,\text{ and } P = 0.0015,\text{ respectively})\) where HIGH-CP emitted more NH\(_3\) than LOW-CP \((P = 0.0042)\), tended to be higher than MED-CP \((P = 0.0562),\)
and there was a difference between MED-CP and LOW-CP ($P = 0.0393$) (Table 2-6 and Figure 2-10). The differences between days are presented in Table 2-6.

Urinary NH$_3$ daily mean concentrations were not affected by diet ($P = 0.1225$), but did change over time in the vessel system ($P < 0.0001$) and were greater when mixed with wheat straw compared to wood shavings ($P = 0.0175$) (Table 2-7 and Figure 2-11). When calculated as cumulative ER, there was an overall effect of day and bedding type ($P < 0.0001$ and $P = 0.0129$) and, a trend for diet ($P = 0.0550$) (Table 2-7 and Figure 2-12). The differences between days are presented in Table 2-7.

**Discussion**

Total daily CP intake increased as dietary CP concentration and DMI increased. The goal when formulating diets was to meet the NRC requirements for maintenance horses in the LOW-CP diet, however, the LOW-CP diet was 110 % of the NRC requirement. While DMI increased and the amount of feces excreted increased across diets, the DMD did not differ across diets. Also, the apparent DMD range reported in this study (32 – 35 %) is lower than previously reported in warm season grass hays (Ott, 1981; Eckert et al., 2010). Eckert and coworkers (2010) reported that the apparent DMD from Coastal Bermudagrass hay and Tifton-85 bermudagrass hay were 53 and 52 %, respectively.

In the current study, apparent total tract CPD ranged from 49 – 59 %. This agrees with previous studies that have investigated the digestion of CP in horses fed warm season grass hays where CPD ranged from 31-64 % (Fonnesbeck et al., 1967; Gibbs et al., 1988). Digestibility of CP is correlated to DM intake as well as CP concentration in the diet (NRC, 2007). True CPD increases with CPI because as intake increases, the endogenous losses represent a smaller portion of total intake and make

64
a relatively smaller contribution to total fecal N losses (Slade et al., 1970). This relationship could explain why the CPD was higher from HIGH-CP compared to the other diets. Forage protein digestibility is related to the relative proportion of the cell content fractions and the cell wall constituents (Van Soest, 1967, Glade 1984). In hindgut fermenters, like horses, cell protein components are considered highly digestible, fiber-bound proteins are only partially digestible with fermentation and lignified nitrogenous compounds completely indigestible (Van Soest, 1967). Muscato and coworkers (1983) examined the AA profile of timothy grass hay fiber components and found over 65 % of the total plant AA were within the neutral detergent fiber insoluble N fraction. Thus, while over half of the amino-N is likely available to the large intestinal microflora, the utilization of this AA pool by the horse remains unknown (Trottier et al., 2016).

Total nitrogen excretion was reduced 10 % in feces and 45 % in urine when dietary CPI was fed close to the requirement (LOW-CP) compared to HIGH-CP diet. These results agree with previous work in ruminants (Cole et al., 2005; Misselbrook et al., 2005; Archibeque et al., 2007; Chiavegato et al., 2015). Misselbrook and coworkers (2005) reported that N excretion in dairy cattle was reduced by 30 % and urinary N excretion by 45 % when dietary N content was lowered from 3.1 to 2.18 %. In the current study, the quantities of TN excreted from feces were 63.74, 70.5, and 71.14 g/d (LOW-CP, MED-CP, and HIGH-CP, respectively), and the quantities of TN excreted from urine were 29.2, 40.88, and 52.92 g/d (LOW-CP, MED-CP, and HIGH-CP, respectively). When feeding similar type diets (warm season grass hays, 787.5 g/d and 987.5 g CP/d) to horses, Eckert and coworkers (2010) reported similar fecal N excreted...
(57 and 65 g/d), but higher urinary N (60 and 65 g/d). Eckert and coworkers (2010) acidified urine (to prevent loss as NH₃) and this could explain why urinary TN excreted in the current study was lower. The primary goal of the current study was to determine the NH₃ emissions from the urine and feces, so it was important to not acidify the urine as then NH₃ would not be emitted into the air. Another study conducted in horses reported similar TN concentration in feces to the current study (0.24-0.28 % vs. 0.23-0.28 %, respectively) (Williams et al., 2011). The proportion of urinary N excreted of the total N excreted (feces + urine) seems to be lower than previously reported in both ruminants and horses (Misselbrook et al., 2005; Eckert et al., 2010), however in the current study, urinary N excreted from the HIGH-CP diet was higher than the other two diets.

The quantity of NH₄⁺ excreted in both feces and urine was not affected by diet, which could be explained by the large variability between samples. Fecal NH₄⁺ ranged from 49.22 – 58.67± 7.21 mg/d and as expected, less than the quantity of NH₄⁺ excreted in urine (299.30 – 408.7 ± 90.33 mg/d). While not statistically significant, it is interesting to note that the amount of NH₄⁺ excreted in urine numerically decreased as dietary CP concentration increased. Urine was collected from harnesses, composited and frozen for further analysis every 8 hours, therefore some loss of NH₃ could have been occurring in the harnesses.

In the current study, the amount of urinary NO₂⁻ excreted was not affected by diet and negligible (ranging from 1.02-2.06 µg/d). This can be explained by denitrification. Denitrification is a microbial facilitated process of nitrate reduction to nitrite (and ultimately N₂). In the horse, this process would not occur until the large intestine, thus
explaining why the amount of nitrite in the urine is so low. Daily excretion of NO$_3^-$ in urine was affected by diet with the horse excreting 0.32 mg/L from the LOW-CP diet, 3 mg/L from the MED-CP diet, and 12.59 mg/L from the HIGH-CP diet. The amount of NO$_3^-$ from the MED-CP and HIGH-CP diets would not be problematic if leached into the ground water, as both concentrations are below the maximum contaminant level of NO$_3^-$ for groundwater (45 mg/L) (WQA, 2013).

The results of this current study confirmed that, like ruminants, the main nitrogenous constituent from urine in horses is urea, and that urinary urea excretion increases as the concentration of dietary CP increases. (Bristow et al., 1992; Bussink and Oenema, 1998; Cole et al., 2005; Reynal and Broderick, 2005; Todd et al., 2006; Archibeque et al., 2007; Cole et al., 2009; Waldrip et al., 2013). The amount of urea-N excreted on a daily basis ranged from 20.05 to 52.21 g/d. Archibeque and colleagues (2007) fed steers 3 different concentrations of dietary CP (9.13, 11.75 and 13.94 % CP) and reported similar results in amount of urea-N excreted on a daily basis (8.2, 19.6, and 39.2 g/d, respectively). Steers fed at the highest CP concentration (13.94 %) excreted more urea-N than horses fed the highest CP concentration on the current study, however this can be explained by the amount of dietary CPI. Bussink and Oenema (1998) summarized existing literature in dairy cattle and reported that urinary urea, as a proportion of total urinary N, ranged from 50 to 90 %. This closely agrees with the findings from the current study (69.6 to 98.8 %). These ranges have also been reported in multiple studies with ruminants (Bristow et al., 1992; Reynal and Broderick, 2005; Cole et al., 2009). Urea is the main source of NH$_3$ volatilization from manure (Bussink and Oenema, 1998).
current study, made up a large proportion of the total urinary N excreted, thus indicating that the majority of the urinary N excreted would be susceptible to NH₃ losses.

The results of the current study confirmed that urinary N (potentially the urea-N portion) is the main source of NH₃ volatilized from equine manure. In ruminants, this relationship is well established (Hristov et al., 2011; Waldrip et al., 2015a; Bougouin et al., 2016). By comparison, fecal NH₃ daily mean concentrations and emissions were quite low (0.07-0.34 ppm and 19.74-39.82 mg/m², respectively). There was not an effect of diet on concentration, but there was an effect of diet on the Cumu ER, due to the NH₃ concentrations increasing on d 4 and d 5 of the 7 d storage period in the HIGH-CP diet. Part of this increase could be related to the increase seen in fecal pH (from 6.62 in LOW-CP diet to 6.8 in HIGH-CP diet), however the fecal contribution is small in comparison to the NH₃ lost from urine. Fecal organic N mineralization (conversion of nitrogenous compounds into NH₄⁺) is a much slower process than urea hydrolysis, and the contribution of feces to NH₃ emissions is generally very low (Waldrip et al., 2015).

In contrast to feces, in vitro NH₃ loss from urine was high and the Cumu ER tended to be higher when dietary CP content increased. While diet CPI did not influence the in vitro NH₃ concentration, the range of NH₃ concentrations (55.48-101.14 ppm) were somewhat similar to other NH₃ concentrations reported from horses (Pratt et al., 2000; Fleming et al., 2008; Fleming, 2009; Williams et al., 2011). Williams and coworkers (2011) reported that NH₃ concentrations in stalls from horses fed lower dietary CP concentrations averaged 25.4 ppm and from horses fed higher dietary CP concentrations averaged 37.8 ppm. A study in which feces and urine were collected from dairy cows fed a diet with high (19.4 %) or low (13.6 %) CP content measured NH₃
emissions over 48 h using an in vitro system (Misselbrook et al., 2005). Similar to the
current study, they found NH$_3$ concentrations were similar across diets despite seeing
differences in urine urea-N concentrations. They hypothesized that this could be due to
urease activity being limited.

The results from the current study support the hypothesis that NH$_3$
concentrations and Cumu ERs in urine are higher when mixed with wheat straw
compared to wood shavings (97.33 vs. 73.53 ppm and 11.10 vs. 6.90 g/m$^2$,
respectively). This agrees well with several studies (Fleming et al., 2008; Garlipp et al.,
2011). Fleming and coworkers (2008) measured the NH$_3$ concentration of horse manure
mixed with different bedding types using an in vitro vessel system. They found that the
mean NH$_3$ concentration was higher on wheat straw (237 ppm) compared to wood
shavings (207 ppm). Another study conducted using a deep litter bedding system,
reported that wheat straw emitted the highest concentration (5.75 ppm) and wood
shavings emitted the lower (2.31 ppm) (Garlipp et al., 2011). They suggested that wood
shavings were smaller in size than wheat straw and therefore had a larger specific
area/unit mass on which the NH$_3$ could be bound compared with straw. Furthermore,
Ward et al. (2000) showed that wood shavings had more than double the ability to bind
water than wheat straw, leading to a better binding of NH$_3$ in the wood shavings. These
factors could potentially explain why the NH$_3$ ER in the current study, from manure
mixed with wood shavings was lower than the ER from wheat straw.

The number of days in the emission vessel system had an effect on NH$_3$
concentration and Cumu ER when measured from feces and urine. The largest
increases occurred from d 3-4 and d 4-5, possibly indicating the beginning of urea
hydrolysis (Misselbrook et al., 2005). Rate of hydrolysis is temperature-dependent, but complete hydrolysis was found to occur within 10 to 15 d at 15°C (Whitehead and Raistrick, 1993). In the current study, the temperature inside the EV could have increased due to increased microbial activity, however that was not determined. Pratt and coworkers (2000) measured the NH$_3$ concentration in horse stalls over a 14 d period. They noted that the concentration increased from 2.5 ppm on d1 to 218.8 ppm on d 14, which is similar to results from the current study (1.94 ppm on d1 to 198.92 ppm on d7).

In conclusion, it is clear that overfeeding CP to horses can lead to higher levels of urinary N and urea-N excretion. This relationship leads to greater risk for loss of NH$_3$ in the atmosphere, which can impact the environment and the health and welfare of the horse. Equine operations that bed with wheat straw may have a higher NH$_3$ ER than those that bed with wood shavings. Further research is needed to test the NH$_3$ ER determined in this study under field conditions.
Table 2-1. Composition of the three experimental diets and mean daily nutrient intake for each diet.

<table>
<thead>
<tr>
<th>Feedstuff or nutrient</th>
<th>LOW-CP</th>
<th>MED-CP</th>
<th>HIGH-CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet composition, % of daily DMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bahiagrass hay</td>
<td>71.0</td>
<td>29.6</td>
<td>-</td>
</tr>
<tr>
<td>Tifton-85 hay</td>
<td>17.8</td>
<td>59.2</td>
<td>89.7</td>
</tr>
<tr>
<td>Triple Crown Lite</td>
<td>8.3</td>
<td>8.3</td>
<td>7.6</td>
</tr>
<tr>
<td>Corn oil</td>
<td>2.3</td>
<td>2.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Salt</td>
<td>0.6</td>
<td>0.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Mean daily nutrient intake

<table>
<thead>
<tr>
<th></th>
<th>LOW-CP</th>
<th>MED-CP</th>
<th>HIGH-CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, kg/d</td>
<td>7.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DE, Mcal/d</td>
<td>20.4</td>
<td>19.4</td>
<td>20.4</td>
</tr>
<tr>
<td>CP, g/d</td>
<td>793.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>934.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1085.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ca, g/d</td>
<td>62.4</td>
<td>56.8</td>
<td>56.3</td>
</tr>
<tr>
<td>P, g/d</td>
<td>27.3</td>
<td>23.8</td>
<td>22.7</td>
</tr>
<tr>
<td>Mg, g/d</td>
<td>24.4</td>
<td>20.5</td>
<td>19.2</td>
</tr>
</tbody>
</table>

<sup>1</sup> Diets contained 110% (LOW-CP), 130% (MED-CP), or 150% (HIGH-CP) of the CP requirement for horses at average maintenance (NRC, 2007).

<sup>2</sup> Mean daily nutrient intake reflects actual intake for all nutrients.

<sup>a,b,c</sup> Means in the same row differ (P < 0.05).
Table 2-2. Daily excretion and pH in feces and urine when horses were fed diets containing different amounts of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC).

<table>
<thead>
<tr>
<th>Item</th>
<th>LOW-CP</th>
<th>MED-CP</th>
<th>HIGH-CP</th>
<th>SEM</th>
<th>Diet</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feces excreted, DM kg</td>
<td>5.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.49&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>5.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.28</td>
<td>0.0025</td>
<td>0.0059</td>
</tr>
<tr>
<td>Fecal pH</td>
<td>6.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.03</td>
<td>0.004</td>
<td>0.0002</td>
</tr>
<tr>
<td>Urine excreted, kg</td>
<td>3.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37</td>
<td>0.0007</td>
<td>0.0123</td>
</tr>
<tr>
<td>Urinary pH</td>
<td>7.39</td>
<td>7.41</td>
<td>7.41</td>
<td>0.03</td>
<td>0.8921</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means in the same row differ ($P < 0.05$).
Table 2.3. Apparent digestibility of dry matter and crude protein and nitrogen balance of horses fed diets containing three different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC).

<table>
<thead>
<tr>
<th>Item</th>
<th>Diet</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LOW-CP</td>
<td>MED-CP</td>
</tr>
<tr>
<td>DM Digestibility, %</td>
<td>31.94</td>
<td>32.93</td>
</tr>
<tr>
<td>CP Digestibility, %</td>
<td>49.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.77&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>N Balance, g/d</td>
<td>33.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means in the same row differ (P < 0.05).
Table 2-4. Concentration of total nitrogen in feces and urine on an as-excreted and DM basis, when horses were fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC).

<table>
<thead>
<tr>
<th>Item</th>
<th>Diet</th>
<th>P- values</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LOW-CP</td>
<td>MED-CP</td>
<td>HIGH-CP</td>
<td>SEM</td>
<td>Diet</td>
<td>Period</td>
</tr>
<tr>
<td>Feces TN, % as-excreted</td>
<td>0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.0007</td>
<td>0.0018</td>
</tr>
<tr>
<td>Feces TN, % DM basis</td>
<td>1.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
<td>0.0406</td>
<td>0.02193</td>
</tr>
<tr>
<td>Urine TN, % as-excreted</td>
<td>0.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04</td>
<td>0.0088</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means in the same row differ (<i>P</i> < 0.05).
Table 2-5. Daily excretion of total nitrogen, ammonium, urea-nitrogen, nitrate and nitrite in feces and urine when horses were fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC).

<table>
<thead>
<tr>
<th>Item</th>
<th>LOW-CP</th>
<th>MED-CP</th>
<th>HIGH-CP</th>
<th>SEM</th>
<th>Diet</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal TN, g/d</td>
<td>63.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.91</td>
<td>0.0345</td>
<td>0.0069</td>
</tr>
<tr>
<td>Urinary TN, g/d</td>
<td>29.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.53</td>
<td>&lt;.0001</td>
<td>0.0031</td>
</tr>
<tr>
<td>Fecal NH&lt;sub&gt;4&lt;/sub&gt;+, mg/d</td>
<td>53.25</td>
<td>49.22</td>
<td>58.67</td>
<td>7.21</td>
<td>0.6586</td>
<td>0.8665</td>
</tr>
<tr>
<td>Urinary NH&lt;sub&gt;4&lt;/sub&gt;+, mg/d</td>
<td>408.57</td>
<td>333.63</td>
<td>299.30</td>
<td>90.33</td>
<td>0.6874</td>
<td>0.7601</td>
</tr>
<tr>
<td>Urea-N, g/d</td>
<td>20.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.08</td>
<td>&lt;.0001</td>
<td>0.0006</td>
</tr>
<tr>
<td>Urinary NO&lt;sub&gt;3&lt;/sub&gt;-, mg/d</td>
<td>1.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.43</td>
<td>&lt;.0001</td>
<td>0.0469</td>
</tr>
<tr>
<td>Urinary NO&lt;sub&gt;2&lt;/sub&gt;-, µg/d</td>
<td>1.02</td>
<td>1.22</td>
<td>2.06</td>
<td>0.4</td>
<td>0.1922</td>
<td>0.5487</td>
</tr>
<tr>
<td>Urea-N as % of Urinary TN</td>
<td>69.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.32</td>
<td>0.0002</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Urinary TN as % of manure TN excreted</td>
<td>31.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.01</td>
<td>0.0062</td>
<td>0.0344</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Means in the same row differ ($P < 0.05$).
Table 2-6. Least square means of ammonia mean concentration and cumulative emissions of feces when incubated over 7 d in emission vessel system from horses fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC).

<table>
<thead>
<tr>
<th>Main effects</th>
<th>NH$_3$ concentration, ppm</th>
<th>NH$_3$ cumulative emission rate, mg/m$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOW-CP</td>
<td>0.071</td>
<td>19.74$^{b}$</td>
</tr>
<tr>
<td>MED-CP</td>
<td>0.10</td>
<td>20.48$^{b}$</td>
</tr>
<tr>
<td>HIGH-CP</td>
<td>0.34</td>
<td>39.82$^{a}$</td>
</tr>
<tr>
<td>SEM</td>
<td>0.07</td>
<td>6.36</td>
</tr>
<tr>
<td>Day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.28</td>
<td>13.32$^{d}$</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>15.52$^{c,d}$</td>
</tr>
<tr>
<td>3</td>
<td>0.02</td>
<td>16.48$^{c,d}$</td>
</tr>
<tr>
<td>4</td>
<td>0.02</td>
<td>17.59$^{c,d}$</td>
</tr>
<tr>
<td>5</td>
<td>0.16</td>
<td>25.27$^{b,c}$</td>
</tr>
<tr>
<td>6</td>
<td>0.33</td>
<td>41.04$^{a,b}$</td>
</tr>
<tr>
<td>7</td>
<td>0.34</td>
<td>57.54$^{a}$</td>
</tr>
<tr>
<td>SEM</td>
<td>0.09</td>
<td>8.07</td>
</tr>
</tbody>
</table>

Fixed effects ($P$-value)

<table>
<thead>
<tr>
<th></th>
<th>$P$-value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>0.5276</td>
<td>0.0049</td>
</tr>
<tr>
<td>Day</td>
<td>0.2468</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diet x Day</td>
<td>0.8867</td>
<td>0.0015</td>
</tr>
</tbody>
</table>

$^{a,b,c,d}$ Means within a column and main effect differ ($P < 0.05$).
Table 2-7. Least square means of ammonia mean concentration and cumulative emissions of urine when mixed with shavings and straw and incubated over 7 d in an emission vessel system from horses fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC).

<table>
<thead>
<tr>
<th>Main effects</th>
<th>NH$_3$ concentration, ppm</th>
<th>NH$_3$ cumulative emission rate, g/m$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOW-CP</td>
<td>55.48</td>
<td>5.87$^y$</td>
</tr>
<tr>
<td>MED-CP</td>
<td>99.67</td>
<td>11.16$^x$</td>
</tr>
<tr>
<td>HIGH-CP</td>
<td>101.14</td>
<td>9.97$^x$</td>
</tr>
<tr>
<td>SEM</td>
<td>10.59</td>
<td>1.28</td>
</tr>
<tr>
<td>Bedding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wood shavings</td>
<td>73.53$^b$</td>
<td>6.90$^b$</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>97.33$^a$</td>
<td>11.10$^a$</td>
</tr>
<tr>
<td>SEM</td>
<td>8.83</td>
<td>1.10</td>
</tr>
<tr>
<td>Day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.94$^d$</td>
<td>0.093$^d$</td>
</tr>
<tr>
<td>2</td>
<td>4.11$^{c,d}$</td>
<td>0.290$^c$</td>
</tr>
<tr>
<td>3</td>
<td>14.42$^c$</td>
<td>0.980$^c$</td>
</tr>
<tr>
<td>4</td>
<td>58.97$^b$</td>
<td>3.8$^b$</td>
</tr>
<tr>
<td>5</td>
<td>131.21$^{a,b}$</td>
<td>10.09$^{a,b}$</td>
</tr>
<tr>
<td>6</td>
<td>188.44$^a$</td>
<td>19.11$^a$</td>
</tr>
<tr>
<td>7</td>
<td>198.92$^a$</td>
<td>28.63$^a$</td>
</tr>
<tr>
<td>SEM</td>
<td>10.59</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Fixed effects (P-value)

<table>
<thead>
<tr>
<th></th>
<th>Diet</th>
<th>Bedding</th>
<th>Day</th>
<th>Diet x Bedding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1225</td>
<td>0.0175</td>
<td>&lt; 0.0001</td>
<td>0.5479</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0550</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0129</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.8311</td>
</tr>
</tbody>
</table>

$^{a,b,c,d}$ Means within a column and main effect differ ($P < 0.05$).

$^{x,y}$ Means within a column and main effect tended to differ ($P < 0.1$).
Figure 2-1. Schematic of the *in vitro* emission vessel system. Courtesy of Hong Li.

Figure 2-2. Mean (± SE) daily excretion of total nitrogen in feces when horses were fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC). Overall effect of diet (*P* = 0.0506), and period (*P* = 0.008). *x,y* Means with different letter tended to differ (*P* < 0.1).
Figure 2-3. Mean (± SE) daily excretion of total nitrogen in urine when horses were fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC). Overall effect of diet ($P = 0.006$), and period ($P = 0.0001$). $a,b,c$ Means with different letters differ ($P < 0.05$).

Figure 2-4. Mean (± SE) daily excretion of ammonium in feces when horses were fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC). Overall effect of diet ($P = 0.6237$), and period ($P = 0.7831$).
Figure 2-5. Mean (± SE) daily excretion of ammonium in urine when horses were fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC). Overall effect of diet ($P = 0.6694$), and period ($P = 0.7268$).

Figure 2-6. Mean (± SE) daily excretion of urea nitrogen in urine when horses were fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC). Overall effect of diet ($P < .0001$), and period ($P = 0.0001$). $^{a,b,c}$Means with different letters differ ($P < 0.05$).
Figure 2-7. Mean (± SE) daily excretion of nitrate in urine when horses were fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC). Overall effect of diet ($P < .0001$), and period ($P = 0.0486$). $^{a,b,c}$ Means with different letters differ ($P < 0.05$).

Figure 2-8. Mean (± SE) daily excretion of nitrite in urine when horses were fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC). Overall effect of diet ($P = 0.2041$), and period ($P = 0.5489$). $^{a,b,c}$ Means with different letters differ ($P < 0.05$).
Figure 2-9. Daily mean ammonia concentration of feces incubated over 7 d in an emission vessel system from horses fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC). Overall effect of diet ($P = 0.5276$), day ($P = 0.2468$) and period ($P = 0.4602$).

Figure 2-10. Cumulative ammonia emissions rate of feces incubated over 7 d in an emission vessel system from horses fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC). Overall effect of diet ($P = 0.0049$), day ($P < 0.0001$) and period ($P = 0.0002$).
Figure 2-11. Daily mean ammonia concentration of urine when mixed with A) shavings and B) straw and incubated over 7 d in an emission vessel system from horses fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC). Overall effect of diet ($P = 0.1225$), day ($P < 0.0001$), period ($P = 0.0002$), and bedding ($P = 0.0175$).
Figure 2-12. Cumulative ammonia emissions rate of urine when mixed with A) shavings and B) straw and incubated over 7 d in an emission vessel system from horses fed diets containing different levels of crude protein: LOW-CP (1.1XNRC), MED-CP (1.3XNRC), or HIGH-CP (1.5XNRC). Overall effect of diet ($P = 0.055$), day ($P < 0.0001$), period ($P = 0.0332$), and bedding ($P = 0.0129$).
CHAPTER 3
AMMONIA EMISSIONS FROM EQUINE FACILITIES IN THE MID-ATLANTIC REGION

Introduction

In the United States, the largest source of NH$_3$ emissions is animal agriculture (National Emission Inventory, 2005). Ammonia is an important pollutant gas that accelerates fine particulate formation in the atmosphere and plays a crucial role in the acidification and the eutrophication of ecosystems (Krupa, 2003). Ammonia is produced as a by-product of the microbial decomposition of the organic nitrogen compounds in manure. According to the American Horse Council (2005), the estimated horse population in the United States is 9.2 million. In 2002, the EPA’s national emissions inventory estimated an NH$_3$ emission factor for horses of 26.9 lb/head/year; therefore, horses produce approximately 123,740 tons of NH$_3$ emissions per year. By 2050, the global emissions of NH$_3$ are expected to double, principally owing to demographic growth, changes in food preferences and the agricultural intensification (Krupa, 2003; Clarisse et al., 2009). Furthermore, large uncertainties remain in the magnitude of NH$_3$ emissions (Reidy et al., 2008). A recent study using satellite monitoring suggests that NH$_3$ emissions have been significantly underestimated (Clarisse et al., 2009). Therefore, the precise knowledge of influencing factors is greatly needed to determine accurate emission factors (Philippe, 2011).

Well-designed, naturally ventilated buildings can provide occupants with good indoor air quality as well as having energy consumption at levels below those recorded in mechanically ventilated systems (Awib, 2003). However, airborne emission of pollutants from natural ventilated livestock buildings has been notoriously difficult to quantify due to the complex connection between the outdoor wind and internal
environment. The CO$_2$-balance method, based on CO$_2$ production by animals, is one of the most commonly used methods to determine ventilation rate in naturally ventilated buildings (CIGR, 1984). Ammonia emissions modeling from naturally ventilated livestock buildings are necessary in order to provide information to develop appropriate mitigation strategies for the future.

While agricultural water quality has been a focus for several decades, focus on agricultural air quality is more recent. Research on mitigating the impact of animal agriculture on air quality in the U.S. has made great strides in recent years, in part due to increased regulatory scrutiny of animal agriculture, litigation regarding public nuisance and interpretation of applicability of community right-to-know laws. However, there is limited information available regarding air emissions (NH$_3$) from equine facilities. Specifically, while it is generally understood that air quality in stables can adversely affect both horse and human health, the effects of different housing systems and the nutritional management of horses on air quality have received little investigation.

Understanding the complex interaction of environment and management on emissions is essential for predicting emission transport and fate. Robust techniques and inexpensive, versatile equipment are needed to link laboratory studies to field-scale verification (Woodbury et al., 2006). Portable flux chambers are an appropriate direct method for assessing fluxes from small individual area sources, such as stalls (Parker et al., 2012).

While some studies in horses have succeeded at determining NH$_3$ concentrations in the barn environment based upon different variables, none have attempted to characterized NH$_3$ emissions based on most of these factors (pH,
temperature, dietary CP intake, bedding, and air velocity). Quantifying NH$_3$ emissions from horse manure, and how diet composition affects N excretion would provide more meaningful information to assess the potential impact of equine operations on air quality. More importantly, horse-specific information is necessary given that regulatory agencies could potentially use such values to determine horse emission factors, or to place restrictions on horse facilities. The objective of this study was to determine air emissions from 4 equine operations as affected by housing type and feeding practices. We hypothesized that stalls of horses fed higher CP diets and housed on wheat straw would emit more NH$_3$ than those fed to closer to the CP requirement and housed on wood shavings.

**Materials and Methods**

**Farm Characteristics**

Four equine operations in Pennsylvania and Maryland were recruited for this project during October 2015 and a brief description of each facility is provided in Table 3-1.

Farm A was a university riding stable located in southeastern Pennsylvania that housed 40 horses (Table 3-1; Figure 3-1). The layout of the barn consisted of a main aisle (where samples were taken), a side aisle, a carriage area with stalls and offices, and an indoor arena. The main aisle was 82.91 m long x 10.06 m wide x 9 m high with concrete flooring. Horse stalls were 3.4 m long x 3.7 m wide x 4.3 m high with rubber mats and wood shavings as the bedding source. All stalls were cleaned daily (removal of fecal material and urine spots) (0730 h) with fecal material being picked up throughout the day. Manure was stored in large dumpsters and removed from the facility weekly.
Farm B was a university breeding facility located in southeastern Pennsylvania that housed 29 horses (Table 3-1; Figure 3-2). The main aisle was 46 m long x 11 m wide x 6.1 m high with concrete flooring. As this was a breeding facility, there were many different stalls for the stallions and foaling, however the stalls that measurements were taken from were 3.4 m long x 3 m wide x 4.3 m high. The stalls had rubber mats and were bedded with wood shavings. Horse were turned out in the evening and stalls were cleaned (removal of fecal material and urine spots) in the morning (0630 h) before horses were brought back inside.

Farm C was a racehorse (Thoroughbred, TB) training facility located in Maryland that housed 39 horses in the main barn (Table 3-1; Figure 3-3). It is common practice to walk horses in training after their workout to cool down. Therefore, the layout of the barn was such that there were stalls to the outside of the walkway and stalls in the center of the walkway. The walkway was made of dirt, while the stalls had concrete flooring with mesh rubber mats covering most of the stall (solid mats under water buckets and the hay rack) and were deeply bedded with wheat straw. The total area of the barn was 39 m long x 25 m wide x 9 m high and each stall was 3.4 m long x 3.5 m wide x 4.9 m high. Stalls were cleaned (removal of all soiled bedding) in the morning (0400 h) before horses were exercised.

Farm D was a large Standardbred (STB) breeding facility located in Maryland. This farm was comprised of approximately 2000 acres with approximately 1,800 horses. Measurements were conducted in one of the barns at the facility which housed 22 broodmares. Horses were turned out during the day and stalls were cleaned in the morning (1030 h). The barn was 56 m long x 12 m wide x 4 m high with concrete
flooring. Stalls had rubber mats, were bedded with wheat straw, and were 3.7 m long x 4.3 m wide x 4 m high. Stalls were cleaned (removal of all soiled bedding) in the morning 0930 h after horses were turned out into pastures.

**Horses and Management Practices**

A questionnaire was administered to the respective farm manager concerning facilities and horse care. The dimensions of the facility and each stall, daily cleaning practices, horse breed and age, exercise schedule, and feeding practices were recorded.

While interested in the daily activities of the entire facility, this study called for the specifics about the horses housed in the stalls from which measurements were taken. Body weight, breed, age, classification of horse, and time spent in the stall were recorded (Table 3-2). Each facility had a livestock scale to measure BW of horses. Horses (n = 4) from Farm A were classified as being in moderate work, as they were ridden 3-5 hours per week (30 % walk, 55 % trot, 10 % canter, and 5 % jumping). At Farm B (n = 4), the stalls from yearlings were used and these yearlings were all approximately 18 months of age. Horses (n = 4) from Farm C were in race-training and thus classified as being in intense work (1 hour per week speed work). Lastly, horses (n = 6) from Farm D were broodmares approximately in the 8th month of gestation. The classification of each horse was important to determine the NRC (2007) requirement for CP. Horses’ daily intake of concentrate and hay was weighed and samples were taken for determination of nutrient composition. Hay samples were analyzed by a commercial laboratory (Equi-Analytical, Ithaca, NY). Concentrate CPI was estimated based upon the manufacturers feed lab. Daily crude protein intake was estimated based on CP concentration in hay and concentrate.
Ammonia Concentrations

For analysis of the NH₃ concentration at various locations in the stalls, air samples were collected from the stall floor using a stainless steel hemispherical dynamic flux chamber (Miller and Woodbury, 2006; Woodbury et al., 2006; Perez et al., 2009). This dynamic flux chamber system consisted of a stainless steel chamber (30 L volume and 0.51 m diameter), a flow meter (Model VFB, Dwyer, Michigan City, IN), an air pump (Model 108, Thomas, Munich, Germany), a photoacoustic NH₃ gas analyzer (Chillgard RT, MSA, Cranberry, PA) and a zeolite filter column (Figure 3-5). Zeolite was used in the filter column to provide NH₃ free air for the flux chamber. The air flow rate was set to 8 LPM and the exposed surface area of the chamber was 0.21 m². Once the chamber was deployed on the floor, air (<1 LPM) was continuously sampled from the top vent of the chamber by the NH₃ analyzer and readings from the analyzer were continuously recorded by a data logger over a 5-min period. The highest readings were used to calculate the NH₃ flux. Two identical flux chamber systems were used so that samples could be taken from 2 stalls concurrently.

Sample Collection and Analysis

In order to achieve a representation of NH₃ emitted from each stall surface, 5 predetermined sampling locations were selected and measurements were taken. Sampling occurred over three days at each facility. If the NH₃ concentration was over 50 ppm, it was classified as a measurement from an area containing urine (wet area), and if the concentration was less than 50 ppm, it was classified as a measurement from an area mostly containing feces and or bedding (dry area). The dry areas of the stall may have contained some old wet bedding, but its contribution to the NH₃ concentration was not large. Sampling times from each facility relative to stall cleaning are shown in Table
3-1. Due to the busy schedule of all farms, measurements had to be taken with minimal interruption to the farm’s regular routine. At Farms A, B, and C, measurements were taken from 4 stalls and from 6 stalls at Farm D. Also, at Farm A measurements were taken in the PM, 6 h post cleaning (Table 3-1).

**Ambient Air**

Ambient temperature and relative humidity (RH) (HOBO Pro Series, Onset, Bourne, MA) were continuously monitored at 5 min intervals with portable data loggers at two different indoor locations (Figures 4-1; 4-2; 4-3; 4-4). Data loggers were positioned 2 m above the barn floor.

**Calculation of Ventilation Rate and Emissions**

Barn ventilation rate (VR) was determined indirectly using the CO₂ mass balance method that has been proven to be relatively accurate as compared to direct ventilation measurement (Li et al., 2005). This method calculates VR by dividing the total CO₂ production rate by the difference of indoor and ambient CO₂ concentrations (Zhao et al., 2013) (Eq. 3-1).

\[
VR = n \cdot \frac{P_{\text{horse}}}{n \cdot ([\text{CO}_2]_{\text{in}} - [\text{CO}_2]_{\text{amb}}) \times 3600}
\]  

where VR = ventilation rate (m³ h⁻¹ horse⁻¹); n = number of horses in barn; \(P_{\text{horse}}\) = CO₂ production by horse (mL s⁻¹ horse⁻¹); \([\text{CO}_2]_{\text{in}}\) = indoor CO₂ concentration (ppm); and \([\text{CO}_2]_{\text{amb}}\) = ambient CO₂ concentration, 390 ppm (Borum et al., 2015).

Production of CO₂ by the horses was calculated based on the horse’s BW and bioenergetics values, i.e., specific total heat production rate (THP) and respiratory quotient (RQ) (Zhao et al., 2013). The THP was calculated based on BW (Eq. 3-2) (CIGR, 2002) and RQ was set to be 0.9 (Pagan et al., 1987), respectively (Eq. 3-3).
\[
THP = (6.1 \times M^{0.75})/M \quad (3-2)
\]
\[
P_{\text{horse}} = THP \times M/ (16.18/RQ + 5.02) \quad (3-3)
\]
where \(M\) is the BW of the horse (kg).

Ammonia emissions rate (ER) was calculated as a mass of \(\text{NH}_3\) emitted from each stall measured and weighted by how much area was considered wet and dry, in the following form (Eq. 3-4):

\[
ER = \frac{[\text{NH}_3] \times m_{\text{NH}_3} \times V \times 1440 \text{min/day}}{A \times 22.414 \text{ L/mol} \times 273.15 + T, K} \quad (3-4)
\]

where \(ER\) is the emission rate, g/m\(^2\)-d horse; \([\text{NH}_3]\) is the weighted concentration of \(\text{NH}_3\) in the stalls (estimated to be 30 % of the stall being wet and 70 % of the stall being dry; \(m_{\text{NH}_3}\) is the molar mass of ammonia, 17.03 g/mol; \(V\) is the air flow in the flux chamber; 8 L/min, \(T\) is temperature in the barn while measurement was taken; \(A\) is surface area of the flux chamber, 0.21 m\(^2\), and \(K\) is temperature in Kelvin.

The daily average \(\text{NH}_3\) ER per horse was estimated as the mean ER from stalls measured and the area of the stalls in each facility.

**Statistical Analysis**

Data was tested for normality and not all variables were normally distributed, therefore, associations between estimated dietary CPI, % daily CPI over NRC requirement, \(\text{NH}_3\) concentrations from dry areas and wet areas, temperature in barn and temperature outside of barn were quantified with Spearman rank correlation coefficients \((r_s)\) using SAS (Version 9.4, SAS Institute Inc., Cary, NC.). Correlations were significant at \(P \leq 0.05\). Data expressed as mean ± SD, unless otherwise noted.
Results

Diets and Management Practices

A brief description of the horses' diets is highlighted in Table 3-3 and the facilities' mean nutrient intakes are shown in Table 3-4. Both Farm A and B were a part of a university equine program in Pennsylvania. On Farm A, all horses received timothy alfalfa mix hay with horses that were considered “hard keepers” receiving a pelleted concentrate and those considered “easy keepers” receiving a ration balancer. On a DM basis, horses ate approximately 2.1 % of BW with approximately 90 % of their diet being hay. At Farm A, horses consumed an average of 1503 g CP/d, which was 162 % of NRC (2007) CP requirement (993.2 g/d). The yearlings from Farm B consumed Alfalfa hay and a pelleted concentrate formulated for growing horses. They ate approximately 2.52 % of BW in DM with forage consumption making up approximately 71 % of their daily intake. These yearlings’ NRC (2007) requirement was 768 g/d and they consumed, on average, 1432 g/d (188 % NRC requirement).

Farm C was an active racehorse training facility and their horses received timothy hay with a custom mixed sweet feed concentrate. On average, horses ate 2.32 % of BW in DM with approximately 38 % of their diet coming from forage. These horses had the highest NRC (2007) requirement for CP (987 g/d) and they consumed 150% of requirement (1472 g/d).

Lastly Farm D was a large Standardbred breeding facility and the barn used in this study housed mostly broodmares. Horses consumed alfalfa hay and a custom mix concentrate specifically formulated for breeding and growing horses. The DM intake for these horses was estimated as 1.98 % of their BW with 67 % of their daily intake
coming from hay. As both the hay and concentrate had a relatively high % CP, horses consumed (1962 g/d) 211 % of NRC (2007) CP requirement (925.8 g/d).

**Ammonia Concentration**

Daily mean NH$_3$ concentrations from dry areas of stalls were 25.5 ± 12.8 ppm over all facilities. The mean barn NH$_3$ concentrations from urine covered areas of stalls were 237.64 ±117.2 ppm with Farm D having the highest NH$_3$ concentrations (400.34 ± 50.2 ppm). Average NH$_3$ concentrations from measurements that were taken 6 h post stall cleaning were lower, 129.3 ± 63.4 ppm (Farm A PM) and 154.35 ± 33.3 ppm (Farm C), compared to those taken 24 h post cleaning: 185.4± 41.4 ppm (Farm A AM), 237.6 ± 59.6 ppm (Farm B), and 400.3 ± 50.2 ppm (Farm D) (Figure 3-6).

Crude protein intake and the NH$_3$ concentration from urine covered areas of stalls were correlated ($r^2 = 0.57$), but CPI and NH$_3$ concentration from the dry areas of the stall were not ($r^2 = 0.23$). When determining if there is a potential correlation between the percent CPI over NRC (2007) requirement and NH$_3$ concentrations, both dry and wet area correlations were significant ($r^5 = 0.499$ and 0.65, respectively) (Figures 3-7 and 3-8).

**Ambient Air and Ventilation Rate**

Daily average indoor T, RH, CO$_2$ concentrations, and VR during the time of measurement in each facility are shown in Table 3-5. Temperatures were higher during the PM (15.4 – 22.8 °C) measurements than in the AM (8.29 – 18.24 °C). Relative humidity was higher in the AM (64.15 – 98.35 %) than in the PM (44.08 – 63.12 %). In each facility, temperature and RH were monitored near the middle of the main aisle (T$_1$, RH$_1$) and near the exterior of the facility (T$_2$, RH$_2$) (Figures 3-1, 3-2, 3-3, 3-4) and were
correlated ($r^2 = 0.98$ and $0.996$, respectively). The $T_2$ and $RH_2$ were negatively correlated ($r^2 = -0.51$), however the $T_1$ and $RH_1$ were not ($r^2 = -0.45, P = 0.0786$).

The daily indoor CO$_2$ concentration mean across facilities was $550.5 \pm 82$ ppm with a range of $469 - 740$ ppm. The VR was highest on Farm D during d1 and lowest on Farm A during d2. Daily CO$_2$ and VR were not correlated to temperature ($r^2 < 0.5$).

**Ammonia Emissions**

Figure 3-9 shows the daily mean NH$_3$ emissions of the 4 horse farms. Ammonia emissions from the 4 horse farms ranged from 18.5 to 124 g d$^{-1}$ horse$^{-1}$ with a mean of 43 g d$^{-1}$ horse$^{-1}$. Farm D appeared to have higher emissions than the other farms. Measurements taken from stalls 6 h post cleaning appeared to be lower than measurements taken from stalls 24 h post cleaning. When standardizing the BW of horses to 1 animal unit (AU, 454 kg), the emissions ranged from 13.7 to 92 g d$^{-1}$ horse$^{-1}$ with a mean of 38 g d$^{-1}$ horse$^{-1}$ (Figure 3-10).

Figure 3-11 shows the daily estimated mean percentage of dietary N intake lost as NH$_3$-N. Across all farms, the average percent lost was 14.2 % of dietary N intake with a range of 6.7 – 21.4 %.

**Discussion**

The results of this study supported previous research showing feeding protein in excess of the requirement is common in the horse industry (Lawrence et al., 2003; Harper et al., 2009). Harper and coworkers (2009) surveyed 11 horse farm owners in the Chesapeake Bay Watershed (same region as the farms in the current study) for their nutrient management practices, including feed management. They reported that on average, horses were overfed CP by 157 % above the NRC (2007) requirement, with a range of 79 – 263 %. These values agree very well with the current results (mean of
182 % over requirement and a range of 139 – 211 %). Feedstuffs that are commonly used for horse rations tend to be relatively high in CP, so it is difficult to feed CP at the NRC (2007) requirement. Determining the daily feeding intake for horses on all farms was an estimation, as all horses had at least 1 h of access to pasture and were therefore also consuming fresh grass.

Ammonia concentrations reported in the current study (mean of 238 ppm from wet areas and 25 ppm from dry areas of the stalls) are higher than those reported in chapter 2 (mean of 85 ppm from urine and < 1 ppm from feces). These differences could be due to a multitude of factors, including how NH₃ was quantified and the dietary CPI. In the current study, a flux chamber was used to determine the NH₃ concentrations from stalls whereas in the previous study, urine and feces were collected from horses and mixed with straw or shavings and measured with an in vitro system. It is common practice in the equine industry to clean stalls every day and to add new bedding when needed, however stalls are only periodically completely stripped of used bedding. This constant mixing of feces, urine, and bedding material could be leading to increased NH₃ concentrations. Also, on average horses in this study consumed more CP (in excess of their NRC (2007) requirement) than those in the previous study. Waldrip and coworkers (2013) evaluated 47 different feeding regimens from 12 beef cattle feeding trials for N excretion characteristics and reported a strong relationship between dietary CP concentration and the proportion of total N excreted in urine. Urinary N represented about 50 % of total excreted N from cattle fed diets containing CP at NRC (2002) recommendations, while as much as 71 % of total N excretion was in urine when diets were in excess of the recommendation. A similar trend was seen in chapter 2, as dietary
CPI increased urinary N as a percent of total N excreted increased from 31 to 43 %. This relationship is important because urinary N is the most susceptible portion to be volatilized as NH₃.

The correlation between dietary CPI and the NH₃ concentration from the stalls is a relationship that is well supported from previous literature (Hristov et al., 2011; Williams et al., 2011; Waldrip et al., 2015; Bougouin et al., 2016). Bougouin and colleagues (2016) performed a meta-analysis on the nutritional and environmental effects on NH₃ emissions from dairy cattle housing. They reported that NH₃ emissions were, in part, influenced by dietary CP content (r = 0.51). The results from chapter 2, determining the effect of dietary CP intake on NH₃ emissions using an in vitro system, showed that NH₃ emissions tended to be higher as dietary CP went from 110 to 150 % over the NRC (2007) CP requirement for maintenance horses (712 g/d). In another study conducted with horses, Williams and coworkers (2011) reported that NH₃ concentrations in stalls of horses fed lower dietary CP concentrations averaged 25.4 ppm and from horses fed higher dietary CP concentrations averaged 37.8 ppm.

The current study was carried out over two weeks at the end of October in the Mid-Atlantic region. Temperatures were low in the morning and higher in the afternoon and early evening. According to the US Climate data (2015), average high and low temperatures in October in the Mid-Atlantic region are 19 and 4 degrees Celsius, respectively. The results from the current study were within or close to this temperature range.

The CO₂ balance method, based on CO₂ production by the animals, is one of the most commonly used methods to determine VR in naturally ventilated buildings (CIGR,
1984). Wang and coworkers (2016) evaluated the CO$_2$ balance method against a direct method. They reported that the differences between the CO$_2$ balance method and a direct method were greater or more erratic when the CO$_2$ concentration between the indoor and outdoor were less than 70 ppm. In the current study, the differences between the indoor CO$_2$ concentration and the outdoor ambient CO$_2$ (estimated at 390 ppm) was at times close to 70 ppm, but never below. In general, conditions of excellent ventilation of NV animal houses results in smaller or narrower concentrations which result in relatively large errors.

The average NH$_3$ emissions over all 4 facilities during the study was 43 g d$^{-1}$ horse$^{-1}$ with a range from 20 – 124 g d$^{-1}$ horse$^{-1}$, which agrees well with previous literature conducted with cattle (McGinn et al., 2007; Hristov et al., 2011; Waldrip et al., 2015; Bougouin et al., 2016). Hristov and coworkers (2011) reviewed the literature and reported that the estimated NH$_3$ emissions from beef cattle have ranged from 50 to 283 g d$^{-1}$ animal$^{-1}$, while Bougouin and colleagues (2013) reported in a meta-analysis that NH$_3$ emissions from dairy cows ranged from 5.8 to 186 g d$^{-1}$ cow$^{-1}$. The higher values for finishing beef cattle compared to dairy cows could be due to a combination of factors including the following: 1) a greater proportion of fed N is retained in dairy than beef cattle; 2) a greater proportion of fed N is lost in urine in beef cattle; and 3) air turbulence and movement is greater in open lot feedlots than dairy barns (Hristov et al., 2011). Another factor to be considered is time since last stall cleaning. It appears that the measurements taken from stalls that were cleaned more recently (6 h prior) had lower NH$_3$ emissions than those that were cleaned 24 h before. Ammonia emissions from Farm D were estimated to be higher than the other facilities, and this could be due to a
few factors. Horses on Farm D were fed CP in the highest excess of NRC (2007) requirement (211 %) and these stalls were bedded with straw. Chapter 2 concluded that equine operations that bed with wheat straw may have a higher NH$_3$ ER than those that bed on wood shavings.

The percent of N lost as NH$_3$-N of dietary N intake reported in the current study was lower compared to previous literature conducted with beef cattle (Cole et al., 2005; Todd et al., 2006; McGinn et al., 2007). McGinn and coworkers (2007) reported approximately 63 % loss of the daily N intake in beef feedlot cattle, and the other studies reported ranges from 45 – 60 % losses (Cole et al., 2005; Todd et al., 2006), whereas the range in the current study was from 6.8 – 32.5 % dietary N lost as NH$_3$-N. In the current study, this calculation was an estimation of the NH$_3$-N loss rather than a direct measurement.

Accurately quantifying data on NH$_3$ emissions from farms is necessary, but very complex. There are many different methods for estimating NH$_3$ emissions, and in this study a dynamic flux chamber system was used. Chamber systems are commonly used for estimating gas emissions because they are generally less expensive and not as complex as other methods (i.e. micrometeorological) (McGinn, 2006; Rochette et al., 2005; Spiehs et al., 2011). Chamber systems are useful for estimating gas emissions from a smaller area (Hu et al., 2014), such as a horse stall. These methods, however, are not appropriate for quantifying actual emissions, but are appropriate for assessing relative emissions (Meisinger et al., 2001). Another limitation of the current study is that measurements were only taken over a very short period of time which does not accurately reflect diurnal or seasonal fluctuations in NH$_3$ emissions. Leytem and
coworkers (2013) reported a strong diurnal trend for NH$_3$, with emissions being lower during the late evening and early morning and then increasing throughout the day in a dairy cow barn. This trend can be associated with wind speed and temperature. The seasonal trend is similar to the diurnal trend in that emissions are the lowest during the winter months and highest in the summer (Leytem et al., 2013). As management practices are different for the equine industry compared to other livestock industries, further research is needed to determine the impact of daily and seasonal fluxes on equine operations.

In conclusion, the NH$_3$ emissions estimated from these 4 equine operations were similar to what has previously been reported in other large livestock species. The mean daily NH$_3$ emissions was 43 g d$^{-1}$ horse$^{-1}$. There seems to be a strong correlation between excess dietary CPI and increased NH$_3$ volatilization. Reducing CP intake may be the most feasible method for mitigating NH$_3$ losses, however since this can be quite challenging given the type of feedstuffs available for horses, further research is needed to assess the most practical method for mitigating NH$_3$ losses. These values are a representation of the relative emissions factors, but give a better understanding of the impact equine operations are having on atmospheric NH$_3$ levels.
Table 3-1. Site descriptions for the four equine operations evaluated.

<table>
<thead>
<tr>
<th>Farm</th>
<th>State</th>
<th># of horses*</th>
<th>Intended use</th>
<th>Bedding used</th>
<th>Time of samples</th>
<th>Time related to stall cleaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>PA</td>
<td>40</td>
<td>University riding stable</td>
<td>Wood shavings</td>
<td>AM &amp; PM</td>
<td>Pre &amp; 6 h post</td>
</tr>
<tr>
<td>B</td>
<td>PA</td>
<td>29</td>
<td>University breeding farm</td>
<td>Wood shavings</td>
<td>PM</td>
<td>Pre</td>
</tr>
<tr>
<td>C</td>
<td>MD</td>
<td>39</td>
<td>Racing stable</td>
<td>Wheat straw</td>
<td>AM</td>
<td>6 h post</td>
</tr>
<tr>
<td>D</td>
<td>MD</td>
<td>22</td>
<td>Breeding farm</td>
<td>Wheat straw</td>
<td>AM</td>
<td>Pre</td>
</tr>
</tbody>
</table>

*number of horses refers to the total number of animals housed in the barn
Table 3-2. Description of horses from which measurements were taken. Data presented as Means ± SE.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Farms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Number of horses*</td>
<td>4</td>
</tr>
<tr>
<td>Breed type</td>
<td>Warmblood type</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>18 ± 0.8</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>608 ± 25.6</td>
</tr>
<tr>
<td>Classification</td>
<td>Moderate work</td>
</tr>
<tr>
<td>Time spent in stall (h/d)</td>
<td>20</td>
</tr>
</tbody>
</table>

* number of horses from which measurements were taken at each barn.
Table 3-3. Description of feeds at the 4 farms.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Hay Type</th>
<th>%CP</th>
<th>Daily intake (kg)</th>
<th>Concentrate Type</th>
<th>%CP</th>
<th>Daily intake (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*</td>
<td>T&amp;A #</td>
<td>9.9</td>
<td>12.5</td>
<td>Pelleted feed</td>
<td>12.5</td>
<td>2.08</td>
</tr>
<tr>
<td></td>
<td>T&amp;A</td>
<td>9.9</td>
<td>12.5</td>
<td>Ration balancer</td>
<td>32</td>
<td>1.06</td>
</tr>
<tr>
<td>B</td>
<td>Alfalfa</td>
<td>15.1</td>
<td>6.6</td>
<td>Pelleted feed</td>
<td>16</td>
<td>2.72</td>
</tr>
<tr>
<td>C</td>
<td>Timothy</td>
<td>7.6</td>
<td>4.8</td>
<td>Custom mix</td>
<td>14</td>
<td>7.8</td>
</tr>
<tr>
<td>D</td>
<td>Alfalfa</td>
<td>14.8</td>
<td>9</td>
<td>Custom mix</td>
<td>14</td>
<td>4.5</td>
</tr>
</tbody>
</table>

* Farm A: Two horses were consuming a pelleted feed and two were consuming a ration balancer. All horses were fed the same hay.
# Timothy Alfalfa mix hay (T&A)
Table 3-4. Estimation of mean daily dry matter and crude protein intake of horses from the 4 farms.

<table>
<thead>
<tr>
<th>Daily nutrient intake</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM intake, kg/d</td>
<td>12.71</td>
<td>8.41</td>
<td>11.39</td>
<td>12.07</td>
</tr>
<tr>
<td>Intake, % of BW</td>
<td>2.1</td>
<td>2.52</td>
<td>2.32</td>
<td>1.98</td>
</tr>
<tr>
<td>NRC CP requirement, g/d</td>
<td>933.2</td>
<td>768.8</td>
<td>987</td>
<td>925.8</td>
</tr>
<tr>
<td>CP intake, g/d</td>
<td>1503</td>
<td>1432</td>
<td>1472</td>
<td>1962</td>
</tr>
<tr>
<td>% NRC CP requirement</td>
<td>162.1</td>
<td>188.3</td>
<td>149.1</td>
<td>211.9</td>
</tr>
</tbody>
</table>
Table 3-5. Ambient weather data during data collection from the 4 farms. Temperature and relative humidity were measured from the middle of the main barn aisle ($T_1$ and $RH_1$) and from the end of the aisle near stalls used for measurement ($T_2$ and $RH_2$).

<table>
<thead>
<tr>
<th>Day</th>
<th>$T_1$, °C</th>
<th>$RH_1$, %</th>
<th>$T_2$, °C</th>
<th>$RH_2$, %</th>
<th>Indoor CO$_{2b}$ ppm</th>
<th>VR, m$^3$ h$^{-1}$ horse$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm A, AM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9.16</td>
<td>81.45</td>
<td>10.77</td>
<td>77.17</td>
<td>667.70</td>
<td>421.01</td>
</tr>
<tr>
<td>2</td>
<td>8.29</td>
<td>83.33</td>
<td>10.24</td>
<td>82.13</td>
<td>739.96</td>
<td>334.08</td>
</tr>
<tr>
<td>3</td>
<td>12.8</td>
<td>74.42</td>
<td>12.69</td>
<td>73.57</td>
<td>626.32</td>
<td>495.74</td>
</tr>
<tr>
<td>Farm A, PM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>22.21</td>
<td>52.23</td>
<td>22.21</td>
<td>54.74</td>
<td>552.63</td>
<td>614.43</td>
</tr>
<tr>
<td>2</td>
<td>22.80</td>
<td>58.52</td>
<td>22.45</td>
<td>63.12</td>
<td>540.24</td>
<td>778.21</td>
</tr>
<tr>
<td>3</td>
<td>16.12</td>
<td>44.08</td>
<td>16.68</td>
<td>45.80</td>
<td>479.34</td>
<td>1309.68</td>
</tr>
<tr>
<td>Farm B, PM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19.99</td>
<td>46.15</td>
<td>21.05</td>
<td>45.54</td>
<td>525.56</td>
<td>554.02</td>
</tr>
<tr>
<td>2</td>
<td>21.80</td>
<td>57.33</td>
<td>21.80</td>
<td>57.33</td>
<td>473.77</td>
<td>896.6</td>
</tr>
<tr>
<td>3</td>
<td>15.44</td>
<td>49.98</td>
<td>15.44</td>
<td>49.98</td>
<td>468.92</td>
<td>951.62</td>
</tr>
<tr>
<td>Farm C, AM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11.03</td>
<td>68.26</td>
<td>12.71</td>
<td>67.48</td>
<td>508.80</td>
<td>718.32</td>
</tr>
<tr>
<td>2</td>
<td>10.49</td>
<td>79.73</td>
<td>11.63</td>
<td>76.97</td>
<td>546.09</td>
<td>600.43</td>
</tr>
<tr>
<td>3</td>
<td>16.16</td>
<td>94.59</td>
<td>16.37</td>
<td>98.35</td>
<td>471.71</td>
<td>1220.82</td>
</tr>
<tr>
<td>Farm D, AM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>17.62</td>
<td>91.06</td>
<td>18.24</td>
<td>95.08</td>
<td>472.15</td>
<td>1426.69</td>
</tr>
<tr>
<td>2</td>
<td>12.97</td>
<td>64.15</td>
<td>13.74</td>
<td>64.23</td>
<td>554.05</td>
<td>461.61</td>
</tr>
<tr>
<td>3</td>
<td>9.56</td>
<td>71.71</td>
<td>9.59</td>
<td>75.19</td>
<td>630.43</td>
<td>471.8</td>
</tr>
</tbody>
</table>
Figure 3-1. Outline of Farm A showing the location of the stalls, aisles, and arena.

*stalls used to measure NH$_3$ concentration;
+temperature, relative humidity, and CO$_2$ detection

Figure 3-2. Outline of Farm B showing the location of the stalls and aisles.

*stalls used to measure NH$_3$ concentration
+temperature, relative humidity, and CO$_2$ detection
Figure 3-3. Outline of Farm C showing the location of the stalls and aisles.

*stalls used to measure NH$_3$ concentration
+temperature, relative humidity, and CO$_2$ detection

Figure 3-4. Outline of Farm D showing the location of the stalls and aisles.

*stalls used to measure NH$_3$ concentration
+temperature, relative humidity, and CO$_2$ detection
Figure 3-5. Schematics of dynamic NH$_3$ flux chamber system. Courtesy of Hong Li.

Figure 3-6. Daily mean NH$_3$ concentrations at the 4 equine operations from A) dry areas in the stalls and B) wet areas of the stalls.
Figure 3-7. The relationship between mean NH₃ concentrations from dry areas of the stall and percentage of daily crude protein intake (CPI) over the NRC (2007) requirement for CP.

Figure 3-8. The relationship between mean NH₃ concentrations from urine covered areas of the stall and percentage of daily crude protein intake (CPI) over the NRC (2007) requirement for CP.
Figure 3-9. Daily ammonia emissions per horse over 3 days using the flux chamber system on 4 horse operations (Farms A, B, C, D).

Figure 3-10. Daily ammonia emissions per animal unit (AU, 454 kg BW) over 3 days using the flux chamber system on 4 horse operations (Farms A, B, C, D).
Figure 3-11. Daily percentage of dietary nitrogen lost as ammonia-nitrogen, averaged over 3 d, on the 4 horse operations (Farms A, B, C, D).
CHAPTER 4
DETERMINING THE AVersion OF HORSES TO DIFFERENT AMMONIA CONCENTRATIONS

Introduction

For the equine industry, concerns about ammonia (NH₃) levels in the barn environment are multifaceted and include issues of animal welfare, animal and human health, and environmental impacts. Ammonia is produced as a by-product of the microbial decomposition of the organic nitrogen compounds in manure. Nitrogen occurs as both unabsorbed nutrients in animal feces and either as urea (mammals) or uric acid (poultry) in urine. The term "manure" refers to the combination of feces and urine that is excreted (USEPA, 2002). Feeding protein in excess of the requirement is common in the horse industry (Lawrence et al., 2003) and leads to increased urinary N excretion. Urea is the main nitrogenous constituent of equine urine. Urea is not volatile, but once it comes in contact with feces it is rapidly hydrolyzed to NH₃ and carbon dioxide by the abundant urease activity in fecal matter (Bussink and Oenema, 1998).

While there are recommendations for proper horse stable ventilation (Wathes, 1989; MWPS, 1971) few stables are built to these specifications. Most horse stables employ natural, rather than mechanical, ventilation due to the relatively low density of mature animals within the building (Clarke, 1987; Golden, 2000). By agricultural engineering definition, stables are "cold housing with no supplemental heat and no, or limited insulation" (Wheeler et al., 2001). In recent history, the trend has been to tighten up construction to a more "residential" standard rather than complying with livestock housing ventilation recommendations (Wheeler et al., 2001). Hence, most modern horse barns are under-ventilated in an attempt to maintain comfortable conditions for handlers and an honest, but misdirected, attempt to provide thermal comfort for the
horse (Sainsbury and Rossdale, 1987). Horses kept indoors can therefore be exposed to ammonia on a regular basis.

Ammonia concentrations have a negative impact on horse health. Chronic and acute respiratory disease is one of the leading causes of wastage in horses used in high performance athletic endeavors and is commonly recognized in pleasure horses as well (Hernandez and Hawkins, 2001). Holcombe et al. (2010) established that stabling is associated with increased airway inflammation and the persistence of upper airway inflammation in young horses. Gerber et al. (2003) found that horses housed in a stable environment while clinically healthy and performing well showed evidence of inflammatory airway disease. Ammonia is the most important gas in the stable air with respect to animal health, particularly of the respiratory tract (Fleming et al., 2009). Various studies have found that ammonia levels are highest near barn and stall floors (Fleming et al., 2009; Pratt et al., 2000). Since horses frequently eat off the floor, have their heads down and/or lie down when stabled for long periods of time, they can be exposed to high levels of ammonia. High levels of ammonia have been associated with foal pneumonia (McMillian, 1986) and may also predispose horses to asthma like conditions (Tanner, 1998). Lawrence (1988) detected ammonia concentrations of 25.3 parts per million (ppm) at the level of the horse's halter following a 16-day period of stall confinement. The concentration of ammonia that is harmful to horses has not been determined; however, in humans, the short-term exposure limit is 35 ppm (ATSDR, 2004).

Intensively housed livestock are exposed to high ambient air concentrations of NH₃ that animals which occupy more extensive environments would not experience.
The adverse effects of NH$_3$ exposure on animal health and productivity is well recognized in pigs (De Boer and Morrison, 1998), poultry (Kristensen and Wathes, 2000), and to some extent in horses (Ivester et al., 2014). However, the impact of NH$_3$ on animal behavior and welfare has not been studied as intensely. These behavioral studies complement physiological investigations by providing an alternative insight into the environmental response of an animal (Wathes et al., 2002).

The majority of studies conducted to determine the aversion of pigs and poultry to ammonia have used environmental preference chambers (EPC). Briefly, these preference chambers consisted of multiple identical compartments kept under positive pressure to control the ventilation rate. The NH$_3$ concentration was maintained independently within each compartment, with all other environment factors kept constant (Jones et al., 2000; Kristensen et al., 2000; Wathes et al., 2002; Green et al. 2008; Sales et al., 2013). Morrison et al. (1993) were the first to study the aversion of pigs and poultry to NH$_3$ in short-term (30 min to 12 h), free choice tests. They found that pigs and poultry show some avoidance behaviors (e.g. less time feeding, etc.) at 60 ppm NH$_3$.

Another study determined the behavioral preferences of laying hens for different concentrations of ammonia found in commercial poultry houses (Kristensen et al., 2000). Six groups, of six laying hens each, were given the choice of three concentrations of ammonia (0, 25, and 45 ppm) in a preference chamber over a period of 6 days and their location and behavior were recorded every 15 minutes. Hens foraged, preened, and rested significantly more in fresh air than in the ammonia-polluted environments. There was a significant difference between the responses in 0
and 25 ppm, but not between 25 and 45 ppm. Thus, suggesting that ammonia may be aversive to hens with a threshold between 0 and 25 ppm. The behavioral responses of pigs to atmospheric ammonia were tested in a chronic choice test (Jones et al., 1996). The pigs also chose to rest, feed, and forage more in the unpolluted compartments. Overall, more feeding behavior was observed in the fresh air and more food was consumed in these compartments of the chamber.

These studies, along with others, show that there is a preference to fresh air rather than NH₃ filled air in other species. However, the use of EPC are implausible with larger species, like the horse, so alternative approaches to determining the impact of NH₃ concentrations on horse behavior and physiology are needed. The objectives of this study were to 1) develop a system to measure horses’ aversion to different NH₃ concentrations and 2) determine horses’ behavioral and physiological responses to acute exposure to ammonia for use as potential indicators to detect when NH₃ levels in equine facilities have become aversive. We hypothesized that horses would show aversion to air with higher concentration of NH₃, and in response, their behavior and physiology would be altered.

**Materials and Methods**

**Horses**

Nine yearling stock type horses (5 geldings and 4 fillies), naïve to stabling, were used to test the aversion of horses to different NH₃ concentrations. Yearlings were group housed in large pens (2 horses per pen) with unlimited access to water and shade at the Institute of Food and Agricultural Sciences (IFAS) Equine Sciences Center in Ocala, FL. All yearlings received routine healthcare, including vaccination, anthelmintic treatment and hoof care established in the standard operating procedures.
for the IFAS Equine Sciences Center. All animal protocols and procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Florida.

**Head Box System**

To test the aversion of horses to different NH$_3$ concentrations, a head box system consisting of two head boxes with gas flow control and a computer-based data recording system was used. The dimensions of each head box were 50.8 cm W x 50.8 cm D x 76.2 cm H. The frame of each head box is made of 2x4 wood with plywood for the top, backside, and bottom panels. Transparent acrylic sheets were used for the two side panels of each box, so that the horses could see while inside the box and to allow viewing of the horses during testing. Transparent weather curtain strips (5 cm width) were attached to the front opening of each head box to minimize air leakage from the head boxes (Figure 4-1).

The fresh air from a blower was measured through a mass flow meter with laminar flow element. The airflow of high NH$_3$ gas (600 ppm) was controlled by a mass flow controller to obtain the desired concentration and injected into the main air stream of each head box. A flexible PVC (5 cm diameter.) pipe delivered the supply air into each head box through PVC distribution pipe with a series of holes (0.5 cm diameter) that surrounded a feed pan placed on the box bottom panel (Figure 4-2). The air flow was adjusted based on the estimated respiration rate of the horses. The airflow rate of each head box was set at 100 LPM. An air exhaust port was located on the top panel of each head box and connected to a vacuum pump that discharged the NH$_3$ air to the outside environment, to avoid accumulation of NH$_3$ in each head box. A fast response photoacoustic NH$_3$ analyzer (MSA Chillgard RT, Cranberry Township, PA) with a
multipoint sampler was used to monitor real time NH$_3$ concentration in each head boxes. An infrared (IR) sensor was mounted on the top of the boxes to detect when the horse was eating inside the box. A computer based software (Labview, National Instruments, Austin TX) was created and modified to record each feeding event and duration, and to measure and control the NH$_3$ concentration in each head box.

The head box system was placed in a 3.7 x 3.7 m constructed box stall. Each head box was equipped with a 38.1 cm x 38.1 cm D x 15.2 cm H square feeding pan that contained an equal amount (1.5 kg) of the horses’ regular concentrate feed.

**Experimental Design**

Prior to the experiment, yearlings were housed in large warm-season grass pastures with free access to water and hay. Yearlings were brought into the large pens close to the testing stall 2 weeks before testing began for an acclimation period. During this acclimation period, morning (0700 h) and evening (1600 h) meals were consumed in the head boxes each day. At the end of the acclimation period, an initial test was conducted to determine if yearlings found the head box system aversive. Since the goal of this study was to determine horses’ aversion to NH$_3$, it was necessary to test whether they found the head box system (sans NH$_3$) aversive.

An experimental timeline is shown in Figure 4-3. During phase 1, the yearlings performed a two-choice aversion test in which they were allowed to select to feed from either NH$_3$ free air or a higher concentration of NH$_3$ (0 vs. 25 ppm, 0 vs. 50 ppm, and 25 vs. 50 ppm NH$_3$) in a 3 X 3 replicated Latin square design. On each test day, 3 horses were randomly assigned to each of the 3 treatments. At the conclusion of each test day, treatments were switched until all horses were exposed to all treatments, giving 9 observations for each treatment. In between each treatment day, there were 3 washout
days to prevent carryover effects. On the second washout day, in between each test day, yearlings were evaluated while eating from head boxes sans NH$_3$ to determine if their aversion to the head boxes changed over the course of the testing period.

In addition to determining if horses showed aversion to higher NH$_3$ concentrations (objective of phase 1), the second goal was to determine if horses would show a physiological or behavioral response to acute ammonia exposure. This was tested during phase 2, where yearlings ate from the head boxes that contained one of two treatments: 25 ppm or 50 ppm NH$_3$ in a randomized, crossover design.

In both phases, testing consisted of a single, 10-minute exposure at both AM and PM feedings. To eliminate directional biases and to reduce the influence of day on NH$_3$ aversion, injection of ammonia versus ammonia-free air into the head boxes was reversed, so that each box received each NH$_3$ concentration, between AM and PM tests. Treatments and box assignment to treatment were randomized.

**Determining Salivary Cortisol**

Saliva samples for cortisol determination were collected within 1 h before each test (pre), immediately after the test (0 post), 30 min post (30 post), and 60 min post (60 post). Saliva was collected using specific cotton rolls (Sarstedt, Nümbrecht-Rommelsdorf, Germany) attached to a chifney bit and placed in the mouth for 5 min. The cotton roll was then returned to the Salivette polypropylene tube and immediately stored on ice until centrifugation 3,000 x $g$ for 10 min at 4°C for saliva collection and subsequent storage at -20°C.

Salivary cortisol was determined using a commercial enzyme immunoassay kit (Salimetrics, State College, PA). The procedure followed the protocol of the
manufacturer. The intra-assay coefficient of variation was 10 %, the inter-assay variation was 7.4 % and the minimal detectable concentration was 0.07 ng/mL.

**Monitoring Heart Rate and Heart Rate Variability**

Before each test, horses were fitted with a heart rate monitoring system (Polar, Kempele, Finland) attached around the heart girth of the horse. Heart rate was recorded for the duration of each test (10 min) and mean HR and HRV were calculated. The Kubios HRV software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) was used for HRV analysis. The software uses a detrending procedure based on the smoothness priors approach (Tarvainen et al., 2002; Tarvainen and Niskanen, 2008). Heart rate was recorded and the HRV variables: R-R interval, standard deviation of R-R interval for the given recording time (SDRR). Root mean square of successive R-R differences (RMSSD), as well as the ratio of the low frequency to high frequency (LF: HF) were calculated. Frequency band thresholds are species specific. In a review conducted by Stucke and coworkers (2016), they recommended that the frequency band thresholds for interpretation of ANS activity in horses were $0.01 \geq 0.07$ Hz for LF and $0.07 \geq 0.6$ for HF.

**Behavioral Analysis**

Two video cameras (GoPro, San Mateo, CA) were installed, one on each side of the head boxes, to allow for detailed analysis of behaviors. Feeding behavior was measured according to how much concentrate each yearling consumed from each head box and the amount of time spent feeding from head boxes.

**Statistical Analysis**

Data were analyzed using one-way ANOVA as part of the PROC MIXED procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC.) unless otherwise noted.
Data were checked for normality and transformed according to their corresponding lambda using a Box-Cox transformation (Box and Cox, 1964). Changes in salivary cortisol concentration were analyzed using a mixed model for repeated measures, with time of sample being repeated. The proportion of time spent feeding from the lower NH$_3$ concentration box was used as a covariate for determining differences in salivary cortisol concentrations. Treatment, day, time of day (AM/PM), and time of sample were fixed effects with yearling serving as the random effect. Time of sample served as the repeated measure with a covariate structure of compound symmetry applied. Feeding behavior, time spent feeding, box preference and heart rate variables were analyzed using a mixed model with treatment and day serving as fixed effects and the random effect was yearling. In order to determine if yearlings behaved differently in response to NH$_3$ exposure compared to exposure to the head box system, the feeding behaviors on the initial and washout days were used as a covariate. In all ANOVA tests, individual treatment means were compared using Tukey’s adjustment of the LSMEANS statement. Significant interactions were included. Differences were considered significant at $P \leq 0.05$ and trends considered at $P \leq 0.1$.

**Results**

**Acclimation to Head Box System**

Yearlings preferred feeding from box 2 (B2) compared to box 1 (B1) (Table 4-1). Feeding behavior variables, such as total amount of feed consumed and percent of time spent feeding, did not change over time ($P = 0.2514$ and $P = 0.6934$, respectively) (Table 4-1), whereas salivary cortisol concentrations were affected by day and time of day ($P = 0.0005$ and $P < 0.0001$) (Figure 4-4). Salivary cortisol concentrations on Washout day 3 were lower than all other control days ($P < 0.01$). The variability in the
salivary cortisol measurements taken immediately after the test (0 post) was high, which resulted in the removal of this time point from the analysis.

**Phase 1: Determining Aversion of Horses to Higher NH$_3$ Concentrations**

**Feeding behavior**

Means were averaged over the day (AM/PM) to control for directional bias. Yearlings did consume more feed in the PM vs. AM ($P < .0001$), but all other feeding behaviors were not different between AM and PM (data not shown).

Total time spent feeding was not affected by treatment ($P = 0.7485$) nor day ($P = 0.5035$) (Table 4-2 and Figure 4-5). When determining time spent feeding in B1 and B2, there was no effect of treatment ($P = 0.3104$) nor day ($P = 0.2475$) on time spent feeding out of B1 (Table 4-2). However, there was an effect of treatment ($P = 0.0116$) and day ($P = 0.0449$) on time spent feeding out of B2, with horses spending more time eating when on the 25 vs. 50 ppm treatment compared to 0 vs. 25 and 0 vs. 50 ppm treatments ($P = 0.0356$). Horses spent more time eating from B2 on test day 1 than test day 2 ($P = 0.0365$), however time spent feeding from B2 on TD1 vs. TD3 ($P = 0.5052$) and TD2 vs. TD3 ($P = 0.3077$) were not different.

Total amount of feed consumed was also not affected by treatment ($P = 0.4629$), but was affected by day ($P = 0.0132$) (Table 4-2 and Figure 4-6). When determining the amount of feed consumed within B1 and B2, there was a trend for treatment to affect feed intake from B1 ($P = 0.0814$), but no effect of treatment on intake from B2 ($P = 0.1375$). Horses tended to eat more from B1 when tested with 25 vs. 50 ppm compared to 0 vs. 25 ppm ($P = 0.0707$).

In an attempt to determine the difference between feeding behaviors when exposed to different NH$_3$ concentrations within treatment, e.g. amount consumed from 0
ppm box compared to 25 ppm box, the proportion of amount of the total feed consumed and time spent feeding from the lower NH₃ box (0 ppm, 0 ppm, and 25 ppm for treatments: 0 vs. 25 ppm, 0 vs. 50 ppm, and 25 vs. 50 ppm, respectively) compared to total consumed or total time spent feeding (L: T) were calculated. There was no effect of treatment on feed consumed ($P = 0.1413$) or time spent feeding ($P = 0.1716$) (Figure 4-7).

**Heart rate and heart rate variability**

Heart rate, RR-interval, and LF: HF ratio underwent the following transformations for statistical analysis, as the data were not normally distributed: the mean RR interval was squared, the inverse of HR was squared, and the LF: HF ratio was log transformed. All transformations were back-transformed to original values after statistical analysis for inclusions in tables and figures. There was no effect of treatment nor day on all HR and HRV variables ($P > 0.05$) (Table 4-3).

**Salivary cortisol**

Salivary cortisol was log transformed for analysis and then back-transformed to original values for inclusion in figures. Salivary cortisol was affected by treatment ($P = 0.0235$), day ($P = 0.0321$), time of day ($P < 0.0001$), and the interaction of day and time of sample ($P = 0.0099$) (Figure 4-8). Salivary cortisol concentration for 0 vs. 50 ppm was greater than 0 vs. 25 ppm ($P = 0.0439$) and 25 vs. 50 ppm ($P = 0.0374$), and there was no difference between 0 vs. 25 ppm and 25 vs. 50 ppm ($P = 0.9975$). Salivary cortisol concentrations were higher in the AM vs. the PM ($P < 0.0001$).

**Phase 2: Determining Physiological Response to Different NH₃ Concentrations**

During the afternoon of test day 4, there was an environmental stressor occurring that was out of the control of the researchers. Therefore, data was not collected for the
PM of test day 4, resulting in only AM samples for that day. Means for feeding behavior and HR were averaged over the day (AM/PM). Similar to Phase 1, yearlings consumed more feed in the PM vs. AM ($P < 0.0001$), but all other feeding behaviors were not different between AM and PM (data not shown).

**Feeding behavior**

Time spent feeding was not affected by treatment ($P = 0.4882$) (Table 4-4 and Figure 4-9). When determining time spent feeding within B1 and B2, there was no effect of treatment on time spent eating from B1 ($P = 0.4499$) nor B2 ($P = 0.3327$) (Table 4-4). Total amount of feed consumed was also not affected by treatment ($P = 0.1728$), (Table 4-4 and Figure 4-9). When determining the amount of feed consumed within B1 and B2, there was no effect of treatment on intake from B1 ($P = 0.1021$), nor B2 ($P = 0.3064$) (Table 4-4).

**Heart rate and heart rate variability indicators**

Heart rate, SDRR, and LF: HF ratio underwent the following transformations for statistical analysis, as the data were not normally distributed: SDRR was log transformed, the inverse of HR was calculated, and the LF: HF ratio was log transformed. All transformations were back-transformed to original values after analysis for inclusion in table. There was no effect of treatment nor day on all HR and HRV variables ($P > 0.05$), except there was an effect of treatment on the LF: HF ratio ($P = 0.0304$) (Table 4-5). The LF: HF ratio was greater for horses exposed to 50 ppm compared to 25 ppm ($P = 0.0281$), but the ratio was not different between 50 ppm and 0 ppm ($P = 0.6903$), nor 25 ppm and 0 ppm ($P = 0.1336$).
Salivary cortisol

In phase 2, salivary cortisol data were not normally disturbed, therefore a square root transformation was performed for salivary cortisol for statistical analysis and then back-transformed to original values for inclusion in figures. Salivary cortisol tended be affected by treatment ($P = 0.0774$), was not affected by day ($P = 0.6448$), and was affected by both time of day ($P = 0.0022$) and time of sample ($P = 0.0138$) (Figure 4-10). Yearlings exposed to the 50 ppm treatment tended to have higher salivary cortisol concentrations than those exposed to 0 ppm ($P = 0.0622$), but was not different from 25 ppm ($P = 0.5011$). Also, there was no difference between 0 ppm and 25 ppm ($P = 0.55$). Salivary cortisol concentrations were higher in the AM vs. the PM ($P = 0.0022$) and were higher from the pre measurement compared to the 30 post measurement ($P = 0.0107$).

Discussion

The main objective of this study was to develop a system to measure the aversion of horses to different NH$_3$ concentrations. This novel head box system represents a method that can be used to determine aversion of horses to NH$_3$. This design overcomes a considerable limitation for conducting aversion experiments in large species, like horses. In smaller species, like poultry, pigs, and laboratory mice, preference studies are conducted using EPC. The head box system developed served as an alternative approach, however, the head box system did present some limitations in the experimental design. Horses were only exposed to NH$_3$ for a short duration (during a meal), so it was not possible to determine if any other behaviors (i.e., sleeping and activity) were affected by exposure to NH$_3$. These limitations make it difficult to compare results from the current study to those obtained in previous studies from other species.
Feeding behavior of the yearling horses did not change over the course of the study, however salivary cortisol concentration was significantly lower on Washout day 3 compared to all other washout days. This could suggest that while yearlings did not change their feeding behavior over the course of the study, the physiological response was not as great, potentially indicating further acclimation to the head box system during the course of the study.

In the current study, feeding behavior did not change due to the different NH$_3$ concentrations. It is important to note that yearlings showed a preference to feed out of B2 compared to B1, and during Phase 1 this preference for B2 was more pronounced when horses were exposed to higher NH$_3$ concentrations (328.5 s in 25 vs. 50 ppm treatment vs. 236.56 s in 0 vs. 25 ppm treatment). This preference for feeding from B2 was still prevalent in Phase 2 (11.02 s in B1 vs. 290.5 s in B2), however this preference was not affected by treatment. As there was the same concentration of NH$_3$ in both B1 and B2 in Phase 2, the yearlings could have become complacent and remained fixed with B2 as that is the one they preferred. B2 was located on the horses’ right when entering the testing area and was located closer to the other horses. This could explain why horses seemed to prefer B2.

Kristensen and coworkers (2000) also reported no differences in feeding behavior in hens when exposed to higher concentrations of NH$_3$. Hens were given the choice of three concentrations of NH$_3$ (0, 25, and 45 ppm) in a preference chamber over a period of 6 days. They showed that hens foraged, rested, and preened more in fresh air compared to NH$_3$ filled environments, but eating behavior was not affected. This finding, and what we found in the current study could be due to the fact that the
motivation of horses to eat is greater than the motivation to avoid higher NH₃ environments. Jones and coworkers (1998) looked at the motivation of pigs to avoid acute exposure to two concentrations of NH₃ using operant conditioning. Pigs did root (desired behavior) more for food (reward) in fresh air compared to 40 or 100 ppm NH₃, however they did not completely avoid the behavior when exposed to 100 ppm NH₃. The authors concluded that while pigs found NH₃ aversive, it was a weak aversion because the motivation to perform the rooting behavior for the food reward was stronger. Also a handful of studies have reported preferences of pigs to avoid areas where NH₃ was ≥ 20 ppm, but this avoidance was delayed and explained by the possible development of a general sense of malaise (Jones et al., 1996; Wathes et al., 2002). In addition, the variation in feeding behavior was higher than expected, thus resulting in some numerical differences, but no significant differences in the current study.

Measuring HR and HRV is a non-invasive technique used in humans and animals to examine the ANS, which is involved in pain and stress reactions (Stucke et al., 2015). The acute exposure to an aversive stimulus, high NH₃ concentrations, in the current study may not have been aversive enough to induce a change in ANS function, as there was no effect of treatment on HR nor HRV indicators during Phase 1. In Phase 2, the LF: HF ratio was higher for the 50 ppm treatment compared to the 25 ppm treatment. In horses, different stressors have been shown to cause a significant decrease in HF power, and thus an increase in the LF: HF ratio (feeding stress test: Bachmann et al., 2003; enforced backward movement: Rietmann et al., 2004). In humans and other animals, when the HF power decreases and the LF: HF ratio
increases after exposure to a stimulus, there is a reduction of parasympathetic activity and thus increased sympathetic activity (von Borell et al., 2007). However, this relationship in horses is unclear (Rietmann et al., 2004; Oel et al., 2010). In the current study, the increased LF: HF ratio when yearlings were exposed to only 50 ppm NH$_3$ may indicate increased sympathetic activity; however, conclusions for cardiac sympathetic tone from the LF: HF ratio should be interpreted carefully (Yamamoto et al., 1991).

The results of the current study showed that salivary cortisol concentrations were higher in the morning compared to concentrations during the evening. These findings agree with previous work conducted in horses that demonstrated a circadian rhythm or pattern in cortisol (Hoffsis et al., 1970; Bottoms et al., 1972; Irvine and Alexander 1994; Aurich et al., 2015). The pre-test salivary cortisol concentrations in the current study seem to be higher than basal salivary cortisol concentrations reported in other studies (853 vs. 250-1000 pg/mL, respectively) (Schmidt et al., 2010a; Schmidt et al., 2010b; Bobel et al., 2016). The increased cortisol in the current study could be due to a few factors, including age, season, and ambient temperature. The current study was conducted in February 2016, and a previous study reported that salivary cortisol concentrations were higher during the winter months compared to the spring and summer months (Aurich et al., 2015). This same study did not find any significant differences between young horses and adult horses; however, Ruis and coworkers (1997) showed that young pigs (< 16 weeks of age) had higher salivary cortisol concentrations compared to adult pigs. Another potential reason for the high salivary cortisol concentrations observed in this study, is the anticipation of the meal due to
horses having been acclimated to the daily feeding routine. Widmann (2010) reported that serum cortisol concentrations were increased 30 minutes prior to receiving a morning meal, which could indicate anticipation of a meal or be due to the time of day the sample was taken.

During Phase 1 of the current study, salivary cortisol concentrations were higher when yearlings were exposed to the 0 vs. 50 ppm treatment compared to the 0 vs. 25 and 25 vs. 50 ppm treatment. There was a similar trend in Phase 2, where salivary cortisol concentrations tended to be higher when yearlings were exposed to 50 ppm NH$_3$ compared to 0 ppm. Similar work has been conducted in pigs with different outcomes (Gustin et al., 1994; von Borell et al., 2007b; O’Connor et al., 2010). Von Borrell and coworkers (2007b) reported that pigs exposed to NH$_3$ (35 ppm and 50 ppm) had higher serum cortisol concentrations than compared to pigs exposed to 0 ppm NH$_3$. However, Gustin et al. (1994) showed no serum cortisol response to exposure of 25 to 100 ppm NH$_3$ over a 6-d period. Interestingly, another study showed that pigs had significantly lower salivary cortisol concentrations when exposed to 20 ppm NH$_3$ compared to pigs exposed to minimal amounts of atmospheric NH$_3$ (O’Connor et al., 2010). Activation of the HPA axis is associated with a generalized response to perceived stressors (Selye 1936; von Borell, 2000), and the interpretation of such measures requires caution (O’Connor et al., 2010). However, the evaluation of the HPA axis, most commonly by measurement of its end-product glucocorticoids, has traditionally been the primary means to make inferences about the stress response in animals. It has been shown that an increase in ANS and HPA activity may simply reflect physiological arousal that does not necessarily denote undesirable experience (Otovic
and Hutchinson, 2014). Therefore, analyzing glucocorticoid concentrations could be providing a partial picture of an animal’s wellbeing and it is important to include other measures of welfare, such as behavior.

In conclusion, the current study did not conclusively show that yearlings were aversive to NH$_3$ concentrations compared to NH$_3$ free air. However, the increased LF: HF ratio and salivary cortisol concentration when yearlings were exposed to 50 ppm NH$_3$ may indicate an increased physiological response to a negative stimulus. The development of the head box system shows promise as a tool to investigate the effects of air quality on horse behavior and physiology. Further research should focus on the horse’s response to chronic exposure of NH$_3$ and look to other potential markers of increased physiological response in addition to HR, HRV, and salivary cortisol.
Table 4-1. Feeding behavior of horses exposed to head box system during initial and washout days.

<table>
<thead>
<tr>
<th></th>
<th>Initial Day</th>
<th>Washout 1</th>
<th>Washout 2</th>
<th>Washout 3</th>
<th>SEM</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed consumed B1 (kg)</td>
<td>0.2</td>
<td>0.07</td>
<td>0.14</td>
<td>0.05</td>
<td>0.07</td>
<td>0.3469</td>
</tr>
<tr>
<td>Feed consumed B2 (kg)</td>
<td>0.71</td>
<td>0.77</td>
<td>0.73</td>
<td>0.83</td>
<td>0.08</td>
<td>0.5737</td>
</tr>
<tr>
<td>Time spent feeding B1 (s)</td>
<td>41.78</td>
<td>21.89</td>
<td>69</td>
<td>17.28</td>
<td>26.28</td>
<td>0.4535</td>
</tr>
<tr>
<td>Time spent feeding B2 (s)</td>
<td>212.39</td>
<td>255.06</td>
<td>197.67</td>
<td>273.28</td>
<td>38.61</td>
<td>0.3791</td>
</tr>
<tr>
<td>Total feed consumed* (kg)</td>
<td>0.91</td>
<td>0.84</td>
<td>0.87</td>
<td>0.87</td>
<td>0.05</td>
<td>0.2514</td>
</tr>
<tr>
<td>Time spent feeding*, % of test time</td>
<td>42.36</td>
<td>45.56</td>
<td>43.66</td>
<td>48.43</td>
<td>7.04</td>
<td>0.6934</td>
</tr>
</tbody>
</table>

*Both boxes combined
Table 4-2. Feeding behavior of horses exposed to NH$_3$ using a head box system during Phase 1. Horses had the choice to feed from two boxes with different concentrations of NH$_3$: 0 vs 25 ppm, 0 vs. 50 ppm or 25 vs. 50 ppm.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 vs. 25</th>
<th>0 vs. 50</th>
<th>25 vs. 50</th>
<th>SEM</th>
<th>Trt</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed consumed B1 (kg)</td>
<td>0.25$^{x,y}$</td>
<td>0.17$^x$</td>
<td>0.028$^y$</td>
<td>0.083</td>
<td>0.0814</td>
<td>0.3308</td>
</tr>
<tr>
<td>Feed consumed B2 (kg)</td>
<td>0.65</td>
<td>0.72</td>
<td>0.84</td>
<td>0.083</td>
<td>0.1375</td>
<td>0.573</td>
</tr>
<tr>
<td>Time spent feeding B1 (s)</td>
<td>75.56</td>
<td>71.39</td>
<td>12.89</td>
<td>32.92</td>
<td>0.3104</td>
<td>0.2475</td>
</tr>
<tr>
<td>Time spent feeding B2 (s)</td>
<td>236.56$^b$</td>
<td>221.33$^b$</td>
<td>328.5$^a$</td>
<td>42.99</td>
<td>0.0116</td>
<td>0.0449</td>
</tr>
<tr>
<td>Total feed consumed (kg)</td>
<td>0.89</td>
<td>0.90</td>
<td>0.87</td>
<td>0.03</td>
<td>0.4629</td>
<td>0.0132</td>
</tr>
<tr>
<td>Time spent feeding, % of test time</td>
<td>54.09</td>
<td>52.95</td>
<td>49.82</td>
<td>4.79</td>
<td>0.7485</td>
<td>0.5035</td>
</tr>
</tbody>
</table>

$^{a,b,c}$ Means within a row differ ($P < 0.05$).

$^{x,y}$ Means within a row tended to differ ($P < 0.1$).
Table 4-3. HR and HRV in horses exposed to NH₃ using a head box system during Phase 1. Horses had the choice to feed from two boxes with different concentrations of NH₃: 0 vs 25 ppm, 0 vs. 50 ppm or 25 vs. 50 ppm.

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>SEM</th>
<th>Trt</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean RR (ms)</td>
<td>0 vs. 25</td>
<td>1021.74</td>
<td>0.5005</td>
<td>0.2659</td>
</tr>
<tr>
<td></td>
<td>0 vs. 50</td>
<td>1017.11</td>
<td>0.2659</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 vs. 50</td>
<td>45.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDRR (ms)</td>
<td>127</td>
<td>124.76</td>
<td>0.2447</td>
<td>0.6596</td>
</tr>
<tr>
<td></td>
<td>110.51</td>
<td>11.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>72.88</td>
<td>59.58</td>
<td>0.4814</td>
<td>0.2076</td>
</tr>
<tr>
<td></td>
<td>62.47</td>
<td>4.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>28.58</td>
<td>32.62</td>
<td>0.5043</td>
<td>0.9635</td>
</tr>
<tr>
<td></td>
<td>30.71</td>
<td>3.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LF/HF (ms)</td>
<td>6.3</td>
<td>5.43</td>
<td>0.4493</td>
<td>0.3095</td>
</tr>
<tr>
<td></td>
<td>5.02</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4-4. Feeding behavior of horses exposed to NH$_3$ using a head box system during Phase 2. Horses had the choice to feed from two boxes with the same concentration of NH$_3$: 0 ppm, 25 ppm, or 50 ppm.

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>$P$-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 ppm</td>
<td>25 ppm</td>
</tr>
<tr>
<td>Feed consumed B1* (kg)</td>
<td>0.048</td>
<td>0</td>
</tr>
<tr>
<td>Feed consumed B2* (kg)</td>
<td>0.8383</td>
<td>0.8144</td>
</tr>
<tr>
<td>Time spent feeding B1 (s)</td>
<td>17.28</td>
<td>0</td>
</tr>
<tr>
<td>Time spent feeding B2 (s)</td>
<td>273.28</td>
<td>330.33</td>
</tr>
<tr>
<td>Total time spent feeding (s)</td>
<td>290.56</td>
<td>330.30</td>
</tr>
<tr>
<td>Total feed consumed (kg)</td>
<td>0.8706</td>
<td>0.8122</td>
</tr>
</tbody>
</table>

*B1 represents head box 1 (located on the horse’s left) and B2 represents head 2 (located on the horse’s right).
Table 4-5. HR and HRV in horses exposed to NH$_3$ using a head box system during Phase 2. Horses had the choice to feed from two boxes with the same concentration of NH$_3$: 0 ppm, 25 ppm, or 50 ppm.

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 ppm</td>
<td>25 ppm</td>
</tr>
<tr>
<td>Mean RR (ms)</td>
<td>1003.56</td>
<td>1042</td>
</tr>
<tr>
<td>SDRR (ms)</td>
<td>148.34</td>
<td>114.44</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>62.65</td>
<td>62.34</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>31.86</td>
<td>33.56</td>
</tr>
<tr>
<td>LF/HF (ms)</td>
<td>4.43$^{a,b}$</td>
<td>3.44$^{b}$</td>
</tr>
</tbody>
</table>

$^{a,b}$ Means within a row differ ($P < 0.05$).
Figure 4-1. Diagram of head box system. A) view of both head boxes, as the horse would see the system upon approach. B) diagram showing different components to the head box. Courtesy of Hong Li.

Figure 4-2. Schematic of the air supply control and data acquisition system. Courtesy of Hong Li.
Figure 4-3. Experimental timeline. Phase 1 consisted of horses choosing between two different NH₃ concentrations at each treatment level: 0 vs. 25 ppm, 0 vs. 50 ppm, or 25 vs. 50 ppm. Phase 2 consisted of horses only having an option of one level of NH₃ concentration per treatment: 0 ppm (Washout 3), 25 ppm, or 50 ppm.
Figure 4-4. Salivary cortisol concentration of horses before and after exposure to head boxes sans-ammonia: A) AM and B) PM. Overall effect of day ($P = 0.00005$), time of day ($P < .0001$), and time of sample ($P = 0.0374$).
Figure 4-5. Time spent feeding in horses exposed to NH$_3$ using a head box system during Phase 1. Horses had the choice to feed from two boxes with different concentrations of NH$_3$: 0 vs 25 ppm, 0 vs. 50 ppm or 25 vs. 50 ppm. Overall effect of treatment ($P = 0.4908$), and day ($P = 0.2288$).

Figure 4-6. Percent of feed consumed by horses exposed to NH$_3$ using a head box system during Phase 1. Horses had the choice to feed out of two boxes with different concentrations of NH$_3$: 0 vs 25 ppm, 0 vs. 50 ppm or 25 vs. 50 ppm. Overall effect of treatment ($P = 0.4629$), and day ($P = 0.0132$).
Figure 4-7. Proportion of feeding behavior performed from box containing the lower NH$_3$ concentration (L: T) during Phase 1. Horses had the choice to feed out of two boxes with different concentrations of NH$_3$: 0 vs 25 ppm, 0 vs. 50 ppm or 25 vs. 50 ppm. Feed consumed L: T overall effect of treatment ($P = 0.1413$), and day ($P = 0.4804$). Time spent feeding L: T overall effect of treatment ($P = 0.1716$), and day ($P = 0.4256$).
Figure 4-8. Salivary cortisol concentration of horses before and after exposure to NH₃ using a head box system during Phase 1 in the A) AM and B) PM. Horses had the choice to feed out of two boxed with different concentrations of NH₃: 0 vs 25 ppm, 0 vs. 50 ppm or 25 vs. 50 ppm. Overall effect of treatment ($P = 0.028$), day ($P = 0.038$), time of day ($P < .0001$), and time of sample ($P = 0.9542$).
Figure 4-9. Percent of time spent feeding and feed offered that was consumed by horses exposed to NH$_3$ using a head box system during Phase 2. Horses had the choice to feed out of two boxes with the same concentration of NH$_3$: 0 ppm, 25 ppm, or 50 ppm. Percent of time spent feeding overall effect of treatment ($P = 0.4882$), and day ($P = 0.0296$). Percent of feed consumed overall effect of treatment ($P = 0.1782$), and day ($P = 0.7504$).
Figure 4-10. Salivary cortisol concentration of horses before and after exposure to NH$_3$ using a head box system during Phase 2 in the A) AM and B) PM. Horses had the choice to feed out of two boxes with the same concentration of NH$_3$: 0 ppm, 25 ppm, or 50 ppm. Overall effect of treatment ($P = 0.077$), day ($P = 0.6948$), time of day ($P = 0.0022$), and time of sample ($P = 0.0137$).
CONCLUDING REMARKS AND FUTURE DIRECTIONS

Overall Conclusions

For the equine industry, concerns about ammonia (NH₃) levels in the barn environment are multifaceted and include issues of animal welfare, animal and human health, and environmental impacts. Ammonia (NH₃) volatilization occurs when excess crude protein (CP) is fed and excreted as urinary nitrogen. Information regarding NH₃ emission from equine facilities is limited, and effects of dietary CP intake (CPI) on NH₃ emission have not been investigated. In addition, NH₃ concentrations are known to have a negative impact on horse health, however, the impact of NH₃ on horse behavior and welfare is not well understood. The work in this dissertation has started to address these areas of concern in order to better understand the impact of the ammonia emitted from equine operations on the environment and horse welfare.

Chapter 2 served as a first step in determining if there is a relationship between dietary CP intake and potential NH₃ losses from feces and urine of horses. The dietary CP intake was significantly different between all diets, however represented a narrow range of daily CPI. It was important to keep the protein quality the same and only change the quantity in this project. In order to do that, two warm-season grass hays were used and their CP concentration ended up being more alike than anticipated. Although dietary CPI was within a narrow range, urinary TN and urea-N excretion increased as dietary CPI increased. This is an important finding because these portions of N excretion are the most susceptible to NH₃ loss. Using an in vitro system to estimate the NH₃ emissions, there was a trend for cumulative ER to increase as dietary CPI increased. Also, this study added to the body of literature reporting that NH₃ emissions
are higher from manure mixed with wheat straw compared to wood shavings. Lastly, NH$_3$ emissions started to increase on d 4 of 7 in the *in vitro* system, thus potentially highlighting the beginning of the hydrolysis of urea.

Chapter 3 extended on chapter 2 by estimating NH$_3$ emissions under field settings. This study took place on 4 working equine operations in the Mid-Atlantic region. Management practices, including feeding practices, barn dimensions, ambient temperature, and barn CO$_2$ concentrations were recorded. Using a flux chamber system, the NH$_3$ concentrations from 5 different sampling points in 4 – 6 stalls in each facility were measured. This study confirmed that in horses, like other large livestock species, there is a strong correlation between overfeeding CP and increased NH$_3$ emissions. The estimated daily NH$_3$ ranged from 20 – 124 g d$^{-1}$ horse$^{-1}$, which agrees with previous research conducted in cattle. This study provides a better understanding of the impact equine operations are having on atmospheric NH$_3$ concentrations.

In addition to understanding the impact equine operations have on the environment, the role NH$_3$ plays in the barn environment on welfare and health is vital. The adverse effects of NH$_3$ exposure on animal health and productivity has been identified in other species and somewhat in horses, however the impact on behavior and welfare is not as well understood. In an attempt to determine the aversion of horses to different NH$_3$ concentrations, a novel head box system was developed. In a test, horses were exposed to 0, 25, or 50 ppm NH$_3$. While feeding behavior was not affected by higher NH$_3$ concentrations, there was an increase in salivary cortisol and LF: HF ratio (HRV) when exposed to 50 ppm NH$_3$. These increases could potentially indicate an increased physiological response to a negative stimulus. The development of this head
box does show promise as a tool to investigate the effects of air quality on horse behavior and physiology.

**Future Perspectives**

Due to the complexities of quantifying NH$_3$ emissions on equine operations, there are a great many directions this area of research can take.

An important next step would be to more directly compare the *in vitro* method used in chapter 2 to the flux chamber system used in the field to see if estimation of NH$_3$ would be similar. Additionally, determining if the relationship between dietary CPI and NH$_3$ emissions ever levels off (NH$_3$ emissions do not increase when dietary CPI increases). Also investigating different dietary interventions, such as feeding zeolite (binds NH$_4^+$), as a means to reduce emissions.

In this work, we looked at quantifying and characterizing NH$_3$ at the stall level, and the next logical step would be to quantify emissions on a whole farm level. This would require determining how much NH$_3$ is lost from the barn, manure storage, and from the pastures. Doing this would add to the current knowledge and give researchers more tools for estimating better NH$_3$ emissions from equine facilities. It is also critical to determine the NH$_3$ flux rates (daily and seasonally) and determine what factors influence these rates.

Due to the difficulty and expense in measuring gaseous emissions from farms, a process based modeling approach has been recommended for quantifying emissions of NH$_3$ in other species. Process based modeling is a procedure in which system processes are mathematically represented at an appropriate level of detail to capture important dynamics and interactions among components (Hristov et al., 2011). This approach is more responsive in predicting the effects of management changes.
compared to more simplistic models. Addressing these challenges, developing effective mitigation techniques and disseminating this information to the equine industry are all important next steps towards reducing the impact of equine operations on impaired air quality.

In regards to the impact of NH$_3$ concentrations on equine welfare, the first important step would be to determine if welfare is compromised when there is long term exposure to NH$_3$ concentrations. Also, short term exposure to higher NH$_3$ concentrations (> 50 ppm) may induce some changes in feeding behavior. To assess if there is an increased physiological response to high NH$_3$ concentrations, measurement of other acute stress biomarkers, such as alpha-amylase and acute phase proteins, would provide a more detailed assessment of the horse’s response.
LIST OF REFERENCES


Stucke, D., M. Große Ruse, and D. Lebelt. 2015. Measuring heart rate variability in


BIOGRAPHICAL SKETCH

Jessie Weir was born in St. Louis, Mo. When she was thirteen years old, she moved to Vero Beach, FL. She attended high school at Vero Beach High School where she was a member of the math and brain bowl team. Jessie was always passionate about horses, and after graduating in 2006 chose to attend the University of Florida to pursue a degree in animal science with a specialization in equine industry.

While attending UF, Jessie particularly enjoyed her animal nutrition classes, especially Equine Nutrition with Dr. Lori Warren. She began her Master of Science program, with Dr. Warren, in the fall of 2010. Jessie’s research focused on the effects of dietary phosphorus on environmentally relevant phosphorus excretion in horses. This is when Jessie developed an interest in studying the impact of equine operations on the environment.

In the fall of 2012, Jessie started her PhD at the University of Delaware under the guidance of Dr. Carissa Wickens and Dr. Hong Li. There she was afforded many different research opportunities that helped lead to her main research projects. In the spring of 2014, Dr. Wickens accepted a position at the University of Florida, so Jessie returned to her alma-mater. After completing her studies in December 2016, Jessie plans to continue her work with nutrient management and nutrition.

Jessie is an avid horse lover who has an intense passion for three-day eventing and polo. Her work in polo has afforded many wonderful opportunities, including spending a summer in Calgary, AB working with high goal polo ponies. Jessie is getting married to Christopher Chouinard on September 9th, 2017, and together they enjoy travelling, watching football, and spending time with their dog, Harvin.