CHEMICAL TREATMENT OF FORAGES WITH CALCIUM OXIDE IN BEEF CATTLE PRODUCTION

By

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To my beloved husband, Darren, and our daughter, Marianna
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# TABLE OF CONTENTS

acknowledgements ........................................................................................................ 4

list of tables .................................................................................................................. 10

list of figures .................................................................................................................. 12

list of abbreviations ....................................................................................................... 13

abstract .......................................................................................................................... 16

Chapter

1  review of the literature ................................................................................................. 18

Differentiation between C_3 and C_4 Grasses ................................................................. 18
Anatomy Comparison of C_3 and C_4 Grasses ................................................................. 19
Plant Cell Wall .................................................................................................................. 22
    Cell Wall Polysaccharides ......................................................................................... 24
    Lignin ......................................................................................................................... 25
Chemical Treatment of Fiber .......................................................................................... 29
    History ....................................................................................................................... 29
    Methods of Chemical Treatment ............................................................................. 30
    Hydrolytic Treatment and Chemicals ..................................................................... 31
    Calcium Oxide ......................................................................................................... 35
Final Remarks ................................................................................................................ 38

2  Ruminal In Situ Degradability and In Vitro Organic Matter
    Digestibility of Roughages Treated with Calcium Oxide .................................... 40

Introduction .................................................................................................................... 40
Materials and Methods ................................................................................................ 41
    Experiment 1 Design and Treatments .................................................................... 41
    Experiment 2 Design and Treatments .................................................................... 42
Ruminal In Situ Degradability ..................................................................................... 43
Laboratory Analyses .................................................................................................... 44
    Chemical composition of roughages ..................................................................... 44
    In vitro OM digestibility ......................................................................................... 44
Statistical Analysis ....................................................................................................... 45
Results and Discussion ................................................................................................. 46
    Experiment 1 .......................................................................................................... 46
    Experiment 2 .......................................................................................................... 49

3  Intake, Ruminal Fermentation Parameters, and Apparent
    Total-Tract Digestibility of Beef Steers Consuming
    Bahiagrass Hay Treated with Calcium Oxide ....................................................... 61
Introduction .................................................................................................................. 61
Materials and Methods ............................................................................................... 62
   Experimental Design, Animals, and Treatments ...................................................... 62
   Treatment of Bahiagrass Hay ............................................................................... 63
   Sampling Procedures .............................................................................................. 63
       Ruminal fluid, pH, and blood sampling .............................................................. 63
       Apparent total tract digestibility .................................................................... 64
Laboratory Analyses ................................................................................................... 65
   Volatile fatty acid profile ...................................................................................... 65
   Ammonia-N and blood urea N concentrations ....................................................... 65
   Concentration of DM, OM, CP, NDF, and ADF .................................................... 66
   Concentration of iNDF ............................................................................................ 67
   In vitro OM digestibility .......................................................................................... 67
Calculations ................................................................................................................ 68
Statistical Analysis ...................................................................................................... 68
Results and Discussion ............................................................................................... 69

4 PERFORMANCE OF GROWING BEEF CATTLE CONSUMING BAHIAGRASS
HAY TREATED WITH CALCIUM OXIDE AND MOLASSES .................................. 83
   Introduction ........................................................................................................... 83
   Materials and Methods .......................................................................................... 84
       Experiment 1 Design, Animals, and Treatments .................................................. 84
       Experiment 2 Design, Animals, and Treatments .................................................. 85
       Treatment of Bahiagrass Hay .......................................................................... 86
       Sampling Procedures ......................................................................................... 87
           Hay samples .................................................................................................... 87
           Body weight and blood samples ................................................................... 87
   Laboratory Analyses ............................................................................................... 87
       Chemical composition of hay and cottonseed meal ........................................... 87
       In vitro OM digestibility .................................................................................... 88
   Statistical Analysis ................................................................................................. 88
Results and Discussion ............................................................................................... 89

5 ENTERIC METHANE PRODUCTION AND APPARENT TOTAL TRACT
DIGESTIBILITY OF BEEF STEERS CONSUMING BAHIAGRASS HAY
TREATED WITH CALCIUM OXIDE ....................................................................... 101
   Introduction ........................................................................................................... 101
   Materials and Methods .......................................................................................... 102
       Experimental Design, Animals, and Treatments ................................................ 102
       Bahiagrass Hay Treatment .............................................................................. 103
       Sampling Procedures ......................................................................................... 103
           Apparent total tract digestibility of nutrients ................................................. 103
           Methane emissions ......................................................................................... 104
   Laboratory Analyses ............................................................................................... 105
       Concentration of DM, OM, CP, NDF, ADF ....................................................... 105
Concentration of iNDF ................................................................. 105
Methane and SF₆ analyses .......................................................... 106
Calculations ................................................................................. 106
Statistical Analysis ....................................................................... 107
Results and Discussion ............................................................... 107

6 SUMMARY AND RECOMMENDATIONS .................................... 115

Summary ......................................................................................... 115
Recommendations ........................................................................... 121

LIST OF REFERENCES .................................................................... 123

BIOGRAPHICAL SKETCH ............................................................. 134
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Nutrient composition of peanut hulls (PHS) after treatment with calcium oxide (CaO) and incubation for either 7 or 14 days.</td>
<td>54</td>
</tr>
<tr>
<td>2-2</td>
<td>Ruminal in situ nutrient degradability of peanut hulls (PHS) treated or not with calcium oxide (CaO) and incubated for either 7 or 14 days.</td>
<td>55</td>
</tr>
<tr>
<td>2-3</td>
<td>Nutrient composition of bahiagrass (<em>Paspalum notatum</em>) hay (BH) after treatment with calcium oxide (CaO) and incubation for either 7 or 14 days.</td>
<td>56</td>
</tr>
<tr>
<td>2-4</td>
<td>Ruminal in situ nutrient degradability and in vitro organic matter digestibility (IVOMD) of bahiagrass (<em>Paspalum notatum</em>) hay (BH) treated or not with calcium oxide (CaO) and incubated for either 7 or 14 days.</td>
<td>57</td>
</tr>
<tr>
<td>2-5</td>
<td>Nutrient composition of Tifton 85 bermudagrass (<em>Cynodon spp.</em>) hay (BM) after treatment with calcium oxide (CaO) and incubation for either 7 or 14 days.</td>
<td>58</td>
</tr>
<tr>
<td>2-6</td>
<td>Ruminal in situ nutrient degradability and in vitro organic matter digestibility (IVOMD) of Tifton 85 bermudagrass (<em>Cynodon spp.</em>) hay (BM) treated or not with calcium oxide (CaO) and incubated for either 7 or 14 days.</td>
<td>59</td>
</tr>
<tr>
<td>3-1</td>
<td>Analyzed(^1) chemical composition of bahiagrass (<em>Paspalum notatum</em>) hay provided to beef steers.</td>
<td>74</td>
</tr>
<tr>
<td>3-2</td>
<td>Effects of bahiagrass (<em>Paspalum notatum</em>) hay treated or not with calcium oxide (CaO) on total dry matter intake (DMI), daily average ruminal fermentation parameters, and blood urea nitrogen (BUN) concentrations of beef steers provided ad libitum intake.</td>
<td>75</td>
</tr>
<tr>
<td>3-3</td>
<td>Effects of bahiagrass (<em>Paspalum notatum</em>) hay treated or not with calcium oxide (CaO) on ruminal VFA molar proportions of beef steers provided ad libitum intake.</td>
<td>76</td>
</tr>
<tr>
<td>3-4</td>
<td>Effects of bahiagrass (<em>Paspalum notatum</em>) hay treated or not with calcium oxide (CaO) on ruminal VFA concentrations of beef steers provided ad libitum intake.</td>
<td>77</td>
</tr>
<tr>
<td>3-5</td>
<td>Effects of bahiagrass (<em>Paspalum notatum</em>) hay treated or not with calcium oxide (CaO) on daily nutrient intake and apparent total tract digestibility of beef steers provided ad libitum intake and in vitro organic matter digestibility (IVOMD).</td>
<td>78</td>
</tr>
</tbody>
</table>
4-1 Analyzed\(^1\) chemical composition of bahiagrass (\textit{Paspalum notatum}) hay treated or not with molasses and calcium oxide (CaO) fed to growing beef cattle (Exp. 1). .......................................................... 94

4-2 Effects of bahiagrass (\textit{Paspalum notatum}) hay treated or not with molasses and calcium oxide (CaO) on performance of growing beef cattle (Exp. 1)............ 95

4-3 Analyzed\(^1\) chemical composition of bahiagrass (\textit{Paspalum notatum}) hay treated or not with molasses plus calcium oxide (CaO) and cottonseed meal (CSM) fed to growing beef cattle (Exp. 2). .......................................................... 96

4-4 Effects of bahiagrass (\textit{Paspalum notatum}) hay treated or not with molasses and calcium oxide (CaO) on performance of growing beef cattle supplemented with cottonseed meal\(^1\) (Exp. 2) .......................................................... 97

4-5 Effects of bahiagrass (\textit{Paspalum notatum}) hay treated or not with molasses and calcium oxide (CaO) on blood urea nitrogen (BUN) concentrations of growing beef cattle (Exp. 1 and Exp. 2)\(^1\) .................................................. 98

5-1 Analyzed chemical composition of bahiagrass (\textit{Paspalum notatum}) hay treated or not with calcium oxide (CaO), cottonseed meal (CSM), and sugar cane molasses provided to beef steers\(^1\) .......................................................... 111

5-2 Effects of bahiagrass (\textit{Paspalum notatum}) hay treated or not with calcium oxide (CaO) on performance of beef steers provided ad libitum hay intake plus supplementation with cottonseed meal (CSM) and molasses\(^1\). ............... 112

5-3 Effects of bahiagrass (\textit{Paspalum notatum}) hay treated or not with calcium oxide (CaO) on daily nutrient intake and apparent total tract digestibility of beef steers provided ad libitum hay intake plus supplementation with cottonseed meal (CSM) and molasses\(^1\) .......................................................... 113

5-4 Effects of bahiagrass (\textit{Paspalum notatum}) hay treated or not with calcium oxide (CaO) on enteric CH\(_4\) production of beef steers provided ad libitum hay intake plus supplementation with cottonseed meal (CSM) and molasses\(^1\) ...... 114
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>In vitro organic matter digestibility (IVOMD) of peanut hulls (PHS) treated or not with 5% calcium oxide (CaO), different DM contents (70 or 50%), and incubated in buckets for 3, 6, 9, 12, or 15 days.</td>
<td>60</td>
</tr>
<tr>
<td>3-1</td>
<td>Effects of bahiagrass (<em>Paspalum notatum</em>) hay treated or not with calcium oxide (CaO) on blood urea nitrogen (BUN) concentration of beef steers provided ad libitum intake.</td>
<td>79</td>
</tr>
<tr>
<td>3-2</td>
<td>Effects of bahiagrass (<em>Paspalum notatum</em>) hay treated or not with calcium oxide (CaO) on molar proportions of acetate of beef steers provided ad libitum intake.</td>
<td>80</td>
</tr>
<tr>
<td>3-3</td>
<td>Effects of bahiagrass (<em>Paspalum notatum</em>) hay treated or not with calcium oxide (CaO) on molar proportions of propionate of beef steers provided ad libitum intake.</td>
<td>81</td>
</tr>
<tr>
<td>3-4</td>
<td>Effects of bahiagrass (<em>Paspalum notatum</em>) hay treated or not with calcium oxide (CaO) on acetate to propionate ratio (A:P) of beef steers provided ad libitum intake.</td>
<td>82</td>
</tr>
<tr>
<td>4-1</td>
<td>Effects of bahiagrass (<em>Paspalum notatum</em>) hay treated or not with calcium oxide (CaO) on blood urea nitrogen (BUN) concentration of beef cattle provided ad libitum intake.</td>
<td>99</td>
</tr>
<tr>
<td>4-2</td>
<td>In vitro organic matter digestibility (IVOMD) of bahiagrass (<em>Paspalum notatum</em>) hay provided to beef cattle in Exp. 1.</td>
<td>100</td>
</tr>
</tbody>
</table>
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>50DM</td>
<td>PHS treated with water (to 50% DM)</td>
</tr>
<tr>
<td>50DMCO</td>
<td>PHS treated with 5% CaO (DM basis) + water (to 50% DM)</td>
</tr>
<tr>
<td>70DM</td>
<td>PHS treated with water (to 70% DM)</td>
</tr>
<tr>
<td>70DMCO</td>
<td>PHS treated with 5% CaO (DM basis) + water (to 70% DM)</td>
</tr>
<tr>
<td>A:P</td>
<td>Acetate to propionate ratio</td>
</tr>
<tr>
<td>ADF</td>
<td>Acid detergent fiber</td>
</tr>
<tr>
<td>ADFD</td>
<td>Ruminal in situ ADF degradability</td>
</tr>
<tr>
<td>ADG</td>
<td>Average daily gain</td>
</tr>
<tr>
<td>ATTD</td>
<td>Apparent total tract digestibility</td>
</tr>
<tr>
<td>BCVFA</td>
<td>Branched-chain VFA</td>
</tr>
<tr>
<td>BH</td>
<td>bahiagrass hay</td>
</tr>
<tr>
<td>BM</td>
<td>Tifton-85 bermudagrass hay</td>
</tr>
<tr>
<td>BUN</td>
<td>Blood urea nitrogen</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>CAO</td>
<td>BH treated with 5% CaO (DM basis) + 10% molasses (DM basis) + water (to 35% DM)</td>
</tr>
<tr>
<td>CC</td>
<td>BH treated with 8.9% CaCO3 (DM basis) + water (to 50% DM)</td>
</tr>
<tr>
<td>CO</td>
<td>BH treated with 5% CaO (DM basis) + water (to 50% DM)</td>
</tr>
<tr>
<td>CO10</td>
<td>BM or BH treated with 10% CaO (DM basis) + water (to 50% DM) and incubated in buckets for 14 d</td>
</tr>
<tr>
<td>CO14</td>
<td>PHS treated with 5% CaO (DM basis) + water (to 50% DM) and incubated in buckets for 7 d</td>
</tr>
<tr>
<td>CO5-14</td>
<td>BM or BH treated with 5% CaO (DM basis) + water (to 50% DM) and incubated in buckets for 14 d</td>
</tr>
<tr>
<td>CO5-7</td>
<td>BM or BH treated with 5% CaO (DM basis) + water (to 50% DM) and incubated in buckets for 7 d</td>
</tr>
</tbody>
</table>
CO7  PHS treated with 5% CaO (DM basis) + water (to 50%DM) and incubated in buckets for 7 d
CP    Crude protein
CPD   Ruminal in situ CP degradability
CSM   Cottonseed meal
D     Dry PHS (as is)
D     Day
DH    Dry BM or BH (as is)
DM    Dry matter
DMD   Ruminal in situ DM degradability
FEF   Feed Efficiency Facility
G:F   Gain to feed ratio
GY    Guaiacyl
h     Hour
HY    Hydroxyphenyl
iNDF  Indigestible NDF
IVDMD In vitro dry matter digestibility
IVOMD In vitro organic matter digestibility
MBW   Metabolic BW
MOL   BH treated with 10% molasses (DM basis) + water (to 35%DM)
NAD-ME Nicotinamide adenine dinucleotide malic enzyme
NADP-ME Nicotinamide adenine dinucleotide phosphate malic enzyme
NDF   Neutral detergent fiber
NDFD  Ruminal in situ NDF degradability
OM    Organic matter
OMD  Ruminal in situ OM degradability
OMDG  OM digested
OMI  OM intake
PCK  Phophoenolpyruvate carboxykinase
PHS  Peanut hulls
PVC  Polyvinyl chloride
RISD  Ruminal in situ degradability
SD  Standard deviation
SEM  Standard error of the mean
SY  Syringyl
TDN  Total digestible nutrients
TRT  Fixed effect of treatment
UF-NFREC BU  University of Florida North Florida Research and Education Center Beef Unit
UF-NFREC FEF  University of Florida North Florida Research and Education Center Feed Efficiency Facility
VFA  Volatile fatty acids
W14  PHS, BH, or BM treated with water (to 50%DM) and incubated in buckets for 14 d
W7  PHS, BH, or BM treated with water (to 50%DM) and incubated in buckets for 7 d
CHEMICAL TREATMENT OF FORAGES WITH CALCIUM OXIDE IN BEEF CATTLE PRODUCTION

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Major: Animal Sciences

With the ever-growing desire to increase efficiency in beef cattle production, researchers have developed strategies, such as treating low-quality forages and low value roughages with chemicals, to increase the digestibility of fiber fractions, consequently increasing their energy value for cattle feeding. Calcium oxide has been proposed as a replacement to chemicals used in the past (e.g. NaOH) and data indicate that it can promote similar and effective outcomes. The primary objective of this dissertation was to test the hypothesis that the alkali CaO would improve digestibility of low-quality forages and roughages commonly found in Florida by increasing the digestibility of fiber fractions. A series of experiments was designed to: 1) evaluate ruminal in situ degradability of nutrients (RISD) and in vitro organic matter digestibility (IVOMD) of peanut hulls (PHS), bermudagrass hay (BM), and bahiagrass hay (BH) treated with CaO and 2) test the effects of bahiagrass hay treated with CaO on ruminal fermentation parameters, apparent total tract digestibility (ATTD) of nutrients, performance, and enteric CH₄ production of beef cattle. Ruminal in situ degradability of nutrients and IVOMD of PHS were not affected by CaO treatment ($P > 0.05$); however, RISD ($P < 0.001$) and IVOMD ($P < 0.001$) of BM and BH were increased with CaO.
treatment. When provided to cattle ad libitum, bahiagrass hay treated with CaO increased ruminal pH \((P < 0.001)\), reduced total volatile fatty acid (VFA) concentration \((P = 0.021)\), and reduced ATTD of fiber \((P \leq 0.034)\). Bahiagrass hay treated with CaO neither increased final BW \((P \geq 0.283)\) nor average daily gain \((P \geq 0.103)\) nor did it reduce CH\(_4\) emissions \((P \geq 0.168)\). As a potential to increase digestibility of forages, data from the first experiment indicated that CaO can effectively increase RISD and IVOMD of warm-season grass hay. However, providing bahiagrass hay treated with CaO as the main dietary ingredient did not increase cattle performance. The reduction in total VFA concentration in the rumen and ATTD indicate that the alkali had a negative effect on ruminal fermentation and digestion of hay, potentially explaining the lack of increased cattle performance.
CHAPTER 1
REVIEW OF THE LITERATURE

Differentiation between C₃ and C₄ Grasses

In the southeastern US, the majority of beef cattle operations rely on forage-based diets because of the abundant forage production in this region (Ciriaco et al., 2015). However, and unfortunately, the predominant forages can be of limited nutritive value for several reasons, including greater concentration of fiber rather than more digestible components. Grasses are grouped into warm-season (C₄) and cool-season (C₃) types based on the photosynthetic pathways they perform and the anatomy of their leaves. For the purpose of this review and to address the production system in beef cattle operations located in the southeastern US, focus will be given to the warm-season grasses with references to the cool-season grasses for basic comparisons.

Warm-season grasses are species that, in the photosynthetic process, fix atmospheric CO₂ into four carbon intermediates, hence, the common term C₄ (Moore et al., 2004). High concentrations of CO₂ and low concentrations of O₂ are the conditions under which the C₃ grasses photosynthetic pathway evolved (Moore et al., 2004). Ribulose-1,5-bisphosphate carboxylase/oxygenase, commonly known as Rubisco, is the initial enzyme in the photosynthesis pathway and it can either carboxylate or oxygenate ribulose-1,5-bisphosphate. Oxygenation results in photorespiration, which can in turn significantly decrease the efficiency of photosynthesis. As atmospheric concentrations of CO₂ declined and concentrations of O₂ increased, the event of photorespiration increased leading to necessary adaptive changes (Cerling, 1999), thus the C₄ grasses evolved from C₃ grasses due to changes in temperature and atmospheric concentrations of CO₂ and O₂. The C₄ photosynthetic pathways are
supplementary to the C₃ pathway, and, therefore, all of the enzymes present in the C₄ pathway existed in their C₃ ancestors. The difference is that evolution required changes in regulation of these enzymes, their location, and consequently leaf anatomy (Monson, 1999).

**Anatomy Comparison of C₃ and C₄ Grasses**

Regardless of their type, it is important to briefly recognize the three tissues present in plants: dermal, ground, and vascular tissues. Each one is comprised of different cell types and proportion of cells (Evert, 2006). In regard to specific cell types, it is also important to address that, in general terms, there are two main classes of cells present in the ground tissue of leaves and stems of grasses.

Parenchyma cells in leaves are thin-walled (primary cell wall only), large, non-specialized cells, without the presence of chloroplasts and are only found in specific areas such as, in the midrib of grasses; however, in the leaf sheath or stem these cells are abundant and significant contributors to poor digestibility because they develop a thick secondary cell wall (1 µm) that can become lignified with maturity (Wilson, 1993).

Sclerenchyma cells, also known as fiber cells, are characterized for being long and narrow, are thicker-walled cells with the presence of a secondary cell wall (2 - 5 µm) and become highly lignified as the cell matures (Wilson, 1993). The cell walls of sclerenchyma cells can make up to 90% of their whole volume. Their presence is significant in grasses, where in the leaves and sheaths, sclerenchyma cells appear in patches on top and bottom of vascular bundles and at the leaf borders. In the stem they may build a ring of tissue around the stem right below the epidermal tissue and are also found close to vascular bundles in patches. Sclerenchyma are dead cells and their most important role is to provide mechanical strength by being part of the girder structure (“I”-
girder in grass leaves and segmented or complete compound girder in stems; Wilson, 1990).

In a more specific description of cell types that are found in leaves and stems of grasses, there are epidermal cells, which are the first layer of cells with only a primary cell wall (secondary wall thickness in C₄ grasses) in the leaves and stem of plants (Wilson, 1994). The outer side of the cell wall (outer side of the plant) is covered with a cuticle and layer or wax, which are indigestible, and varies in thickness and lignin deposition depending if they are in the stem or leaves and plant maturity.

Bundle sheath cells are the main type of cells that can differentiate C₃ and C₄ grasses because these cells present the most different characteristics between the two types of grasses. They are parenchymatous cells and are found in a single layer around the vascular tissue. In C₃ forages they do not contain chloroplasts, have a relatively thin non-lignified primary cell wall (0.18 µm), and are easily digested, similar to mesophyll cells in regards to digestibility (Wilson, 1990). In contrast, bundle sheath cells in C₄ grasses have the presence of chloroplasts and a secondary cell wall thickening (0.58 µm) with weak lignification, which does not prevent digestion but makes it incomplete and slower (Wilson, 1990). Briefly, there are three groups of C₄ grasses, phosphoenolpyruvate carboxykinase (PCK), nicotinamide adenine dinucleotide malic enzyme (NAD-ME), and NAD phosphate ME (NADP-ME), and the bundle sheath cells found in the first two have a structure called suberized lamella, which is totally indigestible by ruminal microorganisms. Thus, the thick wall plus suberized lamella makes the easily digestible contents (e.g. protein and starch) inside of bundle sheath cells inaccessible to microorganisms until rupture of the cell wall.
Mesophyll cells are parenchymatous cells that have the presence of chloroplasts and, for that, they can also be described as clorenchymatous cells. They have only a thin primary cell wall (0.1 – 0.2 µm) and account for most of the volume in the ground tissue of leaves; however, in stems they make up a very discrete volume (Wilson, 1990; Wilson and Mertens, 1995). The main difference in this type of cell between a C₃ grass and a C₄ grass is that in the former they are more loosely arranged in the ground tissue and have two different shapes, the palisade mesophyll (vertically elongated cell) and the spongy mesophyll (smaller, misshaped cell). In C₄ grasses, the mesophyll cells have a more consistent shape and are more organized in layers around the bundle sheath cells around the vascular bundle (Wilson and Mertens, 1995).

Several cell types are present in the vascular tissue. In grasses, vascular tissue contains the phloem and xylem cells and in two of the C₄ types of grasses mentioned previously (PKC and NAD-ME), they are surrounded by mestome sheath cells, while in the NADP-ME type of C₄ the mestome sheath cells has become the bundle sheath cells. Mestome sheath cells are thick-walled cells with the presence of suberized lamella, which makes them highly indigestible (Leegood, 2008).

In C₃ grass leaves (e.g. *Lolium multiflorum* L.), the elongated epidermal cells are straight-sided, parallel veins are further apart of each other, and epidermal tissue is barely attached to the leaf by mesophyll cells, while in warm-season C₄ grasses (e.g. *Cynodon dactylon* and *Paspalum notatum*), the epidermal cells in leaves have sinuous walls, the veins are closer, and the tissue is attached to the vascular bundle by thick-walled sclerenchyma cells (Wilson, 1993; Wilson, 1994; Dengler and Nelson, 1999).
Hence, considering leaf anatomy between $C_3$ and $C_4$ grasses, their difference is mainly due to types of cells present and arrangement of them in the leaf structure. Warm-season species have a leaf anatomy, called Kranz, that has the presence of two types of photosynthetic cells (mesophylls and bundle sheath cells), have a spatial configuration of these cells in a more organized way around the vascular tissue and because of that, the volume of intercellular space in $C_4$ grasses is less than in $C_3$, which provide minimal to no exposure of bundle sheath cells to intercellular air space and microbial attachment (Dengler and Nelson, 1999). Furthermore, bundle sheath cells are larger and have large and abundant chloroplasts, when compared with $C_3$ species. In leaf cross-sectional areas of different species of $C_4$ grasses, 12 to 33% was comprised of bundle sheath cells (Wilson and Hattersley, 1989; Wilson, 1990; Wilson, 1993), whereas in different species of $C_3$ grasses, bundle sheath cells made up of only 4 to 9% (Akin and Burdick, 1975; Akin, 1989). The mesophyll cells in $C_4$ are not much different from $C_3$, but are usually larger and their arrangement allows almost every cell to be in contact with the bundle sheath cells, which also does not allow abundant space for microbial attachment (Dengler and Nelson, 1999). When evaluating the proportion of mesophyll cells, it comprised of 28 to 47% in $C_4$ grasses (Wilson and Hattersley, 1989; Wilson, 1990; Wilson, 1993) and 53 to 65% in $C_3$ grasses (Akin and Burdick, 1975; Akin, 1989).

**Plant Cell Wall**

The plant cell wall can be considered the main structural component of the plant, offering it both protection and strength. The formation of the cell wall happens in a process called cytokinesis (Iiyama et al., 1993; Albersheim et al., 2010) during cell division, when the mother cell undergoes mitosis partitioning the protoplast and forming
a plate cell (small, disk-like wall) inside the cell, between the two daughter nuclei. The plate cell extends outwardly until it reaches and blends with the mother cell wall, cutting the cell in two and forming a brand new cross-wall (beginning point of primary cell wall), which at this time has a simple composition and has a surrounding membrane that is equivalent to the plasma membrane of the mother cell wall. Cellulose that is made at the plasma membrane starts to be deposited on both sides of the cross-wall as cellulose microfibrils, and those from one side do not interact with the ones from the other side, hence, a separation area called middle lamella, which is rich in pectin, is formed in the center of the cross-wall before cellulose deposition starts. Accordingly, the first layer formed in the cell wall growth is the middle lamella, which acts as a cement to hold cells together, attaching their cell walls.

The primary cell wall then is the first cell wall to be formed while cells are still dividing and growing and is composed mainly of cellulose microfibrils, some pectins and proteins, and phenolic acids at the beginning. As the cell keeps growing towards maturity (primary growth), cellulose, pectin, and hemicelluloses continue to be deposited in the cell wall without the deposition of lignin (Jung and Allen, 1995). The same authors also described that, when the plant cells stop elongating (growth is ceased), it starts laying down the secondary wall inside of the primary wall and, during this phase, the cell wall is thickened from the inner part of the primary wall towards the lumen of the cell (Jung and Allen, 1995). Additional polysaccharides are deposited into the cell wall and those are composed more of cellulose than hemicelluloses, while pectin stops being deposited. Lignin deposition in the cell wall starts when secondary wall thickening takes place and it initiates in the middle lamella, moves into the primary
wall and then progresses into the secondary wall (Salisbury and Ross, 1992; Wilson, 1993; Jung and Allen, 1995).

In general, and compared to the secondary cell wall, the primary cell wall alone is thin (0.1 – 0.2 μm in cells that only have a thin primary cell wall; Wilson, 1993; 1- 10 μm in cells that have thicker primary walls; Salisbury and Ross, 1992; Fry, 2001) and is composed of about 9 to 25% cellulose, 20 to 50% hemicelluloses, 10 to 35% pectins, and about 10% of proteins (Salisbury and Ross, 1992). The secondary wall is thicker (up to 20 μm; Fry, 2001) than the primary wall and is composed of 41-45% cellulose, 30% hemicelluloses, and up to 22 to 28% of lignin (Salisbury and Ross, 1992).

**Cell Wall Polysaccharides**

There are three main classes of polysaccharides present in the cell wall: cellulose, hemicelluloses, and pectins. Cellulose is a crystalline structure of β-(1→4)-D-glucose chains, which are synthesized individually and can crystallize into cellulose microfibrils through inter- and intramolecular hydrogen bonds and Van der Waals forces (McFarlane et al., 2014). Cellulose microfibrils form the structural framework of the plant cell wall by being fused with unstructured hemicellulosic materials, and in some walls, lignin (Wilson, 1994). Because cellulose is the main polymer of the cell wall, the angle, degree of polymerization, and crystallinity of cellulose microfibrils are important determinants of the physical characteristics of the cell wall (McFarlane et al., 2014). Cellulose is found in primary and secondary walls; however, the degree of polymerization in each one is different, meaning that the number of units of glucose per chain that forms a sub-unit of cellulose is different (Baker et al., 1959). The degree of polymerization indicates the composition of the cellulose and, as plant matures, the cell walls become thicker, which leads to a greater polymerization of the plant and
construction of the crystalline structure of cellulose (Peterson, 2014). The degree of crystallinity has been reported to affect rate of degradation of cellulose by rumen microorganisms, where the greater the degree of crystallinity, the slower degradation will be (Baker et al., 1959).

Hemicelluloses are polysaccharides in plant cell walls that have a backbone of β-(1→4)-linked xylose with branching sugars such as glucose or mannoses (Scheller and Ulvskov, 2010). Hemicelluloses cross-link cellulose microfibrils through hydrogen bonds influencing cellulose crystallinity and maintaining a strong network of interconnected cellulose microfibrils (McFarlane et al., 2014).

Pectins are a diverse class of soluble polysaccharides that typically have a galacturonic acid–containing backbone. They are closely associated with cellulose and interaction between them can occur via hydrogen bonds (McFarlane et al., 2014).

**Lignin**

Lignin is a general term for a complex organic biopolymer that originates from the polymerization of three different hydroxycinnamyl alcohols (monolignols – i.e. p-coumaryl, coniferyl, and sinapyl) that result in three different phenylpropanoids [i.e. p-hydroxyphenyl (HY), guaiacyl (GY), and syringyl (SY); Baucher et al., 1998], which are the building blocks of lignin and depending on the plant species can be found in different proportions (Vanholme et al., 2010). After cell elongation, in the process of cell maturation, deposition of lignin occurs in cell walls of tissues involved in mechanical support or water conduction of vascular plants (Baucher et al., 1998; Moore and Jung, 2001).

Although lignin has been defined, there are lignin terms that have been used in describing forage lignin that are important to be addressed. Core lignin has been
defined as a highly compressed phenylpropanoid polymer deposited in the cell wall during secondary wall thickening that forms covalent bonds with the cell wall polysaccharide hemicellulose (Jung and Deetz, 1993). Noncore lignin is considered to be all (ester- and ether-linked) p-coumaric and ferulic acids and their dimers, present in the wall that were deposited there during both primary and secondary wall development and can be linked only to cell wall polysaccharides, core lignin, or both (Jung and Deetz, 1993).

The most crucial role of lignin in plants is related to physical structure, where it provides sturdiness and strength to stems and integrity of cell walls (Moore and Jung, 2001). Secondly, lignin shields the cell wall against water loss, which is minimized by making the cells less permeable (Dean and Eriksson, 1992). Even though its evolution in plants occurred to promote plant structure and not as a defense mechanism, lignin also plays a role as a barrier against pathogens (Moore and Jung, 2001).

Because lignin is important to plant survival and function, as mentioned above, reduced lignin in plants can negatively impact their fitness to the environment because in general, although it will depend on environmental conditions and species, crop yields and long-term survival of plants are reduced with significant decreases in lignin content (Pedersen et al., 2005). However, forage nutritive value for herbivores is limited by lignin content because this compound has been recognized as a limiting factor of total cell wall digestibility since it negatively impacts ruminal microorganism degradation of structural polysaccharides by acting as a physical barrier (Jung, 1989; Moore and Jung, 2001). Lignin is essentially indigestible and by being cross-linked with the cell wall
polysaccharides, it makes them less accessible to microbial fermentation, limiting their digestion as well, which in turn will generate less digestible energy.

The rate and extent of cell wall degradation may be influenced by the concentrations of the two distinct lignin fractions, core and noncore lignin, that are covalently bound to forage cell walls, and by composition of core lignin (Jung and Deetz, 1993). It has been described that as lignification of the wall proceeds to secondary thickening, the lignin deposited shifts from GY-type to lignin rich in SY units (Jung and Allen, 1995). This led researchers to assume that lignin that contains more SY units affects cell wall digestibility to a greater extent by being more linear in structure and extending further into the secondary wall, thus shielding a greater proportion of the polysaccharides from digestion (Jung and Allen, 1995).

It has been reported that noncore lignin is readily soluble in alkalis, with ferulic and p-coumaric comprising the majority of phenolic acids recovered (Shreck, 2013). Besides lignin being deposited in the cell wall during secondary wall thickening, an incorporation of ferulic acid esters, from hemicellulose of the primary wall, seems to occur into cross-linkages between hemicellulose and lignin. Along with that, grasses begin to incorporate relatively large amounts of p-coumaric acid esters of lignin into the secondary wall with greater concentrations in C4 grasses compared to C3 (Jung and Allen, 1995). However, most of the p-coumaric acid is linked to core lignin; hence are unlikely to affect cell wall polysaccharide digestion directly and are more of an indicator of lignification of the forage (Jung and Deetz, 1993).

By being esterified to the cell wall polysaccharide, the ferulic acid esters interfere with the hemicellulose digestion because there is an obstruction to the alignment of
xylanases to their substrate (Jung, 1989). However, the reduction in digestibility should only be limited to a reduced rate of digestion and not extent because ruminal bacteria and fungi have been reported to have phenolic acid esterases that are able to break the ester bond that is impeding cell-wall digestion (Akin et al., 1993).

Nonetheless, Jung and Deetz (1993) predicted that, even though only rate of digestion is expected to be affected by esters linkages, when ferulic acid cross-links hemicellulose and lignin via ester and ether linkages, an opposite effect may happen. That is due to the fact that the lignin polymer is kept very close to hemicellulose by the ether link, causing the ester portion of the acid bond to be no longer accessible to breakage because the esterase cannot reach its target anymore. Additionally, besides the fact that the ether linkage becomes inaccessible due to the lignin polymer, anaerobic cleavage of ethers has not been reported (Jung and Allen, 1995). Therefore, different from ferulic acid esters, the effects of ferulic acid ethers are not likely to be overcome during ruminal fermentation, causing a decrease in the extent of cell wall digestion (Jung and Allen, 1995). Moreover, it has been proposed that noncore lignin can reduce cell wall polysaccharide digestibility since, after addition of the phenolics, ferulic and p-coumaric, fiber digestion was inhibited in vitro (Chesson et al., 1982; Martin and Blake, 1989), suggesting that the phenolic acids can cause enzyme activity or microbial growth inhibition. However, p-coumaric or ferulic acid had no effect on in situ or in vitro digestibility when ruminally infused or fed to sheep (Lowry, 1990).

In general, C4 grasses already have lesser concentrations of easily digestible nutrients, such as protein and soluble carbohydrates and greater concentrations of cell wall components. According to Wilson (1994), more than 50% of a leaf’s reserve
carbohydrates and protein are found inside of bundle sheath cells in C₄ grasses. Hence, because of their thick cell walls that are slowly digested, the availability of these nutrients for microbial digestion is limited. Besides, as mentioned before, some C₄ species of grasses also contain a suberized lamella in the bundle sheath cells that is totally indigestible and blocks the access of microorganisms to the secondary wall.

Therefore, in comparison with C₃ species, C₄ grasses have relatively greater proportion of cell-wall material that is potentially digestible but because of the different anatomy, composition of the cell wall, and physical structure, the rate and extent of fermentation in C₄ grasses is relatively slow, negatively affecting their nutritive value.

**Chemical Treatment of Fiber**

Two important items that should be considered before chemical treatment is the plant class (monocotyledon vs. dicotyledon) and the stage of plant maturity, which determines its level of lignification. Chemical treatment is, in general, most beneficial when treating mature, lignified substrates (Fahey et al., 1993). Monocots, which is the class of grasses, have a much greater concentration of esterified phenolic acids (e.g. ρ-coumaric and ferulic acids; Fahey et al., 1993) and a larger number of lignin-polysaccharides linkages taking place across the whole plant, while in dicots those are allocated in more isolated compartments (Fahey et al., 1993). Therefore, besides being most effective in mature, lignified substrates, chemical treatment should promote greater rate and extent of digestion in monocots, thus being more suitable for this class of plants.

**History**

The use of alkali chemicals to treat fiber is not a new concept and it dates back to the paper making industry, where the first chemical pulping process using NaOH to treat
wood pulp and produce paper was patented in 1854 and first commercially used in Sweden in 1884 (Chandra, 2004). From the paper making process, it became well known that the use of a strong alkali such as NaOH softens the fibers in the wood and allows separation of cellulose from lignin. In the 1880’s, in Germany, the chemical treatment of straw with NaOH began with the development of the Beckmann method, which consists of basically soaking the straw in a 1.5% NaOH solution overnight followed by a rinse with fresh water (Wanapat et al., 1985). While this method was effective in increasing organic matter (OM) digestibility by 56%, it also led to losses of dry matter (DM) of approximately 20%, due to washing off excess NaOH, generating a significant amount of environmental pollution (Fingerling and Schmidt, 1919). This concept was adapted by animal scientists in the early 1970’s, and extensive research was conducted to evaluate the effect of different chemicals and methods on fiber digestion.

Methods of Chemical Treatment

Different methods can be grouped by their mode of action: hydrolytic or oxidative. Hydrolytic chemicals, the majority being alkaline compounds, can improve digestibility by action of hydroxide groups that are capable of disruption of the cell wall structure and possible swelling that can result in increased microbial attachment (Fahey et al., 1993). Furthermore, the mode of action of chemical treatment by hydrolytic means have been proposed to include solubilization of hemicellulose and increased extent and rate of cellulose and hemicellulose digestion, the latter possibly by swelling (Klopfenstein, 1978). Moreover, the core lignin contents are generally not reduced by treatment (Klopfenstein et al., 1972), thus it was proposed that the increase in extent of
digestion is probably due to the breakage of the bonds between lignin and hemicellulose rather than actual removal of lignin (Klopfenstein, 1978).

Oxidative methods of treatment involves the use of more caustic and less practical chemicals such as \( \text{H}_2\text{O}_2 \), \( \text{O}_3 \), and \( \text{Na}_2\text{O}_2 \), and it has been proposed that the mode of action for these methods is characterized by attack and degradation of core lignin (Fahey et al., 1993).

**Hydrolytic Treatment and Chemicals**

An ideal chemical for roughage treatment should: be effective in increasing animal intake or digestibility; promote benefits that justify the costs associated with treatment; be non-toxic to animals and safe to the environment when excreted by the animal; provide a nutritive value to the animal or value as fertilizer; and be relatively safe to handle (Owen et al., 1984). Many chemicals have been researched in several laboratory and field experiments for potential to enhance digestibility of roughages mainly from crop residues (Chandra and Jackson, 1971) and, in the 1970’s, four of them \([\text{NaOH}, \text{NH}_4\text{OH}, \text{KOH}, \text{and Ca(OH)}_2]\) were being routinely used in experimentation with animals (Klopfenstein, 1978).

Sodium hydroxide has been the most extensively researched and effective chemical used when compared to \( \text{Ca(OH)}_2 \) or \( \text{NH}_4\text{OH} \) (Klopfenstein, 1978) and, for that reason, became the standard by which other chemicals are compared to. In a review, 24 studies involving crop residues treated with NaOH fed to cattle or sheep were summarized and an average of 22% increase in DMI and 30% increase in DM digestibility were observed (Fahey et al., 1993). Similarly, alfalfa stems, corn cobs, and corn stalks were treated with NaOH and provided to lambs in digestion trials (Klopfenstein et al., 1972). The roughages were mixed with water to reach a 50% DM
content, treated with the chemical, and fed after a 48-h incubation. The authors observed that at a 4% inclusion of NaOH, DM digestibility of alfalfa stems was increased by 6.8%, while corn cobs had an 11.2% increase in DM digestibility (Klopfenstein et al., 1972). Moreover, OM digestibility of corn stalks increased 10.1 and 11.2% for 3 and 5% NaOH, respectively, when provided with only urea, minerals, and vitamins, whereas, when fed with ground alfalfa stems, it had a 20.5% increase in OM digestibility when compared with untreated corn stalks (Klopfenstein et al., 1972). With those results, the authors concluded that poor quality roughages can be treated with 3 to 5% NaOH and, by doing so, the DM digestibility is increased by a satisfactory extent to potentially improve animal performance. Nevertheless, the high Na content of treated forages is one of the major concerns of NaOH use since excess intake of Na can negatively shift the site of fiber digestion to the lower gut due to increased ruminal escape of potentially digestible fiber (Berger et al., 1979), possibly reducing the energetic benefit (Shreck et al., 2015). Moreover, safety concerns have been brought to attention, limiting the application of NaOH treatment and promoting the opportunity for research with other chemicals (Watson et al., 2015).

Ammoniation is another widely used method of treatment, and extensive research has been done evaluating its effectiveness in ruminant nutrition (Males, 1987; Mason et al., 1988). When compared to NaOH, NH₃ has been reported to be easier and less expensive to use with the added benefit of nitrogen supply (Males, 1987), decreasing the amount of supplemental protein needed in diets containing high levels of treated roughages (Sundstøl and Coxworth, 1984). Anhydrous NH₃, the gaseous form of NH₃, reduces physical contact with the chemical, eliminating the need to premix it,
which makes the process of fumigating a bale that is tightly wrapped easier to conduct (Shreck, 2013; Peterson, 2014). Nevertheless, the benefits of NH₃ treatment have been reported to be not as great as with NaOH when it comes to forage digestibility since the average improvement in in vitro DM digestibility (IVDMD) of straws treated with NaOH was observed to be 16% greater than that of straws treated with NH₃ (Males, 1987).

The use of anhydrous NH₃, NH₄OH, thermoammoniation, and use of urea as a source of NH₃ all have been evaluated and the mode of action of ammoniation is assumed to be comparable to that of NaOH (Fahey et al., 1993). Thermoammoniation of barley straw was performed by adding 3% of NH₃ on a DM basis and treatment at 90°C for 16h. The straw was fed ad libitum to sheep and increases in digestibility of DM, OM, and acid detergent fiber (ADF) by 19, 16, and 25%, respectively, were observed as a result of the NH₃ treatment (Fahey et al., 1993). Moreover, 21 studies evaluating crop residues treated with NH₃ showed an average increase in DM intake of 22%, while 32 other studies with the same objective indicated an increase of 15% in DM digestibility as a result of NH₃ treatment (Fahey et al., 1993). As with the use of NaOH, ammoniation can also have its drawbacks. Anhydrous ammonia is maintained under pressure and can be dangerous, causing skin, eyes or throat burn (Brown and Kunkle, 1992).

Moreover, research has shown that animals will refuse roughages treated with NH₃ (Brownson, 2000) unless they aerated or mixed with a fermented feed so the odor will disappear and the acids will neutralize the NH₃, suggesting that aeration should be performed prior to feeding (Romero et al., 2013).

The use of Ca(OH)₂ for treatment was primarily proposed to partially replace NaOH in order to eliminate the negative effects of Na on digestion and excretion, and to
provide supplemental Ca (Rounds et al., 1976). However, in the past, Ca(OH)\textsubscript{2} by itself has been reported to be ineffective in improving digestibility of straw when used at a 5% DM rate plus 10% water, while with 60 or 100% water the straw could not be stored well enough to be suitable for animal feeding (Bass et al., 1982). Moreover, when using IVDMD to evaluate chemical treatments, Ca(OH)\textsubscript{2} appeared to be ineffective and the authors suggested that it could be due to a lower dissociation constant when compared with NaOH, requiring a longer reaction period for complete effectiveness (Rounds et al., 1976). Nevertheless, the same authors reported that, when compared with NaOH alone at 4%, lambs had increased DM intake (DMI), and greater and more efficient gains when consuming corn cobs treated with 3% NaOH plus 1% Ca(OH)\textsubscript{2}, suggesting that the latter appears to complement the former (Rounds et al., 1976). In a review, data from two growth trials using treated wheat straw were summarized and it was reported that, when compared to untreated control, straw treatment with Ca(OH)\textsubscript{2} improved average daily gain (ADG) and feed conversions (Klopfenstein and Owen, 1981). Furthermore, Ca(OH)\textsubscript{2} treatment improved DM and neutral detergent fiber (NDF) digestibility by 9.0 and 9.9%, respectively. With the intent to test different levels of moisture (20, 40, or 60%), crop residues such as wheat straw, corn cobs and corn stalks were treated with 5% Ca(OH)\textsubscript{2} and provided to lambs at 85% of their diets (Paterson et al., 1980). At 40% moisture, no change in DMI, and cell wall digestibility of corn cobs and wheat straw was observed when compared to residues treated at 20 or 60% moisture; however, DMI of corn stalks was increased with increasing moisture contents, while maximal DM and wall cell digestibility was observed at 20% (Paterson et al., 1980). Evidently, the efficacy of chemical treatment can be dependent on several
different factors such as moisture content, rate of application, and type of roughage treated.

**Calcium Oxide**

Calcium oxide, also known as quicklime, is the dehydrated, powdered form of Ca(OH)$_2$. It is readily available in a dry solid form and limited data is available on how competitive CaO (as opposed to Ca(OH)$_2$) is as an alkaline treatment. However, CaO has gained significant attention in recent years (Dahlke and Euken, 2013a; Schroeder et al., 2014; Peterson et al., 2015a; Shreck et al., 2015) as a chemical for treatment of crop residues used as partial replacement in growing and finishing beef cattle diets (Watson et al., 2015).

In vitro digestibility of wheat straw treated with CaO was performed with four different amounts of the chemical tested (0, 4, 8, and 16% CaO; DM basis) and a 2:1 ratio of water:straw was used (Chaudhry, 1998a). Results showed that as CaO inclusion increased, IVDMD of wheat straw increased linearly with an approximately 36% difference between 0 and 16% CaO (Chaudhry, 1998a). In a follow up study, with the objective to investigate the field-scale application of CaO to improve digestion and rumen fermentation of straw in sheep, the greatest amount of CaO (16% DM basis) and the same ratio of water to DM was tested (Chaudhry, 1998b). Plastic bags were filled with the treated straw, tied up, and kept at room temperature for 15 d before feeding. The straw was fed to sheep as approximately 70% of the diet with a supplement containing 42% crude protein (CP) in a 4 x 4 Latin square design. When chemical composition of the treated straw was measured, there was a reduction of 14% in OM, 20% in ash-free NDF (aNDF), 12% in acid detergent lignin, and 89% in hemicellulose. Moreover, the author reported an increase of 23, 49, 41, and 14% in ruminal
degradability and 12, 31, 20, and 26% in total tract digestibility of OM, aNDF, ADF, and cellulose, respectively in sheep consuming CaO treated when compared to those fed untreated straw (Chaudhry, 1998b). When evaluating ruminal fermentation parameters, volatile fatty acid (VFA) molar proportions, total VFA concentration, and ruminal pH were not different between treated and untreated straw fed sheep; however, NH₃-N concentrations decreased in sheep consuming CaO treated straw (7.5 vs 10.1 mM).

As a readily available alternative roughage to cattle feeding, corn crop residues, commonly known as corn stover or stalks have been used to partially replace corn in beef finishing diets (Shreck et al., 2013b; Chapple et al., 2015) or growing calves diets (Shreck et al., 2014; Peterson et al., 2015b). In a study evaluating the effects of replacing corn in feedlot finishing diets with CaO-treated corn stover on growth performance, digestibility, and ruminal metabolism of cattle, corn stover was treated with 5% CaO plus water to reach 50% DM, bagged, and anaerobically stored for a minimum of 21 d before feeding (Chapple et al., 2015). Calcium oxide-treated corn stover replaced 36% of the corn used in the control diet, which contained 35% distillers grains, 55% corn, 5% untreated corn stover, and 5% vitamin mineral supplement. No differences were observed in final body weight (BW), ADG, and gain to feed ratio (G:F); however, DMI was reduced for the animals consuming the diet containing CaO treated corn stover, thus the authors concluded that even though DMI was reduced, animal performance was not affected, suggesting that treated corn residues can replace corn in times of high corn prices, without negative impacts (Chapple et al., 2015). In the same study, total tract digestibility of NDF and ADF were increased by 37 and 23%, respectively and the authors speculated that those effects could have been attributed to
increased ruminal fermentation of the fiber fractions (Chapple et al., 2015). When ruminal fermentation parameters were measured, there was an increase in total VFA and acetate concentrations, with no effect on propionate concentration, while a decrease in ruminal pH was observed (Chapple et al., 2015).

In a similar study, replacing corn and untreated residues with CaO treated corn cobs, wheat straw and corn stover aimed to evaluate beef steers performance, total tract digestibility and ruminal metabolism (Shreck et al., 2015). Crop residues were treated and stored in the same manner reported by Chapple et al. (2015), with the difference that they were fed to steers after at least 30 d after treatment. Calcium oxide treated residues replaced corn and made up of 20 and 25% of the final diet composition in the performance and metabolism trials, respectively. Results showed that when corn cobs or stover were treated with CaO and replaced corn in the diet, there was no difference in final BW of steers; however, diets containing treated wheat straw promoted greater final BW when compared to control. When compared to diets containing non-treated crop residues, steers fed treated straw and corn stover had 9.7 and 12.5% greater ADG, and 10.7 and 5.0% increase in G:F, respectively. For total tract digestibility, all three treated residues promoted greater NDF digestibility when compared to control and non-treated residues. Steers fed diets containing treated wheat straw and corn stover had 26 and 52% greater NDF digestibility, respectively, when compared to their non-treated counterparts (Shreck et al., 2015). Average ruminal pH was not different between steers consuming diets containing treated residues (corn cobs = 5.98; wheat straw = 5.81; corn stover = 6.23) and control (5.99); however, diets containing treated corn stover promoted greater ruminal pH than diets containing non-
treated corn stover (6.23 vs 5.95, respectively). With the results observed, the authors concluded that feeding steers with CaO-treated crop residues, such as wheat straw and corn stover, replacing corn in the diet, supported similar ADG, DMI, and G:F to steers provided with the control diet that contained 10 percentage units more corn (Shreck et al., 2015). Furthermore, differences in observed performance between diets containing treated and non-treated residues were attributed to the increase in fiber digestibility of treated residues when compared to non-treated (Shreck et al., 2015).

**Final Remarks**

Considering the data presented in this literature review regarding lignin composition and its relationship with the cell wall polysaccharides, the main goal of chemical treatment of warm-season C₄ grasses, and for the purpose of this study, with the alkali calcium oxide is then to disrupt the phenolic acid bonds that attach lignin to cell wall polysaccharide and possibly the bonds between phenolic acids and hemicellulose. After reviewing the literature and based on has been done most recently, treatment with CaO has been used as a cheaper and safer alternative to chemicals used in the past in improving the rate and extent of low quality forage digestion.

Most of the research that has been reported in the literature using calcium oxide has tested the effect of the product in crop residues (e.g. corn stover) that have been used as a partial replacement for corn in several kinds of mixed diets and, to our knowledge, no research has been performed by using calcium oxide to treat warm-season C₄ grasses such as Tifton 85 bermudagrass or bahiagrass hay to be used as a sole source of feed to ruminants. This would be of great interest in warm-season C₄ grasses due to their greater proportions of more lignified thick-walled cells in leaves.
such as bundle sheath cells, sclerenchyma, and cells present in the vascular tissue, when compared to C₃ cool-season grasses.
CHAPTER 2
RUMINAL IN SITU DEGRADABILITY AND IN VITRO ORGANIC MATTER DIGESTIBILITY OF ROUGHAGES TREATED WITH CALCIUM OXIDE

Introduction

Ruminants have a great competitive advantage over other domesticated animals: the ability to digest plant cell wall components by a symbiotic relationship with ruminal microorganisms (Van Soest, 1994). The southeastern US has ideal conditions to grow large quantities of forages, and agricultural industries that generate a number of byproducts (e.g. peanut hulls) that have the potential to be used as roughages in cattle diets. Consequently, most of the beef cattle operations located in this region rely on forage-based diets (Ciriaco et al., 2015). However, the predominant forages, and some of the byproducts, can be of limited nutritive value for several different reasons, including high fiber contents when compared to more digestible components. Although ruminants have the advantage mentioned, physical barriers such as lignin attachment to the cell wall polysaccharides (Jung and Deetz, 1993) can make the task more difficult to accomplish.

With the goal to increase forage digestibility, forage breeders have attempted to reduce lignin and cellulose in forages; however, the main function of these compounds in nature is to provide structure and support to plants, making it difficult to improve the digestibility without reducing forage yield (Pedersen et al., 2005). Chemical treatment of low-quality roughages is a promising method of improving fiber digestibility (Klopfenstein et al., 1972; Shreck et al., 2015; Watson et al., 2015) allowing ruminants to more efficiently convert low-quality fibrous feedstuffs into meat, milk, and fiber. Sodium hydroxide has been extensively used in the past and up to 30% improvements in total tract digestibility of DM has been observed (Fahey et al., 1993); however,
compared with NaOH, CaO has been shown to provide similar results and, besides providing a source of Ca in the diet, is considered safer, easier to handle, and less caustic (Shreck et al., 2015). Therefore, it was hypothesized that treating warm-season forages and roughages common to the southeastern US with the alkali CaO would be an effective method of improving the digestibility of nutrients, with major effects on the fiber fraction. Two experiments were designed to test this hypothesis with the following objectives: Exp. 1 – evaluate the effects of CaO on ruminal in situ degradability of nutrients and in vitro organic matter digestibility (IVOMD) of peanut hulls (PHS) at different incubation times and DM contents; Exp. 2 – evaluate the effects of CaO on ruminal in situ degradability of nutrients and IVOMD of Tifton 85 bermudagrass (Cynodon spp.) hay (BM) and bahiagrass (Paspalum notatum) hay (BH) under incubation for 7 or 14 d in buckets.

Materials and Methods

All procedures involving animals were approved by the University of Florida Institutional Animal Care and Use Committee (Protocol # 201508733).

Experiment 1 Design and Treatments

In Exp. 1a, to evaluate the effects of CaO on ruminal in situ degradability of nutrients, PHS were incubated in duplicate in 20-L buckets (n = 2/treatment) in 2 consecutive years (1 bucket/year) after following the treatments: 1) PHS as is (D); 2) PHS + water (to 50% DM) incubated for 7d (W7); 3) PHS + water (to 50% DM) incubated for 14d (W14); and 4) PHS + water (to 50% DM) + 5% CaO (DM basis) incubated for 7d (CO7); and 5) PHS + water (to 50% DM) + 5% CaO (DM basis) incubated for 14d (CO14). After 7 or 14 d of incubation, buckets were opened and contents were dried for 72h at 55°C, ground to pass a 4-mm screen in a Wiley mill
(Thomas Scientific, Swedesboro, NJ) and stored at 4°C for further ruminal in situ degradability analysis.

In Exp. 1b, to evaluate the effects of different DM contents and days of incubation, PHS were incubated in 20-L buckets \((n = 2/\text{treatment})\) following the treatments: 1) PHS + water (to 70% DM; \textit{70DM}); 2) PHS + water (to 70%DM) + 5% CaO (DM basis; \textit{70DMCO}); 3) PH + water (to 50% DM; \textit{50DM}); and 4) PHS + water (to 50%DM) + 5% CaO (DM basis; \textit{50DMCO}). Buckets were opened after 3, 6, 9, 12, and 15 d of incubation when a representative sample was collected. Samples were dried for 72 h at 55°C, ground to pass a 2-mm screen in a Wiley mill and stored at 4°C for further nutrient and IVOMD analysis.

**Experiment 2 Design and Treatments**

Similar to Exp.1a, BM and BH were incubated in 20-L buckets in quadruplicate (2 consecutive years; \(n = 4/\text{treatment}\)) after following the treatments: 1) BM or BH as is (\textit{DH}); 2) BM or BH + water (to 50% DM) incubated for 7d (\textit{W7}); 3) BM or BH + water (to 50% DM) incubated for 14d (\textit{W14}); and 4) BM or BH + water (to 50% DM) + 5% CaO (DM basis) incubated for 7d (\textit{CO5-7}); 5) BM or BH + water (to 50% DM) + 5% CaO (DM basis) incubated for 14d (\textit{CO5-14}); and 6) BM or BH + water (to 50% DM) + 10% CaO (DM basis) incubated for 14 d (\textit{CO10}). After 7 or 14 d of incubation, buckets were opened and contents were dried for 72 h at 55°C. In a Wiley mill, a set of samples was ground to pass a 4-mm screen for further ruminal in situ degradability analysis and another set was ground to pass a 2-mm screen for further nutrient and IVOMD analysis. Samples were stored at 4°C.

The choices of 5% CaO and 50% DM were based on what has been most commonly applied in the literature when treating crop residues (Shreck et al., 2011;
Shreck et al., 2015). The different treatments containing 10% CaO and 30% DM were chosen in an attempt to observe potential additional effects of CaO and to compare effects and practicality of using less water to hydrate the chemical.

**Ruminal In Situ Degradability**

Ruminal in situ degradability of nutrients of PHS, BM, and BH from Exp. 1 and 2 was determined in triplicate in at least two ruminally cannulated steers (323 ± 42 kg BW; average BW ± SD) that were consuming bahiagrass hay for at least 14 d. Dried and ground (4 mm) samples of PHS, BM, and BH after buckets incubations were weighed (5 g) into 10 x 20 cm Ankom in situ bags (R1020, Ankom Technology Corp., Macedon, NY). The bag pore size was 50 μm and the sample size to free bag surface area ratio was 12.5 mg/cm². Bags were heat-sealed and placed in mesh bags, which were zipperred, attached to a rope and clip, and, after soaking in warm (39°C) water for 15 min, incubated in the ventral sac of the rumen for 24, 48, and 72 h. A set (0 h) of bags was prepared in duplicate for each roughage and treatment to account for removal of soluble fraction before ruminal incubation. All the bags (except 0 h) were placed at the same time in the rumen and removal occurred at each time point. After removal, all bags were placed in a cooler with ice-cold water to halt fermentation, subsequently rinsed with cold running water to remove adherent particles and bacteria, frozen overnight, and then washed in a domestic washing machine using a cool-wash regular cycle without soap. The same washing procedure was applied for bags not incubated in the rumen (0 h bags). Washed bags were dried for 48 h at 55°C and weighed. After weighing, residues from the incubation runs were composited by incubation time within steer (3 bags/time point/steer/treatment) and composite samples and original whole
samples were analyzed for determination of DM, OM, CP, NDF, and ADF to calculate ruminal degradability of each nutrient.

**Laboratory Analyses**

**Chemical composition of roughages**

For determination of sample DM and OM in in situ residues and original samples (2 mm) from PHS, BM, and BH after bucket incubation, approximately 0.5 g of samples were weighed in duplicate into ceramic crucibles, dried in a forced-air oven at 105°C for 24 h and subsequently ashed at 650°C for 6 h. Approximately 0.5 g of samples were weighed in duplicate into F57 bags (Ankom Technology Corp., Macedon, NY) and analyzed for NDF, using heat-stable α-amylase and sodium sulfite, and subsequent ADF was performed as described by Van Soest et al. (1991) in an Ankom 200 Fiber Analyzer (Ankom Technology Corp.). For total N concentration and further CP determination, samples were ball-milled and analyzed using a C, H, N, and S analyzer by the Dumas dry combustion method (Vario Micro Cube; Elementar, Hanau, Germany). Crude protein was calculated by multiplying the N concentration of the dry sample by 6.25.

**In vitro OM digestibility**

A modified Tilley and Terry (1963) procedure was used to determine IVOMD of PHS, BM, and BH from Exp. 1 and 2. Briefly, 0.7 g of dried and ground (2 mm) samples after bucket incubations were incubated with 50 mL of a 4:1 McDougall’s buffer:ruminal fluid inoculum in 100-mL plastic centrifuge tubes for 48 h under constant agitation (60 rpm) at 39°C. Two tubes per treatment per roughage and two blank (without substrate or treatment) tubes were incubated in each of the three separate replicate days. After the initial 48 h, 6 mL of HCl was added to the tubes along with 2 mL of a 5% pepsin
solution. Tubes were then incubated for an additional 48 h under constant agitation (60 rpm) at 39°C. Samples were then filtered through P8 filters (Fisherbrand; Thermo Fisher Scientific Inc.). Filters with wet samples were then dried at 105°C in a forced air oven for 24 h to determine IVDMD. Dry filters with residual samples were then placed in a muffle furnace for 6 h at 650°C. The ash was then placed in a 105°C oven for 24 h prior to recording weight. Two ruminally cannulated steers consuming bahiagrass hay for at least 14 d were used as ruminal fluid donors.

**Statistical Analysis**

All data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). In Exp. 1, for ruminal in situ degradability of nutrients of PHS (Exp. 1a), data were analyzed as a randomized complete block design. In Exp. 2, for ruminal in situ degradability of nutrients and IVOMD of BM and BH, data were analyzed as a generalized randomized block design. In both experiments, bucket was considered the experimental unit and the model included the fixed effect of treatment and the random effect of year (block).

In Exp. 1, for IVOMD of PHS (Exp. 1b), data were analyzed as a completely randomized design with repeated measures (3, 6, 9, 12, and 15 d of bucket incubation) and the model included the fixed effects of DM content, CaO, day of incubation, and their interactions. The subject was bucket within DM content and CaO. The covariance structure chosen based on the smallest Akaike information criterion was compound symmetry. Significance was declared at $P \leq 0.05$ and Tukey-Kramer adjustments were utilized on the non-repeated models. Tendencies were considered when $0.05 < P \leq 0.01$ when evaluating IVOMD in Exp. 1.
Results and Discussion

Experiment 1

Peanut production (with hulls) in the US was estimated to be 3.3 million metric tons in 2017 (USDA, 2018). Of the total weight, 21-29% can be assumed to be from hulls (Heuzé et al., 2017); therefore, it can be estimated that approximately 825 thousand metric tons of peanut hulls are produced each year. This byproduct is a major waste in the peanut industry, thus if researchers can develop strategies for its use, environmental impacts potentially caused by waste disposal can be reduced. Peanut hulls are mostly comprised of fiber, with a crude fiber content that can exceed 60% and a lignin content that can vary from 6 to 45% (Barton et al., 1974). Due to their high fiber content, peanut hulls has a great potential to be used as a roughage in beef cattle diets at a limited rate (Utley and McCormick, 1972; Barton et al., 1974).

Nutrient composition of PHS before and after treatment with CaO and incubation for either 7 or 14 d is presented in Table 2-1. No differences among treatments were observed for OM ($P = 0.525$), CP ($P = 0.199$), NDF ($P = 0.593$), and ADF ($P = 0.675$) concentration. Ruminal in situ degradability of nutrients of PHS treated or not with CaO and incubated for either 7 or 14 d is presented in Table 2-2. At all ruminal incubation time points (24, 48, and 72 h), no effect of treatment ($P \geq 0.190$) was observed for ruminal in situ degradability of DM, OM, NDF, and ADF. Although different fiber fraction components (cellulose, hemicellulose, and lignin) were not analyzed in PHS in this experiment, peanut hulls in general have been reported to contain 40.5% cellulose, 14.7% hemicellulose, and 26.4% lignin (López-Rivilli et al., 2012), indicating that of the fiber fraction components, hemicellulose is present in the least amount. Perhaps there was not enough power to be able to detect a difference with only two replicates per
treatment; however, the possible high concentration of lignin present in PHS may explain the lack of effects observed in the present experiment when PHS were treated with CaO, since the increase in extent of digestion is probably due to the breakage of the bonds between lignin and hemicellulose rather than actual removal of lignin. Moreover, when peanut hulls were treated with different chemicals, such as NaOH, NH₃, and Ca(ClO)₂ to improve their digestibility for ruminants, only Ca(ClO)₂ was able to promote an increase in IVDMD from 25 to 45% (Barton et al., 1974). Furthermore, with the exception Ca(ClO)₂, the data indicated that all other treatments actually decreased the digestibility of the hulls (Barton et al., 1974). It was reported that the cellulose fraction of peanut hulls was less digestible than those of certain grasses and the difference could be due to degree of polymerization, crystallinity, sites and number of cross linkages with other polymers, or a combination of these (Kerr et al., 1986). Although there was an increase in digestibility with the Ca(ClO)₂, other researchers suggested that it was still not comparable with the digestibility of residues such as soybean hulls (69.6%) without chemical treatment (Kerr et al., 1986), indicating that peanut hulls have a very low digestibility and are resistant to chemical treatment.

When looking into different DM contents and the effects of days of incubation on IVOMD of PHS treated or not with calcium oxide, there was no effect of DM content \( (P = 0.643) \), CaO \( (P = 0.275) \), or their interaction \( (P = 0.334) \); however, tendencies for CaO × day of incubation \( (P = 0.069) \) and DM × CaO × day of incubation \( (P = 0.099) \) interactions were observed (Figure 2-1). When CaO was added, IVOMD was greatest at 6 d of incubation. When considering DM content and when CaO was present at 70% DM of PHS, IVOMD increased up to 6 d of incubation \( (P = 0.005) \), which after, and up to
15 d, IVOMD was reduced \((P < 0.001)\) by 72\%. At 50\% DM of PHS and with CaO, IVOMD decreased by 38\% as days of incubation increased up to d 15 \((P = 0.038)\).

The majority of recent research recommends addition of water to crop residues in an attempt to reach a final DM of 50\% (Watson et al., 2015) when chemical treatment with CaO is performed. The reasons behind the addition of water are to ensure a proper reaction, since CaO needs hydration to adhere to the forage, be effective, and to provide a heat sink in order to prevent combustion, since the reaction that leads to Ca(OH)\(_2\) is exothermic and produces great amounts of heat (Dahlke and Euken, 2013b). Nevertheless, different moisture contents (25 and 37.5\%) have also been proposed when treating cornstalks, indicating that less water addition could facilitate treatment of roughages with evidence that treatment can still be effective in increasing digestibility, without causing combustion (Euken and Dahlke, 2014). The results observed in the current experiment indicated that when water is added to PHS to reach 50\% DM and CaO is added at 5\% of the total DM, digestibility tends to be reduced as days of incubation increase. However, when the water is added at a lesser amount (to reach 70\% DM), digestibility of PHS can actually be increased up to 6 d of incubation with CaO. Mold growth has been observed when barley straw was treated with Ca(OH)\(_2\) and water to reach 20\% DM (Zaman and Owen, 1995). Therefore, we speculate that greater amounts of water when CaO is present can provide an ideal environment for mold growth starting at the beginning of incubation, with the ideal conditions: high moisture and high pH. Less water perhaps still promotes conditions for the reaction to occur for a short period; however, after more than 6 d of incubation, mold can continue to develop, negatively affecting digestibility (Chaudhry and Miller, 1996). Although mold
count was not performed in the current study, a visual presence of mold was observed, with increased amounts as the days of incubation increased.

**Experiment 2**

Nutrient composition of BH before and after treatment with CaO and incubation for either 7 or 14 d is presented in Table 2-3. Besides CP content, which was not affected by treatment ($P = 0.073$), OM, NDF, and ADF concentrations were all reduced ($P < 0.001$) when CaO was applied at a rate of 10% of the total DM (CO10). The lack of reduction in CP with the reduction in OM is of great interest, leading to believe that the fiber fractions of the forage are being affected the most. There was a treatment difference ($P < 0.001$) for NDF content of bahiagrass hay, where CO10 promoted a reduction of 28% when compared with DH. Although to a lesser extent, when CaO was applied at a 5% rate, there was a significant reduction of 7.1% on NDF content of BH when compared to the non-treated forage (DH), regardless of number of incubation days. Nevertheless, treating BH with 5% CaO did not seem to be effective to reduce ADF content when compared to not treating. As previously mentioned, the proposed mode of action of alkali treatment has been suggested to include some solubilization of hemicellulose and consequently increased extent and rate of cellulose digestion (Klopfenstein, 1978). This could explain the reduction in NDF content when CaO was applied at either 5 or 10% of the total DM, which could have solubilized the hemicellulose, when compared with no treatment of the forage. Moreover, the core lignin contents are generally not reduced by treatment (Klopfenstein et al., 1972), thus it was proposed that the increase in extent of digestion is probably due to the breakage of the bonds between lignin and hemicellulose rather than actual removal of lignin (Klopfenstein, 1978).
Ruminal in situ degradability of nutrients and IVOMD of BH treated or not with CaO and incubated for either 7 or 14 d are presented in Table 2-4. At all ruminal incubation time points, a treatment effect was observed ($P < 0.001$) for degradability of DM, OM, CP, NDF, and ADF, where, in general, CaO treatment promoted greater in situ degradability of BH when compared to the non-treated forage. The treatment difference is more prominent after 48, and up to 72 h of ruminal incubation, where, CO10 promoted the greatest increase in degradability of DM, OM, NDF and ADF, followed by CO5-7 and CO5-14, which were not different. Crude protein degradability was increased after 24 h incubation in the rumen only when CO10 treatment was applied whereas, after 72 h of incubation in the rumen, CP degradability was increased by treating the forage with either 5 or 10% CaO. At 24h of incubation in the rumen, it was observed that OM degradability was similar between CO10 and CO5-7; however, CO10 was greater than CO5-14. It is speculated that, even though mold counts were not performed in the current study, the visual presence of mold after 14 d of bucket incubation and lack of it at 7 d of bucket incubation could explain this difference. This negative effect that could have been caused by mold was probably diluted by the longer incubation in the rumen for 48 or 72 h, as indicated by the greater degradability of OM when CaO was applied at 10% when compared to 5%, regardless of bucket incubation for 7 or 14 d.

In vitro organic matter digestibility of BH increased ($P < 0.001$) with CaO treatment and was greatest when CO10 was applied, promoting a 40% increase when compared to DH and a 31% increase when compared to 5%. Although to a smaller
extent, applying CaO at 5% promoted an increase of 10% in IVOMD of BH when compared to not treating the forage.

Nutrient composition of BM before and after treatment with CaO and incubation for either 7 or 14 d is presented in Table 2-5. Similar to BH, besides CP content, which was not affected by treatment ($P = 0.096$), OM, NDF, and ADF contents were all reduced ($P < 0.001$) when CaO was applied compared to not treating the forage. Greatest reduction (36%) in NDF content was observed when CO10 was applied, followed by 5% CaO (18%), regardless of days of incubation.

Ruminal in situ degradability of nutrients and IVOMD of BM treated or not with CaO and incubated for either 7 or 14 d are presented in Table 2-6. At all ruminal incubation time points, a treatment effect was observed ($P < 0.001$) for degradability of DM, OM, CP, NDF, and ADF. Differently from BH, the effects of CaO can already be noticed after 24 h of ruminal incubation on nutrient degradability. At 24 h, CO10 promoted greater degradability of DM, OM, and CP, when compared to 5% CaO application rate; however, NDF and ADF degradability were not different among CO10, CO5-7, and CO5-14. At 48 and 72 h, no differences were observed on degradability of DM, OM, CP, NDF, and ADF between CO10 and CO5.

Similar to BH, IVOMD of BM was increased ($P < 0.001$) when CaO was applied to the forage by 23%; however, no differences were observed between CO10 and CO5, regardless of days of incubation. These results indicate that the use of a greater amount of CaO is beneficial to improve degradability of nutrients and IVOMD of BH to a greater extent; however, benefits do not apply to BM and 5% CaO should be sufficient.
The results observed in this study with BH and BM are in accordance with previous research where reduction in NDF content, and improved NDF digestibility and in vitro digestibility of corn stalks, oat hulls, and wheat straw were observed when residues were treated with 5% CaO and moistened to reach a 50% DM content (Dahlke and Euken, 2013b). Furthermore, when treating corn cobs, wheat straw, and corn stalks with CaO at 5% of the residue DM and adding water to reach a 50% DM content, increases in in vitro OM digestibility by 26, 49, and 41% were observed, respectively (Shreck, 2013).

The presence of water is necessary in order for the CaO to be converted into Ca(OH)$_2$ thus, the addition of treatments containing just water was necessary to exclude its possible confounding effects. No effects of water addition compared to dry treatment were expected, and a few exceptions that were observed between W treatments and DH are speculated to be attributed to minimal fermentation that might have occurred without the presence of a strong base such as CaO. Length of incubation to allow reaction to occur between forage and chemical treatment has been debated among researchers and 7 or 14 d were chosen in the current study based on the most common reported when treating crop residues (Shreck et al., 2011; Shreck et al., 2012). Research comparing IVDMD of corn stalks treated with 5% CaO at 3, 7, or 14 d after treatment showed that no changes were observed after 3 d of treatment and an increase in digestibility did not occur until 7 d after treatment in most cases; however, a small numerical increase was observed when IVDMD was performed after 14 d of treatment (Euken et al., 2013). These results could indicate that in order for treatment to be effective a minimum of 7 d of incubation is necessary and maybe longer periods can
promote greater effects; however, results were inconsistent since there was considerable variation in the IVDMD of treated samples (Euken et al., 2013). Conversely, when residues were treated with 5% CaO and stored for either 7, 14, or 28 d, no differences were observed between 7 and 14 d for corn cobs; however, incubating the residue for 28 d reduced IVDMD by 6%, whereas for wheat straw, incubation for 14 d increased IVDMD by 11%, when compared to incubation for 7 d, while it was not different from 28 d of incubation (Shreck, 2013). These results indicate that the length of incubation can be variable depending upon the type and composition of forage.

In conclusion, treating peanut hulls with CaO was not effective at improving in situ ruminal degradability of nutrients. Additionally, regardless of moisture content, when peanut hulls were treated with CaO and incubated for more than 6 d, IVOMD was negatively affected. However, CaO treatment of warm-season forages common to the southeastern US, such as bahiagrass and bermudagrass hay, proved to be an efficient method of promoting greater digestibility of nutrients when applied at 5% of the forage DM and in some cases even greater when applied at a 10% rate, which in turn can potentially result in improved animal performance when forage based diets are provided. Moreover, treating the forages for longer than 7 d seemed to be unnecessary, since, in general, no differences between 7 and 14 d were observed. Further in vivo studies will be performed in order to evaluate the effects of treating warm-season forages with CaO on beef cattle ruminal fermentation parameters, total tract digestibility of nutrients, performance, and methane emissions.
Table 2-1. Nutrient composition of peanut hulls (PHS) after treatment with calcium oxide (CaO) and incubation for either 7 or 14 days.

<table>
<thead>
<tr>
<th>Item</th>
<th>D</th>
<th>W7</th>
<th>W14</th>
<th>CO7</th>
<th>CO14</th>
<th>SEM²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM, % DM</td>
<td>94.9</td>
<td>91.9</td>
<td>96.5</td>
<td>90.3</td>
<td>89.8</td>
<td>3.03</td>
<td>0.525</td>
</tr>
<tr>
<td>CP, % DM</td>
<td>8.1</td>
<td>11.1</td>
<td>8.4</td>
<td>8.1</td>
<td>8.7</td>
<td>0.81</td>
<td>0.199</td>
</tr>
<tr>
<td>NDF, % DM</td>
<td>82.5</td>
<td>76.9</td>
<td>82.4</td>
<td>73.2</td>
<td>77.2</td>
<td>4.53</td>
<td>0.593</td>
</tr>
<tr>
<td>ADF, % DM</td>
<td>71.9</td>
<td>68.3</td>
<td>70.4</td>
<td>63.1</td>
<td>66.1</td>
<td>4.45</td>
<td>0.675</td>
</tr>
</tbody>
</table>

¹D = PHS as is (dry); W7 = PHS at 50% DM incubated for 7 d; W14 = PHS at 50% DM incubated for 14 d; CO7 = PHS at 50% DM + 5% CaO incubated for 7 d; and CO14 = PHS at 50% DM + 5% CaO incubated for 14 d.
²n = 2 buckets/treatment.
Table 2-2. Ruminal in situ nutrient degradability of peanut hulls (PHS) treated or not with calcium oxide (CaO) and incubated for either 7 or 14 days.

<table>
<thead>
<tr>
<th>Item&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Treatment&lt;sup&gt;1&lt;/sup&gt;</th>
<th>D</th>
<th>W7</th>
<th>W14</th>
<th>CO7</th>
<th>CO14</th>
<th>SEM&lt;sup&gt;3&lt;/sup&gt;</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMD, %</td>
<td></td>
<td>24h</td>
<td>16.2</td>
<td>16.0</td>
<td>27.7</td>
<td>23.5</td>
<td>5.21</td>
<td>0.472</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48h</td>
<td>16.3</td>
<td>16.5</td>
<td>28.9</td>
<td>24.8</td>
<td>5.56</td>
<td>0.456</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72h</td>
<td>16.4</td>
<td>17.5</td>
<td>29.1</td>
<td>24.3</td>
<td>5.49</td>
<td>0.485</td>
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<tr>
<td>OMD, %</td>
<td></td>
<td>24h</td>
<td>13.1</td>
<td>14.5</td>
<td>22.2</td>
<td>17.8</td>
<td>4.10</td>
<td>0.526</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48h</td>
<td>13.1</td>
<td>14.9</td>
<td>23.5</td>
<td>18.9</td>
<td>4.53</td>
<td>0.500</td>
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<tr>
<td></td>
<td></td>
<td>72h</td>
<td>13.3</td>
<td>15.8</td>
<td>23.4</td>
<td>18.4</td>
<td>4.55</td>
<td>0.545</td>
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<tr>
<td>NDFD, %</td>
<td></td>
<td>24h</td>
<td>3.0</td>
<td>3.3</td>
<td>6.0</td>
<td>5.8</td>
<td>2.25</td>
<td>0.452</td>
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<td>48h</td>
<td>2.8</td>
<td>3.4</td>
<td>7.1</td>
<td>6.6</td>
<td>2.45</td>
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</tr>
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<td></td>
<td></td>
<td>72h</td>
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<td>4.6</td>
<td>7.2</td>
<td>5.7</td>
<td>2.33</td>
<td>0.391</td>
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<td>ADFD, %</td>
<td></td>
<td>24h</td>
<td>1.9</td>
<td>1.6</td>
<td>3.7</td>
<td>4.5</td>
<td>2.02</td>
<td>0.275</td>
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<td></td>
<td></td>
<td>48h</td>
<td>1.9</td>
<td>1.5</td>
<td>4.7</td>
<td>4.9</td>
<td>1.98</td>
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<td>72h</td>
<td>2.1</td>
<td>2.1</td>
<td>4.9</td>
<td>4.2</td>
<td>2.05</td>
<td>0.258</td>
</tr>
</tbody>
</table>

<sup>1</sup>D = PHS as is (dry); W7 = PHS at 50% DM incubated for 7 d; W14 = PHS at 50% DM incubated for 14 d; CO7 = PHS at 50% DM + 5% CaO incubated for 7 d; and CO14 = PHS at 50% DM + 5% CaO incubated for 14 d.

<sup>2</sup>DMD = in situ DM degradability; OMD = in situ OM degradability; NDFD = in situ NDF degradability; and ADFD = in situ ADF degradability.

<sup>3</sup>n = 2 buckets/treatment.
Table 2-3. Nutrient composition of bahiagrass (*Paspalum notatum*) hay (BH) after treatment with calcium oxide (CaO) and incubation for either 7 or 14 days.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>OM, % DM</th>
<th>CP, % DM</th>
<th>NDF, % DM</th>
<th>ADF, % DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DH</td>
<td>W7</td>
<td>W14</td>
<td>CO5-7</td>
<td>CO5-14</td>
</tr>
<tr>
<td>OM, % DM</td>
<td>95.1(^c)</td>
<td>91.9(^b)</td>
<td>94.6(^c)</td>
<td>90.9(^b)</td>
<td>90.6(^b)</td>
</tr>
<tr>
<td>CP, % DM</td>
<td>8.7</td>
<td>9.2</td>
<td>9.1</td>
<td>8.4</td>
<td>8.4</td>
</tr>
<tr>
<td>NDF, % DM</td>
<td>74.3(^c)</td>
<td>74.6(^c)</td>
<td>75.8(^c)</td>
<td>70.3(^b)</td>
<td>67.8(^b)</td>
</tr>
<tr>
<td>ADF, % DM</td>
<td>37.0(^b)</td>
<td>39.4(^d)</td>
<td>38.9(^cd)</td>
<td>38.3(^bcd)</td>
<td>37.4(^bc)</td>
</tr>
</tbody>
</table>

\(^a, b, c, d\) Within a row, means with different superscripts differ, \(P \leq 0.05\)

\(^1\)DH = BH as is (dry); W7 = BH at 50% DM incubated for 7 d; W14 = BH at 50% DM incubated for 14 d; CO5-7 = BH at 50% DM + 5% CaO incubated for 7 d; CO5-14 = BH at 50% DM + 5% CaO incubated for 14 d; and CO10 = BH at 50% DM + 5% CaO incubated for 14 d.

\(^2\)\(n = 4\) buckets/treatment.
Table 2-4. Ruminal in situ nutrient degradability and in vitro organic matter digestibility (IVOMD) of bahiagrass (*Paspalum notatum*) hay (BH) treated or not with calcium oxide (CaO) and incubated for either 7 or 14 days.

<table>
<thead>
<tr>
<th>Item²</th>
<th>Treatment¹</th>
<th>DMD, %</th>
<th>W7</th>
<th>W14</th>
<th>CO5-7</th>
<th>CO5-14</th>
<th>CO10</th>
<th>SEM³</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24h</td>
<td>43.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>35.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.0&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>51.6&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>61.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.07</td>
</tr>
<tr>
<td></td>
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<td>54.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>72.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
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<td>72h</td>
<td>58.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>77.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.87</td>
</tr>
<tr>
<td>OMD, %</td>
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<td>24h</td>
<td>40.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>33.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.2&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>48.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.5&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>58.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.78</td>
</tr>
<tr>
<td>CPD, %</td>
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<td>40.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>53.5&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>78.5&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>54.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.13</td>
</tr>
<tr>
<td>NDFD, %</td>
<td></td>
<td>24h</td>
<td>36.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>30.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>48.6&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>41.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>57.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.14</td>
</tr>
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<td>50.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>55.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>88.0&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>ADFD, %</td>
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<td>24h</td>
<td>38.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>31.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.9&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>44.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.0&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>59.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.0&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>IVOMD, %</td>
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<td>33.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.16</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

<sup>a, b, c, d</sup> Within a row, means with different superscripts differ, \( P \leq 0.05 \).

¹DH = BH as is (dry); W7 = BH at 50% DM incubated for 7 d; W14 = BH at 50% DM incubated for 14 d; CO5-7 = BH at 50% DM + 5% CaO incubated for 7 d; CO5-14 = BH at 50% DM + 5% CaO incubated for 14 d; and CO10 = BH at 50% DM + 5% CaO incubated for 14 d.

²DMD = in situ DM degradability; OMD = in situ OM degradability; CPD = in situ CP degradability; NDFD = in situ NDF degradability; and ADFD = in situ ADF degradability.

³\( n = 4 \) buckets/treatment.
Table 2-5. Nutrient composition of Tifton 85 bermudagrass (*Cynodon spp.*) hay (BM) after treatment with calcium oxide (CaO) and incubation for either 7 or 14 days.

<table>
<thead>
<tr>
<th>Item</th>
<th>DH</th>
<th>W7</th>
<th>W14</th>
<th>CO5-7</th>
<th>CO5-14</th>
<th>CO10</th>
<th>SEM²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM, % DM</td>
<td>93.7</td>
<td>93.3</td>
<td>93.0</td>
<td>86.7</td>
<td>88.2</td>
<td>84.1</td>
<td>0.57</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CP, % DM</td>
<td>11.1</td>
<td>14.1</td>
<td>14.0</td>
<td>14.0</td>
<td>14.4</td>
<td>13.0</td>
<td>0.26</td>
<td>0.096</td>
</tr>
<tr>
<td>NDF, % DM</td>
<td>77.1</td>
<td>74.7</td>
<td>72.0</td>
<td>63.6</td>
<td>63.4</td>
<td>49.2</td>
<td>0.83</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ADF, % DM</td>
<td>42.0</td>
<td>39.4</td>
<td>37.8</td>
<td>36.9</td>
<td>36.8</td>
<td>33.2</td>
<td>0.40</td>
<td>&lt; 0.001</td>
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</tbody>
</table>

Within a row, means with different superscripts differ, $P \leq 0.05$

1DH = BM as is (dry); W7 = BM at 50% DM incubated for 7 d; W14 = BM at 50% DM incubated for 14 d; CO5-7 = BM at 50% DM + 5% CaO incubated for 7 d; CO5-14 = BM at 50% DM + 5% CaO incubated for 14 d; and CO10 = BM at 50% DM + 5% CaO incubated for 14 d.

2$n = 4$ buckets/treatment
Table 2-6. Ruminal in situ nutrient degradability and in vitro organic matter digestibility (IVOMD) of Tifton 85 bermudagrass (*Cynodon* spp.) hay (BM) treated or not with calcium oxide (CaO) and incubated for either 7 or 14 days.

<table>
<thead>
<tr>
<th>Item&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Treatment&lt;sup&gt;1&lt;/sup&gt;</th>
<th>DH</th>
<th>W7</th>
<th>W14</th>
<th>CO5-7</th>
<th>CO5-14</th>
<th>CO10</th>
<th>SEM&lt;sup&gt;3&lt;/sup&gt;</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMD, %</td>
<td>24h</td>
<td>46.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.26</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>48h</td>
<td>54.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>63.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>77.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86.1&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>72h</td>
<td>58.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.92</td>
<td>&lt; 0.0001</td>
</tr>
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<td>44.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.2&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
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<td>53.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.9&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>77.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>85.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.87</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>72h</td>
<td>54.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.4&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>90.4&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>&lt; 0.001</td>
</tr>
<tr>
<td>CPD, %</td>
<td>24h</td>
<td>65.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.2&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>&lt; 0.001</td>
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<td>48h</td>
<td>70.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>75.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>78.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89.2&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>&lt; 0.001</td>
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<td>81.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.1&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>&lt; 0.001</td>
</tr>
<tr>
<td>NDFD, %</td>
<td>24h</td>
<td>38.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.1&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>36.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.0&lt;sup&gt;bc&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>48h</td>
<td>45.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.23</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>72h</td>
<td>48.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>59.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>73.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>78.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.12</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IVOMD, %</td>
<td>50.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.65</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a, b, c</sup> Within a row, means with different superscripts differ, *P* ≤ 0.05

<sup>1</sup>DH = BM as is (dry); W7 = BM at 50% DM incubated for 7 d; W14 = BM at 50% DM incubated for 14 d; CO5-7 = BM at 50% DM + 5% CaO incubated for 7 d; CO5-14 = BM at 50% DM + 5% CaO incubated for 14 d; and CO10 = BM at 50% DM + 5% CaO incubated for 14 d.

<sup>2</sup>DMD = in situ DM degradability; OMD = in situ OM degradability; CPD = in situ CP degradability; NDFD = in situ NDF degradability; and ADFD = in situ ADF degradability.

<sup>3</sup>n = 4 buckets/treatment
Figure 2-1. In vitro organic matter digestibility (IVOMD) of peanut hulls (PHS) treated or not with 5% calcium oxide (CaO), different DM contents (70 or 50%), and incubated in buckets for 3, 6, 9, 12, or 15 days. 70DM = PHS + water (to 70% DM); 70DMCO = PHS + water (to 70% DM) + 5% CaO (DM basis); 50DM = PHS + water (to 50% DM); 50DMCO = PHS + water (to 50% DM) + 5% CaO (DM basis). Tendencies for CaO × day (P = 0.069) and DM × CaO × day (P = 0.098) interaction were observed. Error bars represent SEM; n = 2 buckets/treatment. † At 70DMCO, IVOMD of PHS increased up to 6 d of incubation (P = 0.005); ‡ At 70DMCO, after 6 d of incubation and up to 15 d IVOMD of PHS was reduced (P < 0.001); £ At 50DMCO, IVOMD of PHS decreased as days of incubation increased up to 15 d (P = 0.038).
CHAPTER 3
INTAKE, RUMINAL FERMENTATION PARAMETERS, AND APPARENT TOTAL-TRACT DIGESTIBILITY OF BEEF STEERS CONSUMING BAHIAGRASS HAY TREATED WITH CALCIUM OXIDE

Introduction

Warm-season forages commonly found in Florida and southern states of the US can be of low nutritive value due to a lack of CP and energy, the latter most likely as a consequence of high fiber contents that are of poor digestibility (Vendramini, 2010). Therefore, forages such as bahiagrass hay most often do not offer adequate nutrients to beef cattle when provided as the only ingredient in forage-based diets.

Alkali treatment of low-quality forages to improve the digestibility of the fiber is an effective, but not new, concept that has been extensively researched since the 1970’s, when chemicals such as NaOH were very popular to treat crop residues (e.g. corn cobs, stalks, and wheat straw; Klopfenstein et al., 1972; Klopfenstein, 1978). More recently, researchers have tested different alternatives to NaOH due to animal, environmental, and handling safety concerns, thus chemicals such as Ca(OH)$_2$ and CaO have been given more attention providing similar increases in forage digestibility (Shreck et al., 2011; Dahlke and Euken, 2013; Spore et al., 2015). However, most of the recent research has evaluated the effects of treated forages in mixed finishing and backgrounding diets as a partial replacement for corn (Chapple et al., 2015; Peterson et al., 2015b; Shreck et al., 2015).

Therefore, it was hypothesized that treatment of bahiagrass hay (*Paspalum notatum*) with the alkali CaO would be an effective method to improve the digestibility of the forage fiber, thus increasing total tract digestibility of nutrients; furthermore, increased ruminal forage digestibility would increase total VFA concentrations. The
objective of this experiment was to evaluate the effects of bahiagrass hay treated with 5% CaO on ruminal fermentation parameters, total tract digestibility of nutrients, and intake of beef steers consuming the hay as the only ingredient in the diet ad libitum. An additional objective was to assess effectiveness of CaO treatment by determining IVOMD of the bahiagrass hay treated or not provided to steers.

**Materials and Methods**

All procedures involving animals were approved by the University of Florida Institutional Animal Care and Use Committee (Protocol # 201508733).

**Experimental Design, Animals, and Treatments**

The experiment was conducted at the University of Florida – North Florida Research and Education Center Feed Efficiency Facility (UF – NFREC FEF) in Marianna, FL. Nine ruminally-cannulated Angus-crossbred steers (494 ± 145 kg of BW; average BW ± SD) were used in a triplicated 3 × 3 Latin square design. Steers were housed at the UF - NFREC FEF (3 steers/pen), where daily individual intake was recorded via the GrowSafe system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). The steers had ad libitum access to their diets and were randomly assigned to 1 of 3 treatments: 1) bahiagrass hay as is (n = 8; DH); 2) bahiagrass hay treated with 8.9% CaCO₃ (DM basis) + water (to 50% DM; n = 9; CC); or 3) bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM; n = 8; CO). Calcium carbonate was used in treatment 2 in an attempt to provide the same amount of Ca as treatment 3 and exclude any potential confounding factors. In each of the three 28-d periods, d 0 to 14 were for adaptation to the facility and treatments, d 14 to 21 were for collections and d 21 to 28 for washout, when steers only received untreated bahiagrass hay.
Treatment of Bahiagrass Hay

Bahiagrass hay from round bales was chopped using a Tub Grinder (Haybuster, Jamestown, ND) to reduce particle size into a vertical feed mixer (Roto-Mix VXT-425, Dogde City, Kansas). Using the scale on the mixer, half of the water needed was added with a fire hose and the previously weighed amount of CaCO₃ (CALCOR, minimum 36% Ca, Furst McNess Co., Cordele, GA) or CaO (Standard Quicklime, 94.5% available CaO, Mississippi Lime) was applied to the wet hay. The mixer was turned on and the remainder of the water was added. The hay was mixed for approximately 15 minutes to ensure a proper homogenization of contents. Wet treated hay was square baled (Hesston 4550, Massey Ferguson, Duluth, GA) and bales were placed into 250-L trash bags (2 mil thickness), which were evacuated with the aid of a shop vacuum and zip tied in an attempt to provide an anaerobic environment. Bales were allowed to sit for at least 7 d but no longer than 14 d before feeding to the steers. Dry hay was also chopped and square baled to facilitate feeding process and provide similar particle size to treated hay. Core samples were taken from each bale before feeding to steers and composited within treatment in each period. Samples were sent to a commercial laboratory (Dairy One, Ithaca, NY) for chemical analysis. Chemical composition of hay in each treatment provided to steers is presented in Table 3-1.

Sampling Procedures

All protocols and procedures used for collecting samples and data from animals and laboratory analyses were used in an identical manner throughout all 3 periods.

Ruminal fluid, pH, and blood sampling

Ruminal fluid and blood were collected on d 14 of each period at 0700h and every 3 h after for a total of 24 h. Ruminal fluid was strained from a representative
sample of digesta through 4 layers of cheesecloth and pH was immediately measured using a manual pH meter (Corning Pinnacle M530, Corning Inc., Corning, NY). A 10-mL sample of the ruminal fluid was taken into a 15-mL conical tube and 0.1 mL of a 20% (vol/vol) H$_2$SO$_4$ solution was added to stop fermentation. Samples of ruminal fluid were immediately placed on ice and subsequently frozen and stored at -20°C for further analysis of NH$_3$-N and VFA.

Blood samples were collected via jugular venipuncture into 10-mL evacuated tubes containing Na heparin (BD Vacutainer, Franklin Lakes, NJ), immediately placed on ice, and subsequently centrifuged for 15 min at 1,500 × g at 4°C. Plasma was then transferred to polypropylene vials (12 × 75 mm; Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA) and stored at -20°C for further analysis of blood urea nitrogen (BUN).

**Apparent total tract digestibility**

Apparent total tract digestibility of nutrients was determined using indigestible NDF (iNDF) as an internal marker (Cole et al., 2011; Krizsan and Huhtanen, 2013). Hay samples were collected daily from d 17 to 20 and fecal samples were collected at 0700 and 1600 h from d 18 to 21 either by rectal grab or from the ground, inside the pen, immediately after the animal defecated. Hay and fecal samples were stored at -20°C for further analysis. At the end of the experiment, frozen hay and fecal samples were dried at 55°C for 72 h in a forced-air oven and ground in a Wiley mill (Arthur H. Thomas Co. Philadelphia, PA) to pass a 2-mm screen. Hay was pooled within pen and feces were pooled within steer to determine nutrient content and digestibility marker concentration.
Laboratory Analyses

Volatile fatty acid profile

Concentrations of VFA in the ruminal fluid samples were determined in a water-based solution using ethyl acetate extraction. Samples were centrifuged at 10,000 × g for 10 min at 4°C (Avanti J-E, Beckman Coulter Inc.). Two milliliters of the supernatant was mixed with 0.4 mL (5:1 ratio) of a metaphosphoric:crotonic acid (internal standard) solution and samples were frozen overnight. Samples were then thawed and centrifuged again at 10,000 × g for 10 min at 4°C. Supernatant was transferred into 12 mm × 75 mm borosilicate disposable culture tubes (Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA) and mixed with ethyl acetate to form a 2:1 ethyl acetate:supernatant mixture. After shaking tubes vigorously and a 5-min rest to allow separation, the ethyl acetate fraction (top layer) was transferred to small glass vials and capped. Samples were analyzed with a gas chromatograph (Agilent 7820A GC, Agilent Technologies) using a flame ionization detector and a capillary column (CP-WAX 58 FFAP 25 m × 0.53 mm, Varian CP7767; Varian Inc.). Column temperature was maintained at 110°C, and the injector and detector temperatures were 200 and 220°C, respectively.

Ammonia-N and blood urea N concentrations

Concentrations of NH₃-N in the ruminal fluid were measured following the phenol-hypochlorite technique as described by Broderick and Kang (1980). Briefly, ruminal fluid samples were centrifuged at 10,000 × g for 15 min at 4°C (Avanti J-E, Beckman Coulter Inc.) and 20-μL aliquot of the supernatant was then transferred into 12 × 75 mm borosilicate disposable culture tubes (Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA), where 1 mL of a phenol reagent added. After vortexing, 0.8 mL of a
hypochlorite solution was added to the mixture and vortexed again. The culture tubes were then covered with glass marbles and placed in a water bath at 95°C for 5 min. The only modification to the original protocol was that absorbance was read in 96-well, flat bottom plates at 620 nm using a plate reader (DU 500; Beckman Coulter Inc.).

Plasma was analyzed for BUN using a quantitative colorimetric kit (B7551-120; Pointe Scientific Inc., Canton, MI).

**Concentration of DM, OM, CP, NDF, and ADF**

All hay and fecal samples were placed in a 55°C forced air oven for 72 h to obtain dry samples. Dry, hot weight was used to calculate DM of the sample. The sample was then ground to pass through a 2-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ). To determine OM, 0.5 g of ground sample (in duplicate) was weighed into ceramic crucibles and placed in a 105°C forced air oven for 24 h to determine sample DM. Dried samples were then placed in a 650°C muffle furnace for 6 h before returning to a 105°C forced air oven. Hot, ashed samples were weighed and used to calculate OM.

Samples of hay and feces were weighed (0.5 g in duplicate) into F57 bags (Ankom Technology Corp., Macedon, NY) and analyzed for NDF, using heat stable α-amylase and sodium sulfite. Subsequent ADF analysis was performed sequentially as described by Van Soest et al. (1991) in an Ankom 200 Fiber Analyzer (Ankom Technology Inc.).

Hay and fecal samples were analyzed for total N using a C, H, N, and S analyzer by the Dumas dry combustion method (Vario Micro Cube; Elementar, Hanau, Germany). Crude protein was calculated by multiplying the N concentration of the dry sample by 6.25.
Concentration of iNDF

The concentration of iNDF in hay and feces was determined as described by Gregorini et al. (2008), Cole et al. (2011), and Krizsan and Huhtanen (2013). Briefly, 0.5 g of sample was weighed into Ankom F57 filter bags (Ankom Technology Corp. Macedon, NY) and then incubated into the rumen of a cannulated steer grazing a bahiagrass and bermudagrass mixed pasture for 288 h to ensure complete digestion of potentially digestible NDF. After incubation, samples were rinsed 2 times with tap water followed by four rinses with water filtered through a reverse osmosis system. The rinsed samples were then analyzed for NDF as previously described.

In vitro OM digestibility

In order to test the effectiveness of bahiagrass treatment with CaO, a modified Tilley and Terry (1963) procedure was used to determine IVOMD of bahiagrass hay provided to steers. Briefly, 0.7 g of dried and ground (2 mm) hay samples collected during the 4-d digestibility period from each treatment provided in each of the three periods were incubated with 50 mL of a 4:1 McDougall’s buffer:ruminal fluid inoculum in 100-mL plastic centrifuge tubes for 48 h under constant agitation (60 rpm) at 39°C. Two tubes per treatment per period and two blank (without substrate or treatment) tubes were incubated in each of the three separate replicate days (n = 3/treatment). After the initial 48 h, 6 mL of HCl was added to the tubes along with 2 mL of a 5% pepsin solution. Tubes were then incubated for an additional 48 h under constant agitation (60 rpm) at 39°C. Samples were then filtered through P8 filters (Fisherbrand; Thermo Fisher Scientific Inc.). Filters with wet samples were then dried at 105°C in a forced air oven for 24 h to determine IVDMD. Dry filters with residual samples were then placed in a muffle furnace for 6 h at 650°C. The ash was then placed in a 105°C oven for 24 h prior to
recording weight. Two ruminally cannulated steers consuming bahiagrass hay for at least 14 d were used as ruminal fluid donors.

Calculations

Apparent total tract digestibility of DM, OM, CP, NDF, and ADF were calculated as follows:

\[
\text{Nutrient digestibility (\%) = 100} - \{100 \times [(\text{iNDF concentration in the hay} ÷ \text{iNDF concentration in the feces}) \times (\text{nutrient concentration in the feces} ÷ \text{nutrient concentration in the hay})]\}.
\]

Statistical Analysis

Data were analyzed as a triplicated 3 × 3 Latin square with repeated measures for blood and ruminal fermentation parameters (BUN, NH$_3$-N, ruminal pH, and VFA) using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). For hay DMI and total tract digestibility of nutrients, the model included the fixed effects of treatment, period, and square and the random effect of animal within square. For parameters analyzed using repeated measures, the model included the fixed effects of treatment, time, their interaction, square, and period. Animal within square and animal within treatment were included as random effects, with the latter used to designate the denominator degrees of freedom. Animal within period was considered the subject and the covariance structures used were compound symmetry for pH and molar proportions of propionate and first order autoregressive for all the remainder parameters. Covariance structures were chosen based on the smallest Akaike information criterion value. For IVOMD, the average of six tubes per treatment within day of incubation was considered the experimental unit and were analyzed as a randomized complete block design. The
model included the fixed effect of treatment and the random effect of day of incubation (block). Significance was declared at $P \leq 0.05$.

**Results and Discussion**

Chemical composition of hay provided to steers is presented in Table 3-1. As expected, Ca concentration is notably greater in CC and CO, compared to DH, thus likely diluting others components such as lignin, NDF, and ADF on the DM basis. Therefore, the numerical reductions of those components in the treated forages are not necessarily due to treatment but an artifact of the Ca addition.

Total DMI, daily average ruminal fermentation parameters, and BUN concentrations of beef steers are presented in Table 3-2. There were no differences among treatments on daily DMI in kg ($P = 0.759$) or as a percentage of BW ($P = 0.674$). Nevertheless, intake was considerably low across treatments, likely due to increased retention time in the rumen caused by the poor quality and high fiber content of the hay, thus the animal reached a control of intake via gut fill (Mertens, 2010). Accordingly, it has been reported that DMI of low-quality C$_4$ forages is almost never maximized without protein supplementation and is usually less than 1% of BW (Bohnert et al., 2011), while increases from 30 to 100% have been observed with protein supplementation when comparing to non-supplemented controls (Köster et al., 1996; Mathis et al., 1999).

A treatment effect ($P < 0.001$) was observed for average ruminal pH, where steers consuming bahiagrass treated with CaO had a greater ruminal pH when compared to steers consuming the other two treatments, which were not different. These results were expected since CaO is a strong alkali (pH ~ 11) and most likely promoted a buffering effect leading to the increase in pH. Buffering capacity of CaO has been reported when the chemical was provided in diets containing 60% dried distillers
grains with solubles (Nuñez et al., 2014). The authors hypothesized that the inclusion of CaO would decrease feed acidity and consequently increase ruminal pH; however, what was observed was that animals who were consuming diets with the greatest inclusion of CaO (2.4%) exhibited a stable ruminal pH throughout a 24-h period when compared to steers who did not consume any CaO and had the greatest decline in ruminal pH post feeding (Nuñez et al., 2014). Contrastingly, other research providing 25% crop residues (corn cobs, wheat straw, and corn stalks) treated with CaO as a replacement for corn in finishing diets, reported no differences in ruminal pH when compared to control diets without treated residues (Shreck et al., 2013a). The authors suggested a possible dilution of the alkali in the diets of finishing animals, in which ruminal pH is already more acidic, thus CaO was not present in the rumen in concentrations great enough to act as a strong buffer (Shreck et al., 2013a). Interestingly, reductions in ruminal pH have been reported when feedlot finishing diets containing 20% corn stover treated with 5% CaO as a replacement for corn were fed to steers compared to control diets without the treated residue (Chapple et al., 2015). The authors attributed this decrease in pH to the increase in total VFA in the rumen that was also observed (Chapple et al., 2015).

Ruminal concentrations of NH$_3$-N were not different ($P = 0.059$) among treatments; however, concentrations across treatments (average of 0.45 mM) are well below the value of 3.57 mM repeatedly cited as a minimum concentration to maximize microbial protein synthesis (Satter and Slyter, 1974). Even though it has been suggested that 3.57 mM is more of a margin of excess and, concentrations of NH$_3$-N closer to 1.43 mM are perhaps more realistic to be the precise limiting concentration (Satter and Slyter, 1974), the values observed in the current experiment are still 68%
below this value. A time effect \( (P < 0.001) \) was observed for concentrations of \( \text{NH}_3\)-N; however, there was no treatment \( \times \) time interaction \( (P = 0.522). \) Nitrogen is required for microbial growth and, to determine when it is likely to become limiting for such activity, \( \text{NH}_3\)-N concentration in the rumen, resulting from deamination of amino acids provided by dietary CP, is a better parameter than dietary CP alone (Slyter et al., 1979). When there is a deficiency of dietary CP, ruminal \( \text{NH}_3\) concentrations are relatively low, thus the concentrations of \( \text{NH}_3\)-N observed in the current study indicate that not only the hay provided to the animals did not have adequate CP content but most importantly, protein supplementation would have been beneficial to increase ruminal concentrations of \( \text{NH}_3\)-N to adequate levels required for microbial growth and activity.

Concentrations of BUN were not affected by treatment \( (P = 0.517) \) or time \( (P = 0.319) \); however, a treatment \( \times \) time interaction was observed \( (P = 0.023; \) Figure 3-1). No biological significance was attributed to this interaction. For maximal rates of gain, concentrations of BUN have been reported to be between 11 and 15 mg/dL in growing steers (Byers and Moxon, 1980), while in finishing steers concentrations between 7 and 8 mg/dL have been associated with maximum performance (Preston et al. 1978). Regardless, the values observed in the present study are relatively low and concentrations of BUN are below those reported in the literature, once again indicating that the hay alone did not provide adequate CP and supplementation would have been beneficial.

Molar proportions of VFA are presented in Table 3-3. With the exception of butyrate molar proportions, which was affected by treatment \( (P = 0.002) \), no treatment differences \( (P \geq 0.069) \) were observed on molar proportions of acetate, propionate,
branched-chain VFA (BCVFA), and valerate. Nevertheless, there was a treatment × time interaction on molar proportions of acetate ($P < 0.001$; Figure 3-2), propionate ($P < 0.001$; Figure 3-3), and valerate ($P = 0.010$). Concentrations of VFA are presented in Table 3-4. Total VFA ($P = 0.021$), acetate ($P = 0.035$), propionate ($P = 0.023$), butyrate ($P = 0.008$), BCVFA ($P = 0.028$), and valerate ($P = 0.026$) concentrations were all reduced in steers consuming bahiagrass treated with CaO, when compared to CC, and DH, which were not different. A treatment × time interaction was observed ($P < 0.001$) for acetate to propionate ratio (A:P; Figure 3-4). Although no differences were observed on molar proportions of VFA, other than butyrate, the reduction in VFA concentrations clearly indicate a reduction in ruminal fermentation and is also a plausible explanation for the increase in ruminal pH observed in steers consuming bahiagrass hay treated with CaO.

Daily nutrient intake, apparent total tract digestibility of steers, and IVOMD of hay provided to steers are presented in Table 3-5. With the exception of CP, which was affected by treatment ($P = 0.042$), no treatment differences were observed on intake of DM ($P = 0.385$), OM ($P = 0.628$), NDF ($P = 0.696$), and ADF ($P = 0.613$). Although CP intake was affected by treatment, the difference was observed in steers consuming bahiagrass hay treated with CaCO$_3$ (CC), which had greater CP intake when compared to steers consuming DH and CO, while these were not different. A possible explanation for the difference in CP intake could be that, even though numerically, steers consuming CC had greater intake of DM and OM, and CC hay had a greater CP content when compared to DH and CO, both of which could have caused a significant difference in CP intake, not the actual treating of the forage with CC. No treatment effect was
observed for apparent total tract digestibility of DM ($P = 0.186$), OM ($P = 0.169$), and CP ($P = 0.152$); however, total tract digestibility of NDF ($P = 0.034$) and ADF ($P = 0.003$) were reduced in steers consuming both treated hay (CC and CO), when compared to steers consuming non treated hay (DH). There was no difference between CC and CO, indicating that what caused the reduction in fiber digestibility was not necessarily the treatment with CaO. Nonetheless, the tendency for reduction in ruminal concentration of NH$_3$-N, which could explain lack of ability of fibrolytic microorganisms to digest fiber, and reduction in ruminal concentrations of VFA, which confirms reduction in fermentation, both caused by CaO treatment, point to the fact that CaO could be negatively affecting fiber digestion in the rumen.

Conversely, and similar to results presented and described in Chapter 2, IVOMD of bahiagrass hay treated with CaO (CO) was greater when compared to CC and DH ($P = 0.013$). These results suggest that CaO may be affecting something at the animal level, which we are not able to explain with certainty; however, CaO may still be a valid option to increase digestibility of the forage, since results from a more controlled environment and those observed in Chapter 2 show that increases in IVOMD and ruminal in situ degradability of nutrients are happening when bahiaagrass hay is treated with CaO. Moreover, when CaO treated forages that are of low nutritive value are provided as the sole source of feed, a benefit from protein supplementation should be considered and maybe positive effects of the treated forage can be observed if the diet provided adequate amounts of CP.
Table 3-1. Analyzed\(^1\) chemical composition of bahiagrass (*Paspalum notatum*) hay provided to beef steers.

<table>
<thead>
<tr>
<th>Item</th>
<th>DH</th>
<th>CC</th>
<th>CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM(^3), %</td>
<td>90.1 ± 2.04</td>
<td>51.7 ± 1.41</td>
<td>51.2 ± 1.05</td>
</tr>
<tr>
<td>OM, % DM</td>
<td>92.2</td>
<td>87.3</td>
<td>87.4</td>
</tr>
<tr>
<td>CP, % DM</td>
<td>8.1</td>
<td>8.2</td>
<td>7.6</td>
</tr>
<tr>
<td>NDF, % DM</td>
<td>75.7</td>
<td>66.1</td>
<td>67.1</td>
</tr>
<tr>
<td>ADF, % DM</td>
<td>42.2</td>
<td>37.5</td>
<td>36.5</td>
</tr>
<tr>
<td>Lignin, % DM</td>
<td>5.4</td>
<td>3.6</td>
<td>3.2</td>
</tr>
<tr>
<td>TDN(^4), % DM</td>
<td>53</td>
<td>55</td>
<td>56</td>
</tr>
<tr>
<td>Calcium, % DM</td>
<td>0.47</td>
<td>2.75</td>
<td>3.07</td>
</tr>
</tbody>
</table>

\(^1\)Analyzed by a commercial laboratory using a wet chemistry package (Dairy One, Ithaca, NY).
\(^2\)DH = bahiagrass hay as is (dry); CC = bahiagrass hay treated with 8.9%CaCO3 (DM basis) + water (to 50% DM); CO = bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM).
\(^3\)Analyzed in-house daily to determine DM of hay fed to steers.
\(^4\)TDN = total digestible nutrients.
Table 3-2. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with calcium oxide (CaO) on total dry matter intake (DMI), daily average ruminal fermentation parameters, and blood urea nitrogen (BUN) concentrations of beef steers provided ad libitum intake.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment²</th>
<th>P-value⁴</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>TRT</th>
<th>TIME</th>
<th>TRT × TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DH</td>
<td>CC</td>
<td>CO</td>
<td>SEM³</td>
<td>TRT</td>
<td>TIME</td>
<td>TRT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>4.6</td>
<td>4.8</td>
<td>4.6</td>
<td>0.237</td>
<td>0.759</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI, % BW</td>
<td>1.0</td>
<td>1.1</td>
<td>1.0</td>
<td>0.05</td>
<td>0.674</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruminal pH</td>
<td>6.67a</td>
<td>6.75a</td>
<td>7.06b</td>
<td>0.037</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.181</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₃-N, mM</td>
<td>0.54</td>
<td>0.56</td>
<td>0.25</td>
<td>0.094</td>
<td>0.059</td>
<td>&lt; 0.001</td>
<td>0.522</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td>5.27</td>
<td>5.38</td>
<td>5.05</td>
<td>0.145</td>
<td>0.517</td>
<td>0.319</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹,²Within a row, means with different superscripts differ; treatment effect, \( P \leq 0.05 \).

¹Ruminal fluid and blood samples were collected every 3 h for 24 h.

²DH = bahiagrass hay as is (dry), \( n = 8 \); CC = bahiagrass hay treated with 8.9% CaCO₃ (DM basis) + water (to 50% DM), \( n = 9 \); CO = bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM), \( n = 8 \)

³Largest SEM is provided.

⁴Observed significance levels for treatment (TRT) and TIME effects, and for their interaction (TRT × TIME).
Table 3-3. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with calcium oxide (CaO) on ruminal VFA molar proportions of beef steers provided ad libitum intake.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment $^2$</th>
<th>$P$-value $^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DH</td>
<td>CC</td>
</tr>
<tr>
<td>VFA, mol/100 mol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>74.1</td>
<td>74.4</td>
</tr>
<tr>
<td>Propionate</td>
<td>16.9</td>
<td>17.6</td>
</tr>
<tr>
<td>Butyrate</td>
<td>7.7$^b$</td>
<td>6.8$^a$</td>
</tr>
<tr>
<td>BCVFA$^5$</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Valerate</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

$^a,b$ Within a row, means with different superscripts differ, $P ≤ 0.05$.

$^1$Ruminal fluid samples were collected every 3 h for 24 h.

$^2$DH = bahiagrass hay as is (dry), $n = 8$; CC = bahiagrass hay treated with 8.9% CaCO3 (DM basis) + water (to 50% DM), $n = 9$; CO = bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM), $n = 8$

$^3$Largest SEM is provided.

$^4$Observed significance levels for treatment (TRT) and TIME effects, and for their interaction (TRT × TIME).

$^5$BCVFA = Branched chain VFAs: isobutyrate + isovalerate + 2 methylbutyrate.
Table 3-4. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with calcium oxide (CaO) on ruminal VFA concentrations of beef steers provided ad libitum intake.

<table>
<thead>
<tr>
<th>Item¹</th>
<th>Treatment²</th>
<th>P-value⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DH</td>
<td>CC</td>
</tr>
<tr>
<td>VFA, mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acetate</td>
<td>45.4ᵇ</td>
</tr>
<tr>
<td></td>
<td>Propionate</td>
<td>10.3ᵃ</td>
</tr>
<tr>
<td></td>
<td>Butyrate</td>
<td>4.7ᵇ</td>
</tr>
<tr>
<td></td>
<td>BCVFA⁵</td>
<td>0.4ᵇ</td>
</tr>
<tr>
<td></td>
<td>Valerate</td>
<td>0.3ᵇ</td>
</tr>
<tr>
<td></td>
<td>Total VFA, mM</td>
<td>61.2ᵇ</td>
</tr>
<tr>
<td></td>
<td>A:P⁶</td>
<td>4.4</td>
</tr>
</tbody>
</table>

ᵃᵇWithin a row, means with different superscripts differ, *P* ≤ 0.05.

¹Ruminal fluid and blood samples were collected every 3 h for 24 h.

²DH = bahiagrass hay as is (dry), *n* = 8; CC = bahiagrass hay treated with 8.9% CaCO₃ (DM basis) + water (to 50% DM), *n* = 9; CO = bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM), *n* = 8

³Largest SEM is provided.

⁴Observed significance levels for treatment (TRT) and TIME effects, and for their interaction (TRT × TIME).

⁵BCVFA = Branched chain VFAs: isobutyrate + isovalerate + 2 methylbutyrate.

⁶Acetate to propionate ratio.
Table 3-5. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with calcium oxide (CaO) on daily nutrient intake and apparent total tract digestibility of beef steers provided ad libitum intake and in vitro organic matter digestibility (IVOMD).

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment¹</th>
<th>DH</th>
<th>CC</th>
<th>CO</th>
<th>SEM²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-d Intake, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>4.44</td>
<td>5.06</td>
<td>4.62</td>
<td></td>
<td>0.332</td>
<td>0.385</td>
</tr>
<tr>
<td>OM</td>
<td>4.17</td>
<td>4.47</td>
<td>4.07</td>
<td></td>
<td>0.320</td>
<td>0.628</td>
</tr>
<tr>
<td>CP</td>
<td>0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>0.022</td>
<td>0.042</td>
</tr>
<tr>
<td>NDF</td>
<td>3.44</td>
<td>3.55</td>
<td>3.25</td>
<td></td>
<td>0.253</td>
<td>0.696</td>
</tr>
<tr>
<td>ADF</td>
<td>1.78</td>
<td>1.93</td>
<td>1.77</td>
<td></td>
<td>0.128</td>
<td>0.613</td>
</tr>
<tr>
<td>Total tract digestibility, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>42.3</td>
<td>39.7</td>
<td>36.4</td>
<td></td>
<td>2.18</td>
<td>0.186</td>
</tr>
<tr>
<td>OM</td>
<td>44.6</td>
<td>40.9</td>
<td>38.5</td>
<td></td>
<td>2.24</td>
<td>0.169</td>
</tr>
<tr>
<td>CP</td>
<td>10.9</td>
<td>20.3</td>
<td>8.3</td>
<td></td>
<td>4.57</td>
<td>0.152</td>
</tr>
<tr>
<td>NDF</td>
<td>52.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>1.94</td>
<td>0.034</td>
</tr>
<tr>
<td>ADF</td>
<td>53.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>2.02</td>
<td>0.003</td>
</tr>
<tr>
<td>IVOMD, %</td>
<td>34.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>0.76</td>
<td>0.013</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Within a row, means with different superscripts differ, \( P \leq 0.05 \).

¹DH = bahiagrass hay as is (dry), \( n = 8 \); CC = bahiagrass hay treated with 8.9% \( \text{CaCO}_3 \) (DM basis) + water (to 50% DM), \( n = 9 \); CO = bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM), \( n = 8 \)

²Largest SEM is provided
Figure 3-1. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with calcium oxide (CaO) on blood urea nitrogen (BUN) concentration of beef steers provided ad libitum intake. A treatment × time postfeeding interaction was observed (*P* = 0.02). DH = bahiagrass hay as is (dry), *n* = 8; CC = bahiagrass hay treated with 8.9% CaCO$_3$ (DM basis) + water (to 50% DM), *n* = 9; CO = bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM), *n* = 8. Error bars represent the SEM for treatment × time postfeeding interaction.
Figure 3-2. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with calcium oxide (CaO) on molar proportions of acetate of beef steers provided ad libitum intake. A treatment × time postfeeding interaction was observed (*P* < 0.001). DH: Untreated dry bahiagrass hay, *n* = 8; CC: Bahiagrass hay treated with 8.9% CaCO$_3$ (DM basis) + water (to 50% DM), *n* = 9; CO: Bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM), *n* = 8. Error bars represent the SEM for treatment × time postfeeding interaction.
Figure 3-3. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with calcium oxide (CaO) on molar proportions of propionate of beef steers provided ad libitum intake. A treatment × time postfeeding interaction was observed ($P < 0.001$). DH: Untreated dry bahiagrass hay, $n = 8$; CC: Bahiagrass hay treated with 8.9% CaCO$_3$ (DM basis) + water (to 50% DM), $n = 9$; CO: Bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM), $n = 8$. Error bars represent the SEM for treatment × time postfeeding interaction.
Figure 3-4. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with calcium oxide (CaO) on acetate to propionate ratio (A:P) of beef steers provided ad libitum intake. A treatment × time postfeeding interaction was observed (*P* < 0.001). DH: Untreated dry bahiagrass hay, *n* = 8; CC: Bahiagrass hay treated with 8.9% CaCO₃ (DM basis) + water (to 50% DM), *n* = 9; CO: Bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM), *n* = 8. Error bars represent the SEM for treatment × time postfeeding interaction.
CHAPTER 4
PERFORMANCE OF GROWING BEEF CATTLE CONSUMING BAHIA GRASS HAY TREATED WITH CALCIUM OXIDE AND MOLASSES

Introduction

Many researchers have focused on increasing digestibility of low-quality forages and roughages that would usually go to waste by performing chemical treatment with alkali such as NaOH (Chaudhry and Miller, 1996; Mishra et al., 2000), NH₃ (Mason et al., 1988), and Ca(OH)₂ (Zaman and Owen, 1995; Wanapat et al., 2009), with NaOH being the standard chemical that promoted the greatest effects on fiber degradability. More recently, for animal, environmental, and human safety concerns, attention has been given to the alkali CaO (Euken and Dahlke, 2014; Peterson et al., 2015a), and similar effectiveness and results obtained with NaOH in the past have been observed (Watson et al., 2015).

Beef cattle operations in Florida and the southeastern US rely primarily on grazed and conserved forages to supply dietary nutrients; however, the predominant forages in this region can have elevated fiber contents and may be deficient in both CP and energy, thus needing supplementation to maximize animal performance and reach a desired level of production (Bowman et al., 1995).

Chemical treatment of low-quality forages (e.g. crop residues) with CaO has been researched extensively considering cattle performance when treated forages replaced corn in finishing (Shreck et al., 2013b; Chapple et al., 2015; Shreck et al., 2015) or backgrounding (Shreck et al., 2014; Peterson et al., 2015b) mixed diets. Results observed are varied showing either no negative effects and similar performance in cattle consuming diets with treated residues (Chapple et al., 2015; Shreck et al.,
2015) or increases in ADG and G:F in cattle consuming treated residues in high-forage growing diets (Peterson et al., 2015b).

To the knowledge of this author, no research has been performed by treating low-quality hay, such as bahiagrass hay, with CaO and feeding it as the sole forage component in growing cattle diets to evaluate animal performance. Therefore, it was hypothesized that CaO treatment would improve fiber digestibility of bahiagrass hay and: Exp. 1- with the addition of molasses, beef cattle performance would increase with a combination of increased forage digestibility and additional energy to the diet; Exp. 2 - with the addition of molasses and cottonseed meal, beef cattle performance would increase with a combination of increased forage digestibility, and additional energy and protein to the diet. The objectives were: Exp. 1 - to evaluate the effects of bahiagrass hay treated with 5% CaO and 10% molasses on beef cattle performance; Exp.2 - to evaluate the effects of bahiagrass hay treated with 5% CaO and 10% molasses plus protein supplementation via cottonseed meal on beef cattle performance. An additional objective was to assess effectiveness of CaO treatment by determining IVOMD of the bahiagrass hay treated or not provided to cattle in Exp. 1.

**Materials and Methods**

All procedures involving animals were approved by the University of Florida Institutional Animal Care and Use Committee (Protocol # 201508733).

**Experiment 1 Design, Animals, and Treatments**

Ninety-six growing *Bos taurus* and *Bos indicus* heifers (*n* = 59; 250 ± 29 kg of BW; average BW ± SD) and steers (*n* = 37; 256 ± 45 kg of BW; average BW ± SD) were used in a randomized complete block design at the University of Florida – North Florida Research and Education Center Beef Unit (**UF-NFREC BU**) in Marianna, FL. The
experiment consisted of a 13-d adaptation period followed by 42-d data collection period. On d 0, cattle were stratified by sex, breed, and BW, and blocked by initial BW. Cattle were then allotted to 24 dormant bahiagrass pastures (1.34 ha each; \( n = 4 \) animals/pasture), which were located in 2 different areas of the UF-NFREC BU and were within 0.52 km of each other. The two locations were termed R-pens (\( n = 11 \); pastures per location) and South Circle (\( n = 13 \)). Pastures were stratified by location and randomly assigned (\( n = 8 \) pastures/treatment) to 1 of 3 treatments: 1) bahiagrass hay as is (DH); 2) hay treated with 10% molasses (DM basis) + water (to 35% DM; MOL); or 3) hay treated with 5% CaO (DM basis) + 10% molasses (DM basis) + water (to 35% DM; CAO). The experiment started on December 29, 2017; therefore there was no residual forages in the pastures and bahiagrass hay from treatments was the only forage available, which was provided ad libitum to cattle.

**Experiment 2 Design, Animals, and Treatments**

After completion of Exp. 1, 64 of the 96 growing *Bos taurus* and *Bos indicus* heifers (\( n = 56 \); 249 ± 26 kg of BW) and steers (\( n = 8 \); 249 ± 20 kg of BW) were used in a second experiment in a randomized complete block design at the UF-NFREC BU. The experiment consisted of a 14-d adaptation period followed by 42-d data collection period. On d 0, cattle were stratified by sex, breed, and BW, and blocked by initial BW. Cattle were then allotted to 16 of the same dormant bahiagrass pastures described in Exp. 1 (\( n = 8 \) R-pens and \( n = 8 \) South Circle; \( n = 4 \) animals/pasture). Pastures were stratified by location and randomly assigned (\( n = 8 \) pastures/treatment) to 1 of 2 treatments: 1) hay treated with 10% molasses (DM basis) + water (to 35% DM; MOL) or 2) hay treated with 5% CaO (DM basis) + 10% molasses (DM basis) + water (to 35% DM; CAO). Cottonseed meal (CSM) was provided at a rate of 0.3% of cattle BW/d (as
fed basis). The delivery of the weekly amounts of CSM for all animals in the pasture was performed 3 times a week (Monday, Wednesday, and Friday). Amounts of CSM were adjusted every 2 wk when cattle were weighed. The experiment started on March 7, 2018 when there was still no residual forages in the pastures and bahiagrass hay from treatments was the only forage available, which was provided ad libitum to cattle. However, towards the end of the experiment there was initial growth of spring forage in some of the pastures, which were considered to be minimal and not enough to become a confounding factor.

**Treatment of Bahiagrass Hay**

With a tractor and feed wagon, in Exp. 1, small round bales (average 219 ± 18 kg as is; 178 ± 15 kg DM) and in Exp. 2, small (average 225 ± 16 kg as is; 183 ± 14 kg DM) and large (average 510 ± 16 kg as is; 417 ± 25 kg DM) round bales were individually weighed and core samples were taken of each bale to calculate DM. For both experiments, treatment of hay was performed at the same manner. Amounts of sugar cane molasses (donated by Quality Liquid Feeds, Inc., Dodgeville, WI) and CaO (HI CAL QUICKLIME – FOUNDRY, 89% available CaO; Lhoist North America of Alabama, Calera, AL) were weighed for each bale at 10 and 5% of the bale DM, respectively, in buckets and mixed with the water needed to reach a final bale DM of 35%. The bales were placed on the ground, on their flat side, and the mixture of molasses, CaO, and water was poured as quickly as possible before the bale was placed back on its round side, to minimize liquid run off. Bales were allowed to sit uncovered for at least 7 d before cattle feeding.
Sampling Procedures

Hay samples
In both experiments, before providing a new bale to each pasture, core samples were taken with a hay probe and hand drill to measure DM content before cattle feeding.

Body weight and blood samples
To measure ADG, in Exp. 1 cattle were weighed on d 0, 13, 27, 41, and 56. In Exp. 2 cattle were weighed on d 0, 14, 28, 42, and 56. The following procedures were performed at the same manner for both experiments. On d 0 and 56, cattle were weighed after a 16-h water and feed withdrawal to obtain shrunk initial and final BW. During the same time of BW measurements a blood sample was collected via jugular venipuncture into 10-mL evacuated tubes containing Na heparin (BD Vacutainer, Franklin Lakes, NJ). Tubes were immediately placed on ice, and subsequently centrifuged for 15 min at 1,500 × g at 4°C. Plasma was then transferred to polypropylene vials (12 × 75 mm; Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA) and stored at -20°C for further analysis of BUN.

Laboratory Analyses
All protocols and procedures used for analyzing samples and data from steers were used in an identical manner throughout both experiments.

Chemical composition of hay and cottonseed meal
Subsamples of core samples from hay bales taken before cattle feeding in both experiments were analyzed in-house for DM content in a 105°C forced air oven for 24 h. The remainder of the core samples were dried in a 55°C forced air oven for 72 h, compositied within treatment, ground to pass a 2-mm screen in a Wiley mill (Thomas
Scientific, Swedesboro, NJ), and sent to a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) for analysis of OM, CP, NDF, ADF, lignin, and Ca contents. Chemical composition of bahiagrass hay for all treatments and cottonseed meal from Exp. 2 are presented in Table 4-1 (Exp.1) and Table 4-3 (Exp.2).

**In vitro OM digestibility**

In order to test the effectiveness of bahiagrass treatment with CaO, a modified Tilley and Terry (1963) procedure was used to determine IVOMD of bahiagrass hay provided to cattle in Exp. 1. Briefly, 0.7 g of dried and ground (2 mm) samples from composites of core hay samples taken before feeding were incubated with 50 mL of a 4:1 McDougall’s buffer:ruminal fluid inoculum in 100-mL plastic centrifuge tubes for 48 h under constant agitation (60 rpm) at 39°C. Two tubes per treatment per period and two blank (without substrate or treatment) tubes were incubated in each of the three separate replicate days \( n = 3/\text{treatment} \). After the initial 48 h, 6 mL of a 20% (v/v) HCl solution was added to the tubes along with 2 mL of a 5% pepsin solution. Tubes were then incubated for an additional 48 h under constant agitation (60 rpm) at 39°C. Samples were then filtered through P8 filters (Fisherbrand; Thermo Fisher Scientific Inc.). Filters with wet samples were then dried at 105°C in a forced air oven for 24 h to determine IVDMD. Dry filters with residual samples were then placed in a muffle furnace for 6 h at 650°C. The ash was then placed in a 105°C oven for 24 h prior to recording weight. Two ruminally cannulated steers consuming bahiagrass hay for at least 14 d were used as ruminal fluid donors.

**Statistical Analysis**

For both experiments, all data were analyzed as a randomized complete block design with repeated measures for BUN data. The MIXED procedure of SAS (SAS Inst.
Inc., Cary, NC) was used and for performance and BUN data, pasture was considered the experimental unit. In Exp. 1, the model for performance data included the fixed effects of treatment, sex, and their interaction and the random effects of location and block. Blood urea nitrogen data were analyzed as repeated measures. Pen was considered the subject and the covariance structure chosen was ante-dependence due to unequal spacing between measurements. The model included the fixed effects of treatment, day, and their interaction. Sex, block, location, and pen within treatment were included as random effects, with the latter used to designate the denominator degrees of freedom, which were adjusted using the between-within method. For IVOMD, the average of two tubes per day of incubation was considered the experimental unit and the model included the fixed effect of treatment and the random effect of day of incubation (block). In Exp. 2, the model for performance data included the fixed effects of treatment and sex and the random effects of location and block. Blood urea nitrogen data were analyzed as repeated measures. Pen was considered the subject and the covariance structure chosen was first order autoregressive based on the smallest Akaike information criterion. The model included the fixed effects of treatment, day, and their interaction. Sex, block, location, and pen within treatment were included as random effects, with the latter used to designate the denominator degrees of freedom, which were adjusted using the between-within method. In Exp. 1, the following contrast was for data interpretation: effect of molasses (mean of MOL + CAO vs. DH). Significance was declared at $P \leq 0.05$.

**Results and Discussion**

Performance of cattle consuming bahiagrass treated with CaO and molasses in Exp. 1 is presented in Table 4-2. No differences among treatments ($P = 0.283$),
between sex \((P = 0.495)\), or treatment \(\times\) sex interaction \((P = 0.666)\) were observed for final BW nor was there an effect of treating hay with molasses \((P = 0.131)\).

Consequently, there was no treatment \((P = 0.515)\), sex \((P = 0.868)\) or treatment \(\times\) sex interaction \((P = 0.582)\) on ADG of cattle from d 0 to 56, which had an average loss across treatments of 0.03 kg/d in BW. Moreover, no effect \((P = 0.385)\) of treating hay with molasses was observed on ADG of cattle from d 0 to 56. Similarly, in Exp. 2 (Table 4-4), no differences between treatment \((P = 0.453)\) were observed on final BW, as well as no sex effect \((P = 0.670)\). Treatment \((P = 0.767)\) or sex \((P = 0.285)\) had no effect on ADG from d 0 to 56; however, in this experiment, cattle did not experience weight loss and had an average gain of 0.537 kg/d. Protein supplementation, either as true protein or non-protein nitrogen to low-quality hay-based diets have been reported to improve performance of beef cattle (Bohnert et al., 2002; Currier et al., 2004; Waters et al., 2015). The main difference from Exp. 1 and Exp. 2 was that cattle in the second experiment were supplemented with cottonseed meal (49% CP) at a rate of 0.3% BW/d and that is a plausible explanation for the absence of such a dramatic loss weight observed in the first experiment. Bahiagrass is a warm-season forage that is commonly used for grazing or hay production in Florida and the southern states of US (Chambliss and Sollenberger, 1991); however, when provided as hay, bahiagrass can be of low nutritive value, which is not adequate to support requirements of growing cattle, thus need additional supplementation of energy, protein, or both (Moore et al., 1991; Hersom et al., 2011). In addition to the idea of masking the bitter taste of CaO, the inclusion of molasses to the hay treatment in both experiments was performed intended to provide added energy to the hay; however, only in Exp. 2 protein supplementation was
provided. As it is shown in Table 4-1 and Table 4-3, the TDN content across treatments were fairly similar, indicating that the addition of molasses may not have provided the extra energy that was lacking in the hay, thus potentially explaining the lack in increased performance in animals consuming hay that contained molasses when compared to those consuming the dry hay.

Concentrations of BUN in cattle from Exp. 1 and Exp. 2 are presented in Table 4-5. In both experiments, there was no difference among treatments ($P = 0.789$; Exp. 1 and $P = 0.138$; Exp. 2) in concentrations of BUN; however, a treatment × day interaction was observed ($P = 0.003$; Figure 4-1) in Exp. 1. On d 27, cattle consuming treated hay (MOL and CAO), had greater ($P \leq 0.028$) concentrations of BUN when compared to those consuming untreated hay (DH). The lack of differences in BUN concentrations is expected since, within experiment and across treatments, cattle were receiving the same amounts of CP in the diets, thus CP intake was the expected to be the same.

Any excess of NH$_3$-N in the rumen would be absorbed through the rumen wall into the portal vein and removed by the liver, where it is largely converted to urea (Reynolds and Kristensen, 2008). Urea circulates in the blood and may end up being taken by the kidneys and excreted in the urine or, when dietary protein is not sufficient, can diffuse from the blood back into the rumen, cecum, or into saliva and then back into the rumen. Therefore, BUN concentrations are positively associated with intake of CP, digestible intake protein, and ruminal NH$_3$-N concentrations (Hammond, 1997). Optimal BUN concentrations in growing beef steers were reported to be in the range of 11 - 15 mg/dL to reach maximal rates of gain (Byers and Moxon, 1980). In Exp. 1, BUN concentrations were below this range, which is another indication that protein was not
provided at adequate amounts to those animals leading to weight loss; however, in Exp. 2, where cattle were supplemented with cottonseed meal as a protein source, concentrations of BUN are greater and within the range proposed, demonstrating why cattle in the second experiment did not experience weight loss but rather weight gain.

In order to test if treatment with CaO of the bahiagrass hay provided to cattle in Exp. 1 was being effective at improving the digestibility of the forage, and consequently animal performance, IVOMD was performed with samples taken from the hay bales before feeding to cattle, and results are presented in Figure 4-2. Surprisingly, not only did CaO not improve IVOMD of bahiagrass hay but it actually reduced IVOMD when compared to untreated hay ($P = 0.011$); however, no difference was observed between hay treated with molasses (MOL) only or with molasses and CaO (CAO).

These results can explain the lack of increased performance that was expected in cattle consuming CaO treated hay when compared to non-treated hay and suggest that the method of treatment may not have been the best approach to achieve the results desired. Since, differently from previous experiments described in Chapters 2 and 3, the method of treatment in this study did not involve mixing of the forage with the CaO and addition of water, but soaking of the hay by pouring a mixture of water and CaO, perhaps a proper contact of the CaO with the fiber did not occur. Moreover, it has been suggested that particle size is important for treatment to be effective, where smaller particles would respond better to treatment than larger ones (Shreck et al., 2012; Euken et al., 2013; Peterson et al., 2015b). In this experiment, the whole round bale was soaked with the mixture and in previous studies the hay was chopped before
treatment, promoting greater surface area for adherence of the powder and reaction to occur.
Table 4-1. Analyzed\(^1\) chemical composition of bahiagrass (\textit{Paspalum notatum}) hay treated or not with molasses and calcium oxide (CaO) fed to growing beef cattle (Exp. 1).

<table>
<thead>
<tr>
<th>Item</th>
<th>DH</th>
<th>MOL</th>
<th>CAO</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM(^3), %</td>
<td>90.8 ± 0.3</td>
<td>78.2 ± 1.5</td>
<td>77.7 ± 2.3</td>
</tr>
<tr>
<td>OM, % DM</td>
<td>94.8</td>
<td>94.3</td>
<td>91.1</td>
</tr>
<tr>
<td>CP, % DM</td>
<td>7.7</td>
<td>9.4</td>
<td>9.1</td>
</tr>
<tr>
<td>NDF, % DM</td>
<td>71.9</td>
<td>71.6</td>
<td>67.4</td>
</tr>
<tr>
<td>ADF, % DM</td>
<td>41.3</td>
<td>35.3</td>
<td>38.3</td>
</tr>
<tr>
<td>Lignin, % DM</td>
<td>3.3</td>
<td>2.4</td>
<td>3.6</td>
</tr>
<tr>
<td>TDN, % DM</td>
<td>62</td>
<td>63</td>
<td>59</td>
</tr>
<tr>
<td>Calcium, % DM</td>
<td>0.39</td>
<td>0.50</td>
<td>1.62</td>
</tr>
</tbody>
</table>

\(^1\) Analyzed by a commercial laboratory using a wet chemistry package (Dairy One, Ithaca, NY).

\(^2\) DH = bahiagrass hay as is (dry); MOL = bahiagrass hay treated with 10% molasses (DM basis) + water (to 35% DM); CAO = bahiagrass hay treated with 5% CaO (DM basis) + 10% molasses (DM basis) + water (to 35% DM).

\(^3\) Analyzed in-house to determine DM of hay prior cattle feeding.
Table 4-2. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with molasses and calcium oxide (CaO) on performance of growing beef cattle (Exp.1).

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment¹</th>
<th>SEM²</th>
<th>TRT</th>
<th>Sex</th>
<th>TRT × Sex</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW, kg</td>
<td>DH</td>
<td>247</td>
<td>MOL</td>
<td>5.1</td>
<td>0.362</td>
<td>0.241</td>
</tr>
<tr>
<td></td>
<td>MOL</td>
<td>252</td>
<td>CAO</td>
<td></td>
<td>0.488</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>CAO</td>
<td>258</td>
<td></td>
<td></td>
<td>0.016</td>
<td>0.097</td>
</tr>
<tr>
<td>Final BW, kg</td>
<td>DH</td>
<td>246</td>
<td>MOL</td>
<td>5.0</td>
<td>0.283</td>
<td>0.495</td>
</tr>
<tr>
<td></td>
<td>MOL</td>
<td>256</td>
<td>CAO</td>
<td></td>
<td>0.495</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>CAO</td>
<td>253</td>
<td></td>
<td></td>
<td>0.666</td>
<td>0.011</td>
</tr>
<tr>
<td>ADG⁵, kg</td>
<td>0 to 13 d</td>
<td>1.468</td>
<td>MOL</td>
<td>0.1233</td>
<td>0.1233</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>0 to 27 d</td>
<td>0.453</td>
<td>MOL</td>
<td>0.0687</td>
<td>0.0687</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td>0 to 41 d</td>
<td>0.230</td>
<td>MOL</td>
<td>0.0480</td>
<td>0.0480</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>0 to 56 d</td>
<td>-0.061</td>
<td>MOL</td>
<td>0.0481</td>
<td>0.0481</td>
<td>-</td>
</tr>
</tbody>
</table>

¹DH = bahiagrass hay as is (dry); MOL = bahiagrass hay treated with 10% molasses (DM basis) + water (to 35% DM); CAO = bahiagrass hay treated with 5% CaO (DM basis) + 10% molasses (DM basis) + water (to 35% DM).

²Pooled SE of treatment means, n = 8 pastures/treatment.

³Observed significance levels for effects of treatment (TRT), sex, and their interaction (TRT × Sex).

⁴Orthogonal contrasts: M = effect of molasses (mean of MOL+CAO vs DH).

⁵BW at d 0 and 56 were shrunk while at d 13, 27, and 41 were not..
Table 4-3. Analyzed\(^1\) chemical composition of bahiagrass (*Paspalum notatum*) hay treated or not with molasses plus calcium oxide (CaO) and cottonseed meal (CSM) fed to growing beef cattle (Exp. 2).

<table>
<thead>
<tr>
<th>Item</th>
<th>MOL</th>
<th>CAO</th>
<th>CSM(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM(^4), %</td>
<td>81.5 ± 1.9</td>
<td>82.7 ± 1.5</td>
<td>88.5</td>
</tr>
<tr>
<td>OM, % DM</td>
<td>94.3</td>
<td>91.8</td>
<td>91.8</td>
</tr>
<tr>
<td>CP, % DM</td>
<td>8.3</td>
<td>8.4</td>
<td>49.1</td>
</tr>
<tr>
<td>NDF, % DM</td>
<td>71.8</td>
<td>69.1</td>
<td>25.4</td>
</tr>
<tr>
<td>ADF, % DM</td>
<td>39.2</td>
<td>36.6</td>
<td>18.7</td>
</tr>
<tr>
<td>Lignin, % DM</td>
<td>2.6</td>
<td>3.8</td>
<td>-</td>
</tr>
<tr>
<td>TDN, % DM</td>
<td>63</td>
<td>59</td>
<td>69</td>
</tr>
<tr>
<td>Calcium, % DM</td>
<td>0.46</td>
<td>1.71</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^1\) Analyzed by a commercial laboratory using a wet chemistry package (Dairy One, Ithaca, NY).

\(^2\) MOL = bahiagrass hay treated with 10% molasses (DM basis) + water (to 35% DM); CAO = bahiagrass hay treated with 5% CaO (DM basis) + 10% molasses (DM basis) + water (to 35% DM).

\(^3\) CSM was provided at a rate of 0.3% of total pasture BW/d (as fed basis).

\(^4\) Hay DM was analyzed in-house to determine content prior cattle feeding.
Table 4-4. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with molasses and calcium oxide (CaO) on performance of growing beef cattle supplemented with cottonseed meal\(^1\) (Exp. 2).

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment(^2)</th>
<th>(P)-value(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MOL</td>
<td>CAO</td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>256</td>
<td>254</td>
</tr>
<tr>
<td>Final BW, kg</td>
<td>289</td>
<td>286</td>
</tr>
<tr>
<td>ADG(^5), kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 14 d</td>
<td>2.163</td>
<td>2.163</td>
</tr>
<tr>
<td>0 to 28 d</td>
<td>1.181</td>
<td>1.145</td>
</tr>
<tr>
<td>0 to 42 d</td>
<td>0.918</td>
<td>0.938</td>
</tr>
<tr>
<td>0 to 56 d</td>
<td>0.547</td>
<td>0.526</td>
</tr>
</tbody>
</table>

\(^1\)Cottonseed meal was provided at a rate of 0.3% of total pasture BW/d (as fed basis).

\(^2\)MOL = bahiagrass hay treated with 10% molasses (DM basis) + water (to 35% DM); CAO = bahiagrass hay treated with 5% CaO (DM basis) + 10% molasses (DM basis) + water (to 35% DM).

\(^3\)Pooled SE of treatment means, \(n = 8\) pastures/treatment.

\(^4\)Observed significance levels for effects of treatment (TRT) and sex.

\(^5\)BW at d 0 and 56 were shrunk while at d 14, 28, and 42 were not.
Table 4-5. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with molasses and calcium oxide (CaO) on blood urea nitrogen (BUN) concentrations of growing beef cattle (Exp. 1 and Exp. 2)\(^1\).

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment*2</th>
<th>P-value*4</th>
<th>SEM*3</th>
<th>TRT</th>
<th>day</th>
<th>TRT × day</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp. 1</td>
<td>DH</td>
<td>MOL</td>
<td>CAO</td>
<td>0.359</td>
<td>0.768</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>-</td>
<td>11.97</td>
<td>11.10</td>
<td>0.483</td>
<td>0.138</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

1. In Exp. 2 only MOL and CAO were provided as treatments and cattle were provided cottonseed meal at a rate of 0.3% of total pasture BW/d (as fed basis).
2. DH = bahiagrass hay as is (dry); MOL = bahiagrass hay treated with 10% molasses (DM basis) + water (to 35% DM); CAO = bahiagrass hay treated with 5% CaO (DM basis) + 10% molasses (DM basis) + water (to 35% DM).
4. Observed significance levels for effects of treatment (TRT), day, and their interaction (TRT × day).
Figure 4-1. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with calcium oxide (CaO) on blood urea nitrogen (BUN) concentration of beef cattle provided ad libitum intake. A treatment × day interaction was observed (*P* = 0.003); DH = bahiagrass hay as is (dry); MOL = bahiagrass hay treated with 10% molasses (DM basis) + water (to 35% DM); CAO = bahiagrass hay treated with 5% CaO (DM basis) + 10% molasses (DM basis) + water (to 35% DM); Error bars represent the SEM for treatment × day interaction; *P* ≤ 0.028.
Figure 4-2. In vitro organic matter digestibility (IVOMD) of bahiagrass (*Paspalum notatum*) hay provided to beef cattle in Exp. 1; DH = bahiagrass hay as is (dry); MOL = bahiagrass hay treated with 10% molasses (DM basis) + water (to 35% DM); CAO = bahiagrass hay treated with 5% CaO (DM basis) + 10% molasses (DM basis) + water (to 35% DM). a,b Means with different superscripts differ; $P = 0.011$; Error bars represent SEM; $n = 3$/treatment.
CHAPTER 5
ENTERIC METHANE PRODUCTION AND APPARENT TOTAL TRACT DIGESTIBILITY OF BEEF STEERS CONSUMING BAHIAGRASS HAY TREATED WITH CALCIUM OXIDE

Introduction

The cow-calf sector, where most cattle are provided forage-based diets, has been reported to be associated with approximately 80% of the total greenhouse gases produced by the beef industry (Beauchemin et al. 2010). With that in mind, there is an increasing desire to research strategies to mitigate enteric CH₄ produced by ruminants for the primarily reasons of environmental concerns and the potential to minimize energy losses by the animal, leading towards greater gains.

Methane production is a necessary consequence of forage digestion. Therefore, to reduce the carbon footprint in beef cattle production, an effective strategy would be to increase the production per unit of carbon emitted (Capper, 2011). In forage-based production systems, this may be achieved by increasing the digestibility of forages, which consequently have the potential to increase animal performance.

Chemical treatment of forages with alkali, such as CaO, to improve the digestibility of the fiber fractions has been extensively researched and results are often satisfactory (Klopfenstein et al., 1972; Shreck et al., 2015; Watson et al., 2015). Although an increase in fiber digestion in the rumen can potentially increase total CH₄ production, when ruminants are allowed to utilize low-quality fibrous feedstuffs more efficiently, greater animal performance will likely be observed. Therefore, CH₄ production, when based on feed intake and feed digested, can be actually reduced.

This experiment was designed to test the hypothesis that CaO treatment of bahiagrass hay would promote greater digestibility of the forage fiber, consequently
increasing its energy value that would translate into greater animal performance and reduced nutrient excretion, decreasing the carbon footprint of beef production as a result. The objective was to evaluate the effects of bahiagrass hay treated with 5% CaO on apparent total tract digestibility of nutrients and CH₄ production in beef steers consuming hay ad libitum plus supplemental molasses and cottonseed meal.

**Materials and Methods**

All procedures involving animals were approved by the University of Florida Institutional Animal Care and Use Committee (Protocol # 201508733).

**Experimental Design, Animals, and Treatments**

Twenty *Bos taurus* and *Bos indicus* (314 ± 37 kg of BW; average BW ± SD) steers were used in a generalized randomized block design in a 20-d experimental period. On d -1 and 0, BW was recorded and the average of both days was considered the initial BW. From d 0 to 14, steers underwent adaptation to treatments and facilities. From d 7 to 14 steers were equipped with training CH₄ collection canisters. From d 14 to 17 feed and fecal samples were collected to analyze apparent total tract digestibility. From d 14 to 18, steers were equipped with CH₄ collection canisters to collect breath samples. On d 0, steers were stratified by breed and initial BW, blocked by BW (heavy and light blocks), and randomly assigned to 1 of 2 treatments: 1) bahiagrass hay as is (DH) or 2) bahiagrass hay treated with 5% CaO (DM basis) + water (to 50%DM; CAO). Steers were provided, individually, cottonseed meal and molasses at 0.3% of BW and 0.454 kg/steer/d, respectively.

During the experiment, steers were housed (5 steers/pen) in the UF-NFREC FEF in Marianna, FL. Daily DMI of bahiagrass hay was recorded by the GrowSafe system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). Steers were housed in pens of 108
m² equipped with two GrowSafe feed bunks and a single water trough. Chemical composition of hay in each treatment, cottonseed meal, and molasses provided to steers is presented in Table 5-1.

**Bahiagrass Hay Treatment**

Bahiagrass hay from round bales was chopped using a Tub Grinder (Haybuster, Jamestown, ND) to reduce particle size into a vertical feed mixer (Roto-Mix VXT-425, Dogde City, Kansas). Using the scale on the mixer, half of the water needed was added with a fire hose and the previously weighed amount of CaO (HI CAL QUICKLIME – FOUNDRY, 89% available CaO; Lhoist North America of Alabama, Calera, AL) was sprinkled to the wet hay. The mixer was turned on and the remainder of the water was added. The hay was mixed for approximately 15 minutes to ensure a proper homogenization of contents. Treated hay was spread on a flat covered concrete surface and with a pitchfork mixed daily until a visual assessment of approximately 85% DM was reached. When hay was considered dry enough, it was square-baled (Hesston 4550, Massey Ferguson, Duluth, GA) and bales were stored covered until feeding. Dry hay was also chopped and square-baled to facilitate feeding process and provide similar particle size to treated hay.

**Sampling Procedures**

**Apparent total tract digestibility of nutrients**

Apparent total tract digestibility of nutrients was determined using iNDF as an internal marker (Cole et al., 2011; Krizsan and Huhtanen, 2013). Starting on d 13, hay, cottonseed meal, and molasses samples were collected at time of feeding until d 16. From d 14 to 17 at 0700 and 1600 h, fecal samples were collected either by rectal grab or from the ground, inside the pen, immediately after the steer defecated. Feed and
fecal samples were stored at -20°C for further analysis. Hay and fecal samples were dried at 55°C for 72 h in a forced-air oven and ground in a Wiley mill (Arthur H. Thomas Co. Philadelphia, PA) to pass a 2-mm screen. Hay was pooled within pen and feces were pooled within steer to determine DM, OM, CP, NDF, ADF, iNDF.

**Methane emissions**

Emissions of enteric CH₄ production were measured using the SF₆ tracer technique (Johnson et al., 1994) from d 14 to 18 of each period. Brass permeation tube bodies (length = 4.4 cm, o.d. = 1.43 cm, i.d. = 0.79 cm, inside depth = 3.8 cm, and volume = 1.86 mL), nylon washers, a Teflon membrane (508 μm), secured with a porous (2-μm porosity) stainless steel frit and a brass nut, were used in each steer. Permeation tubes were filled with approximately 2.0 g of SF₆. Permeation tubes were kept at 39°C and weighed 17 times within 60 d. The average SF₆ release rate across steers was of 1.42 ± 0.18 mg/d (average ± SD). Permeation tubes were dosed to steers via balling gun on d 7. Gas collection canisters were constructed of polyvinyl chloride (PVC) pipe to have a final volume of 2 L. The samples were collected by evacuating the collection canisters to 68.6 cmHg and connecting the canister to a halter, which was equipped with a crimped capillary tube positioned to sample, using a loop design, from both nostrils. The volume of the collection canisters and the flow of the capillary tubes were designed to allow half of the vacuum to remain after 24 h. Four collection canisters and capillary tubes were used to determine environmental CH₄ and SF₆ concentrations and were placed, one at each direction (North, South, East, and West), inside the UF-NFREC FEF barn on the outside of the pens where the animals were allocated. It has been proposed by Haisan et al. (2014) to consider any animal with at least 2 d of valid CH₄ measurements. For the current experiment, only steers with at least 3 successful
days of collection and measurement were considered in the final analysis of CH$_4$
variables.

**Laboratory Analyses**

**Concentration of DM, OM, CP, NDF, ADF**

All hay, cottonseed meal, and fecal samples were placed in a 55°C forced air
oven for 72 h to obtain dry samples. Dry, hot weight of the hay was used to calculate
DM of the sample. The sample was then ground to pass through a 2-mm screen in a
Wiley mill (Thomas Scientific, Swedesboro, NJ). To determine OM, 0.5 g of ground
sample (in duplicate) was weighed into ceramic crucibles and placed in a 105°C forced
air oven for 24 h to determine sample DM. Dried samples were then placed in a 650°C
muffle furnace for 6 h before returning to a 105°C forced air oven. Hot, ashed samples
were weighed and used to calculate OM.

Samples of hay, cottonseed meal, and feces were weighed (0.5 g in duplicate)
into F57 bags (Ankom Technology Corp., Macedon, NY) and analyzed for NDF, with
sodium sulfite. Subsequent ADF analysis was performed sequentially as described by
Van Soest et al. (1991) in an Ankom 200 Fiber Analyzer (Ankom Technology Inc.).

Hay, cottonseed meal, and fecal samples were analyzed for total N using a C, H,
N, and S analyzer by the Dumas dry combustion method (Vario Micro Cube; Elementar,
Hanau, Germany). Crude protein was calculated by multiplying the N concentration of
the dry sample by 6.25.

**Concentration of iNDF**

The concentration of iNDF in hay, cottonseed meal, and feces was determined
as described by Gregorini et al. (2008), Cole et al. (2011), and Krizsan and Huhtanen
(2013). Briefly, 0.5 g of sample was weighed into Ankom F57 filter bags (Ankom
Technology Corp. Macedon, NY) and then incubated into the rumen of a cannulated steer grazing a bahiagrass and bermudagrass mixed pasture for 288 h to ensure complete digestion of potentially digestible NDF. After incubation, samples were rinsed 2 times with tap water followed by four rinses with water filtered through a reverse osmosis system. The rinsed samples were then analyzed for NDF as previously described.

**Methane and SF$_6$ analyses**

Methane and SF$_6$ concentrations in collection canisters were analyzed by gas chromatography (Agilent 7820A GC; Agilent Technologies, Palo Alto, CA). A flame ionization detector and electron capture detector were used for CH$_4$ and SF$_6$ analysis, respectively, with a capillary column (Plot Fused Silica 25m by 0.32mm, Coating Molsieve 5A, Varian CP7536; Varian Inc. Lake Forest, CA). Injector, column, and detector temperatures for CH$_4$ analysis were 80, 160, and 200°C, respectively. For SF$_6$, Temperatures were 50, 30, and 300°C for the injector, column, and detector, respectively. The carrier gas for CH$_4$ and SF$_6$ analysis was N$_2$.

**Calculations**

Emission of CH$_4$ produced by steers was determined in relation to the SF$_6$ tracer gas captured in the collection canisters. The following equation was used to quantify CH$_4$ production:

\[
Q_{CH4} = Q_{SF6} \times \left( [CH4]_y - [CH4]_\beta \right) \div \left( [SF6]_y - [SF6]_\beta \right)
\]

in which $Q_{CH4}$ is considered CH$_4$ emissions per animal (g/d), $Q_{SF6}$ is considered SF$_6$ release rate (mg/d), $[CH4]_y$ is considered the concentration of CH$_4$ in the animals collection canister, $[CH4]_\beta$ is considered the concentration of CH$_4$ in the environmental canisters, $[SF6]_y$ is considered the concentration of SF$_6$ in the animals collection
canister, and $[SF_6]_p$ is considered the concentration of $SF_6$ in the environmental collection canister.

Apparent total tract digestibility of DM, OM, CP, NDF, and ADF was calculated as follows:

Nutrient digestibility (%) = 100 – \{100 \times [(iNDF concentration in the feed ÷ iNDF concentration in the feces) \times (nutrient concentration in the feces ÷ nutrient concentration in the feed)]\}.

**Statistical Analysis**

All data were analyzed as a generalized randomized block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Steer was considered the experimental unit. The model included the fixed effect of treatment and the random effects of breed and block. Significance was declared at $P \leq 0.05$.

**Results and Discussion**

Performance of beef steers is presented in Table 5-2. This experiment was not designed to evaluate performance of beef steers since it was performed in a short period and longer periods are more appropriate to collect more accurate results; however, since we had the ability to measure individual animal intake and weight, parameters of performance were analyzed just to provide descriptive statistics. No differences between treatments were observed for final BW ($P = 0.821$), DMI as a percentage of BW ($P = 0.847$), or ADG measured over the 20-d experimental period ($P = 0.103$).

Daily nutrient intake and total tract digestibility of beef steers consuming bahiagrass treated or not with CaO are presented in Table 5-3. No differences between treatments were observed on intake of DM ($P = 0.351$), OM ($P = 0.696$), CP ($P = 0.763$),
NDF \((P = 0.513)\), and ADF \((P = 0.310)\); however, apparent total tract digestibility of DM, OM, CP, NDF, and ADF were reduced \((P \leq 0.001)\) by 19, 16, 32, 7, and 7%, respectively, in steers consuming bahiagrass hay treated with CaO (CAO), when compared to those consuming untreated bahiagrass hay (DH).

No differences between treatments on nutrient intake is in accordance with results presented and described in Chapter 3, where animals consumed bahiagrass hay treated with 5% CaO in a similar manner. Although differences were not detected, these results were valid to demonstrate that steers in this experiment had a greater DMI either as kg/d or as a percentage of BW when compared to steers from the metabolism experiment described in Chapter 3, which were heavier. Steers from both experiments were consuming the exact same treatments, untreated bahiagrass hay and hay treated with 5% CaO. Nonetheless, the main difference between the current experiment and the experiment from Chapter 3, besides animal related differences, was that steers in this study were provided with added energy and protein in their diets that originated from cottonseed meal and molasses supplementation.

Reductions in NDF and ADF digestibility are in accordance with results observed in Chapter 3, where digestibility of fiber was also reduced in steers consuming bahiagrass hay treated with 5% CaO when compared to those consuming non-treated hay. It could be speculated that CaO, besides not being effective in increasing digestibility of fiber as proposed in the literature (Klopfenstein et al., 1972; Shreck et al., 2011; Watson et al., 2015), could be causing animal level effects. This speculation can be supported by the results observed in Chapter 3: reduction in ruminal total VFA concentration, which confirms reduction in fermentation, and tendency for reduction in
ruminal concentration of NH$_3$-N, which could explain lack of ability of fibrolytic microorganisms to digest fiber. These data indicate that CaO could be negatively affecting fiber digestion in the rumen, explaining the reductions in total tract digestibility of NDF and ADF. Differently from the metabolism study, total tract digestibility of CP was reduced by 32% in this study when steers consumed CaO treated bahiagrass hay. Although the forage was treated in a similar manner in both studies, the source of CaO in the current study was different, and it could be speculated that the exothermic reaction between CaO and water released greater amounts of heat that could have caused binding of some of the N to the fiber, thus making it less digestible and able to pass through the gastro-intestinal tract and show up in the feces, overestimating the reduction in total tract digestibility of CP.

Enteric CH$_4$ production of beef steers is presented in Table 5-4. No differences between treatments were observed on total CH$_4$ production (g/d; $P = 0.535$) or grams of CH$_4$ produced per kilogram of OM intake ($P = 0.694$), OM digested ($P = 0.168$), and metabolic BW ($P = 0.717$). Total CH$_4$ production (g/d) observed in the current study are in accordance with data collected in previous studies performed within our laboratory, where steers with similar BW and age consuming bahiagrass hay with supplemental molasses and non-protein nitrogen produced on average 116.8 g/d of CH$_4$ (Henry et al., 2018).

The expectations for this study were to have greater digestibility of the forage fiber promoted by treatment with CaO; however, results obtained here and in the metabolism study described in Chapter 3 indicated that total tract digestibility of fiber was actually reduced. Although an increase in fiber digestibility in the rumen due to
greater fermentation of the fiber fractions would have likely caused a greater total CH$_4$ production, g of CH$_4$ produced per kilogram of OM intake and OM digested could have been reduced. The term emission intensity has been proposed when referring to enteric CH$_4$ production and it is described as CH$_4$ produced per unit of animal product (Hristov et al., 2013). Methane production is a necessary consequence of forage digestion; however, increasing digestibility of forages and digestible forage intake has been reported to be an effective CH$_4$ mitigation strategy (Hristov et al., 2013) since by using the term emission intensity, the effect of a certain practice is based on a combination of feed intake, animal productivity, and CH$_4$ emission. If we had observed greater forage digestibility associated with greater intakes in the present study, it could be assumed that animal performance could have been increased reflected by greater weight gains, thus reducing CH$_4$ emission intensity.
Table 5-1. Analyzed chemical composition of bahiagrass (*Paspalum notatum*) hay treated or not with calcium oxide (CaO), cottonseed meal (CSM), and sugar cane molasses provided to beef steers\(^1\).

<table>
<thead>
<tr>
<th>Item(^2)</th>
<th>Treatment(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DH</td>
</tr>
<tr>
<td>DM, %</td>
<td>89.5 ± 0.4</td>
</tr>
<tr>
<td>OM, % DM</td>
<td>94.8</td>
</tr>
<tr>
<td>CP, % DM</td>
<td>7.7</td>
</tr>
<tr>
<td>NDF, % DM</td>
<td>71.9</td>
</tr>
<tr>
<td>ADF, % DM</td>
<td>41.3</td>
</tr>
<tr>
<td>Lignin(^4), % DM</td>
<td>3.3</td>
</tr>
<tr>
<td>TDN(^4), % DM</td>
<td>62</td>
</tr>
<tr>
<td>Calcium(^4), % DM</td>
<td>0.39</td>
</tr>
</tbody>
</table>

\(^1\)CSM and molasses were provided individually daily at 0.3% of BW and 0.454 kg/steer, respectively.  
\(^2\)DH = bahiagrass hay as is (dry); CAO = bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM).  
\(^3\)Hay and CSM DM, OM, CP, NDF, and ADF were analyzed in-house to determine individual nutrient intake during the 4-d digestibility period.  
\(^4\)Analyzed by a commercial laboratory using a wet chemistry package (Dairy One, Ithaca, NY).
Table 5-2. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with calcium oxide (CaO) on performance of beef steers provided ad libitum hay intake plus supplementation with cottonseed meal (CSM) and molasses$^1$.

<table>
<thead>
<tr>
<th>Item</th>
<th>DH</th>
<th>CAO</th>
<th>SEM$^3$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW, kg</td>
<td>317</td>
<td>316</td>
<td>9.4</td>
<td>0.957</td>
</tr>
<tr>
<td>Final BW, kg</td>
<td>332</td>
<td>329</td>
<td>9.3</td>
<td>0.821</td>
</tr>
<tr>
<td>DMI$^4$, % BW</td>
<td>2.4</td>
<td>2.4</td>
<td>0.10</td>
<td>0.847</td>
</tr>
<tr>
<td>20-d ADG$^5$, kg</td>
<td>0.757</td>
<td>0.642</td>
<td>0.0495</td>
<td>0.103</td>
</tr>
</tbody>
</table>

$^1$CSM and molasses were provided individually daily at 0.3% of BW and 0.454 kg/steer, respectively.

$^2$DH = bahiagrass hay as is (dry); CAO = bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM).

$^3$n = 10 steers/treatment.

$^4$Dry matter intake; calculated using the 4-d intake and the average of initial and final BW.
Table 5-3. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with calcium oxide (CaO) on daily nutrient intake and apparent total tract digestibility of beef steers provided ad libitum hay intake plus supplementation with cottonseed meal (CSM) and molasses\(^1\).  

<table>
<thead>
<tr>
<th>Item</th>
<th>DH</th>
<th>CAO</th>
<th>SEM(^3)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-d intake, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>7.56</td>
<td>8.21</td>
<td>0.365</td>
<td>0.331</td>
</tr>
<tr>
<td>OM</td>
<td>7.19</td>
<td>7.43</td>
<td>0.342</td>
<td>0.696</td>
</tr>
<tr>
<td>CP</td>
<td>0.92</td>
<td>0.93</td>
<td>0.036</td>
<td>0.763</td>
</tr>
<tr>
<td>NDF</td>
<td>5.03</td>
<td>5.35</td>
<td>0.266</td>
<td>0.513</td>
</tr>
<tr>
<td>ADF</td>
<td>2.72</td>
<td>2.96</td>
<td>0.142</td>
<td>0.310</td>
</tr>
<tr>
<td>Digestibility, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>57.73</td>
<td>46.51</td>
<td>0.768</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>OM</td>
<td>59.54</td>
<td>49.73</td>
<td>0.719</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CP</td>
<td>56.28</td>
<td>38.31</td>
<td>1.652</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NDF</td>
<td>61.25</td>
<td>57.14</td>
<td>0.701</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ADF</td>
<td>57.06</td>
<td>52.86</td>
<td>0.767</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(^1\)CSM and molasses were provided individually daily at 0.3% of BW and 0.454 kg/steer, respectively.  
\(^2\)DH = bahiagrass hay as is (dry); CAO = bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM).  
\(^3\)n = 10 steers/treatment.
Table 5-4. Effects of bahiagrass (*Paspalum notatum*) hay treated or not with calcium oxide (CaO) on enteric CH$_4$ production of beef steers provided ad libitum hay intake plus supplementation with cottonseed meal (CSM) and molasses$^1$.

<table>
<thead>
<tr>
<th>Item$^2$</th>
<th>Treatment$^3$</th>
<th>SEM$^4$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_4$ emissions, g/d</td>
<td>DH</td>
<td>119.91</td>
<td>109.66</td>
</tr>
<tr>
<td>CH$_4$ emissions, g/kg OMI$^5$</td>
<td>CAO</td>
<td>15.01</td>
<td>16.10</td>
</tr>
<tr>
<td>CH$_4$ emissions, g/kg OMDG$^6$</td>
<td></td>
<td>25.39</td>
<td>32.92</td>
</tr>
<tr>
<td>CH$_4$ emissions, g/kg MBW$^7$</td>
<td></td>
<td>1.56</td>
<td>1.68</td>
</tr>
</tbody>
</table>

$^1$CSM and molasses were provided individually daily at 0.3% of BW and 0.454 kg/steer, respectively.

$^2$CH$_4$ was determined from the average of at least three out of five 24-h periods of breath sample collection.

$^3$DH = bahiagrass hay as is (dry); CAO = bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM).

$^4$n = 10 steers/treatment.

$^5$OMI = OM intake.

$^6$OMDG = OM digested.

$^7$MBW = BW$^{0.75}$. 
CHAPTER 6
SUMMARY AND RECOMMENDATIONS

Summary

This series of experiments was designed with the primary objective to evaluate the use of the alkali calcium oxide (CaO) as a chemical treatment of low-quality warm-season forages and low nutritive value roughages commonly fed to beef cattle in the Southeastern U.S.

The proposed mode of action involving chemical treatment with alkalis indicate that increases in forage digestibility due to NDF reduction can be a consequence of hemicellulose solubilization and breakage of ester bonds between lignin and hemicellulose (Klopfenstein, 1978; Romero et al., 2013). Nevertheless, chemical treatment of low-quality forages and roughages, in an attempt to improve the digestibility of fiber fractions, is not a new concept in ruminant nutrition and dates back to the 1970's, when several different chemicals and roughages were extensively studied (Klopfenstein, 1978). Since then, NaOH has been considered the “golden standard” when it comes to hydrolytic treatments, promoting increases in digestibility as great as 30% (Fahey et al., 1993). Ammoniation of hay has also been studied (Brown and Kunkle, 1992) due to the ability to treat large quantities of hay at once, plus the additional N to the forage, improving feeding value. However, safety and environmental concerns regarding chemicals previously researched have been brought to attention, and other chemicals, such as CaO, have been tested as alternatives that can promote similar and efficacious results attempting to improve digestibility of forages’ fiber fractions.
To the best of the author’s knowledge, this is the first work conducted in which peanut hulls (PHS), Tifton 85 bermudagrass (*Cynodon spp.*) hay (BM), and Pensacola bahiagrass (*Paspalum notatum*) hay (BH) were treated with CaO and subsequent ruminal in situ degradability of nutrients (RISD) and in vitro organic matter digestibility (IVOMD) were measured. Furthermore, there are little to no data in the literature reporting the specific use of BH treated with CaO and provided to beef cattle as the sole diet ingredient to evaluate its effects on cattle ruminal fermentation parameters, apparent total tract digestibility of nutrients (ATTD), performance, and CH₄ production. Most of the research previously conducted, where CaO was used as a chemical treatment, report its use to treat crop residues such as corn stalks or stover, cobs, and cereal straws, which were used to replace an ingredient, usually corn or other grains, in total mixed rations (e.g. feedlot finishing or backgrounding diets).

Therefore, in the current series of experiments, 4 objectives were developed to test the primary hypothesis that the alkali CaO would improve digestibility of low-quality warm-season forages and roughages commonly found in Florida by increasing the digestibility of fiber fractions. The objectives were: 1) to evaluate the effects of CaO on RISD and IVOMD of PHS, BM, and BH after incubation in buckets; 2) to evaluate the effects of BH treated with 5% CaO on ruminal fermentation parameters, ATTD, and intake of beef steers consuming the hay as the only ingredient in the diet ad libitum; 3) to evaluate the effects of BH treated with 5% CaO and 10% molasses on beef cattle performance with or without protein supplementation via cottonseed meal; and 4) to evaluate the effects of BH treated with 5% CaO on ATTD and CH₄ production in beef steers consuming hay ad libitum plus supplementation of molasses and cottonseed.
meal. An additional objective was to assess effectiveness of CaO treatment by
determining IVOMD of the BH treated or not provided to cattle in the metabolism and
performance studies.

The first objective involved 3 different experiments labeled 1a, 1b, and 2. The
first two experiments evaluated RISD of PHS after treatment with 5% CaO and
incubation in buckets for 7 d or 14 d under 50% DM (Exp. 1a), and IVOMD of PHS after
treatment with 5% CaO and incubation for 3, 6, 9, 12, or 15 d under 70 or 50% DM
(Exp. 1b). The third experiment evaluated RISD and IVOMD of BH and BM after
treatment with either 5 or 10% CaO and incubation in buckets for 7 d or 14 d under 50%
DM (Exp. 2). These experiments generated data indicating that CaO, under the
conditions applied in the current experiment, does not have the ability to improve RISD
and IVOMD of PHS. It can be implied that CaO was not effective in promoting
breakdown of bonds between fiber fractions in PHS, which have a very low digestibility
as it is, and are resistant to chemical treatment, due to the high content of lignin.
Moreover, the tendency for reduction in IVOMD of PHS indicates that with the addition
of CaO, when the DM content is elevated, and as the incubation time increases, the
conditions obtained can create a perfect environment for mold growth, which is a
possible explanation for the reduction. Nevertheless, data obtained in Exp. 2 showed
that in general, CaO treatment promoted increases of up to 53% and 58% in RISD of
OM and NDF, respectively in BH when compared to the non-treated forage. In BM,
increases of up to 53% and 52% were observed for RISD of OM and NDF, respectively.
Greatest increases in ruminal degradability were observed when CaO was used at 10%
in BH, while no differences were observed between 5 or 10% CaO used to treat BM.
Moreover, IVOMD of BH was also increased by 10% with 5% CaO and 31% with 10% CaO treatment. For BM, IVOMD was increased by 31%, when the forage was treated with CaO, with no difference between 5 or 10% CaO. Therefore, with the results obtained from the experiments treating warm-season forages, it can be suggested that CaO is an effective chemical to promote increase fiber digestibility, consequently improving nutritive value of low-quality forages.

The second objective involved a metabolism study with 9 cannulated beef steers, which were provided BH treated or not with 8.9% CaCO$_3$ or 5% CaO as the only ingredient in their diets. The results obtained in this study indicate that although DMI was not affected by treatment, it was considered very low across treatments, implying that when warm-season, low-quality forages are provided as the sole source of feed, protein supplementation should be considered to maximize intake by the animals. Furthermore, BH treated with CaO promoted an increase in ruminal pH, which was expected since CaO is a strong base. Concentrations of NH$_3$-N in the rumen and urea-N in the blood were not different across treatments; however, were considered extremely low, potentially implying N deficiency and also indicating a benefit from protein supplementation. Total and individual concentrations of VFA in the rumen were reduced in steers consuming bahiagrass treated with CaO, partially explaining the increase in ruminal pH. There was a reduction in total tract digestibility of NDF and ADF when bahiagrass was treated with either CaCO$_3$ or CaO, indicating that CaO was not necessarily the culprit of fiber digestibility reduction; however, the reduction of VFA concentration in the rumen caused by CaO, confirms reduction in fermentation, pointing to the fact that CaO can also be negatively affecting fiber digestion in the rumen. The
reasons behind reductions observed in the present study caused by CaO are still undefined, allowing for the opportunity of more in depth research into the effects of CaO on ruminal fermentation, which, in this case, is clearly being negatively affected. However, the data obtained after performing IVOMD of the forage provided to steers are contradictory to the results obtained in vivo since IVOMD of BH treated with CaO was greater when compared to the non-treated forage.

To address the third objective, 2 experiments were performed where growing beef cattle were provided with BH treated with 5% CaO and 10% molasses ad libitum. In Exp. 1, the hay was the only feed consumed and in Exp. 2 cattle were provided with protein supplementation via cottonseed meal. The data generated in Exp. 1 indicated no differences among treatments for final BW or ADG; however, cattle had an average loss in BW across treatments of 0.03 kg/d. Data from Exp. 2 were similar and no differences in final BW and ADG were observed between treatments; however, in this experiment, cattle did not experience weight loss, and had an average gain in BW of 0.537 kg/d. The hay provided in Exp. 1 was tested for IVOMD to evaluate if treatment with CaO was being effective at improving digestibility of the forage, which consequently could improve cattle performance. Surprisingly, not only did CaO not improve IVOMD of BH, but there was a reduction in IVOMD when compared to the non-treated hay. Results from both experiments, in vivo and the in vitro, indicate that, under the conditions of the experiments performed to address the third objective, CaO failed to improve digestibility of the forage, explaining the lack of increased performance that was expected in cattle consuming CaO treated hay.
Finally, the fourth objective proposed was accomplished by feeding BH treated with 5% CaO to 20 beef steers ad libitum plus supplementation with cottonseed meal and molasses. The data generated in this final experiment showed that, similarly to the metabolism study, ATTD of all nutrients measured were reduced by up to 32% in steers consuming BH treated with CaO. Furthermore, CH₄ production was not affected by treatment. It was hypothesized that CaO would promote greater digestibility of the forage, which in turn could translate into increased animal performance reflected by greater weight gains, thus reducing CH₄ emission intensity. Nevertheless, data obtained in the series of experiments in vivo do not support our claim, since total tract digestibility of fiber was reduced and animal performance was not improved. In contrast, data obtained from experiments performed in situ and in vitro still indicate that CaO could be a valid option for treating warm-season forages, since ruminal degradation of nutrients and in vitro organic matter digestibility were increased with CaO treatment. Further research should be performed to address the differences observed.
Recommendations

The data collected from the experiments described in this dissertation create questions that should be addressed, promoting a foundation for future research involving chemical treatment of warm-season forages with CaO and provided to beef cattle.

The results obtained for both warm-season forages (BH and BM) in the first objective indicate that using a greater amount of CaO is beneficial to improve degradability of nutrients and IVOMD of BH to a greater extent; however, benefits do not seem to apply to BM, thus 5% CaO should be recommended for treating BM.

Since using 5% CaO to treat PHS did not seem to promote satisfactory results, perhaps greater amounts, such as 10% or more, could be considered when roughages with greater concentrations of lignin are used. Moreover, it would be interesting to evaluate the CaO treatment of PHS with different concentrations of lignin to evaluate if the lack of results obtained in the present study are strictly due to the high concentration of fiber in the PHS used. Additionally, a greater number of replications should be used when testing PHS, to exclude the potential lack of power.

The three contrasting scenarios described in the first two objectives proposed (in situ vs. in vivo vs. in vitro) indicate that although CaO may be negatively affecting ruminal fermentation, it can still be a valid option to treat low-quality forages, based on the results observed in vitro and in situ. However, animals used as incubators and donors in the experiments addressing the first objective and the additional objective, respectively were not consuming forages treated with CaO. Therefore, it is recommended that the same treatment conditions are applied to the warm-season
forages tested here but forages treated with CaO should be provided to animals used as incubators and ruminal fluid donors to eliminate potential confounding effects.

Based on the results obtained in the metabolism study and the performance study where cattle were only consuming the hay without any supplementation, it is recommended that when low-quality forages such as bahiagrass hay is provided as the only ingredient in the diet, protein supplementation should be provided.

The methods for treatment of the forage in the performance studies were different from the methods applied in the studies addressing the first and second objectives. The main difference was that in the performance studies, the hay was treated by pouring a mixture of CaO, molasses, and water on top of the round bales allowing it to soak through, while for the first two objectives, the CaO and water were both properly mixed with the forage that was previously chopped, allowing for a better contact and homogenization. Therefore, the lack of results in the performance studies could be due to the methodology applied, which did not allow for the CaO to perform properly. It is likely that the lack of proper contact of the CaO with the fiber fractions of the forage did not permit the bonds between lignin and the polysaccharides to be broken down and for that reason fiber digestibility was not improved. It is recommended that further studies are performed where, besides providing protein and energy supplementation, BH is treated exactly like in the metabolism study to evaluate performance of beef cattle.


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BIOGRAPHICAL SKETCH

Francine is originally from the State of São Paulo in Brazil and although agriculture and livestock production were never part of her childhood, she knew at a young age that she had a passion for animals. After graduating from high school, Francine found her interest for livestock, especially beef cattle. In 2011, Francine received her bachelor’s degree in Animal Science from São Paulo State University in Dracena, São Paulo, Brazil and moved to the United States. For a total of eight years, at the North Florida Research and Education Center, in Marianna, FL, Francine performed internships and worked towards her Master of Science and Doctor of Philosophy degrees focusing on beef cattle nutrition. Francine worked under the advisement and mentorship of Dr. Nicolas DiLorenzo throughout both advanced degrees while at the University of Florida. She is now Francine Henry, happily married to her amazing husband, Darren Henry, and they are expecting their first baby. After completing her Doctor of Philosophy in December 2018, Francine pursued a career in the beef cattle industry as a ruminant nutritionist.