



8-2019

## **Impact of nutritional management strategies on semen quality of developing bulls**

Jarret Proctor

*University of Tennessee*, [jprocto7@vols.utk.edu](mailto:jprocto7@vols.utk.edu)

Follow this and additional works at: [https://trace.tennessee.edu/utk\\_gradthes](https://trace.tennessee.edu/utk_gradthes)

---

### **Recommended Citation**

Proctor, Jarret, "Impact of nutritional management strategies on semen quality of developing bulls. " Master's Thesis, University of Tennessee, 2019.  
[https://trace.tennessee.edu/utk\\_gradthes/5538](https://trace.tennessee.edu/utk_gradthes/5538)

This Thesis is brought to you for free and open access by the Graduate School at TRACE: Tennessee Research and Creative Exchange. It has been accepted for inclusion in Masters Theses by an authorized administrator of TRACE: Tennessee Research and Creative Exchange. For more information, please contact [trace@utk.edu](mailto:trace@utk.edu).

To the Graduate Council:

I am submitting herewith a thesis written by Jarret Proctor entitled "Impact of nutritional management strategies on semen quality of developing bulls." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Jason Smith, Major Professor

We have read this thesis and recommend its acceptance:

Justin Rhinehart, Lew Strickland

Accepted for the Council:

Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

**Impact of nutritional management strategies on semen quality of developing  
bulls**

**A Thesis Presented for the**

**Master of Science**

**Degree**

**The University of Tennessee, Knoxville**

**Jarret Allen Proctor**

**August 2019**

## ACKNOWLEDGMENTS

First and foremost, I want to thank my parents for their continued support and encouragement to chase my dreams, wherever that may take me. Secondly, my siblings, along with Shelbi Perry were tremendous supporters throughout this process and I will be eternally grateful for each of them. The friendships I have made throughout my time in Tennessee are those that will last well beyond my years at this institution, and I am thankful for the connections I have developed. Without my research assistants, consisting of Connor Biehler, Kyla-Jo Farmer, Nate Long, Abigayle Pollock, John Robert Snoddy, Kelly Melvin, and Monica Creazzo, these projects would not have been possible.

Additionally, I want to express my gratitude to the Department of Animal Science at the University of Tennessee for funding this opportunity to expand my educational and technical skillset. Additionally, my committee members, Dr. Justin Rhinehart and Dr. Lew Strickland were immensely valuable to my program and I will continue to value their knowledge and insight within their respective fields of study. Most importantly, I want to thank Dr. Jason Smith, my mentor, for allowing me to serve as his first graduate student. I am grateful that his guidance and passion for animal agriculture has pushed my education and development further than I ever thought possible, the knowledge and experience I have accrued under his direction will last a lifetime. Finally, I want to express my gratitude towards the Tennessee Beef Industry Council and Jonathan Perry of Deer Valley Farms for their support of these research projects.

## ABSTRACT

Nutritional management and semen collection method are thought to influence semen quality parameters evaluated during a breeding soundness examination. Therefore, the objective of this experiment was to evaluate the effects of two feeding strategies on growth performance and semen quality of developing bulls. A secondary objective was to quantify the effects of two semen collection methods on semen quality. Angus bulls ( $n = 48$ ;  $332 \pm 47$  d of age) were stratified into one of sixteen drylot pens before random assignment to one of two iso-caloric diets: a total mixed ration (TMR;  $n = 8$  pens) or a component-based ration (COMP;  $n = 8$  pens). Bulls were assigned to either electroejaculation (EE;  $n = 24$ ) or trans-rectal massage (RM;  $n = 24$ ) and each collection method occurred twice throughout an 84-d feeding period. Rumen pH was continuously monitored via rumen boluses and dry matter intake (DMI) was measured daily. Backfat thickness and hoof conformation were measured on d 0 and 84, with body weight measured and semen collected at 21-d intervals. Semen was assessed for progressive motility, total sperm per ejaculate, and sperm cell morphology. Bulls fed the COMP ration had a greater DMI ( $P < 0.01$ ) and ADG ( $P < 0.01$ ), but similar feed efficiency ( $P = 0.19$ ) when compared to TMR-fed bulls. Bulls fed COMP had a lower mean rumen pH ( $P < 0.01$ ). Additionally, COMP-fed bulls had greater backfat thickness ( $P < 0.01$ ) but similar hoof conformation scores ( $P \geq 0.55$ ) when compared to TMR-fed bulls on d 84. Bulls fed the COMP had greater semen volume ( $P < 0.05$ ) but feeding management did not affect progressive motility ( $P = 0.42$ ), total sperm per ejaculate ( $P = 0.86$ ), or percent morphologically-normal sperm ( $P = 0.59$ ). However, bulls collected via RM had greater total sperm per ejaculate ( $P < 0.05$ ), and semen volume ( $P < 0.01$ ), but lower percent morphologically-normal sperm ( $P < 0.05$ ) and progressive motility ( $P < 0.01$ ). Neither diet nor semen collection method affected BSE passage rate ( $P \geq 0.34$ ), suggesting minimal influence on BSE outcomes in developing bulls.

# TABLE OF CONTENTS

|   |    |
|---|----|
| CHAPTER 1: Literature review .....                                      | 1  |
| Introduction.....   | 2  |
| Etiology of SARA.....   | 3  |
| Overview .....  | 3  |
| Energy and starch digestion.....  | 3  |
| Organic acid by-products of ruminal fermentation .....                  | 6  |
| Rumen pH as an indicator of acidosis .....                              | 10 |
| Rumen temperature as an indicator of acidosis .....                     | 13 |
| Rumen osmolality and damage to ruminal papillae .....                   | 14 |
| Identification and implications of SARA .....                           | 15 |
| Parakeratosis, rumenitis, and liver abscesses .....                     | 16 |
| Laminitis/lameness .....  | 18 |
| Fecal composition.....  | 21 |
| Variation in DMI .....  | 22 |
| Treatment and prevention of SARA .....                                  | 25 |
| Ration composition and physically-effective fiber .....                 | 26 |
| Transition diets, ration blending, and bunk and feeding management..... | 28 |
| Ionophores.....   | 30 |

|   |    |
|---|----|
| Buffers.....  | 32 |
| Nutritional influences on bull reproduction.....                                | 33 |
| Pre-weaning nutritional management .....  | 34 |
| Post-weaning development .....  | 39 |
| SARA in developing bulls .....  | 43 |
| References.....   | 46 |
| CHAPTER 2: Impact of feeding management strategies on growth performance, rumen |    |
| conditions, and semen quality of developing bulls .....                         | 66 |
| Abstract.....   | 67 |
| Introduction.....   | 68 |
| Materials and methods .....   | 69 |
| Experimental design and feeding management.....                                 | 70 |
| Feed intake, sampling, re-formulation, and analysis .....                       | 72 |
| Growth performance and hoof conformation .....                                  | 73 |
| Rumen pH and temperature.....   | 74 |
| Scrotal circumference, semen collection, and semen quality .....                | 74 |
| Statistical analyses.....   | 77 |
| Results and discussion .....  | 80 |
| Feed analysis and intake .....  | 80 |
| Growth performance and hoof conformation .....                                  | 82 |

|   |     |
|---|-----|
| Rumen pH and temperature .....  | 84  |
| Semen quality.....  | 89  |
| Conclusions.....  | 100 |
| References.....   | 102 |
| Appendix A: Tables .....  | 109 |
| Appendix B: Figures.....  | 123 |
| CHAPTER 3: Effects of semen collection method on semen quality of developing bulls... |     |
| .....   | 130 |
| Abstract.....   | 131 |
| Introduction.....   | 132 |
| Materials and methods .....   | 133 |
| Experimental design and animal management .....                                       | 133 |
| Sampling and measurements .....   | 134 |
| Statistical analyses.....   | 137 |
| Results and discussion .....  | 138 |
| SC, semen collection volume, and total spermatozoa output .....                       | 138 |
| Spermatozoa morphology and motility.....  | 141 |
| BSE .....   | 143 |
| Conclusions.....  | 144 |
| References.....   | 145 |



|                                       |     |
|---------------------------------------|-----|
| Appendix C: Tables .....              | 148 |
| CHAPTER 4: Conclusion .....           | 150 |
| Conclusions and future research ..... | 151 |
| VITA .....                            | 152 |

## LIST OF TABLES

|  |         |
|--|---------|
| Table 2.1: Ingredient composition, nutrient composition, and cost of rations used to develop Angus bulls over an 84-d period.....  | 109     |
| Table 2.2: Growth performance, feed intake, feed efficiency, and scrotal circumference of Angus bulls developed on one of two iso-caloric rations differing in form .....  | 110-111 |
| Table 2.3: Average rumen pH and temperature of Angus bulls developed on one of two iso-caloric rations differing in form.....  | 112     |
| Table 2.4: Model-adjusted probabilities and subsequent odds ratios of rumen pH below various thresholds for Angus bulls developed on one of two iso-caloric rations differing in form .....  | 113     |
| Table 2.5: Semen quality parameters of Angus bulls developed on one of two iso-caloric rations differing in form.....  | 114-115 |
| Table 2.6: Effect of semen collection day on semen quality parameters of Angus bulls. ....   | 116     |
| Table 2.7: Relationships between the proportion of rumen pH observations below specific pH thresholds and progressive motility, semen collection volume, or percentage morphologically-normal sperm across subsequent semen collection days in Angus bulls ..... | 117     |
| Table 2.8 : Relationships between average rumen pH, average rumen temperature, full trial change in SC, or full trial change in SFT and progressive motility, semen collection volume, and percentage morphologically-normal sperm in Angus bulls .....          | 118     |

|   |     |
|---|-----|
| Table 2.9: Relationships between average rumen pH, average rumen temperature, full trial change in SC, or full trial change in SFT and full trial changes in progressive motility, semen collection volume, or percentage morphologically-normal sperm in Angus bulls.....  | 119 |
| Table 2.10: Relationships between the proportion of rumen pH observations below specific pH thresholds and total sperm in the ejaculate, total morphologically-normal sperm in the ejaculate, and total morphologically-abnormal sperm in the ejaculate across subsequent semen collection days in Angus bulls..... | 120 |
| Table 2.11: Relationships between average rumen pH, average rumen temperature, full trial change in SC, or full trial change in SFT and total sperm per ejaculate, total morphologically-normal sperm per ejaculate, and total morphologically-abnormal sperm per ejaculate in Angus bulls.....                     | 121 |
| Table 2.12: Relationships between average rumen pH, average rumen temperature, full trial change in SC, or full trial change in SC and full trial changes in total sperm per ejaculate, total morphologically-normal sperm per ejaculate, or total morphologically-abnormal sperm per ejaculate in Angus bulls..... | 122 |
| Table 3.1: Least Square means of SC and semen quality parameters.....   | 148 |
| Table 3.2: Least square means of individual classifications of morphological abnormalities.....   | 149 |

## LIST OF FIGURES

|  |     |
|--|-----|
| Figure 2.1: Digestive morbidity of Angus bulls developed on one of two iso-caloric rations differing in form.....          | 123 |
| Figure 2.2: Average daily DMI of Angus bulls developed on one of two iso-caloric rations differing in form.....            | 124 |
| Figure 2.3: Initial and final SFT of Angus bulls developed on one of two iso-caloric rations differing in form.....        | 125 |
| Figure 2.4: Initial and final hoof angle of Angus bulls developed on one of two iso-caloric rations differing in form..... | 126 |
| Figure 2.5: Initial and final claw set of Angus bulls developed on one of two iso-caloric rations differing in form.....   | 127 |
| Figure 2.6: Average hourly rumen pH of Angus bulls developed on one of two iso-caloric rations differing in form.....      | 128 |
| Figure 2.7: Average daily rumen pH of Angus bulls developed on one of two iso-caloric rations differing in form.....       | 129 |

# **CHAPTER 1: Literature review**

## **Introduction**

Subacute ruminal acidosis (SARA) has been established as a pertinent issue in both dairy and beef cattle industries. Annual production losses associated with SARA have been estimated at \$500 million to \$1 billion US dollars annually in lactating dairy cows (Enemark, 2008). However, these estimates did not include the potential economic burden of SARA in beef cattle. Numerous studies have displayed the susceptibility of feedlot cattle to SARA, solidifying the necessity of prevention, identification, and treatment in feedlot/finishing operations. Finishing cattle and lactating dairy cows are fed diets similar in energy composition to realize genetic potential for growth and milk production. Sufficient dietary energy must be supplied to ensure genetic potential for growth and reproduction is achieved (Montaño-Bermudez et al., 1990; Castro Bulle et al., 2007), and inadequate energy has been shown to negatively affect indicators of fertility; specifically the attainment of puberty (Schillo, 1992). Cattle rapidly adjusted to or maintained on high energy diets may experience an increased occurrence of SARA, limiting efficiency in production, and ultimately income. Similarly to finishing cattle, developing bulls, specifically those that are performance-tested, may be vulnerable to SARA as nutritional management practices often resemble those of finishing scenarios.

Sire development is imperative to ensure proper reproductive development and advancement of herd genetics. There is evidence to suggest that SARA may negatively impact developing sire fertility (Callaghan et al., 2016). Failure of a breeding soundness examination (BSE) due to poor semen quality may prevent sires from entering into a breeding herd and thus the advancement of genetics. Moreover, the additional impact on bull development and semen quality may further influence the impact of SARA on the beef industry. It is imperative that beef cattle producers recognize the potential impact of SARA in order to maximize efficiency and

prevent financial loss. This review will discuss the etiology, identification, treatment, and prevention of SARA, with particular emphasis placed on the potential impact that nutrition and SARA have on semen quality in developing bulls.

## **Etiology of SARA**

### ***Overview***

Subacute ruminal acidosis may occur following the consumption of significant amounts of readily fermentable non-structural carbohydrates or starch (Owens et al., 1998), without sufficient physically-effective fiber. Rumen pH is depressed when volatile fatty acids (VFAs) and lactic acid accumulate in the rumen without sufficient buffering (Huber, 1976; Plaizier et al., 2008). Subsequent pH depressions can lead to acidotic conditions in the rumen and create a series of physiological events which have the potential to affect several phenotypic traits. The following sections outline the etiology of SARA as they are specifically related to energy and starch digestion, and organic acid byproducts of ruminal fermentation.

### ***Energy and starch digestion***

Starch-based grains provide a significant source of readily-available energy in ruminant diets (Waldo, 1973). Starch availability and metabolism is highly dependent on grain source, processing, and starch type (Owens et al., 1998). Non-structural carbohydrates, which are more readily fermentable in the rumen, increase the ruminant animal's susceptibility to acidosis but offer a much more bioavailable energy source (Church, 1988; Krause and Oetzel, 2006). Starch content of non-structural carbohydrates serves as a predictor of energy content, and varies between grain sources. Huntington (1997) described the starch content on a dry matter basis of wheat (77%), corn and sorghum (72%), barley (57%), and oats (58%). Heat processing, high-

moisture storage, and particle size reduction increases starch availability in these grain sources (Galvayan et al., 1976). The predominate site of starch digestion is the rumen, and grain processing has been shown to increase overall ruminal digestibility (Theurer, 1986). Of the starch content of known cereal grains, over 90% of oat, barley, and wheat starch is digested in the rumen, with approximately 75% of sorghum starch and 83-88% of corn starch digested in the rumen (Waldo, 1973; Church, 1988). Steam-flaking, high-moisture ensiling, and dry-rolling seem to maximize ruminal digestion and increase overall total tract digestibility of corn (Theurer, 1986). It is important to note that the effects of grain processing on digestibility are dependent upon grain type, moisture, and physical properties (Hale, 1973). Yet, grain processing has been shown to alter rumen fermentation kinetics as enzymatic starch digestion, dry matter disappearance, and VFA production are increased with processing given the greater surface area for the rumen microbial population to act upon (Hale, 1973; Church, 1988). Grain processing is a critical component in cattle feeding and increases availability for the ruminal microbial community to liberate energy from starch granules.

Once feedstuffs reach the reticulo-rumen, starch is primarily fermented by amylolytic bacteria. These bacteria attach to starch granules, and secrete amylase, which is ultimately responsible for the liberation of glucose units (Huntington, 1997; Owens et al., 1997). As identified by Kotarski et al. (1992), 15 strains of amylolytic bacteria produce up to 8 enzymes that are responsible for ruminal starch digestion. Although protozoa and yeast each play roles in starch digestion, ruminal bacteria are the predominate contributors to starch digestion and the release of glucose (Huntington, 1997). Glucose fermented by the microbial population in the rumen results in increased VFA production, including propionate, which serves as the key substrate for glucose synthesis in the liver (Reynolds, 2005). Glucose liberated from starch in the



small intestine is absorbed by both passive and active transport via sodium-glucose cotransporters (SGLT) 1 and 2 from the enterocytes lining the epithelium. Yet, active transport from SGLT1 accounts for more than 90% of total glucose absorption from glucose liberated from starch in the small intestine (Poulsen et al., 2015). Once absorbed, glucose travels via the portal vein to the liver to be metabolized into fatty acids or stored as glycogen, or will remain intact to reach systemic circulation. However, glucose absorption from the small intestine in ruminants accounts for less than 10% of total glucose requirements of the animal (Nafikov and Beitz, 2007).

Blood glucose concentrations in ruminants are generally very low based on tight hormonal regulation from the pancreas, which secretes both insulin and glucagon. Insulin is the key hormone signaling glucose uptake whereas glucagon signals for gluconeogenesis in times of low blood glucose concentrations (Poulsen et al., 2015). Additionally, starch digestion in the small intestine yields minimal amounts of glucose for absorption through SGLT transporters. Limited glucose absorption from ruminal and post-ruminal starch and carbohydrate digestion coupled with hormonal regulation maintains blood glucose at relatively low levels in ruminant animals. However, it has been noted that glucose levels may rise significantly in instances of grain engorgement and acidosis (Owens et al., 1998). In an acidosis challenge by Horn et al. (1979), glucose concentrations 1 hr post-feeding rose to over 160 mg/dL, indicating the rapid breakdown of grain starch to glucose. Following 24 hrs, ruminal glucose levels returned to basal levels at approximately 6-12 mg/dL. The liberation of free glucose in the rumen may be characterized as an increase in starch breakdown and/or a decrease in the utilization of glucose by the rumen microbial population. Once ingested by microbial species, glucose enters glycolysis and has the potential to be interconverted to a number of intermediaries, yet is

generally metabolized to pyruvate, which serves as a precursor for several energy yielding pathways within microbial species in the rumen.

### ***Organic acid by-products of ruminal fermentation***

Excess pyruvate produced by the rapid fermentation of non-structural, grain-derived starches has one of two fates: formation of lactic acid or VFAs. Each of these organic acids are by-products of ruminal fermentation, yet in excess and without sufficient rumen buffering, may induce acidosis in beef cattle (Owens et al., 1998; Krause and Oetzel, 2006). Lactic acid and VFAs may either act in conjunction or individually to alter the ruminal environment, and are highly influenced by ration composition.

Lactic acid is produced in two forms, D- and L-lactic acid, and is a well-known by-product of ruminal fermentation. Lactic acid is predominately converted to acetate, yet has been shown to accumulate in the rumen in animals fed high-concentrate diets (Nakamura and Takahashi, 1971). Both D-lactic acid and L-lactic acid are absorbed through the ruminal epithelium, and at least 50% of -D and 70% of -L forms of lactic acid are absorbed regardless of ruminal concentration (Dunlop and Hammond, 1965). Hibbard et al. (1995) and Oetzel et al. (1999) proposed that lactic acid accumulates in the rumen in low concentrations regardless of diet, at less than 5  $\mu\text{M}/\text{mL}$ . Additionally, it has been found that normal fermentation patterns do not result in lactic acid accumulations above 12  $\text{mM}/\text{mL}$  in the rumen (Hungate, 1975). However, cases of severe acidosis have been associated with rumen lactic acid concentrations of 40  $\text{mM}$  (Owens et al., 1998) and 43  $\text{mM}$  (Goad et al., 1998). Furthermore, it is thought that ruminal concentration of lactic acid may undergo diurnal variation, as Kennelly et al. (1999) showed lactating dairy cows have ruminal lactic acid concentrations upwards of 20  $\text{mM}$  following initial feeding. Interestingly, 12 hours later, following a second feeding of the same

amount previously fed, peak lactic acid production was approximately 5 mM lower, suggesting the possibility of greater absorption rates or conversion to acetate throughout the day to mitigate lactic acid accumulation (Kennelly et al., 1999).

Ruminal L-lactic acid absorbed into portal blood flow is thought to be metabolized by the liver and heart. Yet, D-lactic acid is not metabolized by the liver or heart, and is predominately excreted through urine (Dunlop and Hammond, 1965). There is little support of the prevention and treatment of D-lactic acidosis as it accumulates in the rumen and blood circulation. Thus, D- lactic acid has been loosely associated with SARA but strongly associated with acute forms of acidosis (Dunlop and Hammond, 1965; Mackenzie, 1967; Nocek, 1997; Goad et al., 1998; Ewaschuk et al., 2005). If concentrations of D-lactic acid exceed the ability of the bicarbonate blood buffering system to mitigate the decreases in blood pH, the consequence may be hemoconcentration, coma, or death (Owens et al., 1998).

Bacteria in the rumen fall under one of two classifications: lactate consumers or lactate producers (Russell and Hino, 1985; Brown et al., 2006). As described by Owens et al. (1998), the balance between lactate consumption and production determines lactate concentration in the rumen. Acidosis may occur when high-starch diets are fed and lactate production exceeds consumption (Dunlop and Hammond, 1965; Kleen et al., 2003). Lactate-consuming ruminal bacteria are sensitive to low pH, whereas lactate producers are not disrupted by pH depression (Mackie and Gilchrist, 1979). Bacteria that consume lactate remain active until rumen pH drops below 5.6, and therefore contribute minimally to SARA, but to a greater degree to acute acidosis (Goad et al., 1998; González et al., 2012). Additionally, *Streptococcus bovis*, a lactate-producing bacteria of the *Lactobacillales* order, increases lactate production in acidic culture at pH of 4.7 (Russell and Hino, 1985). As the level of fermentable carbohydrates increases in the diet, *S.*

*bovis* may increase from  $10^7/g$  to as much as  $10^{11}/g$  of ruminal content (Nagaraja and Titgemeyer, 2007), as they appear to proliferate every 20-24 minutes as the proportion of cereal grains increase in the diet (Hungate, 1975; McAllister et al., 1990). Similarly, Slyter (1976) found that cattle adapted to a grain-based diet experience a linear increase in *lactobacilli* and D-lactic acid production *in vitro*. Although lactic acid is predominately used as an energy substrate for acetate production during normal fermentation (Hungate, 1975), it is clear that high-energy diets fed to cattle may induce rapid growth of lactate-producing bacteria in the rumen. There is little question as to whether lactic-acid producing and consuming bacteria contribute to acidosis but more of a question as to when and to what extreme these microorganisms affect fermentation outcomes. The question also remains as to the complexity of the interaction between lactic acid and VFA production, and how they each contribute to acidosis in cattle.

Volatile fatty acids are the principle energy-yielding by-products of ruminal fermentation, with the most abundant being acetate, propionate, and butyrate (Hungate, 1975). While there are varying reports between researchers, VFA concentrations are generally dependent upon several factors. Bergman (1990) suggests that concentrations typically range between  $60-150 \text{ mM} \times \text{mL}^{-1}$  of rumen fluid. It has been documented that VFA concentrations may exceed  $200 \text{ mM} \times \text{mL}^{-1}$  of rumen fluid in cattle adapted to high grain diets (Huber, 1976; Bergman, 1990), with maximum VFA production occurring 2-4 hours following consumption of feed. Balch and Rowland (1957) found that cattle adapted to a high-forage diet did not exceed a total VFA concentration of greater than  $100 \text{ mM} \times \text{mL}^{-1}$  of rumen fluid, yet this is likely due to drastic increases in fermentation time due to high cellulose and hemicellulose concentrations. However, this threshold may be subject to discussion as additional research shows that VFA concentration from forage-adapted cattle may reach 120 mM in the rumen (Goad et al., 1998;

Brown et al., 2006). Furthermore, dietary changes in research by Bergman (1990) suggest changes in the acetate:propionate:butyrate ratio across diet, at 70:19:11 in cows adapted to grass and 46:42:12 in cows adapted to grain. It should be noted that these ratios are susceptible to variation across experiments and are dependent on forage or grain type and quality (Balch and Rowland, 1957; Hungate, 1975; Bergman, 1990). Other VFAs generally contribute less than 5% of the total VFA concentration, and thus often go unreported (Owens et al., 1998). Propionate is the only VFA in which production has been shown to increase when ruminal pH is less than 5.5 (Stock, 2000) and creates an acetate:propionate ratio of close to 1:1 (Davis, 1967). Propionate may play an additional role as a precursor for lactic acid production (Bergman, 1990). Although most literature does not speculate as to which VFA may contribute to ruminal pH depression, Bergman (1990) suggests that while most propionate is absorbed through the ruminal epithelium to be converted to succinyl-CoA and ultimately enter gluconeogenesis, as much as 5% of ruminal propionate may be interconverted to lactic acid, leading to the speculation that it may influence acidotic conditions, specifically below a pH of 5.2.

Although it is thought by some that ruminal pH is strictly influenced by VFA concentration, or lactate, others suggest ruminal pH is dependent on both VFA and lactate concentrations (Briggs et al., 1957; Britton and Stock, 1987; Kleen et al., 2003). Under various feeding conditions, VFAs and bicarbonate serve as the predominate compounds responsible for buffering the bovine rumen (Counotte et al., 1979). It is suggested that hydrogen ion concentration increases from approximately 90 mM of mOsm to nearly 150 mM of mOsm as rumen pH drops from pH 6 to 5 (Owens et al., 1998). As ruminal pH approaches 5.0 from higher pH values, there is an increase in ionization of VFAs which serve as the predominate buffer, thus making lactate de-ionization the primary source of hydrogen ions at this particular pH (Fulton et

al., 1979). The drastic increases in ionization rates results from the known  $pK_a$  of lactate and VFA, which are 3.9 and 4.8, respectively (Krause and Oetzel, 2006). Therefore, as ruminal pH approaches 5.0-5.5, VFAs become un-dissociated, and are more likely to be passively absorbed, pending normal epithelial conditions exist. In an example by Krause and Oetzel (2006), with ruminal pH at 5.0, lactate is 5.2 times more un-dissociated than all VFAs. This suggests that VFAs will contribute to acidosis at a rumen pH of 5.2 – 5.5, where lactate may drive more acute forms of acidosis at a rumen pH around 5.0 (Owens et al., 1998; Krause and Oetzel, 2006). Additionally, Briggs et al. (1957) found that decreases in ruminal pH explained more variation in lactate than VFA concentrations, although both organic acids were linked to the initial pH drop following feed intake. Based on hydrogen ion concentrations at various ruminal pH thresholds, high levels of lactate production are most likely associated with acute acidosis when ruminal pH is around 5.0 to 5.2. The data presented within the bounds of this review support the notion that dissociated VFAs may increase hydrogen ions in the rumen to drive pH below 5.5. However, there is little data to support a threshold of when rumen pH becomes more associated with lactate dissociation as a sink for hydrogen ions to further lower rumen pH. Given the biological influence of the known  $pK_a$  of VFAs and lactate, such a threshold likely exists, and merits further investigation.

### ***Rumen pH as an indicator of acidosis***

Assessment of ruminal pH may offer the most objective indication of SARA, yet remains variable and subjective across researchers, and is not currently applicable across most real-world production scenarios. Nonetheless, rumen pH has become a common measurement used to identify SARA in ruminant nutrition research. Without defined objective pH criteria for acute acidosis and SARA, these events are difficult to identify across research reports. The following

section outlines pH values that have been used as reference values for acidosis in the literature, and describes common sources of variation that may affect measurements of rumen pH.

Although it is well known that decreases in rumen pH may result in SARA, there exists conflict in the literature on a true pH threshold that should be used to accurately identify this event. Cooper et al. (1999) characterized SARA by episodes of ruminal pH between 5.2 and 5.6 in finishing beef steers. Similarly, Beauchemin et al. (2003) established a pH of 5.8 or lower in lactating dairy cows as a threshold for ruminal acidosis, with no specificity for time below threshold pH. Additionally, SARA has been delineated as a pH below 5.5 as is suggested to be the threshold at which lactate begins to contribute to hydrogenation in the rumen in conjunction with VFA becoming un-dissociated and absorbed across the ruminal epithelium (Krause and Oetzel, 2006).

Mode of ruminal pH measurement may alter the pH value, which varies across collection types. Duffield et al. (2004) displayed that pH of rumen fluid samples collected via oral lavage and rumen cannula are 0.33 and 0.35 pH units higher, respectively, in comparison to fluid collected by rumenocentesis, likely due to contamination with salivary buffers. Similarly, Garrett et al. (1999) collected rumen fluid via rumenocentesis that was 0.28 pH units lower than rumen fluid collected through cannulation. Based on their initial data, Duffield et al. (2004) set pH thresholds for SARA at 5.5, 5.8, and 5.9 for collection via rumenocentesis, rumen cannula, and oral lavage, respectively. Similarly, threshold pH for SARA was set at 5.5 for rumenocentesis collection by Garrett et al. (1999) and 6.0 for oral lavage by Plaizier et al. (2008).

It has been established that diurnal variation of rumen pH may alter accuracy of identifying SARA (Keunen et al., 2002; Krause and Oetzel, 2006). Generally it is thought that peak fermentation time is 2-4 hours following feeding (Bergman, 1990), and in order to

accurately assess ruminal pH, it is recommended to implement a standardized collection time. In efforts to account for deviations in rumen pH attributed to time and diurnal variation, Gozho et al. (2005) and Kleen et al. (2003) defined SARA as a ruminal pH of less than 5.5 for 3 h x d<sup>-1</sup> or more. Since diurnal variation in ruminal pH plays a pivotal role in accurate identification of SARA, time of collection must be considered. Keunen et al. (2002) displayed the variation and longevity in rumen pH in response to diet. Researchers showed that a total mixed ration (TMR) fed at 0700 h and again at 1100 h depressed rumen pH from 6.8 to 5.5 until 2100 h the same day. Rumen pH slowly returned to baseline pH (6.3-6.8) overnight and consistently peaked in the morning, just before the next day's feeding. Thus, Plaizier (2004) standardized rumen pH data by collecting fluid samples approximately 4 h following feeding.

Given the drastic changes exhibited following feed intake and normal diurnal variation, a single pH measurement is unable to characterize rumen characteristics beyond that moment in time. More accurate and precise options may be the use of indwelled pH electrode as suggested by Krause and Oetzel (2006) or by a ruminal bolus which offers continuous monitoring of both rumen pH and temperature. It has been suggested that researchers utilize a continuous method of rumen pH monitoring to capture a more comprehensive characterization of rumen pH, while eliminating human error or bias associated with collection method and accounting for diurnal variation (Krause and Oetzel, 2006). Continuous monitoring and a threshold pH of lower than 5.5 for > 3 h x d<sup>-1</sup> seems to be a guideline which combines the most accurate measurement and threshold for rumen pH conditions that are consistent with SARA. It has been widely accepted that a physiological threshold for acute acidosis is a pH below 5.0 (Owens et al., 1998; Krause and Oetzel, 2006; González et al., 2012). These reports suggest the necessity to assign threshold



pH for SARA in accordance with collection method and frequency, which can effectively limit subjectivity and standardize the definition of SARA.

### ***Rumen temperature as an indicator of SARA***

Rumen temperature has historically not been utilized as an indicator of acidosis, but may provide insight to digestive kinetics and fermentation outcomes. Dale et al. (1954) revealed that rumen temperature is consistently 2°C higher than rectal temperature, with little deviation across diet type and composition. In an effort to quantify variation between ambient and rumen temperatures, Beatty et al. (2008) displayed that ruminal temperature was 0.8 – 1.6°C higher than core body temperature with a maximum rumen temperature of 41.4°C recorded throughout the trial. This supports the findings of Dale et al. (1954), given the maximum rumen temperature is around 2.2-3.1°C greater than basal body temperature for bovine (38.3-39.2°C). Thus, it seems rumen temperature may be regulated via physiologic mechanisms, although research is limited at this time.

In an acidosis challenge by AlZahal et al. (2008), SARA-induced dairy cows had a mean average temperature of 39.2°C compared to 38.5°C in control cows. Interestingly, SARA-affected cows spent a greater amount of time (min x day<sup>-1</sup>) above 39.0 and 39.2°C which corresponded with a greater time spent below pH of 5.8 and 5.6, respectively. Regression analysis revealed a strong inverse relationship ( $R^2 = 0.77$ ) between rumen pH and rumen temperature, where a rumen temperature of 40.0°C corresponded with a rumen pH of 5.3 (AlZahal et al., 2008). While the magnitude of difference was just 0.7°C between SARA-induced and control cows, the increase in rumen temperature corresponds with rumen pH indicative of SARA. Thus, even slight variation in rumen temperature may serve as a tool to predict or identify rumen pH indicative of SARA. Although little research has been conducted regarding

the impact of SARA on rumen temperature, constant monitoring of rumen temperatures may prove to be a worthwhile indicator of SARA in cattle, and thus warrants further research.

### ***Rumen osmolality and damage to ruminal papillae***

The introduction of non-structural carbohydrates into ruminant diets has been shown to substantially increase concentrations of ruminal free glucose, and glucose is subsequently fermented into organic acids in the rumen. Such fermentative products have been shown to significantly increase osmotic pressure in the rumen (Nocek, 1997; Owens et al., 1998; Enemark et al., 2002). The following section will outline osmolality changes in response to accretion of glucose, organic acids, and hydrogen ions in the rumen and the subsequent effects on rumen physiology and absorption mechanisms.

Rumen osmolality is dependent on feed composition and management, as roughage based diets induce rumen osmolality in the range of 240 to 265 mOsm x L<sup>-1</sup>. However, as diets become more concentrated with non-structural carbohydrates, rumen osmolality may exceed 300 mOsm x L<sup>-1</sup> (Owens et al., 1998). Rumen osmolality above 300 mOsm x L<sup>-1</sup> negatively impacts dry matter intake and rumen function, and may be indicative of SARA (Carter and Grovum, 1990) and is thought to be a clear indication of SARA, as it alters rumen absorption of fermentative products (Enemark et al., 2002). Rumen osmolality may reach as much as 450-525 mOsm in extreme cases of SARA or acute acidosis (Carter and Grovum, 1990). Yet, Nagaraja and Titgemeyer (2007) suggest that rumen osmolality greater than blood osmolality (285-310 mOsm x L<sup>-1</sup>) will create an inflow of water from circulating blood to the rumen. Rumen osmotic pressure may increase from 95 mM of mOsm to approximately 225 mM of mOsm as the concentrations of lactate, VFA, and glucose in the rumen increase, ultimately resulting in damage to the rumen epithelium and swelling of papillae (Owens et al., 1998). Ruminal papillae

became damaged and decreased in number in beef heifers fed a high concentrate diet, likely from an influx of water into the rumen (Eadie et al., 1970). Consequently, decreases in rumen papillae number results in increased VFA in the rumen. As the papillae begin to heal from water-induced damage, they thicken and become hardened, altering long-term capabilities to absorb fermentation by-products (Eadie et al., 1970; Owens et al., 1998).

Hardened and damaged rumen papillae may create a perfect scenario for SARA. Organic acids and hydrogen ions accumulate in response to digestion of starch-based grains, and prevention of SARA can be achieved through buffering and absorption of acids. Yet, as rumen papillae and epithelial cells are damaged and hardened, their absorptive capabilities are severely reduced, rendering VFA and lactic acid to accumulate in the rumen (Owens et al., 1998). As these acids accumulate in the rumen, pH can be significantly reduced to subacute and acute levels of acidosis. The long-term effects of damaged epithelial cells and papillae may decrease by-product absorption for months or even years (Krehbiel et al., 1995). Even as cattle are adapted to high-energy diets or are transitioned to different diets, the effects of SARA may continue long after the initial insult of organic acids in the rumen. The damage to the ruminal tissue from both influx of water and sustained acidic content may lead to several consequences which may affect production and profitability of cattle.

### **Identification and implications of SARA**

As acidic insult of the rumen occurs and subsequent damage to rumen epithelial cells and papillae ensue, there are a number of consequences that may occur initially and over time. These may have drastic impacts on efficiency, reproduction, and productivity of cattle. Unfortunately, symptoms often indicative of SARA may be delayed days or even months. (Owens et al., 1998;

Krause and Oetzel, 2006; Oetzel, 2007). The most effective way to identify SARA is through ruminal pH, yet this method is rarely feasible for cattle producers outside of the research sector. Therefore, successfully identifying factors that are a direct consequence of ruminal acidosis are necessary to identify SARA and develop proper treatment or preventative measures. These parameters also serve as a confirmation that cattle in acidotic challenges not only meet the ruminal pH requirement for SARA, but other physiological or phenotypic criteria as well. The following section will outline the effects thought to be paired with SARA in both long- and short-term scenarios.

### ***Parakeratosis, rumenitis, and liver abscesses***

Parakeratosis, or the incomplete keratinization of the rumen wall, has been linked to ruminants fed high-grain diets over long periods of time (Hinders and Owen, 1965; Thomson, 1967). The stage between parakeratosis and rumenitis is not well defined, but it appears that rumenitis may occur as a result of parakeratosis and both are a consequence of high-grain diets (Dirksen et al., 1985; Enemark, 2008). Rumenitis has been defined as the inflammatory change in the rumen epithelium in response to high grain diets, and when severe, can lead to focal adhesions and abscesses (Huber, 1976). Parakeratosis occurs when the stratum granulosum layer of the rumen wall is absent, leading to potential effects on ruminal form and function (Hinders and Owen, 1965). Rumen papillae are damaged throughout this process and will become thickened and branched, allowing debris to collect within interpapillary spaces (Thomson, 1967). Hinders and Owen (1965) observed sloughing of the ruminal papillae which led to variation in VFA absorption. Damaged and missing papillae can lead to mucosal lesions in the ruminal wall and may result in bacteria, predominately *Fusobacterium necrophorum* and possibly *Corynebacterium pyogenes* to enter the blood stream, where they are filtered but play an inherent

role in the formation of liver abscesses (Scanlan and Hathcock, 1983; Nagaraja and Titgemeyer, 2007). While there are several bacteria which may reach systemic circulation in times of parakeratosis and liver abscessation, *Fusobacterium necrophorum* and *Corynebacterium pyogenes* are the major bacteria associated with liver abscessation. In order to maintain form and function of the rumen epithelium and prevent parakeratosis or rumenitis, sufficient amounts of VFAs must be present; however, excess VFAs may generate an environment conducive for the development of parakeratosis and rumenitis, and may also yield substantial damage to ruminal papillae.

Growth of ruminal epithelium is stimulated by butyrate, and to a lesser degree, propionate, which increases VFA absorption (Enemark, 2008). However, during acidotic insult, VFA concentrations are too great for absorption, which inherently damages epithelial cells and leads to parakeratosis and further damage to papillae (Thomson, 1967). Inability of the rumen papillae to reabsorb VFA and lactate further decreases rumen pH, potentially increasing rumen osmolality and exaggerating damage to papillae. Moreover, it appears that acid accumulation will also result in thinning of the rumen mucosa, which serves as a barrier between the ruminal environment and bloodstream (Kleen et al., 2003). Mucosal thinning along with crypts derived from parakeratosis/rumenitis, may result in rumen bacteria entering the blood stream, and resulting in severe liver abscesses (Hinders and Owen, 1965; Thomson, 1967). As high performing cattle are fed high energy diets, there is the potential for liver abscessation to occur, and while increased dietary energy maximizes gain and efficiency, there may be a net economic disadvantage due to the economic impact of liver abscesses.

Reports have indicated the presence of liver abscesses in feedlot and dairy cattle (Dirksen et al., 1985; Nordlund et al., 1995; Nagaraja and Chengappa, 1998). The incidence of liver

abscesses in feedlot cattle ranges from 12 to 32% of total cattle on feed (Nagaraja and Chengappa, 1998) and was reported to increase by as much as 25% between 2008 and 2013 (Reinhardt and Hubbert, 2015). SARA-induced parakeratosis may lead to colonization and abscessing of the liver, which may then spread to the heart, lungs, and kidneys (Nordlund and Garrett, 1994; Garrett et al., 1999). Condemned livers produce an estimated carcass loss of \$5-\$6 per animal affected and liver abscesses result in approximately a 10% reduction in ADG and feed efficiency (McKenna et al., 2002). The parakeratosis/liver abscess complex may also decrease dry matter intake (DMI) and body condition (Kleen et al., 2003). Parakeratosis/liver abscess formation may further the economic burden of SARA by deteriorating carcass quality and animal health, expanding the cost of SARA in the United States. While liver abscesses are not an immediate indicator of SARA in beef cattle, the mechanism between the parakeratosis/liver abscess complex and SARA is apparent from the literature, and may serve as an indicator at the terminal end of production to identify cattle that experienced SARA.

### *Laminitis/lameness*

Hoof and leg problems have been reported as the fourth highest reason for culling cows in the dairy industry (Cramer et al., 2008). Lameness has been reported in 26.6% of beef cows and 36.3% of beef bulls in a United States audit of meat packing plants (Roeber et al., 2001). Additionally, it has been estimated that approximately 90% of the cases of lameness in dairy cattle and 70% of cases in feedlot cattle are from hoof issues (Murray et al., 1996). Laminitis contributes to these chronic issues in both beef and dairy production settings, and is a major factor that contributes to herd fallout. It is for these reasons that measurements of hoof conformation serve as a component of breeding soundness examinations (Kleen et al., 2003; Koziol and Armstrong, 2018). Laminitis is most prevalent in early lactation dairy cows and

feedlot cattle, and is also common in performance-tested bulls (Underwood, 1992). Although little definitive evidence exists of a relationship between SARA and laminitis, it is thought that an incidence of laminitis greater than 10% of the total herd may be indicative of SARA in dairy cattle (Nordlund and Garrett, 1994). Acidosis may increase the occurrence of laminitis and can serve as an effective tool in confirming cases of SARA (Nocek, 1997). The potential mechanisms through which SARA and laminitis may be associated are summarized below.

Nutritional management serves as a key component in the development of laminitis; specifically, an increase in fermentable carbohydrates (Vermunt, 1994). However, factors such as body weight, body condition, hoof structure, foot rot, and other infectious diseases can increase the occurrence of laminitis. The development of laminitis is a multi-faceted physiological event which has been described by Nocek (1997). Laminitis is thought to occur from ruminal and subsequent metabolic pH depression that occurs during SARA (Nocek, 1997). Yet it has been shown that elevated concentrations of free glucose resulting from the hydrolysis of non-structural carbohydrates, as is often observed during SARA, may influence the occurrence of laminitis. Free glucose stimulates the growth of coliforms and decarboxylating bacteria, leading to the release of endotoxins and amides, such as histamine. These endotoxins increase blood pressure, which may damage the blood vessel walls of the hoof (Bergsten, 2003). Consequently, damaged vessel walls leak serum resulting in edema, thrombosis, and expansion of the corium. These conditions decrease blood flow and cause damage to the tissue of the hoof, leading to hypoxia, which limits oxygen and nutrient exchange (Nocek, 1997). Eventually, ischemia and regressed vascularization in the hoof cause apoptosis of the epidermal cells, and thus degeneration of the corium and deterioration of the laminar region (Bergsten, 2003). Ultimately, the mechanical damage to the hoof is visualized during the final phase of laminitis

when dorsal and lateral laminar supports of the hoof are broken down, which allows the pedal bone to shift in configuration (Nocek, 1997). Shifts in the pedal bone may cause pressure on and significant damage to the soft tissue between the pedal bone and sole of the hoof (Maclean, 1966). Damage to the soft tissue results in further localized swelling of the hoof, and may eventually result in necrosis and the development of scar tissue leading to permanent damage to the hoof structure that is generally referred to as founder in cattle.

The magnitude and severity of laminitis is highly dependent on endotoxin and subsequent histamine concentrations. Yet, it should be noted that endotoxin concentrations are linearly associated with free glucose concentrations in the rumen. Thus, it would be expected as non-structural carbohydrates are increased in the diet to the degree where free glucose concentrations increase, developing bulls stand a greater risk of becoming laminitic, or potentially foundered (Bergsten, 2003). Laminitis is often associated with ruminal acidosis and can potentially serve as an indicator for SARA given ruminal conditions associated with SARA are often times conducive to elicit laminitis. Laminitis has been shown to delay days or weeks following the initial insult of non-structural carbohydrates in the diet (Nocek, 1997). Thus, laminitis may serve as an indicator of previous instances of SARA and can be used as a tool to evaluate management strategies. Physiological conditions that facilitate laminitis are generally dependent on decreased ruminal pH and elevated free glucose concentrations that stimulate amide and decarboxylating bacteria formation. However, it should be noted that amides and decarboxylating bacteria may flourish in times of disease, and may be present at times when ruminal pH does not meet the threshold for SARA (Vermunt, 1994). Yet, there is a general relationship between dietary level of readily-fermented carbohydrates, ruminal pH and glucose concentrations, and laminitis. Therefore, visual observation of laminitis would suggest that SARA has previously occurred and



can be used as a tool to evaluate management practices and implement nutritional adjustments if necessary.

### ***Fecal composition***

Alterations in fecal composition often go unreported in cases of SARA, but are often a consequence of the condition in dairy and beef cattle. Fecal composition is highly dependent upon rumination time, passage rate, feed particle size, and several other factors (Garrett et al., 1999). However, the distinct fecal changes observed in cases of SARA include color, consistency, pH, smell, and residual particle size (Kleen et al., 2003). Changes in fecal color have been described as brighter with an inherently greater yellow tinge (Kleen et al., 2003) or gray tone. Diarrhea may be a distinct feature of SARA and may be the product of an influx of fermentable carbohydrates. As outlined by Oetzel (2007), increases in post-ruminal fermentation may lead to diarrhea in which manure may be accompanied with froth. Moreover, the increase in osmotic pressure from free glucose and organic acids may prevent water absorption in the omasum, small intestine, and large intestine, which results in soft feces (Garry and Kallfelz, 1983). Osmotic pressure continues to rise as organic acids flow through the gastrointestinal tract and may be exacerbated by increased post-ruminal fermentation. This is thought to change the smell of feces to slightly sweet and acidic (Kleen et al., 2003; Oetzel, 2007). Finally, there has been evidence of forage particle size increases in feces, recorded as 1-2 cm for SARA cattle in comparison to 0.5 cm for animals not experiencing rumen pH or other symptoms of SARA (Hall, 2002; Kleen et al., 2003). Increased forage particle size in manure is likely manifested by both shifts in the microbial population and increased rumen osmolality in animals fed high energy diets. As rumen pH decreases below 5.8, proliferation of fibrolytic bacteria in the rumen decrease, limiting fiber digestibility in the rumen (McAllister et al., 1990). Additionally,

increases in rumen osmolality, often associated with SARA, likely increase passage rate and limit digestive kinetics in affected cattle (Owens et al., 1998). Furthermore, there have been reports of whole cereal grains in feces of acidotic cattle, yet it is unknown if these grains had undergone processing as it was unreported in the literature, and therefore unknown if starch had been ruminally digested or was still intact (Kleen et al., 2003). Regardless, these data support that alterations in fecal composition may be indicative of SARA. Acidotic ruminal conditions may alter the passage rate of feed particles and osmolarity in the rumen and gastrointestinal tract, potentially changing color, composition, and smell of manure.

### ***Variation in DMI***

Subacute ruminal acidosis can produce a rumen environment which may influence DMI of cattle. There have been several reports of decreased DMI in SARA-affected beef cattle (Nocek, 1997; Kleen et al., 2003), although some cases of SARA have corresponded with cyclical DMI patterns (Stock, 2000; Gozho et al., 2005). Although reports are often inconsistent, there is the notion that acidotic cattle will decrease DMI and generally will not return to the previous level of DMI. This section will outline the ways in which SARA can influence DMI as well as offer insight into potential mechanisms for the return to pre-SARA levels of DMI.

In experiments where SARA was induced in both lactating dairy cows and beef steers, intake fluctuated throughout the challenge periods resulting in cyclical DMI (Gozho et al., 2005; Fairfield et al., 2007). As pH decreases to 6.0, fiber digestion is minimally decreased but as pH drops to 5.0- 5.5, consistent with SARA, the magnitude of fiber digestion is substantially decreased when compared to pH 6.0 (Hoover, 1986). Subsequent decreases in fiber digestibility may result from lowered proliferation rates of fibrolytic bacteria. The proliferation of cellulolytic bacteria dramatically decreases below a rumen pH of 6.0 (Russell and Wilson, 1996), which

often corresponds with delayed fiber digestibility as a result of increasing dietary levels of starch (Mertens and Loften, 1980). This decrease in fiber digestibility may impact DMI. Physical distention of the rumen can decrease DMI in cattle, and is thought to be controlled by mechanoreceptors transducing signals to the hypothalamus to indicate satiety (Forbes, 1986). Therefore, as bulky fiber increases in the diet and physical capacity of the rumen is maximized, mechanoreceptor activation will ultimately lead to reductions in feed intake. Moreover, Owens et al. (1998) suggests that acidotic insult may increase osmotic pressure above 300 mOsm x mL<sup>-1</sup>, limiting DMI as a consequence of rumen capacity. Yet, as rumen digestibility increases, as it does with high concentrate diets, intake does not appear to be limited by gut fill (Jones, 1972). It was shown in dairy heifers that physical capacity of the rumen did not affect DMI when digestibilities were 56, 60, 65, and 69%, yet DMI decreased when digestibility of the ration fell below 50% (Montgomery and Baumgardt, 1965). Rations delivered to feedlot cattle and those that are thought to induce SARA are generally devoid of large amounts of bulky roughages, and are generally highly digestible. Thus, chemical control of satiety likely plays an inherent role in DMI in cattle affected by SARA.

Feed intake is predominately controlled by the lateral and ventromedial areas of the hypothalamus. Activity along these neural pathways is mediated by neurotransmitters, such as gamma aminobutyric acid (Baile and McLaughlin, 1987). It was initially postulated that glucose was a key mediator in hypothalamic control of hunger and satiety, yet has been dismissed based upon high regulation of blood glucose in ruminant animals (Jones, 1972). Yet, there has been several proposed chemical factors that may offer explanation to decreased DMI when ration digestibility and roughage amounts do not result in ruminal distention. The literature suggests chemical control is partially driven by ruminal organic acid production. Increases in ruminally-

infused propionate and acetate resulted in 34% and 9% decreases in DMI in beef steers (Theurer and Hale, 1968). Additionally, VFAs may also impact DMI, as infusion of butyrate and propionate into the ruminal vein and systemic blood circulation restricts DMI and elevates satiety (Allen, 2000; Van Winden and Kuiper, 2003). Moreover, lactic acid infusion into the ruminal vein in sheep decreased DMI by 75% (Jones, 1972), and was thought to be based on decreases in rumen pH. Yet, as rumen pH decreases, it increases the propensity for VFA and lactic acid to be passively absorbed across the ruminal epithelium. Thus, as ruminal pH declines, systemic circulation of VFA and lactic acid likely provide a negative feedback to the hypothalamus (Jones, 1972; Owens et al., 1998). Leptin has also been shown to negatively impact DMI in sheep, and is likely to have similar influences in cattle. Therefore, as adiposity increases, there may be chemical control via leptin to reduce intake in cattle (Morrison et al., 2001). Finally, there is a strong influence of cholecystokinin (CCK) on satiety as it originates in the gastrointestinal tract and has been found in the brain (Krieger, 1983). Cholecystokinin plays an inherent role in protein and lipid digestion, as it is the principle stimulus for pancreatic enzymes and bile. Interestingly, CCK receptors have been found on the brain and is proposed to facilitate satiety in ruminants (Krieger, 1983; Baile and McLaughlin, 1987). Furthermore, rumen hypomotility may also affect DMI, as Slyter (1976) reported increases in short-chain fatty acids from fermentation result in reduced rumen motility, resulting in increased rumen retention time, disrupting feed intake. Similarly, it has been thought that bacterial endotoxins and histamine may decrease rumen motility, yet there is limited research to support such claims (Underwood, 1992; Kleen et al., 2003). It is difficult to deduce a specific mechanism for the control of DMI in cattle and likely is influenced by a multitude of factors. Based on the data presented herein, DMI seems to be influenced by both physical and chemical factors that vary based upon ration composition.

As continued decreases in DMI have been reported in response to high levels of non-structural carbohydrates, Owens et al. (1998), Shi and Weimer (1992), and Britton and Stock (1987) suggest decreases in DMI rebound after the rumen environment returns to basal conditions. Cyclical feed intake is thought to be a consequence of SARA (Britton and Stock, 1987; Bevans et al., 2005). Britton and Stock (1987) found cattle return to full feed for a brief period of time before intake decreases again, and Fulton et al. (1979) showed that cattle adjust to a more consistent DMI once rumen and systemic conditions return to basal values. These data provide evidence of cyclical variation in DMI as cattle recover from adverse physical or chemical conditions, regain their appetite, but subsequently overeat if the initial management issue is not corrected. This creates a pattern of repetitive SARA events, and an accompanying overall decrease in DMI. Although there is limited peer-reviewed documentation of cyclical DMI of acidotic cattle, practical experiences in the field suggest that SARA results in inconsistent patterns of DMI.

### **Treatment and prevention of SARA**

Treatment of SARA is uncommon as it often goes unrecognized in cattle. However, the most effective way to minimize the effects of SARA is through prevention. There are several preventative measures that allow cattle to be managed aggressively without the negative effects of SARA. This section will outline common steps and management tools that can be used to aid the prevention of SARA in cattle.

### ***Ration composition and physically-effective fiber***

It has been previously documented in this review and others that grain source and processing play inherent roles in energy availability and nutrient metabolism. One aspect that is key to optimizing production is to maximize grain digestion and energy metabolism without increasing the incidences of ruminal acidosis (Owens et al., 1997; González et al., 2012).

Therefore, it is necessary to be cautious when selecting grain type and processing method as they influence the acidogenic potential of the diet, particularly when grains makes up a large portion of the dietary DM. Increases in the proportion of starch in the diet are generally followed by a subsequent decrease in basal rumen pH (Owens et al., 1998; Krause and Oetzel, 2006).

Additionally, Gonzalez et al. (2012) suggests that a dietary increase in grains and available starch ultimately decreases rumination time. When highly fermentable grain sources are used such as finely ground grains such as wheat, floury corn, and high-moisture or steam-flaked grains, the apparent increase in digestibility may be enough to drive acidosis, especially without an adequate source of fiber (Beauchemin, 2007). Given the enhanced risk for acidosis in cattle fed high energy, concentrate-based rations, the utilization of roughage is a critical component of the ration and feeding management. Roughages utilized as a fiber source should allow sufficient rumination and increase rumen buffering to maintain a healthy rumen environment and potentially mitigate the negative effects of acidosis.

The requirement for neutral detergent fiber (NDF) is critical for cattle as it provides the buffering capacity to maintain the health of the rumen microbial population. Several estimates exist for the dietary level of effective neutral detergent fiber (eNDF) or physically-effective neutral detergent fiber (peNDF) required to maintain normal rumen pH and microbial activity (Fox and Tedeschi, 2002; Mertens, 2002). The most applicable to beef cattle seems to be the

requirement of 7-10% peNDF or 24.5% eNDF of total dietary DM (Fox and Tedeschi, 2002). Physical-effectiveness of NDF is directly related to particle size, and thus peNDF decreases as particle size or stem length decreases, primarily as a result of a decrease in secretion of salivary buffers (Mertens, 2002). There are many effective lengths in forages, which typically range from medium (0.5 in.) to chopped (>2 in.) or any long-stemmed hay (Mertens, 2002). Examples of peNDF include forages cut to the previously-specified lengths, while other examples include feedstuffs with relatively high amounts of peNDF, such as cottonseed hulls, soybean hulls, or other compositionally similar by-products. Collectively, these feed tuffs increase chewing time, leading to an increase in salivary output (Mertens, 2002). Salivary output, resulting from initial ingestion and rumination, has been shown to elicit 7 L of saliva for every hour of chewing (Maekawa et al., 2002). Salivary output in cattle ranges from 40-150 L x d<sup>-1</sup> and serves as the major buffer for the ruminal environment. Bicarbonate (125 mEq x mL<sup>-1</sup>) and phosphate (20 mEq x mL<sup>-1</sup>) serve as the predominate buffers in ruminant saliva, which has a normal pH of 8.2 (Bailey and Balch, 1961; Elrod et al., 1993; González et al., 2012). Of the total hydrogen concentration in the rumen, it is estimated that saliva accounts for 30% of hydrogen elimination with VFA ionization and absorption accounting for 50% and passage through the gastrointestinal tract accounting for 20% of hydrogen elimination (González et al., 2012). Of the 20% of hydrogen elimination that occurs from passage through the gastrointestinal tract, upwards of 10% of ions are bound to phosphates and excreted. Inclusion of 14% peNDF in the diet has been estimated to maintain rumen pH above 6.0, limiting shifts in rumen fibrolytic bacteria and maintain a rumen pH above thresholds associated with SARA (Beauchemin, 2007). The utilization of sufficient peNDF in ruminant diets plays a major role in the mitigation of hydrogen and may reduce the incidence of acidosis.

### ***Transition diets, ration blending, and bunk and feeding management***

Another pivotal aspect of acidosis prevention is ration management, as the diet delivered must be a mirror of the diet formulated. It is important to note that while ration and mixing management is a crucial entity of feeding management, a ration must be formulated to avoid excess hydrogen ion accumulation in the rumen, thus ration formulation with a sufficient buffering capacity is the first step in avoiding SARA. While not all operations utilize a total mixed ration and mixing apparatus, mixer management creates the opportunity for error in systems that do. Proper sequencing of ingredients into the mixer and adequate mixing time is critical to limit feed particle separation and fines (Stone, 2004). In feed particle size analysis conducted by Heinrichs et al. (1999), a Gehl (model 7500) mixer with a 2,720 kg capacity was utilized to mix corn silage, grain premix, high-moisture corn, alfalfa silage, and alfalfa hay. The mixer was filled to approximately 50% capacity and following mixing time of 4 minutes, there was reduced mass of particles above 18 mm, or approximately 0.7 in, by 31%. As mixing time was increased, particle size was dramatically reduced with a significant amount of particles below the 18 mm threshold. Accumulation of fines are thought to induce SARA, as subsequent increases in surface area promote rapid digestion of cereal grains and forage (Owens et al., 1998; González et al., 2012). Functioning and accurate mixer scales, along with adequate mixing are necessary to ensure proper inclusion of ingredients and avoid pockets of cereal grains in the diet, which may induce acidosis (Shaver, 2002). Once feed is properly mixed and ready for delivery, cattle must receive the correct feed call and have appropriate bunk space and consistent time of feeding each day (Pritchard, 1993). There are three predominate options for bunk management when feeding cattle, which include: *ad libitum*, limit feeding, and slick-bunk management (Erickson et al., 2003). Regardless of feeding management strategy, feedlot cattle are generally



recommended to be provided with 18-24 in x head<sup>-1</sup> of bunk space and be fed at consistent times each day (Shaver, 2002). This limits competition and can reduce the occurrence of overeating at the time of feed delivery (González et al., 2012). Feed calls with clean bunk management should be dependent on bunk scores and subsequent adjustments based on previous intake, and it is critical to have a bunk scoring system in place (Pritchard, 1993). Feed calls should be decreased as necessary, as cattle that are off feed may be prone to acidosis if satiety increases and feed calls are not adjusted accordingly (Fulton et al., 1979). As cattle are transitioned onto high energy rations or to *ad libitum*-equivalent levels of intake, it is highly recommended to step cattle up onto full feed over a 14-28 day period (Shaver, 2002). Cattle are prone to acidosis during the adaptation or transition phase, and therefore require strict management (Garza et al., 1989; Kleen et al., 2003).

Apart from proper mixer and bunk management, frequency of feeding provides opportunity to limit SARA in beef and dairy cattle. Higher frequency of feeding favors the synchrony of saliva and organic acid production and is thought to promote an even distribution of organic acid mitigation (González et al., 2012). Feeding twice daily has resulted in higher gain efficiency in some but not all cases, as outlined by Pritchard and Bruns (2003), yet the greatest advantage of increasing feeding frequency may be a reduction in both magnitude of feeding errors and opportunities for cattle to over-consume feed. Robles et al. (2007) compared feeding frequencies and noted that feeding once led to a lower minimum pH when compared to cattle fed twice. All cattle were fed at time point 0, with cattle fed once daily receiving their full feed allotment, and cattle fed twice daily receiving the same total amount, but receiving half of the allotment at time point 0, and the remaining half four hours later. Following the initial feeding, there was a rumen pH difference of 0.85 units after twelve hours, in favor of twice-fed cattle to

display a higher rumen pH value. The difference in rumen pH may be the result of synchrony between organic acid production and salivary output, which allows the buffering system in the rumen to neutralize acids more effectively (Robles et al., 2007). Often times, the model to induce acidosis in research cattle is to withhold feed or delay feed time to increase hunger, followed by an increase in the amount of dry matter offered compared to the previous feed allotment (Nagaraja and Titgemeyer, 2007). Feeding management has a dramatic impact on rumen conditions in cattle, and thus proper feed mixing, feedbunk management, and consistent feed times are all feeding management practices that should aid in the prevention of acidosis in cattle.

### ***Ionophores***

One of the most common categories of drug used in U.S. beef cattle production is the ionophores. These antimicrobial drugs are utilized to promote growth and increase feed efficiency through altering fermentation, and include monensin (Rumensin), lasalocid (Bovatec), and laidlomycin propionate (Cattlyst). The resulting shift in fermentation affects VFA production by reducing acetate production, which brings acetate:propionate ratios closer to 1:1 (Bergen and Bates, 1984). Ionophores act to alter ruminal VFA output through lysis of gram-positive microbes in the rumen, as these microbes are predominately responsible for the production of acetate, butyrate, hydrogen, and formate (Bergen and Bates, 1984). Ionophores penetrate the peptidoglycan layer of gram-positive bacteria, protozoa, and fungi and facilitate the influx of potassium into the cell and sodium out of the cell as mediated by adenosine triphosphate. This results in the cell becoming hypotonic which eventually leads to cellular membrane rupture (Bergen and Bates, 1984). These events allow gram-negative bacteria to flourish in the rumen and increase propionate production, ultimately increasing feed efficiency. Additionally, lasalocid

and monensin are approved as a coccidiostat to prevent the growth and reproduction of coccidian parasites.

Ionophores, such as monensin, are thought to alter feeding behavior, where cattle consume smaller meals much more frequently and thus lead to less variable fermentation patterns (Erickson et al., 2003). In a steer metabolism study conducted by Burrin and Britton (1986), steers fed monensin had a higher rumen pH 8 and 16 hours post feeding along with increases in propionate and a corresponding decrease in acetate. There was speculation that increases in rumen pH were associated with a decrease in lactate concentration within the rumen. There is a link between gram-positive bacteria and the potential to reduce lactate concentrations in the rumen (Russell, 1987; Krause and Oetzel, 2006). Both lasalocid and monensin inhibit several lactate-producing rumen bacteria including *Butyrivibrio fibrisolvens*, *Eubacterium cellulosolvens*, *E. ruminantium*, *Lacbnospira multiparus*, *Lactobacillus ruminis*, *L. vitulinus*, *Ruminococcus albus*, *R. flavefaciens*, and *Streptococcus bovis* (Dennis et al., 1981). However, laidlomycin propionate does not appear to alter rumen pH or rumen acid concentrations in general (Bauer et al., 1995). When compared to monensin, laidloymcin propionate is not as potent of an inhibitor of gram-positive bacteria and lactate production in the rumen (Domesicik and Martin, 1999). Additionally, in-vitro culture of rumen contents supplemented with 5 mg/mL of monensin reduced *Streptococcus bovis* growth (Russell, 1987). Furthermore, Nagaraja et al. (1982) showed monensin-fed cattle had higher pH than control cattle, yet neither pH was within standard SARA thresholds. The potential for ionophores, specifically monensin and lasalocid to reduce lactic acid production is apparent from the research. While the specific rumen pH where hydrogen ion contribution shifts from VFA to lactate is not strictly known, these data suggest that ionophores can serve as a preventative

measure against rumen pH to dropping below 5.2 to 5.0, which is likely driven by lactate dissociation (Krause and Oetzel, 2006). There is the potential that VFAs can still drive SARA in cattle at rumen pH around 5.5, yet ionophores may be able to synchronize organic acid production and neutralization through salivary buffering and absorption mechanisms by regulating meal size and frequency (Erickson et al., 2003; González et al., 2012).

### ***Buffers***

Another option to decrease the risk of SARA is through the addition of feed additives that increase the buffering capacity of the rumen. Bicarbonate, typically found in saliva, sequesters hydrogen ions and may account for as much as 30% of acid neutralization in the rumen (González et al., 2012). Generally, when feeding a bicarbonate-based buffer, mineral ions are a common carrier and some options include: potassium bicarbonate, magnesium bicarbonate, and sodium bicarbonate (Miller et al., 1965). Yet, the inclusion of any bicarbonate-based buffer has been shown to have similar results on organic acid concentrations when comparing each buffer (Miller et al., 1965). The addition of monobasic sodium phosphate, magnesium bicarbonate, or sodium bicarbonate each resulted in increased rumen fluid pH *in-vitro* when fed to dairy steers (Herod et al., 1978). Phosphate has been shown to account for upwards of 10% of hydrogen ion sequestration in ruminants and may prove beneficial as a feed-through buffer, and merits further investigation (González et al., 2012). Additionally, calcium carbonate (limestone) is included in a substantial amount of rations to help maintain calcium:phosphorus ratios, yet the added benefit for buffering is not well documented (Wise et al., 1965). However, it has been observed that inclusion of 1.0% coarse or fine ground calcium carbonate on a DM-basis increased rumen pH 0.07 to 0.10 units in Holstein cows (Keyser et al., 1985). There is conflicting literature as to the efficacy of sodium bicarbonate and the influence on rumen conditions. Ghorbani et al. (1989)

found that diets buffered with sodium bicarbonate and sodium sesquicarbonate increased rumen pH, and neither buffer affected VFA production. Interestingly, in Holstein heifers, inclusion of sodium bicarbonate at 1.25% of dietary DM increased total daily DMI ( $1.44 \text{ kg} \times \text{day}^{-1}$ ) when compared to 2.5% ( $0.98 \text{ kg} \times \text{day}^{-1}$ ) or 5% inclusion ( $0.52 \text{ kg} \times \text{day}^{-1}$ ). It was proposed that heifers fed 1.25% of sodium bicarbonate decreased meal size but increased feeding frequency, which led to a rumen pH 0.45 units higher than control heifers (González et al., 2008).

Additionally, inclusion of 1.25% of sodium bicarbonate of dietary DM reduced the time below rumen pH of 5.8 from 12 to 3 hr  $\times \text{d}^{-1}$ , with higher inclusion rates displaying no additional benefit. However, it has been shown that inclusion of 10 g of sodium bicarbonate  $\times \text{kg}^{-1}$  of dietary DM may reduce rumination due to a proposed increase from osmolality based on ion accumulation in the rumen (Mooney and Allen, 2007). Yet, as it seems sodium bicarbonate provides benefit at specific inclusion in the diet, general recommendations are to include sodium bicarbonate at 0.8-1.0% of DMI, which has been shown to stabilize ruminal pH above the SARA threshold of ruminal pH at 5.5 (Stone, 2004). Moreover, sodium bicarbonate must be mixed in the ration as Keunen et al. (2003) showed dairy cows will not consume sodium bicarbonate on a free-choice basis to attenuate SARA. It is recommended that sodium bicarbonate is included in diets at approximately 1% of DM or even upwards of 1.25% of DM, as this level has been shown to strike the optimum balance between total DM intake, meal size and frequency, and buffering of hydrogen ions in order to minimize variation in rumen pH.

### **Nutritional influences on bull reproduction**

Bull development is a necessary and vital entity of beef production, as bulls account for a substantial portion of genetic influence in a cow herd. The utilization of bulls at an early age following rapid development is thought to reduce production costs, decrease generation intervals,

and presents the opportunity to implement genetics faster within the herd (Barth, 2008). Rapid development of bulls has historically been a common strategy utilized in beef cattle production. Bulls targeted to enter the breeding herd will often be fed high-energy diets to meet growth requirements while simultaneously evaluating genetic merit for growth and other economically-relevant traits. It is commonly thought across the industry that post-weaning nutrition will control the fate of semen quality of developing bulls, yet there is compelling evidence to suggest that pre-weaning management may play a substantial role in sexual development (Brito et al., 2007; Barth, 2008; Dance et al., 2015; Byrne et al., 2018). Additional evidence suggests that energy content in pre- and post-pubertal phases may significantly impact semen quality in developing bulls. Nutrition is a critical component to bull development as early stages of sexual development may be heavily influenced by nutritional management. This section will review the impact of pre- and post-weaning nutrition on sexual development and indicators of fertility of bulls, and evaluate the potential influence of nutritional mismanagement and SARA.

### ***Pre-weaning nutritional management***

There is a pre-conceived notion that bull calves will receive the nutrients they need to maintain growth and initiate the onset of puberty from their dams. Yet, Lunstra et al. (1988) noted that bull calves raised by multiparous dams had a significantly greater testis size at the time of weaning compared to bulls raised by first-parity dams. It is not uncommon for seedstock producers to creep feed bull calves while nursing dams to maintain adequate growth. In early work conducted by Bratton et al. (1956), Holstein bull calves, removed from their dams at birth, were fed rations to meet 75, 100, and 140 % of their nutrient requirements for growth from 1 to 80 weeks of age. Interestingly, medium (100 %) and high (140 %) nutrient intake dramatically

decreased age at puberty, increased weight at puberty, and increased the number of sperm per ejaculate when compared to low nutrient intake (75 %).

The attainment of puberty and apparent rise in semen quality in bulls fed high-energy diets may be directly related to complex hormonal signaling. As suggested by Barth (2008), development of the neonatal bull occurs in three distinct phases: infantile, pre-pubertal, and pubertal, with each stage dependent on gonadotropin levels. Gonadotropins that are necessary for reproduction in bulls are luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and their secretion is dependent upon gonadotropin-releasing hormone (GnRH). Low gonadotropin levels are commonly observed in the infantile stage, and hormonal levels generally remain low until around 8 weeks of age. The pre-pubertal phase is characterized by increases in gonadotropins and subsequently testosterone at around 8-20 weeks of age. Finally, as the pubertal period is reached, gonadotropin levels decrease, although testosterone levels remain elevated. Luteinizing hormone is thought to be lower in the pre-weaning phase of development in bulls that are destined to have smaller testes and mature at an older age (Aravindakshan et al., 2000). Additionally, supplementation of exogenous LH at 200 ng for 14 days between 4 to 6 weeks of age has been shown to hasten the onset of puberty in Hereford bulls, increasing testosterone at 24 weeks of age in LH-treated bulls (Chandolia et al., 1997). Furthermore, FSH is thought to be a master regulator, along with LH, to control Sertoli cell proliferation, seminiferous tubule size, and final testes size (Bagu et al., 2004; Barth, 2008). Sertoli cell proliferation ceases at 25 weeks of age, therefore it is thought that maximum testicular mass is pre-determined by this age and bulls with more FSH secretion during calfhood are thought to have greater testes size post-development (Barth, 2008). On this basis, it appears that calfhood development is

instrumental in the development of seminiferous tubule size and Sertoli cell number, which play pivotal roles in spermatogenesis.

Metabolic hormones that have been shown to influence reproductive hormones include leptin, insulin, and insulin-like growth factor-I (Evans et al., 1995; Brito et al., 2007; Barth, 2008; Dance et al., 2015). Low leptin levels following nutrient-restricted conditions in beef heifers have been shown to decrease LH concentrations, which return to basal levels following exogenous infusion of leptin (Amstalden et al., 2002). Insulin-like growth factor-I receptors have been found on Leydig and Sertoli cells, which are responsible for the production of testosterone and support of spermatogenesis, respectively (Borland et al., 1984; Barth, 2008). Proliferation of Leydig cells increases when exposed to IGF-I and LH, with introduction of insulin leading to elevated production of testosterone in cultured cells (Bernier et al., 1986; Rouiller-Fabre et al., 1998). Moreover, Brito et al. (2007) found lower concentrations of IGF-I resulting from nutrient restriction decreased LH pulses in peri-pubertal bulls, which led to decreases in overall testosterone production. Interestingly, IGF-I concentrations accounted for a significant portion of the variation in scrotal circumference ( $R^2 = 0.72$ ) and paired-testis volume ( $R^2 = 0.67$ ) at any age, verifying the influence of nutrition on reproductive development in bulls (Bravo et al., 2007). In an experiment conducted by Dance et al. (2015), bulls were fed low (62.9% TDN and 12.2% CP), medium (66.0% TDN and 17.0% CP), and high (67.9% TDN and 20.0% CP) energy and protein diets from 2 to 31 weeks of age, and then all bulls were subjected to a medium energy diet from 31 to 72 weeks of age. Bulls fed high levels of energy and protein achieved puberty quicker, had larger testes, and experienced greater levels of IGF-I secretion at a younger age when compared to medium and low energy and protein treatments. Elevated IGF-I levels were associated with earlier rises in LH in the high protein and energy bulls, which is expected to



result in elevated testosterone and would offer an explanation for the earlier onset of puberty (Dance et al., 2015). Nutrition-related hormones warrant further research as there seems to be an apparent influence on reproductive hormones, attainment of puberty, and scrotal size in developing bulls. Nutrition and hormones have a substantial impact on bull development, yet these nutrition-related hormones are often ignored in bull-related research. These reports confirm that early nutritional management may have long term impacts on sire development, and can be confirmed by several other projects.

In research conducted by Byrne et al. (2018), combinations of high- and low-energy diets were fed to Holstein bull calves during the pre-weaning phase (0 to 24 weeks of age) and post-weaning phase (24 weeks to puberty) of development. Bulls assigned to the high-energy diet were fed 1,200 g of a commercially-available milk replacer (216.3 g/kg of CP; 20.3 MJ/kg of GE), whereas bulls assigned to the low-energy diet were fed 450 g of the same milk replacer. Milk replacer was fed until the time of weaning at 24 weeks of age. Additionally, bulls fed the high-energy treatment were fed a concentrate mix *ad libitum* (167.9 g/kg of CP; 16.1 MJ/kg of GE) with low-energy bulls offered a maximum of 1 kg of the same concentrate per head per day. All bulls were offered hay *ad-libitum* (107.5 g/kg of CP; 16.1 MJ/kg of GE) along with the other components of the diet until 24 weeks of age. Thereafter, bulls were weaned and reassigned within treatment to either remain on the same diet or switch to the opposite diet until attainment of puberty. Following attainment of puberty, bulls were assigned to a moderate plane of nutrition until 60 wk of age, followed by *ad libitum* access to concentrate prior to slaughter at 72 wk of age. High-energy diets fed in the early development, pre-weaning phase hastened puberty by around 25 days compared to low-energy in the early development, and this subsequent onset of puberty was not affected by lower energy diets following weaning. Moreover, bulls fed low-

energy diets in the pre-weaning phase were restricted in the attainment of puberty, which was not reversed by feeding high-energy diets during the post-weaning phase. Bulls fed high-energy diets throughout their entire development period sustained a 2-cm increase in scrotal circumference at the time of puberty and a numerical increase in number of spermatozoa per ejaculate at 68 wk of age compared to any other treatment group, with no differences in total semen volume observed across dietary treatments. Thus, these data suggest that pre-pubertal energy restriction may have long-term impacts on semen quality as it slows sexual maturation, and such energy restriction cannot be compensated for following weaning (Byrne et al., 2018). Barth (2008) conducted a series of experiments in which Angus x Charolais bull calves were fed high-, medium-, and low-energy diets through distinct phases of development. Diets were distinguished based on concentrate level which were 0%, 6.6%, and 37% concentrate for low-, medium-, and high-energy diets, respectively. Bull calves were weaned at 8 weeks of age and were transitioned onto their respective diets. Bulls fed high-energy diets from 10 to 70 weeks of age attained puberty at a younger age (292 d of age) compared to medium- (305 d of age) or low- (327 d of age) energy levels. Researchers defined puberty as the first time that an ejaculate had at least  $50 \times 10^6$  spermatozoa that were over 10% progressively motile. Paired testis weights in the high-energy group were 131 g heavier than low-energy and 103 g heavier than medium-energy treated bulls. Nonetheless, these authors observed no effect on average percentages of morphologically-normal spermatozoa throughout the trial. Bulls in the high-energy group had an earlier rise in gonadotropin concentrations, characterized by an increase in LH and FSH, which was likely mediated by elevated IFG-I concentrations observed between 10-22 weeks of age (Barth, 2008). In another research project conducted by Barth (2008), Angus x Charolais bulls were fed a medium-nutrition diet on an *ad libitum* basis from 8 to 26 weeks of age, followed by either low-,

medium-, or high-energy diets, containing 0%, 6.6%, and 37% concentrate, respectively. Bulls fed high-energy diets following 26-weeks of age attained puberty at 299 d of age compared to 328 d of age for medium-energy and 302 d of age for low energy. Interestingly, bulls fed low-energy following week 26 had the greatest paired testis weight (619 g), followed by high-energy (611 g), and medium-energy (574 g). However, there was no impact of dietary energy composition on percentage morphologically-normal spermatozoa. Collectively, this experiment suggests that the influence of diet on puberty and testicular mass may be more critical prior to 26 weeks of age, as early nutrient restriction appears to have a greater influence on physical and sexual development. These data suggest that calfhood nutrient restriction may have long-term consequences for sexual development and indicators of fertility, regardless of post-weaning nutritional management. Furthermore, these findings support the notion that pre-weaning nutrition is an important, albeit often overlooked component of bull development.

### ***Post-weaning development***

Post-weaning development is generally dependent on high-energy diets as they provide the greatest potential for growth and evaluation of genetic merit. Previous research has indicated the effects of high-energy diets on semen quality and scrotal development in bulls. Although calfhood nutrition plays an inherent role in this stage of development, high-energy diets may continue to elicit changes in development and subsequent semen quality in growing bulls.

High-energy diets have been proven to reduce spermatozoa mobility and morphologically-normal spermatozoa in bulls when compared to diets that are compositionally lower in energy. In a study by Coulter et al. (1987), 143 bull calves of Angus and Hereford lineage were fed either a high- or medium-energy diet from the time of weaning through slaughter at 15 months of age. High-energy diets consisted of 80% grain and 20% forage

whereas medium-energy diets consisted of 100% forage, and the experiment was replicated over the course of two years. Bulls developed on the high-energy diet had a greater scrotal circumference at 12 months of age, but not 15 months of age, suggesting delayed but compensatory scrotal growth for bulls fed medium-energy diets. The total number of spermatozoa produced per day was 9% greater in year one and 30% greater in year two for bulls fed the medium-energy diet. Caput-corporis epididymal sperm reserves were 76% greater in year one and 89% greater in year two for bulls fed the medium-energy diet when compared to bulls fed high-energy diets. Additionally, it was found that cauda epididymal spermatozoa reserves were 52% greater for bulls fed medium-energy and epididymal transit time from corpus to cauda epididymis was 0.5 d faster than bulls fed high energy diets. Collectively, these data suggest that high-energy diets may increase testicular mass faster, but may elicit negative effects on total spermatozoa production and result in the maintenance of fewer epididymal spermatozoa. In a two-year feedlot development study conducted by Mwansa and Makarechian (1991), 112 weaned bulls were developed over two 77-d performance test intervals on one of four sequential combinations of diets that contained either high or low levels of energy. In this experiment, bulls were fed either high- or low-energy diets during the first 77-d test phase, and two of the four groups were adjusted over a 14-day adaptation to the other ration for the remaining 77-day test. High energy diets contained 3.39 Mcal/kg of dry matter of digestible energy and low-energy diets consisted of 2.33 Mcal/kg of dry matter of digestible energy. This created four dietary energy treatments: high-high, high-low, low-high, low-low. Bulls maintained on high-energy diets throughout the development period had the greatest scrotal size (38.3 cm) compared to high-low (36.4 cm), low-high (36.4 cm), and low-low (35.3 cm). However, bulls in the high-high treatment had 24.2% head-defected spermatozoa compared to 15.2% for high-low, 17.1% for

low-high, and 16.4% for low-low (Mwansa and Makarechian, 1991). Although, it should be noted that the percentage of normal spermatozoa would allow passage of a breeding soundness examination in all groups when compared to current standards (Koziol and Armstrong, 2018). In a two year study conducted by Coulter et al. (1997), 72 Angus, Angus x Simmental, or Hereford x Simmental bulls were developed over the course of a 168-d feeding period in which bulls were fed either high-energy (80% grain, 20% forage) or moderate-energy (100% forage) diets. Following the trial, two ejaculates were evaluated and semen quality was evaluated. Bulls fed the high-energy diets yielded less motile (53.4 vs 44.5%) and morphologically-normal spermatozoa (68.8 vs 62.5%) but a higher scrotal surface temperature (3.9 vs 3.4°C) when compared to bulls treated with moderate-energy diets. Finally, Pruitt and Corah (1985) fed varying amounts of the same diet to achieve high, medium, and low levels of energy intake in Simmental and Hereford bulls. Over a 200-d feeding period 29 Simmental bulls were fed 14.6, 19.2, and 23.8 Mcal per day and 27 Hereford bulls were fed 13.4, 17.5, and 22.2 Mcal per day for high-, medium-, and low-energy treatments. Simmental bulls fed the highest amount of energy had a greater scrotal circumference, yet there were no differences in scrotal size in Herefords, and neither breed nor energy level affected attainment of puberty or semen quality (Pruitt and Corah, 1985). Although there exists conflicting literature on the effect of feeding high-energy diets post-weaning on semen quality, it seems there is a trend towards high-energy diets influencing scrotal growth in developing bulls, which may result from adiposity and each of these studies showed the commonality of elevated scrotal growth reducing semen quality in bulls.

Elevated dietary energy has been shown to increase scrotal fat deposits, which may in turn have significant impacts on bull reproduction (Mwansa and Makarechian, 1991; Barth, 2008). Bull testes must be 4-6°C cooler than core body temperature to produce fertile sperm

(Kastelic and Thundathil, 2008). As scrotal fat increases, a subsequent rise in temperature is expected to follow, as the accumulation of adipose tissue around the vascular cone limits the ability of the pampiniform plexus to act as a countercurrent heat exchanger. Restriction of this mechanism from elevated dietary energy and scrotal adiposity offer one explanation to the substantial decreases in sperm quality. Although high-energy post-weaning diets generally maximize growth rate, they also seem to negatively affect spermatogenesis and may impact attainment of puberty and libido. The effect of nutrition on age at sexual maturity and attainment of puberty is subjectively defined and inconsistent across research. While bulls fed high-energy (80% grain, 20% forage) or moderate-energy (100% forage) diets resulted in earlier attainment of puberty (Coulter et al., 1997), bulls fed high-energy diets (23.8 and 22.2 Mcal) by Pruitt and Corah (1985) did not differ in age at attainment of puberty. However, based on data presented herein, it seems early calfhod management may impact the attainment of puberty whereas post-weaning management may play a minor role, pending energy contents are suitable to meet dietary requirements (Barth, 2008).

Libido data is scarce throughout the literature, especially those linking libido to nutritional influences. Limited reports are likely a function of the degree of subjectivity and difficulty in assessing such parameters in bulls. Yet, Mwansa and Makarechian (1991) reported no significant differences in libido, in which bulls were fed in combinations of high and low energy throughout two 77-d feeding periods. High- and low-energy diets contained 3.39 Mcal x kg<sup>-1</sup> of dry matter of digestible energy and 2.33 Mcal x kg<sup>-1</sup> of dry matter, respectively. The limited information on the effects of nutrition and libido merits further investigation. While reductions in semen quality may be a function of scrotal fat and subsequent scrotal temperature, bulls maintained on high-energy diets have the potential to become acidotic, in acute or sub-

acute forms. Therefore, SARA is of interest as it may affect physiological parameters that ultimately influence reproduction in developing bulls.

### ***SARA in developing bulls***

As the data reported herein support, high energy diets have the potential to create a rumen environment which may induce SARA in developing bulls. Although research on the effects of SARA on bull reproduction is limited, initial discoveries suggest SARA may negatively impact semen quality in bulls. In a study by Callaghan et al. (2016), bulls were challenged with oligofructose (OFF) or a sham dose of water administered via oral lavage. Bulls induced into SARA had a lower average rumen pH (6.27 vs 7.19) and higher rectal temperature (39.2 vs 38.6°C). Semen was collected over the course of 88 days following the challenge period. Percentage morphologically-normal spermatozoa was lower in bulls treated with OFF and such decreases were observed throughout the duration of the 88-d trial. As a result, the percentage of morphologically-abnormal spermatozoa was greater in semen collected from OFF-treated bulls with sustained increases that were dominated by distal mid-piece reflections, proximal droplets, and vacuole and teratoid morphological abnormalities. Bulls induced into SARA had lower serum testosterone and tended to have elevated cortisol concentrations. Cortisol concentrations peaked 5 days following OFF treatment. Although, it should be noted that elevations in cortisol concentrations may be a result of stress induced by several bouts of oral lavage in order to administer OFF and could also be driven by ambient temperatures. While animal number was limited (n = 8), this study offers insight into the effects of SARA in bulls, and suggests a link between stress-induced hormone signaling and semen quality.

As documented by Callaghan et al. (2016), elevated cortisol concentrations influenced several morphological abnormalities in spermatozoa and gonadotropin concentrations at various

time points in their trial. Follicle-stimulating hormone was decreased on d 7, 23, and 32 in OFF treated bulls. Increases in cortisol and depression of FSH were thought to account for the increase in distal mid-piece reflections at these times. Similarly, researchers found that vacuole and other spermatozoa head defects did not occur until d 67, 74, and 88, which was over 30-days after the rise in cortisol and drop in FSH concentration. This signifies that stress induced from the experiment most likely interrupted the spermatogenic cycle but also affected spermatozoa stored in the epididymis. Regarding gonadotropins, Thibier and Rolland (1976) showed that dexamethasone decreases LH and testosterone in post-pubertal bulls; likely a result of stress. Similarly, Barth and Bowman (1994) showed that infusion of dexamethasone upregulated cortisol and decreased testosterone concentrations, leading to increases in the prevalence of morphologically-abnormal spermatozoa. It has long been established that cortisol has a strong negative feedback on LH and FSH secretion, and therefore an influence on testosterone concentrations and reproduction (Welsh and Johnson, 1981; Stoebel and Moberg, 1982). Testosterone is a critical component to the attainment of puberty and spermatogenesis, and factors that raise cortisol concentrations are of interest in bulls. It has been previously established that SARA may influence cortisol, yet it has been shown that secretion of histamine in response to an influx of glucose into the rumen may also increase cortisol concentrations (Majzoub, 2006). Histamine plays a complex role in SARA, as it has been shown to flourish in the rumen, induce laminitis, and has the potential to serve a role in the suppression of testosterone and indicators of fertility in bulls through increases in cortisol concentrations. (Bergsten, 2003). There are countless other factors that may illicit stress in developing bulls, yet SARA remains of interest due to the known consequences in dairy and feedlot cattle. Objectively indicating fertility



parameters in developing bulls is a complex and multi-faceted process that can be heavily influenced by nutritional factors and mismanagement.

## References

- Allen, M. S. 2000. Effects of Diet on Short-Term Regulation of Feed Intake by Lactating Dairy Cattle. *Journal of Dairy Science* 83(7):1598-1624. doi: [https://doi.org/10.3168/jds.S0022-0302\(00\)75030-2](https://doi.org/10.3168/jds.S0022-0302(00)75030-2)
- AlZahal, O., E. Kebreab, J. France, M. Froetschel, and B. McBride. 2008. Ruminant Temperature May Aid in the Detection of Subacute Ruminant Acidosis. *Journal of Dairy Science* 91(1):202 - 207. doi: <https://doi.org/10.3168/jds.2007-0535>
- Amstalden, M., M. Garcia, R. Stanko, S. Nizielski, C. Morrison, D. Keisler, and G. Williams. 2002. Central infusion of recombinant ovine leptin normalizes plasma insulin and stimulates a novel hypersecretion of luteinizing hormone after short-term fasting in mature beef cows. *Biology of Reproduction* 66(5):1555-1561. doi: <https://doi.org/10.1095/biolreprod66.5.1555>
- Aravindakshan, J., A. Honaramooz, P. Bartlewski, A. Beard, R. Pierson, and N. Rawlings. 2000. Pattern of gonadotropin secretion and ultrasonographic evaluation of developmental changes in the testis of early and late maturing bull calves. *Theriogenology* 54(3):339-354. doi: [https://doi.org/10.1016/S0093-691X\(00\)00353-8](https://doi.org/10.1016/S0093-691X(00)00353-8)
- Bagu, E., S. Madgwick, R. Duggavathi, P. Bartlewski, D. Barrett, S. Huchkowsky, S. Cook, and N. Rawlings. 2004. Effects of treatment with LH or FSH from 4 to 8 weeks of age on the attainment of puberty in bull calves. *Theriogenology* 62(5):861-873. doi: <https://doi.org/10.1016/j.theriogenology.2003.12.021>
- Baile, C. A., and C. L. McLaughlin. 1987. Mechanisms Controlling Feed Intake in Ruminants: A Review. *Journal of Animal Science* 64(3):915-922. doi: [10.2527/jas1987.643915x](https://doi.org/10.2527/jas1987.643915x)

- Bailey, C., and C. Balch. 1961. Salivary secretion and its relation to feeding in cattle. Part I: The composition and rate of secretion of parotid saliva in a small steer. *Brit. J. Nut* 15:371. doi: <https://doi.org/10.1079/BJN19610048>
- Balch, D., and S. Rowland. 1957. Volatile fatty acids and lactic acid in the rumen of dairy cows receiving a variety of diets. *British Journal of Nutrition* 11(3):288-298. doi: <https://doi.org/10.1079/BJN19570046>
- Barth, A. D., L.F.C Brito, J.P Kastelic. 2008. The effect of nutrition on sexual development of bulls. *Theriogenology* 70(3):485 - 494. doi: <https://doi.org/10.1016/j.theriogenology.2008.05.031>
- Bauer, M. L., D. W. Herold, R. A. Britton, R. A. Stock, T. J. Klopfenstein, and D. A. Yates. 1995. Efficacy of laidlomycin propionate to reduce ruminal acidosis in cattle. *Journal of Animal Science* 73(11):3445-3454. doi: 10.2527/1995.73113445x
- Beatty, D. T., A. Barnes, E. Taylor, and S. K. Maloney. 2008. Do changes in feed intake or ambient temperature cause changes in cattle rumen temperature relative to core temperature? *Journal of Thermal Biology* 33(1):12 - 19. doi: <https://doi.org/10.1016/j.jtherbio.2007.09.002>
- Beauchemin, K. A. 2007. Ruminal acidosis in dairy cows: Balancing physically effective fiber with starch availability. In: *Florida Ruminant Nutrition Symposium*, January. p 30-31.
- Beauchemin, K. A., W. Z. Yang, and L. M. Rode. 2003. Effects of Particle Size of Alfalfa-Based Dairy Cow Diets on Chewing Activity, Ruminal Fermentation, and Milk Production1. *Journal of Dairy Science* 86(2):630-643. doi: [https://doi.org/10.3168/jds.S0022-0302\(03\)73641-8](https://doi.org/10.3168/jds.S0022-0302(03)73641-8)

- Bergen, W. G., and D. B. Bates. 1984. Ionophores: their effect on production efficiency and mode of action. *Journal of animal science* 58(6):1465-1483. doi: <https://doi.org/10.2527/jas1984.5861465x>
- Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews* 70(2):567-590. doi: [10.1152/physrev.1990.70.2.567](https://doi.org/10.1152/physrev.1990.70.2.567)
- Bergsten, C. 2003. Causes, Risk Factors, and Prevention of Laminitis and Related Claw Lesions. *Acta Veterinaria Scandinavica* 44(1):S157. doi: [10.1186/1751-0147-44-S1-S157](https://doi.org/10.1186/1751-0147-44-S1-S157)
- Bernier, M., P. Chatelain, J. Mather, and J. Saez. 1986. Regulation of gonadotropin receptors, gonadotropin responsiveness, and cell multiplication by somatomedin-C and insulin in cultured pig Leydig cells. *Journal of cellular physiology* 129(2):257-263. doi: <https://doi.org/10.1002/jcp.1041290218>
- Bevans, D. W., K. A. Beauchemin, K. S. Schwartzkopf-Genswein, J. J. McKinnon, and T. A. McAllister. 2005. Effect of rapid or gradual grain adaptation on subacute acidosis and feed intake by feedlot cattle<sup>12</sup>. *Journal of Animal Science* 83(5):1116-1132. doi: <https://doi.org/10.2527/2005.8351116x>
- Borland, K., M. Mita, C. Oppenheimer, L. Blinderman, J. Massague, P. Hall, and M. Czech. 1984. The actions of insulin-like growth factors I and II on cultured Sertoli cells. *Endocrinology* 114(1):240-246. doi: <https://doi.org/10.1210/endo-114-1-240>
- Bratton, R., S. Musgrave, H. Dunn, R. Foote, and C. Henderson. 1956. Semen Production and Fertility of Young Bulls Raised on 3 Different Levels of Feed Intake. In: *Journal of Animal Science*. p 1296-1297.

- Briggs, P., J. Hogan, and R. Reid. 1957. Effect of volatile fatty acids, lactic acid and ammonia on rumen pH in sheep. *Australian Journal of Agricultural Research* 8(6):674-690. doi: <https://doi.org/10.1071/AR9570674>
- Brito, L. F., A. D. Barth, N. C. Rawlings, R. E. Wilde, D. H. Crews Jr, P. S. Mir, and J. P. Kastelic. 2007. Effect of nutrition during calthood and peripubertal period on serum metabolic hormones, gonadotropins and testosterone concentrations, and on sexual development in bulls. *Domestic animal endocrinology* 33(1):1-18. doi: <https://doi.org/10.1016/j.domaniend.2006.04.001>
- Britton, R., and R. Stock. 1987. Acidosis, rate of starch digestion and intake. 0361-8722, Stillwater, OK.
- Brown, M. S., C. H. Ponce, and R. Pulikanti. 2006. Adaptation of beef cattle to high-concentrate diets: Performance and ruminal metabolism<sup>1</sup>. *Journal of Animal Science* 84(13\_suppl):E25-E33. doi: 10.2527/2006.8413\_supplE25x
- Burrin, D., and R. Britton. 1986. Response to monensin in cattle during subacute acidosis. *Journal of animal science* 63(3):888-893. doi: <https://doi.org/10.2527/jas1986.633888x>
- Byrne, C., S. Fair, A. English, M. Cirot, C. Staub, P. Lonergan, and D. Kenny. 2018. Plane of nutrition before and after 6 months of age in Holstein-Friesian bulls: I. Effects on performance, body composition, age at puberty, and postpubertal semen production. *Journal of dairy science* 101(4):3447-3459. doi: <https://doi.org/10.3168/jds.2017-13719>
- Callaghan, M. J., P. McAuliffe, R. J. Rodgers, J. Hernandez-Medrano, and V. E. A. Perry. 2016. Subacute ruminal acidosis reduces sperm quality in beef bulls<sup>1</sup>. *Journal of Animal Science* 94(8):3215-3228. doi: 10.2527/jas.2015-0235

- Carter, R. R., and W. L. Grovum. 1990. A review of the physiological significance of hypertonic body fluids on feed intake and ruminal function: salivation, motility and microbes. *Journal of animal science* 68(9):2811-2832. doi: 10.2527/1990.6892811x
- Castro Bulle, F. C. P., P. V. Paulino, A. C. Sanches, and R. D. Sainz. 2007. Growth, carcass quality, and protein and energy metabolism in beef cattle with different growth potentials and residual feed intakes<sup>12</sup>. *Journal of Animal Science* 85(4):928-936. doi: 10.2527/jas.2006-373
- Chandolia, R., A. Honaramooz, P. Bartlewski, A. Beard, and N. Rawlings. 1997. Effects of treatment with LH releasing hormone before the early increase in LH secretion on endocrine and reproductive development in bull calves. *Reproduction* 111(1):41-50. doi: <https://doi.org/10.1530/jrf.0.1110041>
- Church, D. C. 1988. *The Ruminant Animal: Digestive Physiology and Nutrition* Prentice Hall, Englewood Cliffs, New Jersey.
- Cooper, R. J., T. J. Klopfenstein, R. A. Stock, C. T. Milton, D. W. Herold, and J. C. Parrott. 1999. Effects of imposed feed intake variation on acidosis and performance of finishing steers. *Journal of animal science* 77(5):1093-1099. doi: 10.2527/1999.7751093x
- Coulter, G. H., T. D. Carruthers, R. P. Amann, and G. C. Kozub. 1987. Testicular Development, Daily Sperm Production and Epididymal Sperm Reserves in 15-mo-old Angus and Hereford Bulls: Effects of Bull Strain Plus Dietary Energy. *Journal of Animal Science* 64(1):254-260. doi: 10.2527/jas1987.641254x
- Coulter, G. H., R. B. Cook, and J. P. Kastelic. 1997. Effects of dietary energy on scrotal surface temperature, seminal quality, and sperm production in young beef bulls. *Journal of Animal Science* 75(4):1048-1052. doi: 10.2527/1997.7541048x

- Counotte, G., A. T. Van't Klooster, J. Van der Kuilen, and R. Prins. 1979. An Analysis of the Buffer System in the Rumen of Dairy Cattle<sup>1</sup>. *Journal of animal science* 49(6):1536-1544. doi: <https://doi.org/10.2527/jas1979.4961536x>
- Cramer, G., K. D. Lissemore, C. L. Guard, K. E. Leslie, and D. F. Kelton. 2008. Herd- and Cow-Level Prevalence of Foot Lesions in Ontario Dairy Cattle. *Journal of Dairy Science* 91(10):3888-3895. doi: <https://doi.org/10.3168/jds.2008-1135>
- Dale, H., R. Stewart, and S. Brody. 1954. Rumen temperature. 1. Temperature gradients during feeding and fasting. *Cornell veterinarian* 44:368-374.
- Dance, A., J. Thundathil, R. Wilde, P. Blondin, and J. Kastelic. 2015. Enhanced early-life nutrition promotes hormone production and reproductive development in Holstein bulls. *Journal of dairy science* 98(2):987-998. doi: <https://doi.org/10.3168/jds.2014-8564>
- Davis, C. L. 1967. Acetate Production in the Rumen of Cows Fed Either Control or Low-Fiber, High-Grain Diets. *Journal of Dairy Science* 50(10):1621-1625. doi: [https://doi.org/10.3168/jds.S0022-0302\(67\)87683-5](https://doi.org/10.3168/jds.S0022-0302(67)87683-5)
- Dennis, S. M., T. G. Nagaraja, and E. E. Bartley. 1981. Effect of Lasalocid or Monensin on Lactate-Producing or Using Rumen Bacteria. *Journal of Animal Science* 52(2):418-426. doi: [10.2527/jas1981.522418x](https://doi.org/10.2527/jas1981.522418x)
- Dirksen, G., H. Liebich, and E. Mayer. 1985. Adaptive changes of the ruminal mucosa and their functional and clinical significance. *Bovine Pract* 20:116-120.
- Domescik, E. J., and S. A. Martin. 1999. Effects of laidlomycin propionate and monensin on the in vitro mixed ruminal microorganism fermentation. *Journal of Animal Science* 77(8):2305-2312. doi: [10.2527/1999.7782305x](https://doi.org/10.2527/1999.7782305x)

- Duffield, T., J. C. Plaizier, A. Fairfield, R. Bagg, G. Vessie, P. Dick, J. Wilson, J. Aramini, and B. McBride. 2004. Comparison of Techniques for Measurement of Rumen pH in Lactating Dairy Cows. *Journal of Dairy Science* 87(1):59-66. doi: [https://doi.org/10.3168/jds.S0022-0302\(04\)73142-2](https://doi.org/10.3168/jds.S0022-0302(04)73142-2)
- Dunlop, R. H., and P. B. Hammond. 1965. D-LACTIC ACIDOSIS OF RUMINANTS\*†. *Annals of the New York Academy of Sciences* 119(3):1109-1132. doi: 10.1111/j.1749-6632.1965.tb47466.x
- Eadie, J. M., J. Hyldgaard-Jensen, S. Mann, R. Reid, and F. Whitelaw. 1970. Observations on the microbiology and biochemistry of the rumen in cattle given different quantities of a pelleted barley ration. *British Journal of Nutrition* 24(1):157-177. doi: <https://doi.org/10.1079/BJN19700018>
- Elrod, C., M. Van Amburgh, and W. Butler. 1993. Alterations of pH in response to increased dietary protein in cattle are unique to the uterus. *Journal of Animal Science* 71(3):702-706. doi: <https://doi.org/10.2527/1993.713702x>
- Enemark, J. M. D. 2008. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. *The Veterinary Journal* 176(1):32-43. doi: <https://doi.org/10.1016/j.tvjl.2007.12.021>
- Enemark, J. M. D., R. Jorgensen, and P. S. Enemark. 2002. Rumen acidosis with special emphasis on diagnostic aspects of subclinical rumen acidosis: a review. *Veterinarija ir zootechnika* 20(42):16-29.
- Erickson, G., C. Milton, K. Fanning, R. Cooper, R. Swingle, J. Parrott, G. Vogel, and T. Klopfenstein. 2003. Interaction between bunk management and monensin concentration on finishing performance, feeding behavior, and ruminal metabolism during an acidosis



- challenge with feedlot cattle. *Journal of animal science* 81(11):2869-2879. doi:  
<https://doi.org/10.2527/2003.81112869x>
- Evans, A., F. Davies, L. Nasser, P. Bowman, and N. Rawlings. 1995. Differences in early patterns of gonadotrophin secretion between early and late maturing bulls, and changes in semen characteristics at puberty. *Theriogenology* 43(3):569-578. doi:  
[https://doi.org/10.1016/0093-691X\(94\)00062-Y](https://doi.org/10.1016/0093-691X(94)00062-Y)
- Ewaschuk, J. B., J. M. Naylor, and G. A. Zello. 2005. D-Lactate in Human and Ruminant Metabolism. *The Journal of Nutrition* 135(7):1619-1625. doi: 10.1093/jn/135.7.1619
- Fairfield, A. M., J. C. Plaizier, T. F. Duffield, M. I. Lindinger, R. Bagg, P. Dick, and B. W. McBride. 2007. Effects of Prepartum Administration of a Monensin Controlled Release Capsule on Rumen pH, Feed Intake, and Milk Production of Transition Dairy Cows. *Journal of Dairy Science* 90(2):937-945. doi: [https://doi.org/10.3168/jds.S0022-0302\(07\)71577-1](https://doi.org/10.3168/jds.S0022-0302(07)71577-1)
- Forbes, J. M. 1986. *The voluntary food intake of farm animals*. Butterworth-Heinemann, London, England.
- Fox, D., and L. Tedeschi. 2002. Application of physically effective fiber in diets for feedlot cattle. In: *Proceedings of Plains Nutrition Conference*. (The Plains Nutrition Council: San Antonio, TX). Available at <http://www.cncps.cornell.edu/papers/ModUse/FoxandTedeschiPNC2002.pdf> [Verified 26 November 2009]
- Fulton, W. R., T. J. Klopfenstein, and R. A. Britton. 1979. Adaptation to High Concentrate Diets by Beef Cattle. I. Adaptation to Corn and Wheat Diets. *Journal of Animal Science* 49(3):775-784. doi: 10.2527/jas1979.493775x

- Galyean, M. L., D. G. Wagner, and R. R. Johnson. 1976. Site and Extent of Starch Digestion in Steers Fed Processed Corn Rations<sup>1,2</sup>. *Journal of Animal Science* 43(5):1088-1094. doi: 10.2527/jas1976.4351088x
- Garrett, E. F., M. N. Pereira, K. V. Nordlund, L. E. Armentano, W. J. Goodger, and G. R. Oetzel. 1999. Diagnostic Methods for the Detection of Subacute Ruminant Acidosis in Dairy Cows. *Journal of Dairy Science* 82(6):1170-1178. doi: [https://doi.org/10.3168/jds.S0022-0302\(99\)75340-3](https://doi.org/10.3168/jds.S0022-0302(99)75340-3)
- Garry, F., and F. Kallfelz. 1983. Clinical aspects of dietary buffers for dairy-cattle. Compendium on continuing education for the practicing veterinarian Princeton Junction, New Jersey.
- Garza, F., F. Owens, and J. Breazile. 1989. Effects of diet on ruminal liquid and on blood serum osmolality and hematocrit in feedlot heifers. Miscellaneous publication-Agricultural Experiment Station, Oklahoma State University (USA)
- Ghorbani, G., J. Jackson, and R. Hemken. 1989. Effects of sodium bicarbonate and sodium sesquicarbonate on animal performance, ruminal metabolism, and systemic acid-base status. *Journal of dairy science* 72(8):2039-2045. doi: [https://doi.org/10.3168/jds.S0022-0302\(89\)79327-9](https://doi.org/10.3168/jds.S0022-0302(89)79327-9)
- Goad, D. W., C. L. Goad, and T. G. Nagaraja. 1998. Ruminal microbial and fermentative changes associated with experimentally induced subacute acidosis in steers. *Journal of animal science* 76(1):234-241. doi: 10.2527/1998.761234x
- González, L., A. Ferret, X. Manteca, and S. Calsamiglia. 2008. Increasing sodium bicarbonate level in high-concentrate diets for heifers. I. Effects on intake, water consumption and ruminal fermentation. *animal* 2(5):705-712. doi: <https://doi.org/10.1017/S1751731108001675>

- González, L. A., X. Manteca, S. Calsamiglia, K. S. Schwartzkopf-Genswein, and A. Ferret. 2012. Ruminant acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). *Animal Feed Science and Technology* 172(1):66-79. doi: <https://doi.org/10.1016/j.anifeedsci.2011.12.009>
- Gozho, G. N., J. C. Plaizier, D. O. Krause, A. D. Kennedy, and K. M. Wittenberg. 2005. Subacute Ruminant Acidosis Induces Ruminant Lipopolysaccharide Endotoxin Release and Triggers an Inflammatory Response. *Journal of Dairy Science* 88(4):1399-1403. doi: [https://doi.org/10.3168/jds.S0022-0302\(05\)72807-1](https://doi.org/10.3168/jds.S0022-0302(05)72807-1)
- Hale, W. H. 1973. Influence of Processing on the Utilization of Grains (Starch) by Ruminants. *Journal of Animal Science* 37(4):1075-1080. doi: [10.2527/jas1973.3741075x](https://doi.org/10.2527/jas1973.3741075x)
- Hall, M. B. 2002. Rumen acidosis: carbohydrate feeding considerations. In: Proc. 12th Int. Symp. on Lameness in Ruminants. JK Shearer, ed. Orlando, FL. p 51-61.
- Heinrichs, A., D. Buckmaster, and B. Lammers. 1999. Processing, mixing, and particle size reduction of forages for dairy cattle. *Journal of animal science* 77(1):180-186. doi: <https://doi.org/10.2527/1999.771180x>
- Herod, E. L., R. Bechtel, E. Bartley, and A. Dayton. 1978. Buffering ability of several compounds in vitro and the effect of a selected buffer combination on ruminal acid production in vivo. *Journal of Dairy Science* 61(8):1114-1122. doi: [https://doi.org/10.3168/jds.S0022-0302\(78\)83695-9](https://doi.org/10.3168/jds.S0022-0302(78)83695-9)
- Hibbard, B., J. P. Peters, S. T. Chester, J. A. Robinson, S. F. Kotarski, W. J. Croom, and W. M. Hagler. 1995. The effect of slaframine on salivary output and subacute and acute acidosis in growing beef steers. *Journal of animal science* 73(2):516-525. doi: [10.2527/1995.732516x](https://doi.org/10.2527/1995.732516x)

- Hinders, R., and F. Owen. 1965. Relation of ruminal parakeratosis development to volatile fatty acid absorption. *Journal of dairy science* 48(8):1069-1073. doi: [https://doi.org/10.3168/jds.S0022-0302\(65\)88393-X](https://doi.org/10.3168/jds.S0022-0302(65)88393-X)
- Hoover, W. H. 1986. Chemical factors involved in ruminal fiber digestion. *Journal of Dairy Science* 69(10):2755-2766. doi: [https://doi.org/10.3168/jds.S0022-0302\(86\)80724-X](https://doi.org/10.3168/jds.S0022-0302(86)80724-X)
- Horn, G., J. Gordon, E. Prigge, and F. Owens. 1979. Dietary buffers and ruminal and blood parameters of subclinical lactic acidosis in steers. *Journal of animal science* 48(3):683-691. doi: <https://doi.org/10.2527/jas1979.483683x>
- Huber, T. L. 1976. Physiological Effects of Acidosis on Feedlot Cattle<sup>1</sup>. *Journal of Animal Science* 43(4):902-909. doi: [10.2527/jas1976.434902x](https://doi.org/10.2527/jas1976.434902x)
- Hungate, R. 1975. The rumen microbial ecosystem. *Annual Review of Ecology and Systematics.*, Davis, California
- Huntington, G. B. 1997. Starch utilization by ruminants: from basics to the bunk<sup>2</sup>. *Journal of Animal Science* 75(3):852-867. doi: [10.2527/1997.753852x](https://doi.org/10.2527/1997.753852x)
- Jones, G. M. 1972. Chemical factors and their relation to feed intake regulation in ruminants: A Review. *Canadian Journal of Animal Science* 52(2):207-239. doi: [10.4141/cjas72-026](https://doi.org/10.4141/cjas72-026)
- Kastelic, J., and J. Thundathil. 2008. Breeding soundness evaluation and semen analysis for predicting bull fertility. *Reproduction in Domestic Animals* 43(s2):368-373. doi: <https://doi.org/10.1111/j.1439-0531.2008.01186.x>
- Kennelly, J. J., B. Robinson, and G. R. Khorasani. 1999. Influence of Carbohydrate Source and Buffer on Rumen Fermentation Characteristics, Milk Yield, and Milk Composition in Early-Lactation Holstein Cows. *Journal of Dairy Science* 82(11):2486-2496. doi: [https://doi.org/10.3168/jds.S0022-0302\(99\)75500-1](https://doi.org/10.3168/jds.S0022-0302(99)75500-1)

- Keunen, J., J. Plaizier, I. Kyriazakis, T. Duffield, T. Widowski, M. Lindinger, and B. McBride. 2003. Effects of subacute ruminal acidosis on free-choice intake of sodium bicarbonate in lactating dairy cows. *Journal of dairy science* 86(3):954-957. doi: [https://doi.org/10.3168/jds.S0022-0302\(03\)73678-9](https://doi.org/10.3168/jds.S0022-0302(03)73678-9)
- Keunen, J. E., J. C. Plaizier, L. Kyriazakis, T. F. Duffield, T. M. Widowski, M. I. Lindinger, and B. W. McBride. 2002. Effects of a Subacute Ruminal Acidosis Model on the Diet Selection of Dairy Cows. *Journal of Dairy Science* 85(12):3304-3313. doi: [https://doi.org/10.3168/jds.S0022-0302\(02\)74419-6](https://doi.org/10.3168/jds.S0022-0302(02)74419-6)
- Keyser, R., C. Noller, L. J. Wheeler, and D. Schaefer. 1985. Characterization of limestones and their effects in vitro and in vivo in dairy cattle. *Journal of dairy science* 68(6):1376-1389. doi: [https://doi.org/10.3168/jds.S0022-0302\(85\)80974-7](https://doi.org/10.3168/jds.S0022-0302(85)80974-7)
- Kleen, J. L., G. A. Hooijer, J. Rehage, and J. P. T. M. Noordhuizen. 2003. Subacute Ruminal Acidosis (SARA): a Review. *Journal of Veterinary Medicine Series A* 50(8):406-414. doi: 10.1046/j.1439-0442.2003.00569.x
- Kotarski, S. F., R. D. Waniska, and K. K. Thurn. 1992. Starch Hydrolysis by the Ruminal Microflora. *The Journal of Nutrition* 122(1):178-190. doi: 10.1093/jn/122.1.178
- Koziol, J. H., and C. L. Armstrong. 2018. *Society for Theriogenology Manual for Breeding Soundness Examination of Bulls.*
- Krause, K. M., and G. R. Oetzel. 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. *Animal Feed Science and Technology* 126(3):215-236. doi: <https://doi.org/10.1016/j.anifeedsci.2005.08.004>
- Krehbiel, C. R., R. A. Stock, D. H. Shain, C. J. Richards, G. A. Ham, R. A. McCoy, T. J. Klopfenstein, R. A. Britton, and R. P. Huffman. 1995. Effect of level and type of fat on

- subacute acidosis in cattle fed dry-rolled corn finishing diets. *Journal of Animal Science* 73(8):2438-2446. doi: 10.2527/1995.7382438x
- Krieger, D. T. 1983. Brain peptides: what, where, and why? *Science* 222(4627):975-985. doi: 10.1126/science.6139875
- Lofgreen, G., and W. Garrett. 1968. A system for expressing net energy requirements and feed values for growing and finishing beef cattle. *Journal of animal science* 27(3):793-806. doi: <https://doi.org/10.2527/jas1968.273793x>
- Lunstra, D., K. Gregory, and L. Cundiff. 1988. Heritability estimates and adjustment factors for the effects of bull age and age of dam on yearling testicular size in breeds of bulls. *Theriogenology* 30(1):127-136. doi: [https://doi.org/10.1016/0093-691X\(88\)90270-1](https://doi.org/10.1016/0093-691X(88)90270-1)
- Mackenzie, D. D. S. 1967. Production and Utilization of Lactic Acid by the Ruminant. A Review. *Journal of Dairy Science* 50(11):1772-1786. doi: [https://doi.org/10.3168/jds.S0022-0302\(67\)87715-4](https://doi.org/10.3168/jds.S0022-0302(67)87715-4)
- Mackie, R., and F. M. Gilchrist. 1979. Changes in lactate-producing and lactate-utilizing bacteria in relation to pH in the rumen of sheep during stepwise adaptation to a high-concentrate diet. 0099-2240.
- Maclean, C. 1966. Observations on laminitis in intensive beef units. 0042-4900.
- Maekawa, M., K. Beauchemin, and D. Christensen. 2002. Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. *Journal of Dairy Science* 85(5):1165-1175. doi: [https://doi.org/10.3168/jds.S0022-0302\(02\)74179-9](https://doi.org/10.3168/jds.S0022-0302(02)74179-9)
- Majzoub, J. A. 2006. Corticotropin-releasing hormone physiology. *European Journal of Endocrinology* 155(suppl 1):S71-S76. doi: <https://doi.org/10.1530/eje.1.02247>

- McAllister, T. A., K.-J. Cheng, L. M. Rode, and C. W. Forsberg. 1990. Digestion of barley, maize, and wheat by selected species of ruminal bacteria. *Applied and environmental microbiology* 56(10):3146-3153.
- McKenna, R. D., D. L. Roebert, P. K. Bates, T. Schmidt, D. S Hale, D. B Griffin, J. W Savell, J. Brooks, J. B. Morgan, T. H. Montgomery, K. Belk, and G. C. Smith. 2002. National Beef Quality Audit-2000: Survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers.
- Mertens, D., and J. Loften. 1980. The effect of starch on forage fiber digestion kinetics in vitro. *Journal of Dairy Science* 63(9):1437-1446. doi: [https://doi.org/10.3168/jds.S0022-0302\(80\)83101-8](https://doi.org/10.3168/jds.S0022-0302(80)83101-8)
- Mertens, D. R. 2002. Measuring fiber and its effectiveness in ruminant diets. In: *Proc. Plains Nutr. Cncl. Spring Conf. San Antonio, TX.* p 40-66.
- Miller, R., R. Hemken, D. Waldo, M. Okamoto, and L. Moore. 1965. Effect of feeding buffers to dairy cows fed a high-concentrate, low-roughage ration. *Journal of Dairy Science* 48(11):1455-1458. doi: [https://doi.org/10.3168/jds.S0022-0302\(65\)88498-3](https://doi.org/10.3168/jds.S0022-0302(65)88498-3)
- Montaño-Bermudez, M., M. K. Nielsen, and G. H. Deutscher. 1990. Energy requirements for maintenance of crossbred beef cattle with different genetic potential for milk. *Journal of animal science* 68(8):2279-2288. doi: [10.2527/1990.6882279x](https://doi.org/10.2527/1990.6882279x)
- Montgomery, M. J., and B. R. Baumgardt. 1965. Regulation of Food Intake in Ruminants. 1. Pelleted Rations Varying in Energy Concentration1, 2. *Journal of Dairy Science* 48(5):569-574. doi: [https://doi.org/10.3168/jds.S0022-0302\(65\)88286-8](https://doi.org/10.3168/jds.S0022-0302(65)88286-8)

- Mooney, C., and M. Allen. 2007. Effect of dietary strong ions on chewing activity and milk production in lactating dairy cows. *Journal of dairy science* 90(12):5610-5618. doi: <https://doi.org/10.3168/jds.2007-0008>
- Morrison, C., J. Daniel, B. Holmberg, J. Djiane, N. Raver, A. Gertler, and D. Keisler. 2001. Central infusion of leptin into well-fed and undernourished ewe lambs: effects on feed intake and serum concentrations of growth hormone and luteinizing hormone. *Journal of Endocrinology* 168(2):317-324. doi: <https://doi.org/10.1677/joe.0.1680317>
- Murray, R., D. Downham, M. Clarkson, W. Faull, J. Hughes, F. Manson, J. Merritt, W. Russell, J. Sutherst, and W. Ward. 1996. Epidemiology of lameness in dairy cattle: description and analysis of foot lesions. *Veterinary record* 138(24):586-591. doi: <http://dx.doi.org/10.1136/vr.138.24.586>
- Mwansa, P. B., and M. Makarechian. 1991. The effect of postweaning level of dietary energy on sex drive and semen quality of young beef bulls. *Theriogenology* 35(6):1169-1178. doi: [https://doi.org/10.1016/0093-691X\(91\)90363-I](https://doi.org/10.1016/0093-691X(91)90363-I)
- Nafikov, R. A., and D. C. Beitz. 2007. Carbohydrate and Lipid Metabolism in Farm Animals. *The Journal of Nutrition* 137(3):702-705. doi: [10.1093/jn/137.3.702](https://doi.org/10.1093/jn/137.3.702)
- Nagaraja, T., T. Avery, E. Bartley, S. K. Roof, and A. D. Dayton. 1982. Effect of lasalocid, monensin or thiopeptin on lactic acidosis in cattle. *Journal of Animal Science* 54(3):649-658. doi: <https://doi.org/10.2527/jas1982.543649x>
- Nagaraja, T., and E. Titgemeyer. 2007. Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook1, 2. *Journal of Dairy Science* 90:E17-E38. doi: <https://doi.org/10.3168/jds.2006-478>



- Nagaraja, T. G., and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: a review. *J Anim Sci* 76(1):287-298. doi: <https://doi.org/10.2527/1998.761287x>
- Nakamura, K., and H. Takahashi. 1971. Role of lactate as an intermediate of fatty acid fermentation in the sheep rumen. *The Journal of General and Applied Microbiology* 17(4):319-328. doi: <https://doi.org/10.2323/jgam.17.319>
- Nocek, J. E. 1997. Bovine Acidosis: Implications on Laminitis. *Journal of Dairy Science* 80(5):1005-1028. doi: [https://doi.org/10.3168/jds.S0022-0302\(97\)76026-0](https://doi.org/10.3168/jds.S0022-0302(97)76026-0)
- Nordlund, K., and E. Garrett. 1994. Rumenocentesis: a technique for collecting rumen fluid for the diagnosis of subacute rumen acidosis in dairy herds. *Compendium on continuing education for the practicing veterinarian.*
- Nordlund, K. V., E. F. Garrett, and G. R. Oetzel. 1995. Herd-based rumenocentesis- A clinical approach to the diagnosis of sub acute rumen acidosis. *Compendium on continuing education for the practicing veterinarian.*
- Oetzel, G., K. Nordlund, and E. Garrett. 1999. Effect of ruminal pH and stage of lactation on ruminal lactate concentration in dairy cows. *Journal of Dairy Science* (82:38)
- Oetzel, G. R. 2007. Subacute ruminal acidosis in dairy herds: physiology, pathophysiology, milk fat responses, and nutritional management. In: 40th Annual Conference, American Association of Bovine Practitioners. p 89-119.
- Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1997. The effect of grain source and grain processing on performance of feedlot cattle: a review. *Journal of Animal Science* 75(3):868-879. doi: [10.2527/1997.753868x](https://doi.org/10.2527/1997.753868x)
- Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1998. Acidosis in cattle: a review. *Journal of animal science* 76(1):275-286. doi: [10.2527/1998.761275x](https://doi.org/10.2527/1998.761275x)

- Plaizier, J. C. 2004. Replacing Chopped Alfalfa Hay with Alfalfa Silage in Barley Grain and Alfalfa-Based Total Mixed Rations for Lactating Dairy Cows. *Journal of Dairy Science* 87(8):2495-2505. doi: [https://doi.org/10.3168/jds.S0022-0302\(04\)73374-3](https://doi.org/10.3168/jds.S0022-0302(04)73374-3)
- Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. *The Veterinary Journal* 176(1):21-31. doi: <https://doi.org/10.1016/j.tvjl.2007.12.016>
- Poulsen, S. B., R. A. Fenton, and T. Rieg. 2015. Sodium-glucose cotransport. *Current opinion in nephrology and hypertension* 24(5):463. doi: 10.1097/MNH.0000000000000152
- Pritchard, R., and K. Bruns. 2003. Controlling variation in feed intake through bunk management. *Journal of Animal Science* 81(14\_suppl\_2):E133-E138. doi: [https://doi.org/10.2527/2003.8114\\_suppl\\_2E133x](https://doi.org/10.2527/2003.8114_suppl_2E133x)
- Pritchard, R. H. 1993. Bunk management. In: Land O'Lakes Beef Seminar, Cedar Rapids, IA, Columbus, NE, and Storm Lake, IA. Land O'Lakes Inc., St. Paul, MN. p 4-15.
- Pruitt, R. J., and L. R. Corah. 1985. Effect of Energy Intake after Weaning on the Sexual Development of Beef Bulls. I. Semen Characteristics and Serving Capacity<sup>2</sup>. *Journal of Animal Science* 61(5):1186-1193. doi: 10.2527/jas1985.6151186x
- Reinhardt, C. D., and M. E. Hubbert. 2015. Control of liver abscesses in feedlot cattle: A review<sup>11</sup>Contribution no. 10-205-J from the Kansas Agric. Exp. Stn., Manhattan 66506. *The Professional Animal Scientist* 31(2):101-108. doi: <https://doi.org/10.15232/pas.2014-01364>
- Reynolds, C. 2005. Glucose balance in cattle. In: Proceedings of the 2005 Florida Ruminant Nutrition Conference, Gainesville. p 143-154.

- Robles, V., L. González, A. Ferret, X. Manteca, and S. Calsamiglia. 2007. Effects of feeding frequency on intake, ruminal fermentation, and feeding behavior in heifers fed high-concentrate diets. *Journal of Animal Science* 85(10):2538-2547. doi: <https://doi.org/10.2527/jas.2006-739>
- Roeber, D. L., P. D. Mies, C. D. Smith, K. E. Belk, T. G. Field, J. D. Tatum, J. A. Scanga, and G. C. Smith. 2001. National market cow and bull beef quality audit—1999: a survey of producer-related defects in market cows and bulls. *Journal of Animal Science* 79(3):658-665. doi: 10.2527/2001.793658x
- Rouiller-Fabre, V., L. Lecerf, C. Gautier, J. Saez, and R. Habert. 1998. Expression and effect of insulin-like growth factor I on rat fetal Leydig cell function and differentiation. *Endocrinology* 139(6):2926-2934. doi: <https://doi.org/10.1210/endo.139.6.6035>
- Russell, J. B. 1987. A proposed mechanism of monensin action in inhibiting ruminant bacterial growth: Effects on ion flux and protonmotive force. *Journal of Animal Science* 64(5):1519-1525. doi: <https://doi.org/10.2527/jas1987.6451519x>
- Russell, J. B., and T. Hino. 1985. Regulation of Lactate Production in *Streptococcus bovis*: A Spiraling Effect That Contributes to Rumen Acidosis. *Journal of Dairy Science* 68(7):1712-1721. doi: [https://doi.org/10.3168/jds.S0022-0302\(85\)81017-1](https://doi.org/10.3168/jds.S0022-0302(85)81017-1)
- Russell, J. B., and D. B. Wilson. 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? *Journal of dairy science* 79(8):1503-1509. doi: [https://doi.org/10.3168/jds.S0022-0302\(96\)76510-4](https://doi.org/10.3168/jds.S0022-0302(96)76510-4)
- Scanlan, C., and T. Hathcock. 1983. Bovine rumenitis-liver abscess complex: a bacteriological review. *Cornell Vet* 73(3):288-297.

- Schillo, K. K. 1992. Effects of dietary energy on control of luteinizing hormone secretion in cattle and sheep. *Journal of animal science* 70(4):1271-1282. doi: 10.2527/1992.7041271x
- Shaver, R. 2002. Rumen acidosis in dairy cattle: Bunk management considerations, University of Wisconsin – Madison.
- Shi, Y., and P. Weimer. 1992. Response surface analysis of the effects of pH and dilution rate on *Ruminococcus flavefaciens* FD-1 in cellulose-fed continuous culture. *Applied and environmental microbiology* 58(8):2583-2591.
- Slyter, L. L. 1976. Influence of Acidosis on Rumen function1. *Journal of Animal Science* 43(4):910-929. doi: 10.2527/jas1976.434910x
- Stock, R. 2000. Acidosis in cattle: an overview. In: Thrity-third Annual Conference, American Association of Bovine Practitioners, Rapid City, South Dakota. p 30-37.
- Stoebel, D. P., and G. P. Moberg. 1982. Effect of Adrenocorticotropin and Cortisol on Luteinizing Hormone Surge and Estrous Behavior of Cows. *Journal of Dairy Science* 65(6):1016 - 1024. doi: [https://doi.org/10.3168/jds.S0022-0302\(82\)82303-5](https://doi.org/10.3168/jds.S0022-0302(82)82303-5)
- Stone, W. C. 2004. Nutritional Approaches to Minimize Subacute Ruminant Acidosis and Laminitis in Dairy Cattle. *Journal of Dairy Science* 87(Supplement):E13-E26. doi: [https://doi.org/10.3168/jds.S0022-0302\(04\)70057-0](https://doi.org/10.3168/jds.S0022-0302(04)70057-0)
- Theurer, B., and W. Hale. 1968. Effect of volatile fatty acids on intake of high-grain rations by steers. In: *JOURNAL OF ANIMAL SCIENCE*. p 1178-&.
- Theurer, C. B. 1986. Grain Processing Effects on Starch Utilization by Ruminants. *Journal of Animal Science* 63(5):1649-1662. doi: 10.2527/jas1986.6351649x

- Thibier, M., and O. Rolland. 1976. The effect of dexamethasone (DXM) on circulating testosterone (T) and luteinizing hormone (LH) in young postpubertal bulls. *Theriogenology* 5(2):53 - 60. doi: [https://doi.org/10.1016/0093-691X\(76\)90168-0](https://doi.org/10.1016/0093-691X(76)90168-0)
- Thomson, R. 1967. Rumenitis in cattle. *The Canadian Veterinary Journal* 8(8):p 189-192.
- Underwood, W. 1992. Rumen lactic acidosis: 1. Epidemiology and pathophysiology. *The compendium on continuing education for the practicing veterinarian Princeton Heights, New Jersey.*
- Van Winden, S. C., and R. Kuiper. 2003. Left displacement of the abomasum in dairy cattle: recent developments in epidemiological and etiological aspects. *Veterinary research* 34(1):47-56. doi: <https://doi.org/10.1051/vetres:2002060>
- Vermunt, P. R. G. 1994. Predisposing factors of laminitis in cattle. *British Veterinary Journal* 150(2):151 - 164. doi: [https://doi.org/10.1016/S0007-1935\(05\)80223-4](https://doi.org/10.1016/S0007-1935(05)80223-4)
- Waldo, D. R. 1973. Extent and Partition of Cereal Grain Starch Digestion in Ruminants<sup>1</sup>. *Journal of Animal Science* 37(4):1062-1074. doi: [10.2527/jas1973.3741062x](https://doi.org/10.2527/jas1973.3741062x)
- Welsh, T., and B. H. Johnson. 1981. Stress-Induced Alterations in Secretion of Corticosteroids, Progesterone, Luteinizing Hormone, and Testosterone in Bulls\*. *Endocrinology* 109(1):185-190. doi: [10.1210/endo-109-1-185](https://doi.org/10.1210/endo-109-1-185)
- Wise, M. B., T. N. Blumer, H. B. Craig, and E. R. Barrick. 1965. Influence of Rumen Buffering Agents and Hay on Performance and Carcass Characteristics of Steers Fed All-Concentrate Rations. *Journal of Animal Science* 24(1):83-88. doi: [10.2527/jas1965.24183x](https://doi.org/10.2527/jas1965.24183x)

**CHAPTER 2: Impact of feeding management strategies on growth performance, rumen conditions, and semen quality of developing bulls**

## Abstract

The use of high-energy diets to develop bulls is perceived to influence semen quality, yet little research has been conducted using iso-caloric diets that differ in form or feeding management strategy. Therefore, the objective of this experiment was to evaluate the effects of two feeding strategies on growth performance and semen quality of developing bulls. A secondary objective was to evaluate the relationship between phenotypic traits of interest and semen quality parameters. Angus bulls ( $n = 48$ ;  $332 \pm 47$  d of age) were stratified into one of sixteen drylot pens before pens were randomly assigned to one of two iso-caloric diets: a total mixed ration (TMR;  $n = 8$  pens) or a component-based ration (COMP;  $n = 8$  pens) fed for 84 d. Rumen pH was continuously monitored via rumen boluses and dry matter intake (DMI) was measured daily throughout the experiment. Subcutaneous fat thickness and hoof conformation were measured on d 0 and 84, with body weight measured and semen collected at 21-d intervals. Semen was assessed for progressive motility, total sperm per ejaculate, and sperm morphology. Data were analyzed utilizing JMP 13.0. Bulls fed the COMP ration had a greater DMI ( $P < 0.01$ ) and ADG ( $P < 0.01$ ), but similar feed efficiency ( $P = 0.21$ ) when compared to TMR-fed bulls. Bulls fed COMP had a lower mean rumen pH ( $P < 0.05$ ) and higher probability to display rumen pH below 5.8 ( $P < 0.01$ ) and 5.2 ( $P < 0.01$ ). Additionally, COMP-fed bulls had greater backfat thickness ( $P < 0.01$ ) but similar hoof conformation scores ( $P = 0.54$ ) when compared to TMR-fed bulls on d 84. Feeding strategy did not affect progressive motility ( $P = 0.34$ ), total sperm per ejaculate ( $P = 0.81$ ), or percent morphologically-normal sperm ( $P = 0.34$ ). Change in scrotal circumference between d 0 and 84 was directly related to change in percent morphologically-normal sperm ( $R^2 = 0.12$ ;  $P < 0.05$ ), but no other semen quality characteristics ( $P \geq 0.17$ ). Change in subcutaneous fat thickness throughout the experiment tended to be inversely related to change in percent morphologically-normal sperm ( $R^2 = 0.06$ ;  $P < 0.10$ ), but was directly related to changes in semen volume ( $R^2 = 0.10$ ;  $P < 0.05$ ) and total sperm per ejaculate ( $R^2 = 0.10$ ;  $P < 0.05$ ),

but not change in motility ( $P = 0.24$ ). Mean rumen pH tended to be directly related to changes in semen volume ( $R^2 = 0.07$ ;  $P < 0.10$ ) and total sperm per ejaculate ( $R^2 = 0.07$ ;  $P < 0.10$ ). Mean rumen temperature was not related to any semen quality characteristics ( $P \geq 0.15$ ). These results suggest that the feeding strategies utilized in this experiment affected growth performance, rumen pH, and adiposity without influencing semen quality of developing bulls, and therefore should not be expected to influence breeding soundness examination outcomes.

## **Introduction**

Rapid development of beef bulls is common in seedstock beef cattle production, as it hastens the time required before an animal's genetics can be implemented in a herd, and decreases overall input costs when compared to prolonged development. This process is generally made possible through the use of high-energy diets, which accelerate physical and sexual development, and allow for objective evaluation of a bull's genetic merit for growth performance, feed efficiency, and carcass traits.

Nonetheless, the use of high-energy diets to develop bulls has been shown to negatively affect semen quality (Coulter and Kozub, 1984; Mwansa and Makarechian, 1991; Coulter et al., 1997). Corresponding reductions in semen quality have historically been attributed to elevated testicular temperature resulting from high levels of scrotal adiposity (Mwansa and Makarechian, 1991; Vogler et al., 1993). These concerns have driven many seedstock producers to develop bulls using low-energy diets, in an attempt to avoid excess accumulation of scrotal fat, and preserve semen quality.

Recent evidence suggests that experimentally-induced subacute ruminal acidosis (SARA) reduces semen quality, and does so independently of scrotal adiposity (Callaghan et al., 2016).



Feeding high-energy diets that are comprised of an abundance of non-structural, readily-fermentable carbohydrates, primarily from corn or other cereal grains, to ruminants has the potential to result in SARA without proper management (Owens et al., 1998; Oetzel, 2007). In addition to the presence of readily-fermentable, non-structural carbohydrates, insufficient levels of physically-effective neutral detergent fiber (peNDF) in the diet limit rumination and rumen buffering capacity, and result in the aggregation of hydrogen ions (González et al., 2012).

The majority of previous research in the field of bull development has predominately focused on the effects of pre- and post-weaning dietary energy level on the sexual development and semen quality of developing bulls. Effects of feeding rations that are similar in protein and energy content, but differ in nutrient form or feeding management strategies have, to this point, been relatively unexplored. Based upon review of the existing literature, it was hypothesized that rations fed to developing bulls that differ in nutrient form or feeding management strategy, but not in the level of energy or protein, may lead to ruminal conditions indicative of SARA, alter performance characteristics, and ultimately affect semen quality. Therefore, an experiment was conducted to evaluate the effects of two iso-caloric and iso-nitrogenous diets of different form fed using a different feeding management strategy on growth performance, feed efficiency, rumen conditions, and semen quality of developing bulls. Additionally, a secondary objective of this experiment was to quantify relationships between rumen conditions, adiposity, and semen quality.

## **Materials and methods**

This experiment and all procedures reported herein were approved by the University of Tennessee Institutional Animal Care and Use Committee, and adhered to the criteria outlined by

the Federation of Animal Science Societies Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010).

### **Experimental design and feeding management**

Angus bulls (n = 48;  $332 \pm 47$  d of age ) with an average initial live weight of  $376.5 \pm 60.7$  kg were provided by Deer Valley Farms of Fayetteville, TN, and transported 360 km to the East Tennessee Research and Education Center (ETREC) – Blount Unit in Alcoa, TN on d -10. Bulls were provided with *ad-libitum* access to processed *Festuca arundinacea* (tall fescue) and *Trifolium repens* (white clover) mixed grass and legume hay [16.1% crude protein, 59.9% neutral detergent fiber (NDF), 36.3% acid detergent fiber (ADF), 4.62% acid detergent lignin (ADL), 2.93% ether extract (EE), 7.93% ash, 1.32 Mcal of NEm x kg<sup>-1</sup>, and 0.75 Mcal of NEg x kg<sup>-1</sup>; DM-basis] for the 11 days leading up to the initiation of the experiment. Bulls received 0.2 kg x head<sup>-1</sup> x day<sup>-1</sup> from d -9 to -6, 0.4 kg x head<sup>-1</sup> x day<sup>-1</sup> from d -5 to -3, and 0.6 kg x head<sup>-1</sup> x day<sup>-1</sup> from d -2 to 0 of a custom-formulated micro-ingredient premix (Table 2.1) containing 300 mg of monensin x kg<sup>-1</sup> [as-is basis (Rumensin; Elanco Animal Health, Greenfield, IN)] to begin the transition to rations that contained monensin. Additionally, bulls received 10 mg of amprolium (Corid 1.25 % Crumbles; Boehringer Ingelheim Animal Health USA, Inc., Duluth, GA) x kg of BW<sup>-1</sup> x d<sup>-1</sup> from d -9 to -5. On d -5, bulls received an oral dose of 4.5 mg x kg<sup>-1</sup> of body weight (BW) of oxfendazole (Synanthic, Bovine Dewormer Suspension 22.5%; Boehringer Ingelheim Animal Health USA, Inc.).

Bulls were weighed on d 0 and stratified before being randomly assigned to one of sixteen dry-lot pens. Pens were then alternately assigned in a randomized complete block design to one of two iso-caloric and iso-nitrogenous diets: a total mixed ration (TMR; n = 8 pens, n = 24 bulls) or a component-based feeding system (COMP; n = 8 pens, n = 24 bulls) that consisted of a

pelleted concentrate (Table 2.1) and processed mature *Secale cereale* (cereal rye) hay (6.6% CP, 74.1% NDF, 48.9% ADF, 7.7% ADL, 1.6% EE, 4.8% ash, 1.09 Mcal of NEm x kg<sup>-1</sup>, and 0.54 Mcal of NEg x kg<sup>-1</sup>; DM-basis) fed separately.

Individual hay boxes were placed in each feed bunk to separate rye hay from pelleted concentrate in COMP-fed pens. Ingredient addition to the TMR was sequenced in the order of corn silage, dry-rolled corn, micro-ingredient premix, dried corn gluten feed, processed mixed grass hay, and water into a mixing apparatus (WIC MDR-48; WIC, Wickham, Quebec) with a target total mixing time of 8 min beginning with the addition of the first ingredient in order to ensure ration homogeneity prior to delivery. Pens were fed once daily at 0715 ± 0015 h, with all bunks receiving an initial allotment of 14.7 kg of DM, and the remainder of the daily feed allotment delivered within 30 minutes thereafter. Cattle were provided with *ad-libitum* access to water throughout the duration of the experiment, and automatic waterers were cleaned or flushed on a daily basis.

Bunk scores and feed calls were assigned to each pen as described by Pritchard (1993) to promote appropriate bunk and feeding management. On d 1, pens assigned to TMR received 1.50% of pen BW in DM from the TMR, and COMP-assigned pens received 1.75% of pen BW in DM from the concentrate-based pellet and were provided with *ad-libitum* access to processed mature cereal rye hay. Additionally, pens fed the TMR were provided with *ad-libitum* access to processed grass hay throughout the duration of the 21-d transition onto full feed. The amount of daily offered DM was then increased by 5% no more frequently than once every three days for TMR-fed pens and no more frequently than once every two days for COMP-fed pens throughout an initial 21-d transition period. The purpose of the variation in percentage of BW in DM on d 1 and a more aggressive feeding strategy for COMP-fed pens was to elicit SARA in COMP-fed

bulls through a more rapid transition onto full feed. Following the 21-d transition onto full feed, TMR-fed pens were managed on an *ad-libitum* but slick-bunk basis throughout the remainder of the 63 d on feed. Pens fed the COMP ration were fed on an *ad libitum* basis with a 1-5% targeted feed refusal until d 63 of the experiment. At this time, COMP-fed bulls were adjusted to *ad-libitum* slick-bunk management throughout the remainder of the experiment to mitigate the prevalence of digestive morbidity

### **Feed intake, sampling, re-formulation, and analysis**

Pen feed intake was measured daily, calculated by difference of daily offerings and refusals weighed to the nearest 0.1 kg immediately prior to delivering each day's feed allotment, and expressed in kg of DM intake (DMI). Samples of each individual feedstuff and the TMR were collected weekly in triplicate and stored in a -20°C freezer until further processing and analysis. Additionally, a fourth sample of each ingredient was collected and dried at 105°C for 24 h in a forced-air oven in order to determine DM content, which was then used to adjust inclusion rates of each feedstuff in order to achieve a common DM-inclusion rate in the TMR and COMP.

Following the completion of the experiment, TMR, concentrate-based pellet, hay, and other ingredient samples were dried at 55°C for 48-72 h in a forced-air drying oven. Cereal rye, processed mixed grass hay, and corn silage were ground through a 1- or 2-mm screen using a Wiley Mill (Udy Corporation, Fort Collins, CO), then were cyclone ground using a Udy Mill (Udy Corporation, Fort Collins, CO) to pass through a 1-mm screen. The pelleted concentrate, dry-rolled corn, micro-ingredient premix, and dried corn gluten feed were initially ground in a coffee grinder before being cyclone ground using a Udy Mill to pass through a 1-mm screen. Samples were then composited based on average weekly DMI before being shipped to an

independent contractual analytical laboratory (Cumberland Valley Analytical Services, Cumberland Valley, PA) for determination of nutrient content via wet chemistry.

### **Growth performance and hoof conformation**

The experiment was divided into four 21-d intervals where following each interval, sample collections were conducted on two consecutive days. Bulls were weighed on two consecutive days at each of four 21-d intervals, which included d 0 and 1, d 20 and 21, d 41 and 42, d 62 and 63, and d 83 and 84. Respective consecutive-day weights were averaged for each bull and considered to be the true un-shrunk BW on d 0, 21, 42, 63, and 84. Average daily gain (ADG) was calculated at individual 21-d intervals as well as for the entire 84-d experiment. Average daily gain and average daily DMI data were then used to express feed efficiency as the ratio of ADG to daily DMI.

Immediately after being weighed, bulls were restrained in a squeeze chute, and hoof conformation was assessed as described by Jeyaruban et al. (2012). In brief, hoof angle and claw set were each analyzed on a 1-9 scale with 5 and 6 considered to be the most ideal hoof conformation for both hoof angle and claw set. Hoof scores were assigned by each of two trained analysts, and then averaged for each bull.

Backfat measurements were collected by measuring the 12<sup>th</sup>-rib subcutaneous fat thickness (SFT) on d 0 and 84, and used to determine change in SFT over the 84-d experiment. In brief, duplicate ultrasound images were collected by a trained technician utilizing an ultrasound machine (Ibex EVO; E.I. Medical Imaging, Loveland, CO) equipped with a 3-6 MHz linear transducer (L3ASE; E.I. Medical Imaging, Loveland, CO). Images were saved at the time of capture, and linear measurements were later performed using the ultrasound interface.

## **Rumen pH and temperature**

Rumen boluses (eBolus; eCow, Devon, UK) were orally administered via balling gun on d 0 and used to continuously monitor rumen pH and temperature at 15-min intervals. Bolus data were downloaded at each 21-d interval immediately following evaluation of hoof conformation. Prior to administration, rumen boluses were calibrated in pH 4 and 7 buffer solutions and calibration information was stored in accordance with manufacturer-recommended guidelines. Once collected, bolus data were plotted with a quadratic best-fit line to evaluate the data for evidence of drift. If linear drift of pH readings occurred, the inflection point in which linear increase of pH occurred was identified and all data thereafter were removed and considered to be missing observations. Additionally, rumen temperature observations of  $< 35.3^{\circ}\text{C}$  and  $> 41.3^{\circ}\text{C}$  were removed from further analysis and recorded as missing observations, as adapted from Beatty et al. (2008).

## **Scrotal circumference, semen collection, and semen quality**

A breeding soundness examination (BSE) that adhered to the guidelines set forth by Koziol and Armstrong (2018) (accepted by the Society for Theriogenology for 2019), was conducted on one of two d that corresponded with other chute-side data collection at each of the 21-d intervals, and included measurements of scrotal circumference (SC) and palpation of the reproductive tract, as well as semen collection and semen quality analysis. Scrotal circumference was measured at the beginning of each BSE as previously described by Barth (2007). In brief, the scrotal neck was grasped and the testicles were pushed to the ventral portion of the scrotal sac before an 80-cm inelastic scrotal tape was utilized to measure the scrotum at the widest portion. Pressure was applied to the scrotal tape and tightened three times to guarantee a secure fit around the scrotal sac before recording the measurement. Scrotal circumference will be

reported as a growth performance measure, yet will remain a facet of the BSE. Testicles were also visually examined and palpated to evaluate size, shape, and consistency.

Immediately following measurement of SC and testicular palpation, semen was collected utilizing either electroejaculation or transrectal massage. Collection method and d of collection were alternated for each bull across sample collections, resulting in a total of two semen collections from each bull by each collection method, but on different days of each collection. Regardless of method, semen collection was performed immediately following palpation of the reproductive tract. Transrectal massage was performed by a trained technician using a technique adapted from Palmer et al. (2005). Briefly, a gentle massage and cranial-to-caudal raking motion was applied to the reproductive tract via the ventral portion of the rectal wall in concert with urethralis muscle contractions to elicit semen emission and ejaculation. Electroejaculation was accomplished by a trained technician using a 60-mm probe with two ventrally-located electrodes attached to a pre-programmed electroejaculator (Pulsator V; Lane Manufacturing, Denver, CO). If semen samples were not obtained from the programmed function of the ejaculator, semen was collected using the manual function of the machine or via transrectal massage. Similarly, when semen samples were not obtained through transrectal massage, semen was collected via electroejaculation. If these combined semen collection attempts were unsuccessful, data was considered to be a missing observation.

Semen was collected into a 15-mL disposable vial attached to a plastic disposable director cone adhered to a semen collection handle. In the case of low ambient temperature (< 21°C), the semen collection tube was submerged into warm water to prevent chilling of the sample. Semen collection was initiated when pre-ejaculatory fluid became white or cloudy, indicating the presence of spermatozoa in the fluid. Collection time was standardized at 15 s for

electroejaculation and 120 s for transrectal massage following the start of semen collection in order to ensure an accurate comparison of semen quality parameters.

Semen samples were then analyzed immediately following semen collection by a board certified theriogenologist. Percentage progressive motility was quantified by one of two trained technicians utilizing light-phase microscopy at 10x magnification (EVOS XL Core; ThermoFisher Scientific, Waltham, MA). One drop (approximately 60  $\mu\text{L}$ ) of semen was placed on a pre-warmed slide, covered with a pre-warmed cover slip, and placed on a warmed stage for analysis. If the semen sample was concentrated to the degree where progression of individual spermatozoon could not be evaluated, one drop of pre-warmed phosphate-buffered saline was placed directly onto the drop of semen as a diluent. Progressive motility was defined as the estimated percentage of spermatozoa traveling into and leaving the objective field of view in a linear path. Semen samples were weighed to the nearest 0.0001 g. Semen weights were then used to calculate semen volume using an adjustment factor of 1.053 g  $\times$  mL<sup>-1</sup> of semen (Kocks and Broekhuijse, 2014). Spermatozoa concentration (number of spermatozoa  $\times$  mL<sup>-1</sup>) was determined in duplicate using light photometry (SpermaQ-II; MOFA Global, Madison, WI), which was then multiplied by semen volume to quantify the total number of spermatozoa  $\times$  ejaculate<sup>-1</sup>.

Duplicate slides were then prepared for spermatozoa morphological analysis utilizing an eosin-nigrosin smear. Once prepared, slides were stored at ambient temperature until further analysis. Morphological analysis of spermatozoa was performed with the inverted function of the previously-described microscope. In brief, a drop of immersion oil was placed on the slide and the stage was adjusted to immerse the 40x objective in oil prior to adjusting focus and brightness. Morphological analyses were conducted by each of three trained technicians, resulting in a total of six counts of spermatozoa morphology  $\times$  ejaculate<sup>-1</sup>. Each morphological evaluation assessed



100 spermatozoa which were classified as either morphologically-normal or -abnormal, and counts were averaged across technicians. Intra- and inter-slide coefficients of variation (CV) for morphologically-normal spermatozoa were 9.8% and 15.0%, respectively. Fractional percentages of morphologically-normal and -abnormal spermatozoa were multiplied by the total number of spermatozoa x ejaculate<sup>-1</sup> to quantify the total number of morphologically-normal and -abnormal spermatozoa x ejaculate<sup>-1</sup>.

Following quantification of semen quality parameters, BSE results were assigned to each bull using the guidelines previously established by Koziol and Armstrong (2018), where bulls were classified as either pass or fail. In brief, bulls were required to pass a physical examination evaluating eye, hoof, accessory sex gland, penile, and testicular structure and health. Additionally, semen quality must achieve thresholds of percentage progressive motile spermatozoa  $\geq 30\%$  along with percentage morphologically-normal spermatozoa  $\geq 70\%$  for a bull to qualify as satisfactory.

### **Statistical analyses**

Analysis of variance (ANOVA) was conducted utilizing the Fit Model procedure of JMP 13.0 (SAS Institute Inc., Cary, NC) to determine the main effect of diet on metrics of growth performance, DMI, feed efficiency, rumen pH, rumen temperature, SFT, hoof conformation, and scrotal circumference. Body weight on d 1 accounted for a portion of the variation ( $P < 0.05$ ) in at least one model and was included in all analyses of growth performance. Pen served as the experimental unit for growth performance, DMI, and feed efficiency data, whereas bull was considered the experimental unit for SFT, hoof conformation, and scrotal circumference. Average daily DMI was analyzed by Standard Least Squares with the Fit Model program in JMP 13.0. Repeated measures of DMI were analyzed with the main effect of diet evaluated on a pen-

basis. Additionally, ANOVA was conducted using the Fit Model procedure to determine the main and interaction effects of diet and day on parameters of semen quality, where individual bull served as the experimental unit. The interaction between diet and day was not significant ( $P > 0.10$ ) and was removed from the model. Additionally, blocking factors of semen collection method, d of experiment, and calving ease direct EPD accounted for a significant ( $P < 0.05$ ) portion of the variation in at least one response variable, and therefore were included in all final ANOVA models. Visual observation of plots of the residual vs. predicted values indicated that continuous data were normally distributed, of approximately equal variance, and contained no outliers. Where applicable, least square means were separated using Tukey-Kramer adjustments for multiple comparisons.

Rumen pH and temperature were analyzed using PROC Univariate in SAS 9.4 and the histogram function was used to obtain descriptive statistics. PROC Means was utilized to summarize data into mean bull averages over the entire study, each 21-d interval, individual d of experiment, and h within d, separately. These outputs were exported into separate files for individual analyses of interest. To test if there was a diet, h within d, d of experiment, diet by hour interaction, or diet by d interaction on mean rumen pH and body temperature, mixed model ANOVA were performed in PROC GLIMMIX of SAS 9.4. Fixed effects included diet, h within d, d of experiment, and the two-way interaction of diet and h or diet and d. Random effects included diet nested within pen and the interaction between pen, h, and treatment. For significant interactions, the “slicediff” option was used to assess treatment differences within each h or d. Tukey-Kramer adjustments for multiple comparisons were used for experiment-wise type I error control.

Logistic regression was utilized to quantify the probabilities of rumen pH below specific pH thresholds from observed data. Prior to analysis, data were sorted and summarized using PROC SORT and PROC MEANS into the number of events (number of observations where a bull displayed a pH below the specific threshold) for pH less than 6.0, 5.8, 5.5, 5.2, and 5.0 and number of trials (total number of observations for each bull). Separate multi-level multi-variable logistic regressions were performed using PROC Glimmix of SAS within each 21-d interval to determine the main effect of treatment on the probability for a bull to experience a rumen pH that would fall below each specific pH threshold. Logit link and binomial distributions were indicated and the fixed effect of treatment was assessed. The random residual effect of d and the subject of bull were included in the model as to account for the repeated measures taken on each bull over time. Compound symmetry covariance structure was found to be best fitting according to generalized chi-square/df fit statistics.

Simple linear regression was conducted utilizing the Fit Y by X procedure of JMP 13.0 to determine the relationship between the proportion of observations below specific rumen pH thresholds and all semen quality parameters within the respective 21-d interval and subsequent 21-d intervals. Additionally, simple linear regression was conducted to quantify relationships between average rumen pH, average rumen temperature, as well as full-trial changes (d 0 to 84) in SFT and SC and all semen quality parameters, and full-trial changes in semen quality parameters. Furthermore, logistic regression was conducted to determine the main effect of diet on the likelihood of BSE outcome and the incidence of digestive morbidity. One bull fed the COMP diet experienced an omasal impaction and was removed from the project following d-42 sample collections.

Least square mean differences, as well as linear and logistic regression models were considered statistically significant if  $P < 0.05$ , and considered to have a tendency toward significance if  $0.05 \leq P < 0.10$ .

## **Results and discussion**

### **Feed analysis and intake**

Nutrient composition of both COMP and TMR can be found in Table 2.1. Variation in commercial feed manufacturing practices for COMP and within individual TMR batches caused slight deviations between formulated and as-delivered nutrient composition. Yet, given weekly ration re-formulation of TMR ingredients based upon DM content, nutrient composition between COMP and TMR closely resemble targeted quantities, and resulted in diets that were nearly identical in caloric and nitrogenous values.

Average daily DMI was greater for COMP-fed compared to TMR-fed bulls between d 1 and 21 (8.17 vs 6.37 kg x day<sup>-1</sup>;  $P < 0.01$ ), d 22 and 42 (11.25 vs 9.73 kg x day<sup>-1</sup>;  $P < 0.01$ ), d 43 and 63 (12.65 vs 11.51 kg x day<sup>-1</sup>;  $P < 0.05$ ), and d 64 and 84 (12.84 vs 11.85 kg x day<sup>-1</sup>;  $P < 0.05$ ). Thus, average DMI throughout the duration of the feeding period was greater ( $P < 0.01$ ) for COMP (11.13 kg x day<sup>-1</sup>) compared to TMR (9.81 kg x day<sup>-1</sup>). Variation in DMI was influenced by the management of initial feeding and feeding strategy thereafter. Pens fed the COMP diet were initiated onto feed at 1.75% of pen BW on a DM basis whereas TMR pens were initiated onto feed at 1.50% of pen BW on a DM basis. Thereafter, COMP bulls were stepped onto feed more aggressively compared to TMR. Therefore, management strategy offers a partial explanation for the differences ( $P < 0.01$ ) in DMI between d 1 and 21 for bulls fed the COMP and TMR (1.93 vs. 1.51% of BW, respectively). Additionally, the difference in feeding

management likely also influenced DMI for bulls fed the COMP and TMR between d 22 and 42 (2.37 vs 2.08% of BW, respectively;  $P < 0.01$ ) and d 43 to 63 (2.40 vs 2.28% of BW;  $P < 0.05$ ). Differences in physical form between the COMP and TMR may have also influenced DMI, as the COMP ration had approximately 4-fold greater bulk density than the TMR (observational data that was not statistically evaluated). Therefore, differences in bulk density, and thus gut fill, likely also contributed to differences in DMI between bulls fed COMP and TMR. Additionally, a drastic increase in digestive morbidity and subsequent change in bunk management that occurred beginning on d 63 likely drove similarities between DMI for COMP- and TMR-fed bulls (2.20 vs. 2.14% of BW, respectively;  $P = 0.42$ ) between d 64 and 84. Although DMI was similar when expressed as a percentage of BW, the amount of DMI ( $\text{kg} \times \text{day}^{-1}$ ) differed between d 64 and 84 for COMP-fed and TMR-fed bulls. We hypothesize that the difference was likely driven by BW, as COMP-fed bulls were 22 kg heavier on d 63 and approximately 30 kg heavier on d 84 when compared to TMR-fed bulls, and thus likely able to consume more feed. Therefore, it is likely that DMI was influenced as a function of bulk density of the rations, feeding management, and BW as the feeding trial progressed. Diet influenced the likelihood that bulls would experience digestive morbidity ( $X^2 = 5.93$ ;  $P < 0.05$ ). As shown in Figure 2.1, bulls fed the COMP ration were approximately 10-times more likely than bulls fed the TMR to experience digestive morbidity, and thus a decrease in DM offering. Nonetheless, this increased incidence of digestive morbidity appeared to decrease average daily DMI of COMP-fed bulls between d 64 and 84, but may be of further interest and warrant further investigation regarding the influence of feed form on digestive distress and overall DMI.

Figure 2.2 outlines average daily DMI. While there was slight variation in DMI, COMP-fed bulls had or tended to have greater DMI than TMR-fed bulls for a total of 64 d of the 84-d

experiment. Bulls fed the COMP ration were likely able to quickly adapt to the diet, effectively mitigating the negative affects generally associated with an acidogenic diet compositionally similar to the COMP (Brown et al., 2000). The forced inclusion of a high level of bulky ingredients that had a relatively low bulk density in the TMR likely limited DMI through rumen kinetics and gut fill. Yet, COMP-fed bulls consumed more rye hay than expected (Table 2.1; 7.63 vs. 4.75% of DMI for observed vs. expected, respectively) and may have consumed a sufficient amount of low quality roughage to slow digestion and limit drastic decreases in DMI from adverse rumen conditions. It is worthy of mentioning, based on the intake patterns outlined in Figure 2.2, it appears that it took approximately 42 d of the 84-d on feed for the TMR-fed bulls to completely transition onto the ration. However, during the final half of the experiment, COMP-fed bulls maintained a DMI that was on average 1-1.5 kg of DM greater per d. This further confirms the previously denoted theory that gut fill was likely the limiting factor in DMI for the TMR-fed bulls, and along with decreased BW compared throughout the trial, account for the reduction in DMI across time.

### **Growth performance and hoof conformation**

Least square means for un-shrunk BW and ADG can be found in Table 2.2. Un-shrunk BW was similar between COMP- and TMR-fed bulls on d 21 (Table 2.2;  $P = 0.76$ ) and 42 ( $P = 0.10$ ), but COMP-fed bulls had a greater BW than TMR-fed bulls on d 63 (526.3 vs 504.3 kg;  $P < 0.01$ ) and 84 (583.3 vs 553.6 kg;  $P < 0.01$ ). Similarly, ADG did not differ between COMP- and TMR-fed bulls between d 1 and 21 ( $P = 0.76$ ), but differed throughout the remainder of the trial between d 22 and 42 (2.51 vs 2.08 kg x day<sup>-1</sup>;  $P < 0.05$ ), d 43 and 63 (2.51 vs 1.91 kg x day<sup>-1</sup>;  $P < 0.01$ ), and d 64 and 84 (2.72 vs 2.35 kg x day<sup>-1</sup>;  $P < 0.05$ ). Therefore, total ADG over the entire 84-d trial was greater ( $P < 0.01$ ) for COMP (2.46 kg x day<sup>-1</sup>) compared to TMR (2.13 kg x day<sup>-1</sup>)

<sup>1</sup>). Observed increases in ADG are likely a direct result of elevated DMI in COMP-fed bulls compared to TMR-fed bulls.

Diets capable of inducing rumen conditions indicative of SARA are often considered to be responsible for cyclical patterns of DMI that decrease overall DMI, and thus decrease feed efficiency in cattle (Britton and Stock, 1987; Nocek, 1997; Gozho et al., 2005). Bulls fed the COMP ration were managed using a relatively aggressive feeding management program, and it was expected that DMI and ADG would be more variable than what was observed in the present experiment. Nonetheless, TMR-fed bulls had greater feed efficiency when compared to COMP-fed bulls between d 1 and 21 (0.326 vs 0.260 kg of BW x kg of DM<sup>-1</sup>;  $P < 0.01$ ), and were less efficient between d 43 and 63 (0.199 vs. 0.167 kg of BW x kg of DM<sup>-1</sup> for TMR vs. COMP, respectively;  $P < 0.05$ ), but did not differ between d 22 and 42 ( $P = 0.39$ ), d 64 and 84 ( $P = 0.13$ ), or when evaluated across the entire 84-d trial ( $P = 0.19$ ). Variation in feed efficiency may also be attributed to rumen kinetics and gut fill. Bulls fed the TMR ration likely did not reach ruminal capacity consuming the bulkier TMR ration between d 1 and 21, yet the physical capacity of the rumen may have been reached following the first 21-d interval. Thus, as intake was limited from gut fill, increases in ADG and BW were limited by rumen kinetics and offers potential insight into the variation in feed efficiency presented herein.

Elevated DMI and increased ADG for bulls fed the COMP ration offer an explanation for a 0.25-cm increase in SFT on d 84 that was observed in comparison to bulls fed the TMR (Figure 2.3;  $P < 0.01$ ). While increases in adiposity and intake were observed for COMP-fed bulls, it was not to the extent to alter SC ( $P = 0.45$ ), hoof angle (Figure 2.4;  $P = 0.63$ ), or claw set (Figure 2.5;  $P = 0.54$ ) following the 84-d feeding period. Hoof and leg problems, especially laminitis, have commonly been characterized as a consequence of high inclusion of starch and energy in the

diet, and are often associated with SARA (Nocek, 1997). In contrast, the observations of the present experiment were that neither diet nor energy level within each diet impacted hoof conformation. Although scrotal adiposity was not directly assessed in this experiment, it can be assumed that scrotal adiposity did not differ between dietary treatments, primarily due to the lack of a difference in SC. Further research is warranted to quantify the optimal degree of SFT to ensure bulls are neither over- nor under-conditioned for a breeding season and to maximize reproductive parameters.

### **Rumen pH and temperature**

In total, 47 of 48 boluses recorded rumen pH and temperature data, resulting in the possibility of 379,008 data observations for both rumen pH and temperature, of which 27.4% of rumen pH observations along with 33.7% of rumen temperature observations were missing. Of the functional boluses, 12.8% (6 boluses of 47 total) displayed rumen pH data that followed a pattern of linear drift, which resulted in the exclusion of 9.6% of the existing data observations. Additionally, 6.8% of the existing observations for rumen temperature were excluded as they were beyond the acceptable range adapted from Beatty et al. (2008). This resulted in a total of 251,104 rumen pH observations and 234,317 rumen temperature observations utilized in the analysis. Least square means for average rumen pH and temperature are reported in Table 2.3, and it was observed that average rumen pH was lower for COMP-fed bulls at all times ( $P < 0.01$ ). Average rumen pH was 0.17 units lower ( $P < 0.01$ ) for bulls fed the COMP ration when compared to those fed the TMR (6.05 vs. 6.22, respectively). Average rumen pH for COMP was 6.18 between d 1 and 21, 5.97 between d 22 and 42, 5.98 between d 43 and 63, and 6.06 between d 64 and 84. Compared to reports from SARA-affected animals, average rumen pH between d 22 and 42, d 43 and 63, and d 64 and d 84 are similar to previous reports of 5.84 (AlZahal et al.,



2008), 5.94 (Colman et al., 2010), and 5.97 (Khafipour et al., 2009). Thus, it begins to confirm the dietary influence of the COMP diet on rumen pH values indicative of SARA. Furthermore, it may offer insight into the impact that diets compositionally lower in roughage have on DMI and subsequent rumen pH. However, to maintain consistency within the literature, and to provide a better indication of dietary influence on rumen conditions, rumen pH should be quantified under specific pH thresholds (Kleen et al., 2003; Krause and Oetzel, 2006).

Rumen pH is the most accurate and effective means of identifying SARA in cattle, but average rumen pH is rarely utilized as an objective indicator generally due to the influence of diurnal variation on the estimate (Keunen et al., 2002). Figure 2.6 outlines average rumen pH by h, allowing for an objective evaluation of fermentation patterns and subsequent changes to rumen pH. Pens were fed within 0015 of 0715 h, which led to the initial rumen pH depression at 0700 h for both COMP- and TMR-fed bulls. Rumen pH was higher ( $P < 0.05$ ) for TMR-fed bulls at 0800 and 0900 h, but then similar ( $P > 0.05$ ) from 1000 to 1600h, which can be assumed to be the peak fermentation time for bulls, regardless of dietary treatment. Yet, at 1600 h, bulls fed the TMR experienced rumen pH increases which peaked just before feeding at 0700 h, much like fermentation patterns outlined by Keunen et al. (2002). However, COMP-fed bulls maintained a relatively low rumen pH until 1900, in which a gradual rise in pH was observed until feeding at 0700 h. This slow return of rumen pH in COMP-fed bulls led to decreases ( $P < 0.05$ ) in average rumen pH from 17 to 23 h, through 1 h ( $P < 0.10$ ) the following d. It is likely that rumen pH was lower in COMP-fed compared to TMR-fed bulls as a function of increased DMI and thus elicited greater levels of fermentation to facilitate lower rumen pH.

Objective measures of rumen temperature are uncommon in SARA-related research. Yet, as characterized by AlZahal et al. (2008), rumen temperatures above 39.0 and 39.2° correspond

with a rumen pH indicative of SARA, and average rumen temperature for dairy cows within their study was 0.67°C higher for SARA compared to control cows, respectively. Additionally, AlZahal et al. (2008) found a strong negative relationship between rumen pH and temperature. Comparatively, rumen temperatures observed in the present experiment were higher for both COMP and TMR (39.7 vs. 39.5°C, respectively;  $P = 0.12$ ) than the findings of AlZahal et al. (2008), with a very weak ( $R^2 = 0.01$ ;  $P < 0.01$ ) relationship between rumen pH and temperature. Thus, the findings of the present experiment are inconsistent with those of AlZahal et al. (2008), as rumen temperature did not serve as a reliable predictor of rumen pH, and had little utility as a descriptor of rumen conditions indicative of SARA.

Reports of rumen pH thresholds as indicators of SARA vary throughout the literature, and have been reported as 5.8 (Beauchemin et al., 2003), 5.6 (Cooper et al., 1999; Colman et al., 2010), and 5.5 (Kleen et al., 2003; Krause and Oetzel, 2006). While these estimates are variable, they offer the opportunity to quantify various pH thresholds and identify the threshold that is best suited for a particular group of cattle managed under a particular set of conditions. Table 2.4 outlines the model-adjusted probabilities for COMP or TMR to display pH below each assigned threshold of 6.0, 5.8, 5.5, 5.2, and 5.0, and outlines calculated odds ratios. Compared to TMR, rumen pH of bulls fed the COMP ration were 2.1-, 2.0-, 2.2-, 3.6-times more likely ( $P < 0.01$ ) to be below the rumen pH threshold of 6.0 between d 1 and 21, d 22 and 42, d 43 and 63, and d 64 and 84, respectively, and 2.2-times more likely ( $P < 0.01$ ) at any time point throughout the trial. Additionally, the odds for rumen pH below 5.8 were increased ( $P < 0.05$ ) for COMP-fed compared to TMR-fed bulls as they were 2.7-, 2.2-, 2.1-, and tended ( $P < 0.10$ ) to be 2.8-times more likely to display rumen pH below threshold between d 1 and 21, d 22 and 42, d 43 and 63, and d 64 and 84, respectively, and 2.5-times more likely ( $P < 0.01$ ) at any time point during the

feeding period. Ruminant pH threshold values of 6.0 and 5.8 were utilized based on findings of Hoover (1986) and Russell and Wilson (1996) which suggest rumen pH below 6.0 limits fiber digestion as a result of decreased proliferation rates of fibrolytic bacteria. Additionally, Beauchemin et al. (2003) noted the incidence of SARA increases when ruminal pH falls below 5.8 as the decrease in microbial proliferation and digestibility of fiber is exacerbated compared to pH 6.0. Based on previous data, it may be expected that increased probability of rumen pH below 6.0 or 5.8 may be indicative of decreases in fiber digestibility and DMI. Yet, given the rumen conditions and consistent DMI in this study, reduced fiber digestion as a function of decreased proliferation rates of fibrolytic bacteria did not seem to be a limiting factor in DMI within the current study.

Compared to TMR, rumen pH of bulls fed the COMP ration were 4.1-, 2.4-, 2.7-, and 4.2-times greater ( $P < 0.05$ ) to display a rumen pH below the rumen pH threshold of 5.5 between d 1 and 21, d 22 and 42, d 43 and 63, and d 64 and 84, respectively and 3.0-times more likely ( $P < 0.01$ ) at any time point throughout the trial. Similarly, bulls fed the COMP were 5.2-, 1.9-, 4.9-, and 19.6-times greater ( $P < 0.05$ ) to display a rumen pH below the rumen pH threshold of 5.2 between d 1 and 21, d 22 and 42, d 43 and 63, and d 64 and 84, respectively and 4.4-times more likely ( $P < 0.01$ ) at any time point throughout the trial. Rumen pH of 5.6 and 5.5 are often used interchangeably as a threshold for SARA, and seems to be a function of acidification of the rumen based on hydrogen sources. It is speculated that rumen pH 5.5 is driven by dissociation of VFA as a source of hydrogen ions to decrease rumen pH, and the contribution of hydrogen ions from lactate dissociation does not seem to influence rumen pH at this point. This is supported by Krause and Oetzel (2006) based on ionization rates and known  $pK_a$  values of VFA (4.8) and lactate (3.9). Yet, as rumen pH declines, a shift occurs from VFA to lactate as the predominate

source of hydrogen ions for acidification. When rumen pH falls to 5.0, lactate is 5.2-times less dissociated than VFA (Krause and Oetzel, 2006). Although lactate levels have been variable across research (Hungate, 1975; Hibbard et al., 1995; Owens et al., 1998), it seems to play a role in acute forms of acidosis and possibly low pH points associated with SARA. This idea is further supported by proliferation rates of *Streptococcus bovis*, which are lactate-producing bacteria, and have been found to nearly double in concentration as acidification of the rumen occurs (Russell and Hino, 1985; McAllister et al., 1990) as a function of increased levels of fermentable carbohydrates in the diet (Nagaraja and Titgemeyer, 2007). Therefore, early stages of SARA are likely driven by VFA dissociation, with pH decline further exacerbated by dissociation of lactate. Thus, it appears that as rumen pH decreases, the influence of hydrogenation in the rumen from lactate increases, leading to the evaluation of pH threshold of 5.2 as a metric of SARA. Lastly, a threshold of more acute stages of acidosis at pH 5.0 was previously described by Owens et al. (1998). Compared to TMR, rumen pH of bulls fed the COMP ration were 6.4-, 2.9-, 5.9-, and 84.5-times greater ( $P < 0.01$ ) to display a rumen pH below the rumen pH threshold of 5.0 between d 1 and 21, d 22 and 42, d 43 and 63, and d 64 and 84, respectively, and 6.7-times more likely ( $P < 0.01$ ) at any time point throughout the trial.

To our knowledge, this is the first objective evaluation of rumen conditions utilizing model-adjusted probabilities and subsequent odds ratios as a metric to define rumen conditions or acidosis. Given the 15-min intervals between data collection of the rumen boluses, probabilities and odds ratios provide the most accurate objective measures of rumen conditions without over- or under-estimating times spent above or below a particular threshold. Interestingly, bulls fed the COMP ration had or tended to have greater odds of displaying pH below each of the five rumen pH thresholds during each of the 21-d intervals and throughout the

duration of the 84-d feeding period. It is concluded from this data that the COMP ration increased the risk of rumen pH dropping below any of the aforementioned thresholds that are indicative of SARA or more acute forms of ruminal acidosis in cattle. Although this research does not explicitly denote SARA based on time below pH thresholds, it offers insight into heightened risk of the COMP and compositionally-similar rations to elicit SARA. Interestingly, the probabilities were greater for pH below specific thresholds for COMP, yet it did not hinder DMI within 21-d intervals, given the statistical increases in DMI throughout the trial. High-performing cattle such as lactating dairy cattle, feedlot and finishing cattle, and performance-tested bulls often experience pH below 6.0 (Russell and Wilson, 1996; Keunen et al., 2002), yet rumen conditions may rapidly adjust to prevent the negative physiologic repercussions generally associated with SARA. It is determined from these data that although COMP increases the probability for pH below specific thresholds associated with SARA, the impact was not to the degree to affect DMI and ADG in these cattle. Further research may be warranted to evaluate relationships between SARA, or rumen conditions indicative of SARA, and performance metrics in cattle. These data suggest that lower rumen pH does not decrease DMI, ADG, or adiposity and has limited repercussions on skeletal and structural integrity of developing bulls within the bounds of this experiment.

### **Semen quality**

Least square means for percent progressive motility, semen collection volume, percentage morphologically-normal spermatozoa, as well as the number of spermatozoa x ejaculate<sup>-1</sup>, and the number of morphologically-normal and-abnormal spermatozoa x ejaculate<sup>-1</sup> can be found in Table 2.5. Given the similar ( $P = 0.45$ ) SC observed between COMP-fed and TMR-fed bulls, scrotal mass and adiposity was not expected to influence semen quality parameters.

Ration did not affect percentage progressive motility on d 21 ( $P = 0.15$ ), d 42 ( $P = 0.26$ ), d 63 ( $P = 0.49$ ), d 84 ( $P = 0.25$ ), or throughout the full trial ( $P = 0.34$ ). Independently of ration, day of experiment affected (Table 2.6) percentage progressive motility as d 42 (56.6%) differed ( $P < 0.05$ ) from d 84 percentage progressive motility (44.9%), but neither differed ( $P > 0.05$ ) from d 21 (50.4%) and d 63 (50.1%). There is little objective evidence to support an increase in percentage progressive motility over time, and a continuous numerical decrease in progressive motility was observed throughout the feeding period in the current experiment. As described by Palmer et al. (2005), progressive motility is a subjective measurement that is highly variable across semen samples and technicians. As the feeding trial progressed, ambient temperatures declined, and while measures were taken to prevent sample chilling, there is the possibility that percentage progressive motility may have been affected prior to analysis. Additionally, semen volume accounted for a small portion of the variation ( $R^2 = 0.03$ ;  $P < 0.05$ ) in progressive motility. Although a weak relationship was observed, it brings about question the additional influence fluid dynamics and substrates within the seminal fluid may have on percentage progressive motility of spermatozoa. While the influence of such factors were not assessed in the current study, they merit further investigation.

As shown in Table 2.7, the proportion of rumen pH observations below a threshold of pH 5.8 between d 1 and 21 tended to be directly related to percentage progressive motility on d 21 ( $R^2 = 0.08$ ;  $P < 0.10$ ) and d 63 ( $R^2 = 0.08$ ;  $P < 0.10$ ), but not d 42 ( $P = 0.73$ ) or 84 ( $P = 0.12$ ). The proportion of rumen pH observations below a threshold of pH 5.2 between d 1 to 21 was directly related to percent progressively motile sperm on d 84 ( $R^2 = 0.16$ ;  $P < 0.05$ ), but no other time points ( $P \geq 0.29$ ). Additionally, the proportion of rumen pH observations below a threshold of pH 5.2 between d 22 to 42 was inversely related to percent progressively motile sperm on d 42

( $R^2 = 0.10$ ;  $P < 0.05$ ), but no other time points ( $P \geq 0.27$ ). The proportion of rumen pH observations below thresholds of 5.2 or 5.8 in the remaining 21-d intervals were not related to progressive motility within or across any other subsequent semen collection days ( $P \geq 0.32$ ). Moreover, average rumen pH and temperature, along with changes in SC and SFT throughout the trial were unrelated to progressive motility on any of the collection days (Table 2.8;  $P \geq 0.35$ ) as well as the change in progressive motility across the trial (Table 2.9;  $P \geq 0.17$ ). Given the few weak relationships that were observed between the proportion of rumen pH observations below assigned thresholds, and the inability to identify discernable trends, it is denoted that rumen conditions should not be expected to influence progressive motility.

Feeding management strategy did not affect semen volume on d 21 (Table 2.5;  $P = 0.52$ ) or d 42 ( $P = 0.74$ ). Yet, there tended to be a 34% increase in semen volume for COMP-fed compared to TMR-fed bulls on d 63 ( $P < 0.10$ ), and there was a 48% increase in semen volume for COMP-fed bulls on d 84 ( $P < 0.05$ ). Day of experiment had an effect ( $P < 0.01$ ) on semen volume as d 84 semen volume (8.9 mL) was greater than d 21 (4.2 mL), 42 (6.2 mL), and 63 (5.8 mL), although d 21, 42, and 63 semen volumes did not differ from one another ( $P > 0.05$ ). The exact reason for the increase in semen volume for COMP-fed bulls is not fully understood, but may be a function of greater DMI of carbohydrates available for ruminal digestion in the COMP ration. In rams, diet energy density has been found to influence semen volume (Tilton et al., 1964). Based on increases in adiposity and the elevated probability that COMP bulls would display rumen pH below various thresholds indicative of SARA, it can be assumed that the amount of ruminally digested carbohydrates was greater in COMP-fed bulls as opposed to TMR-fed bulls. The full trial change in SFT was directly related to semen volume from d 0 to 84 ( $R^2 = 0.06$ ;  $P < 0.01$ ) and the change in semen volume from d 0 to 84 ( $R^2 = 0.10$ ;  $P < 0.05$ ). Although

weak relationships, it offers support for the notion that the amount of ruminally digested carbohydrates was elevated in bulls fed the COMP ration, which also increased SFT. Further support is provided by average daily pH values (Figure 2.7), in which the average rumen pH of COMP-fed bulls was lower ( $P < 0.05$ ) than TMR-fed bulls from d 46 to 50 and on d 52, and tended ( $P < 0.10$ ) to be lower on d 55 and 56, suggesting greater amounts of carbohydrate digestion in the days leading up to semen collection on d 63. Similar trends were found prior to d 84 semen collection as COMP-fed bulls had lower ( $P \leq 0.05$ ) average rumen pH between d 75 and 83, and tended ( $P \leq 0.10$ ) to be lower on d 73, 74, and 84. Additionally, there tended to be a direct relationship between average rumen pH and the change in semen volume between d 0 and 84 ( $R^2 = 0.07$ ;  $P < 0.10$ ). Moreover, the proportion of rumen pH observations below a threshold of pH 5.8 between d 1 and 21 tended to be directly related to semen volume on d 63 ( $R^2 = 0.09$ ;  $P < 0.10$ ), and the proportion of rumen pH observations below a threshold of pH 5.2 between d 1 and 21 was directly related to semen volume on d 63 ( $R^2 = 0.16$ ;  $P < 0.05$ ). Additionally, the proportion of rumen pH observations below a threshold pH 5.8 between d 22 and 42 was directly related to semen volume on d 63 ( $R^2 = 0.11$ ;  $P < 0.05$ ), yet no other threshold pH value had a relationship within or between subsequent collection days (Table 2.7;  $P \geq 0.11$ ). Together, these data suggest that factors that increase SFT and lower rumen pH may have been responsible for the effects of ration form on semen collection volume.

Carbohydrate digestion in the rumen and small intestine facilitates glucose absorption through the small intestine and glucose synthesis in the liver. It was observed in a challenge conducted by Khafipour et al. (2009) that SARA significantly increased blood glucose levels. It should also be noted that propionate serves as the major gluconeogenic substrate in ruminants, and has been shown to be the only volatile fatty acid to increase in ruminal concentration below



a rumen pH of 5.5 (Stock, 2000). Glucose is a key metabolite in the seminal vesicles, as it is interconverted to fructose and serves as the main energy substrate for spermatozoa in semen (Mann, 1974; Prendergast and Veneziale, 1975). Fructose has been positively correlated with semen volume in the man (Harvey, 1948), and increased fructose has been proposed to increase the secretion of seminal fluid by the seminal vesicles in the bull (Branton et al., 1952), likely from increases in osmolality (Mann, 1974). Additionally, the seminal vesicles contribute the highest volume of fluid into the ejaculate (Austin et al., 1961). From the data presented herein, we postulate that the COMP diet may have augmented carbohydrate digestion and led to higher concentrations of blood glucose, subsequently enhancing substrate availability for conversion of glucose to fructose in the seminal vesicles. It is not fully understood as to why semen volume was similar on d 21 and 42 but greater on d 63 and 84 for COMP bulls but may be a function of age (Hahn et al., 1969a) and the amount of ruminal carbohydrate digestion. Leading up to semen collection on d 21, COMP bulls had a lower ( $P \leq 0.05$ ) average rumen pH on d 1 and 3 and tended ( $P \leq 0.10$ ) to be different on d 2 with no other significantly different time points, leading to the speculation that carbohydrate digestion and thus blood glucose was likely similar throughout that 21-d interval. There were several time points leading up to the d-42 semen collection in which average rumen pH was lower for COMP-fed bulls. The difference in average rumen pH between d 22 and 42 was 0.15 pH units lower for COMP-fed compared to TMR-bulls, yet was 0.20 pH units lower between d 43 and 63, and 0.37 pH units lower between d 64 and 84 for COMP-fed bulls. This suggests that variation in the amount of carbohydrate digestion in the rumen and thus rumen pH may not have been to the degree for COMP-fed and TMR-fed bulls between d 22 and 42 to alter blood glucose and subsequently semen volume. Although the

specific reason for similar semen volume is not known, it may be related to nutrient utilization and allocation of metabolites away from certain reproductive functions in growing bulls.

As shown in Table 2.5, percentage morphologically-normal spermatozoa was similar for COMP- and TMR-fed bulls on d 21 ( $P = 0.58$ ), d 42 ( $P = 0.76$ ), d 63 ( $P = 0.62$ ), d 84 ( $P = 0.78$ ), and throughout the entire feeding period ( $P = 0.34$ ). However, d of collection had an independent effect on percentage morphologically-normal spermatozoa as d 84 percentage morphologically-normal spermatozoa (64.3%) differed ( $P < 0.05$ ) from d 21 percentage morphologically-normal spermatozoa (54.8%) but neither differed ( $P > 0.05$ ) from d 42 (58.5%) or d 63 (61.5%) percentage morphologically-normal spermatozoa values. The proportion of rumen pH observations below pH thresholds was unrelated to the percentage of morphologically-normal spermatozoa (Table 2.7;  $P \geq 0.18$ ). Similarly, average rumen pH, average rumen temperature, and the change in SFT from d 0 to 84 were each unrelated to the percentage of morphologically-normal spermatozoa between d 0 and 84 ( $P \geq 0.23$ ) or change in the percentage of morphologically-normal spermatozoa from d 0 to 84 ( $P \geq 0.10$ ). However, change in SC from d 0 to 84 was indirectly related to morphologically-normal spermatozoa between d 0 and 84 ( $R^2 = 0.07$ ;  $P < 0.01$ ) but directly related to the change in percentage morphologically-normal spermatozoa from d 0 to 84 ( $R^2 = 0.12$ ;  $P < 0.05$ ). Bulls with larger SC are more likely to produce more than 70% morphologically-normal spermatozoa (Coe, 1999). Based on these data, it supports the notion that bulls must attain puberty earlier but also have sufficient scrotal growth to achieve semen quality worthy of passing a BSE (Barth, 2008). Attainment of puberty was not evaluated in this study, but these data support the notion that bulls must grow and mature to a certain degree before semen quality as a whole begins to approach the standards to pass a BSE. Following the 84-d feeding trial, there was approximately a 10% increase in percentage

morphologically-normal spermatozoa, at approximately 64%. The linear increase in percentage morphologically-normal spermatozoa suggests that semen quality increases with age, as percentage morphologically-normal spermatozoa approached the threshold set forth by (Koziol and Armstrong, 2018), and supports claims that semen quality improves with both age and frequency of ejaculation (Hahn et al., 1969a; Foote et al., 1977; Brito et al., 2002). In the present experiment, changes in SC, but not adiposity (measured as SFT), rumen conditions, or diet, influenced the development of morphological abnormalities in spermatozoa.

Total spermatozoa x ejaculate<sup>-1</sup> was similar between COMP and TMR on d 21 ( $P = 0.31$ ), d 42 ( $P = 0.43$ ), d 63 ( $P = 0.55$ ), d 84 ( $P = 0.72$ ), and throughout the feeding period ( $P = 0.81$ ). However, there was an effect of day of experiment (Table 2.6), as d 21 total spermatozoa x ejaculate<sup>-1</sup> ( $1.88 \times 10^9$  x ejaculate<sup>-1</sup>) differed from d 84 total spermatozoa x ejaculate<sup>-1</sup> ( $3.42 \times 10^9$  x ejaculate<sup>-1</sup>;  $P < 0.05$ ), yet neither differed ( $P < 0.05$ ) from d 42 ( $2.64 \times 10^9$  x ejaculate<sup>-1</sup>) or d 63 ( $2.80 \times 10^9$  x ejaculate<sup>-1</sup>). Previous research has indicated an increase in total spermatozoa x ejaculate<sup>-1</sup> as bulls age (Hahn et al., 1969; Foote et al., 1977; Brito et al., 2002), and there is a strong positive correlation between SC and spermatozoa output (Hahn et al., 1969b). There was no relationship between the proportion of rumen pH observations below threshold pH values and total spermatozoa x ejaculate<sup>-1</sup> at any time point (Table 2.10;  $P \geq 0.12$ ) in the present experiment. There tended to be a direct relationship between average rumen pH and changes in total spermatozoa x ejaculate<sup>-1</sup> between d 0 and 84 ( $R^2 = 0.07$ ;  $P < 0.10$ ). Additionally, average rumen temperature was unrelated to both total spermatozoa x ejaculate<sup>-1</sup> between d 0 and 84 (Table 2.11;  $P = 0.75$ ) and the change in total spermatozoa x ejaculate<sup>-1</sup> from d 0 to 84 (Table 2.12;  $P = 0.87$ ). However, there tended to be an inverse relationship between change in SC from d 0 to 84 and total spermatozoa x ejaculate<sup>-1</sup> between d 0 and 84 ( $R^2 = 0.02$ ;  $P < 0.10$ ) but not change in

total spermatozoa x ejaculate<sup>-1</sup> from d 0 to 84 ( $P = 0.56$ ). There was a direct relationship between change in SFT from d 0 to 84 and both total spermatozoa x ejaculate<sup>-1</sup> between d 0 and 84 ( $R^2 = 0.05$ ;  $P < 0.01$ ) and change in spermatozoa x ejaculate<sup>-1</sup> from d 0 to 84 ( $R^2 = 0.12$ ;  $P < 0.05$ ). Interestingly, diet and rumen conditions had minimal influence on spermatozoa production in these bulls, yet changes in SFT may have influenced spermatozoa production, although relationships in this study were weak. Total spermatozoa production was greater when SFT was increased, and although often associated to be a negative relationship (Mwansa and Makarechian, 1991; Barth, 2008), these data show that SFT may be beneficial to spermatozoa production. There is little published evidence that establishes a link between SFT and total spermatozoa x ejaculate<sup>-1</sup>, or general increases in semen quality. Nonetheless, since SFT is an indicator of overall energy reserves, these reserves, or overall nutrient status may influence the availability of nutrients for spermatozoa production, although the specific mechanism responsible for these effects has yet to be identified (Coulter, 1994). Although the relationship between change in SC from d 0 to 84 and spermatozoa x ejaculate<sup>-1</sup> was weak, the observed numerical increases in SC between d 0 and 84 correspond with increases in spermatozoa x ejaculate<sup>-1</sup> between d 0 and 84, and support the notion that increases in SC correspond with increases in spermatozoa x ejaculate<sup>-1</sup> (Hahn et al., 1969a).

Total morphologically-normal spermatozoa x ejaculate<sup>-1</sup> was unaffected by diet on d 21 ( $P = 0.29$ ), d 42 ( $P = 0.48$ ), d 63 ( $P = 0.82$ ), d 84 ( $P = 0.76$ ), and throughout the duration of the trial ( $P = 0.32$ ). Yet, there was a day of experiment effect with d 84 ( $2.20 \times 10^9$  x ejaculate<sup>-1</sup>) total morphologically-normal spermatozoa x ejaculate<sup>-1</sup> differing ( $P < 0.05$ ) from d 21 ( $1.02 \times 10^9$  x ejaculate<sup>-1</sup>), although neither were different ( $P < 0.05$ ) from d 42 ( $1.54 \times 10^9$  x ejaculate<sup>-1</sup>) or d 63 ( $1.72 \times 10^9$  x ejaculate<sup>-1</sup>). As expected, increases in total spermatozoa x ejaculate<sup>-1</sup>, along

with increases in percentage morphologically-normal spermatozoa, led to increases in the total number of morphologically-normal spermatozoa x ejaculate<sup>-1</sup> as the experiment progressed. Yet, there was no effect of diet on total normal spermatozoa x ejaculate<sup>-1</sup> and the proportion of rumen pH observations below specific pH thresholds was unrelated to the total number of morphologically-normal spermatozoa x ejaculate<sup>-1</sup> (Table 2.10;  $P \geq 0.30$ ). Average rumen pH and average rumen temperature were not related to the total number of morphologically-normal spermatozoa x ejaculate<sup>-1</sup> between d 0 and 84 (Table 2.11;  $P \geq 0.58$ ) or the change in total number of morphologically-normal spermatozoa x ejaculate<sup>-1</sup> from d 0 to 84 (Table 2.12;  $P \geq 0.10$ ). Similar to the total number of spermatozoa x ejaculate<sup>-1</sup>, there was an inverse relationship between change in SC between d 0 and 84 and the total number of morphologically-normal spermatozoa x ejaculate<sup>-1</sup> between d 0 and 84 ( $R^2 = 0.04$ ;  $P < 0.01$ ) but not change in the total number of morphologically-normal spermatozoa x ejaculate<sup>-1</sup> from d 0 to 84 ( $R^2 < 0.01$ ;  $P = 0.52$ ). Additionally, there tended to be a direct relationship between the change in SFT between d 0 and 84 and the total number of morphologically-normal spermatozoa x ejaculate<sup>-1</sup> between d 0 and 84 ( $R^2 = 0.02$ ;  $P < 0.10$ ), but not change in the total number of morphologically-normal spermatozoa x ejaculate<sup>-1</sup> from d 0 to 84 ( $P = 0.61$ ). Given the similar relationships and continual numeric increase similar to total spermatozoa x ejaculate<sup>-1</sup>, it seems that an increase in total spermatozoa led to a continued increase in the total number of morphologically-normal spermatozoa x ejaculate<sup>-1</sup>. These data suggest that neither diet nor ruminal conditions were detrimental to the number of morphologically-normal spermatozoa delivered through an ejaculate. Similar results were observed for the total number of morphologically-abnormal spermatozoa x ejaculate<sup>-1</sup> as diet did not affect d 21 ( $P = 0.71$ ), d 42 ( $P = 0.64$ ), d 63 ( $P = 0.25$ ), d 84 ( $P = 0.32$ ), or the total number of morphologically-abnormal spermatozoa x ejaculate<sup>-1</sup>

throughout the full trial ( $P = 0.21$ ). However, there was an effect of day of experiment, leading to differences ( $P < 0.05$ ) between d 84 ( $1.22 \times 10^9 \times \text{ejaculate}^{-1}$ ) and d 21 ( $0.85 \times 10^9 \times \text{ejaculate}^{-1}$ ) total morphologically-abnormal spermatozoa  $\times \text{ejaculate}^{-1}$ , yet neither differed ( $P > 0.05$ ) from d 42 ( $1.10 \times 10^9 \times \text{ejaculate}^{-1}$ ) or d 63 ( $1.08 \times 10^9 \times \text{ejaculate}^{-1}$ ). Although the proportion of rumen pH observations below a threshold pH of 5.8 between d 22 and 42 tended to be related to the total number of morphologically-abnormal spermatozoa  $\times \text{ejaculate}^{-1}$  on d 63 ( $R^2 = 0.10$ ;  $P < 0.10$ ) there were no other significant relationships ( $P \geq 0.13$ ), and likely has little biological relevance. Moreover, neither average rumen pH, average rumen temperature, nor changes in SC were related to the total number of morphologically-abnormal spermatozoa  $\times \text{ejaculate}^{-1}$  between d 0 and 84 ( $P \geq 0.70$ ) or change in the total number of morphologically-abnormal spermatozoa  $\times \text{ejaculate}^{-1}$  between d 0 and 84 ( $P \geq 0.42$ ). Yet, there was a direct relationship between changes in SFT and the total number of morphologically-abnormal spermatozoa  $\times \text{ejaculate}^{-1}$  between d 0 and 84 ( $R^2 = 0.07$ ;  $P < 0.01$ ) and change in the total number of morphologically-abnormal spermatozoa  $\times \text{ejaculate}^{-1}$  from d 0 to 84 ( $R^2 = 0.29$ ;  $P < 0.01$ ). It seems that change in SFT between d 0 and 84 share a common relationship with increases in the total number of spermatozoa  $\times \text{ejaculate}^{-1}$ , total number of morphologically-normal spermatozoa  $\times \text{ejaculate}^{-1}$ , and the total number of morphologically-abnormal spermatozoa  $\times \text{ejaculate}^{-1}$ . Backfat thickness was previously thought to negatively influence semen quality (Coulter and Kozub, 1989), likely due to hormonal signaling or scrotal adiposity. Yet given the similar SC of bulls fed the TMR and COMP in the current study, scrotal adiposity is an improbable cause and potential hormonal mechanisms are not well defined at this time. While there may be an adipose-related hormonal mechanism that increases the total number of morphologically-abnormal spermatozoa  $\times \text{ejaculate}^{-1}$ , it appears to be independent of diet or rumen conditions. Backfat seems to be related

to the production of spermatozoa, and limited research in this area calls for further investigation of the influence, if any, of SFT on semen quality and testicular function.

Collectively, these data suggest that diet did not negatively impact semen quality in developing bulls, and it was found that diet did not influence the likelihood of BSE outcome ( $X^2 = 0.87$ ;  $P = 0.65$ ). Given the overall weak relationships and indiscernible trends between proportion of rumen pH observations below specific thresholds that serve as unique qualifiers for SARA and semen quality, it can be concluded that rumen conditions displayed by bulls in this study had minimal impact on semen quality. While a link between experimentally-induced SARA and reduced semen quality in bulls has been previously established (Callaghan et al., 2016), it seems that feeding strategies that naturally support SARA do not elicit similar reductions in semen quality. Thus, we can conclude that SARA, or rumen conditions similar to, do not reduce semen quality parameters in a real-world bull development scenario. Additional work has suggested that feeding high-energy diets to bulls reduces semen quality (Coulter and Kozub, 1984; Mwansa and Makarechian, 1991; Coulter et al., 1997), yet these experiments are difficult to compare to the current study as diet composition, nutrient profiles, and performance data were not well documented. The data presented herein refutes the argument that elevated energy intake decreases semen quality, as semen quality was improved throughout the duration of the feeding period. Semen volume increased in COMP-fed bulls on d 63 and 84, and while it is unknown as to whether high semen volume is advantageous, it warrants further investigation as increased semen volume may increase fructose availability for spermatozoa, which serves as the predominate energy substrate for the spermatozoon. There were weak relationships between changes in SFT and SC and semen quality parameters, and these results support that increases in SC and SFT may facilitate increases in semen quality over time. The influence of SC and SFT on

specific semen quality parameters should be further investigated as they could potentially be used as indicators of reproductive parameters in bulls.

## **Conclusions**

Bulls fed the COMP ration had greater DMI, ADG, and thus SFT following the 84-d feeding trial, which led to decreases in average rumen pH and greater probabilities of rumen pH to fall below thresholds associated with SARA when compared to bulls fed the TMR. Future research is warranted to advance the understanding of the relationship between organic acid production, such as volatile fatty acids and lactate, and the odds below each rumen pH threshold. Diet influenced the likelihood of digestive morbidity with a large portion of the bulls fed the COMP ration experiencing signs of digestive distress. Yet, neither COMP nor TMR diets negatively impacted hoof conformation or semen quality as a whole, and it was observed that the COMP diet elicited a greater semen volume on d 63 and 84, and may offer insight into the influence of amount of diet consumption on semen volume production. Greater research emphasis should be placed on the influence of semen volume on spermatozoa quality and viability, as possible increases in energy substrates within seminal fluid may prove to be beneficial. Additionally, influences of SFT and spermatozoa production merits further investigation given the relationships that were observed with the total number of spermatozoa x ejaculate<sup>-1</sup>, and total number of morphologically-normal and -abnormal spermatozoa x ejaculate<sup>-1</sup>. Additionally, diet did not influence the BSE outcomes when assessed against current standards. Given these results, it can be concluded that rumen conditions indicative of SARA did not negatively impact semen quality or growth performance in a real-world bull development scenario. Both diets, and those compositionally similar, seem to be suitable to maintain growth



and semen quality, however the COMP diet should be fed with caution given the increases in digestive morbidity, and the higher propensity for adiposity.

## References

- AlZahal, O., E. Kebreab, J. France, M. Froetschel, and B. W. McBride. 2008. Ruminal Temperature May Aid in the Detection of Subacute Ruminal Acidosis. *Journal of Dairy Science* 91(1):202-207. doi: <https://doi.org/10.3168/jds.2007-0535>
- Austin, J. W., E. Hupp, and R. Murphree. 1961. Comparison of quality of bull semen collected in the artificial vagina and by electroejaculation. *Journal of Dairy Science* 44(12):2292-2297. doi: [https://doi.org/10.3168/jds.S0022-0302\(61\)90057-1](https://doi.org/10.3168/jds.S0022-0302(61)90057-1)
- Barth, A. D. 2007. Evaluation of potential breeding soundness of the bull, Current therapy in large animal Theriogenology. Elsevier. p. 228-240.
- Barth, A. D., L.F.C Brito, J.P Kastelic. 2008. The effect of nutrition on sexual development of bulls. *Theriogenology* 70(3):485 - 494. doi: <https://doi.org/10.1016/j.theriogenology.2008.05.031>
- Beatty, D. T., A. Barnes, E. Taylor, and S. K. Maloney. 2008. Do changes in feed intake or ambient temperature cause changes in cattle rumen temperature relative to core temperature? *Journal of Thermal Biology* 33(1):12 - 19. doi: <https://doi.org/10.1016/j.jtherbio.2007.09.002>
- Beauchemin, K. A., W. Z. Yang, and L. M. Rode. 2003. Effects of Particle Size of Alfalfa-Based Dairy Cow Diets on Chewing Activity, Ruminal Fermentation, and Milk Production1. *Journal of Dairy Science* 86(2):630-643. doi: [https://doi.org/10.3168/jds.S0022-0302\(03\)73641-8](https://doi.org/10.3168/jds.S0022-0302(03)73641-8)
- Branton, C., G. D'Arensbourg, and J. Johnston. 1952. Semen production, fructose content of semen and fertility of dairy bulls as related to sexual excitement. *Journal of Dairy Science* 35(10):801-807. doi: [https://doi.org/10.3168/jds.S0022-0302\(52\)93759-4](https://doi.org/10.3168/jds.S0022-0302(52)93759-4)

- Brito, L., A. Silva, L. Rodrigues, F. Vieira, L. Deragon, and J. Kastelic. 2002. Effects of environmental factors, age and genotype on sperm production and semen quality in *Bos indicus* and *Bos taurus* AI bulls in Brazil. *Animal reproduction science* 70(3-4):181-190. doi: [https://doi.org/10.1016/S0378-4320\(02\)00009-X](https://doi.org/10.1016/S0378-4320(02)00009-X)
- Britton, R., and R. Stock. 1987. Acidosis, rate of starch digestion and intake. 0361-8722, Stillwater, OK.
- Brown, M. S., C. R. Krehbiel, M. L. Galyean, M. D. Remmenga, J. P. Peters, B. Hibbard, J. Robinson, and W. M. Moseley. 2000. Evaluation of models of acute and subacute acidosis on dry matter intake, ruminal fermentation, blood chemistry, and endocrine profiles of beef steers. *Journal of animal science* 78(12):3155-3168. doi: 10.2527/2000.78123155x
- Callaghan, M. J., P. McAuliffe, R. J. Rodgers, J. Hernandez-Medrano, and V. E. A. Perry. 2016. Subacute ruminal acidosis reduces sperm quality in beef bulls<sup>1</sup>. *Journal of Animal Science* 94(8):3215-3228. doi: 10.2527/jas.2015-0235
- Coe, P. H. 1999. Associations among age, scrotal circumference, and proportion of morphologically normal spermatozoa in young beef bulls during an initial breeding soundness examination. *Journal of the American Veterinary Medical Association* 214(11):1664-1667.
- Colman, E., W. Fokkink, M. Craninx, J. Newbold, B. De Baets, and V. Fievez. 2010. Effect of induction of subacute ruminal acidosis on milk fat profile and rumen parameters. *Journal of dairy science* 93(10):4759-4773. doi: <https://doi.org/10.3168/jds.2010-3158>

- Cooper, R. J., T. J. Klopfenstein, R. A. Stock, C. T. Milton, D. W. Herold, and J. C. Parrott. 1999. Effects of imposed feed intake variation on acidosis and performance of finishing steers. *Journal of animal science* 77(5):1093-1099. doi: 10.2527/1999.7751093x
- Coulter, G., and G. Kozub. 1984. Testicular development, epididymal sperm reserves and seminal quality in two-year-old Hereford and Angus bulls: Effects of two levels of dietary energy. *Journal of Animal Science* 59(2):432-440. doi: <https://doi.org/10.2527/jas1984.592432x>
- Coulter, G., and G. Kozub. 1989. Efficacy of methods used to test fertility of beef bulls used for multiple-sire breeding under range conditions. *Journal of Animal Science* 67(7):1757-1766. doi: <https://doi.org/10.2527/jas1989.6771757x>
- Coulter, G. H. 1994. Beef bull fertility: factors affecting seminal quality, *Factors Affecting Calf Crop*. CRC Press, Inc., Boca Raton, FL. p. 312-320.
- Coulter, G. H., R. B. Cook, and J. P. Kastelic. 1997. Effects of dietary energy on scrotal surface temperature, seminal quality, and sperm production in young beef bulls. *Journal of Animal Science* 75(4):1048-1052. doi: 10.2527/1997.7541048x
- FASS. 2010. Federation of Animal Science Societies: Guide for the Care and Use of Agricultural Animals in Research and Teaching.
- Foote, R., G. Seidel Jr, J. Hahn, W. Berndtson, and G. Coulter. 1977. Seminal quality, spermatozoal output, and testicular changes in growing Holstein bulls. *Journal of dairy science* 60(1):85-88. doi: [https://doi.org/10.3168/jds.S0022-0302\(77\)83832-0](https://doi.org/10.3168/jds.S0022-0302(77)83832-0)
- González, L. A., X. Manteca, S. Calsamiglia, K. S. Schwartzkopf-Genswein, and A. Ferret. 2012. Ruminant acidosis in feedlot cattle: Interplay between feed ingredients, rumen

- function and feeding behavior (a review). *Animal Feed Science and Technology* 172(1):66-79. doi: <https://doi.org/10.1016/j.anifeedsci.2011.12.009>
- Gozho, G. N., J. C. Plaizier, D. O. Krause, A. D. Kennedy, and K. M. Wittenberg. 2005. Subacute Ruminal Acidosis Induces Ruminal Lipopolysaccharide Endotoxin Release and Triggers an Inflammatory Response. *Journal of Dairy Science* 88(4):1399-1403. doi: [https://doi.org/10.3168/jds.S0022-0302\(05\)72807-1](https://doi.org/10.3168/jds.S0022-0302(05)72807-1)
- Hahn, J., R. Foote, and G. Seidel Jr. 1969a. Quality and freezability of semen from growing and aged dairy bulls. *Journal of Dairy Science* 52(11):1843-1848. doi: [https://doi.org/10.3168/jds.S0022-0302\(69\)86851-7](https://doi.org/10.3168/jds.S0022-0302(69)86851-7)
- Hahn, J., R. Foote, and G. Seidel Jr. 1969b. Testicular growth and related sperm output in dairy bulls. *Journal of animal science* 29(1):41-47. doi: <https://doi.org/10.2527/jas1969.29141x>
- Harvey, C. 1948. Relation between the volume and fructose content of human semen. *Nature* 162(4125):812. doi: <https://doi.org/10.1038/162812a0>
- Hibbard, B., J. P. Peters, S. T. Chester, J. A. Robinson, S. F. Kotarski, W. J. Croom, and W. M. Hagler. 1995. The effect of slaframine on salivary output and subacute and acute acidosis in growing beef steers. *Journal of animal science* 73(2):516-525. doi: [10.2527/1995.732516x](https://doi.org/10.2527/1995.732516x)
- Hoover, W. H. 1986. Chemical factors involved in ruminal fiber digestion. *Journal of Dairy Science* 69(10):2755-2766. doi: [https://doi.org/10.3168/jds.S0022-0302\(86\)80724-X](https://doi.org/10.3168/jds.S0022-0302(86)80724-X)
- Hungate, R. 1975. The rumen microbial ecosystem. *Annual Review of Ecology and Systematics.*, Davis, California

- Jeyaruban, G., B. Tier, D. Johnston, and H. Graser. 2012. Genetic analysis of feet and leg traits of Australian Angus cattle using linear and threshold models. *Animal production science* 52(1):1-10. doi: <https://doi.org/10.1071/AN11153>
- Keunen, J. E., J. C. Plaizier, L. Kyriazakis, T. F. Duffield, T. M. Widowski, M. I. Lindinger, and B. W. McBride. 2002. Effects of a Subacute Ruminant Acidosis Model on the Diet Selection of Dairy Cows. *Journal of Dairy Science* 85(12):3304-3313. doi: [https://doi.org/10.3168/jds.S0022-0302\(02\)74419-6](https://doi.org/10.3168/jds.S0022-0302(02)74419-6)
- Khafipour, E., D. Krause, and J. Plaizier. 2009. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. *Journal of dairy science* 92(3):1060-1070. doi: <https://doi.org/10.3168/jds.2008-1389>
- Kleen, J. L., G. A. Hooijer, J. Rehage, and J. P. T. M. Noordhuizen. 2003. Subacute Ruminant Acidosis (SARA): a Review. *Journal of Veterinary Medicine Series A* 50(8):406-414. doi: 10.1046/j.1439-0442.2003.00569.x
- Kocks, J. E., and M. L. Broekhuijse. 2014. The practical use of density of whole bull semen to determine the volume of an ejaculate at AI laboratories. *Animal reproduction science* 146(3-4):98-102. doi: 10.1016/j.anireprosci.2014.02.015
- Koziol, J. H., and C. L. Armstrong. 2018. *Society for Theriogenology Manual for Breeding Soundness Examination of Bulls*.
- Krause, K. M., and G. R. Oetzel. 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. *Animal Feed Science and Technology* 126(3):215-236. doi: <https://doi.org/10.1016/j.anifeedsci.2005.08.004>

- Mann, T. 1974. Secretory function of the prostate, seminal vesicle and other male accessory organs of reproduction. *Reproduction* 37(1):179-188. doi:  
<https://doi.org/10.1530/jrf.0.0370179>
- McAllister, T. A., K.-J. Cheng, L. M. Rode, and C. W. Forsberg. 1990. Digestion of barley, maize, and wheat by selected species of ruminal bacteria. *Applied and environmental microbiology* 56(10):3146-3153.
- Mwansa, P. B., and M. Makarechian. 1991. The effect of postweaning level of dietary energy on sex drive and semen quality of young beef bulls. *Theriogenology* 35(6):1169-1178. doi:  
[https://doi.org/10.1016/0093-691X\(91\)90363-I](https://doi.org/10.1016/0093-691X(91)90363-I)
- Nagaraja, T., and E. Titgemeyer. 2007. Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook1, 2. *Journal of Dairy Science* 90:E17-E38. doi:  
<https://doi.org/10.3168/jds.2006-478>
- Nocek, J. E. 1997. Bovine Acidosis: Implications on Laminitis. *Journal of Dairy Science* 80(5):1005-1028. doi: [https://doi.org/10.3168/jds.S0022-0302\(97\)76026-0](https://doi.org/10.3168/jds.S0022-0302(97)76026-0)
- Oetzel, G. R. 2007. Subacute ruminal acidosis in dairy herds: physiology, pathophysiology, milk fat responses, and nutritional management. In: 40th Annual Conference, American Association of Bovine Practitioners. p 89-119.
- Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1998. Acidosis in cattle: a review. *Journal of animal science* 76(1):275-286. doi: 10.2527/1998.761275x
- Palmer, C., L. Brito, A. Arteaga, L. Söderquist, Y. Persson, and A. Barth. 2005. Comparison of electroejaculation and transrectal massage for semen collection in range and yearling feedlot beef bulls. *Animal reproduction science* 87(1-2):25-31. doi:  
<https://doi.org/10.1016/j.anireprosci.2004.09.004>

- Prendergast, F. G., and C. M. Veneziale. 1975. Control of fructose and citrate synthesis in guinea pig seminal vesicle epithelium. *Journal of Biological Chemistry* 250(4):1282-1289.
- Pritchard, R. H. 1993. Bunk management. In: Land O'Lakes Beef Seminar, Cedar Rapids, IA, Columbus, NE, and Storm Lake, IA. Land O'Lakes Inc., St. Paul, MN. p 4-15.
- Russell, J. B., and T. Hino. 1985. Regulation of Lactate Production in *Streptococcus bovis*: A Spiraling Effect That Contributes to Rumen Acidosis. *Journal of Dairy Science* 68(7):1712-1721. doi: [https://doi.org/10.3168/jds.S0022-0302\(85\)81017-1](https://doi.org/10.3168/jds.S0022-0302(85)81017-1)
- Russell, J. B., and D. B. Wilson. 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? *Journal of dairy science* 79(8):1503-1509. doi: [https://doi.org/10.3168/jds.S0022-0302\(96\)76510-4](https://doi.org/10.3168/jds.S0022-0302(96)76510-4)
- Stock, R. 2000. Acidosis in cattle: an overview. In: Thrity-third Annual Conference, American Association of Bovine Practitioners, Rapid City, South Dakota. p 30-37.
- Tilton, W., A. Warnick, T. Cunha, P. Loggins, and R. Shirley. 1964. Effect of low energy and protein intake on growth and reproductive performance of young rams. *Journal of Animal Science* 23(3):645-650. doi: <https://doi.org/10.2527/jas1964.233645x>
- Vogler, C., J. Bame, J. DeJarnette, M. McGilliard, and R. Saacke. 1993. Effects of elevated testicular temperature on morphology characteristics of ejaculated spermatozoa in the bovine. *Theriogenology* 40(6):1207-1219. doi: [https://doi.org/10.1016/0093-691X\(93\)90291-C](https://doi.org/10.1016/0093-691X(93)90291-C)



## Appendix A: Tables

Table 2.1: Ingredient composition, nutrient composition, and cost of rations used to develop Angus bulls over an 84-d period

| Ingredient, % of DM                            | COMP <sup>1</sup> |        | TMR <sup>1,2</sup> |        |
|--|-------------------|--------|--------------------|--------|
|  | Formulated        | Actual | Formulated         | Actual |
| Custom-blended pellet <sup>3,4</sup>           | 95.25             | 92.37  | --                 | --     |
| Processed cereal rye hay                       | 4.75              | 7.63   | --                 | --     |
| Dry-rolled corn                                | --                | --     | 39.10              | 39.12  |
| Processed mixed grass hay                      | --                | --     | 25.00              | 24.99  |
| Corn silage                                    | --                | --     | 15.00              | 14.97  |
| Dried corn gluten feed                         | --                | --     | 13.75              | 13.75  |
| Micro-ingredient premix <sup>5</sup>           | --                | --     | 7.15               | 7.17   |
| Nutrient                                       | --                | --     | --                 | --     |
| DM, % as-fed                                   | 89.20             | 88.63  | 65.00              | 64.85  |
| CP, % of DM                                    | 13.46             | 13.43  | 13.47              | 13.40  |
| NDF, % of DM                                   | 30.74             | 31.65  | 31.15              | 30.10  |
| ADF, % of DM                                   | 18.89             | 19.88  | 16.07              | 16.50  |
| EE, % of DM                                    | 3.42              | 3.89   | 3.72               | 3.64   |
| Starch, % of DM                                | 36.20             | 34.26  | 36.24              | 35.55  |
| NE <sub>m</sub> , Mcal/kg of DM                | 1.74              | 1.70   | 1.76               | 1.76   |
| NE <sub>g</sub> , Mcal/kg of DM                | 1.12              | 1.08   | 1.12               | 1.12   |
| Ration cost <sup>6</sup> , \$/kg               | --                | 0.33   | --                 | 0.27   |
| Bulk density, kg of DM x cubic m <sup>-1</sup> | --                | 564.68 | --                 | 143.50 |

<sup>1</sup>Rations were formulated to contain 9.51 and 6.80 mg/lb of monensin (as-fed basis) for COMP and TMR rations, respectively.

<sup>2</sup>Water was included as a conditioning and mixing aid in the TMR to decrease DM content to 65 % as-fed.

<sup>3</sup>Custom-blended pellet contained (DM-basis) 39.56 % ground corn, 15.75 % soybean hulls, 14.94 % dried corn gluten feed, 6.02 % wheat middlings, 5.14 % dried distiller's grains, 4.96 % ground wheat, 3.93 % micro-ingredient premix, 3.90 % rice hulls, 3.55 % cottonseed hulls, 1.22 % liquid pellet binder (Pellbind), and 1.03 % soybean meal.

<sup>4</sup>Micro-ingredient premix included in custom-blended pellet contained (DM-basis) 63.34 % limestone, 11.49 % soybean oil, 10.60 % urea, 8.34 % white salt, 3.64 % magnesium oxide, 0.81 % vitamin E premix (125 KIU/lb as-included), 0.43 % vitamin ADE micro-mix (10,000 KIU/lb vitamin A, 800 KIU/lb vitamin D, and 4.5 KIU/lb vitamin E, as-included), 0.41 % zinc sulfate, 0.31 % Rumensin 90, 0.28 % selenium yeast (Sel-Plex 600), 0.20 % manganous oxide, 0.13 % copper sulfate, and 0.02 % EDDI 40.

<sup>5</sup>Micro-ingredient premix provided to all bulls from d -10 to d 0 and included in the TMR contained (DM-basis) 51.65 % dried distiller's grains, 26.72 % limestone, 7.16 % sodium bicarbonate, 5.34 % urea, 4.27 % white salt, 2.19 % corn oil, 1.33 % magnesium oxide, 0.44 % vitamin E premix (125 KIU/lb as-included), 0.23 % vitamin ADE micro-mix (10,000 KIU/lb vitamin A, 800 KIU/lb vitamin D, and 4.5 KIU/lb vitamin E, as-included), 0.19 % zinc sulfate, 0.16 % Rumensin 90, 0.16 % selenium yeast (Sel-Plex 600), 0.11 % manganous oxide, 0.04 % copper sulfate, and 0.01 % EDDI.

<sup>6</sup>Ration cost included ingredient, freight, shrink, storage, labor, and equipment depreciation costs.

Table 2.2: Growth performance, feed intake, feed efficiency, and scrotal circumference of Angus bulls developed on one of two iso-caloric rations differing in form<sup>1</sup>

| Item <sup>2</sup>                                  | Ration             |                    | Pooled SEM | P – value |
|--|--------------------|--------------------|------------|-----------|
|  | COMP               | TMR                |            |           |
| D 1 to 21  | --                 | --                 | --         | --        |
| BW, kg   | 423.4              | 422.5              | 2.1        | 0.76      |
| DMI, kg x day <sup>-1</sup>                        | 8.17 <sup>a</sup>  | 6.37 <sup>b</sup>  | 0.07       | < 0.01    |
| DMI, % of BW                                       | 1.93 <sup>a</sup>  | 1.51 <sup>b</sup>  | 0.01       | < 0.01    |
| ADG, kg x day <sup>-1</sup>                        | 2.13               | 2.09               | 0.09       | 0.76      |
| Feed efficiency, kg of BW x kg of DM <sup>-1</sup> | 0.260 <sup>b</sup> | 0.326 <sup>a</sup> | 0.012      | < 0.01    |
| SC, cm   | 35.2               | 35.3               | 0.4        | 0.75      |
| D 22 to 42   | --                 | --                 | --         | --        |
| BW, kg   | 476.1              | 466.1              | 4.0        | 0.10      |
| DMI, kg x day <sup>-1</sup>                        | 11.25 <sup>a</sup> | 9.73 <sup>b</sup>  | 0.25       | < 0.01    |
| DMI, % of BW                                       | 2.37 <sup>a</sup>  | 2.08 <sup>b</sup>  | 0.04       | < 0.01    |
| ADG, kg x day <sup>-1</sup>                        | 2.51 <sup>c</sup>  | 2.08 <sup>d</sup>  | 0.11       | < 0.05    |
| Feed efficiency, kg of BW x kg of DM <sup>-1</sup> | 0.223              | 0.213              | 0.008      | 0.39      |
| SC, cm   | 36.2               | 35.7               | 0.4        | 0.41      |
| D 43 to 63   | --                 | --                 | --         | --        |
| BW, kg   | 526.3 <sup>a</sup> | 504.3 <sup>b</sup> | 3.2        | < 0.01    |
| DMI, kg x day <sup>-1</sup>                        | 12.65 <sup>a</sup> | 11.51 <sup>b</sup> | 0.23       | < 0.01    |
| DMI, % of BW                                       | 2.40 <sup>c</sup>  | 2.28 <sup>d</sup>  | 0.04       | < 0.05    |
| ADG, kg x day <sup>-1</sup>                        | 2.51 <sup>a</sup>  | 1.91 <sup>b</sup>  | 0.09       | < 0.01    |
| Feed efficiency, kg of BW x kg of DM <sup>-1</sup> | 0.199 <sup>c</sup> | 0.167 <sup>d</sup> | 0.009      | < 0.05    |
| SC, cm   | 37.1               | 36.4               | 0.4        | 0.26      |

<sup>1</sup>Rations consisted of a component-based feeding system that included a concentrate-based pellet and processed cereal rye hay fed separately (COMP; n = 24) or a total mixed ration consisting of dry-rolled corn, processed mixed grass hay, corn silage, dried corn gluten feed, and a micro-ingredient premix (TMR; n = 24) fed throughout an 84-d development period.

<sup>2</sup>Body weight (BW) represents the paired-day un-shrunk BW measurements on sample collection d 0, 21, 42, 63, and 84. DMI = dry matter intake, ADG = average daily gain, and SC = scrotal circumference.

<sup>a,b</sup>Means within a row without common superscripts differ ( $P < 0.01$ ).

<sup>c,d</sup>Means within a row without common superscripts differ ( $P < 0.05$ ).

Table 2.2 continued : Growth performance, feed intake, feed efficiency, and scrotal circumference of Angus bulls developed on one of two iso-caloric rations differing in form<sup>1</sup>

| Item <sup>2</sup>                                  | Ration             |                    | Pooled SEM | P – value |
|--|--------------------|--------------------|------------|-----------|
|  | COMP               | TMR                |            |           |
| D 64 to 84   | --                 | --                 | --         | --        |
| BW, kg   | 583.3 <sup>a</sup> | 553.6 <sup>b</sup> | 4.3        | < 0.01    |
| DMI, kg x day <sup>-1</sup>                        | 12.84 <sup>c</sup> | 11.85 <sup>d</sup> | 0.30       | < 0.05    |
| DMI, % of BW                                       | 2.20               | 2.14               | 0.05       | 0.42      |
| ADG, kg x day <sup>-1</sup>                        | 2.72 <sup>c</sup>  | 2.35 <sup>d</sup>  | 0.10       | < 0.05    |
| Feed efficiency, kg of BW x kg of DM <sup>-1</sup> | 0.212              | 0.199              | 0.006      | 0.13      |
| SC, cm   | 37.7               | 37.3               | 0.4        | 0.45      |
| D 0 to 84  | --                 | --                 | --         | --        |
| Initial BW, kg                                     | 377.8              | 375.1              | 2.6        | 0.88      |
| Final BW, kg                                       | 583.3 <sup>a</sup> | 553.6 <sup>b</sup> | 4.3        | < 0.01    |
| DMI, kg x day <sup>-1</sup>                        | 11.13 <sup>a</sup> | 9.81 <sup>b</sup>  | 0.17       | < 0.01    |
| DMI, % of BW                                       | 2.33 <sup>a</sup>  | 2.11 <sup>b</sup>  | 0.03       | < 0.01    |
| ADG, kg x day <sup>-1</sup>                        | 2.46 <sup>a</sup>  | 2.13 <sup>b</sup>  | 0.05       | < 0.01    |
| Feed efficiency, kg of BW x kg of DM <sup>-1</sup> | 0.221              | 0.215              | 0.003      | 0.19      |
| SC, cm   | 35.7               | 35.5               | 0.2        | 0.53      |

<sup>1</sup>Rations consisted of a component-based feeding system that included a concentrate-based pellet and processed cereal rye hay fed separately (COMP; n = 24) or a total mixed ration consisting of dry-rolled corn, processed mixed grass hay, corn silage, dried corn gluten feed, and a micro-ingredient premix (TMR; n = 24) fed throughout an 84-d development period.

<sup>2</sup>Body weight (BW) represents the paired-day un-shrunk BW measurements on sample collection d 0, 21, 42, 63, and 84.

DMI = dry matter intake, ADG = average daily gain, and SC = scrotal circumference.

<sup>a,b</sup>Means within a row without common superscripts differ ( $P < 0.01$ ).

<sup>c,d</sup>Means within a row without common superscripts differ ( $P < 0.05$ ).

Table 2.3: Average rumen pH and temperature of Angus bulls developed on one of two iso-caloric rations differing in form<sup>1,2</sup>

| Item <sup>3</sup> | Ration            |                   | Pooled SEM | <i>P</i> – value |
|-------------------|-------------------|-------------------|------------|------------------|
|                   | COMP              | TMR               |            |                  |
| D 1 to 21         | --                | --                | --         | --               |
| pH                | 6.18 <sup>b</sup> | 6.25 <sup>a</sup> | 0.08       | < 0.01           |
| Temperature, °C   | 40.0              | 40.0              | 0.1        | 0.99             |
| D 22 to 42        | --                | --                | --         | --               |
| pH                | 5.97 <sup>b</sup> | 6.14 <sup>a</sup> | 0.08       | < 0.01           |
| Temperature, °C   | 39.5              | 39.3              | 0.1        | 0.18             |
| D 43 to 63        | --                | --                | --         | --               |
| pH                | 5.98 <sup>b</sup> | 6.15 <sup>a</sup> | 0.08       | < 0.01           |
| Temperature, °C   | 39.7              | 39.6              | 0.1        | 0.51             |
| D 64 to 84        | --                | --                | --         | --               |
| pH                | 6.06 <sup>b</sup> | 6.35 <sup>a</sup> | 0.09       | < 0.01           |
| Temperature, °C   | 39.5              | 39.1              | 0.2        | 0.12             |
| D 0 to 84         | --                | --                | --         | --               |
| pH                | 6.05 <sup>b</sup> | 6.22 <sup>a</sup> | 0.07       | < 0.01           |
| Temperature, °C   | 39.7              | 39.5              | 0.1        | 0.12             |

<sup>1</sup>Rumen pH and temperature were continuously measured via rumen bolus (eBolus; eCow, Devon, UK) at 15-min intervals throughout an 84-d feeding trial.

<sup>2</sup>Diets consisted of a component-based feeding system that included a concentrate-based pellet and processed cereal rye hay fed separately (COMP; n = 24) or a total mixed ration consisting of dry-rolled corn, processed mixed grass hay, corn silage, dried corn gluten feed, and a micro-ingredient premix (TMR; n = 24) fed throughout an 84-d development period.

<sup>3</sup>Sample collections occurred on d 21, 42, 63, and 84 and rumen pH and temperature values were averaged within 21-d intervals and throughout the duration of the trial.

<sup>a,b</sup>Means within a row without common superscripts differ (*P* < 0.01).

Table 2.4: Model-adjusted probabilities and subsequent odds ratios of rumen pH below various thresholds for Angus bulls developed on one of two iso-caloric rations differing in form<sup>1,2</sup>

| Item       | Ration             |                    | Odds ratio,<br>COMP vs. TMR | 95% confidence<br>interval | Pooled<br>SEM | P –<br>value |
|------------|--------------------|--------------------|-----------------------------|----------------------------|---------------|--------------|
|            | COMP               | TMR                |                             |                            |               |              |
| D 1 to 21  | --                 | --                 | --                          | --                         | --            | --           |
| pH 6.0     | 0.399 <sup>a</sup> | 0.242 <sup>b</sup> | 2.1                         | 1.8, 2.4                   | 0.012         | < 0.01       |
| pH 5.8     | 0.306 <sup>a</sup> | 0.141 <sup>b</sup> | 2.7                         | 1.5, 4.9                   | 0.040         | < 0.01       |
| pH 5.5     | 0.174 <sup>a</sup> | 0.049 <sup>b</sup> | 4.1                         | 1.8, 9.6                   | 0.031         | < 0.01       |
| pH 5.2     | 0.061 <sup>a</sup> | 0.003 <sup>b</sup> | 5.2                         | 3.2, 8.3                   | 0.050         | < 0.01       |
| pH 5.0     | 0.022 <sup>a</sup> | 0.004 <sup>b</sup> | 6.4                         | 2.8, 14.                   | 0.003         | < 0.01       |
| D 22 to 42 | --                 | --                 | --                          | --                         | --            | --           |
| pH 6.0     | 0.514 <sup>a</sup> | 0.348 <sup>b</sup> | 2.0                         | 1.7, 2.3                   | 0.012         | < 0.01       |
| pH 5.8     | 0.418 <sup>a</sup> | 0.245 <sup>b</sup> | 2.2                         | 1.3, 3.8                   | 0.043         | < 0.01       |
| pH 5.5     | 0.230 <sup>c</sup> | 0.113 <sup>d</sup> | 2.4                         | 1.2, 4.5                   | 0.035         | < 0.05       |
| pH 5.2     | 0.060 <sup>a</sup> | 0.032 <sup>b</sup> | 1.9                         | 1.3, 2.8                   | 0.049         | < 0.01       |
| pH 5.0     | 0.026 <sup>a</sup> | 0.009 <sup>b</sup> | 2.9                         | 1.5, 5.6                   | 0.019         | < 0.01       |
| D 43 to 63 | --                 | --                 | --                          | --                         | --            | --           |
| pH 6.0     | 0.545 <sup>a</sup> | 0.354 <sup>b</sup> | 2.2                         | 1.8, 2.7                   | 0.018         | < 0.01       |
| pH 5.8     | 0.429 <sup>c</sup> | 0.269 <sup>d</sup> | 2.1                         | 1.1, 3.9                   | 0.053         | < 0.05       |
| pH 5.5     | 0.244 <sup>c</sup> | 0.106 <sup>d</sup> | 2.7                         | 1.2, 6.3                   | 0.046         | < 0.05       |
| pH 5.2     | 0.103 <sup>a</sup> | 0.023 <sup>b</sup> | 4.9                         | 2.9, 8.3                   | 0.012         | < 0.01       |
| pH 5.0     | 0.056 <sup>a</sup> | 0.010 <sup>b</sup> | 5.9                         | 2.8, 12.6                  | 0.009         | < 0.01       |
| D 64 to 84 | --                 | --                 | --                          | --                         | --            | --           |
| pH 6.0     | 0.500 <sup>a</sup> | 0.218 <sup>b</sup> | 3.6                         | 2.8, 2.7                   | 0.022         | < 0.01       |
| pH 5.8     | 0.367 <sup>e</sup> | 0.170 <sup>f</sup> | 2.8                         | 0.9, 8.5                   | 0.085         | 0.06         |
| pH 5.5     | 0.223 <sup>c</sup> | 0.064 <sup>d</sup> | 4.2                         | 1.0, 18.2                  | 0.072         | < 0.05       |
| pH 5.2     | 0.133 <sup>a</sup> | 0.008 <sup>b</sup> | 19.6                        | 7.8, 49.2                  | 0.015         | < 0.01       |
| pH 5.0     | 0.085 <sup>a</sup> | 0.001 <sup>b</sup> | 84.5                        | 10.4, 689.9                | 0.011         | < 0.01       |
| D 0 to 84  | --                 | --                 | --                          | --                         | --            | --           |
| pH 6.0     | 0.480 <sup>a</sup> | 0.295 <sup>b</sup> | 2.2                         | 2.0, 2.4                   | 0.008         | < 0.01       |
| pH 5.8     | 0.380 <sup>a</sup> | 0.199 <sup>b</sup> | 2.5                         | 1.6, 3.9                   | 0.035         | < 0.01       |
| pH 5.5     | 0.210 <sup>a</sup> | 0.081 <sup>b</sup> | 3.0                         | 1.7, 5.5                   | 0.028         | < 0.01       |
| pH 5.2     | 0.081 <sup>a</sup> | 0.020 <sup>b</sup> | 4.4                         | 3.4, 5.6                   | 0.004         | < 0.01       |
| pH 5.0     | 0.040 <sup>a</sup> | 0.006 <sup>b</sup> | 6.7                         | 4.4, 10.2                  | 0.034         | < 0.01       |

<sup>1</sup>Rumen pH was continuously measured at 15-min intervals via rumen bolus (eBolus; eCow, Devon, UK) and used to calculate the probability of rumen pH below assigned thresholds of 6.0, 5.8, 5.5, 5.2, and 5.0.

<sup>2</sup>Rations consisted of a component-based feeding system that included a concentrate-based pellet and processed cereal rye hay fed separately (COMP; n = 24) or a total mixed ration consisting of dry-rolled corn, processed mixed grass hay, corn silage, dried corn gluten feed, and a micro-ingredient premix (TMR; n = 24) which were fed throughout an 84-d development period.

<sup>a,b</sup>Means within a row without common superscripts differ ( $P < 0.01$ ).

<sup>c,d</sup>Means within a row without common superscripts differ ( $P < 0.05$ ).

<sup>e,f</sup>Means within a row without common superscripts tend to differ ( $P < 0.10$ ).

Table 2.5: Semen quality parameters of Angus bulls developed on one of two iso-caloric rations differing in form<sup>1</sup>

| Item <sup>2</sup>   | Ration           |                  | Pooled SEM | P – value |
|---|------------------|------------------|------------|-----------|
|   | COMP             | TMR              |            |           |
| D 0   | --               | --               | --         | --        |
| Progressive motility, %   | 49.4             | 51.2             | 3.4        | 0.71      |
| Volume, mL  | 3.3              | 3.4              | 0.4        | 0.89      |
| Morphologically-normal sperm, %   | 45.6             | 54.8             | 6.3        | 0.31      |
| Total sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup>                    | 1.91             | 2.05             | 0.4        | 0.81      |
| Morphologically-normal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup>   | 0.87             | 1.12             | 0.30       | 0.38      |
| Morphologically-abnormal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> | 1.04             | 0.93             | 0.15       | 0.25      |
| D 21  | --               | --               | --         | --        |
| Progressive motility, %   | 53.5             | 47.6             | 2.8        | 0.15      |
| Volume, mL  | 4.4              | 3.9              | 0.6        | 0.52      |
| Morphologically-normal sperm, %   | 52.6             | 57.2             | 5.7        | 0.58      |
| Total sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup>                    | 1.64             | 2.10             | 0.31       | 0.31      |
| Morphologically-normal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup>   | 0.86             | 1.20             | 0.25       | 0.29      |
| Morphologically-abnormal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> | 0.78             | 0.90             | 0.11       | 0.71      |
| D 42  | --               | --               | --         | --        |
| Progressive motility, %   | 54.0             | 59.0             | 3.1        | 0.26      |
| Volume, mL  | 6.0              | 6.4              | 0.8        | 0.74      |
| Morphologically-normal sperm, %   | 59.8             | 57.3             | 5.6        | 0.76      |
| Total sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup>                    | 2.38             | 2.87             | 0.42       | 0.43      |
| Morphologically-normal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup>   | 1.42             | 1.64             | 0.35       | 0.48      |
| Morphologically-abnormal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> | 0.96             | 1.23             | 0.19       | 0.64      |
| D 63  | --               | --               | --         | --        |
| Progressive motility, %   | 48.7             | 51.4             | 2.7        | 0.49      |
| Volume, mL  | 6.7 <sup>c</sup> | 5.0 <sup>d</sup> | 0.7        | 0.08      |
| Morphologically-normal sperm, %   | 59.3             | 63.4             | 5.7        | 0.62      |
| Total sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup>                    | 3.04             | 2.59             | 0.53       | 0.55      |
| Morphologically-normal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup>   | 1.80             | 1.64             | 0.45       | 0.82      |
| Morphologically-abnormal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> | 1.24             | 0.95             | 0.18       | 0.25      |

<sup>1</sup>Rations consisted of a component-based feeding system that included a concentrate-based pellet and processed cereal rye hay fed separately (COMP; n = 24) or a total mixed ration consisting of dry-rolled corn, processed mixed grass hay, corn silage, dried corn gluten feed, and a micro-ingredient premix (TMR; n = 24) fed throughout an 84-d development period.

<sup>2</sup>Semen quality was measured on d 0, 21, 42, 63, and 84, and also reported as a full trial average.

<sup>a,b</sup>Means within a row without common superscripts differ ( $P < 0.05$ )

<sup>c,d</sup>Means within a row without common superscripts tend to differ ( $P < 0.10$ )

Table 2.5 continued: Semen quality parameters of Angus bulls developed on one of two iso-caloric rations differing in form<sup>1</sup>

| Item <sup>2</sup>   | Ration            |                  | Pooled SEM | P – value |
|---|-------------------|------------------|------------|-----------|
|   | COMP              | TMR              |            |           |
| D 84  | --                | --               | --         | --        |
| Progressive motility, %   | 42.4              | 47.0             | 2.8        | 0.25      |
| Volume, mL  | 10.8 <sup>a</sup> | 7.3 <sup>b</sup> | 1.2        | < 0.05    |
| Morphologically-normal sperm, %   | 63.4              | 65.2             | 4.5        | 0.78      |
| Total sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup>                    | 3.58              | 3.30             | 0.55       | 0.72      |
| Morphologically-normal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup>   | 2.27              | 2.15             | 0.39       | 0.76      |
| Morphologically-abnormal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> | 1.31              | 1.15             | 0.31       | 0.32      |
| D 0 to 84   | --                | --               | --         | --        |
| Progressive motility, %   | 49.0              | 50.8             | 1.3        | 0.34      |
| Volume, mL  | 6.6 <sup>a</sup>  | 5.5 <sup>b</sup> | 0.4        | < 0.05    |
| Morphologically-normal sperm, %   | 55.6              | 59.2             | 2.5        | 0.34      |
| Total sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup>                    | 2.58              | 2.65             | 0.20       | 0.81      |
| Morphologically-normal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup>   | 1.43              | 1.57             | 0.16       | 0.32      |
| Morphologically-abnormal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> | 1.15              | 1.08             | 0.10       | 0.21      |

<sup>1</sup>Rations consisted of a component-based feeding system that included a concentrate-based pellet and processed cereal rye hay fed separately (COMP; n = 24) or a total mixed ration consisting of dry-rolled corn, processed mixed grass hay, corn silage, dried corn gluten feed, and a micro-ingredient premix (TMR; n = 24) fed throughout an 84-d development period.

<sup>2</sup>Semen quality was measured on d 0, 21, 42, 63, and 84, and also reported as a full trial average.

<sup>a,b</sup>Means within a row without common superscripts differ ( $P < 0.05$ )

<sup>c,d</sup>Means within a row without common superscripts tend to differ ( $P < 0.10$ )

Table 2.6: Effect of semen collection day on semen quality parameters of Angus bulls<sup>1</sup>

| Item  | Day                 |                     |                    |                    |                   | Pooled SEM | P - value |
|---|---------------------|---------------------|--------------------|--------------------|-------------------|------------|-----------|
|   | 0                   | 21                  | 42                 | 63                 | 84                |            |           |
| Progressive motility, %   | 47.2 <sup>b</sup>   | 50.4 <sup>a,b</sup> | 56.6 <sup>a</sup>  | 50.1 <sup>ab</sup> | 44.9 <sup>b</sup> | 2.3        | < 0.01    |
| Volume, mL  | 5.1 <sup>b</sup>    | 4.2 <sup>b</sup>    | 6.2 <sup>b</sup>   | 5.8 <sup>b</sup>   | 8.9 <sup>a</sup>  | 0.6        | < 0.01    |
| Morphologically-normal sperm, %   | 47.5 <sup>d</sup>   | 54.8 <sup>d</sup>   | 58.5 <sup>cd</sup> | 61.5 <sup>cd</sup> | 64.3 <sup>c</sup> | 3.5        | < 0.05    |
| Total sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup>                    | 2.34 <sup>a,b</sup> | 1.88 <sup>b</sup>   | 2.64 <sup>ab</sup> | 2.80 <sup>ab</sup> | 3.42 <sup>a</sup> | 0.30       | < 0.01    |
| Morphologically-normal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup>   | 0.92 <sup>d</sup>   | 1.02 <sup>d</sup>   | 1.54 <sup>cd</sup> | 1.72 <sup>cd</sup> | 2.20 <sup>c</sup> | 0.27       | < 0.05    |
| Morphologically-abnormal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> | 1.02 <sup>d</sup>   | 0.85 <sup>d</sup>   | 1.10 <sup>cd</sup> | 1.08 <sup>cd</sup> | 1.22 <sup>c</sup> | 0.14       | < 0.05    |

<sup>1</sup>Semen samples were collected and analyzed on d 21, 42, 63, and 84.

<sup>a,b</sup>Means within a row without common superscripts differ ( $P < 0.01$ ).

<sup>c,d</sup>Means within a row without common superscripts differ ( $P < 0.05$ ).



Table 2.7: Relationships between the proportion of rumen pH observations below specific pH thresholds and progressive motility, semen collection volume, or percentage morphologically-normal sperm across subsequent semen collection days in Angus bulls<sup>1</sup>

| Item           | Progressive motility, % |        |                |           | Collection volume, mL |       |                |           | Morphologically-normal sperm, % |        |                |           |
|----------------|-------------------------|--------|----------------|-----------|-----------------------|-------|----------------|-----------|---------------------------------|--------|----------------|-----------|
|                | Intercept               | Slope  | R <sup>2</sup> | P – value | Intercept             | Slope | R <sup>2</sup> | P – value | Intercept                       | Slope  | R <sup>2</sup> | P – value |
| D 21           | --                      | --     | --             | --        | --                    | --    | --             | --        | --                              | --     | --             | --        |
| D 1-21 pH 5.8  | 45.88                   | 19.02  | 0.08           | 0.09      | 3.66                  | 1.77  | 0.02           | 0.44      | 58.51                           | -21.69 | 0.02           | 0.35      |
| D 1-21 pH 5.2  | 48.80                   | 39.78  | 0.03           | 0.29      | 3.67                  | 10.85 | 0.05           | 0.15      | 56.46                           | -80.50 | 0.03           | 0.30      |
| D 42           | --                      | --     | --             | --        | --                    | --    | --             | --        | --                              | --     | --             | --        |
| D 1-21 pH 5.8  | 56.16                   | 4.79   | < 0.01         | 0.73      | 6.52                  | -1.01 | < 0.01         | 0.74      | 55.40                           | 0.34   | < 0.01         | 0.99      |
| D 1-21 pH 5.2  | 58.07                   | -23.12 | < 0.01         | 0.62      | 6.45                  | -4.33 | < 0.01         | 0.69      | 54.97                           | 14.16  | < 0.01         | 0.86      |
| D 22-42 pH 5.8 | 59.18                   | -7.32  | 0.01           | 0.57      | 5.97                  | 0.23  | < 0.01         | 0.93      | 51.22                           | 12.30  | < 0.01         | 0.56      |
| D 22-42 pH 5.2 | 59.37                   | -44.59 | 0.10           | < 0.05    | 5.75                  | 5.19  | 0.04           | 0.24      | 57.00                           | -32.76 | 0.02           | 0.38      |
| D 63           | --                      | --     | --             | --        | --                    | --    | --             | --        | --                              | --     | --             | --        |
| D 1-21 pH 5.8  | 53.01                   | 17.32  | 0.08           | 0.08      | 4.80                  | 5.49  | 0.09           | 0.06      | 62.78                           | -3.06  | < 0.01         | 0.88      |
| D 1-21 pH 5.2  | 49.34                   | -6.79  | < 0.01         | 0.84      | 5.18                  | 24.12 | 0.16           | < 0.05    | 62.41                           | -8.83  | < 0.01         | 0.90      |
| D 22-42 pH 5.8 | 46.35                   | 3.81   | < 0.01         | 0.68      | 4.43                  | 5.24  | 0.11           | < 0.05    | 64.20                           | -7.54  | < 0.01         | 0.67      |
| D 22-42 pH 5.2 | 47.33                   | 4.10   | < 0.01         | 0.80      | 5.86                  | 4.29  | 0.02           | 0.37      | 62.81                           | -17.58 | < 0.01         | 0.58      |
| D 43-63 pH 5.8 | 45.74                   | 8.13   | 0.03           | 0.32      | 5.18                  | 3.06  | 0.05           | 0.21      | 52.59                           | 29.96  | 0.10           | 0.18      |
| D 43-63 pH 5.2 | 47.71                   | 9.02   | 0.01           | 0.50      | 6.15                  | -0.44 | < 0.01         | 0.91      | 61.32                           | 8.20   | < 0.01         | 0.77      |
| D 84           | --                      | --     | --             | --        | --                    | --    | --             | --        | --                              | --     | --             | --        |
| D 1-21 pH 5.8  | 40.33                   | 17.85  | 0.06           | 0.12      | 10.35                 | -6.33 | 0.03           | 0.30      | 63.58                           | 1.34   | < 0.01         | 0.94      |
| D 1-21 pH 5.2  | 41.00                   | 94.22  | 0.16           | < 0.05    | 8.94                  | -0.52 | < 0.01         | 0.98      | 62.41                           | 41.49  | 0.01           | 0.51      |
| D 22-42 pH 5.8 | 46.55                   | -1.05  | < 0.01         | 0.92      | 9.19                  | -1.62 | < 0.01         | 0.78      | 64.31                           | -1.63  | < 0.01         | 0.93      |
| D 22-42 pH 5.2 | 44.97                   | 21.61  | 0.04           | 0.27      | 8.00                  | 11.79 | 0.04           | 0.25      | 64.16                           | -6.34  | < 0.01         | 0.84      |
| D 43-63 pH 5.8 | 46.25                   | 0.72   | < 0.01         | 0.93      | 10.62                 | -6.94 | 0.08           | 0.11      | 59.32                           | 15.98  | 0.04           | 0.28      |
| D 43-63 pH 5.2 | 47.12                   | -11.29 | 0.03           | 0.36      | 8.84                  | -6.05 | 0.02           | 0.40      | 64.45                           | -3.72  | < 0.01         | 0.88      |
| D 64-84 pH 5.8 | 47.15                   | 2.40   | < 0.01         | 0.75      | 10.08                 | -8.14 | 0.11           | 0.14      | 60.58                           | 17.04  | 0.05           | 0.33      |
| D 64-84 pH 5.2 | 48.21                   | -7.63  | 0.02           | 0.54      | 8.73                  | -9.37 | 0.05           | 0.30      | 64.69                           | -0.45  | < 0.01         | 0.99      |

<sup>1</sup>Proportion of rumen pH observations below pH threshold 5.8 and 5.2 was calculated from rumen pH values collected via rumen bolus (eBolus; eCow, Devon, UK) at 15-minute intervals throughout an 84-d feeding trial and semen samples were collected on d 21, 42, 63, and 84 and utilized in the regression analysis.

Table 2.8 : Relationships between average rumen pH, average rumen temperature, full trial change in SC, or full trial change in SFT and progressive motility, semen collection volume, and percentage morphologically-normal sperm in Angus bulls<sup>1,2,3</sup>

| Item <sup>1</sup>             | Progressive motility, % |         |                |         | Collection volume, mL |        |                |         | Morphologically-normal sperm, % |         |                |         |
|-------------------------------|-------------------------|---------|----------------|---------|-----------------------|--------|----------------|---------|---------------------------------|---------|----------------|---------|
|                               | Intercept               | Slope   | R <sup>2</sup> | P-value | Intercept             | Slope  | R <sup>2</sup> | P-value | Intercept                       | Slope   | R <sup>2</sup> | P-value |
| Average rumen pH              | 6.23                    | > -0.01 | < 0.01         | 0.35    | 6.07                  | < 0.01 | 0.01           | 0.23    | 6.20                            | > -0.01 | 0.01           | 0.25    |
| Average rumen temperature, °C | 39.68                   | < 0.01  | < 0.01         | 0.93    | 39.67                 | < 0.01 | < 0.01         | 0.74    | 39.73                           | > -0.01 | < 0.01         | 0.84    |
| Change in SC, cm              | 5.41                    | -0.01   | < 0.01         | 0.55    | 5.03                  | 0.02   | < 0.01         | 0.51    | 6.22                            | -0.02   | 0.07           | < 0.01  |
| Change in SFT, cm             | 0.42                    | > -0.01 | < 0.01         | 0.85    | 0.33                  | 0.01   | 0.06           | < 0.01  | 0.36                            | < 0.01  | 0.01           | 0.23    |

<sup>1</sup>Average rumen pH and temperature were calculated from values collected via rumen bolus (eBolus; eCow, Devon, UK) at 15-min intervals throughout an 84-d feeding trial.

<sup>2</sup>Change in scrotal circumference (SC) and ultrasound-estimated 12<sup>th</sup>-rib subcutaneous fat thickness (SFT) were calculated as the difference between measurements collected on d 0 and 84.

<sup>3</sup>Semen samples were collected on d 21, 42, 63, and 84. Change in a respective measurement of semen quality was calculated as the difference between measurements collected on d 0 and 84.

Table 2.9: Relationships between average rumen pH, average rumen temperature, full trial change in SC, or full trial change in SFT and full trial changes in progressive motility, semen collection volume, or percentage morphologically-normal sperm in Angus bulls<sup>1,2,3</sup>

| Item                          | Change in progressive motility, % |         |        |            | Change in collection volume, mL |       |       |            | Change in morphologically-normal sperm, % |         |        |            |
|-------------------------------|-----------------------------------|---------|--------|------------|---------------------------------|-------|-------|------------|---|---------|--------|------------|
|                               | Intercept                         | Slope   | $R^2$  | $P$ -value | Intercept                       | Slope | $R^2$ | $P$ -value | Intercept                                 | Slope   | $R^2$  | $P$ -value |
| Average rumen pH              | 6.13                              | < 0.01  | 0.03   | 0.28       | 6.05                            | 0.01  | 0.07  | 0.08       | 6.11                                      | < 0.01  | < 0.01 | 0.94       |
| Average rumen temperature, °C | 39.69                             | < 0.01  | < 0.01 | 0.65       | 39.58                           | 0.02  | 0.05  | 0.14       | 39.70                                     | > -0.01 | < 0.01 | 0.70       |
| Change in SC, cm              | 5.26                              | 0.02    | 0.04   | 0.17       | 4.97                            | 0.03  | 0.02  | 0.37       | 4.81                                      | 0.02    | 0.12   | < 0.05     |
| Change in SFT, cm             | 0.39                              | > -0.01 | 0.03   | 0.24       | 0.34                            | 0.01  | 0.10  | < 0.05     | 0.44                                      | > -0.01 | 0.06   | 0.10       |

<sup>1</sup>Average rumen pH and temperature were calculated from values collected via rumen bolus (eBolus; eCow, Devon, UK) at 15-min intervals throughout an 84-d feeding trial.

<sup>2</sup>Change in scrotal circumference (SC) and ultrasound-estimated 12<sup>th</sup>-rib subcutaneous fat thickness (SFT) were calculated as the difference between measurements collected on d 0 and 84.

<sup>3</sup>Semen samples were collected on d 21, 42, 63, and 84. Change in a respective measurement of semen quality was calculated as the difference between measurements collected on d 0 and 84.

Table 2.10: Relationships between the proportion of rumen pH observations below specific pH thresholds and total sperm in the ejaculate, total morphologically-normal sperm in the ejaculate, and total morphologically-abnormal sperm in the ejaculate across subsequent semen collection days in Angus bulls<sup>1,2</sup>

| Item           | Total sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> |       |                |           | Morphologically-normal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> |       |                |           | Morphologically-abnormal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> |       |                |           |
|----------------|--|-------|----------------|-----------|---|-------|----------------|-----------|---|-------|----------------|-----------|
|                | Intercept  | Slope | R <sup>2</sup> | P – value | Intercept   | Slope | R <sup>2</sup> | P – value | Intercept   | Slope | R <sup>2</sup> | P – value |
| D 21           | --   | --    | --             | --        | --  | --    | --             | --        | --  | --    | --             | --        |
| D 1-21 pH 5.8  | 1.89   | -0.05 | < 0.01         | 0.97      | 1.23  | -0.25 | < 0.01         | 0.81      | 0.66  | 0.21  | < 0.01         | 0.68      |
| D 1-21 pH 5.2  | 1.95   | -2.02 | < 0.01         | 0.65      | 1.24  | -1.86 | < 0.01         | 0.60      | 0.71  | -0.16 | < 0.01         | 0.92      |
| D 42           | --   | --    | --             | --        | --  | --    | --             | --        | --  | --    | --             | --        |
| D 1-21 pH 5.8  | 3.04   | -1.22 | 0.01           | 0.49      | 1.79  | -0.89 | 0.01           | 0.53      | 1.25  | -0.33 | < 0.01         | 0.72      |
| D 1-21 pH 5.2  | 2.94   | -5.03 | 0.02           | 0.39      | 1.70  | -3.09 | 0.01           | 0.51      | 1.24  | -1.94 | 0.01           | 0.53      |
| D 22-42 pH 5.8 | 3.00   | -0.54 | < 0.01         | 0.74      | 1.79  | -0.37 | < 0.01         | 0.78      | 1.21  | -0.17 | < 0.01         | 0.83      |
| D 22-42 pH 5.2 | 2.89   | -1.12 | < 0.01         | 0.70      | 1.79  | -2.09 | 0.02           | 0.36      | 1.10  | 0.97  | 0.01           | 0.49      |
| D 63           | --   | --    | --             | --        | --  | --    | --             | --        | --  | --    | --             | --        |
| D 1-21 pH 5.8  | 2.55   | 0.69  | < 0.01         | 0.73      | 1.66  | 0.36  | < 0.01         | 0.81      | 0.89  | 0.33  | < 0.01         | 0.67      |
| D 1-21 pH 5.2  | 2.41   | 8.09  | 0.04           | 0.23      | 1.58  | 4.51  | 0.02           | 0.39      | 0.84  | 3.58  | 0.05           | 0.17      |
| D 22-42 pH 5.8 | 1.83   | 2.89  | 0.07           | 0.12      | 1.28  | 1.52  | 0.03           | 0.30      | 0.55  | 1.38  | 0.10           | 0.05      |
| D 22-42 pH 5.2 | 2.55   | 3.42  | 0.03           | 0.30      | 1.68  | 1.49  | < 0.01         | 0.33      | 0.87  | 1.93  | 0.07           | 0.13      |
| D 43-63 pH 5.8 | 2.55   | 0.97  | 0.01           | 0.57      | 1.59  | 1.06  | 0.02           | 0.46      | 0.95  | -0.09 | < 0.01         | 0.87      |
| D 43-63 pH 5.2 | 2.74   | 1.84  | 0.01           | 0.50      | 1.87  | 0.88  | < 0.01         | 0.70      | 0.87  | 0.96  | 0.04           | 0.28      |
| D 84           | --   | --    | --             | --        | --  | --    | --             | --        | --  | --    | --             | --        |
| D 1-21 pH 5.8  | 3.85   | -1.89 | 0.02           | 0.40      | 2.46  | -1.20 | 0.02           | 0.44      | 1.39  | -0.69 | < 0.01         | 0.59      |
| D 1-21 pH 5.2  | 3.43   | -0.15 | < 0.01         | 0.98      | 2.12  | 1.97  | < 0.01         | 0.71      | 1.31  | -2.12 | < 0.01         | 0.62      |
| D 22-42 pH 5.8 | 4.06   | -1.89 | 0.02           | 0.37      | 2.66  | -1.37 | 0.03           | 0.34      | 1.41  | -0.52 | < 0.01         | 0.66      |
| D 22-42 pH 5.2 | 3.58   | -2.09 | < 0.01         | 0.58      | 2.29  | -1.24 | < 0.01         | 0.63      | 1.29  | -0.85 | < 0.01         | 0.69      |
| D 43-63 pH 5.8 | 3.71   | -1.21 | 0.01           | 0.51      | 2.35  | -0.62 | < 0.01         | 0.63      | 1.36  | -0.59 | 0.01           | 0.57      |
| D 43-63 pH 5.2 | 3.39   | -0.93 | < 0.01         | 0.75      | 2.19  | -0.56 | < 0.01         | 0.79      | 1.20  | -0.37 | < 0.01         | 0.82      |
| D 64-84 pH 5.8 | 4.01   | -2.38 | 0.04           | 0.35      | 2.59  | -1.44 | 0.04           | 0.40      | 1.42  | -0.94 | 0.02           | 0.52      |
| D 64-84 pH 5.2 | 3.65   | -3.19 | 0.03           | 0.61      | 2.38  | -2.04 | 0.03           | 0.46      | 1.27  | -1.15 | 0.01           | 0.44      |

<sup>1</sup>Proportion of rumen pH observations below pH threshold 5.8 and 5.2 was calculated from rumen pH values collected via rumen bolus (eBolus; eCow, Devon, UK) at 15-min intervals throughout an 84-d feeding trial.

<sup>2</sup>Semen samples were collected on d 21, 42, 63, and 84.

Table 2.11: Relationships between average rumen pH, average rumen temperature, full trial change in SC, or full trial change in SFT and total sperm per ejaculate, total morphologically-normal sperm per ejaculate, and total morphologically-abnormal sperm per ejaculate in Angus bulls<sup>1,2,3</sup>

| Item                          | Total sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> |       |                |         | Morphologically-normal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> |        |                |         | Morphologically-abnormal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> |         |                |         |
|-------------------------------|--|-------|----------------|---------|---|--------|----------------|---------|---|---------|----------------|---------|
|                               | Intercept  | Slope | R <sup>2</sup> | P-value | Intercept   | Slope  | R <sup>2</sup> | P-value | Intercept   | Slope   | R <sup>2</sup> | P-value |
| Average rumen pH              | 6.10   | 0.01  | < 0.01         | 0.55    | 6.11  | < 0.01 | < 0.01         | 0.58    | 6.11  | 0.01    | < 0.01         | 0.70    |
| Average rumen temperature, °C | 39.73  | -0.01 | < 0.01         | 0.75    | 39.73   | -0.02  | < 0.01         | 0.70    | 39.71   | > -0.01 | < 0.01         | 0.95    |
| Change in SC, cm              | 5.44   | -0.11 | 0.02           | 0.06    | 5.49  | -0.20  | 0.04           | < 0.01  | 5.10  | 0.04    | < 0.01         | 0.74    |
| Change in SFT, cm             | 0.34   | 0.02  | 0.05           | < 0.01  | 0.37  | 0.02   | 0.02           | 0.05    | 0.35  | 0.05    | 0.07           | < 0.01  |

<sup>1</sup>Average rumen pH and temperature were calculated from values collected via rumen bolus (eBolus; eCow, Devon, UK) at 15-min intervals throughout an 84-d feeding trial.

<sup>2</sup>Change in scrotal circumference (SC) and ultrasound-estimated 12<sup>th</sup>-rib subcutaneous fat thickness (SFT) were calculated as the difference between measurements collected on d 0 and 84.

<sup>3</sup>Semen samples were collected on d 21, 42, 63, and 84. Change in a respective measurement of semen quality was calculated as the difference between measurements collected on d 0 and 84.

Table 2.12: Relationships between average rumen pH, average rumen temperature, full trial change in SC, or full trial change in SC and full trial changes in total sperm per ejaculate, total morphologically-normal sperm per ejaculate, or total morphologically-abnormal sperm per ejaculate in Angus bulls<sup>1,2</sup>

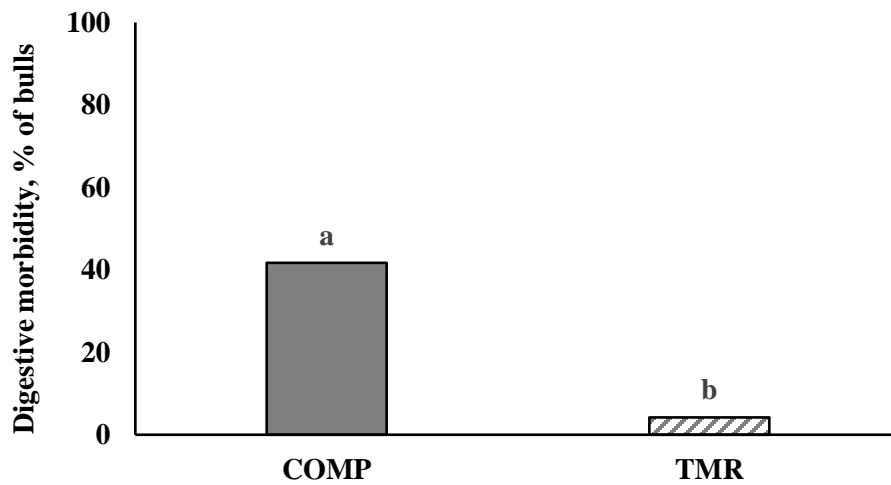
| Item                          | Change in total sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> |         |                |         | Change in morphologically-normal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> |        |                |         | Change in morphologically-abnormal sperm, 10 <sup>9</sup> x ejaculate <sup>-1</sup> |       |                |         |
|-------------------------------|--|---------|----------------|---------|---|--------|----------------|---------|---|-------|----------------|---------|
|                               | Intercept  | Slope   | R <sup>2</sup> | P-value | Intercept   | Slope  | R <sup>2</sup> | P-value | Intercept   | Slope | R <sup>2</sup> | P-value |
| Average rumen pH              | 6.07   | 0.03    | 0.07           | 0.08    | 6.07  | 0.04   | 0.06           | 0.10    | 6.10  | 0.03  | 0.01           | 0.42    |
| Average rumen temperature, °C | 39.69  | > -0.01 | < 0.01         | 0.87    | 39.71   | -0.02  | < 0.01         | 0.68    | 39.68   | 0.03  | < 0.01         | 0.71    |
| Change in SC, cm              | 5.05   | 0.07    | < 0.01         | 0.56    | 5.03  | 0.10   | < 0.01         | 0.52    | 5.13  | 0.03  | < 0.01         | 0.88    |
| Change in SFT, cm             | 0.35   | 0.03    | 0.12           | < 0.05  | 0.39  | < 0.01 | < 0.01         | 0.61    | 0.36  | 0.10  | 0.29           | < 0.01  |

<sup>1</sup>Average rumen pH and temperature were calculated from values collected via rumen bolus (eBolus; eCow, Devon, UK) at 15-min intervals throughout an 84-d feeding trial.

<sup>2</sup>Change in scrotal circumference (SC) and ultrasound-estimated 12<sup>th</sup>-rib subcutaneous fat thickness (SFT) were calculated as the difference between measurements collected on d 0 and 84.

<sup>3</sup>Semen samples were collected on d 21, 42, 63, and 84. Change in a respective measurement of semen quality was calculated as the difference between measurements collected on d 0 and 84.

## Appendix B: Figures

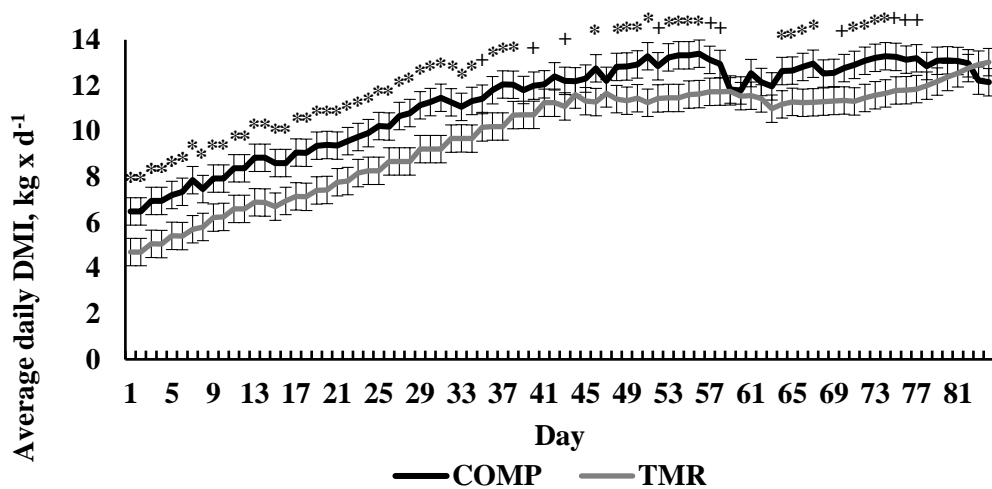


**Figure 2.1: Digestive morbidity of Angus bulls developed on one of two iso-caloric rations differing in form<sup>1,2</sup>**

<sup>1</sup>Diets consisted of a component-based feeding system that included a concentrate-based pellet and processed cereal rye hay fed separately (COMP; n = 24) or a total mixed ration consisting of dry-rolled corn, processed mixed grass hay, corn silage, dried corn gluten feed, and a micro-ingredient premix (TMR; n = 24) which were fed throughout an 84-d development period.

<sup>2</sup>Incidences of digestive morbidity were recorded when observed.

<sup>a,b</sup>Bars without a common letter differ ( $\chi^2 = 5.93$ ;  $P < 0.01$ ).



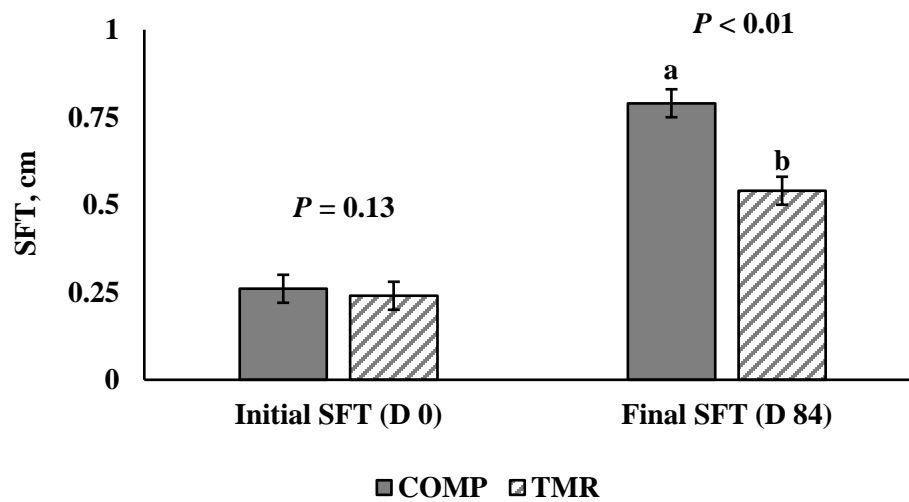
**Figure 2.2: Average daily DMI of Angus bulls developed on one of two iso-caloric rations differing in form<sup>1</sup>**

<sup>1</sup>Rations consisted of a component-based feeding system that included a concentrate-based pellet and processed cereal rye hay fed separately (COMP; n = 24) or a total mixed ration consisting of dry-rolled corn, processed mixed grass hay, corn silage, dried corn gluten feed, and a micro-ingredient premix (TMR; n = 24) fed throughout an 84-d development period.

\*Denotes least square means that differ ( $P < 0.05$ ).

+Denotes least square means that tend to differ ( $P < 0.10$ ).



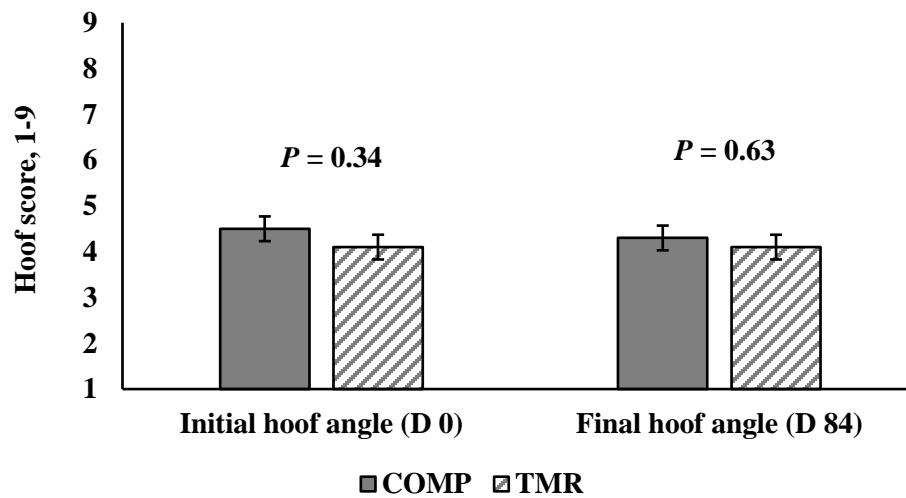


**Figure 2.3: Initial and final SFT of Angus bulls developed on one of two iso-caloric rations differing in form<sup>1,2</sup>**

<sup>1</sup>12<sup>th</sup>-rib subcutaneous fat thickness (SFT) was measured via ultrasound on d 0 (initial) and 84 (final).

<sup>2</sup>Diets consisted of a component-based feeding system that included a concentrate-based pellet and processed cereal rye hay fed separately (COMP; n = 24) or a total mixed ration consisting of dry-rolled corn, processed mixed grass hay, corn silage, dried corn gluten feed, and a micro-ingredient premix (TMR; n = 24) fed throughout an 84-d development period.

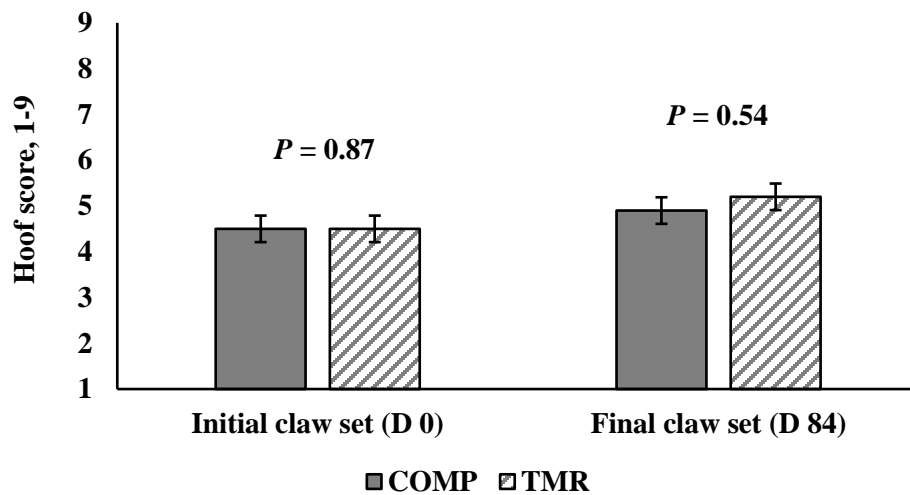
<sup>a,b</sup>Bars within a comparison without common letters differ ( $P < 0.05$ ).



**Figure 2.4: Initial and final hoof angle of Angus bulls developed on one of two iso-caloric rations differing in form<sup>1,2</sup>**

<sup>1</sup>Hoof angle was assessed on d 0 and 84 of an 84-d feeding trial using a scale of 1-9 scale as previously described by Jeyaruban et al. (2012) with scores of 5-6 considered to be ideal.

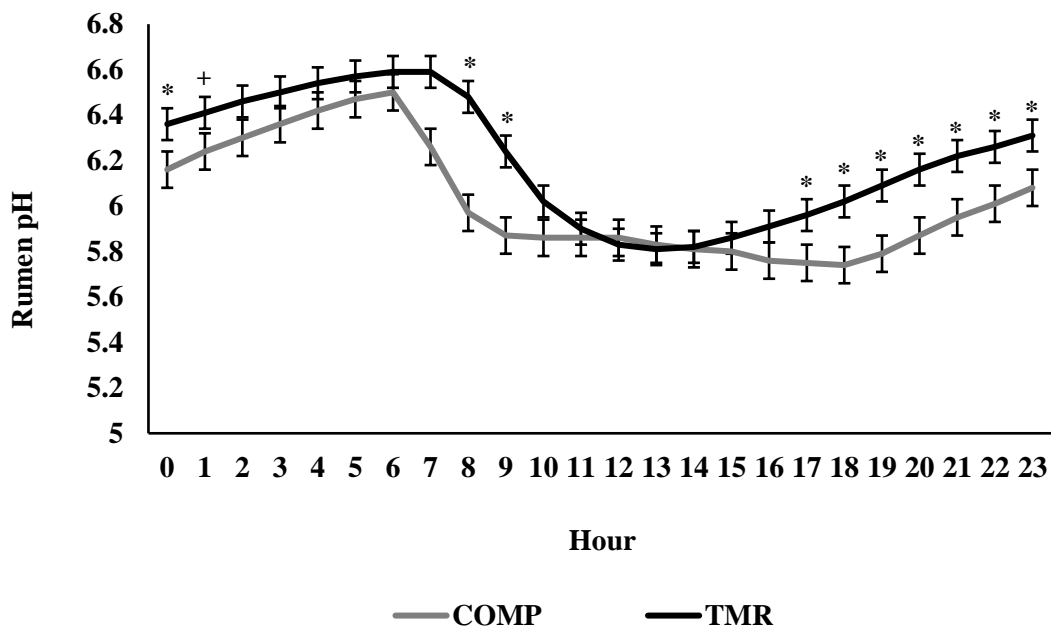
<sup>2</sup>Diets consisted of a component-based feeding system that included a concentrate-based pellet and processed cereal rye hay fed separately (COMP; n = 24) or a total mixed ration consisting of dry-rolled corn, processed mixed grass hay, corn silage, dried corn gluten feed, and a micro-ingredient premix (TMR; n = 24) fed throughout an 84-d development period.



**Figure 2.5: Initial and final claw set of Angus bulls developed on one of two iso-caloric rations differing in form<sup>1,2</sup>**

<sup>1</sup>Claw set was assessed on d 0 and 84 of an 84-d feeding trial using a scale of 1-9 as previously described by Jeyaruban et al. (2012), with scores of 5-6 considered ideal.

<sup>2</sup>Rations consisted of a component-based feeding system that included a concentrate-based pellet and processed cereal rye hay fed separately (COMP; n = 24) or a total mixed ration consisting of dry-rolled corn, processed mixed grass hay, corn silage, dried corn gluten feed, and a micro-ingredient premix (TMR; n = 24) which were fed throughout an 84-d development period.



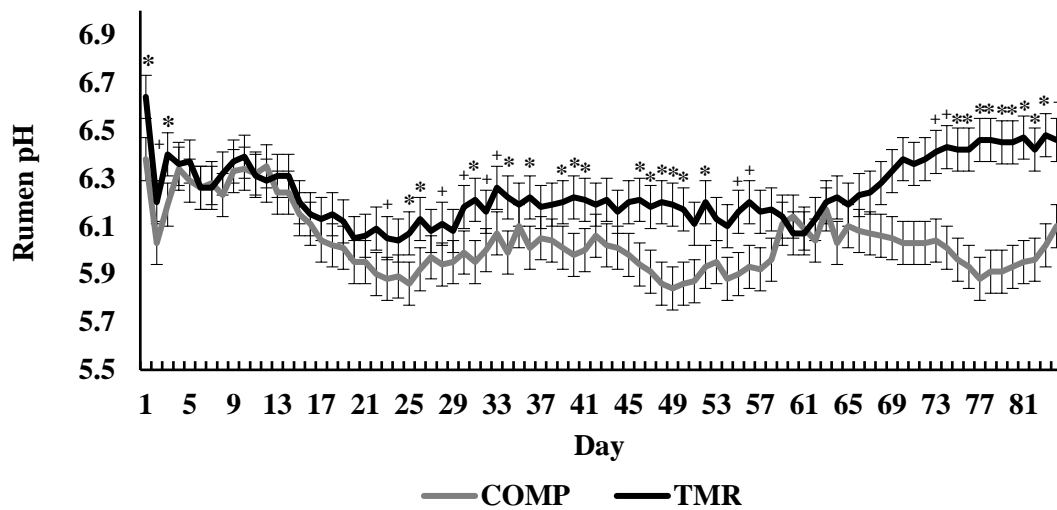
**Figure 2.6: Average hourly rumen pH of Angus bulls developed on one of two iso-caloric rations differing in form<sup>1,2</sup>**

<sup>1</sup>Rumen pH was continuously measured via rumen bolus (eBolus; eCow, Devon, UK) at 15-min intervals throughout an 84-d feeding trial.

<sup>2</sup>Diets consisted of a component-based feeding system that included a concentrate-based pellet and processed cereal rye hay fed separately (COMP; n = 24) or a total mixed ration consisting of dry-rolled corn, processed mixed grass hay, corn silage, dried corn gluten feed, and a micro-ingredient premix (TMR; n = 24) fed throughout an 84-d development period.

\*Denotes least square means that differ ( $P < 0.05$ ) within an hour.

†Denotes least square means that tend to differ ( $P < 0.10$ ) within an hour.



**Figure 2.7: Average daily rumen pH of Angus bulls developed on one of two iso-caloric rations differing in form<sup>1,2</sup>**

<sup>1</sup>Rumen pH was continuously measured via rumen bolus (eBolus; eCow, Devon, UK) at 15-min intervals throughout an 84-d feeding trial.

<sup>2</sup>Diets consisted of a component-based feeding system that included a concentrate-based pellet and processed cereal rye hay fed separately (COMP; n = 24) or a total mixed ration consisting of dry-rolled corn, processed mixed grass hay, corn silage, dried corn gluten feed, and a micro-ingredient premix (TMR; n = 24) fed throughout an 84-d development period.

\*Denotes least square means that differ ( $P < 0.05$ ) within a day.

+Denotes least square means that tend to differ ( $P < 0.10$ ) within a day.

**CHAPTER 3: Effects of semen collection method on semen quality of  
developing bulls**

## Abstract

Breeding soundness examinations (BSE) are commonly utilized to provide an objective indication of sire fertility. While semen collection method is thought to influence BSE outcomes, little evidence supports the use of one method over another. Therefore, the objective of this experiment was to quantify the effects of two common semen collection methods on semen quality, and test the hypothesis that semen quality and BSE outcomes are affected by semen collection method. Angus bulls ( $n = 48$ ;  $332 \pm 47$  days of age) enrolled in an 84-day development program were randomly assigned to one of two initial collection methods [transrectal massage (RM;  $n = 24$ ) or electro-ejaculation (EE;  $n = 24$ )] performed by a trained technician. Semen was collected on day 21 utilizing the initial assigned method. Thereafter, semen collection method was alternated within bull for each of three proceeding semen collections that occurred on days 42, 63, and 84 ( $416 \pm 47$  days of age). Duration of each collection procedure was standardized at 120 seconds of trans-rectal stimulation for RM and 15 seconds of pulsed electro-stimulation utilizing a programmed electro-ejaculator for EE. Immediately following collection, progressive motility was determined via phase-contrast microscopy. Duplicate slides were prepared for morphological analysis and analyzed in duplicate by each of three technicians. Semen samples were weighed and total sperm cells were quantified via photometry using a standard sample volume, and expressed as total sperm cells per ejaculate. Data were analyzed using JMP 13.0. Electro-ejaculation resulted in collection of less total volume ( $P < 0.01$ ) and fewer total sperm in the ejaculate ( $P < 0.05$ ), but a greater percentage of progressively motile sperm ( $P < 0.01$ ) when compared to RM. Electro-ejaculation also decreased the percentage of abnormal sperm ( $P < 0.05$ ), which corresponded with an increased percentage of normal sperm ( $P < 0.05$ ) when compared to RM. Semen collected via RM resulted in more

total abnormal sperm cells ( $P < 0.01$ ) when compared to EE but neither semen collection method affected total normal sperm cells ( $P = 0.54$ ) or the likelihood of BSE failure ( $X^2 = 0.92$ ;  $P = 0.34$ ). Based upon these findings, semen collection method should not be expected to influence BSE outcomes.

## **Introduction**

Breeding soundness examinations (BSE) are a multi-faceted assessment of the ability of a bull to successfully breed females throughout a breeding season. Semen evaluations are a necessary entity of such examinations and provide an objective indication of fertility. Semen is generally collected via artificial vagina, transrectal massage, or electroejaculation. Use of the artificial vagina was common prior to the invention of the electroejaculator, however management, time, and labor restrictions have limited its common use during BSE in modern beef cattle production (Salisbury and VanDemark, 1961; Palmer, 2005). Transrectal massage has become a popular alternative method to electroejaculation in response to welfare concerns (Mosure et al., 1998). However, anecdotal evidence across the industry suggests that method of semen collection contributes to variation in semen quality of bulls, and thus potentially influences BSE outcomes. While there is limited scientific evidence to support such claims, Palmer et al. (2005) found no differences in semen quality of semen collected from 15-month-old bulls via electroejaculation or transrectal massage. However, it was denoted that the results may have been influenced by electroejaculation which occurred immediately following transrectal massage, with no replication of semen collection over time. Moreover, quantification of semen quality is crucial to bull development programs as objective indicators of fertility provide information to both buyers and sellers that can be used to assess the potential reproductive performance and longevity of a sire.



Failure of a breeding soundness examination in a recently developed bull is costly to the beef cattle producer and it is unclear if outcomes are influenced by semen collection method. Therefore, an experiment was conducted under the hypothesis that semen collection method (electroejaculation or transrectal massage) impacts semen quality of yearling bulls. The objectives of this study were to evaluate the effects of two common semen collection methods (electroejaculation and transrectal massage) on semen quality in developing bulls, and determine if semen collection method influences the probability of breeding soundness examination passage or failure.

## **Materials and methods**

This experiment and the procedures reported herein were approved by and conducted in accordance with the University of Tennessee Institutional Animal Care and Use Committee. Additionally, this experiment adhered to standards delineated by the Federation of Animal Science Societies Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010).

### **Experimental design and animal management**

Angus bulls ( $n = 48$ ) with an initial age of  $332 \pm 47$  d and weight of  $377 \pm 61$  kg were transported 360 km from Deer Valley Farms in Fayetteville, TN to the East Tennessee Research and Education Center – Blount Unit in Alcoa, TN. Upon arrival, bulls were stratified into one of sixteen replicated dry-lot pens and fed one of two iso-caloric and iso-nitrogenous diets with *ad libitum* access to water. Bulls were then randomly assigned in a completely randomized design to one of two initial semen collection methods: transrectal massage (RM;  $n = 24$ ) or electroejaculation (EE;  $n = 24$ ). Bulls were developed over the course of an 84-d feeding period

with a final age of  $416 \pm 47$  d and weight of  $569 \pm 50$  kg. Breeding soundness examinations were conducted at 21-d intervals and semen was first collected on d 21 utilizing the initial assigned semen collection method. Samples were collected on one of two paired days within each collection period and each bull was processed through the working facility each day. Each collection day, semen was collected via RM ( $n = 12$ ) and EE ( $n = 12$ ) and the remaining bulls within each semen collection method were collected the following day. Thereafter, semen collection method and d of collection within the sampling period were alternated with subsequent BSE conducted on d 42, 63, and 84. This resulted in semen collection occurring twice for both EE and RM within each bull over the course of the experiment.

### **Sampling and measurements**

Guidelines for BSE were established by Koziol and Armstrong (2018) (approved by the Society for Theriogenology for 2019) and included a physical examination including hoof, eye, penile, reproductive tract, and testicular structure and health. As a part of the BSE, scrotal circumference (SC) was measured as described by Barth (2007) where a trained technician first grasped the scrotal neck and pushed the testicles into the ventral portion of the scrotal sac before the testicles were palpated and visually assessed for health and normal structure. An 80-cm inelastic scrotal tape was then secured around the scrotum and tightened three times to ensure an appropriate fit, and the SC was recorded.

Following scrotal evaluation, EE-collected bulls were rectally-palpated by a trained technician for removal of fecal material from the rectum, examination of the reproductive tract, and to sexually stimulate the bulls. It should be noted that sexual stimulation via rectal palpation has been shown to have no effect on time to penile protrusion and facilitation of ejaculation (Koziol and Moor, 2018), and thus should not be expected to influence semen quality parameters

in the current study. Electroejaculation was then accomplished utilizing a 60-mm probe with two ventrally-located electrodes powered by a programmed electroejaculator (Pulsator V; Lane Manufacturing, Denver, CO). All RM collections were facilitated by a trained technician that used a rectal palpation method adapted from Palmer et al. (2005). In brief, pressure was initially applied to the ampullae, followed by repeated application of sustained cranial to caudal pressure using a raking motion in concert with contractions of the urethralis muscle in order to facilitate semen emission and ejaculation. If EE semen collection was not accomplished via the manufacturer-programmed setting of the electroejaculator, a manual setting on the electroejaculator was utilized, or RM if manual electroejaculation was unsuccessful. Similarly, if RM semen collection was unsuccessful in bulls assigned to RM, EE was utilized. If semen collection was attempted three times without success, bulls were considered a no-collect.

Semen was collected into a 15-mL disposable vial attached to a plastic disposable director cone which was placed within a semen collection handle. If ambient temperatures were low (approximately  $< 21^{\circ}\text{C}$ ), semen collection vials were submerged into warmed water within the jacket of the semen collection handle to prevent the sample from chilling. Semen collection began upon the appearance of white or cloudy pre-ejaculatory seminal fluid which indicated the presence of spermatozoa. Once collection was initiated, collection time was standardized at 15 s for EE and 120 s for RM. The purpose for the standardized collection times was to ensure an accurate analysis of semen output and quality parameters between collection method.

Immediately following collection, semen samples were translocated to a chute-side laboratory for semen analysis. Percentage progressive motility was quantified by a board certified theriogenologist utilizing light-phase microscopy (EVOS XL Core; ThermoFisher Scientific, Waltham, MA) at 10x magnification. A drop (approximately 60  $\mu\text{L}$ ) of the semen

sample was placed onto a pre-warmed slide, covered with a cover slip, and placed onto a pre-warmed stage for analysis. Progressive motility was determined as the estimated percentage of spermatozoa traveling into and leaving the objective field of view in a linear path. If semen samples were concentrated to the degree where linear movement of individual spermatozoon could not be evaluated, a drop of pre-warmed phosphate-buffered saline was placed directly onto a drop of the semen sample as a diluent.

Semen samples were weighed on certified scales (TC-204; Denver Instrument Company, Arvada, CO) to the nearest 0.0001 g and converted to mL based on a semen volume conversion factor of 1.053 g x mL<sup>-1</sup> of semen (Kocks and Broekhuijse, 2014). Concentration of spermatozoa (spermatozoa x mL<sup>-1</sup> of semen) was determined via light photometry (SpermaQ-II; MOFA Global, Madison, WI) in duplicate. Concentration of spermatozoa was then multiplied by calculated semen volume to determine the total number of spermatozoa contained within the ejaculate.

Slides were then prepared in duplicate for morphological analysis utilizing an eosin-nigrosin smear, and stored at ambient temperature prior to analysis at a later date. Morphology of spermatozoa was assessed at 40x magnification using an inverted function of the previously-described microscope. A drop of immersion oil was placed on each slide and the 40x objective was immersed in the droplet before brightness and focus were adjusted as necessary. Morphological analysis of each duplicated slide was conducted by each of three trained technicians, resulting in a total of six morphology counts x ejaculate<sup>-1</sup>. One hundred spermatozoa were counted for each morphological evaluation, which were each classified as morphologically-normal or -abnormal spermatozoa, and counts were averaged across technicians for each ejaculate. Additionally, abnormal spermatozoa were sub-classified on a percentage basis as

either head-, midpiece-, or tail-defected spermatozoa, in accordance with the guidelines established by Koziol and Armstrong (2018). Intra- and inter-slide coefficients of variation (CV) for morphologically-normal spermatozoa were 9.8% and 15.0%, respectively. Once percentages of morphologically-normal and -abnormal spermatozoa were quantified, the total number of morphologically-normal, morphologically-abnormal, and specific-defected spermatozoa x ejaculate<sup>-1</sup> were calculated by multiplying the respective fractional percentage by the total number of spermatozoa x ejaculate<sup>-1</sup>.

Results of each BSE were assigned following a physical examination and quantification of semen quality parameters. The guidelines set forth by Koziol and Armstrong (2018) were strictly adhered to, with semen quality requirements including a minimum of 30% progressively motile spermatozoa and 70% morphologically-normal spermatozoa. Bulls were classified as either satisfactory or unsatisfactory.

### **Statistical analyses**

Statistical analyses were performed using JMP 13.0 (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was conducted using the Fit Model procedure to determine the main effect of semen collection method on all continuous response variables. Bull served as the experimental unit for all response variables. Day of collection and individual bull accounted for a significant portion ( $P < 0.01$ ) of the variation in each response variable, and thus were included in the model as blocking factors. Continuous data were assessed for normality and equality of variance by visually evaluating plots of the residual vs. predicted values. No outliers were observed, and all continuous data appeared to be normally distributed. Additionally, logistic regression was conducted to determine the main effect of collection method on the likelihood of BSE outcome. One bull was removed from the project following d 42 sample collections due to

reasons unrelated to the experiment. Furthermore, if semen was not collected via the intended collection method, data from the individual bull within that sampling day was excluded from analysis. Differences between least square means and logistic regression models were considered statistically significant if  $P < 0.05$ , and considered to have a tendency toward significance if  $0.05 \leq P < 0.10$ .

## **Results and discussion**

### **SC, semen collection volume, and total spermatozoa output**

Least square means of SC, semen collection volume, and total sperm output are reported in Table 3.1. Scrotal circumference was unaffected (Table 3.1;  $P = 0.35$ ) by semen collection method, suggesting that any influence of scrotal mass on semen output or quality was likely similar across semen collection methods. Therefore, observed differences in semen quality can be assumed to be a direct result of collection method and not influenced by parenchymal tissue mass.

Method of collection influenced semen volume, as the ejaculate of RM bulls contained nearly twice the volume of EE bulls ( $P < 0.01$ ). While the present experiment did not quantify penile protrusion rates, a limited number of RM-collected bulls achieved full erection and relaxation of the retractor penis muscle to completely protrude the penis from the sheath, which is similar to the observation of Palmer et al. (2005). While limited penile protrusion from RM not only limits the ability to visualize the penis for completion of the BSE, it may also offer an explanation for the observed increase in semen volume. Contents of the preputial pouch are generally expelled as the penis protrudes from the sheath. This process limits the amount of contaminants, such as urine, bacteria, or other preputial fluids that may collect within the

ejaculate. Thus, it is likely that preputial fluid made a large contribution to the total volume of the ejaculate in RM collections, and to a much lesser degree to EE collections. Additionally, ejaculation in the beef bull is initiated from neural hypothalamic stimulation. Hypothalamic neurons directly innervate the smooth muscle surrounding the prostate gland and male reproductive tract. Stimulation of these muscle groups is expected to influence production of seminal plasma by the accessory sex glands. Once ejaculation is initiated in the bull, a neuroendocrine reflex is activated by stimuli on afferent nerves leading to the paraventricular region of the hypothalamus, signaling oxytocin release from the posterior lobe of the pituitary gland. Oxytocin receptors are present throughout the reproductive tract in the male and have been found to be present on the seminal vesicles (Filippi et al., 2002) and on the prostate of the man, guinea pig, dog, and rat (Bodanszky et al., 1992). Therefore, it is likely that oxytocin contributes to stimulation and secretion of seminal fluid from the accessory sex glands in the bull. Previous research by Sharma and Hays (1973) found that oxytocin release just minutes after stimulation from RM ranged from 2-640  $\mu\text{U}/\text{plasma}$  with a range of 7-280  $\mu\text{U}/\text{plasma}$  in EE-stimulated bulls. While this data is variable between each bull, it suggests that RM has the potential to elicit higher responses of oxytocin release when compared to EE. Moreover, physical contact with the prostate and vesicular glands during RM may also stimulate the release of seminal fluid. Previous authors have shown that the seminal vesicles account for the greatest contribution to seminal plasma (Austin et al., 1961) and their increased stimulation may increase semen volume (Persson et al., 2007).

Ejaculation and the contribution of seminal fluid to total semen volume is multi-faceted and dependent upon several physiological processes. While specific physiologic mechanisms were not evaluated in this study, it is possible that RM may elevate neural reflexes and oxytocin

to hyper-stimulate the accessory sex glands. It remains in question as to why RM may increase neural stimulation of the accessory sex glands but not elicit penile erection and protrusion. It is possible that the nonadrenergic, noncholinergic parasympathetic neurons responsible for penile erection are stimulated directly from electrical current from EE, which is absent during RM. This theory remains to be investigated. Nonetheless, it is postulated that direct neural stimulation and oxytocin release, in concert with an increase in preputial fluid led to the increase in semen volume during RM collections that were observed in the present experiment.

Semen from bulls collected by RM contained approximately 25 % more total spermatozoa ( $P < 0.05$ ) than that of bulls collected by EE. Similarly to semen volume, variation in total sperm output may be linked to neural stimulus and the influence of preputial fluid. As the tail of the epididymis reaches storage capacity, spermatozoa are released into the ductus deferens to be excreted in urine via the pelvic urethra. Therefore, the preputial pocket has the potential to serve as a collection site for spermatozoa suspended in urine, influencing both volume of the ejaculate and total sperm output. In addition, oxytocin has been shown to facilitate spermatozoa transport from the tail of the epididymis into the ductus deferens and pelvic urethra in the boar and ram (Maggi et al., 1987; Nicholson et al., 1999; Whittington et al., 2001) and it is likely that the bull expresses epididymal oxytocin receptors as well. Therefore, in the circumstance of elevated neural stimulation, oxytocin may increase smooth muscle contraction around the epididymis and increase total spermatozoa in the ejaculate. A series of afferent neurons exists in the rectal wall of the bull, which may be stimulated by both electrical and tactile stimuli (Peeters et al., 1983). Thus, tactile stimuli may lead to a stronger neuroendocrine response during RM semen collections. Based upon these proposed physiological mechanisms, it is possible that RM increased cauda epididymal contractions, and when coupled with suspension of spermatozoa in



the preputial fluid, may explain the differences in total spermatozoa output between collection methods.

### **Spermatozoa morphology and motility**

Least square means of semen volume, percentage progressive motility, percentages normal and abnormal spermatozoa, and the total number of spermatozoa within the ejaculate, along with the prevalence of morphologically-normal and -abnormal spermatozoa can be found in Table 3.1. Additionally, the prevalence and total number of classified morphologically-abnormal spermatozoa are reported in Table 3.2. Semen collected from EE bulls contained 5.2% more morphologically-normal spermatozoa ( $P < 0.05$ ), but a similar total number of morphologically-normal spermatozoa ( $P = 0.54$ ) when compared to RM-collected bulls, which may have contributed to the 6.0 % increase in progressive motility ( $P < 0.01$ ) that was observed for EE-collected bulls. This change in morphological abnormalities, and consequently progressive motility, may have been influenced by erection intensity and flow of ejaculate from the glans penis. Consistent with the report of Palmer et al. (2005), although not objectively measured, it was observed that ejaculates resulting from RM occurred as a continuous drip with a considerable amount of ejaculate remaining on the plastic director cone prior to dripping into the semen collection vial. Thus, any attempts to prevent chilling of the ejaculate with warm water in the collection handle were likely mitigated. As the penis fully extended, as it did in the majority of EE collections, it was observed that the glans penis was positioned near the opening of the semen collection vial, preventing long-term exposure to ambient temperatures. Additionally, semen collection time may have influenced exposure of semen to ambient temperatures. Collection times were standardized at 15 s for EE and 120 s for RM in order to promote consistency in evaluation of semen quality parameters. However, prolonged collection time for

RM likely increased exposure to ambient temperature, and possibly influenced measures of morphology and motility. Exposure to ambient temperature can cause rapid chilling of spermatozoa and lead to increased prevalence of midpiece and tail defects (Barth and Oko, 1989). While the percentage of midpiece-defected spermatozoa were similar between semen collection methods ( $P = 0.93$ ), semen collected by RM contained  $9.0 \times 10^7$  more midpiece-defected spermatozoa ( $P < 0.05$ ) than semen collected by EE. Conversely, both percentage ( $P = 0.61$ ) and number ( $P = 0.14$ ) of tail-defected spermatozoa were unaffected by semen collection method. Exposure to the preputial cavity may increase the instance of midpiece and tail defects in spermatozoa, driven by both temperature and pH of the preputial fluid (Barth and Oko, 1989). Although preputial contents were not analyzed in the present experiment, future research may offer insight into the influence of the preputial cavity and fluid on viability, morphology, and mobility of the spermatozoon. Interestingly, RM resulted in approximately a 24 % increase in percentage ( $P < 0.01$ ) and 55 % increase in number ( $P < 0.01$ ) of head-defected spermatozoa when compared to EE, which are generally considered to be a product of irregular spermatogenesis (Barth and Oko, 1989), and thus should be unaffected by collection method. It is our speculation that the increase in head-defected spermatozoa is a function of the drastic increase in total sperm output during RM, and the physiological cause, if any, is not known.

Variation in percentage progressive motility between EE and RM is likely highly influenced by alterations in spermatozoa morphology and the incidence of cold shock. As previously suggested, spermatozoa stored in the preputial pocket would be expected to undergo morphological changes that coincide with reduced motility as a result of damage to flagella. Additionally, cold shock is thought to reduce spermatozoa progressive motility and may affect analysis of progressive motility as a facet of the BSE (Barth and Oko, 1989). While semen

volume and fluid dynamics likely contribute to progressive motility, the impacts of composition of seminal fluid and fluid dynamics on progressive motility were not evaluated as part of the objectives within the current study. Further efforts are warranted to assess the influence of cold shock and preputial fluid on estimates of motility in order to provide an adjustment factor that could be applied to ejaculates collected via RM.

## **BSE**

In total, there were 91 successful semen collections for EE and 92 successful semen collections for RM. Of the RM collections, 56.5 % resulted in failure of the BSE compared to 50.5 % for EE. For both RM and EE collections, 100 % of bulls that failed a BSE had percentage morphologically-normal spermatozoa below the passing threshold, yet 2.1 % of RM collections resulted in percentage progressive motility below passing threshold compared to 1.1 % for EE. It should be noted that no bulls failed a BSE due to physical examination. Semen collection method did not influence the likelihood of the outcome of a BSE ( $P = 0.34$ ;  $X^2 = 0.92$ ) in the present experiment. Although statistically-significant differences that can be attributed to semen collection method were observed regarding morphology and progressive motility, the small magnitude of these differences was not sufficient to influence the likelihood of a particular BSE outcome when adhering to the criteria defined by Koziol and Armstrong (2018). While BSE outcomes were unaffected by semen collection method in the current study, ejaculates obtained via RM clearly contained more total spermatozoa and a lower percentage of morphologically-normal spermatozoa in the ejaculate, yet a similar total number of morphologically-normal spermatozoa. Consequently, regardless of collection method, if a BSE is conducted using current guidelines that require reaching a certain threshold for percentage of morphologically-normal spermatozoa, bulls with lower percentages of morphologically-normal spermatozoa risk failing.

However, evaluating spermatozoa morphology based upon a threshold for total number of morphologically-normal spermatozoa may reduce the risk of falsely identifying a bull as sub-fertile. Further research is warranted to identify metrics that can be used to most accurately and objectively assess the morphological aspects of the BSE.

## **Conclusions**

Semen collected via RM had a greater volume, total number of spermatozoa, percentage morphologically-abnormal spermatozoa, and total number of morphologically-abnormal spermatozoa than semen collected via EE, which corresponded with a lower percentage of morphologically-normal spermatozoa and progressive motility. Despite these effects, method of semen collection did not influence the likelihood of BSE results in the yearling bulls included in this experiment. Decreases in semen quality reported herein may be linked to low instances of full erection and penile protrusion from the sheath during RM collections, which is likely influenced by neural stimuli, or a lack thereof. While RM negatively affected certain parameters of semen quality, these effects were accompanied by an increase in the total number of morphologically-normal spermatozoa. Additionally, emphasis that is currently placed on percentage of morphologically-normal spermatozoa neglects compensability that may be attributed to a greater total number of morphologically-normal spermatozoa delivered through an ejaculate. This use of a percentage threshold may result in a bull failing the BSE that may have greater fertility than a bull that passes, and warrants further investigation. Nonetheless, semen collection method should not be expected to influence BSE outcomes when conducted in accordance with current guidelines, but may impact semen quality parameters to a small degree.

## References

- Austin, J. W., E. Hupp, and R. Murphree. 1961. Comparison of quality of bull semen collected in the artificial vagina and by electroejaculation. *Journal of Dairy Science* 44(12):2292-2297. doi: [https://doi.org/10.3168/jds.S0022-0302\(61\)90057-1](https://doi.org/10.3168/jds.S0022-0302(61)90057-1)
- Barth, A. D. 2007. Evaluation of potential breeding soundness of the bull, Current therapy in large animal Theriogenology. Elsevier. p. 228-240.
- Barth, A. D., and R. Oko. 1989. Abnormal morphology of bovine spermatozoa. Iowa State University Press, Ames, IA.
- Bodanszky, M., H. Sharaf, J. B. Roy, and S. I. Said. 1992. Contractile activity of vasotocin, oxytocin, and vasopressin on mammalian prostate. *European journal of pharmacology* 216(2):311-313. doi: [https://doi.org/10.1016/0014-2999\(92\)90376-F](https://doi.org/10.1016/0014-2999(92)90376-F)
- FASS. 2010. Federation of Animal Science Societies: Guide for the Care and Use of Agricultural Animals in Research and Teaching.
- Filippi, S., G. Vannelli, S. Granchi, M. Luconi, C. Crescioli, R. Mancina, A. Natali, S. Brocchi, L. Vignozzi, and E. Bencini. 2002. Identification, localization and functional activity of oxytocin receptors in epididymis. *Molecular and cellular endocrinology* 193(1-2):89-100. doi: [https://doi.org/10.1016/S0303-7207\(02\)00101-6](https://doi.org/10.1016/S0303-7207(02)00101-6)
- Kocks, J. E., and M. L. Broekhuijse. 2014. The practical use of density of whole bull semen to determine the volume of an ejaculate at AI laboratories. *Animal reproduction science* 146(3-4):98-102. doi: [10.1016/j.anireprosci.2014.02.015](https://doi.org/10.1016/j.anireprosci.2014.02.015)
- Koziol, J. H., and C. L. Armstrong. 2018. Society for Theriogenology Manual for Breeding Soundness Examination of Bulls.

- Koziol, J. H., and G. E. Moor. 2018. Transrectal massage of the accessory glands in bulls prior to EEJ does not affect interval to penile extension and ejaculation. *Clinical Theriogenology* 10(2)
- Maggi, M., S. Malozowski, S. Kassis, V. Guardabasso, and D. Rodbard. 1987. Identification and characterization of two classes of receptors for oxytocin and vasopressin in porcine tunica albuginea, epididymis, and vas deferens. *Endocrinology* 120(3):986-994. doi: <https://doi.org/10.1210/endo-120-3-986>
- Mosure, W. L., R. A. Meyer, J. Gudmundson, and A. D. Barth. 1998. Evaluation of possible methods to reduce pain associated with electroejaculation in bulls. *The Canadian Veterinary Journal* 39(8):504-506 p. 779
- Nicholson, H., T. Parkinson, and K. Lapwood. 1999. Effects of oxytocin and vasopressin on sperm transport from the cauda epididymis in sheep. *Reproduction* 117(2):299-305. doi: <https://doi.org/10.1530/jrf.0.1170299>
- Palmer, C., L. Brito, A. Arteaga, L. Söderquist, Y. Persson, and A. Barth. 2005. Comparison of electroejaculation and transrectal massage for semen collection in range and yearling feedlot beef bulls. *Animal reproduction science* 87(1-2):25-31. doi: <https://doi.org/10.1016/j.anireprosci.2004.09.004>
- Palmer, C. W. 2005. Welfare aspects of theriogenology: Investigating alternatives to electroejaculation of bulls. *Theriogenology* 64(3):469 - 479. doi: <https://doi.org/10.1016/j.theriogenology.2005.05.032>
- Peeters, G., J. Legros, C. Piron-Bossuyt, R. Reynaert, R. V. Driessche, and E. Vannieuwenhuysse. 1983. Release of neurophysin I and oxytocin by stimulation of the genital organs in bulls. *Journal of endocrinology* 99(2):161-171. doi: <https://doi.org/10.1677/joe.0.0990161>

- Persson, Y., G. Strid, M. Håård, and L. Söderquist. 2007. Comparison of semen samples collected from beef bulls by transrectal massage or artificial vagina. *Veterinary Record* 161(19):662. doi: 10.1136/vr.161.19.662
- Salisbury, G. W., and N. L. VanDemark. 1961. Physiology of reproduction and artificial insemination of cattle. *Physiology of reproduction and artificial insemination of cattle.*: p. 779.
- Sharma, O., and R. Hays. 1973. Release of an oxytocic substance following genital stimulation in bulls. *Reproduction* 35(2):359-362. doi: <https://doi.org/10.1530/jrf.0.0350359>
- Whittington, K., S. Assinder, T. Parkinson, K. Lapwood, and H. Nicholson. 2001. Function and localization of oxytocin receptors in the reproductive tissue of rams. *REPRODUCTION-CAMBRIDGE-* 122(2):317-325. doi: 10.1530/rep.0.1220317

## Appendix C: Tables

Table 3.1: Least square means of SC and semen quality parameters<sup>1</sup>

| Item <sup>2</sup>  | EE                | RM                | Pooled SEM | <i>P</i> – value |
|--|-------------------|-------------------|------------|------------------|
| SC, cm   | 36.4              | 36.3              | 0.1        | 0.35             |
| Volume, mL   | 4.7 <sup>a</sup>  | 8.0 <sup>b</sup>  | 0.4        | < 0.01           |
| Progressive motility, % of total                                       | 53.0 <sup>a</sup> | 47.0 <sup>b</sup> | 1.3        | < 0.01           |
| Normal spermatozoa, % of total   | 61.0 <sup>c</sup> | 55.8 <sup>d</sup> | 1.5        | < 0.05           |
| Abnormal spermatozoa, % of total                                       | 39.0 <sup>c</sup> | 44.2 <sup>d</sup> | 1.5        | < 0.05           |
| Total sperm output, 10 <sup>9</sup> x ejaculate <sup>-1</sup>          | 2.37 <sup>c</sup> | 2.97 <sup>d</sup> | 0.18       | < 0.05           |
| Total normal sperm output, 10 <sup>9</sup> x ejaculate <sup>-1</sup>   | 1.45              | 1.66              | 0.13       | 0.54             |
| Total abnormal sperm output, 10 <sup>9</sup> x ejaculate <sup>-1</sup> | 0.92 <sup>a</sup> | 1.31 <sup>b</sup> | 0.09       | < 0.01           |

<sup>1</sup>Semen was collected via electroejaculation (EE) or trans-rectal massage (RM) in feedlot developed bulls (n = 48) and semen was collected twice using each collection method technique. Semen collections occurred on d 21, 42, 63, and 84 throughout an 84-d development period.

<sup>2</sup>Scrotal circumference (SC) was measured utilizing an 80-cm inelastic tape. Semen volume calculated based on an adjustment factor (Kocks and Broekhuijse, 2014). One of two trained technicians assessed progressive motility which was defined as a linear movement of spermatozoa into and out of the objective field of view. Percentages morphologically-normal and -abnormal spermatozoa were determined from spermatozoa morphological analysis which was averaged across 100 cell counts on duplicated slides by each of three trained technicians. Total spermatozoa x ejaculate<sup>-1</sup> was calculated from spermatozoa concentration x mL<sup>-1</sup> (SpermaQ-II; MOFA Global, Madison, WI) and multiplied by semen volume. Percentages morphologically-normal and -abnormal spermatozoa were used along with metrics of total spermatozoa x ejaculate<sup>-1</sup> to quantify total morphologically-normal and – abnormal spermatozoa x ejaculate<sup>-1</sup>.

<sup>a,b</sup>Least square means without a common superscript differ (*P* < 0.01).

<sup>c,d</sup>Least square means without a common superscript differ (*P* < 0.05).



Table 3.2: Least square means of individual classifications of morphological abnormalities<sup>1</sup>

| Item <sup>2</sup>  | EE                | RM                | Pooled SEM | <i>P</i> – value |
|--|-------------------|-------------------|------------|------------------|
| Head defects, % of total spermatozoa   | 21.5 <sup>b</sup> | 26.7 <sup>a</sup> | 1.2        | < 0.01           |
| Midpiece defects, % of total spermatozoa                                       | 16.7              | 16.6              | 1.0        | 0.93             |
| Tail defects, % of total spermatozoa   | 0.8               | 0.9               | 0.2        | 0.61             |
| Total head-defected spermatozoa, 10 <sup>9</sup> x ejaculate <sup>-1</sup>     | 0.51 <sup>b</sup> | 0.79 <sup>a</sup> | 0.06       | < 0.01           |
| Total midpiece-defected spermatozoa, 10 <sup>9</sup> x ejaculate <sup>-1</sup> | 0.40 <sup>d</sup> | 0.49 <sup>c</sup> | 0.04       | < 0.05           |
| Total tail-defected-spermatozoa, 10 <sup>9</sup> x ejaculate <sup>-1</sup>     | 0.02              | 0.03              | 0.01       | 0.14             |

<sup>1</sup>Semen was collected via electroejaculation (EE) or trans-rectal massage (RM) in feedlot-developed bulls (n = 48) and semen was collected twice using each collection method technique. Semen collections occurred on d 21, 42, 63, and 84 throughout an 84-d development period.

<sup>2</sup>Percentages morphologically head-, midpiece-, and tail-defected spermatozoa were determined from spermatozoa morphological analyses which were averaged across 100 cell counts on duplicated slides by each of three trained technicians. Percentages morphologically head-, midpiece-, and tail-defected spermatozoa were used along with metrics of total spermatozoa x ejaculate<sup>-1</sup> to quantify total morphologically head-, midpiece-, and tail-defected spermatozoa x ejaculate<sup>-1</sup>.

<sup>a,b</sup>Least square means without a common superscript differ (*P* < 0.01).

<sup>cd</sup>Least square means without a common superscript differ (*P* < 0.05).

## **CHAPTER 4: Conclusion**

## Conclusions and future research

Beef bulls are a valuable commodity in the beef cattle industry, as they have the ability to advance herd genetics and ultimately increase the profitability of beef cattle producers. Nutritional management of bulls is challenging as prolonged development is costly, yet rapid development has long been thought to induce scrotal adiposity or SARA that may reduce semen quality. However, in a real-world bull development scenario, it does not seem that rations differing in form or rumen conditions indicative of SARA reduce semen quality in developing bulls. Further research is warranted in the area of bull development to determine the optimal degree of adiposity in bulls to both maximize semen quality parameters and ensure success throughout a breeding season. Additionally, the influence of semen volume and energy substrates within the ejaculate may be influenced by energy content of the ration and rumen conditions, and the subsequent impacts on the spermatozoon merit further research.

Evaluation of semen quality as a facet of the BSE is an objective evaluation that determines a bull's preparedness to perform successfully throughout the breeding season. It has been previously thought that semen collection method impacts BSE results in bulls. However, neither electroejaculation nor trans-rectal massage influenced the outcomes of a breeding soundness examination in bulls. However, trans-rectal massage resulted in a greater amount of morphologically-normal spermatozoa, although a decreased percentage of morphologically-normal spermatozoa. The current guidelines for a BSE place emphasis on percentages rather than amounts of morphologically-normal spermatozoa, and may increase the risk of a bull being classified as sub-fertile. Discovering a threshold for an acceptable amount of morphologically-normal spermatozoa warrants further investigation to improve objective measures of fertility in bulls.

## **VITA**

Jarret Allen Proctor was born in New Castle, Indiana on March 3, 1996 to Joe and Laura Proctor. Jarret grew up on a family-operated row crop operation and was actively involved in youth livestock projects. His early interests and love for livestock led him to pursue his Bachelor of Science degree in Animal Sciences from Purdue University in West Lafayette, Indiana. While there, he developed an appreciation for meat-animal research and elected to pursue his Master of Science degree in Nutritional Physiology and Bull Development from the University of Tennessee.