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STRESS, DNA METHYLATION, AND THEIR IMPLICATIONS IN CATTLE PRODUCTION

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Summary

Epigenetic modifications such as DNA methylation may be one of the ways that environmental stressors manifest on a biological level and affect beef cattle performance. Calves born from dams who experienced transportation stress exhibited differential methylation patterns at 28 days of age in genes active in immune response and behavior. However, minimal differences were present at 5 years of age. Although the methylation changes did not persist over time, critical developmental periods could still be influenced by such alterations. By understanding and accounting for stress-induced methylation changes, more accurate estimations of heritability and genetic merit can be achieved. In addition to its role in understanding the impact of stress on cattle performance, DNA methylation has the potential to serve as a valuable marker for previous stressors experienced by cattle. This information can lead to precise management decisions that optimize animal performance and ultimately improve profitability in cattle production.

Introduction

Genetics are vital for beef cattle performance, but the environment can exert a substantial influence. Stressors are known to hinder beef cattle performance. While the genetic sequence remains unchanged by a stressful environment, it can affect gene expression and, subsequently, the production of specific proteins crucial for growth, development, and overall performance. Environmental influences begin during fetal development, and stress-induced epigenetic modifications serve as one avenue through which the prenatal environment impacts gene expression. One common epigenetic modification involves adding a methyl group ($-CH_3$) to a DNA base called cytosine. The addition or removal of these methyl groups can influence gene expression (Figure 1). Changes in gene expression due to DNA methylation can affect cell function and result in variations of traits important to the industry. This research aimed to examine how prenatal transportation stress influences DNA methylation patterns in Brahman cattle and the implications of stress-induced DNA methylation alterations.

Experimental Procedures

Pregnant Brahman cows were assigned to two groups based upon treatment groups with respect to age, parity, and temperament score. One group was transported for a duration of 2 hours every 20 days ($\pm$ 5 days) from 60 to 140 days of gestation. A group of non-transported cows was maintained as controls. At 28 days of age, blood was harvested from bull and heifer calves born from the transport group (PNS) and

from those born from the non-transport group (Control). Heifer calves then entered a development regimen typical of cows in the herd, which included exposure to bulls at 1 year of age and annually after that. Of the females remaining when the cows were 5 years old, 8 Control and 6 PNS nonpregnant cows were slaughtered, and tissue that play a role in behavior and stress response. Methylation of cytosines across the genome was compared between the PNS and control group for the blood cells and tissues. Additionally, gene expression within the tissues was compared at 5 years of age. Genes containing cytosines that had more (or less) methylation were further investigated to gain insight into what cellular functions could be impacted by the alterations in methylation patterns.

Results and Discussion

Prenatal Transportation Stress

There were substantial differences in DNA methylation patterns between the PNS and Control group in the blood cells harvested at 28 days of age for the bull and the heifer calves (Littlejohn et al., 2018, Baker et al., 2020). In bull calves there were roughly 16,745 differentially methylated cytosines across the genome and 17,298 for the heifer calves. Of those differentially methylated cytosines 1,205 and 1,137 were located in regulatory regions of genes of the bull and heifer calves respectively. A slightly higher number of sites exhibited decreased methylation in PNS bull calves compared to the control group. Conversely, in PNS heifer calves, more sites displayed increased methylation than the control group. Many of the cytosines were located within genes active in biological pathways that could affect behavior and immune response including the “Corticotropin Releasing Hormone Signaling Pathway” and the “Interleukin 8 Signaling Pathway”. Bull calves that experienced prenatal transportation stress showed altered temperament and immune response (Littlejohn et al., 2016; Littlejohn et al., 2019). These differences could potentially be attributed to the alterations in DNA methylation patterns that were observed.

While there were substantial differences in DNA methylation patterns at 28 days of age, analysis in tissues harvested from the same females at 5 years of age revealed minimal differences between the PNS and Control groups. The same pattern was observed when comparing gene expression within the tissues between the two groups (Table 1) (Baker et al., 2022, Earnhardt-San, 2023). Methylation patterns shift over time, meaning any differences observed at a younger age, such as those found in blood cells at 28 days old, may not persist to later stages of life. This is supported by comparing methylation patterns of blood cells at 5 years of age. It was observed that there was a substantial decrease in the number of cytosines exhibiting differential methylation between the PNS and Control groups at 5 years of age compared to the amount observed at 28 days of age (Cilkiz et al., 2020). It is also possible that prenatal transportation stress is not severe enough to cause lasting differences. In cases where changes in methylation patterns have persisted into later stages of life, the stress experienced has typically been more severe, such as nutrient restriction throughout gestation (Heijmans et al., 2008).

Implications in Cattle Production

There are numerous reasons why the effect stress has on DNA methylation patterns is important to consider in cattle production. Methylation patterns have a potential role in important production traits such as meat tenderness and milk production. Varied methylation profiles have been observed in cows

with high and low milk production and in Angus steers with differences in tenderness of the longissimus dorsi muscle (Dechow et al., 2018; Zhao et al., 2020). Stress-induced alterations in the regions associated with favorable performance could have an adverse effect. These effects could be observed in numerous generations due to the transgenerational inheritance of epigenetic marks (Gudex et al., 2014). Stress-induced DNA methylation changes can introduce new sources of variation. Accounting for these changes and considering their inheritance can contribute to a more accurate estimation of heritability and genetic merit (Clarke et al., 2022).

Stress-induced epigenetic modifications might also have a role in adaptation and stress resilience. Recent work showed that Nellore and Angus cattle differ in methylation response before and after exposure to sun and high temperatures. Many genes in Nellore cattle that exhibited shifts in methylation were involved in stress response and cellular defense, while Angus cattle had a less proactive response (Del Corvo et al., 2021). There are also distinct differences in methylation patterns between tropically adapted cattle and their ancestors. (Saven et al., 2019). The findings suggest that methylation patterns and how they change in response to stress might affect adaptation to heat. The relationship between methylation patterns, stress response, and adaptation to environmental challenges provides valuable insights into the specific genes and pathways underlying stress resilience in cattle. Identifying these mechanisms is critical for developing innovative approaches to enhance the adaptability and performance of cattle as the climate continues to shift.

Unlike other physiological responses to stressors, epigenetic alterations can be retained and maintained over time. This presents a unique opportunity to use DNA methylation patterns as biomarkers to investigate an animal's previous environment and exposure to stressors, providing valuable insights into the animal's overall well-being, health, and behavior (Caulton et al., 2020). Using DNA methylation patterns as biomarkers opens opportunities for targeted interventions, such as nutritional adjustments or environmental modifications (Whelan et al., 2022). This approach not only considers the animal's current state but also considers its past experiences, allowing for tailored strategies that prioritize animal health, welfare, and overall productivity.

Conclusions

This research was the first to investigate the impact of prenatal transportation stress on DNA methylation patterns in tropically adapted beef cattle. It yielded valuable insights, indicating that transportation stress can influence the DNA methylation of the fetus. These findings are the foundation for future investigations aiming to understand the dynamics of stress-induced methylation alterations over time and their potential ramifications on biological processes. While the field of epigenetics in beef cattle is still evolving, ongoing research holds promise for utilizing epigenetic markers as valuable tools for tracking and assessing animal performance, health, welfare, and the impact of environmental factors.

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Table 1: Number of differentially methylated cytosines and differentially expressed genes

Tissue	Cytosines With Increased Methylation	Cytosines With Decreased Methylation	Up-Regulated[2] Genes	Down-Regulated Genes
Paraventricular nucleus	2	3	6	0
Anterior Pituitary	93	64	25	24
Adrenal Cortex	90	99	5	0
Adrenal Medulla	36	37	0	0
Amygdala	8	21	1	1

between the Prenatally Stressed Group and Control group.

[1]Increased methylation (decreased) indicates increased (decreased) methylation in the prenatally stressed group compared to the control

[2] Up-regulated (down-regulated) indicates increased (decreased) gene expression in the prenatally stressed group compared to the control

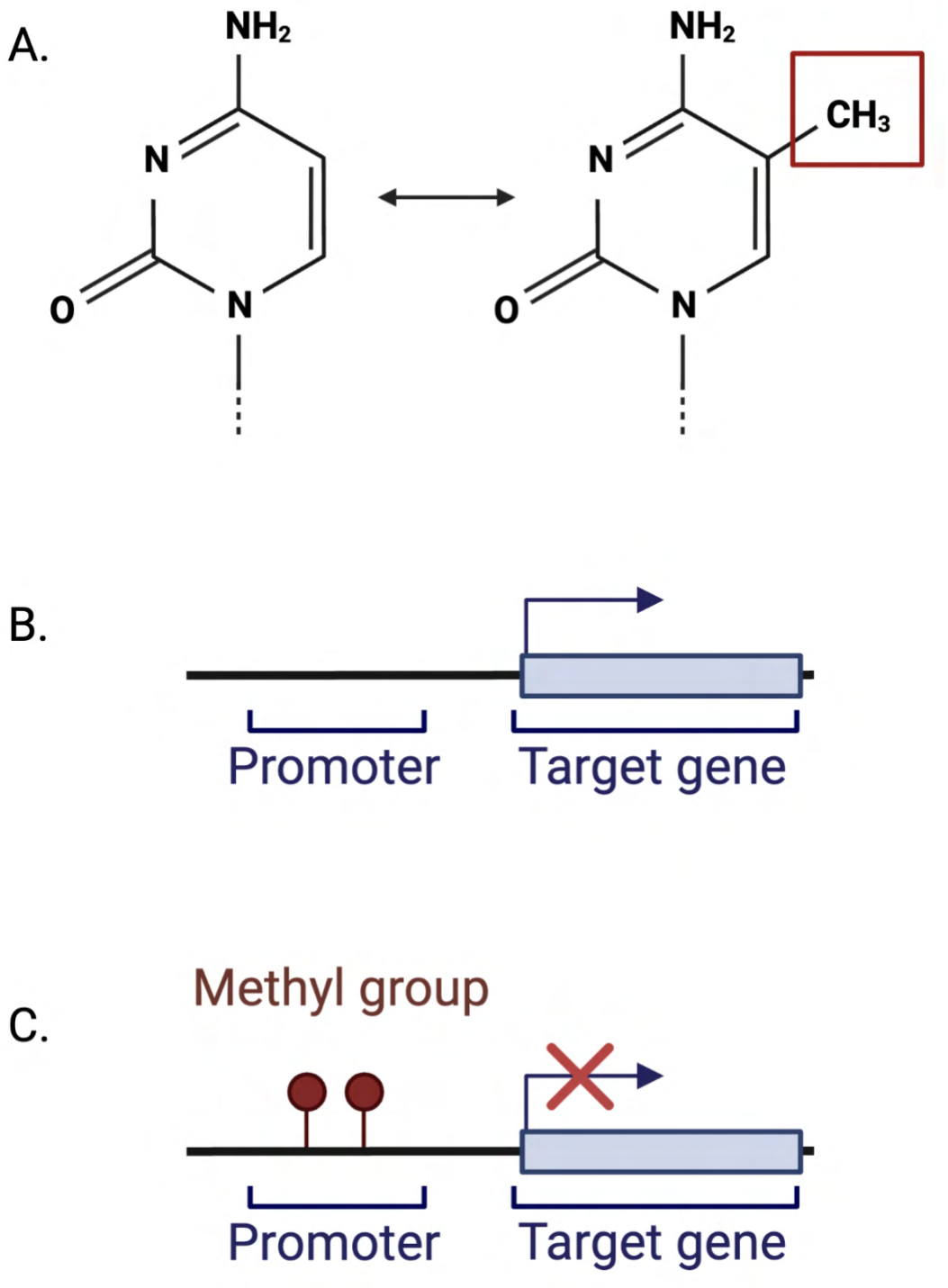

Figure 1. Methylation is the addition of a methyl group (-CH_3) to the base cytosine (A). Typically, when the promoter region of a gene is free of methylation, the gene will be expressed (B). When methyl groups are added within the promoter region of a gene, expression is typically inhibited (C).

TRANSCRIPTOMIC COMPARISON BETWEEN BOVINE SIRE WITH DIFFERING FIELD FERTILITY

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Summary

Pregnancy loss is considered one of the main causes of reproductive inefficiency in cattle. Results from this study could lead us to identify sub-fertile bulls. The objective was to determine the differentially abundant genes (DEGs) between the conceptus and the uterus of pregnancies produced by sires of known fertility. Our hypothesis is that the paternal genome will have a significant effect on transcript abundance between these tissues. The sires used have been previously reported to have different levels of field fertility; however, they had passed all normal standards for frozen thawed semen. Heifers (n=45) were subjected to estrus synchronization and embryo transfer with *in vitro* produced embryos from either a high fertility (HF) or low fertility sire (LF). Samples were collected for RNA sequencing. Gene ontology analysis reported DEGs were associated with immunology and reproduction. Of interest were the *MHC-I* gene and the *Spermatogenesis Associated Gene-22*. These data suggest that field fertility records might be an inaccurate assessment for bull selection.

Introduction

Bull fertility is the degree of sperm's ability to fertilize and activate the egg and support embryo development, this is critical for herd reproductive performance (Kutchy et al., 2017). Fertility in general has been studied intensely across multiple species, however most of this research has been focused on the female. It is known that paternal genetics contribute significantly to pregnancy establishment and maintenance, specifically placenta formation, and contributes to the incidence of pregnancy loss (Franco et al., 2020). Currently, methods being used to determine bull fertility are either by breeding many normal fertile females and assessing pregnancy rates (Kastelic et al., 2008) or by determining sperm characteristics such as motility and morphology (Correa et al., 1997) through a soundness breeding exam (BSE). Despite the results that we can attain while using these tools, a standard breeding soundness evaluation identifies bulls with substantial deficits in fertility, but does not consistently identify sub-fertile bulls (Kastelic et al., 2008)

Experimental Procedures

Bos indicus (Nelore) heifers (N=45) were synchronized to receive an embryo seven days after estrus expression (D7). The embryos utilized in this study were frozen in-vitro produced of excellent quality according to the International Embryo Transfer Society guidelines. The embryos transferred were from one of two sires: High Fertility or Low Fertility, pregnancies were later confirmed at slaughter. Approximately 0.5 g of tissue was collected from the endometrium (Caruncle) and trophectoderm on gestational days 25 and 36. The tissues were immediately disposed in cryogenic vials and stored in -80 °C until further RNA sequencing processing.

RNA Sequencing

RNA-seq studies to provide knowledge about the quantitative and qualitative aspects of transcriptomes in both prokaryotes and eukaryotes (Sharma et al., 2014). Samples were collected from the trophectoderm and uterus on day 25 and 36 of gestation to attain samples for RNA

sequencing. Total RNA was isolated from tissue samples using the RNeasy kit (QIAGEN; Hilden, Germany) per manufacturer's instructions. The RNA sequencing was conducted using an Illumina platform. Differentially expressed genes (DEGs) between sires and by tissue were determined using edge-R package from R. The false discovery rate used in this case was 0.05.

Progesterone
Blood samples were harvested by venipuncture from the coccygeal vein on D25 and D36 for determination of plasma progesterone concentrations. Plasma progesterone concentrations were quantified using a solid phase 125 P4 double-antibody radioimmunoassay kit (MP biomedicals, Irvine, CA, USA) on samples from D25 and D36. The assay was performed using an in-house sandwich ELISA, as described by Reese et al. (2018). Progesterone levels were measured in both High and Low fertility pregnancies.

Results and Discussion

On day 25 15,755 genes were identified in the trophectoderm between the two sires. Of those, 11 genes were downregulated and 6 genes were upregulated in the Low fertility sire. Moreover, 16,044 genes were identified within the caruncle where the Low Fertility Sire resulted in 2 downregulated genes and no upregulated genes. Interestingly the *Phospholipase A2* (PLA-2) gene resulted downregulated, this gene has been described to play important roles in the late maturational events of spermatozoa, the acrosomal reaction and sperm-egg fusion (Kumar et al., 2012). Not only this but, the expressions of PLA2 are more in the accessory sex gland fluid of high fertility bulls than in low fertility bulls (Moura et al., 2006). Additionally, there is also evidence that PLA-2 stimulates immune cells (Moura et al., 2007).

Of particular interest, MHC-I gene was found to be downregulated in the trophectoderm on day 36, out of a total of 17,080 observed. This gene is known for promoting maternal tolerance of the conceptus in mammals during the first trimester of gestation. Research has shown that Interferon Tau (ITF- τ), the signal for maternal recognition of pregnancy in cattle, promoted MHC-1 expression and then alleviated the inflammatory response in Bovine Endometrial Epithelial cells (Wu et al., 2018). Recently, it has been observed that MHC-I is involved in pre-implantation embryo development and fetal-maternal interactions. If the mother "misunderstands" the signal sent by the fetus during pregnancy, the fetus will be miscarried. (Zhao et al., 2018), leading to embryonic mortality. This makes sense considering that this gene was not found to be highly expressed in the low fertility bull.

In the caruncle sample of day 36 17,843 genes were observed resulting in 8 downregulated genes for the Low Fertility Sire whereas 21 genes were upregulated. Additional to the other findings, Caltrin was found to be upregulated in the caruncle sample. Caltrin's function in bull fertility has been described by Ashwitha and others in 2023 as a protein that enhances sperm motility and it is usually found in the spermatozoa of bulls with a high Ejaculate Rejection Rate. The way this works is by Caltrin inhibiting calcium uptake in mature spermatozoa and thereby facilitates sperm motility (Ashwitha et al., 2023). This upregulated finding is of importance considering that not only does Caltrin assists motility, but it also serves as a bactericide (Viana, 2022).

Interestingly, found in the trophectoderm sample of gestational day 36 was the transferrin gene (TF**),** known to be responsible for the transport of iron from sites of absorption and heme degradation to those of storage and utilization, but it is also known for its role in stimulating cell proliferation. In closer relation to fertility and while considering data from several sources, it is suggested that four phenomena affecting reproductive performance are associated with transferrin polymorphism in cattle (Ashton et al., 1965). Although most research on this particular gene has not

been conducted in recent years, it should still be taken into account while studying bull fertility considering that this is still an area that hasn't received much attention in comparison to female fertility.

A finding in the caruncle sample of gestational day 36 was the *Spermatogenesis Associated 22* (SPATA 22) gene responsible for gamete generation, homologous chromosome pairing at meiosis, meiotic DNA repair synthesis, this was also found downregulated in between sires. Meiosis is critical for reproduction, it's special mode of cell division, which makes haploid cells from a diploid cell. It is essential for sexual reproduction in eukaryotes and diploid organisms and produces gametes, such as eggs and sperm (Ohkura et al., 2015). This gene has also been found to be relevant in fertility in both males and females across different species.

In the present study, circulating progesterone concentrations were not significantly different ($P > 0.05$) between the High and Low fertility sires on heifers slaughtered on D25 or D36 (Figure 1). The assessment of luteolysis is a good predictor of pregnancy; still, it is not efficient in monitoring conceptus viability or predicting embryonic mortality since decreased progesterone only occurs together or after embryonic death (Pohler et al., 2016). Progesterone is a required hormone for pregnancy initiation, implantation, and embryo development (Driver et al., 2009). Progesterone measurements are absolutely necessary when estimating the respective fiecluencies of Early Embrionic Mortality (EEM) and Late Embryonic Mortality (LEM) from field data because a high proportion of cows although found non pregnant (or no longer pregnant) by Day 21 when measuring progesterone, express a late return in estrus due to bad heat detection and/or silent estrus (Humblot et al., 2001).

Conclusions

Genes listed above could possibly be used as fertility markers in beef cattle, more research needs to be conducted specially in different breeds. Ultimately these genes could be applied to develop a tool that can lead us to identify subfertile bulls within a herd that could be easily implemented as routinely as current fertility tests are and more importantly to accurately select high fertility bulls more efficiently. These data suggest that other underlying factors are at work regarding conception rate and current methods are insufficient for sire selection and fertility testing. Different paternal genome results in differences at both trophectoderm and uterus level in *bos indicus* beef cows.

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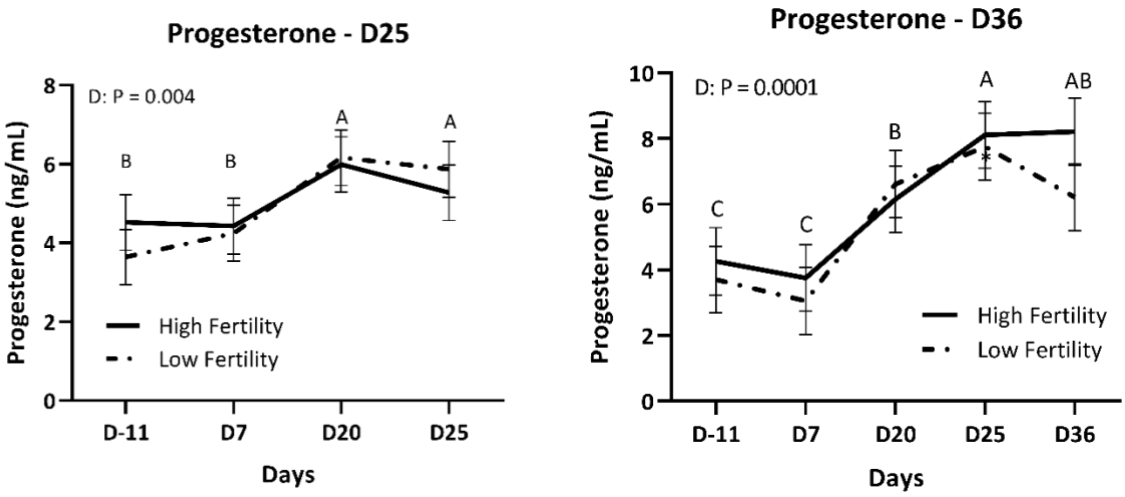

Figure 1. Progesterone concentrations (ng/mL) on days 25 and 36 between the groups of High and Low fertility in heifers slaughtered on days 25 or 36 of gestation.

ANALYSIS OF THE NASAL MICROBIOTA IN NEWLY RECEIVED FEEDLOT HEIFERS ACCORDING TO SUBSEQUENT INCIDENCE OF BOVINE RESPIRATORY DISEASE

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Summary

Bovine respiratory disease (**BRD**) is the most common disease in feedlot cattle, and costs the US cattle industry more than $2 billion annually. Such economical losses include mortality, wasted feed resources, pharmaceutical inputs, and decreased performance of morbid cattle. Hence, research to understand the etiology of BRD are critical to lessen the incidence and productive impacts of this disease in feedlot systems. The upper respiratory tract is home to a plethora of bacteria associated with BRD in cattle, whereas the composition and stress-related imbalances in this microbiota can lead to the disease. Based on this rationale, this experiment evaluated the microbiota composition in the nasal cavity of newly receiving feedlot heifers, and contrasted with subsequent prevalence of BRD. In general, heifers that develop BRD had altered nasal microbiota at the time of feedlot arrival compared with heifers that remained healthy. Such differences in microbiota were heightened in heifers that developed BRD shortly after arrival, or heifers that required multiple antimicrobial treatments upon disease occurrence.

Introduction

Bovine respiratory disease (**BRD**) is a multifactorial disorder caused by a combination of microbial pathogens, impaired host immunity, and environmental factors (Gershwin et al., 2015). Such economical losses include mortality, wasted feed resources, pharmaceutical inputs, and decreased performance of morbid cattle (Loerch and Fluharty, 1999). Despite recent advances in management systems to mitigate BRD, new approaches are still warranted as BRD incidence remains elevated during feedlot receiving (Johnson and Pendell, 2017; Galyean et al., 2022). To our knowledge, limited or no studies have attempted to evaluate the nasal microbiota at feedlot entry in cattle that develop or not BRD later in the feeding period. Therefore, this experiment compared the relative abundance of bacteria in the nasal cavity of beef heifers at feedlot arrival according to incidence of BRD during a 56-d receiving period. The hypothesis is that the nasal microbiota of heifers that develop BRD is already altered at feedlot arrival compared with cohorts that remain healthy.

Experimental Procedures

This study was conducted at the New Mexico State University, Clayton Livestock Research Center, Clayton, NM. All procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee (IACUC #2020-028).

Heifers evaluated in this experiment were part of a companion project focused on nutritional management during feedlot receiving (Smithyman et al., 2022). Pertaining to the current experiment, 76 Angus-influenced recently-weaned heifers (age and weaning date unknown) sourced from commercial auctions were road-transported for 1,100 km (11-h haul) to the Clayton Livestock Research Center. Upon arrival (d 0), heifers were immediately weighed [initial shrunk body weight (BW) = 234 ± 15 kg) and a nasal cavity swab was collected as in Bringhenti et al. (2021). Briefly, a 20-cm DNA-free sterile swab (Puritan Medical Products; Guilford, ME) was aseptically introduced (15 cm) into each nostril and rotated around the sides of the nasal passage. Swabs were placed into sterile RNAse, DNAse, pyrogen-free polypropylene tubes, kept on ice and stored at −80°C within 24 h of collection.

After nasal sampling on d 0, heifers were managed as a single group with free-choice access to feed and water. On d 1, heifers were vaccinated against Clostridium (Covexin 8; Merck Animal Health, Madison, NJ), Mannheimia haemolytica, bovine respiratory syncytial virus, bovine herpesvirus-1, bovine viral diarrhea virus 1 and 2, and parainfluenza-3 virus (Vista Once SQ; Merck Animal Health), administered an anthelmintic (Dectomax; Zoetis,

Parsippany, NJ), and received a growth-promoting implant (Synovex H; Zoetis) as typically done in commercial US feedlots (USDA-APHIS, 2012). No antimicrobial metaphylaxis was administered to heifers during the experiment. Heifers were then ranked by initial shrunk BW and allocated into 6 soil-surfaced pens (12 × 35 m) with 11 m of bunk space. Pens were assigned to receive 1 of 3 the treatments described by Smithyman et al. (2022), which evaluated the inclusion of additional water tanks or rehydration solution to pens during the initial 4 d of feedlot receiving. From d 0 to 55, all heifers were fed the same free-choice receiving diet containing (as-fed basis) 35.9% steam-flaked corn, 30.0, 17.0% Sweet Bran (Cargill Corn Milling, Blair, NE), 15.0% dried distillers' grain, 1.60% limestone, 0.33% salt, and 0.22% of a commercial mineral-vitamin premix (Livestock Nutrition Center; Chickasha, OK). Final shrunk BW was collected from all heifers on day 56 after a 16-h feed and water deprivation. Heifer average daily gain (ADG) was calculated based on initial and final shrunk BW.

Heifers were observed daily for BRD signs according to the DART system (Zoetis) and received antimicrobial treatment as in Colombo et al. (2020). Briefly, heifers diagnosed with BRD signs received florfenicol antibiotic with flunixin meglumine (Resflor Gold, Merck Animal Health) at 1 mL/7.6 kg of BW subcutaneously as the first antimicrobial administered, followed by a 5-d moratorium. Heifers diagnosed with BRD signs after first antimicrobial treatment were administered ceftiofur crystalline free acid (Excede; Zoetis) at 1 mL/30.3 kg of BW, followed by another 5-d moratorium. Heifers diagnosed with BRD signs after the second antimicrobial treatment were administered oxytetracycline (Bio-Mycin 200; Boehringer Ingelheim, Ridgefield, CT) at 1 mL/10 kg of BW. Heifers were classified according to the number of BRD treatments received during the experiment [0 treatments (BRD0), 1 treatment (BRD1), or ≥2 treatments (BRD≥2)], and according to time of first incidence of BRD signs [no incidence (NOBRD), early incidence (EARLY), or late incidence (LATE). This latter classification was based on BRD signs observed prior to (EARLY) or after (LATE) the mean observed in this experiment (6.5 ± 0.9 d upon arrival).

Results and Discussion

Heifers evaluated in this experiment were considered high-risk as their previous management and health history were not fully known, and they experienced the stress of transport, commingling, vaccination against BRD pathogens, and exposure to a new environment within a 48-h period (Cooke, 2017). Accordingly, the BRD incidence observed herein is above the values reported in the latest NAHMS Beef Feedlot Study (16.2%; NAHMS, 2013), but is equivalent to the BRD incidence observed in recent research with high-risk receiving cattle (Wilson et al., 2017; Colombo et al., 2020; Theurer et al., 2021). Mortality rate was also comparable to Theurer et al. (2021), which evaluated nearly 200 pens and 16,000 animals in a commercial US feedlot and reported 4.83% mortality in high-risk cattle. The 4 heifers that died were classified as EARLY as well as BRD≥2 because they received 3 antimicrobial treatments before mortality occurred, but their initial BW and nasal microbiota results were maintained in all analyses. Theurer et al. (2021) reported that the inflection point for BRD incidence in high-risk cattle occurred at 7 d upon arrival, which agrees with the mean time of BRD incidence noted in this experiment. Sanderson et al. (2008) also observed that BRD incidence was the highest during the first week on feed in a study evaluating 122 feedlot pens. Colombo et al. (2020) reported that 82% of BRD incidence occurred during the initial 7 days on feed, corroborating the distribution of EARLY (84%) and LATE heifers (16%). Hence, this experiment fully represents the typical management and health challenges that high-risk cattle experience during feedlot receiving (Duff and Galyean, 2007). The occurrence of BRD during the first week (EARLY heifers) was concentrated within a 3-d period, but dispersed over an 18-d period later in the experiment (LATE heifers) as similarly observed by others (Snowder et al., 2006; Colombo et al., 2020; Theurer et al., 2021).

Heifer BW at arrival did not differ according to number of BRD treatments received, as previously reported (Holland et al., 2010; Waggoner et al., 2007). In turn, the negative effects of BRD incidence on ADG and final BW feedlot cattle are well known, and were heightened according to recurrence of the disease (Wilson et al., 2017; Blakebrough-Hall et al., 2020). When performance data was analyzed according to time of BRD incidence, heifers classified as EARLY were lighter at arrival compared with NOBRD heifers, suggesting greater risk of BRD of lighter cattle during the initial days on feed (Sanderson et al., 2008; Taylor et al., 2010). Heifer ADG and final BW were also less in EARLY and LATE heifers, although such decrease in ADG was more pronounced in LATE heifers. Reduced feed intake is one of the primary consequences of BRD (Gifford et al., 2012), and feed intake is typically deficient during the initial week upon feedlot arrival (Loerch and Fluharty, 1999). Perhaps the negative effects of BRD on ADG were lessened in EARLY heifers because the disease occurred when feed intake was still low, and these heifers had more

time to compensate for performance losses until completion of the experiment. The main goal of this study, however, was not to characterize performance differences according to BRD incidence these outcomes have already been well established in the literature (Galyean et al., 2022).

The most common bacterial phylum found in the nasal cavity of heifers in this experiment were Proteobacteria, Tenericutes, Firmicutes, Bacteroidetes, and Actinobacteria as reported by others (Holman et al., 2015; Zeineldin et al., 2017). In general, the abundance of Tenericutes increased according to the number of BRD treatments received, resulting in decreased diversity in the bacterial phyla colonizing the nasal cavity of heifers. Tenericutes is one of the most prevalent phyla in the nasopharynx and trachea of feedlot cattle, and may comprise more than 40% of the total bacterial community (Timset et al., 2018; Strobel et al., 2018). This phylum includes the genus *Mycoplasma*, which is a major pathogen associated with BRD in cattle (Czuprynski et al., 2004; Caswell and Archambault, 2007). Perhaps BRD1 and BRD≥2 heifers were already sick and their nasal cavity mostly colonized by Tenericutes at feedlot arrival, although signs of BRD were only noted beginning 3 d later. Indeed, the abundance of Tenericutes was greater in EARLY heifers that compared with NOBRD cohorts, but intermediate values were noted in LATE heifers. Nonetheless, Tenericutes was the prevailing bacterial phylum in the nasal cavity of newly-received heifers that developed BRD shortly after arrival, and also in heifers that required ≥ 2 antimicrobial treatments during the 56-d receiving period.

The most common bacterial genus in the nasal cavity of heifers evaluated herein were *Mycoplasma* and *Mannhemia* as also reported by others (Holman et al., 2015; Zeineldin et al., 2017), and these genera belong to Tenericutes and Proteobacteria phylum, respectively. In accordance with bacterial phyla analyses, the abundance of *Mycoplasma* increased while bacterial genera diversity decreased according to the number of BRD treatments received. *Mycoplasma* genus is a major contributor to morbidity and mortality in feedlot cattle (Caswell et al., 2010), and include 13 species that constitute the normal flora of the bovine URT or that can cause BRD (Dudek et al., 2020). Conversely, the abundance of other bacterial genera associated with BRD did not differ at arrival according to BRD incidence during the experiment, such as *Mannhemia* and *Pasteurella*. Heifers classified as EARLY also had greater *Mycoplasma* abundance and thus reduced bacterial genera diversity in the nasal cavity at arrival compared with NOBRD cohorts. The abundance of *Mycoplasma* in the nasal cavity of LATE heifers was intermediate compared with EARLY and NOBRD, although increased by nearly 2-fold compared with NOBRD heifers. As consequence, LATE heifers also had reduced abundance of *Corynebacterium*, *Jeotgalicoccus*, *Dietzia,* and *Planomicrobium* at arrival despite similar bacterial genera diversity compared with NOBRD. Hence, Mycoplasma was the prevailing bacterial genus in the nasal cavity of newly-received heifers that developed BRD during the experiment, including those diagnosed with BRD later in the experimental period.

Conclusions

Collectively, this experiment provides novel insights on the relationship between the nasal microbiota in high-risk heifers at feedlot arrival, and subsequent incidence of BRD during a 56-d receiving period. Heifers that developed BRD had altered nasal microbiota at the time of feedlot arrival compared with heifers that remained healthy, particularly increased prevalence of Tenericutes phylum and Mycoplasma genus. Such differences in nasal microbiota were heightened in heifers that developed BRD shortly after arrival, or that required multiple antimicrobial treatments. Nonetheless, Mycoplasma was the genus with greatest abundance in the nasal cavity of heifers that required only 1 treatment against BRD, and also those diagnosed with BRD later in the receiving period. These results suggest the potential of using the nasal microbiota of feedlot cattle at arrival to identify those at higher risk of developing BRD, allowing for early treatment of morbid cattle and management interventions to prevent the establishment of the disease. Further research is warranted to replicate the outcomes from this experiment, which can be used to develop predictive tools that mitigate the impacts and incidence of BRD in commercial feedlots.

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Table 1. Differences in bacterial composition (relative abundance, %) and diversity [Shannon diversity (**SD;** Kim et al., 2017)] in the nasal cavity at feedlot arrival, as well as growth performance of beef heifers according to number of BRD treatments received.

	Number of BRD treatments				*P-value*	
	0	**1**	**≥2**	**SEM**	**Linear**	**Quadratic**
n	26	35	15	-	-	-
Initial BW	228	220	222	3	0.20	0.14
Final BW	327	312	308	5	0.03	0.29
ADG	1.77	1.61	1.51	0.08	0.04	0.74
Bacterial phyla						
Proteobacteria	34.3	28.7	30.0	5.2	0.59	0.56
Tenericutes	13.5	27.3	40.4	6.4	< 0.01	0.96
Firmicutes	27.3	23.1	16.0	2.9	0.01	0.67
Bacteroidetes	11.1	10.6	6.54	1.40	0.03	0.28
Actinobacteria	10.8	7.05	5.43	1.80	< 0.01	0.46
Verrucomicrobia	0.693	0.660	0.421	0.089	0.05	0.31
Spirochaetes	0.743	0.590	0.396	0.107	0.04	0.86
Euryarchaeota	0.388	0.288	0.187	0.049	0.01	0.98
Chlorobi	0.300	0.266	0.187	0.039	0.05	0.61
Chloroflexi	0.279	0.237	0.157	0.033	0.02	0.61
SD index	1.28	1.29	1.05	0.09	0.10	0.25
Bacterial genera						
Mycoplasma	13.9	28.3	42.9	6.6	< 0.01	0.98
Mannhemia	21.7	18.8	19.7	5.4	0.80	0.76
Bacteroides	3.33	3.43	2.11	0.52	0.12	0.22
Corynebacterium	3.32	2.13	1.62	0.40	< 0.01	0.46
Ruminococcus	2.31	2.47	1.69	0.34	0.24	0.23
Blautia	2.39	2.34	1.52	0.30	0.05	0.28
Psychrobacter	2.34	1.53	2.52	0.65	0.85	0.53
Jeotgalicoccus	2.63	1.59	1.35	0.34	0.01	0.31
Clostridium	2.15	1.87	1.18	0.25	0.01	0.49
Oscillospira	1.66	1.79	1.10	0.23	0.12	0.13
Dietzia	1.89	1.06	0.885	0.240	< 0.01	0.24
Pedobacter	1.34	1.39	0.912	0.199	0.16	0.26
Prevotella	1.45	1.21	0.470	0.527	0.22	0.69
Pasteurella	1.15	1.51	0.310	0.880	0.52	0.43
Planomicrobium	1.50	0.948	0.772	0.228	0.03	0.47
SD index	3.38	2.93	2.24	0.28	0.01	0.72

[1] Heifers were weighed and sampled for nasal microbiota analysis via nasal swab (Bringhenti et al., 2021) immediately upon feedlot arrival (d 0). Heifers were observed daily for BRD signs and received antimicrobial treatment as in Colombo et al. (2020) during a 56-d receiving period. Heifers were classified according to the number of BRD treatments received [0 treatments (**BRD0**), 1 treatment (**BRD1**), or ≥2 treatments (**BRD≥2**)]. Final shrunk BW was collected on d 56 for ADG calculation.

Table 2. Differences in bacterial composition (relative abundance, %) and diversity [Shannon diversity (**SD;** Kim et al., 2017)] in the nasal cavity tract at feedlot arrival, as well as growth performance of beef heifers according to time of first incidence of BRD signs.

	Time to first BRD incidence				
	NOBRD	**EARLY**	**LATE**	**SEM**	***P-value***
n	26	42	8	-	
Initial BW	228[a]	220[b]	224[ab]	3	0.09
Final BW	327[a]	312[b]	306[b]	5	< 0.01
ADG	1.77[a]	1.62[ab T]	1.49[b]	0.08	0.02
Bacterial phyla					
Proteobacteria	34.3	29.2	28.3	6.0	0.70
Tenericutes	13.5[b]	33.1[a]	22.3[ab]	7.5	0.04
Firmicutes	27.3[a]	19.8[b]	26.7[ab]	3.3	0.05
Bacteroidetes	11.1	8.66	12.8	1.62	0.17
Actinobacteria	10.8[a]	6.47[b]	6.87[b]	1.45	0.01
Verrucomicrobia	0.693	0.567	0.791	0.103	0.22
Spirochaetes	0.743	0.498	0.697	0.124	0.16
Euryarchaeota	0.388[a]	0.241[b]	0.338[a]	0.057	0.04
Chlorobi	0.300	0.227	0.323	0.046	0.21
Chloroflexi	0.279[a]	0.196[b]	0.296[a]	0.038	0.05
SD index	1.28	1.18	1.39	0.10	0.40
Bacterial genera					
Mycoplasma	13.9[b]	34.6[a]	23.5[ab]	7.6	0.04
Mannhemia	21.7	18.9	19.9	6.2	0.91
Bacteroides	3.33	2.77	4.32	0.60	0.25
Corynebacterium	3.32[a]	1.94[b]	2.12[b]	0.46	0.02
Ruminococcus	2.31	2.02	3.32	0.38	0.14
Blautia	2.39	1.94	2.81	0.35	0.22
Psychrobacter	2.34	1.90	1.48	0.75	0.78
Jeotgalicoccus	2.63[a]	1.50[b]	1.61[b]	0.39	0.02
Clostridium	2.15[a]	1.54[b]	2.28[a]	0.29	0.05
Oscillospira	1.66	1.43	2.31	0.27	0.14
Dietzia	1.89[a]	1.01[b]	0.976[b]	0.273	0.01
Pedobacter	1.34	1.11	1.91	0.22	0.12
Prevotella	1.45	0.956	1.08	0.610	0.72
Pasteurella	1.15	1.34	0.020	1.01	0.72
Planomicrobium	1.50[a]	0.934[b]	0.703[b]	0.270	0.04
SD index	3.38[a]	2.61[b]	3.23[ab]	0.33	0.04

[1] Heifers were weighed and sampled for nasal microbiota analysis via nasal swab (Bringhenti et al., 2021) immediately upon feedlot arrival (d 0). Heifers were observed daily for BRD signs and received antimicrobial treatment as in Colombo et al. (2020) during a 56-d receiving period. Heifers were classified according to time of first incidence of BRD signs [no incidence (**NOBRD**), early incidence (**EARLY**; 4.1 ± 0.1 d), or late incidence (**LATE**; 18.5 ± 9.6 d).

DIFFERENCES IN PROTEIN SUPPLEMENTATION RESPONSE IN *BOS TAURUS TAURUS* (ANGUS) AND *BOS TAURUS INDICUS* (BRAHMAN)

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Summary

Globally cattle convert low-quality forage to beef; therefore, understanding the differences in protein utilization between *Bos taurus taurus* (Angus) and *Bos taurus indicus* (Brahman) is critical to enhancing sustainability as over and under-supplementation of protein are economically and environmentally costly. We determined the effect of ruminal degradability and level of protein supplementation on forage utilization in Angus and Brahman steers fed hay (3.5% crude protein). Steers were provided protein supplements containing 43% CP, with two levels of protein degradability (35 or 70% RDP) fed at two levels of supplementation, equivalent to 1.5 or 3 lbs in a 1200 lb cow. A control treatment providing no supplemental protein was also included. In Angus, increasing protein supplementation increased forage OMI (FOMI) and total digestible OMI (TDOMI). In brahman steers increasing supplementation increased total OMI and TDOMI. In both angus and brahman steers FOMI was greater when supplements contained 70% RDP versus 35% RDP. Differences in steer forage utilization were extrapolated to simulate differences between bred cows of the corresponding breed. This extrapolation is an attempt to translate our results into a clear illustration of the effects of protein supplementation.

Introduction

Optimizing forage utilization is important for the success and profitability of cattle producers. Multiple factors, (i.e. forage maturity, species, fertilization, and weather), affect the forage quality potentially limiting utilization by decreasing intake and/or digestion. When forage quality is low, typically less than 7% CP, ruminal microbes lack the N required to support microbial growth. Addressing microbial requirements is important as they are responsible for digesting fiber and allowing forage to move out of the rumen. When forage digestion decreases, energy available to the animal is reduced. Further exacerbating this problem is a reduction in forage intake. Reduced forage utilization results in an energy deficit and ultimately body condition score (BCS) loss. Providing supplemental N allows ruminal microbes to function more effectively, resulting in greater digestibility and intake, and ultimately more energy to the cow.

Experimental Procedures

Model

Our objective was to determine the effect of protein degradability (i.e. how much the microbes can access) and level of supplementation on forage utilization in *Bos taurus taurus* (Angus) and *Bos taurus indicus* (Brahman) cattle. Cattle were provided a base diet of low-quality King Ranch Bluestem hay (3.5% CP). Four protein supplements were provided, two levels of ruminal degradability (35 and 70% RDP), at two levels of supplementation, and a fifth treatment received no supplemental protein.

Ruminal degradability of the supplemental protein determines the extent to which microbes can access the N required for proper digestion. These two levels of ruminal degradability were accomplished by providing a similar amount of total protein and including feedstuffs with varying degrees of degradability. Soybean meal was used as the baseline supplement, being 70% degradable. Corn gluten meal (58%) and soybean hulls (42%) were mixed to provide a supplement supplying the same amount of total protein as soybean meal with only 35% ruminal degradability. Protein that is not degradable by ruminal microbes can still be of benefit to meeting the microbial N requirements, although indirectly and less efficiently. Two levels of supplementation were established to correspond to 1.5 or 3 lbs of supplement in a 1200 lb cow.

Five, 14-d periods, consisted of 9 d adaptation and 4 d to measure intake, digestion, and N balance, and 1 d for ruminal fermentation. During adaptation, steers were housed in individual metabolism pens. For the remainder of the experimental period, cattle were housed in metabolism crates to allow for the total collection of urine and feces for intake and digestibility determination.

Application

These data collected from Angus and Brahman steers were used to estimate the intake and digestion of mature cows of the same breeds (1200 lbs). Differences in steer forage utilization were extrapolated to simulate differences between bred cows of the corresponding breed. This extrapolation is an attempt to translate our results into a clear illustration of the effects of protein supplementation.

Using digestion coefficients observed in the steer model, the amount of net energy used for maintenance by each breed was calculated. When coupled with the intakes observed in the steer model, the expected total energy used for maintenance per day in 1200lb cows of each breed were calculated. Cow requirements were estimated using the NASEM (2016) with a 10% adjustment for breed, as *Bos taurus indicus* have lower maintenance energy requirements than *Bos taurus taurus*. Using maintenance requirements and projected energy consumption, calculations of expected energy deficiencies were translated to changes in body condition score. With estimated body condition score changes, expected pregnancy rates for each treatment were calculated and compared to a baseline pregnancy rate at BSC 5 adapted from Rasby et al. (2007).

Results and Discussion

Model

In Angus steers, protein supplementation increased all measures of intake such as, forage organic matter intake (FOMI) and total digestible OMI (TDOMI). In addition, level of supplementation affected all measures of intake with the high level being greater than the low. Protein supplements with 70% RDP increased FOMI 28% versus control when supplemented at low levels, and 47% at the high level. Supplementation with 35% RDP increased FOMI 23% at the low level and 28% at the high level indicating the lower degradability supplement was less effective at improving forage utilization that the 70% RDP supplement.

Similarly, Brahman steers provided supplemental protein had increased TDOMI compared to those consuming only hay. Total digestible OMI was greater in Brahman steers receiving 70% RDP than the 35% RDP. This response resulted from greater FOMI with 75% RDP, that was not observed with 35% RDP. Despite differences in FOMI, TDOMI was improved with both 35 and 70% RDP, partially the result of providing a highly digestible supplement. In contrast to angus, provision of 35% RDP supplement did not improve FOMI. Supplementation with 70% RDP improved FOMI 19 and 20% compared to the hay only diet at the low and high levels, respectively. A portion of this relatively modest response to protein supplementation could be attributed to greater intake by Brahman steers when no protein supplement was provided.

Despite the effect of protein supplementation on intake, OM digestion (OMD) of angus steers was not affected by protein supplementation regardless of degradability or level; however, in Brahman steers OMD increased as level of supplementation increased.

Application

Forage used to feed the steers was low in protein (3.5% CP), leading to low intake of hay. Low voluntary forage intake is expected to lead to predictions of significant BCS loss in both subspecies that may be greater than observed with other low-quality forages. Due to greater total digestible organic matter intake in brahman steers, brahman cows would be expected to be better at maintaining body condition score when consuming low-quality forage, with or without protein supplementation compared to Angus cows. In both breeds, supplementation would be expected to reduce body condition losses. When angus cows were fed a high level of protein supplementation, BCS losses were even less than when supplemented at a low level. Even at the highest level of supplementation, Angus cows continued to respond to supplementation suggesting supplementing over 3 lbs/day may result in further reduction of BCS losses. In general, the 70% RDP supplement is expected to prevent BCS losses better than the 35% RDP supplement in both breeds. Brahman cows would be expected to have less

response to the 35% RDP treatment at low levels; however, when fed at the highest level of supplementation, BCS losses would be similar to the 70% RDP supplement.

Pregnancy rate reductions would be expected to follow a similar pattern. When consuming low-quality forage, maintaining BCS close to BSC 5 will improve pregnancy rates, therefore supplementation strategies that maintain BCS can be impactful. Compared to a BSC 5, Angus cows consuming low-quality forage without supplemental protein would be expected to have a 24% reduction in pregnancy rates. Providing the low level of supplemental protein can minimize the reduction in pregnancy rate to 18 and 15% for 35 and 70% RDP supplements, respectively. Providing the highest level of supplementation would further improve pregnancy rates 8% compared to the low level of supplementation for both the 35 and 70% RDP supplements.

Due to their ability to maintain body condition score when consuming low-quality forage, Brahman cows are predicted to have higher pregnancy rates than angus cows, with and without supplemental protein. When consuming low-quality forage without supplemental protein, brahman cows would be expected to have 4% greater pregnancy rate compared to angus cows. When provided 35% RDP protein supplement, pregnancy rates would increase 4 and 17% for the low and high levels of supplementation, respectively over the cows not receiving a protein supplement. Providing the 70% RDP supplement would increase pregnancy rates 10 and 15% for the low and high levels, respectively. This means that supplementing brahman cows with the 35% RDP supplement would not be particularly advantageous unless supplemented at the high level (3 lbs per day). In contrast, supplementing with the 70% RDP over the low level (1.5 lbs per day) would not provide much additional benefit.

Conclusions

In general, the 70% RDP supplement is expected to prevent BCS losses better than the 35% RDP supplement. This suggests that supplements with higher RDP should be chosen over lower RDP supplements when costs are comparable. Lastly, Brahman cows should not be supplemented based on the same recommendations as Angus cattle. Accurate knowledge about the protein and energy requirements in brahman cattle is lacking. Our ongoing research is attempting to quantify the differences between Brahman and Angus to improve our ability to sustainably feed brahman influenced cattle. Additionally, we aim to better understand N metabolism in all cattle to reduce the over and under feeding of protein in all classes of cattle.

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Rasby, R.J., L.A. Stalker, and R.N. Funston. 2007. EC07-281 Body Condition Scoring Beef Cows: A Tool for Managing the Nutrition Program for Beef Herds. Historical Materials from University of Nebraska-Lincoln Extension. 3541.

Table 1: Diet Composition

Item	Hay	35% RDP[1]	70% RDP[2]
Chemical composition, % of DM			
OM	92.0	91.9	91.9
CP	3.5	43.0	43.0
NDF	71.0	37.5	12.8
ADF	42.6	27.1	9.4

[1]35% RDP = 58% Corn gluten meal and 42% Soybean hulls
[2]70% RDP = soybean meal

Table 2. Intake and digestibility in *Bos taurus taurus* (Angus and *Bos taurus indicus*

	Treatments[1]						Contrast *P*-value[3]	
		35% RDP		70% RDP				
Item	CON	Low	High	Low	High	SEM[2]	35 vs 70%	Low vs High
Supplement OMI, g/kg MBW[4]								
Angus	0	5.0	9.9	4.8	9.6	0.09	<0.01	<0.01
Brahman	0	5.3	10.5	5.1	10.2	0.09	<0.01	<0.01
Forage OMI								
Angus	54.0	66.3	69.0	69.1	79.5	2.66	0.01	0.01
Brahman	61.9	63.2	68.1	74.5	73.5	5.28	0.05	0.59
Total OMI								
Angus	54.0	71.3	79.0	73.8	89.1	2.67	0.02	<0.01
Brahman	61.9	68.5	78.6	79.7	83.7	4.97	0.06	0.07
TDOMI								
Angus	31.6	39.3	46.0	41.8	50.3	2.02	0.09	<0.01
Brahman	33.5	37.7	48.5	45.2	48.4	2.90	0.29	0.04
Total Tract OM Digestion, %								
Angus	58.6	55.8	58.5	56.8	56.6	2.53	0.81	0.56
Brahman	54.4	55.0	61.1	55.0	57.9	2.22	0.40	0.03

[1]CON= no supplement; 35% RDP = 58% Corn gluten meal and 42% Soybean hulls; 70% RDP = soybean meal; Low = 1.26 g/kg BW; High = 2.53 g/kg BW
[2]SEM = standard error of the mean
[3]Contrasts: 35 vs 70% = Degradability level; Low vs High = Level of supplementation
[4]MBW = metabolic body weight (initial BW^0.75)

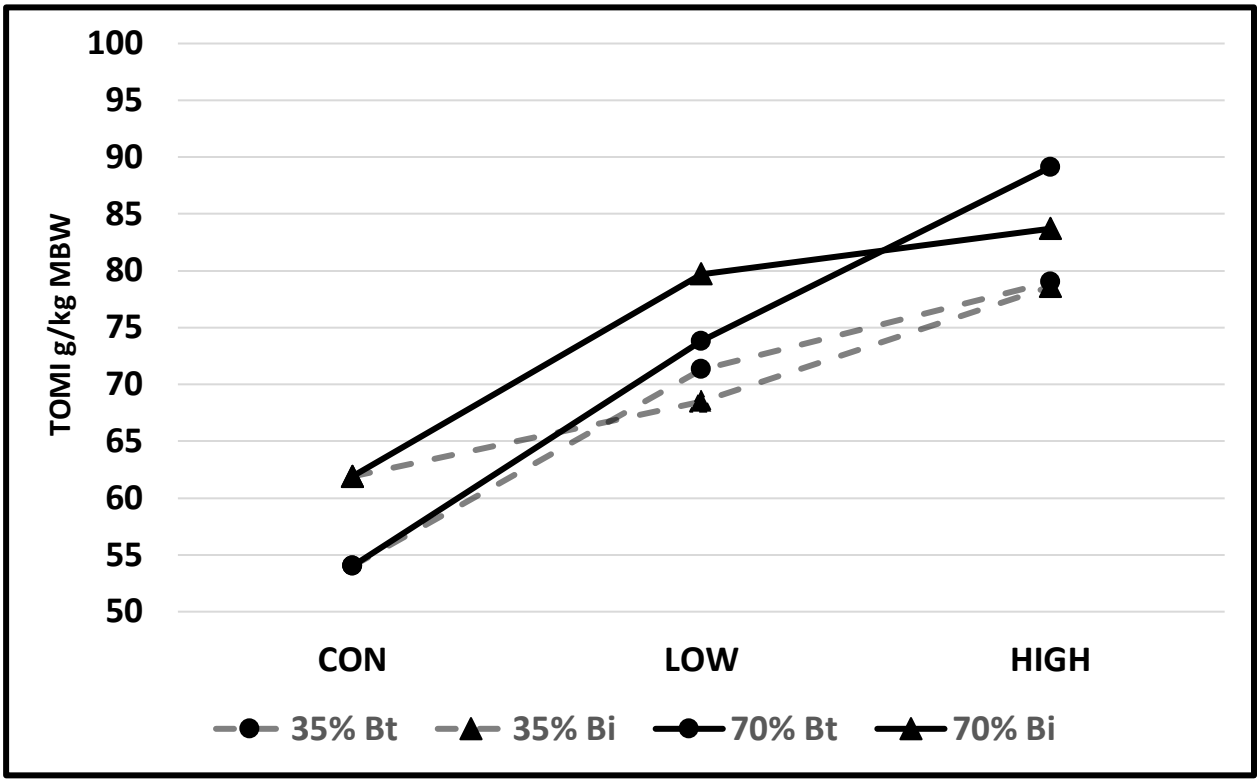


Figure 1: Effect of intake level and degradability on TOMI

CON= no supplement; 35% RDP = 58% Corn gluten meal and 42% Soybean hulls; 70% RDP = soybean meal; Low = 1.26 g/kg BW; High = 2.53 g/kg BW

MBW = metabolic body weight (initial BW^0.75)

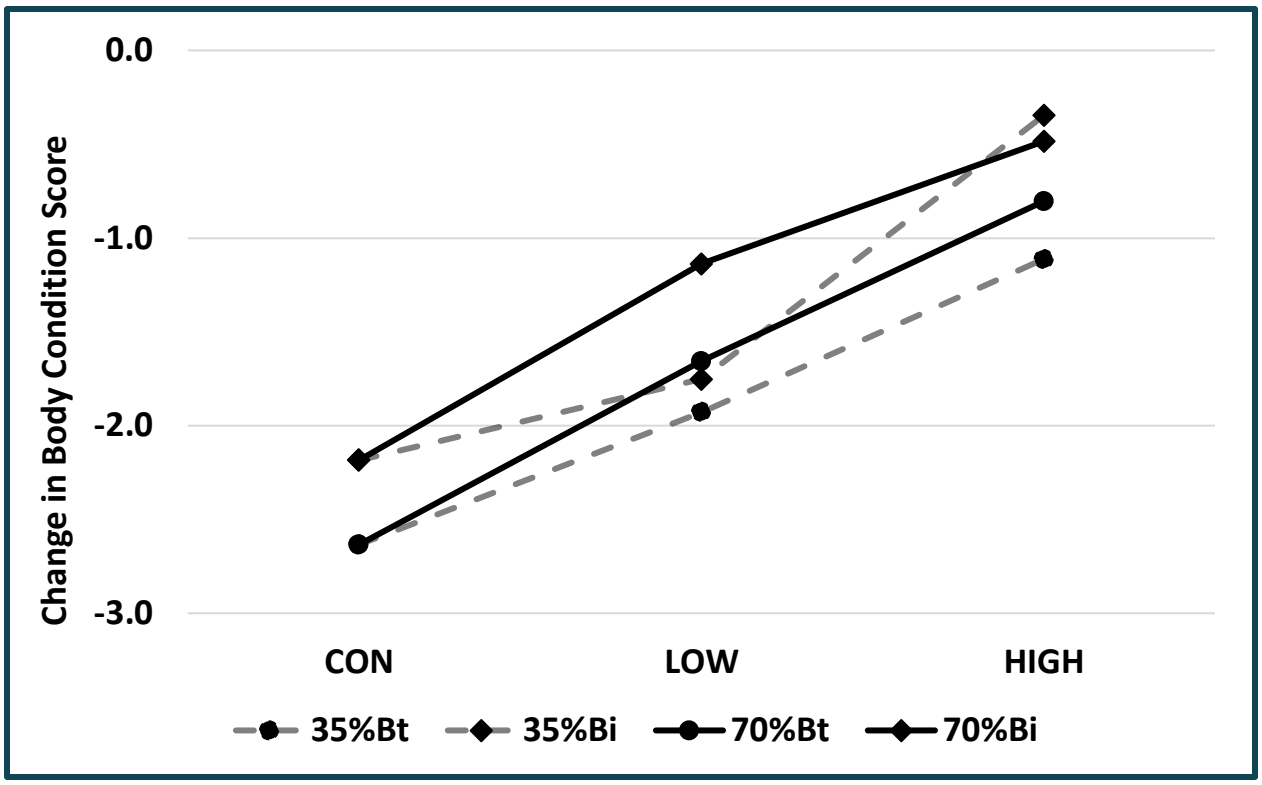
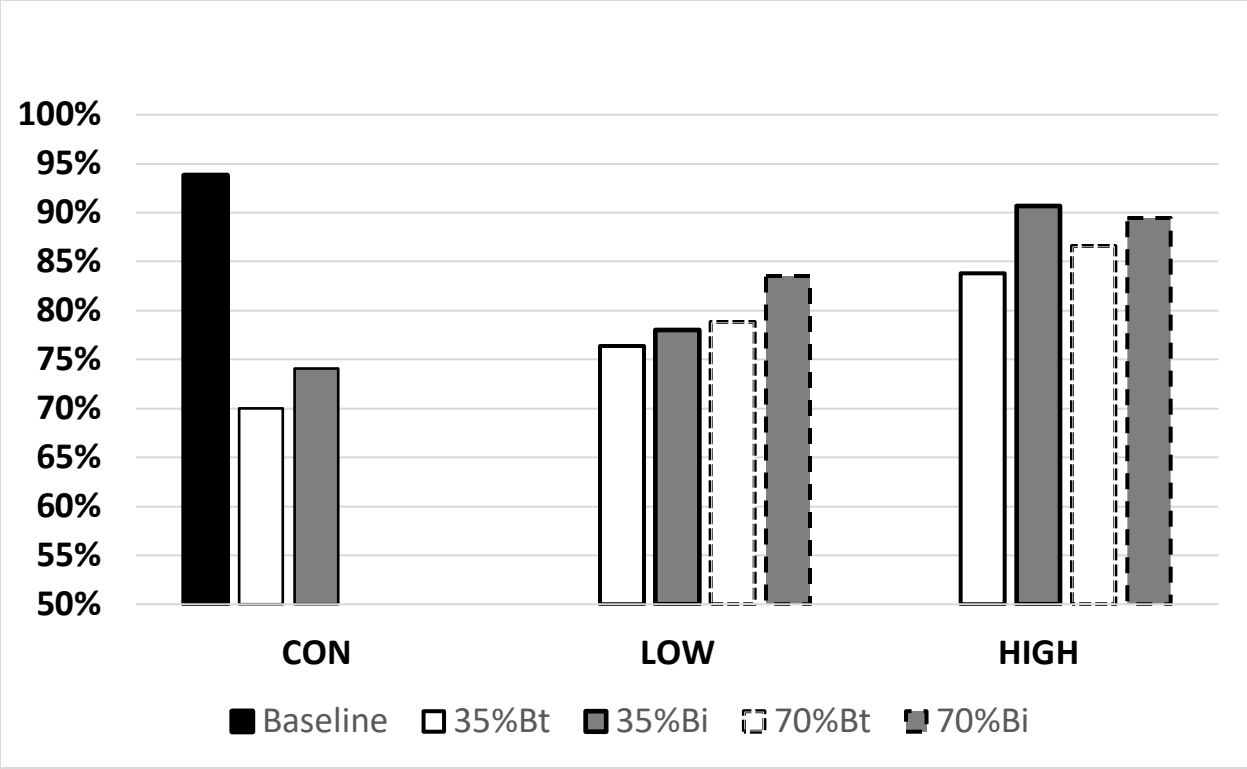


Figure 2: Change in Body Condition Score

CON= no supplement; 35% RDP = 58% Corn gluten meal and 42% Soybean hulls; 70% RDP = soybean meal; Low = 1.26 g/kg BW; High = 2.53 g/kg BW

MBW = metabolic body weight (initial BW^0.75)



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MBW = metabolic body weight (initial BW^0.75)

HOW CONSUMER'S MOUTH BEHAVIOR IMPACTS PERCEPTIONS OF BEEF

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Summary

Consumer acceptance of beef has been closely tied to tenderness. Since the early 1930's tenderness has been measured similarly. We utilized a new tool for texture called mouth behavior that has been used by the food industry to meet consumer demands. Mouth behavior classifies consumers into four classes based on how the chew and manipulate food in their mouth. These categories are chewers, crunchers, smooshers, and suckers. We found that consumers that differ in mouth behavior perceive ground beef and steaks differently. In fact, their ideal ground beef patty and steak do not have the same texture attributes and tenderness is perceived differently by consumers across mouth behaviors.

Introduction

Texture of foods has been shown to impact consumer perceptions. For beef, tenderness has been the main texture attribute studied, but are there other aspects of texture that are important to consumers? Mouth behavior (MB), how consumers manipulate food in their mouths as they eat, has been shown to affect food preferences (Jeltema et al., 2015). The mouth behavior tool created by Jeltema et al. (2015) identified consumers as crunchers, chewers, smooshers, and suckers. Chewers and crunchers prefer to use their teeth to break down foods while smooshers and suckers manipulate food between the roof of their mouth and tongue. Crunchers prefer foods that break or fracture easily. Chewers like products that require increased mastication. Consumers have increased levels of satisfaction when consuming foods categorized into an individuals preferred mouth behavior type. Our objective was to understand if consumers from the four mouth behavior groups responded differently to ground beef and steaks that differed in texture attributes.

Experimental Procedures

Three studies were conducted where ground beef was evaluated in Phases 1 and 2, and steaks were evaluated in Phase 3. For each study, consumers were selected from the Bryan/College Station area that were classified as Crunchers, Chewers, Smooshers and Suckers using the using the JBMB® graphic tool (Figure 1). Consumers within mouth behavior group participated in a workshop that began with discussing participants good and bad burger eating or steak experiences. Consumers then were asked what attributes in a ground beef patty or steak were important. Key words were reported to begin framing texture attributes for each mouth behavior type. Each Mouth Behavior session had varying attributes that were deemed as most important, resulting in unique attributes for each MB group. The two most critical factors were defined and used to develop a landscape map for Phase 1. For Phase 2, the landscape maps were standardized based on results from Phase 1. For steaks in Phase 3, consumers from Phase 2 across MB groups defined what were the most important texture for steaks and those attributes were used for landscape maps. Before being served any patties or steaks, consumers identified where their ideal burger or steak would fall on the landscape maps.

In Phase 1, four 227 g ground beef patty treatments (three treatments were machine formed patties containing either 7, 20 or 27% chemical lipid; and bowl chopped, machine formed, 20% chemical lipid patties) and two 110 g patty treatments (bowl chopped and either hand formed or formed into balls and smashed during cooking) were served. Each consumer received a ground beef patty from each of the six treatments. Consumers were randomly selected across each MB category (crunchers n=7, chewers n=5, smooshers n=5, suckers n=2). In the Phase II, seven foodservice (Wayback Burgers, Five Guys, Koppe Bridge, Whataburger, McDonald's, Sonic, and Freddy's) commercially prepared patties weighing approximately 110 g and six ground beef products (hand formed round, sirloin and chuck that were purchased in chubs; hand formed brisket and chuck patties purchased in over-wrap trays; and chuck patties machine formed at the retail location) were purchased from H-E-B. Patties were presented as in Phase I (crunchers n=4, chewers n=7, smooshers n=3, suckers n=7); however, responses were identified on a bi-plot landscape map with common axis that were derived from Phase I. For steaks in Phase 3, consumers were served Choice ribeye, tenderloin, top loin, and top sirloin butt steaks; Select, Certified Angus Beef, and Prime top loin steaks; and Choice top loin steaks that were aged 45 days, cooked using sous vide and then seared, and 21 day aged top loin steaks. Consumer responses were identified on a landscape map developed by consumers from Phase 2 on what were important attributes for texture of beef steaks.

Differences in ground beef texture attributes were evaluated by Texture profile analysis (TPA) where hardness 1, adhesion, hardness 2, cohesiveness, springiness, gumminess, and chewiness were calculated. Descriptive texture attributes of surface roughness, firmness, springiness, hardness, initial juiciness, mouthcoating, connective tissue amount, cohesiveness, cohesiveness of mass, particle size, particle amount, chewiness, toothpacking, and sustained juiciness were evaluated by a 5-member expert descriptive attribute panel. Steaks were evaluated using descriptive texture attributes of juiciness, muscle fiber tenderness, connective tissue amount, and overall tenderness. Warner-Bratzler shear force was determined for each steak type.

Results and Discussion

In Phases I and 2, ground beef patties differed ($P<0.05$) in descriptive texture attributes of surface roughness, firmness, connective tissue amount, cohesiveness of mass, particle size, and chewiness; and TPA values of hardness 1, adhesion, gumminess, chewiness, and hardness 2. Additionally, in Phases 1 and 2, treatments differed ($P<0.05$) in descriptive sensory attributes indicating that patties in both phases differed in texture and were acceptable patties to use to understand if consumer perceptions differed based on their mouth behavior. Steaks in Phase 3 also differed in descriptive sensory attributes and Warner-Braztler shear force indicating that these steaks provided sufficient range for the objectives of this study.

During Phase 1 and 2, consumers identified factors that influenced their texture evaluation of ground beef (Figure 2). Chewers must have a burger that is flavorful, the bun cannot be soggy, the texture has to differ across the bite and not be rubbery with no gristle, not be dry and not be too greasy. Crunchers must have a burger that is not too dry or raw, not chewy, crumbly or chunky; the bun cannot be soggy; and the meat cannot stick to their teeth. Smoochers have different must haves in their burgers. They want a juicy, well-seasoned, not too big patty with no gristle, not congealed or sludgy with no residue or film in the mouth, and is a patty that does not fall apart in the mouth during chewing, and that is not too greasy or dry. Suckers defined must haves in their burger as the burger has to be juicy, not too chewy, but does not fall apart or cannot be crumbly, and they prefer the burger seasoned before cooking and it

cannot be bland. These results indicated that consumers differing in mouth behavior perceived beef patties differently or had different drivers of liking.

The six patties in Phase 1 were served to understand how fat content (73, 80, and 93% fat) affected consumers perception. Chewers and smooshers found higher fat patties less tough and chewy. Crunchers found 93% lean ground beef patties to be too dry, but they indicated that juiciness was only slightly different in 73% and 80% lean ground beef patties. Higher fat was associated with higher tenderness, but the lower fat sample, though dry, was deemed "fixable" via toppings and condiments. Suckers found little differences in juiciness between the fat levels. Juiciness was more impacted for them based on whether the patty was chopped or ground. However, higher fat samples evoked comments about "different in every bite", which was also important to suckers. Juiciness was important to consumers across the four mouth behavior groups and fat content affected juiciness perception, but in different ways. Also, fat content affected tenderness, but this was only important to crunchers, and while fat content affected texture of ground beef patties, they were more interested in differences in texture across multiple bites. These results indicate that ground beef fat content is important to consumers, but for different reasons.

When consumers evaluated chopped ground beef patties versus ground beef patties at the same fat level (80%) that were ground using traditional methods, consumers within mouth behavior groups responded differently. Chewers said that the lean chopped patties were more tough, whereas crunchers indicated that the lean chopped patties were slightly less juicy. Smooshers indicated that the chopped patties were more 'greasy' and suckers said that the chopped patties were more dry. When consumers evaluated the hand-formed versus machine-formed ground beef patties that also differed in thickness, crunchers liked the thinner hand-formed patties, smoochers said that these patties were less greasy, and suckers indicated that these patties were less dry. When consumers evaluated smashed versus not smashed patties, crunchers said that the smashed patties were too thin and too dry whereas chewers indicated that these same patties were less tough. These results showed that depending on mouth behavior, patties that differed in texture were perceived differently.

To further understand what aspects of ground beef patties that were important to consumers across mouth behaviors, consumers were asked about their perception of the ideal burger (Figure 3). It is apparent that foodservice and casual dining burgers differed in preference across mouth behavior groups. For example, crunchers identified Freddy's as furthest from ideal whereas smooshers rated Freddy's burger closer to ideal. These data were used to select patties for use in Phase 2.

In Phase 2, consumers across mouth behavior groups evaluated commercially available foodservice patties identified in Figure 3. Also, ground beef was purchased at retail to represent the major ground beef blends available. In this phase, consumers across mouth behavior groups evaluated the texture of ground beef patties based on two criteria, how the patty stays together while eating and how tough or tender the patty was or how hard or easy the patty was to bite through (Figure 4). Consumers across mouth behaviors indicated their ideal and while there was some overlap between suckers and smooshers, chewers and crunchers differed in their preferences.

During Phase II consumer perceptions of foodservice ground beef patties differed from their perception of casual dining burgers across the four mouth behaviors (Figure 5). Chewers rated ground beef patties differently than consumers from the other three mouth behavior groups derived from foodservice, casual dining, and retail ground beef patties. Chewers and Crunchers together make up the biggest proportion of the US population (~75%), and they rated burgers differently as indicated by no overlap of

their evaluations in Figure 5. These results indicate that there is no "one-size fits all" for ground beef patties. Understanding this allows companies to provide ground beef patties that meet the preferences of consumers with different mouth behaviors. For example, a burger that has a little bit of fight and somewhat falls apart during chewing may be acceptable to smooshers, suckers and crunchers, but chewers want a burger with a little bit more fight.

Steaks were presented to consumers across the four mouth behavior groups. To start each session, consumers defined their ideal steak differently. Crunchers consider a steak's "ease of chew" to be important, along with how well the meat breaks down, the gumminess of the meat, and the result of the chew size as the steak breaks down. Chewers paid close attention to the chewiness of the meat, along with the consistency of its cooking, its flavor, how it's seasoned, and if the meat melts in the mouth during chewing. Smooshers, by contrast, do not want meat that is chewy; they want their teeth to sink into the meat, for the flavor to persist, and for it not to be dry or gristly. Suckers assess a steak's fiber tenderness, how easily it cuts, the flavor, and how long it takes to chew and then swallow the meat. It was obvious that the "ideal" steak was different for consumers across mouth behavior groups and that there were different drivers of what is desired for steak texture.

Consumers were provided 12 steaks served in five flights to understand perceptions of cut, marbling, degree of doneness, and cooking method. One of the biggest take home messages was that consumers defined tenderness differently. Based on differences in their ideal steak, it was not surprising that they responded to steak treatments differently. Suckers, for instance, want the meat to dissolve, while smooshers want the sensation of their teeth sinking into the meat. Chewers, meanwhile, like to experience multiple textures as they chew, and crunchers want a tenderness that breaks down well, but is not gummy.

Marbling impacted texture perceptions across mouth behavior groups. The Certified Angus and Prime steaks were popular with the majority of consumers. The Certified Angus steaks were close to the ideal for some smooshers, crunchers, and chewers. For suckers, it broke down too easily. For some crunchers and smooshers, the Prime top loin steak required too much chewing time, but suckers and chewers were satisfied with the chewing time. The Select top loin steaks required too much chewing time for all consumers, but the chewers liked the chewing time.

For whole muscle steak, these preliminary findings indicate that a steak that was easy to chew, breaks down relatively fast when chewing, has small pieces when swallowing, and if more or less mouth chewing was required than ideal, at least one mouth behavior group rejected the steak.

Conclusions

These results indicated that mouth behavior classification impacted consumer acceptance of ground beef patties based on difference in beef patty and steak texture. For ground beef patties, a higher fat content reduced toughness and chewiness more for chewers and smooshers than other groups. Changing the texture of ground beef patties by chopping, changed the toughness of ground beef patties for chewers, but juiciness and greasiness was changed for others. Ground beef patties derived from the chuck may be less polarizing across the mouth behavior groups compared to ground beef patties made from other lean sources. While hand-formed ground beef patties may be a great marketing strategy, chewers do not like the resultant texture and the eating experience. For steaks, higher marbled steaks were liked by consumers across each mouth behavior group, but for different reasons. Tenderness was defined differently by consumers across mouth behavior groups. If the beef industry is going to increase

consumer satisfaction and increase beef demand, further research on the impact of consumer mouth behavior preferences is needed.

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Jeltema, M., Beckley, J., & Vahalik, J. (2015). Model for understanding consumer textural food choice. Food Science & Nutrition, 3(3), 202-212. doi: 10.1002/fsn3.205

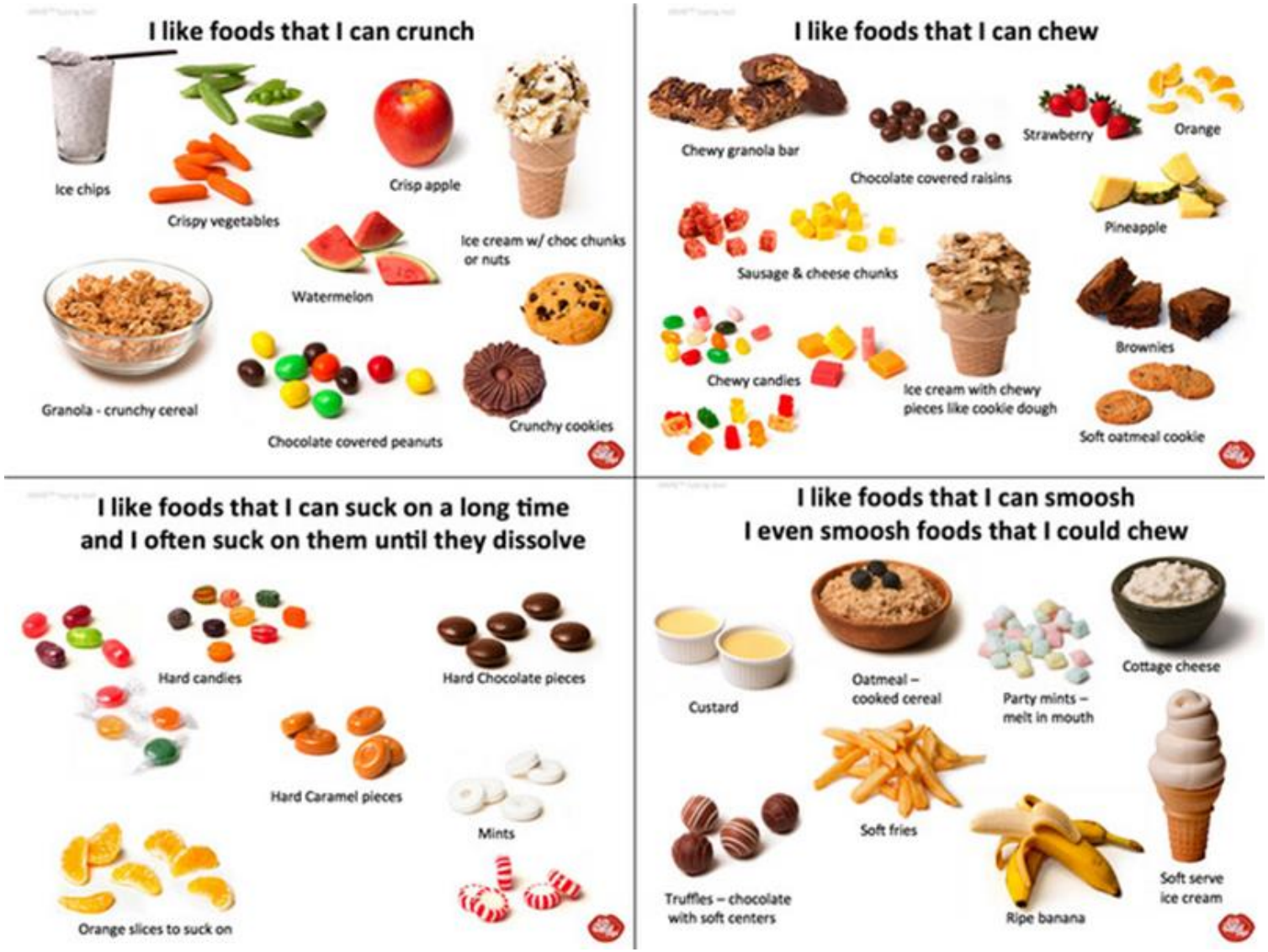

Figure 1. JBMB® graphic tool (Jeltema et al., 2015) used for identifying consumer classifications for mouth behavior.

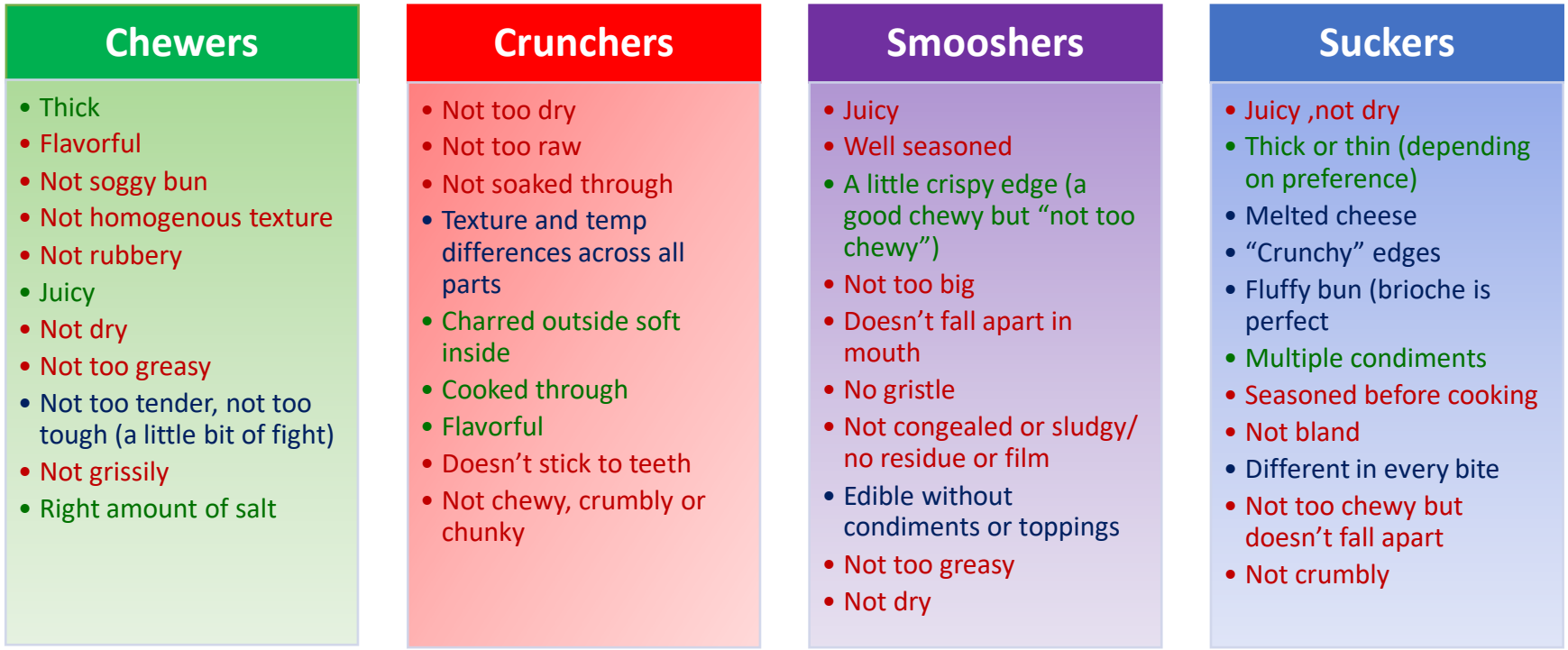

Figure 2. Summary of what a good burger should be across mouth behavior groups where red text denotes Must Have; green text is for Optimizer; and blue text is for Delighter.

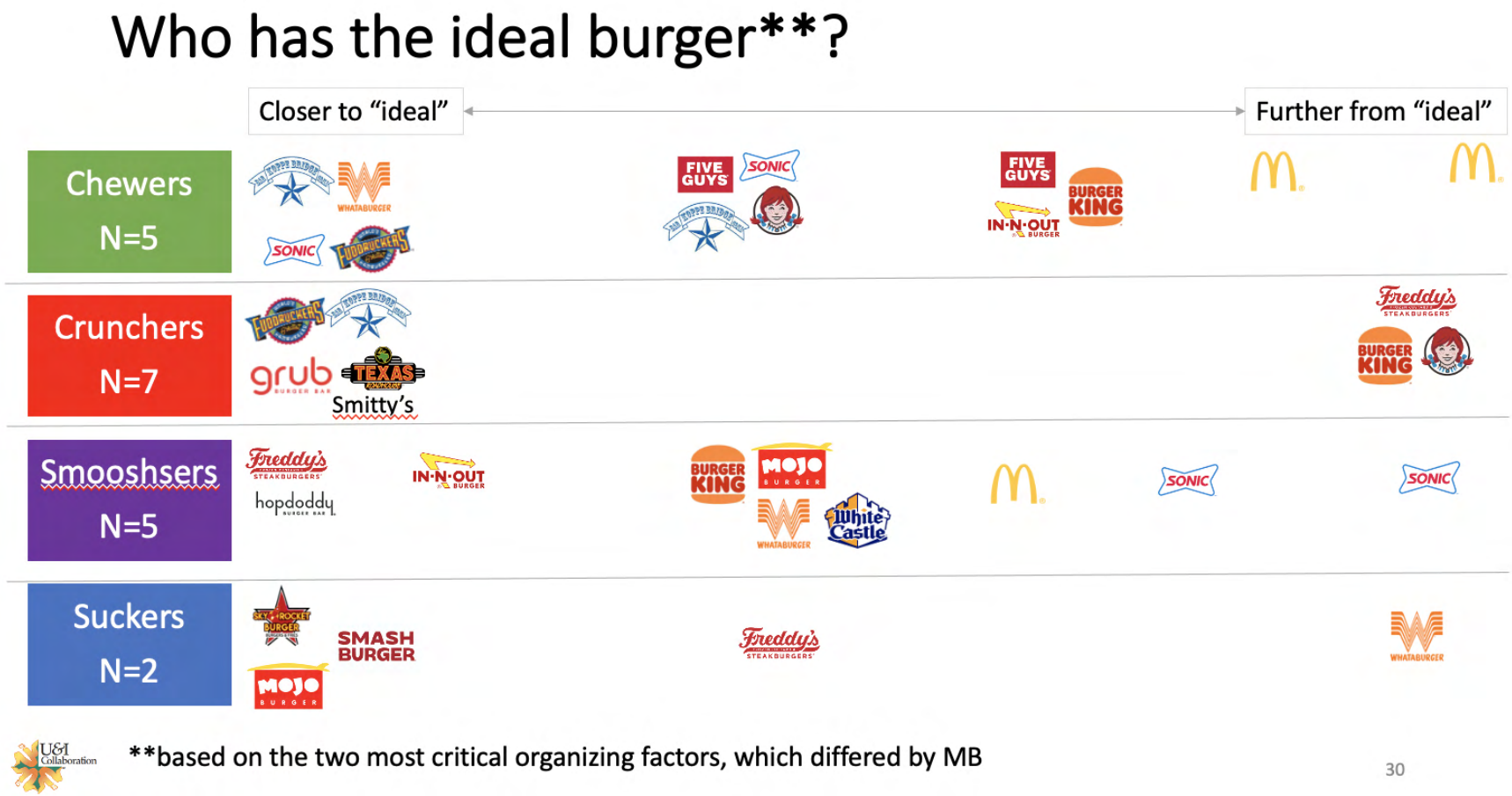

Figure 3. Consumer responses across four mouth behavior groups for who has the ideal burger.

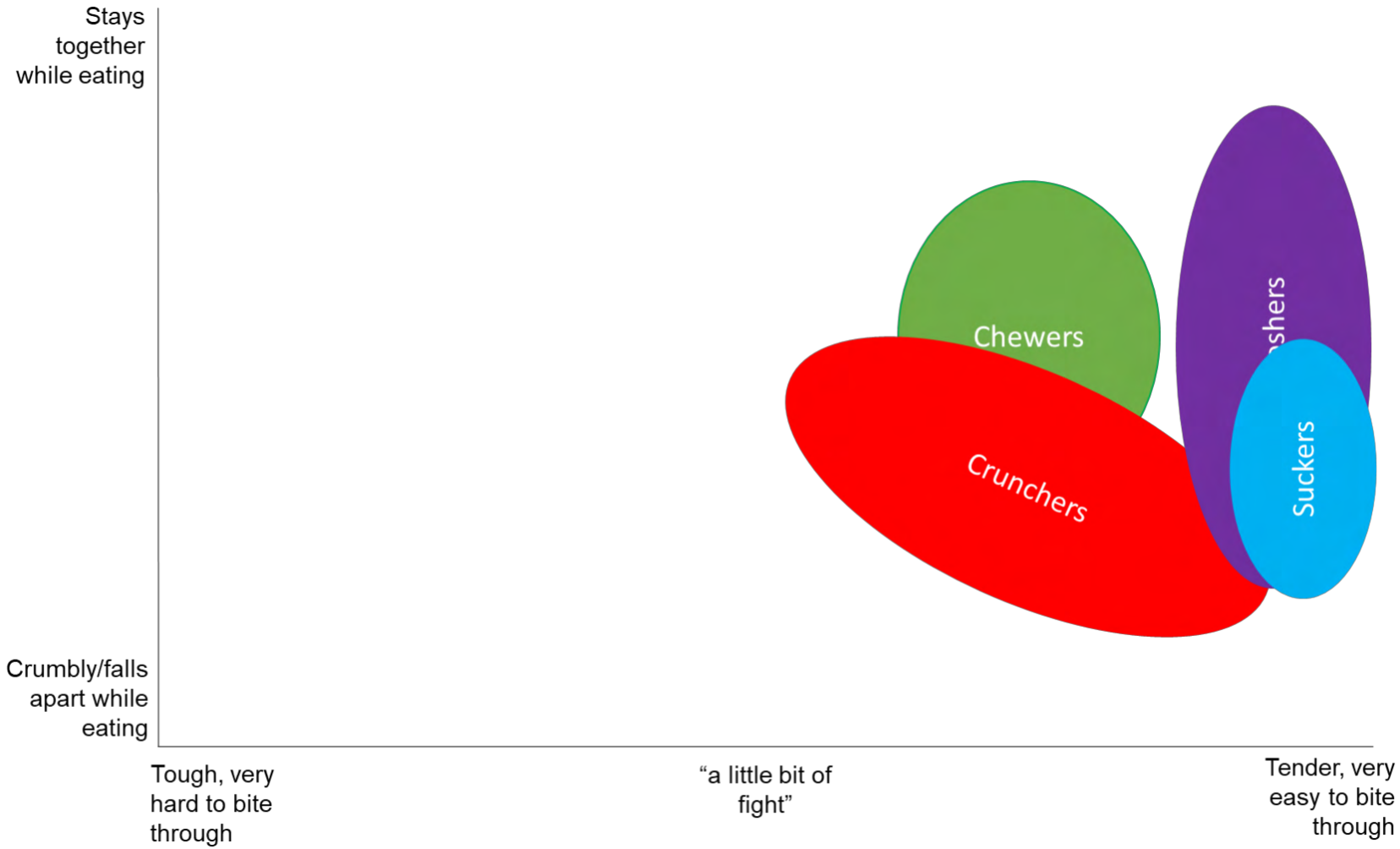

Figure 4. Biplot of optimal food service or casual dining burgers for consumers across four mouth behavior groups.

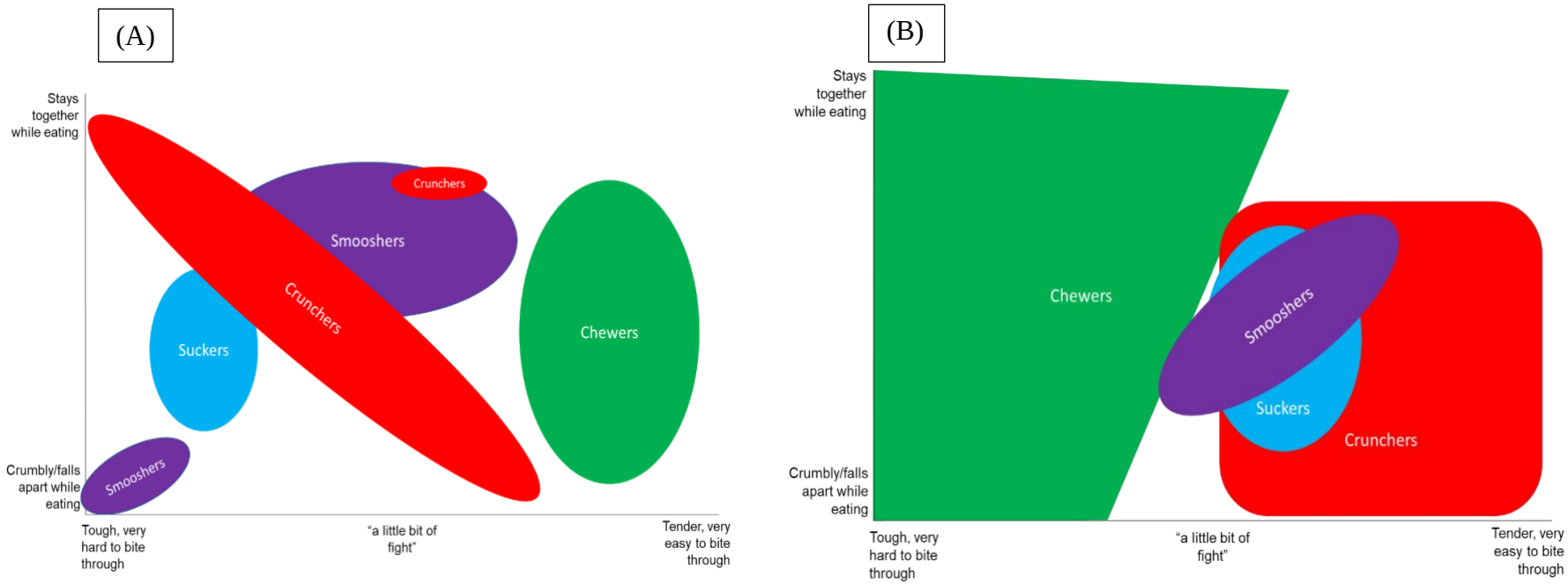

Figure 5. Biplots for consumers across four mouth behavior categories for (A) food service burgers; and (B) for retail ground beef patties.

CURING MEAT PRODUCTS WITHOUT SODIUM NITRITE? A NOVEL APPROACH TO MEAT CURING

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Summary

A series of studies evaluated the feasibility of utilizing the endothelial nitric oxide synthase system (eNOS) in post rigor skeletal muscle as an innovative method to generate nitric oxide and residual nitrite to cure meat products. We hypothesize that the addition of the amino acid L-arginine activates the eNOS system in post rigor skeletal muscle to generate nitric oxide (NO) for cured meat color development and residual nitrite for product shelf life and safety as an antimicrobial.

This research indicates that the addition of L-arginine to post rigor beef that is subsequently thermally processed can generate NO to cure beef products. This research will improve our knowledge and understanding of the chemical, physical, and biological properties of meat products cured via this novel amino acid based alternative curing method by eliminating the need for sodium nitrite. It is expected that this innovative alternative curing system will be similar to the safety, quality, shelf-life, convenience, nutrient profile and sensory attributes of conventionally (sodium nitrite) cured meat products.

Introduction

Sodium nitrite acts as a curing agent for meat products which increases product shelf life and stability. This curing reaction results in the formation of the nitric oxide (NO) molecule which can bind to the meat pigment myoglobin to from the traditional cured pink color and when two NO molecules combine, they form residual nitrite which provides antimicrobial and antioxidant products to improve product safety and shelf life. Recent concerns regarding the potential carcinogenic compounds (i.e., nitrosamines) (Sindelar and Milkowski, 2012) that can be formed from cured meat products has resulted in the development of alternative curing methods where nitrite is indirectly added to meat via a "high nitrite source" (i.e., vegetable/celery powder).

The Nitric Oxide Synthase (NOS) system is vital for muscle function due to its ability to use L-arginine to produce nitric oxide (NO). In the presence of oxygen, NO is oxidized to nitrite and then to nitrate. Little to no research has been conducted to evaluate the NOS system's ability to generate NO and nitrite in post-rigor meat. The overall objective of this research was to determine the feasibility of the endothelial nitric oxide synthase (eNOS) system to generate NO as an alternative curing system.

Experimental Procedures

Beef samples (25g) containing 2% salt, 10% water and either Prague powder (6.25% sodium nitrite) to achieve 120, 156 or 200 ppm sodium nitrite, or L-arginine to achieve 1000, 2000, 3000, 4000, or 5000 ppm were cooked to either 55.6, 70.0, or 73.9°C. From these results, commercial beef frankfurters were manufactured containing either 156 ppm sodium nitrite or 3000, 4000 or 5000 ppm L-arginine. Based on the frankfurter study results, beef samples were treated with either L-arginine (2000 or 4000 ppm) or a combination of L-arginine and L-citrulline (2000/2000 or 4000/4000 ppm). All samples for each study were analyzed for residual nitrite (RNO_2 by UV/VIS spectrophotometry) and cured color (nitrosylhemochromagen) as indicators that the beef products were cured. Each experiment was conducted as a randomized complete block design with a factorial arrangement of treatments replicated three times. Tukey's HSD was used with a predetermined significance of $P<0.05$.

Results and Discussion

Study 1

For the first study, sodium nitrite treated beef samples (Table 1) a C x T ($P<0.05$) interaction indicated that residual nitrite (RNO_2) tended to decrease as temperature increased at each sodium nitrite concentration. L-arginine treated beef sample RNO_2 values decreased as temperature ($P<0.0001$) increased. As L-arginine concentration increased, the RNO_2 values were observed to be lower at 55.6°C. At higher temperatures, the 3000 and 4000 ppm L-arginine samples tended to have lower RNO_2 values. All L-arginine concentrations and endpoint temperatures for each species generated similar, but generally lower RNO_2 values compared to sodium nitrite treated beef samples at concentrations of 120, 156 and 200 ppm. Nitric oxide heme values (NO-H) for nitrite treated beef samples indicated that as concentration increased, NO-H values decreased (less intense cure color). For L-arginine treated beef samples a C x T interaction indicated that as concentration increased, NO-H values tended to increase at lower endpoint temperatures (55.6°C). L-arginine concentrations of 1000-4000 ppm for beef samples generated RNO_2 values comparable to sodium nitrite treated samples at concentrations of 120, 156 and 200 ppm. Higher endpoint temperatures tended to decrease RNO_2 levels. Using RNO_2 as a baseline identified L-arginine concentrations that generated similar RNO_2 values compared to samples treated with 120, 156 or 200 ppm sodium nitrite at different endpoint temperatures.

Study 2

Based on this data we evaluated the efficacy of an amino acid-based "no added sodium nitrite" alternative curing system compared to a conventional curing system (direct addition of sodium nitrite) in the manufacture of a beef frankfurter. Beef (90/10) was used to create frankfurters salt (1.8%), sodium tripolyphosphate (3500 ppm), seasonings, added water (10%) containing either 156 ppm sodium nitrite (6.25% Prague powder, conventional curing) or 3000, 4000 or 5000 ppm L-arginine. Frankfurters were stuffed into 26 mm cellulosic casings thermally processed using a standard frankfurter schedule until an internal temperature of 72.2°C was reached then chilled (4.4°C) peeled and vacuum packaged. The frankfurters were analyzed at 1, 14, 28 and 56 days post manufacture and were evaluated for physiochemical properties such as proximate composition, pH, water activity, residual nitrite, internal and external color and the shelf life attributes of lipid oxidation, and aerobic plate counts.

A concentration x day interaction ($p<0.001$) was observed for residual nitrite values. Treatment frankfurters were lower in residual nitrite than the sodium nitrite control across all L-arginine concentrations at day 1 and 7 of refrigerated storage. From day 14 to 56 days of storage, residual nitrite values for L-arginine treated frankfurters were similar to (Day 14) or slightly higher (Day 28 and 56) than. Internal a* values (redness) were approximately 50% less intense than the sodium nitrite control frankfurters for all storage days (Figure 1B). Additionally, there was a noticeable difference in external surface color between treatment and control frankfurters (Figure 1A). The results from this study indicate that L-arginine can be used as a substrate to activate the nitric oxide synthase system to generate residual nitrite and nitric oxide in a similar fashion to conventionally cured (sodium nitrite) beef frankfurters. The potential exists for L-arginine to be used to generate sodium nitrite as an alternative curing system for processed meat products. However, further investigation needs to be conducted with respect to color development and color stability of products manufactured using this system.

Study 3

We observed from the second study that the effects temperature and time on the ability of the eNOS system to generate NO should be investigated. Beef samples were prepared as previously described for Study 1. Beef samples contained either 120, 156 or 200 ppm sodium nitrite ($NaNO_2$) or 2000 or 4000 ppm L-arginine, or 2000/2000 or 4000/4000 ppm of a combination of L-arginine and L-citrulline. L-citrulline was added due to its ability to convert to L-arginine within the eNOS system under appropriate conditions. Additionally, beef samples (25 g) were placed in tubes and heated (water bath) to 37°C. At 37°C samples were removed at 0 min or held for 45 min (Ti) at 37°C then heated to a final endpoint temperature (T) of 70°C. Samples were cooled, vacuum packaged and held at 3°C for seven days, then tested for residual nitrite (RNO_2) and nitrosylhemochromagen (NO-H) formation.

For amino acid treated beef, RNO_2 levels increased at higher C ($P<0.05$) and longer Ti ($P<0.01$, Table 4). A C x T and Ti x T interaction ($P<0.0005$) was observed for $NaNO_2$ treated samples. C x Ti and Ti x T interactions were observed for NO-H values of amino acid treated beef. NO-H levels tended to be higher at 0 min and higher C, and lower at 45 min at lower T, respectively (Table 5). In summary, amino acid AA treated beef samples showed higher RNO2 and NO-H levels and were similar to sodium nitrite treated samples. Greater concentrations of L-arginine or L-arginine/L-citrulline combinations and longer times increased RNO_2 and NO-H levels in amino acid treated beef samples.

Conclusions

These studies provided data to support our hypothesis of utilizing L-arginine as the substrate to activate the endothelial nitric oxide synthase (eNOS) system in post rigor skeletal beef muscle to generate nitric oxide and residual nitrite to cure meat products. Addition of 1000 to 4000 ppm L-arginine and holding these amino acid treated products at 37°C for 45 min, appears to enhance the ability of the eNOS system to generate NO and develop cured meat color. Further studies will investigate the eNOS system's ability to generate NO, and how it impacts product shelf life, sensory properties, nutrient composition and safety.

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Sindelar, J. J., and A. L. Milkowski. 2012. Human safety controversies surrounding nitrate and nitrite in the diet. Nitric Oxide 26: 259-266.

Table 1. Study 1. Summary least squares means for main effect of concentration on residual nitrite levels in sodium nitrite and l-arginine beef samples.

	Residual Nitrite (ppm)[1]					
Sodium Nitrite	120 ppm	156 ppm	200 ppm			Significance
	73.56	68.51	64.30			C x T P<0.05
L-Arginine	1000 ppm	2000 ppm	3000 ppm	4000 ppm	5000 ppm	NS[4]
	57.74	51.42	54.98	56.04	51.93	

[1]ppm = absorbance level x standard curve slope (1.7438) x dilution factor (200)
[2]SEM: Standard error of the mean
[3]With 547 ppm sodium erythorbate
[4]NS=Not significant

Table 2. Study 2. Least squares main effect and two-way interaction means for internal color a* values of sodium nitrite and l-arginine treated frankfurters stored for 56 days.

n=240	Sodium Nitrite	L-Arginine Treatments					
Concentration	156 ppm	3000 ppm	4000 ppm	5000 ppm		SEM[2]	p-value
a*	14.67[a]	7.07[b]	6.89[b]	6.89[b]		0.07	<0.0001
Day	1	7	14	28	56		
a*	8.70[bc]	8.52[c]	8.86[b]	8.83[b]	9.40[a]	0.08	<0.0001
Conc x Day	1	7	14	28	56		
a*							
156 ppm	15.09[a]	14.51[a]	14.66[a]	14.72[a]	14.38a	0.17	<0.0001
3000 ppm	6.68[de]	6.81[de]	7.01[cde]	6.92[cde]	7.96b		
4000 ppm	6.55[e]	6.32[e]	6.82[de]	7.01[cde]	7.76bc		
5000 ppm	6.47[e]	6.45[e]	6.95[cde]	7.08[cde]	7.51bcd		

[1]a* = measure of redness Red (100) to Green (0)
[2]SEM: Standard error of the mean (largest) of the least squares means
[a-e]LSMeans with different superscripts are significantly different (P < 0.05)

Table 3. Study 2. Least squares main effect and two-way interaction means for residual nitrite values of sodium nitrite and l-arginine treated frankfurters stored for 56 days.

n=438	Sodium Nitrite	L-Arginine Treatments					
Concentration	156 ppm	3000 ppm	4000 ppm	5000 ppm		SEM[1]	p-value
	61.34[a]	54.62[a]	58.80[a]	58.93[a]		2.37	0.1737
Day	1	7	14	28	56		
	60.36[a]	60.14[a]	54.29[a]	59.13[a]	58.20[a]	2.10	0.4233
Conc x Day	1	7	14	28	56		
156 ppm	70.34[abc]	70.93[ab]	60.41[a]	51.19[abc]	53.83[abc]	4.62	<0.0001
3000 ppm	44.26[c]	55.83[abc]	59.61[abc]	49.81[abc]	63.60[abc]		
4000 ppm	62.17[abc]	59.13[abc]	50.36[abc]	63.24[abc]	59.11[abc]		
5000 ppm	64.65[abc]	54.66[abc]	46.80[bc]	72.27[a]	56.27[abc]		

[1]SEM: Standard error of the mean
[a-c]LSMeans with different superscripts are significantly different (P < 0.05)
[a-c]Two-way interaction means within each row/column with different superscripts are different (P < 0.05)

Table 4. Study 3. Least squares main effect means for residual nitrite levels of L-arginine and L-citrulline treated beef samples

Residual Nitrite (ppm)[1]						
		Beef n=192				
Conc (ppm)	2000[3]	4000[3]	2000/2000[4]	4000/4000[4]	SEM[2]	p-value
	78.75[b]	94.29[a]	96.01[a]	86.35[a]	4.98	0.0494
Time (min)	0	45				
	82.23[b]	95.47[a]			3.52	0.0062
Temp (°C)	37	70			SEM[2]	p-value
	93.37	84.33			4.04	0.0611

[1]ppm = absorbance level x standard curve slope (1.7438) x dilution factor (200)
[2]SEM: Standard error of the mean (largest) of the least squares means
[3]L-arginine concentrations
[4]Combination L-arginine and L-citrulline concentrations
[a-b]LSMeans within a row with different superscripts are different (P < 0.05)

Table 5. Study 3. Least squares means for main effects and two-way interactions of NO-heme values for L-arginine and L-citrulline treated beef samples

NO Heme (ppm)[1]						
		Beef n=192				
Conc (ppm)	2000	4000	2000/2000	4000/4000	SEM[2]	p-value
	95.24	99.78	88.34	93.49	3.11	0.0522
Time (min)	0	45			SEM[2]	p-value
	97.61[a]	90.81			2.14	0.0245
Temp (°C)	37	70			SEM[2]	p-value
	119.85[a]	68.57[b]			2.53	<0.001
Conc x Time	2000	4000	2000/2000	4000/4000	SEM[2]	p-value
0	93.23[ab]	108.13[a]	88.92[b]	100.15[ab]	4.40	0.0487
45	97.24[ab]	91.43[ab]	87.76[b]	86.83[b]		
Conc x Temp	2000	4000	2000/2000	4000/4000	SEM[2]	p-value
37	120.99[ab]	132.98[a]	106.65[b]	118.78[ab]	3.25	0.0054
70	69.48[c]	66.59[c]	70.02[c]	68.20[c]		
Time x Temp	0	45			SEM[2]	p-value
37	119.93[a]	119.76[a]			3.62	0.0281
70	75.29[b]	61.86[c]				

[1]NO-heme pigment concentration (ppm acid hematin) = sample A540 × 290
[2]SEM: Standard error of the mean
[3]L-arginine concentrations
[4]Combination L-arginine and L-citrulline concentrations
[a-b]LSMeans for each main effect within a row with different superscripts by row different (P < 0.05)
[a-c]LSMeans for each significant main effect and two way interaction within each row and column with different superscripts are different (P < 0.05)

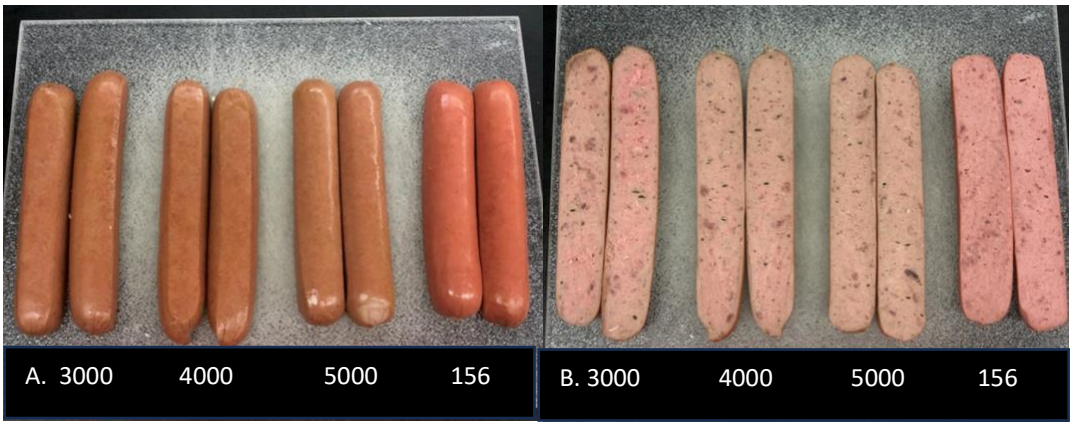

Figure 1. Study 2. External (A) and internal (B) color of beef frankfurters with sodium nitrite (156 ppm control) or L-arginine (3000, 4000, 5000 ppm).

A SATELLITE-BASED DECISION-SUPPORT TOOL TO OPTIMIZE PROFITABILITY AND ENVIRONMENTAL STEWARDSHIP OF GRAZING COW-CALF OPERATIONS

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Summary

A computer program to optimize weaning weight (**WW**), profitability, and environmental sustainability of cow-calf operations was developed by combining an existing nutrition model with pasture biomass estimated using satellite images. Data from McGregor Research Center was the model testbed. Pasture forage data was collected via satellite imagery, and predicted pasture biomass was adjusted based on third-party algorithms. Nutrient requirements were estimated using the Ruminant Nutrition System, and constant pasture nutritive value was assumed. The model used initial herd size to estimate potential dry matter intake (**DMI**) and actual average WW to predict cow milk yield and energy requirements, which were used to estimate the required DMI (**DMR**). Actual forage allowance correlated to DMR simulated forage allowance to support increased DMI and milk yield for a targeted average WW. Simulations demonstrated that increasing target WW with fewer mature cows produced heavier calves, decreased calf gain, reduced carbon emissions, and increased net income.

Introduction

As Texas beef production is predominantly based on grazing systems (Asem-Hiablie et al., 2015), cow-calf production, as the first sector of the supply chain, is critical in assuring the sustainability of the entire beef supply chain. However, Texas pastureland conditions have exhibited increased degradation in the last five years (USDA-NASS, 2022). Although variable annual precipitation can partially explain changes in pasture conditions, overgrazing can result in substantial loss of pasture stand (Rouquette Jr., 2017). Ultimately, pasture degradation could have detrimental environmental and economic impacts.

Sustainable grazing systems necessitate judicious resource utilization to balance animal stocks and forage supply properly. Precision agriculture is a means of optimizing productivity via whole-farm management for resource conservation, but proper decision-support tools are required for deployment (Tedeschi et al., 2021). Thus, our objective was to demonstrate how technology, based on a precision nutrition model and satellite data, can improve grazing cattle systems' environmental and economic sustainability.

Experimental Procedures

Data acquired from Texas A&M University McGregor Research Center, a pasture-based cow-calf operation located in McLennan County, Texas, was used as a testbed for the model. The region has subtropical climatic conditions, with average temperatures ranging from 35.5 to 76˚F and year-round, but highly variable, rainfall. The operation spans 6,372 acres, of which 3,611 acres are native grass pastures, 1,184 acres are improved pastures, 964 acres are used for row crops, and 613 acres are used for hay production. Approximately 40% of the pasture area has mild-to-high weed infestation. The predominant soils are Slidell silty clay, Sanger clay, McLennan clay loam, and Crawford silty clay (USDA-NRCS, 2022).

The current herd inventory includes approximately 700 mature cows, 118 first-calf heifers, 150 heifers, and 60 bulls. Calving generally occurs from February to April, and calves are weaned at 525 lb, on average, in October or November.

Pasture forage data from 2016 to 2022 was collected via satellite imagery and predicted pasture biomass was adjusted based on third-party algorithms (Sigfarm Intelligence LLC). Maps obtained by satellite imagery demonstrated spatiotemporal variations of available forage mass in all paddocks. Spatiotemporal changes in forage mass were equivalent to expected changes in pasture vegetation, as driven by forage development, management strategies, and weather conditions.

The Ruminant Nutrition System (**RNS**) was used to estimate animal nutrient requirements (Tedeschi and Fox, 2020). Pasture nutritive value was assumed to be constant, with 10% crude protein, 70% neutral detergent fiber, and 55% digestibility (dry matter basis). Potential dry matter intake (**DMI**) was estimated based on the actual herd size for each animal category. Actual forage allowance was calculated by dividing total forage mass by total herd live weight. Actual average weaning weight was used to predict cow milk yield and total metabolizable energy requirements, which was used to estimate the required DMI (**DMR**). Actual forage allowance was correlated to DMR, and we simulated the forage allowance needed to support increased DMI and, consequently, increased milk yield to achieve a targeted average weaning weight.

Based on Intergovernmental Panel on Climate Change (**IPCC**) methodology (IPCC, 2019), environmental impact was assessed using greenhouse gas emission estimates from enteric and fecal methane (**CH_4**), nitrous oxide (**N_2O**) from manure and fertilizer, and carbon dioxide (**CO_2**) from feed and fertilizer production. All emissions were converted to their 100-year global warming potential (**CO_2e**), with values of 28 and 265 for CH_4 and N_2O, respectively. Emissions were allocated as a function of live weight production (lb CO_2e/lb live weight).

Assumptions to simulate financial conditions were based on actual hay and calf prices (USDA-NASS, 2022), variable costs of $400, $500, and $600/cow, and income of $0.91/lb of weaned live weight sold.

Each scenario in the simulation represented a different weaning weight (441-657 lb/calf) input to yield a different cow herd size. Additional inputs included available forage in 2022, the 2016-2021 average, and hay supplementation for 90 days/year. Simulations were evaluated based on total weaned live weight, total CO_2e emissions, and net income.

Results and Discussion

The annual green forage mass and accumulated rainfall from 2016 to 2022 are depicted in Figure 1 and display moderate to high variability among the years studied. Slightly decreased forage availability during the 2018 and 2019 production seasons may have been the result of overstocking, while the depletion of forage mass observed in 2022 was due to a period of severe drought, which decreased forage mass by approximately 30% compared to the previous 5-year average production.

The trade-off between cow herd size and calf weaning weight is depicted in Figure 2. Increasing the weaning weight input resulted in the output of a smaller herd size with fewer mature cows but heavier calves. Our simulations indicated that the average available forage from 2016 to 2021 would support a herd with approximately 1,034 mature cows (Figure 2A), while the 2022 available forage would support about 713 mature cows (Figure 2B) for the actual weaning weight of 510 lb/hd. The reduction in available forage, and consequently herd size, demonstrated the vulnerability of long-term grazing systems to unforeseeable periods of drought. Nevertheless, the decrease in the number of weaned

calves was partially offset by increased weaning weight per calf, resulting in a 4% decrease in calf gain per area (Figure 3). Otherwise, the net income increased in scenarios with heavier weaned calves (Figure 3). In addition, the required hay supplementation was reduced when the targeted weaning weight was increased (Figure 4). From an environmental standpoint, the enhanced productivity due to heavier weaned calves and fewer mature cows resulted in a lower carbon emission intensity (Figure 5).

Based on our simulations using the average available forage from 2016 to 2021, we predicted the ideal management strategies to optimize the operation's weaning weight, profitability, and environmental stewardship in 2023. Reducing the herd size from 1,034 to 812 mature cows would increase the average target weaning weight from 510 to 620 pounds per calf. Although the recommended increase in average target weaning weight would result in a 5 lb/acre reduction in calf gain, the operation would be more profitable with an approximately $70,600 increase in net returns per year. In addition, the increased average target weaning weight would reduce hay supplementation by 23 days and reduce carbon emissions by 2.5 lb of CO_2e per lb of carcass weight.

Diminishing pasture conditions are typically offset by increasing hay supplementation. However, increased hay supplementation could be detrimental to the economic sustainability of cow-calf production due to the increased cost of hay production from rising fuel, fertilizer, and equipment costs (Beck et al., 2017). Cow-calf producers in the southeastern United States generally provide supplemental hay to their herd for over 130 days per year (Troxel et al., 2014). Still, we predicted that only 102 days of hay supplementation per year would be needed if the studied operation reduced their herd size. Overall, grazing capacity and intensity are key factors that should be monitored to optimize feeding resources in cow-calf operations (Ali et al., 2016).

From an environmental standpoint, the loss of pasture conditions could harm climate change because overgrazing exacerbates the risk of soil organic carbon losses (Zou et al., 2007). However, the adoption of adequate management strategies and grazing intensities have demonstrated the ability to improve soil organic carbon and potentially contribute to carbon sequestration (Crème et al., 2020). In a study that evaluated the impacts of grazing intensity on greenhouse gas emissions, da Silva Cardoso et al. (2017) determined that pasture management strategies that allow for lighter stocking intensity and prevent overgrazing can reduce N_2O emissions and improve carbon sequestration in soil. The recommended management strategies predicted from our simulations support this concept, as greenhouse gas emissions were reduced by nearly 600 tons per year when grazing intensity was reduced.

Conclusions

Monitoring forage mass can be helpful for herd planning and is an innovative decision-support tool during periods of extreme drought for the establishment of feeding management strategies. Our results implied that this satellite-based decision-support tool could improve profitability, benefit society, and protect the environment, contributing to beef production systems' sustainability (Tedeschi et al., 2015). Overall, this computer model provides the fundamental framework for a decision-support tool for producers to optimize their cow-calf operations while producing ideal weaned calves.

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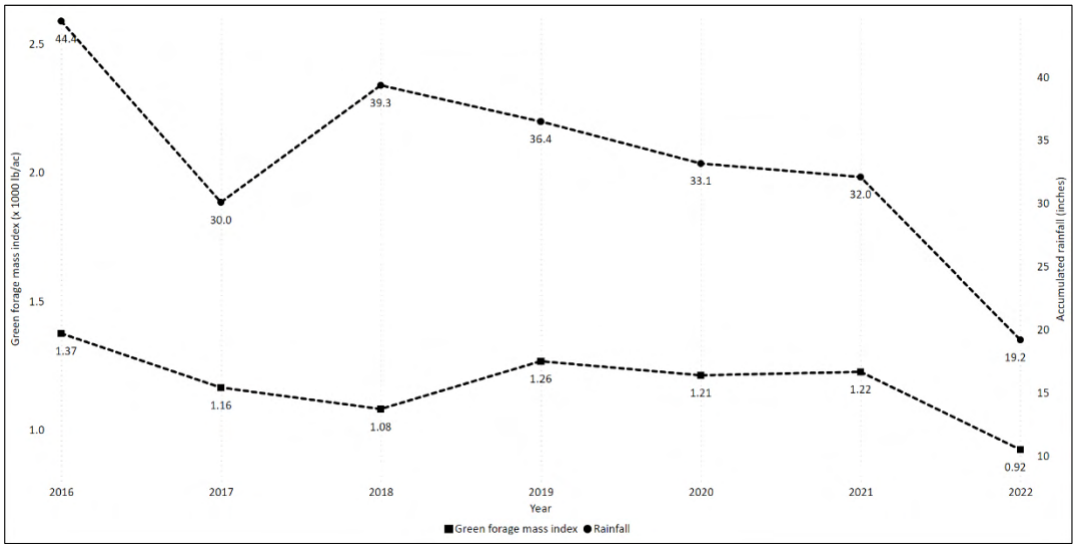

Figure 1. Relationship between average annual green forage mass and accumulated rainfall from 2016 to 2022.

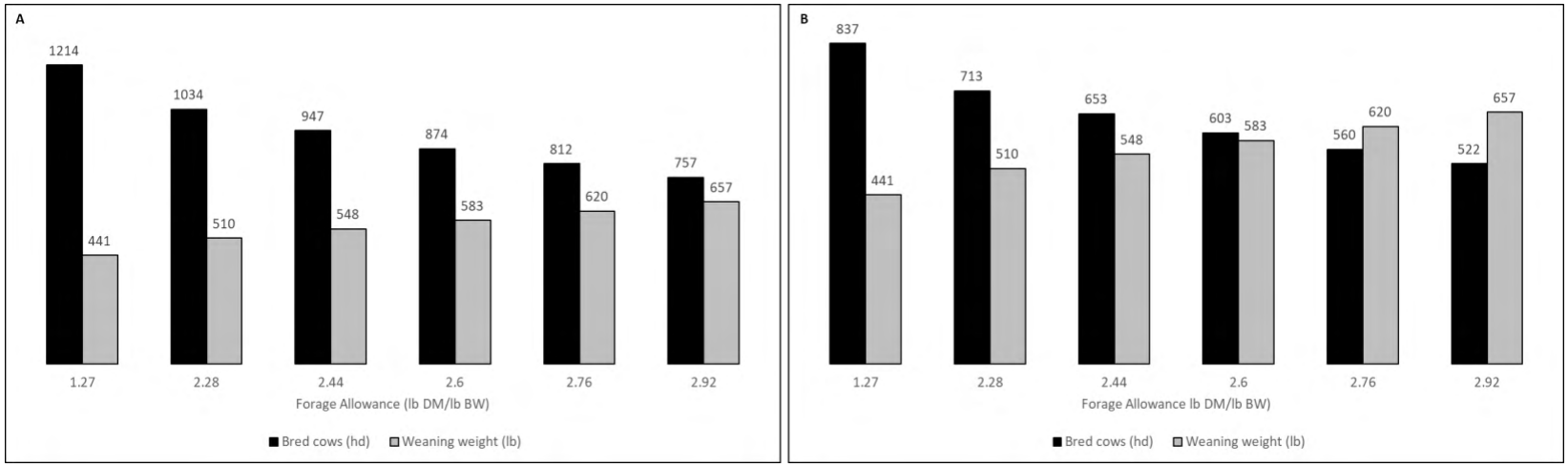

Figure 2. Simulated cow herd size according to expected weaning weight based on actual measurement of available forage from the 2016 to 2021 average (**A**) and from 2022 (**B**).

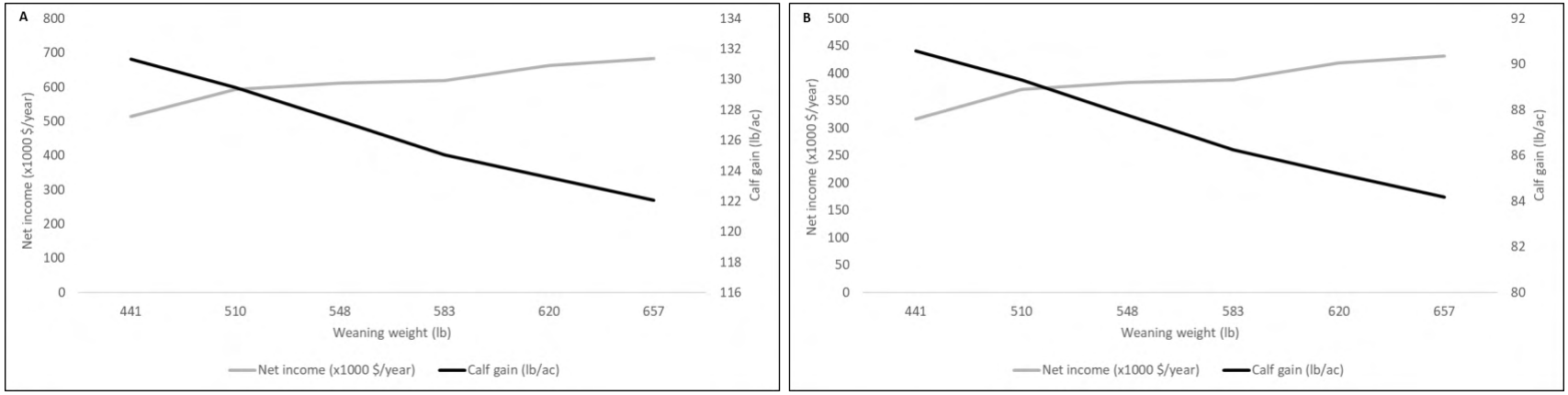

Figure 3. Simulated calf gains and net income according to expected weaning weight based on actual measurement of available forage from the 2016 to 2021 average (**A**) and from 2022 (**B**).

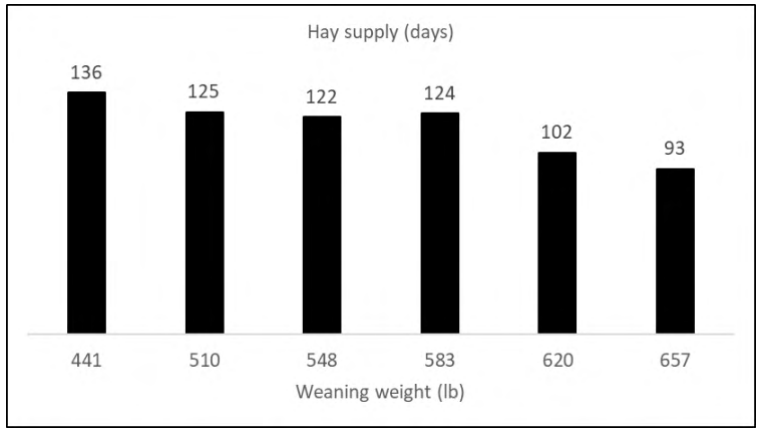

Figure 4. Simulated hay supplementation required according to expected average weaning weight based on actual measurement of available forage.

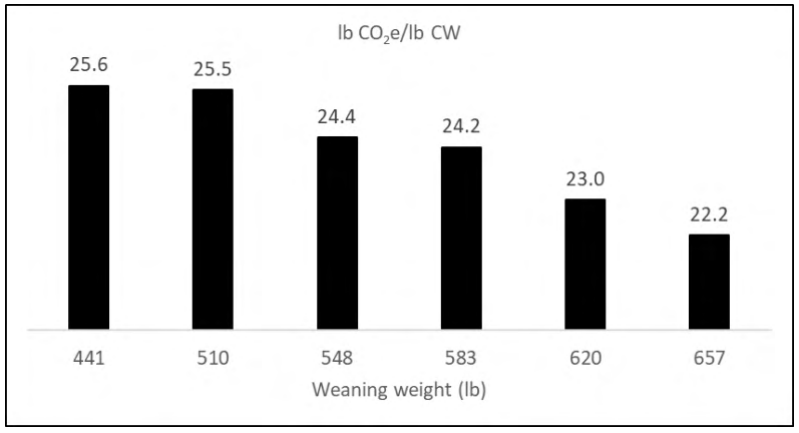

Figure 5. Simulated carbon emission intensity according to expected weaning weight. CO_2e, carbon dioxide equivalent; CW, carcass weight.