MOO-VING METRIÇS: ADVANCING BOVINE NUTRITIONAL EFFICIENCY THORUGH GUSTATORY ANALYSIS AND TASTE BUD PAPILLAE INSIGHT

by

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(Under the Direction of Todd Callaway)

ABSTRACT

Beef producers in the U.S. face the Sisyphean challenge of producing more protein using less land requiring fewer of their limited resources. Improving cattle feed efficiency is an important way to maximize profitability and sustainability, and taste is an important driver in increasing feed intake. The sense of taste plays a crucial role in mammals' food choices and eating habits. With around 25,000 taste buds, cattle have significantly more receptors than humans, making them more sensitive to taste. Taste receptors, located in specific tongue areas, correlate to sweet, salty, bitter, sour, and umami tastes, helping cattle avoid harmful food sources. The present study analyzed the type and number of cattle taste buds on the tongue along with cattle feed intake data to identify potential drivers of feed intake, aiming to optimize feed strategies while also improving cattle health and productivity. The abundance of taste buds allows cattle to select and sample forages and feedstuffs, optimizing their dietary choices.

This study addressed three objectives. The first goal was to compare feed intake data from Angus influenced steers and correlated with taste bud counts and types. Specific focus was placed on examining both circumvallate and fungiform taste buds to identify significant

relationships their populations with animal performance metrics. The second objective was to quantify the types of taste bud cells in the apex, intermediate region, and lingual area of the bovine tongue to understand distribution and density. The final objective of this study was to determine volatile fatty acid concentrations in cattle saliva to determine if it could be used as a proxy for ruminal volatile fatty acid content or if the VFA concentrations could be correlated with taste bud type and geographic distribution. This study provided further insight into the measurements and concentrations of Volatile Fatty Acid (VFA) found in cattle saliva to understand the role in digestion and energy production. Saliva sampling offers a potential noninvasive method to assess digestion kinetics and end products. By analyzing VFA concentrations in cattle saliva, this study aims to enhance understanding of diet's impact on animal welfare and feed efficiency. The findings could significantly advance knowledge of ruminal microbial ecology and its implications for animal nutrition and welfare.

INDEX WORDS: efficiency, cells, volatile fatty acids, ruminant nutrition

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DEDICATION

This work is dedicated to the cattle, for their unwavering dedication to chewing cud and taking this all in tongue and cheek. To anyone who mistakenly asked me about my research, you know more about cattle tongues and volatile fatty acids that you ever wanted to. And to coffee, for fueling me and the cattle through this rather long journey. Oh, and to my Rachie Bug, you can do anything you want in life—unless it's illegal. I'll always be your #1 fan and your biggest pain.

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CHAPTER 1

INTRODUCTION

Beef producers in the US are facing a unique challenge, they must create more protein while using less land and utilizing fewer affordable resources. Improving cattle feed efficiency is an important way to maximize producer profitability and enhance cattle production sustainability. This study analyzed the type and number of cattle taste buds along with cattle feed intake data to identify potential drivers of feed intake, aiming to optimize feed strategies while also improving cattle health and productivity.

The sense of taste plays a crucial role in mammals' food choices and eating habits. Cattle have a a variety of taste buds which are distributed across the tongue. Through the analysis of cattle taste buds and comparison to feed intake data, we can begin to draw correlations between potential drivers of cattle feed intake. This is especially important because cattle feed intake is correlated with feed efficiency and producer profitability. This study aimed to provide insights that could optimize feed strategies and improve overall cattle health and productivity, thereby impacting producer profitability.

The main objectives of this study were:

1. **Compare Feed Intake Data**: Analyze feed intake data from Angus-influenced steers and correlate it with taste bud counts and types, specifically focusing on circumvallate and

fungiform taste buds. This comparison aims to identify any significant relationships between taste bud characteristics and feed intake patterns.

2. **Quantify Taste Bud Cells**: Quantify the types of taste bud cells located within each region of the bovine tongue. The tongue was divided into three regions: the apex, intermediate region, and lingual area. This quantification will help understand the distribution and density of taste bud cells in different tongue regions.
3. **Determine Volatile Fatty Acid Concentration in Saliva**: Measure the Volatile Fatty Acid (VFA) concentration found in cattle saliva. VFAs are crucial for understanding the metabolic processes and overall health of cattle, as they play a significant role in digestion and energy production.

This project aimed to uncover valuable data that can lead to more efficient feeding practices, ultimately benefiting both beef producers and the cattle industry. The findings could pave the way for innovative approaches to cattle nutrition, ensuring sustainable and productive beef production in the face of growing challenges.

CHAPTER 2

LITERATURE REVIEW

Feed Efficiency in Beef Cattle

Beef producers in the United States face an important challenge: producing more of a wholesome food product (beef) while using less land and reducing their environmental impact. Meeting this complex demand is a challenge that is more important than ever and applies across all food-animal species. However, the public perception in the zeitgeist is that "beef cattle are destroying the world," which means that our producers feel an increased pressure to improve. The world faces an impending global food crisis; it will be necessary to feed 10 billion people by 2050 (Myer et al., 2018). By 2050, the world's population is expected to exceed 9 billion people; with that, meat consumption is expected to increase by more than 70% compared to 2010 (FAO, 2011). To ensure a world where people can access adequate animal protein products, beef producers must be more innovative than ever before. A critical method to solve this crisis is to look at behavioral characteristics associated with feeding efficiency to help optimize resource use and minimize input waste. Producers can enhance the sustainability of beef production by focusing on selective breeding, improved feed formulations, and advanced management practices. Embracing technology and innovation will be crucial in meeting the growing demand for meat while mitigating environmental impacts. This multifaceted approach addresses the immediate challenges and ensures a resilient and sustainable future for beef production.

Challenges associated with beef production

Feed costs account for an estimated 65% of total input costs in a cattle production system. (Lancaster et al., 2009). The term efficiency implies a ratio of outputs to inputs (Carstens & Tedeschi, 2006). Therefore, feed efficiency refers to an animal's ability to convert feed into body weight. Cattle convert feed at a 4-6:1 ratio, meaning it takes an estimated 4-6 lb. of input to convert to 1 lb. of output. Feed Efficiency helps the producer determine which animal is eating the most and can be combined with carcass data to see which animal is producing the optimal amount of meat product most efficiently, which equals improved profitability.

Several measures can be used to determine feed efficiency, such as residual feed intake or conversion ratios. Feed Conversion Ratios (FCR) can be calculated by dividing the average daily weight gain by the average dry matter intake. Residual feed intake is a measure of efficiency that can be used to provide insight into various physiological and genetic factors affecting beef production. It is used to identify animals that consume less feed than expected for their maintenance and growth. Thus, low RFI animals are considered more feed efficient, consuming less feed for the same weight as high RFI animals (Vincent et al., 2015). Some studies show that RFI has a genetic basis that differs in feed efficiency as it relates to their genetics. Therefore, RFI is a valuable trait for selective breeding programs to improve feed efficiency (Herd & Arthur, 2009).

Feed efficiency measurements in beef cattle

Overall, Feed efficiency describes the relationship between feed inputs and growth outputs. High-efficiency animals grow well but consume less feed than other animals within their cohorts (Haskell et al., 2019). Several factors can contribute to individual feed efficiency differences within the cohorts, including feed intake, digestion, activity, and thermoregulation (Herd et al., 2004). There are a variety of ways to quantify feed efficiency. Average Daily Gain

(ADG), Residual Feed Intake (RFI), and Residual Average Daily Gain (RADG) are a few ways to look at feed efficiency in cattle. FCR associates the amount of feed required per unit of body growth with the animal. These ratios can be calculated as the average dry matter intake (DMI) per day divided by the Average Daily Gain (ADG). (FCR=Average Dry Matter Intake/Average Daily Gain). Feed Conversion ratios vary among animal species. Fish convert at a 1:1 ratio, chickens 2:1, swine 3:1, and cattle 5-8:1. The poultry industry has increased its efficiency by 250% over the last 50 years (Shike, 2013). In the 1950s, the FCR of cattle was 10:1, suggesting that while not as great as poultry, there are improvements in the species. Through genetic selection, producers rely on EPDs (Expected Progeny Differences) which contribute to producing more efficient animals over time. The use of EPDs made possible through genetic predictions, can help improve population-wide genetic evaluation of beef cattle traits as they summarize all real-world information into a prediction index of genetic merit for an individual producer to make decisions (Thrift & Thrift 2006).

Average Daily Gain

Average Daily Gain (ADG) is a traditional measure of efficiency used for more than 50 years to compare gain rates. It is measured as the weight gained divided by a specified period, measured in days. Not all gain is equal in terms of energetic cost, and cattle gain muscle, bone, fat, and organ tissues as they grow. Many producers calculate ADG because it requires only a scale to measure the weight gained and the amount of feed given to the animal, which allows producers to select their breeding cattle based upon growth and feed efficiency or FCR. One negative impact of selecting based on FCR is that selecting breeding females based on growth merit will increase the mature size of females, thus resulting in an increased cost of maintaining the herd (Herd and Bishop, 2000).

Residual Feed Intake

Residual Feed Intake (RFI) is a measure that represents the difference between an animal's actual feed intake and the expected amount of intake, calculated as:

RFI = Actual Intake - Predicted Intake

This concept was first introduced by Koch in 1963 and has since been recognized for its ability to reduce an animal's feed intake without compromising its growth performance (Bingham et al., 2009). Unlike other feed efficiency metrics, RFI is independent of production traits such as Dry Matter Intake (DMI), Body Weight (BW), and milk production. This independence makes RFI a unique and valuable trait for evaluating feed efficiency. Residual feed intake can be calculated as the deviation of the actual DMI (kg/day) from the DMI predicted based on the linear regression of actual DMI on Average Daily Gain (ADG) (Haskell et al., 2019). Essentially, this calculation determines the intake required for growth and maintenance. Animals that consume less feed than predicted are considered more efficient and exhibit a negative RFI value (Haskell et al., 2019). A negative RFI value indicates that the animal is consuming less feed than expected for its production level, making it more feed efficient.

RFI is a moderately heritable trait, meaning it can be passed down from generation to generation (Arthur et al., 2001). Consistent cattle selection for low RFI values can lead to significant improvements in feed efficiency, resulting in more pounds of gain per pound of feed consumed. This makes RFI a valuable trait for breeding programs aimed at improving the overall efficiency of cattle production (Arthur et al., 2001). One of the notable features that distinguish RFI from other feed efficiency traits is its phenotypic independence from production traits used to compute expected intake (Carstens et al., 2006). This means that RFI is not influenced by the same factors that affect traits like DMI, BW, and milk production, allowing for a more accurate

assessment of an animal's feed efficiency. By focusing on RFI, producers can identify and select inherently more efficient animals, leading to more sustainable and cost-effective cattle production systems.

Residual Average Daily Gain

The residual average daily gain (RADG) EPD was created by the American Angus Association in 2010 (Detweiler et al., 2019). This calculated EPD considers the weaning weights, postweaning gain, ultrasound subcutaneous fat thickness, and genomic dry matter intake (Northcutt, 2010; MacNeil et al., 2011; Nielsen et al., 2013). RADG is expressed in pounds of weight per day where a higher value RADG indicates a more feed efficient animal (Detweiler et al., 2019).

Quantification of Feed Intake through Electronic Devices

The most common way to monitor individual animal feeding behavioral patterns is through a monitoring device on a bunk feed sensor (Schwartzkopf-Genswein et al., 2004). The most common forms of these bunk feeders include Growsafe (now Vytalle, Lenexa, Kansas, USA) and C-Lock (Rapid City, South Dakota, USA), which are automated feed monitoring systems that use different technical approaches to measuring time-consuming feeds. These systems contain bunks equipped with load bars that measures feed disappearance and an antenna for each bunk that record the presence of the animal using a unique RFID tag (Parsons et al., 2020).

Utilization of these feed monitoring systems allows producers to more accurately monitor and measure feed intake in growing cattle through the electronic recording system. This system may help to reduce the number of inputs needed in a cattle production system and reduces the time and money associated with recording feed intake (Wang et al., 2006). GrowSafe systems

utilize a gate and radio transponder frequencies to measure cattle feed intake. Animals are tagged with an electronic identification device (EID) that records animal activity data upon radio frequency (RF) contact with the sensor. When animals are within 20 inches of the sensor, the RFID and EID combination allows producers to capture how often animals visit a feeder and how much they consume each time. Monitoring of intake and feeding pattern behavior helps with calculations related to gain. The most common use of the feed intake data is for RFI related to animal efficiency (Wang et al., 2006). These major behaviors of cattle can be correlated to feeding behaviors associated with measuring feed intake.

Behavioral Patterns and Feed Efficiency

Cattle's behavioral patterns significantly influence their feeding habits. Feeding traits encompass a wide variety of behaviors and characteristics related to how and why cattle consume feed. As herd animals, cattle thrive in larger groups and prefer to eat together when given the choice. Hafez and Lindsey (1965) emphasized the importance of understanding an animal's behavior under various environmental conditions to form an intelligent analysis of research results on physiology, nutrition, breeding, and management (Arave et al.). Key feeding traits to examine include feeding frequency, feeding duration, residual feed intake (RFI), feed conversion ratio (FCR), head-down duration, and flight speed. Understanding these traits can provide insights into cattle temperament, which affects handling, liability, beef quality assurance, and performance.

Cattle possess the five senses of vision, hearing, smell, taste, and touch, contributing to their temperament. Temperament can impact flight speed (FS), which influences feeding behavior. Cattle with a calmer temperament are more likely to have more prolonged feeding bouts and higher intake (Gibbons et al., 2011). FS is defined as an objective measure of the

behavioral response of cattle to handling procedures. Most research has focused on the beef industry, with little done in the dairy sector (Gibbons et al., 2011). Highly excitable cattle have lower ADG and carcass quality grades compared to those with a gentle temperament, which also affects the shedding of *E. coli* O157 (Brown-Brandl et al., 2009). A study by Voisinet et al. (1997) concluded that sex plays a role in temperament and ADG. The study assessed 292 steers and 144 heifers, finding that calmer heifers had higher average daily gains. Additionally, cattle with Brahman breeding exhibited higher temperaments than those without Brahman influence. The results suggest that selecting cattle for calmer temperaments could improve feed efficiency and increase weight gain.

The mammalian olfactory system is complex and consists of multiple subsystems that work together to serve various purposes. Mammals will utilize these complex networks to survey their environment for food, water, and social interactions (Breer et al., 2006). Cattle have an acute sense of smell and can detect odors many miles away (Rorvang et al., 2017). This highly versatile chemoreceptor that cattle possess can receive a network of scents that cattle will then use to select areas of particular interest for feeding, resting, or ruminating (Breer et al., 2006). Smells heavily influence feeding and foraging, and cattle use the odor to indicate food preference. Olfaction can have a significant impact on the flavor of food (Nielson et al., 2015). Cattle often avoid eating in adverse-smelling areas that are ridden with the smell of feces, chemicals, or urine (Dohi et al., 1991).

Cattle are very social creatures that thrive within a herd. However, as with any group, they must determine a leadership order. Leadership in cattle refers to an individual's generalized movements and ability to manipulate and influence movement patterns within the herd. Interactions establish the social status of cattle and determine the roles of leader and follower

(Sueur et al., 2018). This concept of leadership or dominance within a herd or group is often established within a few minutes or no more than two hours (Arave & Albright, 1997). This sense of herd leadership will remain intact until a more dominant animal is introduced into the herd, or the current dominant animal is removed. The leader is defined as the individual who is consistently the one who initiates long-distance spontaneous group movements toward a new feeding site and (ii) long-distance spontaneous group movements are movements that happen when an animal changes activity and location and are immediately followed by a similar change in activity and location by other members of the group (Dumont et al., 2005).

Herd leaders are often allowed by their peers to graze or eat first and typically ingest more feed with higher nutrients than those who follow behind (Teague & Kreuter, 2020). Herd leaders show a higher ADG than other herd members due to better access to nutrient-rich forages, which can produce faster growth. Additionally, they tend to have higher feed intake as they consume higher-quality forages compared to those grazing later (Teague & Kreuter, 2020). Leaders within the herd often have a greater bite-size than followers (Hirata et al., 2022). Exercise is often included in maintenance energy evaluations and the amount of movement over the course of a 24-hr period can also impact FE. For example, a study by Richardson et al. (2000) suggested that steers with higher ADG had higher feed intakes and performed more standing bouts. Collectively, results suggest that more active cattle tend to have better growth rates.

Cattle with better FCR values performed more standing bouts, which indicates increased activity, including more steps, and can indicate a more efficient animal (Haskell et al., 2019). Steers with more consistent feeding times, meaning they consistently ate for around the same time each meal, had better RFI data. Overall, the study implies that cattle taking more steps are

more efficient and have better growth rates than their sedentary counterparts (Haskell et al., 2019). Luiting et al. (1994) suggested that higher activity and lower RFI have been reported in other species, such as poultry and swine. Cattle spend more time eating for longer periods of time at an uninterrupted pace than the cattle identified as followers. They may consume concentrates more quickly than their counterparts (Arave & Albright, 1997) and so effectively may select their diet based on their tastes.

The most common way to quantify feeding behavior traits is by examining meal frequency, total meal duration, average meal duration, meal size, feeding time, and feeding rate (Holtshausen et al., 2011). Feeding events are often pooled into meals using the meal criterion that considers a meal the most extended nonfeeding interval (Lamb & Maddock, 2009). Non feeding intervals in cattle feeding behavior refer to the periods when cattle are not consuming feed. These intervals can occur between feeding bouts and often will vary in length (Van der Werf, 2002). Feeding time refers to the duration of time that cattle spend consuming their food and it plays a vital role in feed efficiency (Lamb & Maddock, 2009). This can be an indication of the efficiency of how cattle convert feed into body mass. Studies have shown that cattle with higher feed efficiency tend to have a longer feeding time implying that they have spent more time eating, which can lead to better nutrient absorption and utilization (Lamb & Maddock, 2009). As mentioned earlier, RFI is a measure of feeding efficiency that represents the difference between an animal's actual feed intake and their expected intake. Cattle who have a lower RFI number are considered more efficient because they consume less feed that predicted (Lamb & Maddock, 2009). Thus, animals with a shorter feeding time have a more negative RFI value, meaning that these animals are more efficient, in terms of FCR, than their counterparts (Kriese-Anderson, 2016).

The Rumen Microbial Population and Feed Efficiency

The rumen microbial population converts feeds into volatile fatty acids (VFA), which the host animal uses for energy, underscoring the rumen's critical role in feed efficiency (Hungate, 1966). The rumen, a pregastric compartment, is where feed particles encounter microbes and produce metabolites (Hungate 1966). The rumen contains a diverse and dense population of microorganisms (bacteria, protozoa, and fungi) that ferment dietary feedstuffs to produce VFAs (e.g., acetate, propionate, and butyrate), leading to proper growth and performance of the animal (Reynolds et al., 2017). Paz et al. (2018) aimed to identify the predominant rumen bacterial groups correlated with variations in feed efficiency across traits using simple linear regression models. In their study, commercial steers and heifers were fed a growing diet for a specified number of days, after which average daily feed intake (ADFI) and average daily gain (ADG) were calculated. Heifers were fed a diet of 70% corn silage and 30% alfalfa hay for 84 days. Steers were fed a diet comprising 57.6% dry-rolled corn, 30% wet distillers' grain with solubles, 8% alfalfa hay, and 4.4% vitamin and mineral mix on a dry matter basis for 78 days. At the end of the trial, ADFI and ADG were calculated for each group. The study found that steers fed the 57.6% dry-rolled corn diet showed improved feed efficiency, requiring less feed to achieve the same gain as other diets. Additionally, these steers exhibited a higher ADG, indicating better growth performance, and the diet also positively impacted carcass characteristics, resulting in greater hot carcass weight and a larger Longissimus dorsi muscle area.

The Gastrointestinal Tract Microbial Population Impact on Feed Efficiency

Research regarding microbial communities and feed efficiency has primarily focused on the rumen; however, increasing attention is being given to other regions of the digestive tract (e.g., the hindgut). The gastrointestinal (GI) tract in cattle also hosts secondary fermentation of

feedstuffs, opening the door for further research on the interactions between efficiency and the ruminant hindgut (Oh et al., 1972). The duodenum in ruminants is the first section of the small intestine. It plays a vital role in digestions (Lopes et al., 2019). It receives the bile and pancreatic juices for enzymatic breakdown of fats, proteins, and carbohydrates. It also neutralizes the acidic chyme from the stomach to create an optimal pH for enzymatic activity. Furthermore, it begins nutrient absorption by taking in some vitamins and minerals (Heda et al., 2023). The jejunum, a part of the small intestine, serves as a critical site for digestion and absorption of dietary nutrients into the bloodstream. This highly specialized area is best known for absorbing carbohydrates and proteins. The jejunal inner surfaces are covered with villi (e.g., finger-like projections), which increase the surface area, allowing for more nutrient absorption. The colon is responsible for water absorption but also plays a significant role in volatile fatty acid absorption (Xu et al., 2021). The ileum, the final section of the small intestine, helps absorb vitamin B12, bile salts and products of digestion that are not absorbed by the jejunum (Shi et al., 2024). The cecum serves as a secondary post gastric fermentation chamber where feed digestion is performed by a specialized consortium of microorganisms (Siciliano-Jones & Murphy, 1989).

Bacteria that colonize the hindgut include the phyla *Proteobacteria, Actinobacteria, Bacteroidetes,* and *Tenericutes. Ruminococcus, Butyrivibrio, Lactobacillus, Bulledia, Mogibacterium,* and *Mitsuokella* have all been detected at the genus level. Other taxa present include *Clostridiaceae, Ruminococcaceae, Micrococcaceae*, and *Lachnospiraceae* (Myer et al, 1988.). These microbial populations in the gastrointestinal ecosystem, especially in the hindgut, are essential for the overall well-being and maintenance of the animal (Myer et al., 2015).

The Process of Taste in Beef Cattle

Taste is "sensations mediated by specific gustatory systems with its own anatomical and physiological particularities. (Bachmanov & Beauchamp, 2007). Food substances in the oral cavity activate specialized sensory cells of the taste bud cells, which will then convey signals to the ruminant's brain. Taste buds are the peripheral organs of gustation and are located on the epithelium of the tongue (Roper & Chaudhari, 2017). They are composed of clusters of columnar sensory cells that are embedded in the stratified epithelium of the tongue, palate, and epiglottis (Roper & Chaudhari, 2017). The taste call determines the perceived quality of the receptor rather than the receptor itself (Mueller et al., 2005). Notable papillae types on the tongue's surface in cattle include fungiform and circumvallate.

Fungiform papillae are mushroom shaped with a slender neck and enlarged head. They resemble fungi, thus gaining the name fungiform (Dorland, 1950). Vallate, or circumvallate papillae, are round and measure 2 mm-8mm in diameter (Tizzano et al., 2015). The pores of circumvallate papillae open to trenches around the base structure. The von Ebner's glands are specialized salivary glands found in the mouths of cattle, which are located near the circumvallate and help flush out the taste buds and allow them to respond more rapidly to new stimuli (Fukami & Bradley, 2007). The Von Ebner’s glands are also known as posterior deep lingual glands which empty directly into circumvallate trenches (Sbarbati, A. et al., 2002; Lee, M. J. et al., 2006; Suzuki, Y., 2006).

Taste Papillae in Cattle

There are several primary tastes in cattle which have been identified: sweet, salty, bitter, savory (umami), and acidic (Ftuwi et al., 2021). It is speculated that cattle have 25,000-35,000 taste buds (Davies et al., 1979). Interestingly, this number in cattle is also about 20% of the taste

buds found in fish (Grover-Johnson & Farbman, 1976). Catfish are the most feed efficient animals, with a 1:1 feeding ratio and approximately 175,000-200,00 taste buds (Morais, 2016). This number is three times the amount found in humans. Therefore, they are more sensitive to tastes than a human.

Mammals have four different types of taste bud cells (TBC), which are organized into taste buds within structures known as the circumvallate, foliate, and fungiform papillae (Shaikh et al., 2023). Cattle have lingual and non-lingual taste papillae which are classified as circumvallate and fungiform. Each of the lingual taste buds contain approximately 13,000-20,000 taste bud receptors (Shaikh, 2023). This number is astounding, considering humans contain only about 10,000 taste buds in their lingual and non-lingual structures (Miller & Reedy, 1990). Humans also possess fungiform, circumvallate and foliate in all four taste bud cell types (Liu, 2019).

The ruminant tongue can be geographically divided into the root, the body, and the apex (Gilbert et al., 2006). The prominent swelling along the dorsal surface that helps push food against the hard palate is known as the torus linguae, and the groove located along the dorsal surface can be described as the transverse lingual (Sakr, 2022). Other muscles that compose this structure include the dorsal and ventral longitudinal muscles and the transverse and vertical muscles that help aid in precise movements (Dotiwala & Samra, 2023). Finally, the extrinsic muscles of the tongue known as the styloglossus, genioglossus, hyoglossus, and geniohyoideus all help with protraction, retraction, and the protrusion and depression of the tongue (Nazih, 2019). The epithelial surface of the tongue contains the filiform, fungiform, and vallate (circumvallate) papillae (Sakr, 2022). The Cranial Nerves (CN) controls portions of taste. The hypoglossal nerve system (CN XII) controls the overall movement of the tongue, and the

trigeminal nerve (CN V) provides the sensory for touch, temperature, and pain (Sakr, 2022). Finally, the taste is transmitted to the brain of the animal via the chorda tympani of the facial and glossopharyngeal nerve and glossopharyngeal nerve (CN IX) (Gibbons, 2011). The taste of feed significantly influences cattle's feed intake and overall well-being.

Type II and Type III taste bud cells often act as insulation providing functionality and integrity of the taste bud allowing them to effectively detect and transmit taste signals (Roper & Chaudhari, 2017). Type II and III cells are spindle-shaped and protrude with long brushlike structures with wing-like cytoplasmic extensions. These hair-like projections, or microvilli, extend into the taste pore and increase the surface area for detecting tastants. Furthermore, these extensions help to connect the cell to the taste pit and provide structural support (Murray, 1993 & Pumplin et al., 1997). Additionally, by regulating the ionic environment and clearing neurotransmitters, Type I cells contribute to the overall homeostasis of the taste bud and ensure that taste cells can respond appropriately to different stimuli (Roper & Chaudhari, 2017).

Approximately 30% of the cells in a taste bud are identified as Type II cells (Roper & Chaudhari, 2017). Type II are more commonly referred to as the taste bud receptor cell, which are peripheral and have a more rounded nucleus than Type I cells (Roper & Chaudhari, 2017). There are two types of taste bud cells located in the taste bud: Type II (the receptor) and Type III (the presynaptic) (Yee et al., 2001; Clapp et al., 2006; DeFazio et al., 2006). Other literature suggests that more mature mammalian taste bud cells can be classified into three broad subcategories: Type I, Type II, and Type III (Roper & Chaudhari, 2017). Type I TBCs are glia-like receptor cells that are the most frequent in appearance and comprise around one-half of the number of cells in a taste bud. They have a notable irregularly shaped nucleus. Although they are some of the more numerous types, little is known about them (Rodriguez et al., 2021). Guarascio

et al. (2021) suggest that Type I taste bud cells have been proposed to help regulate the ionic environment in taste buds concerning the concentrations of K+ and Cl-. This is important as proper ionic concentration is essential for the generation and transmission of electrical signals in taste cells (Rodriguez et al., 2021).

G protein-coupled receptors (GPCRs) are a large family of membrane receptors that play a crucial role in many physiological processes, including taste perception. In the context of taste, GPCRs are found in specialized taste receptor cells within taste buds. These receptors are responsible for detecting different taste modalities such as sweet, umami, and bitter. There are two main types of taste GPCRs:Type I and Type II. Type I Taste GPCRs (TAS1R): These form heterodimeric complexes that function as sweet (TAS1R2/TAS1R3) or umami (TAS1R1/TAS1R3) taste receptors. Type II Taste GPCRs are the monomeric receptors that detect bitter tastes and include receptors like TAS2R (Ahmad & Dalziel, 2020). When a tastant (a substance that can be tasted) binds to these receptors, it activates downstream signaling pathways. Type II and III taste cells are also larger in dimension than Type I (Roper & Chaudhari, 2017). Type II has G-coupled protein receptors (GCPRs) tuned to taste sweet, bitter, and umami. These tastes are sensed by dedicated taste cells that express specific taste sensor molecules. Most of these express one receptor type, either Type 1 (T1R) or Type 2 (TR2). Each of these types will correspond to only one taste quality such as sweet or salty. When a molecule, such as sugar, contacts the sweet receptor, it triggers an electrical signal perceived as "sweet" by the brain REF. The sweet taste most likely indicates the presence of soluble carbohydrates in the diet (Roper & Chaudhari, 2017). Carbohydrates are the primary source of energy for fermentation in a cattle's diet and make up 60%-70% of their overall diet (National Academies of Sciences, Engineering, and Medicine, 2021).

Type III taste cells are presynaptic cells that have synaptic contacts with intragemmal nerve fibers (Kataoka et al., 2008). The dense core vesicles near the nucleus of the cell help channel the sour and salty taste (Kruetzburger et al., 2019). These channels can more commonly be referred to as ion channels that help play a critical role in gustation or taste perception as they are involved in the transduction of taste signals that convert the chemical signal from food into electrical signals that the brain can process (Katoaka et al., 2008). Ion channels are part of the complex process that allows us to perceive and differentiate from the five basic tastes (Kruetzburger et al., 2019). These Type III are more easily distinguished from other types by their pronounced depolarization-dependent calcium influx (DeFazio et al., 2006; Huang et al., 2007).

Finally, Type IV taste cells are basal precursor cells and are very small compared to the other types (Adpaikar et al., 2022). These undifferentiated cells are found on the basal lamina of the taste bud epithelium and assist with replacing aged or damaged cells (Stone et al., 2002). These basal cells may promote taste cell differentiation (Miura & Barlow, 2010). This means that Type IV cells are essential for the continuous renewal and maintenance of taste buds. By differentiating into mature taste cells, they ensure that the taste buds remain functional and responsive to various taste stimuli. Essentially, they act as a reserve pool of cells that can replenish the taste bud population, maintaining the integrity and sensitivity of the taste system.

Conclusion

Feed efficiency is a critical factor in addressing the increasing demand for meat production while minimizing environmental impact. By focusing on selective breeding, improved feed formulations, and advanced management practices, beef producers can enhance the sustainability of beef production. Measures such as Feed Conversion Ratio (FCR), Residual

Feed Intake (RFI), and Average Daily Gain (ADG) provide valuable insights into an animal's ability to convert feed into body weight, helping producers identify and breed more efficient animals. Embracing technology and innovation will be crucial in meeting the growing demand for meat while ensuring a resilient and sustainable future for beef production. Overall, a multifaceted approach addressing behavioral characteristics associated with feeding efficiency is essential to optimize resource use and minimize input waste in beef production systems.

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CHAPTER 3

BEHAVIORAL CHARACTERISTICS ASSOCIATED WITH FEEDING EFFICIENCY IN ANGUS INFLUENCED STEERS SELECTED FOR RESIDUAL AVERAGE DAILY GAIN[1]

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To be submitted to Translational Animal Science

Behavioral characteristics and factors associated with feed efficiency in cattle

ABSTRACT

To meet the demand of a growing global human population, technological advancements and efficiency improvements are essential, particularly in the production of animal-derived protein. U.S. beef producers face the challenge of increasing production while minimizing land use and environmental impact. Innovative solutions are required to enhance feed efficiency and nutrient intake in cattle. Ruminant animals, through their unique digestive systems and symbiotic relationship with gastrointestinal microbes, convert otherwise unusable substrates into valuable protein sources. Understanding the intricacies of ruminant digestion, including the role of the rumen and microbial populations, is crucial for improving feed efficiency. Advances in microbiome research offer noninvasive methods to study these processes, potentially leading to more sustainable livestock production. The tongue's role in feed intake and taste perception also influences feeding behavior and efficiency. By optimizing these factors, it is possible to address the impending global food crisis and ensure sufficient protein supply for the growing population. 48 Angus-influenced steers were assessed over a two-year period. By utilizing ImageJ for detailed image analysis, we were able to accurately assess the epithelial tissue of the tongue. The findings provide valuable insights into the distribution and characteristics of taste bud cells and papillae across different regions of the tongue, including the apex, intermediate region, and areas lateral to the lingual prominence. These results contribute to a deeper understanding of bovine taste physiology and may have implications for improving cattle feeding strategies and overall animal health.

Introduction

The world populace faces an impending global food crisis; it is necessary to feed more than 10 billion people by 2050 (Meyer et al., 2017). Increasing the global population's standard of living will require 100 times more calories and protein, and 70% of the increase must come from improvements in technology and efficiency (United Nations, 2017). United States beef producers face several impending critical challenges, such as how to produce more animal-derived protein products while using less land while simultaneously reducing the environmental impact. Meeting these contradictory demands creates novel solutions that can be applied to all food-animal species. To ensure affordable and sufficient protein access, food animal producers must be more innovative and precise in rearing animals than ever. One way to resolve this crisis is to look at animal nutrient intake and understand cattle feeding efficiency and the drivers of feed intake.

Ruminant animals are unique creatures that transform sunlight and otherwise unusable substrates into meat, milk, and energy via a symbiotic relationship with a resident gastrointestinal microbial population, especially that of the rumen (Hungate, 1966). Ruminant animals evolved about 50 million years ago and were initially small omnivorous creatures. Almost 200 living species of ruminants are found in six families (Church, 1993) and are members of the suborder Ruminantia and the order Artiodactyla (Church, 1993). Ruminants are defined by ruminating their feed or cud-chewing when animals regurgitate and remasticate their previously chewed feed (Mississippi State University Extension Service, n.d.). The cud comprises larger pieces of forage or grains from the rumen that are not initially degradable prior to microbial action in the rumen (Weimer, 2022).

Ruminants use their tongue as a prehensile grabbing mechanism to bring feedstuff into the oral cavity before grinding the feed against the dental pad, composed of connective tissue covered with epithelium (Mississippi State University Extension Service, n.d.). Using their incisors, ruminants physically disrupt the structure of the forage to allow microbial access, as well as the saliva that contains some enzymes such as amylase, lipase and lysozyme (Matthews et al., 2019). Mastication is crucial to the ruminal microbial catabolic processes that allow animals to degrade and utilize feedstuffs otherwise unusable by mammals. Because of their gastrointestinal physiology and the symbiotic relationship with the native ruminal microbial population, ruminants must spend a substantial proportion of their day chewing their cud to supplement the process of rumination, and the length of time is linked to the NDF/ADF found in the forage and feedstuffs (Perez-Barberia, 2020).

Ruminant animals cannot digest cellulose directly due to their lack of the production of the cellulase enzyme that can break down cellulose (Church, 1993). Cellulase is a microbially-produced enzyme that catabolizes the individual disaccharide units that comprise cellulose (cellobiose) (Akula et al., 2023). The rumen is an anaerobic fermentation chamber that contains a large and diverse microbial ecosystem that utilizes cellulolytic feedstuffs (Russell, 2002). Ruminant animals capture dietary energy from plant materials through the fermentative action of the native microbial population of the rumen, which ferments feedstuffs to produce endproducts (e.g., volatile fatty acids and microbial crude protein) that are used by the animal (Hungate, 1966; Russell, 2002; Bryant & Small, 1954). Fiber degradation occurs due to the presence and activity of fiber-degrading (e.g., cellulolytic and hemicellulolytic) bacteria (Weimer, 2022) cellulolytic. The ruminal fermentation also produces carbon dioxide and hydrogen, which

ruminal methanogens (archaea) use as substrates to produce methane, which acts as an electron sink that supports the actions of this microbial ecosystem (Matthews et al., 2019).

MATERIALS AND METHODS

All animals involved in this study were treated humanely in strict accordance with the guidelines set forth by the University of Georgia Animal Care and Use Committee (AUP #A2012 11-0060R1).

Experimental Design

Over a six-year period, a purebred Angus cow herd from the Northwest Georgia Research and Education Center in Calhoun, GA was bred by random assignment to Angus sires within their respective breeding lines. The Angus sires were carefully selected based on Residual Average Daily Gain (RADG) and intramuscular marbling (MARB) Expected Progeny Difference (EPD), resulting in a randomized complete block design with a 2x2 factorial arrangement. This rigorous selection process was designed to determine the effect of RADG and MARB on growth, feed efficiency, temperament and carcass traits in Angus steers.

GrowSafe Feed Trial

Shortly after the yearling phase, the steers began preparation to start the feedlot trial by undergoing a 14-day acclimation period. During this period, they were given a grain-based finishing diet (Table 1) with *ad libidum* access. Following this acclimation period, the steers were moved into pens with the GrowSafe (GrowSafe Systems, Ltd, Airdrie, Alberta, Canada) bunks where their feed intake was tested over a 70-day period using the GrowSafe Beef system. Steers were weighed at the start and end of the feeding trial. The following formula calculate the residual feed intake of the animals:

$$\text{RFI} = \text{Actual Intake} - \text{Predicted Intake}$$

Average Daily Gain (ADG) over the feed intake trial was used to predict intake.

Tissue Collection and processing

Cattle were harvested under USDA inspection at the University of Georgia Meat Science and Technology Center in Athens following an overnight hold with access to water. Samples were collected from 48 Angus-Influenced steers over two years on four separate harvest dates in 2021 and 2022. The animals were harvested at an optimal market weight of approximately 1300 lbs. Tongues were collected immediately at harvest and then stored at 4.4°C for 48 hours. The epithelial tissue was removed for further analysis.

After washing the tongues with deionized water, they were vacuum-sealed and stored at -26.7°C to preserve the tissues. The tissues were later thawed slowly at 4.4°C and photographed for analysis. Using ImageJ software, each animal's types and counts of taste bud papillae were quantified. After thawing the tongue tissues, they were photographed to capture a more detailed image of the taste buds and papillae. Images were processed to highlight the taste bud papillae. The tongue images were divided into the apex (tip of the tongue), intermediate region, and area lateral to the lingual prominence. Taste bud papillae were manually counted and recorded in an Excel data sheet. Feed intake data was then compared using RStudio Analysis (R studio, Boston, MA) and analyzed using the Pearson method. Pearson's correlation coefficient ® is a statistical measure that quantifies the strength and direction of the linear relationship between two continuous variables. It ranges from -1 to 1, where 1 indicates a perfect positive correlation, -1 indicates a perfect negative correlation, and 0 indicates no correlation. The formula for Pearson's r involves the covariance of the variables divided by the product of their standard deviations. This method assumes that the variables are normally distributed, the relationship between them is linear, and the variance of one variable is stable at all levels of the other variable (Kirch, 2008).

RESULTS

All cattle tongues (Figure 3.1) had an average of 15.27 circumvallate and 101.90 fungiform papillae per animal. The greatest concentrations of the fungiform papillae were on the apex lingue or the tip of the tongue. The greatest circumvallate papillae concentrations were located behind the torus lingue, suggesting that the tip of the tongue is more sensitive to taste due

to the higher concentration of fungiform papillae, which are known for their role in taste perception. The circumvallate papillae, being fewer in number and located further back, likely contribute to the detection of different taste modalities and play a role in the overall taste experience. Results indicate a relationship between the number of circumvallate taste buds and various performance metrics. Specifically, a higher number of circumvallate taste buds were associated with a lower ADG (P=0.01; Figure 3.4), lower feed intake as-is (P=0.044; Figure 3.3), and DMI (P=0.047; Figure 3.4). There was no correlation (P > 0.1) between fungiform taste buds and any cattle production or growth metric. Additionally, there was a tendency for a higher number of circumvallate taste buds to correlate with a lower RFI (P=0.085). Collectively, our findings suggest that the number of circumvallates were correlated with cattle's feed efficiency and growth performance, which could have significant implications for animal breeding and management.

DISCUSSION

Feed costs account for an estimated 65% of total input costs in a cattle operation system (Lancaster et al., 2009); and cattle production efficiency is driven by FI and/or DMI (Berry & Crowley, 2013). The cattle mouth has a dental pad at the top of the mouth in place of upper incisors and incisors on the bottom jaw that use side-to-side chewing motions that assist in grinding and chewing, opening the plant tissue to microbial attack (Church, 1993). Animals will repeat the cycle of chewing, breaking down the particles into smaller pieces that can pass through the reticulo-omasal orifice, mixing it with saliva and regurgitation; collectively, this process is known as rumination (Church, 1993). Rumination is a voluntary process in cattle where the frequency of rumination is controlled by diet and management techniques such as particle size, environmental stressors, and dietary fiber type and composition (Paudyal, 2021). The ruminant animal's feed efficiency ultimately depends on its diet composition, which will

determine the amount of time it spends ruminating daily. Cattle spend 35% to 45% of their day chewing and ruminating. Cattle prefer to ruminate while lying down (Cooper et al., 2007) and can spend up to 550 minutes (about 9 hours) daily ruminating. Some studies suggest that the saliva flow rate is correlated with the REM sleep cycle of cattle in that as they become more comfortable and relaxed, they are better able to process their feedstuffs.

Because of the decrease in sequencing costs, there has been an increase in the number of studies examining the diversity of the microbiome structure present in the rumen, the ruminant oral cavity, and the saliva. Thus, the oral microbiome has been shown to serve as a noninvasive proxy of the rumen microbiome, including as a method to sample foodborne pathogenic bacteria (Tapio et al., 2016). Kittlemann et al. suggested numerous benefits associated with using saliva as a noninvasive rumen proxy; however, limitations remain. One significant challenge is that the microbial communities present in saliva may not perfectly represent those in the rumen. Saliva contains microbes from the oral cavity, which might not be as metabolically active or relevant to ruminal processes as those directly sampled from the rumen. Additionally, the composition of saliva can be influence by factors such as diet, health status and environmental conditions (Mortazavi et al., 2024). The microbiome data found in the saliva must be further interpreted cautiously when used in this way. Therefore, saliva can be a noninvasive way to examine volatile fatty acids and the microbial contents of the animal's digestive system and rumination process (2015).

Feeding mechanisms, such as how feed is ingested can be a key driver of determining the success of vertebrates' adaptation to their environment (Darwish, 2012). The tongue of cattle is long and prehensile, and it is used to bring feedstuffs (e.g., forage or grain) into the oral cavity to begin the rumination process. The shape and structure of the tongue differ significantly between

animal species, which reflects the various functions of the respective tongues based on the evolution of each species (Iwasaki, 2002; Santos et al., 2011). Tongues of animals are often studied for their participation in the assessment of food, suckling, intake of liquids, mastication, rumination, mixing food with saliva digestion, and speech (Stevens & Lowe, 2005; Kulawik & Zdrojewksa, 2006).

Ruminants are categorized as browsers or grazers (Church, 1993). Browsers typically consume woody stems or leaves, including deer and goats among the domestic species (Church, 1993). Grazers instead tend to graze on forages and will spend 1/3 of their day grazing, and cattle and sheep are grazers (Church, 1993). In all ruminants, the tongue is an essential organ, the prehensile mechanism for the intake of nutrients. However, the sense of taste mediated by taste buds is an essential function of the tongue that can dramatically impact feed intake (Forbes, 2007). Taste perception is activated when chemicals or feedstuffs encounter the taste bud cells (TBCs) on the tongue, and specific sub-populations can be found in the gut and brain (Shin, 2008).

In mammals, four types of lingual papillae are located on the dorsal surface of the tongue (Roper & Chaudhari, 2017). Taste buds are the sensory end organs for gustation and are on the tongue epithelium in mammals (Roper & Chaudhari, 2017). The receptors found on the chemosensitive apical tips respond directly to gustatory stimuli from the diet. Taste buds in mammals are located along the tongue but can also be on the soft palate, tonsillar pillars, but also on the posterior pharyngeal wall, epiglottis, and larynx (Farbman, 1988; Stinson & Calhoun, 1993). In addition to being on the tongue, taste buds can also be found in some species of animals' hard and soft palates (Roper & Chaudhari, 2017).

Taste receptor cells (TRCs) are arranged in onion-shaped groups of 50-100 cells that make up an individual taste bud (Sullivan et al., 2010). The TRCs comprise epithelial cells in taste papillae (e.g., fungiform, foliate, and circumvallate) (Davies et al., 1979). Taste receptor cells are characterized as four types: Type I, Type II, Type III, and Type IV. There are two prominent types of cells exist in the taste bud: receptor cells and presynaptic cells. Type I tastebud cells help interpret salty tastes because they are class C GPCRs (G protein-coupled receptor) with a large N-terminal. Category C GPCRs contain three taste receptor subunits (T1R1, T1R2, and T1R3). They form sweet and umami receptors (Jeon et al., 2012). About 50% of TBCs are type I cells that maintain the supporting structure of the taste bud. These cells have distinct electrophysiological features that allow small voltage-gated outward K+ and inward Na2+ currents but no voltage-gated Ca2+ currents (Calvo & Eglan, 2006). Type II cells are receptor cells and Type III are presynaptic cells (Ahmad & Dalziel, 2020). Type II cells recognize umami (savory), which has a sweet and bitter taste and is often referred to as receptor cells (Ftuwi, et al., 2021). The sweet taste of sugar indicates the presence of soluble carbohydrates and other sweet stimuli utilizing the heterodimer formed from two GPCRs. Sweet receptors comprise Type I receptors, with Type III being presynaptic cells that pick up the sour taste. They contain high-voltage Ca^{2+} channels that release GABA when depolarized (Jeon et al., 2012). These are the only cell types to contain conventional neuronal synapses. Sour tastes are perceived when protons enter Type III cells, causing cellular acidification. Proton influx results in the closure of resting K^{+} channels, membrane depolarization, and the release of classic neurotransmitters (Calvo & Egan, 2006). Sweet taste most likely indicates carbohydrates and uses the heterodimer of a T1R2 and T1R3. These respond to sucrose, fructose, artificial sweeteners, and some D-amino acids that help elicit a sweet taste (Roper & Chaudhari, 2017).

T1R2 and T1RC belong to the family of GCPR that possess an extended extracellular amino terminus forming a Venus flytrap module (VFTM). The VFTMs of the class C GPCRs are responsible for ligand recognition and binding and share sequence similarity with bacterial periplasmic amino acid binding proteins (PBPs) (Nie et al., 2005). Additionally, they function as a heterodimer and have multiple ligand binding sites. The purified cellular domain of the T1R2 and T1R3 enables the capabilities of binding sugars with alcohols (Nie et al., 2005). Channels typically found in axonal membranes are on the basolateral aspect of taste cells and include voltage-gated Na^+, K^+, and Ca^{2+} channels that produce depolarizing potentials when taste cells interact with chemical stimuli. The resulting receptor potential raises Ca^{2+} to levels sufficient for synaptic vesicle fusion and synaptic transmission, thus eliciting action potentials in the afferent axons. In general, the greater the tastant concentration, the greater the depolarization of the taste cell (Purves et al., 2001).

The taste of amino acids protein falls into both the sweet (d-amino acids) and bitter categories (l-isomers) and uses various transduction mechanisms (Kawai et al., 2012). An exception is the amino acid l-glutamate (and its sodium salt), which elicits a different taste (see above). The effects of l-glutamate on taste cells involve both ionotropic receptors that activate ion channels and unusual taste-specific metabotropic glutamate receptors (mGluR4) that are less sensitive to glutamate and that close ion channels through a cAMP-dependent pathway (Purves et al., 2001).

It is currently unknown whether Type III taste bud cells exist in cattle. These presynaptic gustatory cells are known for detecting sour tastes (Perea-Martinez et al., 2013). Yu et al. suggest that the Type III cell is immunohistochemically distinguished using PGP 9.5, 5HT, and NCAM (Yee et al., 2019). Further studies are needed to immunohistochemically demonstrate whether

the Type III cell exists in the bovine taste buds. Type IV cells serve as basal cells, which are round-shaped and reside at the base of the taste bud, which will eventually mature into another type of taste bud cell.

G protein coupled receptors (GPCRs) are a large family of cell surface receptors that play a critical role in cellular level communication and transduction, specifically as it relates to sensory perception within taste (Purves et al., 2001). Sweet, bitter, and umami bind to the G protein-coupled receptors. Salty and sour are associated with ion channel membranes and need more linkage or info in the sentence. Most sour tastes are associated with acids; this is presented to the animal as a spoiled food item or a lack of ripeness. The sour taste is also due to protons' low pH (Frank et al., 2022). Bitter tastes serve as a warning from the ingestion of toxins as well. There are many chemically distinct classes of bitter-tasting compounds. Some are alkaloids, like quinine and caffeine; others are l-amino acids, urea, and even salts like $MgSO_4$. (Chandrashekar, 2006). Not all these bitter tastants use the same receptor or transduction pathways. Bitter-tasting organic compounds typically bind to GPCRs that activate gustducin, which is a G-protein found in taste cells homologous to transducing in photoreceptors, which in turn activates phosphodiesterase, thus lowering the cyclic nucleotide concentration and closing cyclic nucleotide-gated channels on the basolateral membranes of taste cells (Purves et al., 2001).

Cattle tongues contain taste pores on the tongue covered in saliva that solubilizes and feeds in the oral cavity. Cattle have several primary tastes: sweet, salty, bitter, savory, and acidic. The taste receptors for each of these are located within different geographic regions of the tongue (Roper & Chaudani, 2017). Food taste chemicals activate specialized sensory cells of the taste bud cells (TBCs) that convey signals to the brain. The nerve impulse enters an intracellular pathway where the neurotransmitter release occurs via afferent nerves located in the ganglia of

the peripheral nervous system. The anatomical units of taste detection are taste receptor cells (TRCs) assembled into taste buds distributed across different tongue and palate epithelium papillae (Jakubowski & Flatt, 2020). Taste processing is first achieved at the level of TRCs that are activated by specific tastants (Ahmad & Dalziel, 2020), which transmit information via sensory afferent fibers to the gustatory cortex in the brain for taste perception (Ahmad & Dalziel, 2020). Three different morphologic subtypes of TRCs in taste buds sense the different tastes we perceive (Ahmad & Dalziel, 2020). Type I glial-like cells detect salty taste, while type II cells expressing GPCRs detect sweet, umami, and bitter tastes, and Type III cells sense sour stimuli (Janssen & Depoortere, 2013). Through tongue-based chemoreceptors, cattle can identify palatable feedstuffs (Chapman, 2008).

Cattle have an estimated 25,000-35,000 taste buds compared to the 10,000 in humans (Miller & Reedy, 1990). Therefore, cattle are thought to be more sensitive to tastes than are humans. The sense of taste determines which feedstuffs will enter the digestive tract of the animal (Kare & Beauchamp, 1984). Taste is activated when certain classes of chemicals encounter taste receptor cells located on epithelial tissue of the tongue, palate, throat, or near the epiglottis and upper esophagus (Breslin & Spector, 2001). The sense of taste in cattle helps determine which feedstuff is palatable (often based upon the nutrient content) and can also include aversions from previous bad experiences (Baumont, 1996).

Differences in structures signal variability in morphological features associated with dietary preferences in herbivores and carnivores. Ishimaru et al., (1988) suggested a relationship between the feeding habits and the development of the papillae. The distribution of these specifics may depend upon species and rely upon the taste bud to help transduce the sense of taste, rely on the mouth to the brain, and contribute to feeding mechanisms. Foliate papillae are

short tongue protrusions separated from parallel grooves associated with lateral taste buds (Sakr, 2022). Fungiform papillae are fungi-shaped structures in the distal portion of the tongue. Circumvallate papillae are the largest and dome-shaped papillae.

Cattle saliva is a buffer for food intake

As cattle chew, they add saliva to the feed particles. Cattle can produce up to 80 liters of saliva daily, serving as a liquid buffer and nutrient supply for the gastrointestinal microbes to begin degrading feedstuffs (Karisch, 2024). Cattle saliva primarily contains water, minerals, mucus, and urea. Specifically, cattle saliva contains phosphate, nitrogen, potassium, and sodium bicarbonate, which helps create a buffered environment that aids in the fermentation process by helping to stabilize ruminal pH (Xu et al., 2021). It is essential to note that Urea nitrogen makes up ¾ of the total Nitrogen in saliva, which is crucial in helping to facilitate the microbial fermentation process (Church, 1993). Salivary urea provides a basal level of readily available Nitrogen in the rumen to ensure that sufficient Nitrogen is available temporally along with carbon in the ruminal fermentation to support Microbial Crude Protein (MCP) synthesis (Vyas & Amaro, 2020). The addition of saliva helps soften food and provides lubrication for swallowing and solubilizing feedstuffs (Sakr, 2022). Saliva contains a more extensive and diverse set of proteins that can perform multiple functions such as taste and digestion, lubrication, pH buffering, and general health maintenance by controlling the oral microbiota (Akula et al., 2023).

Saliva can also help reduce bloating in cattle, which is potentially fatal. Cattle that are shown to have lower levels of saliva are prone to chronic bloat in which death can occur (Boyles, 2019). Comparisons of cattle and human saliva show that cow saliva has a simpler proteome than human saliva regarding significant components. Different salivary glands in cattle produce

different types and amounts of saliva compared to humans. The number of markers may differ between humans and cattle; therefore, the protein composition differs (Akula et al., 2023).

The mammalian olfactory system is complex and consists of multiple subsystems that work together to serve various purposes. Mammals will utilize these complex networks to survey their environment for food, water, and social interactions (Breer et al., 2006). Cattle have an acute sense of smell and can detect odors many miles away (Rorvang et al., 2017). This highly versatile chemoreceptor that cattle possess can receive a network of scents that cattle will then use to select areas of particular interest for feeding, resting, or ruminating (Breer et al., 2006). Smells heavily influence feeding and foraging, and cattle use the odor to indicate food preference. Olfaction can have a significant impact on the flavor of food (Nielson et al., 2015). Cattle often avoid eating in adverse-smelling areas that are ridden with the smell of feces, chemicals, or urine (Dohi et al., 1991).

The senses of taste and smell combine to create flavor (Idris, 2023). Flavor preferences by animals can increase feed intake (Harper et al., 2016). Nombekela et al. (1994) suggested that flavor preferences in cattle can be short-term, meaning that there is the potential for their palates to develop over time. Consequently, cattle may develop affinities and aversions for certain feedstuffs, which can modify their intake preferences and subsequent weight gain. Albright (1993) stated that feeding cattle a corn silage diet may increase their subsequent affinity for eating sweeter feeds, decreasing their sensitivity to sour taste preferences.

It is assumed that behavioral patterns of cattle (e.g., eating meals primarily early in the day) often play a more significant role in their overall feeding traits, such as meal size and amount of time spent feeding (Llonch et al., 2018). Cattle are social/herd animals who thrive in larger groups and prefer eating in large groups. The importance of understanding an animal's

behavior under various environmental conditions has been underscored (Arave & Albright., 1997). The temperament of cattle affects handling, liability, beef quality assurance, and feedlot performance (Haskell, 2014). Highly excitable cattle are more likely to have lower average daily gains in carcass quality grades compared to those with a gentle temperament, and it can also impact fecal shedding of *E. coli* O157:H7 (Brown-Brandl et al., 2009).

The rumen is a large fermentation vat in cattle and other ruminants that is home to a highly dense and diverse population of microbes that ferment feedstuffs to produce Volatile Fatty Acids (VFA) and Microbial Crude Protein, which are used by the animal as a source of energy and amino acids, respectively (Hungate, 1966). Because the rumen microbial population converts feeds into energy and protein sources, the rumen plays a critical role in cattle's feed efficiency and growth (Reynolds et al., 2017). The rumen is a pre-gastric anaerobic compartment where feed particles are mixed with saliva and resident microbes to produce metabolites via fermentation (Xu et al., 2021). The rumen's microbial population acts in many ways as a "black box" of anaerobic catabolic processes, providing the animal the unique ability to convert otherwise indigestible forages into usable byproducts such as meat, milk, fiber, and entertainment sources (Liu et al., 2021). Ruminal microbial conversion of carbohydrates to propionate, acetate, and butyrate creates energy sources for the animal. The ruminal microbial ecosystem comprises fungi, protozoa, and bacteria, with bacteria and protozoa comprising more than 90% of the ruminal biomass (Matthews et al., 2019). Together, this microbial consortium degrades almost 80% of the dry matter in the rumen (Maxin et al., 2013).

Additionally, these microbial populations produce ammonia from amino acid fermentation, which other ruminal microbes can use to synthesize microbial protein (Lu et al., 2019). Bacteria comprise 60% protein by weight, making them the primary source of protein for

the animal as they leave the rumen and are degraded and digested in the abomasum and small intestine (Hackmann, 2015). A non-beneficial (from the animal's perspective) byproduct of rumen fermentation is gases, such as methane, which wastes carbon and energy and represents a loss of up to 12% of Digestible Energy (Johnson & Johnson, 1995).

The reticulo-rumen is the largest compartment by volume (40-70 liters) of the four-chambered ruminant stomach, providing the ideal anaerobic conditions for fermentation. This warm (near 39 C), near-neutral pH environment is home to one of the wealthiest microbial habitats in the world. The reticulum is most noted for its honeycomb tissue appearance and contains the reticulo-omasal orifice, which sorts feeds based on size before they can leave the reticulo-rumen (Church, 1993). The rumen can be viewed as an anaerobic and methanogenic fermentation chamber that contains microorganisms that can utilize, and increase the productivity of, cellulolytic feeds (i.e. straw, hay, silage and grass) (Matthews et al., 2019).

There are various ways to quantify feed efficiency, but feed efficiency encompasses the relationship between feed inputs and growth outputs. High-feed efficiency animals grow well but consume less feed than other animals within their cohorts (Haskell et al., 2019). Several factors can contribute to individual feed efficiency differences within any feeding cohort, including feed intake, digestion, activity, and thermoregulation (Herd et al., 2004). Feed Conversion Ratio is a standard method that associates the amount of feed required per unit of body growth to the animal. Feed conversion ratios can be calculated by dividing the average dry matter intake (DMI) per day by the Average Daily Gain (ADG).

Advancements in precision agriculture feeding systems such as the C-Lock (Rapid City, SD, USA) or GrowSafe (now Vytalle Systems, Lenexa, KS, USA) systems have helped precisely quantify animal feed intake data along with feeding times, feeding rates, meal durations, bite-

size, and amounts of feed consumed. Various RFID-based systems have been used to monitor feeding behavior, including those that measure bunk attendance from feed alleys (Quimby et al., 2001; Urton et al., 2005) and bunk attendance from open (Dobos & Herd, 2008; Lancaster et al., 2009) or gated (Chapinal et al., 2007) feed bunks. Feeding behavior data are based on recording in-to-out visits to the feed bunk, separated by nonfeeding intervals of variable lengths that can be clustered into meals once an appropriate meal criterion has been applied (Yeates et al., 2001). The system's design and resolution will affect the definition of individual bunk visit (BV) events.

Improving feed efficiency can be achieved through prescribed feeding, resulting in changes in body composition and weight per unit of feed papers (Andreini et al., 2020). Improving feed efficiency improves overall farm profitability. RFI is measured so that the more efficient animals have a more negative RFI (Kerley, 2014). Cattle that consume less feed than predicted for their body weight and average daily gain are assigned a negative RFI calculation. This lower RFI results in animals being more efficient as they are consuming less feed to achieve a higher, more productive gain (Lamb & Maddock, 2009). When evaluating cattle's economic value, the cost of inputs is essential to a profit margin. Animals must be selected for genetic merits and feeding efficiency, as they offer considerable economic and environmental benefits (Carstens & Kerley, 2009). Understanding the relationship between feeding behaviors and traditional units of measure, such as average daily gain (ADG), can help select animals with a better residual feed intake index (RFI) and perform better in the feedlot.

Growth and feed intake measures

Average Daily Gain (ADG) is a traditional measure of efficiency used for over 50 years to compare gain rates and is simply weight gain over a specified period of time, measured in days. Not all gain is equal in energetic cost, and cattle gain muscle, bone, fat, and organ tissues

as they grow. ADG requires only a scale to measure the weight gained and the amount of feed given to the animal. One negative impact of selecting cattle based on FCR is that breeding females based strictly using growth will increase the mature size of females, thus resulting in an increased cost of maintaining the herd (Herd & Bishop, 2000).

Residual Feed Intake:

Residual Feed Intake (RFI) is the difference between the animals' actual feed intake and the expected amount of intake. Koch first proposed this theory in 1963, and it reduces an animal's feed intake without compromising growth performance (Bingham et al., 2009). RFI is independent of production traits such as DMI, Body Weight (BW), and milk production. It is calculated as the deviation of the actual DMI (kg/day) from the DMI predicted based on the linear regression of actual DMI on ADG (Haskell et al., 2019). An animal that eats less would be considered more efficient and have a negative RFI value (Haskell et al., 2019). RFI is a moderately heritable trait, and consistent selection of cattle for low RFI values can result in more pounds of gain per pound of feed (Lamb et al., 2013) A notable feature distinguishing RFI from other feed efficiency traits is that it is phenotypically independent of production traits used to compute expected intake (Carstens et al., 2006).

CONCLUSIONS

Our results demonstrate that producers must look at various factors to understand nutrient intake in cattle. No single driving factor dominates cattle taste or flavor preferences. A closer look at their feeding behavioral patterns and microbial population data could give us greater insight into how their taste preferences affect animal performance. By assessing their preferences in taste and diet, we can further understand drivers of dry matter intake, which can impact RFI and ADG which determines animal efficiency and growth performance. This will help create a

more sustainable beef production system that helps us accomplish the goal of more meat on less land.

Figure 3.1 Fungiform and Circumvallate populations on cattle tongues collected at four different collection times (n=48 cattle collected on 4 collection dates; n = 12 cattle/date). Tongues were collected from commercial angus cattle undergoing feed intake monitoring (Krause 2019)

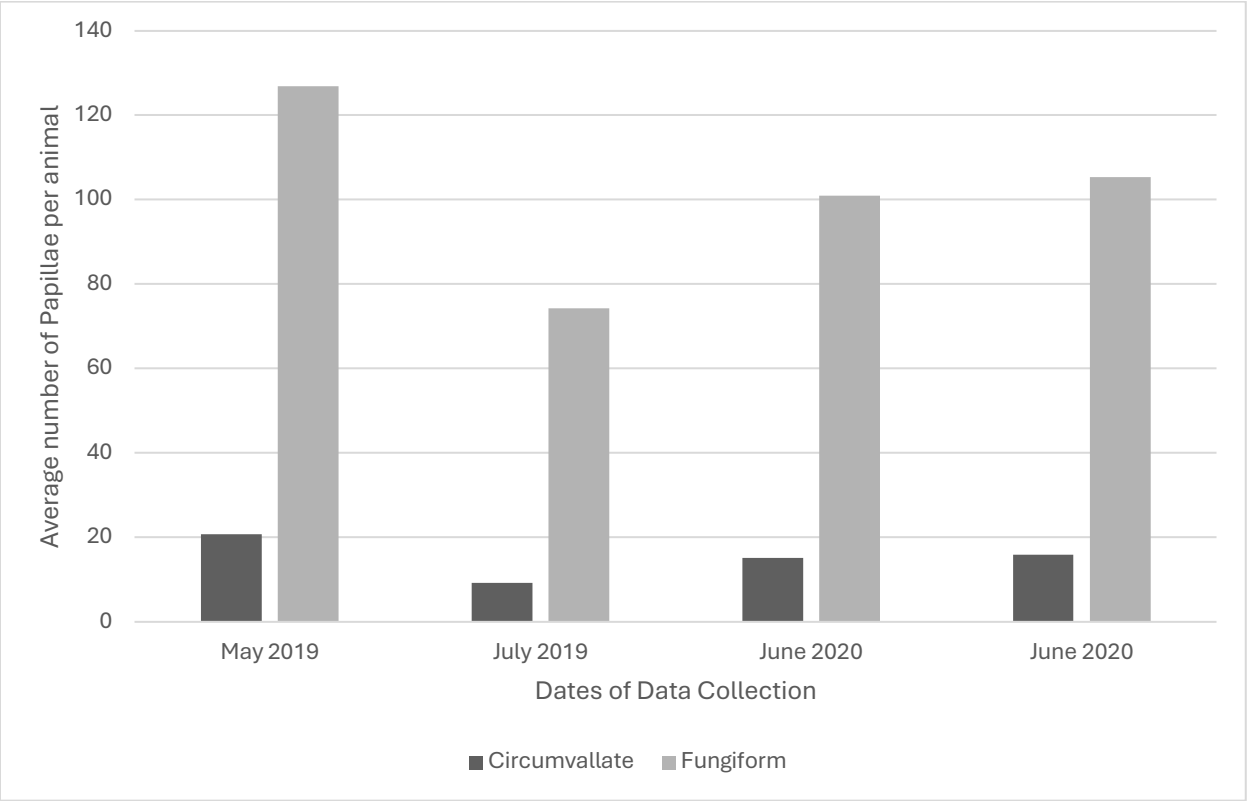


Figure 3.2 Circumvallate papillae from cattle tongues collected at four different collection times (n=48 cattle collected on 4 collection dates; n = 12 cattle/date). Tongues were collected from commercial angus cattle undergoing feed intake monitoring (Krause, 2019) to feed intake as-is

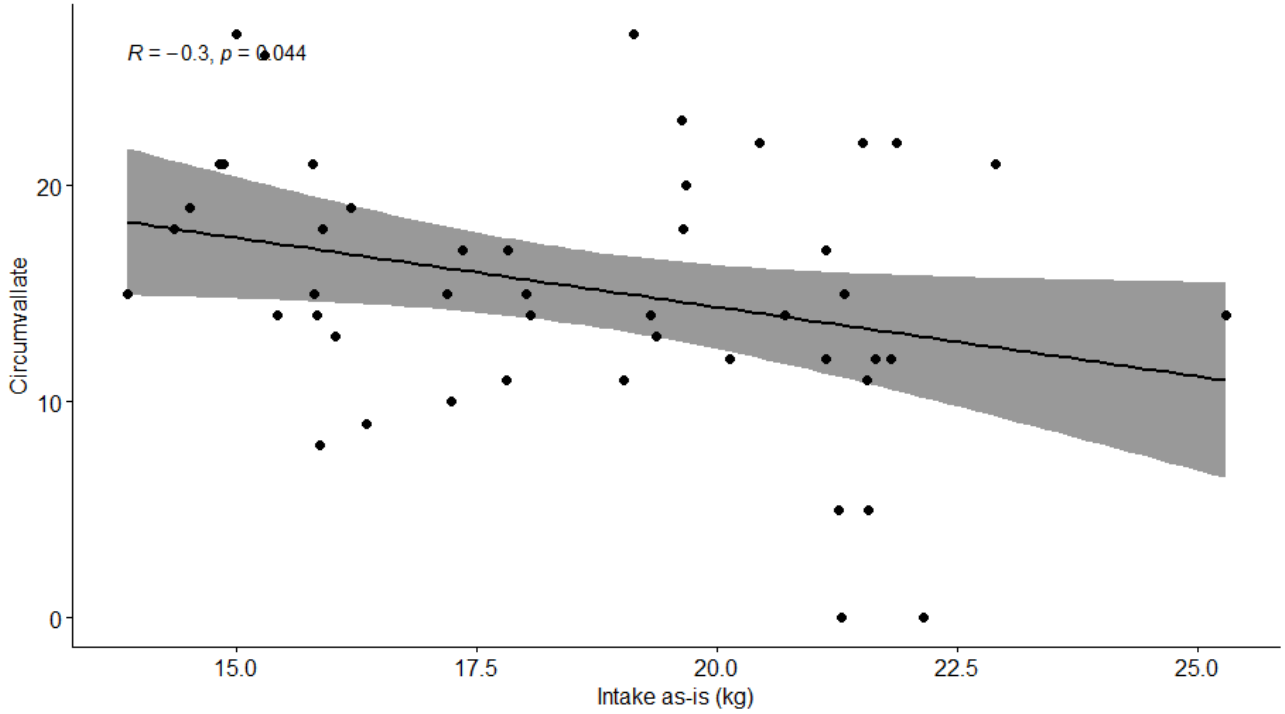


Figure 3.3 Comparison of circumvallate papillae populations from cattle tongues collected at four different collection times (n=48 cattle collected on 4 collection dates; n = 12 cattle/date). Tongues were collected from commercial angus cattle undergoing feed intake monitoring (Krause 2019) Dry Matter Intake (DMI)

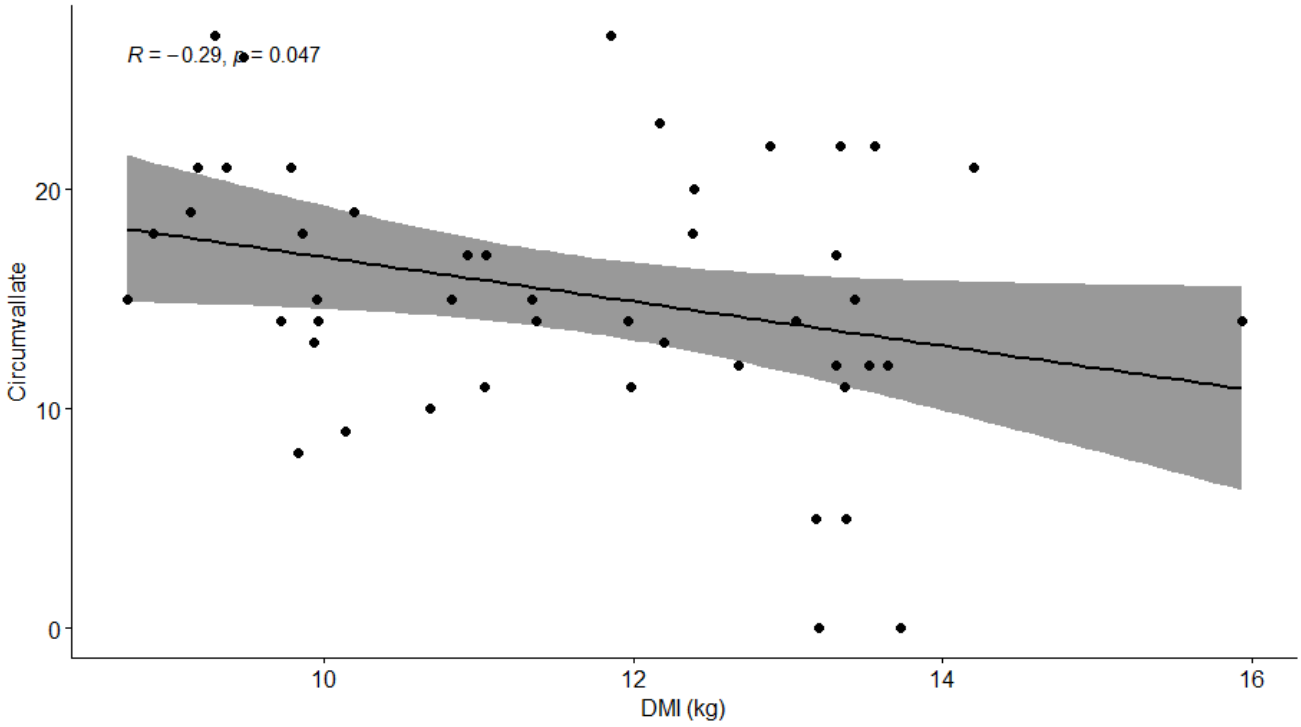


Figure 3.4 Comparison of Circumvallate and Dry Matter Intake (DMI) papillae from cattle tongues collected at four different collection times (n=48 cattle collected on 4 collection dates; n = 12 cattle/date). Tongues were collected from commercial angus cattle undergoing feed intake monitoring (Krause, 2019) to feed intake as-is

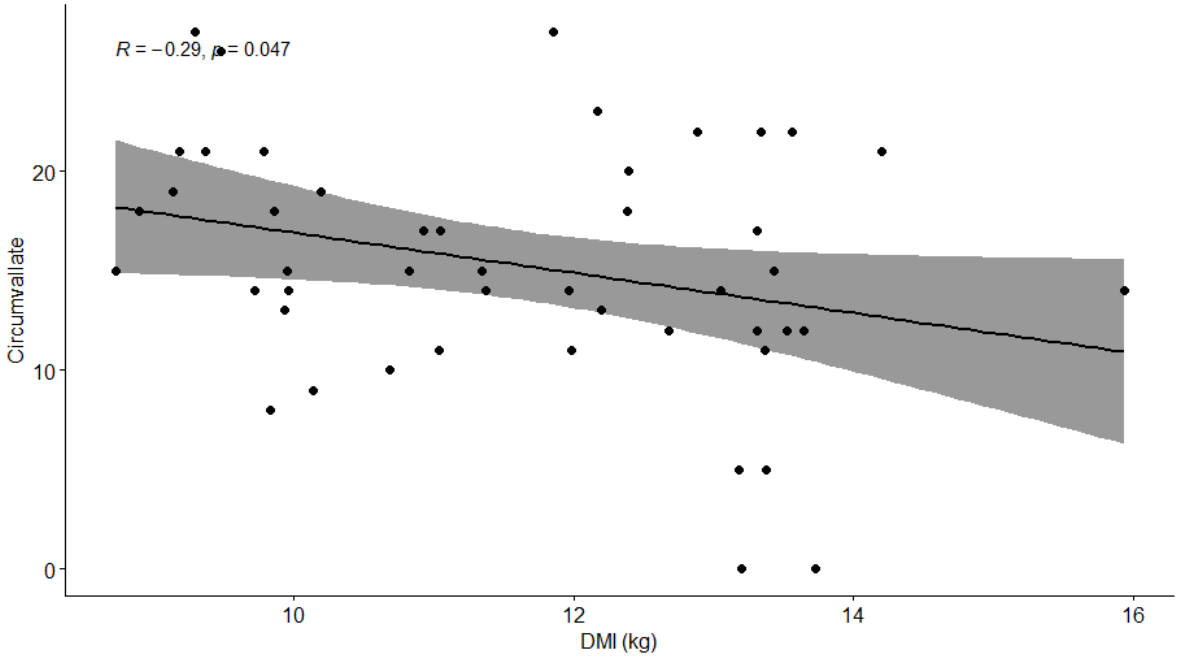


Figure 3.5 Comparison of Circumvallate papillae to Average Daily Gain (ADG). Circumvallate papillae from cattle tongues collected at four different collection times (n=48 cattle collected on 4 collection dates; n = 12 cattle/date). Tongues were collected from commercial angus cattle undergoing feed intake monitoring (Krause, 2019) to feed intake as-is

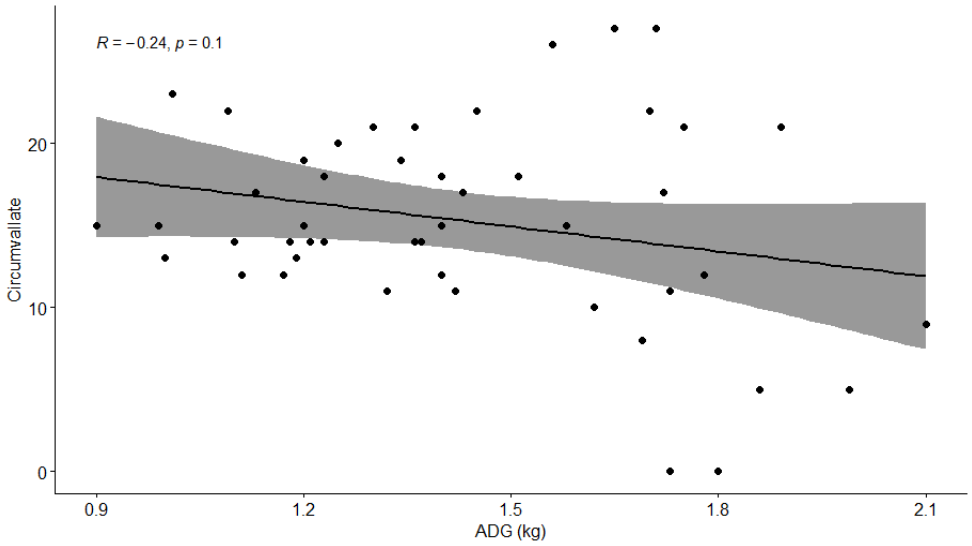


Figure 3.6 Correlation of Fungiform papillae to Average Daily Gain (ADG) from cattle tongues collected at four different collection times (n=48 cattle collected on 4 collection dates; n = 12 cattle/date). Tongues were collected from commercial angus cattle undergoing feed intake monitoring (Krause, 2019) to feed intake in terms of Average Daily Gain (ADG)

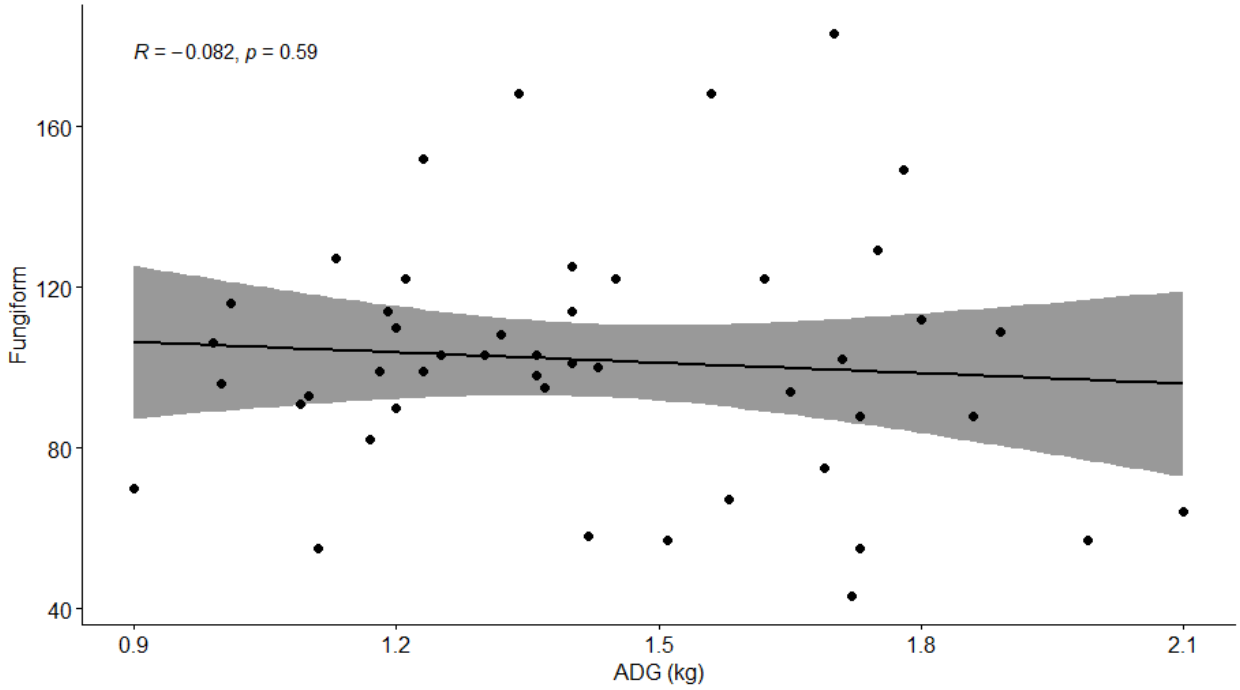


Figure 3.7 Comparison of Fungiform papillae on the tongues of 48 angus steers collected over a 2 year period on As-Fed intake. Fungiform papillae from cattle tongues collected at four different collection times (n=48 cattle collected on 4 collection dates; n = 12 cattle/date). Tongues were collected from commercial angus cattle undergoing feed intake monitoring (Krause, 2019) to feed intake as-is.

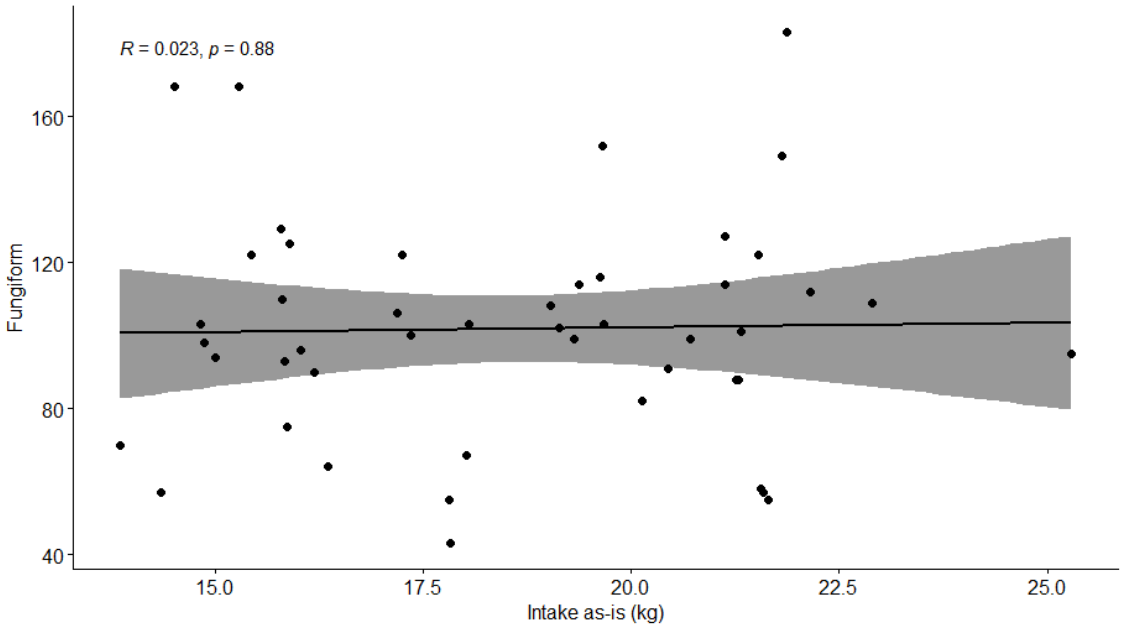
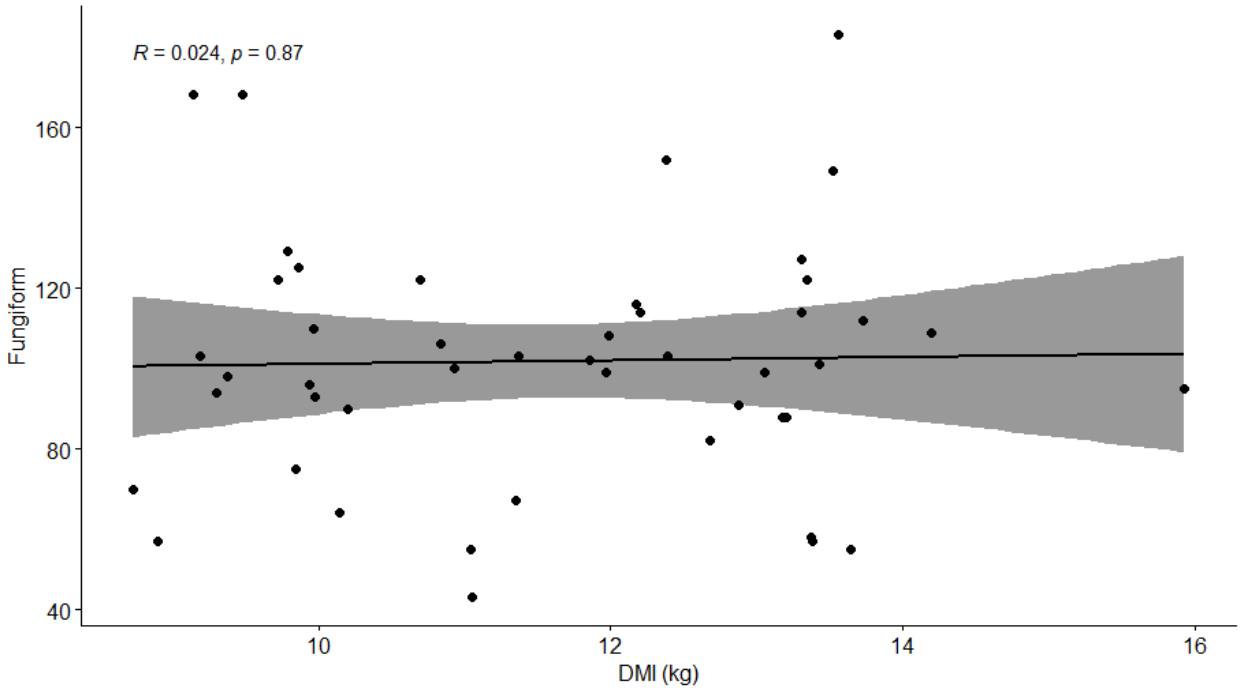


Figure 3.8 Comparison of Fungiform papillae to Dry Matter Intake (DMI). Fungiform papillae from cattle tongues collected at four different collection times (n=48 cattle collected on 4 collection dates; n = 12 cattle/date). Tongues were collected from commercial angus cattle undergoing feed intake monitoring (Krause, 2019) to feed intake in terms of Dry Matter Intake (DMI).



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CHAPTER 4

REGIONAL DISTRIBUTION AND QUANTIFICATIONS OF TASTE BUD PAPILLAE ON BOVINE TONGUES IN GRAIN FED ANGUS CATTLE [1]

[1] R. Fitzpatrick, H. Liu, M. Burnham, A. Osoria Doblado, T. Callaway, and T. Pringle

To be submitted to *Translational Animal Science*

Abstract

The sense of taste plays the most crucial role in mammals' food choices and food consumption habits. The abundance of taste receptors allows cattle to select carefully and sample forages and feedstuffs, optimizing their dietary choices. These taste receptors in specific tongue areas may correlate to sweet, salty, bitter, sour, and umami tastes. They serve as a crucial mechanism that enables cattle to avoid adverse or harmful food sources, thus playing a vital role in cattle feeding behavior (Spence, 2022). Tongues were collected from 48 Angus-influenced steers over two years, on 4 separate harvest dates in 2021 and 2022. The animals were harvested at an optimal market weight of approximately 1300 lbs. Tongues were extracted upon harvest by rapid removal, and the entire tongue and epithelial tissue was removed for further analysis. The findings provide valuable insights into the distribution and characteristics of taste bud cells and papillae across different tongue regions, including the apex, intermediate region, and areas lateral to the lingual prominence. Within the sectioned areas, there were an average of 101.42 fungiform found on the apex, 26.25 fungiform found on the intermediate region and 20.83 circumvallate found. Overall, there was an average of 101.9 fungiform found on cattle tongues and 15.27 circumvallate. A deeper understanding of this physiology can provide valuable insights into their dietary preferences and health management. This study was designed to understand the distribution and types of taste buds on the tongues of commercial cattle fed a feedlot type ration.

Introduction

Cattle are ruminant animals, meaning that they have a four-chambered stomach. While rumination requires the microbial population to synthesize and break down feedstuffs, cattle rely on their sense of taste to acquire the feedstuffs necessary to fuel their bodies. The sense of taste plays the most crucial role in mammals' food choices and eating habits (Ftuwi et al., 2021). With around 25,000 taste buds, cattle have a significantly higher number of receptors than humans, making them more sensitive to taste (Liu, H. lectures ADSC 8230, 2020). The abundance of taste receptors allows cattle to select carefully and sample forages and feedstuffs, optimizing their dietary choices (Peng et al., 2015).

The cow's tongue is a highly specialized organ that plays a crucial role in prehension, mastication, and deglutition (Karisch, 2024). The tongue can be geographically divided into the root, the body, and the apex (Dotiwala & Samra, 2023). The prominent swelling along the dorsal surface that helps push food against the hard palate is known as the torus linguae, and the groove located along the dorsal surface can be described as the transverse lingual fossa (Dotiwala & Samra, 2023). Other muscles that compose this structure include the dorsal and ventral longitudinal muscles and the transverse and vertical muscles that help in precise movements (Dotiwala & Samra, 2023). Finally, the extrinsic muscles known as the styloglossus, genioglossus, hyoglossus, and geniohyoideus all help with protraction, retraction, and the protrusion and depression of the tongue (Nazih, 2019). The epithelial surface of the tongue contains filiform, fungiform, and vallate (circumvallate) papillae (Sakr, 2022). The hypoglossal nerve system controls the overall movement of the tongue, and the trigeminal nerve (CN V) provides the sensory for touch, temperature, and pain. Finally, the taste is transmitted to the

animal brain via the chorda tympani of the facial and glossopharyngeal nerve and glossopharyngeal nerve (CN IX) (Gibbons, 2023).

Taste is defined as “sensations mediated by specific gustatory systems with its own anatomical and physiological particularities” (Bachmanov & Beauchamp, 2007). Food substances activate specialized sensory cells of the taste bud cells, which will then convey signals to the ruminant's brain. Taste buds are the peripheral organs of gustation and are located on the epithelium of the tongue (Roper & Chaudhari, 2017). They comprise clusters of columnar sensory cells embedded in the stratified epithelium of the tongue, palate, and epiglottis (Roper & Chaudhari, 2017). The taste call determines the perceived quality of the receptor rather than the receptor itself (Mueller et al., 2005). Notable papillae types on the tongue's surface in cattle include fungiform and circumvallate. Fungiform are mushroom-shaped with a slender neck and enlarged head, which physically resemble fungi, thus gaining the name fungiform (Dorland, 1950). Vallate, or circumvallate, are round and measure 2 mm-8mm in diameter. Their pores open to trenches around the base structure. The von Ebner's glands, also known as posterior deep lingual glands, empty directly into circumvallate trenches (Sbarbati A. *et al.*, 2002; Lee et al. *et al.*, 2006; Suzuki Y., 2006).

There are two types of taste bud cells located in the taste bud: Type II (the receptor) and Type III (the presynaptic) (Yee et al., 2001; Clapp et al., 2006; DeFazio et al., 2006). More mature mammalian taste bud cells can be classified into three broad subcategories: Type I, Type II, and Type III (Roper & Chaudhari, 2017). Type I TBCs are glia-like receptor cells that are the most frequent in appearance and comprise around one-half of the number of cells in a taste bud. They have a notable irregularly shaped nucleus. Although they are some of the more numerous types, little is definitively known about them (Rodriguez et al., 2021). Guarascio et al. (2021)

suggested that Type I taste bud cells have been proposed to help regulate the ionic environment in taste buds concerning the concentrations of K^+ and Cl^-, which are essential for the generation and transmission of electrical signals in taste cells (Rodriguez et al., 2021)

Type II and Type III often act as insulation providing structural support within the taste bud which helps to insulate and protect the sensory cell (Roper, 2022). These cells are spindle-shaped and protrude with long brush-like structures with wing-like cytoplasmic extensions connecting into the taste pit (Murray, 1993; Pumplin et al., 1997). Furthermore, by regulating the ionic environment, Type I cells contribute to the overall homeostasis of the taste bud and ensure that taste cells can respond appropriately to different stimuli (Roper & Chaudhari, 2017)

Approximately 30% of the cells in a taste bud are Type II cells (Roper & Chaudhari, 2017), and are commonly referred to as the taste bud receptor cell. Type II cells are peripherally shaped and have a more rounded nucleus than Type I. These receptor cells express taste GCPRs and can transduce sweet, bitter, or umami stimuli but cannot form traditional synapses (Tomchik et al., 2007). They are also larger than Type I (Roper & Chaudhari, 2017). These tastes are sensed by dedicated taste cells that express specific taste sensor molecules. Most of these express one receptor type, either Type 1 (T1R) or Type 2 (TR2) (Roper & Chaudhari, 2017). When a molecule, such as a soluble sugar, contacts the sweet receptor, it triggers an electrical signal perceived as "sweet" by the brain. The sweet taste most likely indicates to the animal the presence of energy-rich carbohydrates in the diet (Roper & Chaudhari, 2017). Carbohydrates are the primary source of energy in a cattle's diet and comprise 60%-70% of their overall diet (National Academies of Sciences, Engineering, and Medicine, 2021). More than 25 receptors, T2Rs specifically, are responsible for bitter taste transduction (Ftuwi et al., 2021).

Type III cells are presynaptic cells with synaptic contacts with intragemmal nerve fibers. The dense core vesicles near the nucleus of the cell help channel sour and salty taste and are more commonly referred to as ion channels.

The present study was designed to understand the distribution and types of taste buds on the tongues of commercial angus cattle fed a feedlot type ration.

Materials and Methods

Samples were collected from 48 Angus-influenced steers over two years, on 4 separate harvest dates in 2021 and 2022. The cattle were harvested at an optimal market weight of approximately 1300 lbs. Tongues were extracted upon harvest by rapid removal, and the entire tongue and epithelial tissue was removed for further analysis. After washing the tongues with deionized water, they were vacuum-sealed and stored at -80°F to preserve the tissues. The tissues were thawed slowly at 5°C and photographed for analysis a Canon 70D camera 20.2 Megapixel, 300 pixels per inch (PPI).

Images were processed and examined using ImageJ location image processing software to determine each animal's types and counts of taste bud cells were quantified visually. After thawing the tongues, they were photographed to capture a more detailed image of 300 PPI of the taste buds and papillae. Images were then imported into ImageJ software and processed to highlight the taste bud cells. Additionally, the tongue images were divided into the apex (tip) intermediate region and areas lateral to the lingual prominence. Taste buds were manually counted by two independent observers and recorded in an Excel data sheet. Means of observations from both observers are presented.

Results

Figure 4.1 illustrates the physiological geographic landmarks of the tongue used in the present study, including: Apex, intermediate region and area lateral to the lingual prominence. Taste bud papillae populations in each region of tongue were counted from photographs, and the total counts of taste bud papillae were found per animal (Figure 4.2). Each animal exhibited approximately 15.3 circumvallate papillae and 101.9 fungiform papillae on each tongue, and 80% of the total fungiform papillae were predominantly concentrated on the apex of the tongue, highlighting a clustering in this region. In contrast, 90% of the circumvallate papillae were primarily located behind the torus lingue, indicating a distinct geographic pattern of taste bud papillae distribution. Figure 4.3 shows the quantities and distributions of the fungiform and circumvallate papillae among cattle tested. From this figure, we determined that the majority of the fungiform taste bud papillae on all cattle were primarily located in the apical region.

Discussion

Taste is perceived by either an ion channel-linked or a GCPR (G-coupled protein receptor) linked taste bud on the tongue (San Gabriel, 2015). Ion channels are associated with sour and salty tastes, while GCPRs are associated with bitter, sweet, and umami. The GCPRs are integral membrane proteins that contain an extracellular amino terminus, seven transmembrane a-helical domains, and an intracellular carboxyl terminus (Rehman et al., 2023). Most Type II cells express one class of GCPR—taste receptor types being T1R or T2R but not both. T1Rs detect sweet and umami receptors and are also known as GPCRs with long N terminals that contain bi-lobed domains, also known as the Venus Fly Trap model (Chandrasheker et al., 2006). This gains its name from its shape, which resembles a Venus fly trap plant and are found in both circumvallate and fungiform papillae. The “trap” closes around the taste molecule and activates

the receptor. Once the tastant binds to the receptor, the VFT undergoes the conformational change leading to the activation of the G Cellular proteins (San Gabriel, 2015). The data found in the present study helps to draw a correlation between feed intake and taste bud papillae types.

Cattle select different feed types and discriminate against certain flavors based on their taste preference which could be related to their density of papillae on the tongue. By determining the density of taste papillae, especially in relation to cattle feed intake, we can formulate diets preferred by cattle. This diet modification could not only impact overall feed intake but could help producers create diets that are more economically feasible with less feed loss waste. Cattle can select different feed types and discriminate against certain flavors based on their taste preference which could be related to their density of cells on the tongue. By determining the density of tastebuds, especially in relation to cattle feed intake, we are better able to formulate diets that are more preferential to cattle. This diet modification could not only impact overall feed intake but could help producers create diets that are more economically feasible with less feed loss waste.

Ion channels help play a critical role in gustation or taste perception as they are involved in the transduction of taste signals that convert the chemical signal from food into electrical signals that the brain can process; these ion channels are part of the complex process that allows us to perceive and differentiate from the five basic tastes. Type III are more easily distinguished from other types by their pronounced depolarization-dependent calcium influx (DeFazio et al., 2006; Huang et al., 2007). Type IV cells are basal precursor cells and are very small compared to the other types. These undifferentiated cells are found on the basal lamina of the taste bud epithelium and assist with replacing aged/damaged cells (Stone et al., 2002). These basal cells may promote taste cell differentiation meaning they are essential for ongoing renewal and cell

maintenance of the taste buds (Miura & Barlow, 2010). This differentiation ensures that the cells can respond to a variety of stimuli.

Ion channels play a crucial role in taste perception by facilitating the transduction of chemical signals from tastants to the electrical signals that the ruminant's brain picks up on. Sodium ions (Na+) enter the taste cells through epithelial sodium channels (ENaCs), which lead to depolarization and signal transmission (Bradbury, 2004). Sour tastes involve detecting the proton H+ channels that respond to the acidic substance (Taruno et al., 2021). In either case, when the tastant binds to the receptor on the taste cell, the ion channels open or close, leading to the cell's membrane potential change. This change then elicits an electrical signal transmitted to the brain via the gustatory nerves (Bradbury, 2004).

As described above, type II cells are most notable for expressing the sweet receptor. These type II taste bud cells most notably detect sweetness related to the presence of carbohydrates. (Roper & Chaudhari, 2017). T2R is most notably associated with bitter taste receptors. In conclusion, this study successfully quantified the types and counts of taste bud cells and papillae in 48 Angus-influenced steers over two years. By utilizing ImageJ processing software for detailed image analysis, we accurately assessed the papillae counts on the tongue. The findings provide valuable insights into the distribution and characteristics of taste bud papillae across different tongue regions, including the apex, intermediate region, and areas lateral to the lingual prominence. These results contribute to a deeper understanding of bovine taste physiology and may have implications for improving cattle feeding strategies and overall animal health.

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Figure 4.1 Image of Angus influence steer tongue used for papillae counts. Steers (n=48/4 collection dates) were selected for Residual Average Daily Gain.

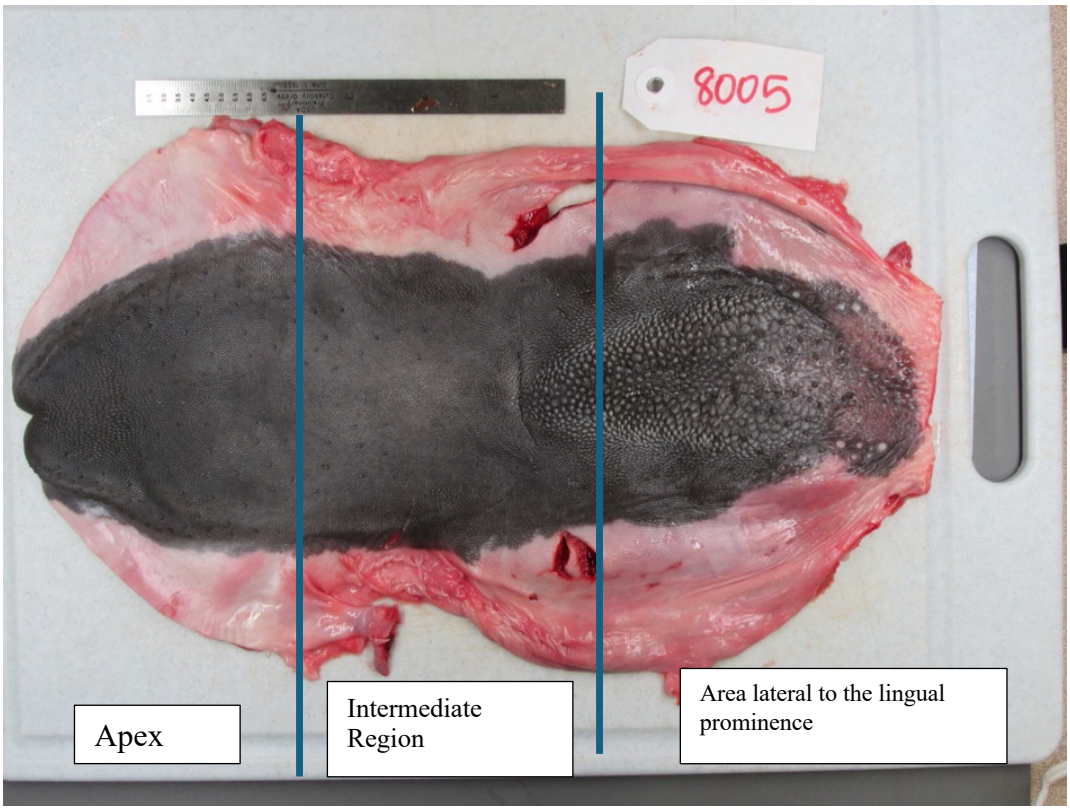


Figure 4.2 Fungiform and Circumvallate populations on cattle tongues collected at four different collection times (n=48 cattle/4 collection dates). Tongues were collected from Angus cattle undergoing feed intake monitoring.

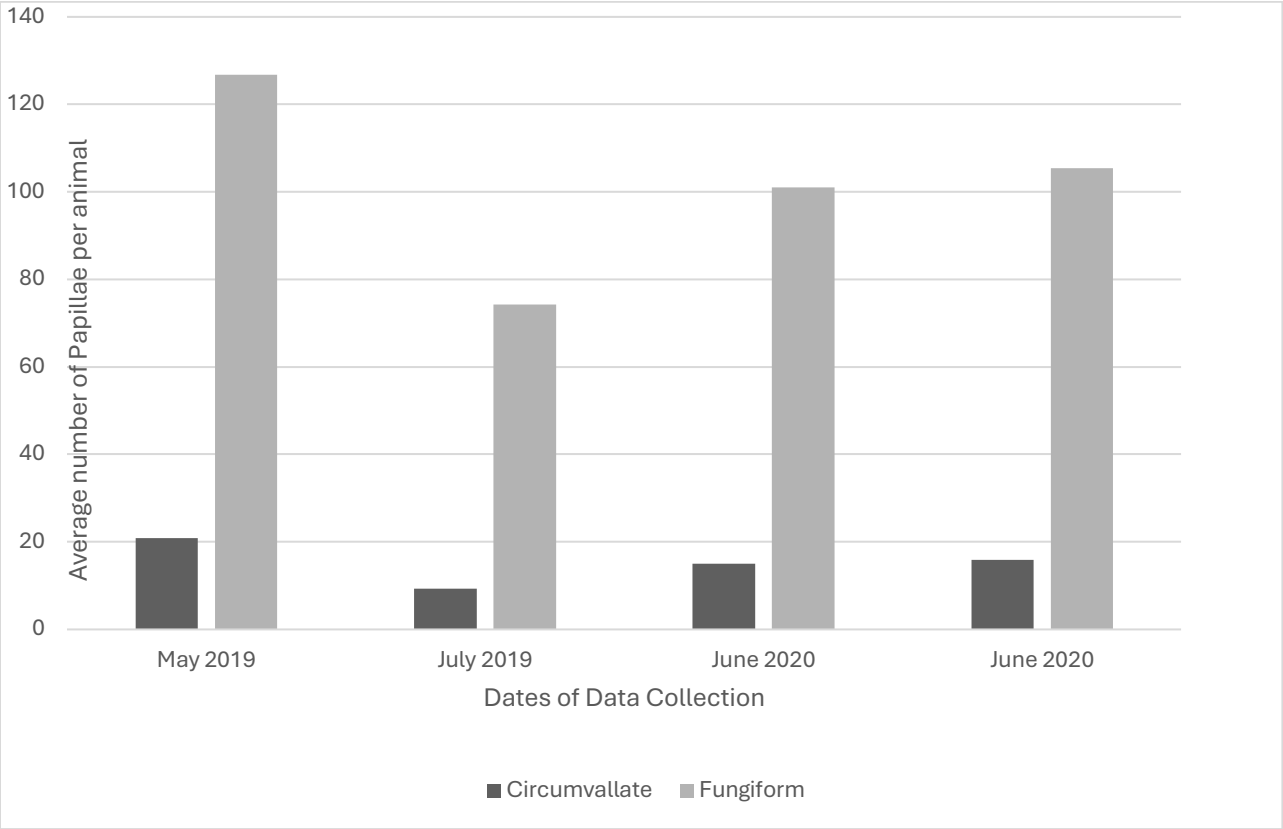
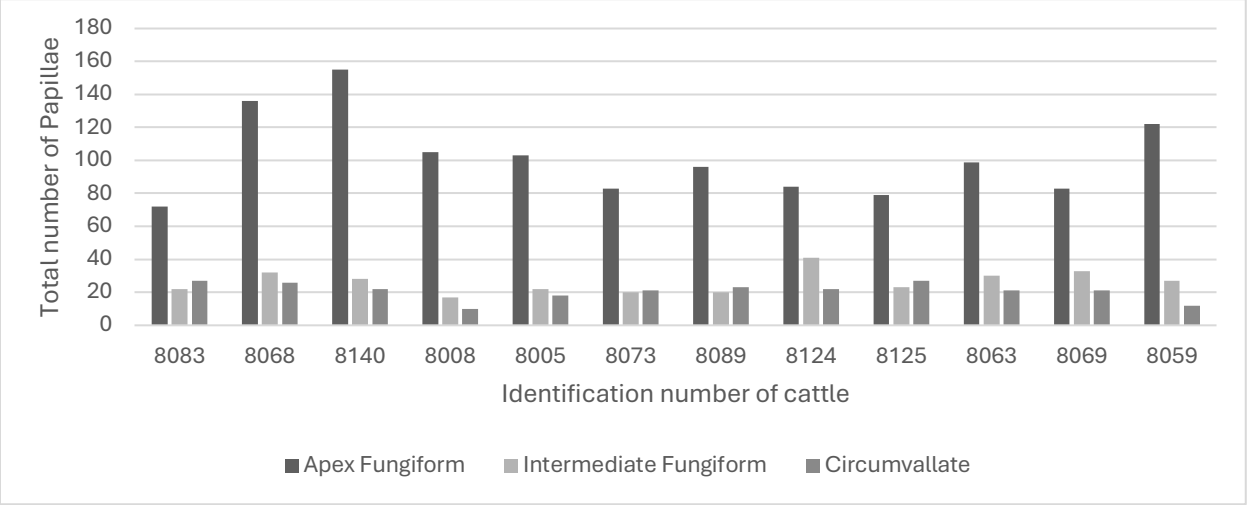


Figure 4.3 Regional Distributions of tastebuds found in 12 Angus Steers selected for Residual Average Daily Gain



CHAPTER 5

VOLATILE FATTY ACIDS FOUND IN CATTLE SALIVA AS A POTENTIAL PREDICTOR OF FEEDING EFFICIENCY AND BEHAVIOR[1]

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ABSTRACT

The ruminal microbial ecosystem activity is critical to the productivity, sustainability, and safety of ruminant animal production. While examining the composition of the microbial population has become more affordable and available, it remains challenging and time-consuming to collect a representative ruminal microbial sample for analysis. Methods of collection of ruminal contents all have significant limitations to their applicability in research settings and field studies. Saliva sampling can give a noninvasive insight into ruminant digestion kinetics and end products. Volatile fatty acid (VFA) concentrations as a predictor of animal productivity and gut integrity have gained popularity recently. This study examined the VFA concentrations found in cattle saliva in a separate feed intake study to determine if salivary VFA concentrations using mouth swabs could be an proxy estimate of ruminal VFA concentrations. Saliva analysis is a noninvasive and harmless method of assessing animal welfare, and this study further sought to explore its potential connection to VFA content in cattle related to taste bud counts. Results of this study showed that Acetate was the most common volatile fatty acid (86% of the total) found in the saliva of the cattle sampled. However, salivary VFA concentrations were relatively low (< 3 mM). By analyzing VFA concentrations in cattle saliva, we hoped to contribute to a better understanding of the impact of diet on animal welfare and feed efficiency. However, these findings, demonstrate that the potential use of saliva sampling as a proxy for ruminal VFA concentrations in cattle in the study conditions did not advance our understanding

of ruminal microbial ecology and its implications for ruminant nutrition and welfare in the field of animal science and agriculture.

INTRODUCTION

Ruminant animals evolved with a sizeable microbial population in their forestomach (the rumen) and hindgut that break down the plant polysaccharides found in their feed (Hungate, 1966). Because the rumen is anaerobic and highly reduced, ruminal microorganisms ferment feedstuffs and produce volatile fatty acids (VFAs), which cattle absorb and use as their primary energy source for maintenance and production (Russell, 2002). Acetate (A), butyrate (B), and propionate (P) are the VFA produced at the highest concentrations in the rumen, and propionate is the only glucogenic VFA (Bergman, 1990). Volatile fatty acids, also known as short-chained fatty acids (SCFA), are ruminants' main glucogenic and fat precursors and provide energy to the animal, particularly propionate and Acetate (Church, 1993). Bergman (1990) reported that VFAs provided up to 75% of the total metabolizable energy in cattle, and it is evident that ruminal VFA concentrations play an integral role.

Acetate is an essential precursor for fatty acid synthesis, participating in the TCA cycle, and is involved in lipogenesis, particularly in subcutaneous and milk fat. If the diet lacks sufficient fiber, the levels of acetic acid will decrease (Ishler et al., 2016). When the diet contains a large amount of starch or heat-treated starch, such as in the case of pelleted, steam-crimped, or steam-flaked feeds, the acetate proportion produced in the rumen will be reduced. Consuming large amounts of oil can also reduce ruminal acetic acid (Ishler et al., 2016). Propionic acid typically constitutes 18 to 20 percent of the overall VFAs, but it can be higher when the diet is rich in fermentable starch (de Assis Lange et al., 2020). Its concentration peaks when a diet high

in grains is consumed. Propionate is most notably recognized as a critical substrate in gluconeogenesis in cattle. This process contributes to the synthesis of fatty acids in the intramuscular fat, which is essential for marbling (Ladeira et al., 2018). Propionate is the glucogenic VFA and is converted into blood glucose in the liver, providing energy to the host animal (Church, 1993). Additionally, propionate plays a role in synthesizing lactose or milk sugar (Ishler et al., 2016). Butyrate is converted to ketones during absorption through rumen epithelial tissue. (Church, 1993). Butyric acid, 12 to 18 percent of the total VFAs, supplies energy to the rumen wall (Ishler et al., 2016). Butyrate is primarily transformed into ketones following absorption across the rumen epithelium (Church, 1993). More than 80 percent of these ketones comprise B-hydroxy-butyric acid (B-HBA), creating fatty acids in adipose tissues and the mammary gland (Ishler et al., 2016).

VFAs are absorbed across the ruminal epithelium, where ruminal veins carry them to the portal vein and through the liver (Na & Guan, 2022). The continuous flow of VFAs from the rumen is essential for energy distribution. It prevents the excessive and damaging drop in pH of the rumen fluid that can lead to acidosis (Hernandez et al., 2014). Production of VFAs also contributes to changes in the rumen pH, which is a critical measurement that contributes to the overall health of the rumen and, in turn, can select for or against specific microbial populations (Karisch, 2024; Clemmons, 2020). VFA is a known inhibitor of the growth of gram-negative bacterial species such as *E. coli* and *Salmonella* (Wolin et al., 1969). VFAs, produced by fermentation of organic matter in the rumen, can significantly affect animal production and tissue composition in ruminants (Pokhrel & Jiang, 2024; Choirunnisa et al., 2017). The relative proportions in which VFAs are produced are influenced by several factors (Dijkstra, 1994; Kyriazopoulou, 2021).

VFA concentrations in the rumen are essential to understanding how the microbial population impacts animal physiology and growth, but collecting the samples can be difficult and costly. Additionally, collecting ruminal fluid can be very invasive and can significantly stress cattle. Ruminal samples are typically via either rumen cannulation or "stomach tubing" by passing a tube from the mouth to the rumen (Ramos-Morales et al., 2014). Both methods are feasible but have significant differences in cost and time required. Rumen cannulation is a more invasive surgical procedure typically requiring a relatively small sample size (de Assis Lange et al., 2020). Stomach tubing allows more animal testing on producer farms in multiple locations (Nocek, 1997; de Assis Lange et al., 2020). However, there is a risk of sample contamination with saliva in the esophagus, and it may only represent small areas in the rumen geographically (Shen et al., 2012). There are additional discrepancies that exist between the comparable nature of these tests about VFA and pH (Guishauser & Gitzel, 1996; Duffield et al., 2004; Wang et al., 2016). Additionally, it is essential to note that comparison can be challenging as they vary across breeds, diets fed, feeding efficiency, and sample timing. However, this process is time-consuming and does not allow collecting samples from many cattle on the same day. Therefore, this study was designed to determine if saliva samples would be a suitable proxy to estimate ruminal VFA concentrations in cattle so that more samples could be collected.

MATERIALS AND METHODS

All experimental procedures involving live animals during this study were conducted at the University of Georgia – Beef Cattle and Sheep Center located in Double Bridges Farm in Winterville, GA. Animals were handled in compliance with the regulations of the University of Georgia Institutional Animal Care and Use Committee (AUP# A2023 07-011-Y1-A0) and the UGA's NIH Animal Welfare Assurance (# D16-00276/A3437-01).

45 Saliva samples were collected from Angus-influenced steers and heifers from a larger study. The experiment followed a randomized complete block design, with animals consuming a high-concentrate diet for 35 days with the following treatments: (1) 0% DDGS (CTRL), (2) 20% DDGS (20DDG), (3) 40% DDGS (40DDG), and (4) a positive control with 40% DDGS (PCTRL; all treatments on a DM-basis). All diets were isonitrogenous and isocaloric. The animals were adapted to the dietary treatments following the protocol by Fluharty (2017) starting with a DMI of 1.7% of their BW. Feed allotments were increased by 0.1% every two days, only when animals consumed more than 95% of their daily allotment, until they reached 2.2% of BW in DMI. Once animals consistently consumed over 95% of their daily allotment, DMI was increased by 5% of the previous day's feed offered, increments were performed every two days. Diets were formulated to meet nutrient requirements as specified by NASEM (2016).

Animals were fed daily at 0800 h, with treatments individually mixed in batches at a commercial feed mill (Oglethorpe Feed and Hardware Supply, Crawford, GA) using a KUHN Knight stationary mixer (142 Reel Augie®; Kuhn, Brodhead, WI). The feed was delivered to the Beef Cattle and Sheep Center and stored in separate holding feed bins (Brock, Milford, IN) for each dietary treatment. Each day, feed was removed from the bins, transported to the animals using a feed cart, and delivered to their respective SmartFeed Pro systems.

After a 35-day pre-switch period, all animals, except those on the PCTRL treatment, were abruptly switched to a 100% endophyte-free tall fescue and alfalfa mixed hay diet for 18 days. Additionally, high-concentrate pellets were provided through the GreenFeed system to encourage visits and facilitate daily measurements of enteric CH_4 emissions, throughout the trial.

Samples were collected using 2x2 inch Band-Aid sterile gauze rubbed against the cheeks and complex palate of cattle by attempting to rub three downward motions within the oral cavity.

Samples were stored in 20 mL of NaCl and frozen at –80 °C for further analysis. Further analysis for VFA was used in the methodology described by Lourenco et al. (2020). Briefly, 2 mL of each sample was centrifuged at 10,000× *g* for 10 min, and 1 mL of the supernatant was mixed with 0.2 mL of 25% (wt/vol) meta-phosphoric acid, vortexed, and frozen overnight. Subsequently, the samples were thawed and centrifuged (10,000× *g*, 10 min) before 1 mL of supernatant was transferred to screw-thread vials that contained 2 mL of ethyl acetate. The samples were vortexed and allowed to settle for at least 5 min. The upper layer was transferred (1 mL) into gas chromatography vials for VFA analysis using a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector and capillary column (Zebron ZB-FFAP; 30 m × 0.32 mm × 0.25 µm; Phenomenex Inc., Torrance, CA, USA). The sample injection volume was 1.0 µL, and helium was used as the carrier gas. The starting temperature of the column was set at 110 °C and gradually increased to 200 °C, the injector temperature was set at 250 °C, and the detector temperature was set at 350 °C. The output variables recorded were the acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, and caproic acid levels (Lourenco et al., 2020).

Samples were prepared in duplicate, and VFA means were average by treatment, and across treatments. Significant differences were determined at $P < 0.05$.

RESULTS & DISCUSSION

The analysis of volatile fatty acids (VFAs) in the saliva of cattle revealed that acetate was the predominant VFA detected. Specifically, the average concentration of acetate was found to be 2.625 mmol/Liter in each sample (Table 5.4). This concentration is notably low, representing less than 5% of the typical ruminal concentrations of acetate (Figure 5.1). The low levels of

acetate in saliva compared to the rumen can be attributed to the primary site of VFA production and absorption being the rumen, with only a small fraction entering the saliva.

In addition to acetate, only trace amounts of other VFAs such as propionate, butyrate, and isovalerate were detected. These VFAs were present in less than 10% of the sampled population, indicating their minimal presence in the saliva. The low detection rates of these VFAs suggest that their primary absorption and utilization occur within the rumen and other parts of the digestive system, rather than being excreted in significant amounts through saliva. Due to the insufficient concentrations of propionate in the saliva samples, it was not possible to calculate the acetate:propionate ratio. This ratio is often used as an indicator of fermentation patterns and metabolic processes within the rumen. The inability to assess this ratio in saliva highlights the limited role of saliva in reflecting the detailed VFA profile of the rumen.

Many ruminant nutrition studies have found associations among types of feed, VFA concentrations, and ruminal pH. The relationship between the host, VFA, and the rumen microbiota has been extensively explored and previously reviewed (Russell, 2002; Clemmons et al., 2018). Although VFA production and pH have been well-studied in cattle, there is still much knowledge to gain regarding manipulating the microbiome and its effects on these factors or vice versa and how that will, in turn, modify energy substrate and nutrient production (Clemmons et al., 2018). VFA's absorption at their production site is rapid, and the ruminal or large intestinal epithelium metabolizes large quantities before reaching the portal blood. Intestinal epithelial cells use much of the energy in butyrate and convert most of the butyrate to ketone bodies or CO_2, and the liver utilizes the rest of the ruminally produced butyrate (Bergman, 1990). Propionate is similarly removed by the liver but is converted to glucose. Although species

differences exist among ruminants, acetate is used principally by peripheral tissues, especially fat and muscle (Bergman, 1990).

Volatile fatty acid production is a significant job of the gastrointestinal tract of ruminants. They serve as the primary energy source for cattle and are the major product of microbial fermentation from carbohydrates. Other end products consist of methane and carbon dioxide. The production rate depends directly on the diet fed, thus impacting the energy of the animal and their ability to reduce feed inputs. The proportions of VFA production also determine fat and milk content. If acetate is low, milk production may be depressed. A lower proportion of acetate can be seen in diets high in grain and lower in forage. Diets higher in fiber and lower in energy lead to microbial populations that produce high ratios of acetate to propionate (Apajalahti et al., 2019).

In cattle, the breakdown of both fibrous and non-fibrous carbohydrates in the rumen is essential for energy production (Church, 1993). Fibrous carbohydrates, such as cellulose and hemicellulose, are slowly digested by rumen microbes, resulting in the production of volatile fatty acids (VFAs) like acetic acid, butyric acid, and a smaller amount of propionic acid. These VFAs are absorbed through the rumen wall and transported to the liver. Non-fibrous carbohydrates, including starches and sugars, are more rapidly digestible and primarily produce propionic acid, along with acetic and butyric acids, through microbial fermentation (Hall, 2000). The VFAs from non-fibrous carbohydrates are also absorbed and transported to the liver. Acetic acid, mainly from fibrous carbohydrates, is crucial for milk fat synthesis, while propionic acid, primarily from non-fibrous carbohydrates, is a key precursor for glucose synthesis in the liver (Church, 1993). Butyric acid, produced from both types of carbohydrates, serves as an energy

source for the rumen wall and other tissues. Balancing the diet with an optimal mix of fibrous and non-fibrous carbohydrates is essential for maintaining rumen health and maximizing production performance (Hall, 2000)

As cattle chew, they produce up to 80 liters of saliva daily (Akula et al., 2023), serving as a liquid buffer and nutrient supply for the gastrointestinal microbes to begin degrading feedstuffs. This large amount of saliva is produced from eight types of salivary glands (Xu et al., 2021). Cattle saliva is primarily composed of water, minerals, mucus, and urea; the secretions can range from serous to mucous and, in some cases, mixed (Boyles, 2019). Specifically, cattle saliva contains phosphate, Nitrogen, potassium, and sodium bicarbonate, which in high concentrations helps to create a buffered environment that aids in the fermentation process by helping to maintain ruminal pH (Xu et al., 2021). It is essential also to note that Urea nitrogen makes up 75% of the total Nitrogen in saliva, which is crucial in helping to facilitate a constant ability for ruminal microbes to synthesize microbial crude protein de novo (Church, 1993). Sodium and bicarbonate make up the bulk of saliva with 126 mEq/l. Phosphate comprises 26 mEq/l, and chloride and potassium contain six mEq/l and seven mEq/l, respectively (Bailey & Balch, 1961).

Salivary urea provides a basal level of readily available Nitrogen in the rumen to ensure that sufficient Nitrogen is available temporally with carbon in the ruminal fermentation to support Microbial Crude Protein (MCP) synthesis. The addition of saliva helps to soften food and provides lubrication for swallowing. This saliva contains a more extensive and more diverse set of proteins that can perform multiple functions, such as taste and digestion, lubrication, pH buffering, and general health maintenance by controlling the oral microbiota.

The rate at which an animal produces saliva is heavily influenced by the type of feed consumed and the amount of time the animal spends ruminating. Cattle saliva is a vital component of the ruminant digestive system and plays a crucial role in nutrient breakdown, pH regulation, and rumen health. Saliva helps reduce bloating in cattle, which is a potentially fatal condition. Cattle that have lower levels of saliva are prone to chronic bloat in which death can occur (Boyles, 2019). Different salivary glands produce cow saliva compared to humans. The number of markers may differ between humans and cattle; therefore, the protein composition differs (Akula et al., 2023).

Acetate is a microbially produced VFA that serves as a source of energy in cattle (Church, 1993). A small amount of acetate is absorbed through the rumen wall and is converted to ketone bodies, which are carried by a portal to the liver unchanged (Church, 1993). Acetate was found in all our samples. Based on our results, under these conditions collecting saliva samples under these conditions was not a valid proxy measure to determine ruminal VFA concentrations.

Overall, VFA play a pivotal role in the energy metabolism of cattle, serving as a primary energy source and contributing significantly to their overall health and productivity. The intricate balance of ruminal VFA production and absorption is crucial for maintaining a stable ruminal environment and preventing conditions such as ruminal acidosis (Penner, 2014). Strategies to optimize VFA levels and mitigate the risks associated with their fluctuation are essential for enhancing cattle performance. As research unravels the complexities of VFA dynamics, it becomes increasingly clear that understanding and managing these compounds is critical to advancements in beef production and understanding of feeding efficiencies.

A follow-up study is needed using animals which are not assessed for rumen fluid sampling. Stresses increase salivation in cattle (Contreras-Aguilar et al., 2022), and could alter the saliva type, amount, and composition that are found in the oral cavity. Thus, the collection process may have inadvertently diluted the salivary VFA prior to collection. While it would be expected that acetate would be the most common, the resting state of the animal, stress level, and mixing of rumen fluid samples in the oral cavity altered the state of samples.

CONCLUSIONS

While this was an easy sample collection to make, the low levels of VFA found in saliva were too low to be useful as a proxy for determining the ruminal VFA concentrations. The added stress of collecting rumen fluid may have altered these saliva concentrations giving inaccurate insight into the true VFA concentrations of saliva in cattle. Cattle tend to salivate more when excited therefore there was an excess saliva produced. At the time of sample collection, cattle were very excited and over stimulated due to having rumen fluid samples taken. Additional data should be collected to compare our data to samples in a lower-stress situation. It is also noted that a diet change will alter the VFA concentration. Finally, cotton fiber swabs have been used widely to sample the microbial oral populations of cattle (Parks et al., 2015). In the present study, the cotton swabs used for VFA sample collection, potentially caused a decrease in concentrations of propionate in salivary fluid due to binding hydrogen bonding of propionate to cotton. Cotton fibers contain hydroxyl groups which interact with propionate through hydrogen bonding (Adamowicz et al., 2014). This potential binding lead to the retention of propionate on the sterile cotton gauze pad, instead of in the fluid phase of the sample. This differential binding likely created an artificially reduced level of propionate, as well as an elevated acetate:propionate ratio in saliva sample. If this study were to be replicated in the future, it would be suggested to

use either other sampling methods of specialized gauze or swabs or to use a synthetic sponge to collect saliva (Medeiros & Medeiros, 2023).

Understanding the VFA composition in cattle saliva can provide insights into their digestive health and metabolic status. The predominance of acetate and the trace presence of other VFAs in saliva suggest that while saliva plays a role in buffering the rumen and aiding digestion, it does not significantly contribute to the overall VFA pool utilized by the animal. This information can be useful for developing nutritional strategies and managing feeding practices to optimize rumen health and overall productivity in cattle.

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Table 5.1 Feedstuff the 0% DDG Diet (Control), 20% DDG Diet and 40% DDG Diet fed to cattle examined (n=48 cattle)

Ingredient	% Total DM (0% DDG Diet)	% Total DM (20% DDG Diet)	% Total DM (40% DDG Diet)
Ground Ear Corn	53.2	47.5	44
Low Fat DDG	0	20	40
Soy Hulls	26.42	20.21	11.42
Soybean Meal Expeller Cost	16.93	8.39	0
Pulverized Calcium Limestone	.65	1.1	1.78
TM Salt	.8	.8	.8

Figure 5.2 Volatile Fatty Acid Concentration from saliva samples of cattle fed 0% DDG (n=94)

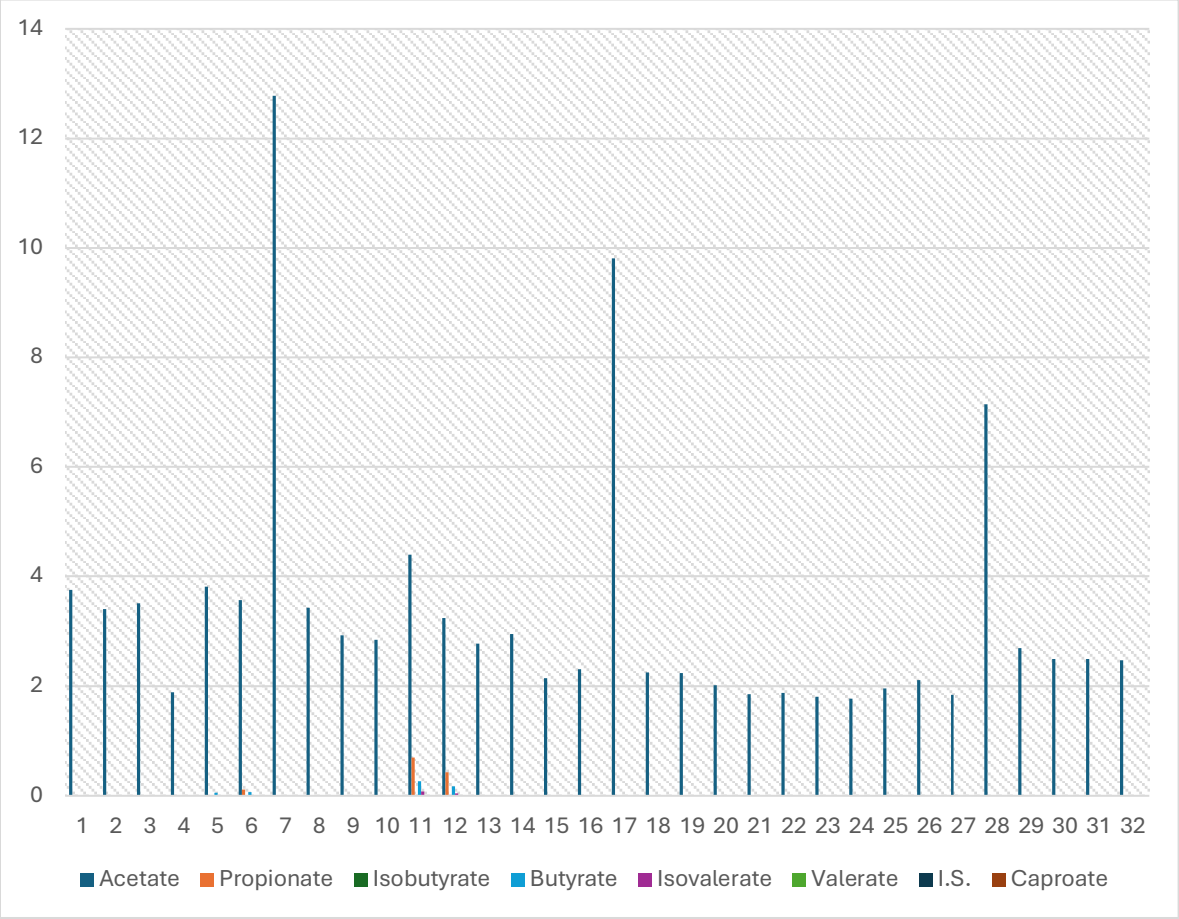


Figure 5.3 Volatile Fatty Acid Concentration from saliva samples of cattle fed 20% DDG (n=94)

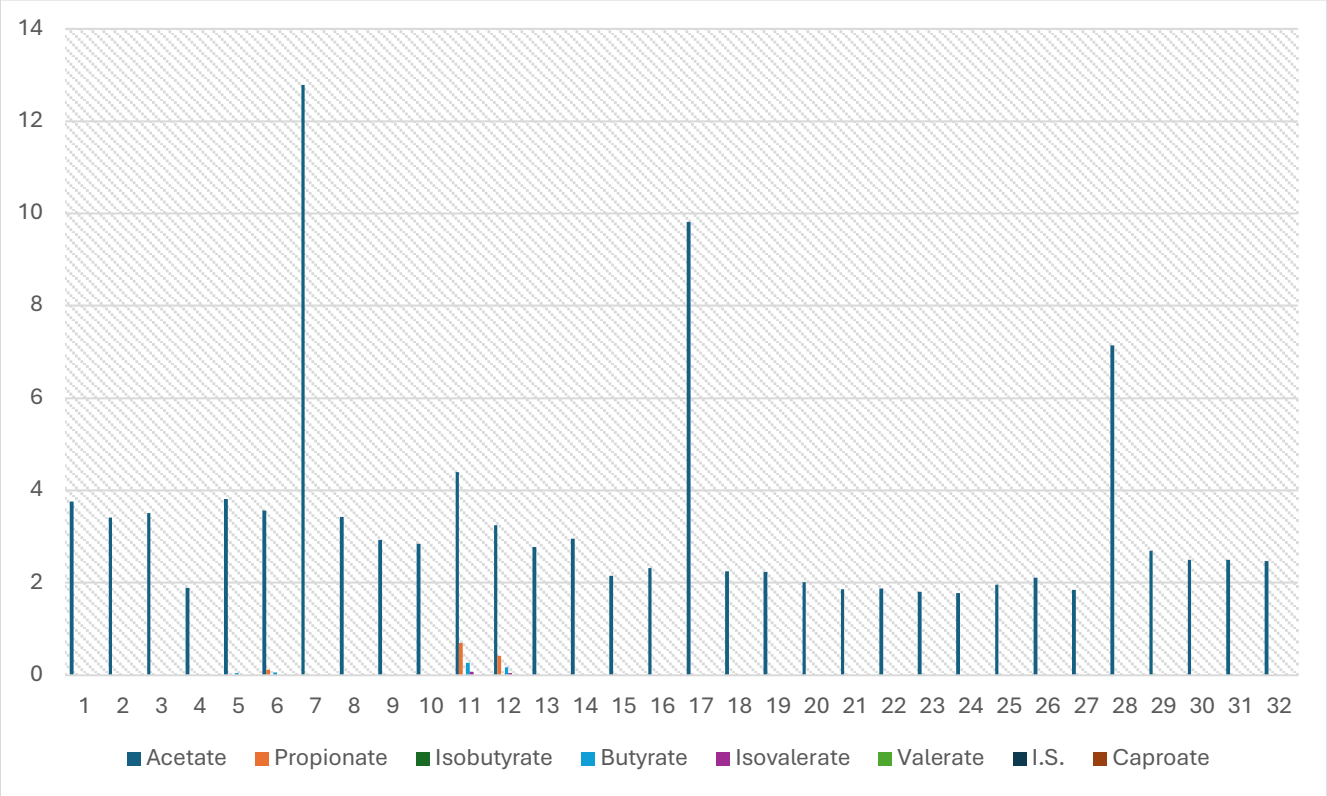


Figure 5.4 Volatile Fatty Acid Concentration from saliva samples of cattle fed 40% DDG (n=94)

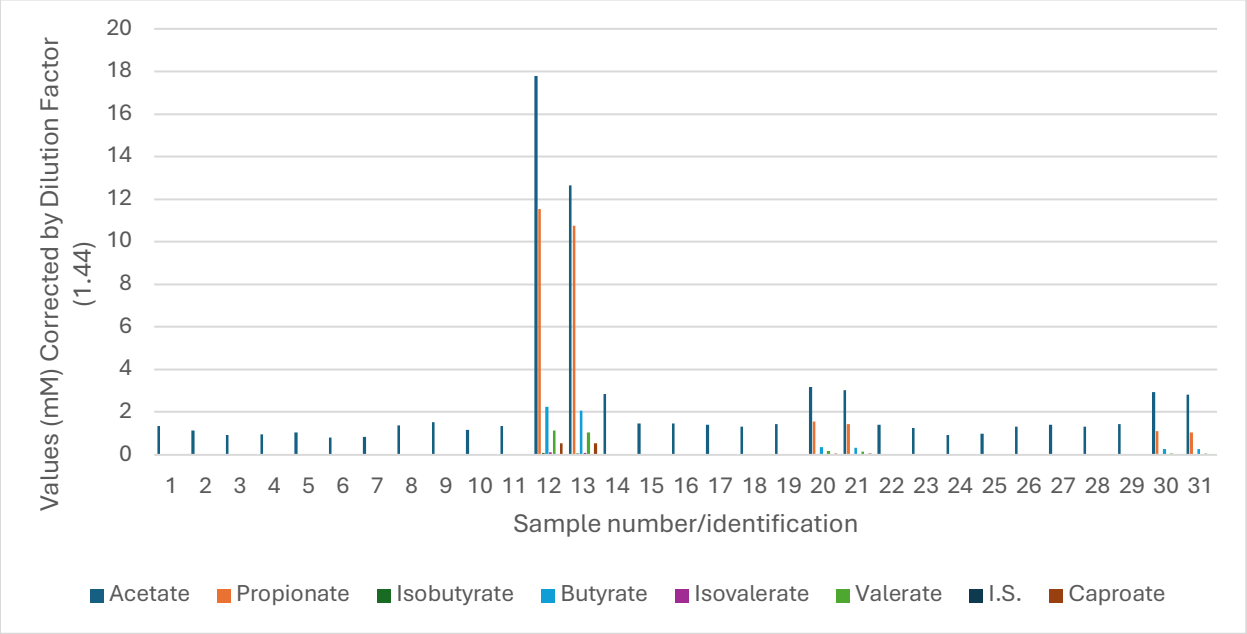
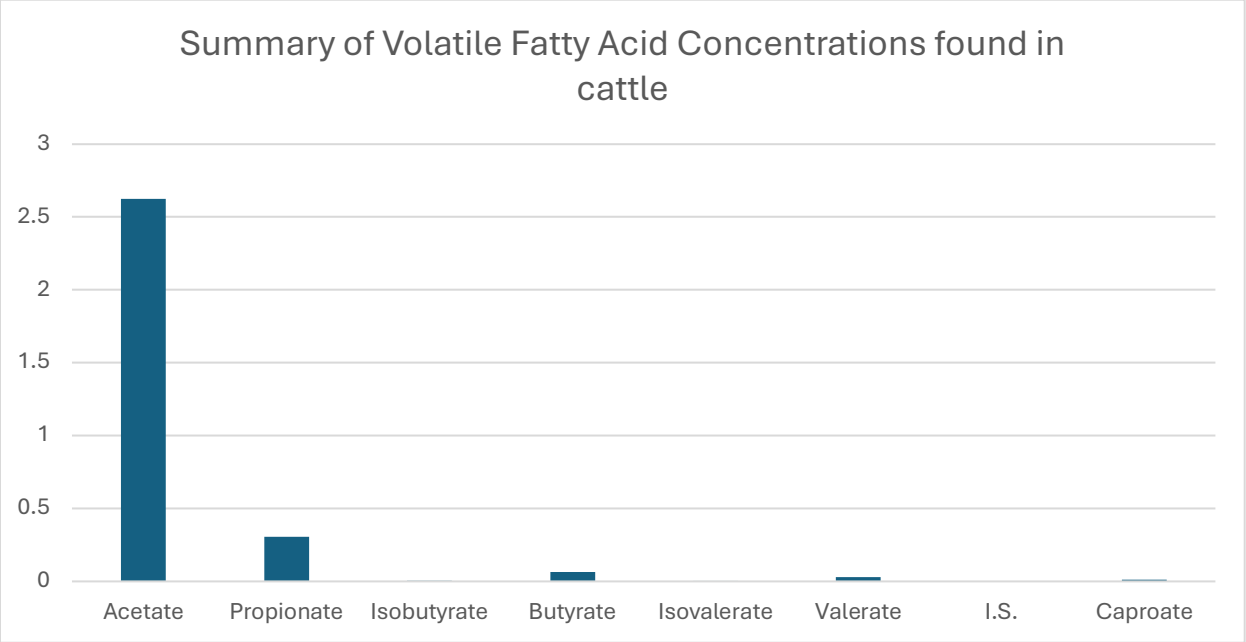


Figure 5.5 Summary of salivary VFA Concentrations (mM)in Angus cattle (n = 94) pooled across 0, 20, and 40% Distiller's grain containing rations.



CHAPTER 6

CONCLUSION

Understanding the oral environment is important to understanding cattle feed intake and consequently feed efficiency, productivity, and sustainability. This dissertation explored the potential relationship between feeding efficiencies and taste buds found in cattle. Through three studies, we found a significant correlation between cattle taste buds and feed intake, suggesting that the taste buds can be a predictor for feed intake. Chapter 3 investigates the correlation between feeding behaviors and taste buds as a predictor of feed intake in cattle. Chapter 4 discusses the regional distribution and quantification of taste bud types. Chapter 5 aims to look at the relationship between Volatile Fatty Acid (VFA) concentrations found in cattle saliva on cattle fed a high grain diet.

In the first study, cattle were fed a high grain diet for a 70-day test period while having their feed intake monitored by the GrowSafe feeding system. After harvest, tongue tissue samples were evaluated to determine the number and type of taste buds on the tongue which were correlated to feed intake data. The study reveals significant correlations between the number of circumvallate papillae and various performance metrics in animals. A higher number of circumvallate papillae is associated with a tendency for lower Average Daily Gain (ADG) ($P=0.01$), indicating that animals with more circumvallate papillae tend to grow at a slower rate. Additionally, there is a significant negative correlation between circumvallate papillae and feed intake, both as-is ($P=0.044$) and Dry Matter Intake (DMI) ($P=0.047$), suggesting that animals with more circumvallate papillae consume less feed. Furthermore, a tendency for lower Residual

Feed Intake (RFI) (P=0.085) is observed with greater numbers of circumvallate papillae, implying that these animals are less efficient in converting feed into body mass. These findings highlight the potential impact of circumvallate papillae on growth performance and feed efficiency. The objective of the second study was to look at regional distribution and quantification of taste buds in different physical regions of the tongues of cattle. Results served as a learning mechanism to help better understand the taste bud types and geographic location along the epithelial tissue on the bovine tongue. It was discovered that the majority of the fungiform are distributed on the apex of the tongue. Circumvallate are largely concentrated on the area lateral to the lingual prominence.

The objective of the third study was to look at the volatile fatty acid concentrations found in cattle saliva. Acetate was proven to be the most prominent VFA found in saliva. It was also determined that the collection method may have played a part in the overall results. Saliva was demonstrated to not be a useful proxy to determine ruminal VFA concentration due to low concentrations. It is possible that the use of less stressed cattle might be more beneficial in future studies.

Across the three studies, it is apparent that there is a correlation between cattle taste buds and could serve as a predictor or driver for feed intake in cattle. While a correlation among circumvallate and feed intake exists, more comprehensive studies should be done in order to achieve a solid conclusion. A more in-depth study beginning at birth could give us better insight to taste bud growth and development.

Recommendations

1. Consider fetal programming as a method to further explore the relationship between taste buds and feeding preferences.

2. Consider conducting MRIs in cattle prior to starting a grain-based diet and compare to the results of the counts of taste buds post-harvest. This should be done in on cattle of similar genetics with altered diets as well.
3. Continue to take a more invasive look at cattle taste buds to determine the precise types that cattle may possess.

The findings underscore the importance of taste buds in influencing feed intake and feeding behaviors in cattle. By further investigating these relationships, particularly through advanced techniques like fetal programming and MRI studies, we can enhance our understanding and potentially improve cattle feeding efficiencies.