

Management-driven strategies to mitigate environmental impacts and improve productivity of  
beef systems using precision technologies and nutritional interventions

by

Logan Edward Diller

B.S., The Pennsylvania State University, 2024

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences & Industry  
College of Agriculture

KANSAS STATE UNIVERSITY  
Olathe, Kansas

2026

Approved by:

Co-Major Professor  
Dr. Logan Thompson

Approved by:

Co-Major Professor  
Dr. Haley Larson

# Copyright

© Logan Diller 2026.

## Abstract

Beef production systems face pressure to improve environmental efficiency while maintaining animal performance and welfare. Nutritional management and environmental monitoring represent two complementary strategies to enhance sustainability in both confined and grazing systems. The objectives of the thesis were to evaluate (1) precision monitoring technologies for assessing feedlot pen-surface nutrient heterogeneity and (2) nutritional interventions aimed at reducing enteric methane (CH<sub>4</sub>) emissions in grazing cattle, with emphasis on practical implementation under commercial conditions. Objective 1 was evaluated using UAV-mounted thermal cameras to characterize and predict nutrient distribution within feedlot pens managed under limit-fed (LOW) or ad libitum (HIGH) feeding programs. Six outdoor pens housing crossbred steers were subdivided into 15 quadrants for sample collection and measurement of moisture, organic matter, fiber fractions, and nitrogen content of the pen surface material. Thermal orthomosaics were generated and color metrics were extracted to evaluate associations with measured nutrient components. On a single day evaluation, moisture differed by feeding program ( $P = 0.01$ ), averaging 44.0% in HIGH pens and 34.0% in LOW pens. Moisture to organic matter ratio was similarly greater in HIGH compared to LOW pens (98.0 vs 71.0;  $P = 0.02$ , respectively). Nitrogen concentration (dry matter basis) was greater in LOW pens (21.5%) compared to HIGH pens (18.5%;  $P < 0.01$ ). Quadrant significantly influenced moisture and nutrient distribution ( $P < 0.01$ ), confirming structured spatial heterogeneity within pens. When evaluated across multiple days, moisture remained greater in HIGH pens compared to LOW (52.1 vs 42.1%;  $P < 0.01$ , respectively), demonstrating consistent feeding program effects despite precipitation variability. Several thermal derived color metrics (R, B, mean RGB, Lab\*L;  $P \leq 0.04$ ) differed by quadrant over time, and pen-level thermal color

metrics demonstrated predictive capability for moisture and organic matter. These findings support UAV-derived thermal imaging as a promising precision management tool for pen-level environmental assessment, though refinement in time of sampling and sensor calibration may enhance sub-pen resolution. Objective 2 examined a nutrient management strategy for grazing cattle systems by evaluating supplementation of an essential oil blend (Agolin; Alltech Inc., Nicholasville, KY) in 63 grazing beef heifers (BW =  $274 \pm 42$  kg) over a 70-day triticale pasture trial beginning mid-winter. Supplement intake averaged approximately 0.30 kg/head/day, resulting in an actual Agolin exposure of 484.6 mg/head/day. Average daily gain (ADG) did not differ between treatments ( $P = 0.18$ ), with Agolin heifers gaining 0.69 kg/day compared to 0.62 kg/day in control heifers. Despite variable supplement intake, heifers supplemented with Agolin resulted in measured enteric CH<sub>4</sub> emissions of 162.6 g/d compared to 178.6 g/day in control heifers ( $P = 0.05$ ), corresponding to a 16 g/d absolute reduction and approximately 8.96% mitigation. A decrease in emission intensity (g CH<sub>4</sub>/kg ADG) was found for heifers supplemented with Agolin compared to heifers without Agolin supplementation (242.81 and 293.50;  $P = 0.03$ , respectively). Carbon dioxide emissions remained unchanged, potentially supporting the interpretation that CH<sub>4</sub> reduction occurred through altered hydrogen disposal rather than suppressed overall fermentation. Collectively, observed findings demonstrate that both environmental monitoring technologies and nutritional interventions can improve sustainability outcomes in beef production systems. Integration of precision technologies with targeted nutritional management represents a scalable pathway to improve nutrient efficiency, reduce emission intensity, and maintain productivity across diverse beef production environments.

**Keywords:** Pen condition, essential oil supplementation, methane emissions, drone

# Table of Contents

|                                                                                                                  |      |
|------------------------------------------------------------------------------------------------------------------|------|
| List of Figures .....                                                                                            | vii  |
| List of Tables .....                                                                                             | viii |
| Acknowledgements .....                                                                                           | ix   |
| Dedication .....                                                                                                 | x    |
| Chapter 1 - Literature review of relevant topics .....                                                           | 1    |
| Part I – UAV assessment of cattle pen conditions and cattle health impacts .....                                 | 1    |
| Introduction .....                                                                                               | 1    |
| Nutrient effects on manure composition and volume .....                                                          | 2    |
| Spatial variation of nutrient components on cattle pen surfaces .....                                            | 6    |
| Role of bedding on seasonality and pen-surface moisture mitigation .....                                         | 6    |
| Current metrics used to assess pen condition .....                                                               | 8    |
| Influence of pen condition on cattle health .....                                                                | 9    |
| Influence of pen condition on cattle performance .....                                                           | 10   |
| Thermal imaging technologies to detect surface moisture .....                                                    | 12   |
| Applications of UAV thermal infrared technologies in cattle production .....                                     | 14   |
| Transition to the current study .....                                                                            | 15   |
| Part II – Essential oil supplementation and its effects on beef cattle performance and enteric emissions .....   | 17   |
| Introduction .....                                                                                               | 17   |
| Essential oils as modifiers of rumen microbial fermentation .....                                                | 18   |
| Rationale for combining essential oils into blends .....                                                         | 20   |
| Diet context for essential oil supplementation .....                                                             | 21   |
| Essential oils in feedlot and high concentrate diets .....                                                       | 22   |
| Essential oils in forage-based and grazing systems .....                                                         | 24   |
| Role of nitrogen in essential oil effects across feeding systems .....                                           | 27   |
| Previous work associated with Agolin essential oil blend .....                                                   | 28   |
| Development and formulation of Agolin .....                                                                      | 28   |
| Impacts of Agolin on dairy cattle performance .....                                                              | 28   |
| Impacts of Agolin in beef and beef x dairy systems .....                                                         | 29   |
| Synthesis of Agolin responses .....                                                                              | 30   |
| Transition to the current study .....                                                                            | 31   |
| Literature Cited .....                                                                                           | 32   |
| Chapter 2 - Use of unmanned aerial vehicle thermal imagery to assess the nutrient landscape of cattle pens ..... | 50   |
| Introduction .....                                                                                               | 50   |
| Materials and methods .....                                                                                      | 52   |
| Animal care and use .....                                                                                        | 52   |
| Animals, experimental design, and feeding programs .....                                                         | 52   |
| Sample collection and UAV thermal imaging .....                                                                  | 53   |
| Sample and thermal image processing .....                                                                        | 54   |
| Calculations .....                                                                                               | 57   |
| Statistical Analysis .....                                                                                       | 58   |
| Objective 1 .....                                                                                                | 58   |
| Objective 2 .....                                                                                                | 59   |

|                                                                                             |     |
|---------------------------------------------------------------------------------------------|-----|
| Objective 3 .....                                                                           | 60  |
| Results and discussion .....                                                                | 61  |
| Objective 1: Characterize pen-surface nutrient composition and color metrics .....          | 61  |
| Objective 2: Multi-day pen-surface nutrient components and color metrics.....               | 64  |
| Objective 3: Pen-level thermal color metric predictive capabilities .....                   | 68  |
| Association of pen-level thermal color metrics to pen-surface nutrient composition .....    | 68  |
| Agreement of actual pen-level nutrient composition to color-metric-predicted values ...     | 70  |
| Implications.....                                                                           | 72  |
| Tables.....                                                                                 | 73  |
| Figures .....                                                                               | 92  |
| Literature Cited .....                                                                      | 95  |
| Chapter 3 - Growth performance and enteric gas emissions in grazing beef heifers fed Agolin | 101 |
| Introduction.....                                                                           | 101 |
| Materials and methods .....                                                                 | 104 |
| Animal care and use.....                                                                    | 104 |
| Animals, treatments, and experimental design .....                                          | 104 |
| Supplement delivery and intake monitoring .....                                             | 105 |
| Forage and body weight data collection .....                                                | 105 |
| Enteric gas emissions measurement.....                                                      | 107 |
| Data preparation.....                                                                       | 107 |
| Statistical analysis .....                                                                  | 108 |
| Results and discussion .....                                                                | 109 |
| Supplement intake and treatment exposure .....                                              | 109 |
| Performance recordings .....                                                                | 110 |
| Enteric emissions .....                                                                     | 111 |
| Implications.....                                                                           | 113 |
| Tables.....                                                                                 | 114 |
| Literature Cited .....                                                                      | 119 |

## List of Figures

|                                                                                                                                                                                      |    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Figure 2.1 Virtual map of pen quadrants (n = 15) used for manure and UAV thermal-derived color metric sampling. ....                                                                 | 92 |
| Figure 2.2 Visual depiction of terms used to describe relative pen orientation labeled with general pen infrastructure landmarks. ....                                               | 93 |
| Figure 2.3 (a, b, c) Visual representation of the process of confounding object removal and color metric extraction from Adobe Photoshop using example pen thermal orthomosaic. .... | 94 |

## List of Tables

|                                                                                                                                                                                       |     |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Table 2.1 Nutrient composition (% of DM) of diets fed in high-intake and low-intake feeding programs. ....                                                                            | 73  |
| Table 2.2 Ingredient composition of diets fed in high-intake and low-intake feeding programs. ....                                                                                    | 74  |
| Table 2.3 Weather and environmental conditions relevant to experimental sampling period.....                                                                                          | 75  |
| Table 2.4 Effects of feeding program (HIGH vs. LOW) and pen-surface quadrant on nutrient components and UAV thermal-derived color metrics for a single day (Objective 1). ....        | 76  |
| Table 2.5 Effect of feeding program (HIGH vs. LOW) on nutrient components for a singular day (Objective 1). ....                                                                      | 77  |
| Table 2.6 Effect of feeding program (HIGH vs. LOW) on thermal-derived color metrics for a singular day (Objective 1).....                                                             | 78  |
| Table 2.7 Estimated nutrient components by quadrant for a singular day (Objective 1). ....                                                                                            | 79  |
| Table 2.8 Estimated UAV thermal-derived color metrics by quadrant for a singular day (Objective 1). ....                                                                              | 80  |
| Table 2.9 Effects of feeding program (HIGH vs. LOW) and quadrant on nutrient components and UAV thermal-derived color metrics using data from all collection days (Objective 2). .... | 81  |
| Table 2.10 Effect of feeding program (HIGH vs. LOW) on nutrient components using data from all collection days (Objective 2). ....                                                    | 82  |
| Table 2.11 Effect of feeding program (HIGH vs. LOW) on thermal-derived color metrics using data from all collection days (Objective 2). ....                                          | 83  |
| Table 2.12 Estimated nutrient components by quadrant using data from all collection days (Objective 2). ....                                                                          | 84  |
| Table 2.13 Estimated UAV thermal-derived color metrics by quadrant using data from all collection days (Objective 2). ....                                                            | 85  |
| Table 2.14 Association of pen-level nutrient components to UAV thermal imagery-derived color metrics and estimated nutrient levels from all collection days (Objective 3). ....       | 86  |
| Table 2.15 Pen-level nutrient predictions using UAV thermal-derived color metrics from all collection days (Objective 3). ....                                                        | 88  |
| Table 2.16 Pen-level nutrient predictions using UAV thermal-derived color metrics from data with precipitation outlier day removed (Objective 3). ....                                | 90  |
| Table 3.1 Formulated ingredient inclusion and nutrient composition of the Agolin and control supplements.....                                                                         | 114 |
| Table 3.2 Nutrient composition of triticale forage during early and late periods of the grazing trial. ....                                                                           | 115 |
| Table 3.3 Manufacturer-guaranteed nutrient composition of pasture mineral supplement formulated for a target intake of 56.7 g/head/day. ....                                          | 116 |
| Table 3.4 Performance and enteric emissions of grazing beef heifers supplemented with dried distillers grains with or without Agolin. ....                                            | 117 |
| Table 3.5 Target and average supplement intake on a dry matter basis, zero-intake frequency, and resulting Agolin dose calculations during the 70-day grazing trial. ....             | 118 |

## Acknowledgements

My passion for agriculture is a result of growing up immersed in a farming family. I would not be the person I am today if it was not for the love and generosity of my late grandfather, Eddie, and grandmother Betty Hessong. Thank you for always supporting me and teaching me what it means to be a farmer, a leader, and a great person.

I want to thank my parents, Ryan and Sarah, for grounding me in my faith, their support, and confidence in me to reach my goals whatever they may be. Thank you also to my brothers, Brady, Blake, and Chase for the countless laughs and random phone calls. A big thank you to my wife, Madison Bemisderfer, for not only supporting me throughout my bachelor's and graduate school programs but also for inspiring me to be a great scholar and a better man as we live our lives together.

A sincere thank you to my co-advisors, Dr. Haley Larson and Dr. Logan Thompson. It has been a pleasure learn under Dr. Larson—I truly admire her ability to push the boundaries of science and research to bring a higher level of understanding to the beef cattle industry. Likewise, being mentored by Dr. Thompson has brought me a level of education that goes beyond the basics—he pushed me to think deeper and to balance the science with producer practicality. I am truly grateful to have been supported by my co-advisors and my committee members, Dr. Phillip Lancaster and Dr. Dale Blasi, throughout my graduate program. Thank you to my fellow lab mates, Madison Bemisdefer and Ashlee Gibbons, for the laughs, project support, and time spend at the feedyard—they made my time in Kansas memorable and fun.

Finally, I would like to thank Clark County Feeders and Alltech Inc. for their continued support and guidance in many projects that I have had the pleasure to be a part of.

## **Dedication**

To Eddie Hessong,

Your passion for agriculture, mentorship, and generosity throughout your life is the reason that I am who I am today.

# **Chapter 1 - Literature review of relevant topics**

## **Part I – UAV assessment of cattle pen conditions and cattle health impacts**

### **Introduction**

The feedlot environment plays a critical role in cattle performance, health, and overall welfare (Grandin, 2016). The physical characteristics of the pen surface microclimate, such as manure accumulation, moisture content, surface temperature, and dust generation directly affect both animal well-being and the operational sustainability of feedlots (Cortes et al., 2021; Mader et al., 2007). Environmental challenges such as excessive mud, dust, and heat stress are persistent concerns in feedlot management. Mud accumulation increases cattle energy expenditure, reduces feed efficiency, and may predispose animals to lameness or other health issues (Cortes et al., 2021; Mader, 2011; National Academies of Sciences, Engineering, and Medicine, 2016). Dust emissions from dry pen surfaces not only impair local air quality and cattle respiratory health (Urso et al., 2021; Wilson et al., 2002) but also contribute to elevated levels of particulate matter in ambient air, especially around concentrated animal feeding operations (Emert et al., 2023). Heat stress further compounds animal health concerns, particularly in the absence of adequate shade or cooling strategies, leading to reduced performance and higher risk of morbidity (Miller et al., 2024). By integrating unmanned-aerial-vehicle-based assessments with ground measurements, researchers and producers can develop more precise, real-time monitoring systems to mitigate these risks. Traditional methods of assessing pen conditions rely heavily on manual ground sampling and subjective scoring systems, which are labor-intensive, time-consuming, and often lack spatial resolution (Bastos et al., 2024).

In recent years, there has been an increase in the application of unmanned aerial vehicles (UAVs) as tools to optimize agricultural practices. Unmanned aerial vehicles equipped with high-resolution cameras, multispectral sensors, and/or thermal infrared imaging technology offer a non-invasive means of collecting large-scale data across pens with minimal labor and disturbance to cattle (Abdulai et al., 2021; Shao et al., 2020). Adoption of UAV technologies in feedlot settings aligns with broader trends in precision livestock farming, which leverage automation, remote sensing, and machine learning to improve efficiency and animal care (Jiang et al., 2023). While UAVs have been successfully applied in crop agriculture for mapping soil moisture, plant health, and nutrient dynamics, their application in feedlots remains relatively new and underexplored (Olson & Anderson, 2021). A UAV approach provides the opportunity to quantify spatial heterogeneity in pen surface conditions, capture changes due to weather or management interventions, and link environmental factors to cattle health outcomes. Addressing limitations of calibration, data interpretation, and integration with existing management systems is crucial for translating UAV-derived measurements into actionable tools for producers.

This review synthesizes current knowledge on the nutritional and environmental drivers of cattle pen manure accumulation, pen surface variability, effects on cattle health and performance, and evaluates the potential of UAV technologies to enhance assessment and management of feedlot conditions.

### **Nutrient effects on manure composition and volume**

Nutrition is a fundamental determinant of the volume and composition of manure excreted by cattle. Dietary intake of nutrients, specifically crude protein, fiber, and minerals, influences not only the quantity of excreta, but also the nutrient profile of the manure: nitrogen, phosphorus, potassium, etc. (Nennich et al., 2005). The nutrient content of manure is often

modeled as the difference between intake and utilization plus endogenous losses (*Nutrient Management Technical Note No. 190-Nm-1. Effects of Diet and Feeding Management on Nutrient Content of Manure*, 2020).

Protein content of the diet contributes to the amount of urinary and fecal nitrogen excretion. Nitrogen excretion is especially elevated for diets with protein levels exceeding the metabolizable requirement of the animal. For example, Cole et al. (2005) reported that lowering dietary crude protein concentration from 13.0% to 11.5% of dry matter reduced urinary nitrogen output by approximately 25% and decreased potential ammonia emissions by 30 to 44% in finishing cattle. Excreted nitrogen from urine can rapidly volatilize as ammonia, particularly under conditions of high temperature and pH, contributing to air quality degradation and greenhouse gas emissions (Miller & Berry, 2005). Chemical characteristics of manure, including ammonia-nitrogen concentration, have been shown to respond strongly to crude protein supply (Cole et al., 2005). Driedger & Loerch (1999) found nitrogen excretion to be 22% greater in nonlactating Holsteins fed an ad libitum, high-forage ration (11.2% CP DM-basis) compared to a limit-fed, high-corn ration (15.4% CP DM-basis). Miller et al. (2003) observed that fecal and urine nitrogen levels varied seasonally by as much as 15-20%.

Dietary phosphorus inclusion level has shown a direct effect on fecal phosphorus excretion. Erickson et al. (2002) demonstrated that increasing dietary phosphorus above requirements, through supplementation with monosodium phosphate to achieve dietary concentrations ranging from 0.16 to 0.40% phosphorus (dry matter basis) resulted in increased phosphorus excretion. Fecal phosphorus concentrations increased from approximately 0.30 to 0.50% of fecal dry matter in finishing cattle. Elevated phosphorus excretion from cattle can raise concerns in certain regions where cattle manure is applied to crop ground creating a potential for

phosphorus loading in soils and subsequent downstream effects on sediment/surface water runoff resulting in higher levels of eutrophication (McDowell et al., 2000).

Dietary fiber content strongly influences fecal output. Neutral detergent fiber (NDF) increases fecal mass due to its limited digestibility compared to starch and its capacity to hold water as it passes through the digestive tract (Huhtanen et al., 2016). Hales et al. (2014) demonstrated that increasing alfalfa hay from 2% to 14% of diet dry matter in finishing cattle linearly increased fecal organic matter and NDF excretion, with fecal energy losses rising by nearly 20% of gross energy intake. The increase in fecal energy loss occurred because higher alfalfa inclusion increased dietary NDF and reduced total tract digestibility, resulting in greater excretion of undigested organic matter and fiber-derived energy in the feces. Additionally, fiber inclusion in the diet influences manure volume primarily through reduced digestibility, which increases the amount of undigested material excreted, and through diet-dependent changes in ruminal passage dynamics. Across ruminant studies, diets higher in NDF consistently yield greater fecal output. Huhtanen et al. (2016) reported that a 10% increase in dietary NDF raised fecal dry matter output by ~0.2 kg/day, while Pickinpaugh et al. (2022) observed a quadratic increase in fecal output as roughage inclusion rose from 10% to 30% in finishing beef diets.

Intake level, specifically dry matter intake (DMI) is a direct contributor to total fecal output and can be influenced by limit-feeding or ad libitum feeding programs (Sip & Pritchard, 1991). Cattle offered restricted quantities of high-concentrate diets consumed less dry matter but maintained similar or improved growth efficiency compared with cattle fed ad libitum (Sip & Pritchard, 1991). Multiple studies demonstrated that limit-feeding (10-30% of ad libitum) improved nutrient utilization and reduced excreted nitrogen and organic matter (Loerch, 1990; Murphy & Loerch, 1994). Sip & Pritchard (1991) reported that steers limit-fed high-concentrate

diets produced approximately 25-30% less fecal output compared with higher-intake controls due to reduced DMI and greater digestibility.

When cattle consume diets formulated with highly digestible ingredients at levels aligned with animal requirements, nutrient absorption and retention is maximized; however, oversupply of digestible nutrients results in increased fecal or urinary excretion rather than improved utilization (Huhtanen et al., 2016). Satter et al. (2002) highlighted the idea of improved dietary digestibility in reducing manure output through greater nutrient utilization, a relationship later quantified by Huhtanen et al., (2016). In the meta-analysis, Huhtanen et al., (2016) reported that each 1% increase in dietary organic matter digestibility was associated with a reduction in fecal dry matter output of approximately 8 g per kg of feed consumed. Organic matter digestibility across the included studies ranged from approximately 27-86%, indicating that the relationship reflects an effect across diverse feeding systems rather than responses to narrowly defined diet types. These findings demonstrate that improvements in digestibility can substantially reduce manure volume while highlighting the importance of biologically realistic formulation targets. Hales et al. (2014) demonstrated that increasing alfalfa hay from 2 to 14% of diet dry matter, replacing dry-rolled corn in finishing diets containing 25% wet distillers grains, linearly reduced total-tract digestibility by approximately 3-6%, increased fecal neutral detergent fiber excretion by ~59%, and numerically increased OM fecal output by ~30%. As a result, fecal energy losses increased from approximately 21% to 27-28% of gross energy intake. These findings illustrate that substituting lower-digestibility roughage for highly digestible concentrate reduces nutrient utilization and increases fecal output and manure solids.

## **Spatial variation of nutrient components on cattle pen surfaces**

Accumulation of manure in cattle pens can introduce spatial heterogeneity in moisture and nutrient distribution across the pen surface, generally due to cattle behavior (Gilley et al., 2008). The variation in manure deposition variability has been observed for nitrogen, phosphorus, potassium, and other compounds relevant to the pen microclimate. Regions near waterers and bunks typically accumulate greater moisture and manure depth, with nutrient concentrations often two to three times higher than in drier mound areas (Miller & Woodbury, 2003). Miller & Berry (2005) found that soil nitrate-nitrogen concentrations were greater in wet pen zones (~80 mg/kg) compared to dry, mounded areas (<30 mg/kg). Elevated moisture also alters biological activity within those areas; manure exceeding 28.6% moisture was found to stimulate microbial activity and shift gas flux dynamics, in turn increasing odor potential and suppressing dust generation. Consistent with this relationship, Berry & Miller (2005) further demonstrated that *E. coli* O157:H7 persistence in feedlot pen surfaces was greater in wet, manure-rich zones compared to drier areas. The patterns reported by the discussed literature underscore the importance of spatial heterogeneity in pen surfaces for cattle health, environmental emissions, and downstream field application.

### **Role of bedding on seasonality and pen-surface moisture mitigation**

Environmental conditions, such as rain, snow, freeze-thaw cycles, heat, and dust have the potential to stress pen surfaces and cattle behavior (Grandin, 2016). The addition of bedding can mitigate cattle pen environmental extremes by enhancing surface drainage, reducing mud formation, providing thermal insulation, and maintaining a more stable substrate for pen integrity (Grandin, 2016; Miller et al., 2003). Field studies have shown that bedding materials improve water infiltration and limit standing water (Olson et al., 2006). Bedded pack zones absorbed 23 -

33% more moisture than adjacent pen floors and bedding delayed runoff onset by 3 - 7 minutes compared with un-bedded surfaces. In a two-year rainfall simulation study conducted at a research feedlot, Miller et al., (2006) evaluated how bedding and within pen location influenced runoff characteristics. Rainfall was applied at a constant rate and time at each sampling location to generate runoff analyzed for nutrient and chemical composition. Bedding pack areas consistently produced greater concentrations of nitrogen and soluble salts compared to pen-surface material locations, indicating that bedded regions serve as nutrient reservoirs within cattle pens. Spiehs et al. (2010) measured ammonia emissions at 56 different locations within deep-bedded feedlot pens and found that ammonia concentrations significantly varied ( $\sim 15$ - $100$  mmol/L) by bedded pack depth, moisture, and pH. Regions with high ( $\sim 100$  mmol/L) ammonia concentrations occurred on and around the bedded pack and on the concrete apron; regions with low and moderate ( $\sim 15$ - $57$  mmol/L) ammonia concentrations occurred in drainage areas. Jaderborg et al. (2021) further observed that in hot climates ( $\sim 30^{\circ}\text{C}$ ), bedded manure packs exhibited elevated ammonia and greenhouse gas concentrations on the pen surface compared to cool/ambient temperature ( $\sim 15^{\circ}\text{C}$ ), suggesting that temperature influences ammonia and greenhouse gas volatilization. A broad review from Lee et al. (2025) synthesized average ammonia emissions measurements from studies conducted in feedyards primarily in Texas, Colorado, and Alberta, Canada across all times of year. Reported average ammonia emissions was approximately 119 g/head/day (range of 24-318 g) and fluxes around  $58 \mu\text{g}/\text{m}^2/\text{s}$  ( $2$ - $185 \mu\text{g}/\text{m}^2/\text{s}$ ). A significant range in emissions data indicates a potential for greenhouse gas mitigation via pen surface management (seasonal, bedding, diet) in the feedyard environment. Understanding pen-surface-related emissions and the spatial and temporal factors governing

manure moisture, organic matter accumulation, and microbial activity is essential for interpreting feedlot-scale emission measurements and identifying targeted mitigation opportunities.

Wilkes et al. (2022) quantified water absorption capacity across multiple bedding materials in feedlot pens and reported sawdust and peat moss retained two to three times more moisture than woodchips, while straw held approximately half relative to woodchips. Tait et al. (2023) demonstrated that woodchip bedding in irrigated pens reduced pen substrate moisture and penetrability compared to unbedded controls, thus improved surface dryness in wet seasons. While bedding is a tool for moisture mitigation in cattle pens, its effectiveness is influenced by other management factors. For example, Mader & Colgan (2007) reported that a reducing stocking density (~23 vs 46 m<sup>2</sup>/head) decreased mud severity more effectively than straw bedding alone, with fewer pens exhibiting severe mud (>17 cm) compared with high-density, unbedded pens. Applying bedding to cattle pens can help to preserve pen integrity, improve drainage, reduce runoff, and modulate moisture levels of cattle pens. However, these effects are dynamic and influenced by weather conditions, animal traffic, and manure accumulation, resulting in changes to pen moisture.

### **Current metrics used to assess pen condition**

In practice, pen condition is evaluated via a combination of visual scoring (mud depth, wetness, pen slope, footprint impressions; Grandin, 2016; Neville et al., 2020; Olson et al., 2006), spatial heterogeneity of manure vs. base material (Pratt et al., 2016), and surface temperature measurements (Arias & Mader, 2023). Animal-oriented pen condition assessments also include hide cleanliness and panting scores (Van Donkersgoed et al., 1997; Grandin, 2016; Hagenmaier et al., 2016; Neville et al., 2020). Quantification of physical and chemical metrics of animals link to animal health and performance outcomes to determine the effect of pen condition

on the animals. Arias & Mader (2023) demonstrated that pen surface temperature is a reliable predictor of animal water intake ( $r^2 = 0.86$ ) and explained 79% of the tympanic temperature variance in finishing steers during the day. Dickson et al. (2022) quantified behavioral responses to increasing mud depth and found that severe mud conditions (defined in the study as, mud above cattle fetlock) significantly reduced lying time, lying bout frequency, and step counts within the feedlot, although preference for feedlot versus pasture access was not altered.

### **Influence of pen condition on cattle health**

Variance in pen surface conditions have been shown to influence cattle health and welfare by impacting locomotion, resting behavior, pathogen exposure, heat/cold stress, and overall stress load (Grandin, 2016). Locomotion and joint health are particularly sensitive to pen surface conditions (Dickson et al., 2022). Cortes et al. (2021) reported that in pens with more mud than bedding or excessive mud levels increased the risk of digital dermatitis (odds ratio = 8.45 and 13.9, respectively) relative to dry pens. Digital dermatitis is a contagious hoof disease caused by a bacterial infection and is characterized by painful inflammatory lesions as the heel bulbs, strongly associated with prolonged exposure to wet and manure-dense conditions (Cortes et al., 2021). Cortes et al. (2021) reported that the incidence of digital dermatitis resulted in ~0.14 kg lower average daily gain (ADG) and lower hot carcass weight compared to healthy cattle. Macitelli et al. (2020) conducted a study in which increasing stocking density resulted in greater mud depths resulting in more frequent instances of coughing, sneezing, nasal discharge, diarrhea, and locomotion alteration. Increasing pen stocking density, subsequently increasing mud depth, also resulted in higher instances of macroscopic signs of bronchitis, pulmonary emphysema, nephritis and urinary cysts. Dickson et al. (2022) found a positive association with low mud depth and an increase in lying behavior. Tait et al. (2023) demonstrated that cattle

housed in pens with added bedding to improve pen surface conditions tended to display lower adrenal gland weights relative to unbedded controls, indicating lower chronic stress loads in cattle provided bedding. In a multi-season study, Berry & Miller (2005) demonstrated that moisture levels (~20-40%) supported *E. coli* O157:H7 persistence for approximately six weeks, indicating that wet, manure-rich pen conditions can promote prolonged pathogen survival. Dry surfaces (< ~10% moisture) resulted in rapid population decline. Miller et al. (2003) showed that season exerted a strong influence on bacterial populations in feedlot pen surface manure, with *E. coli* and total coliform concentrations significantly higher during summer months compared to winter. In contrast, bedding material (straw vs. wood chips) had minimal effects on pathogen abundance, although both bedding and season altered manure chemical composition on the pen-surface relevant to nutrient management and environmental factors.

### **Influence of pen condition on cattle performance**

Accumulation of moisture and mud on the pen surface directly alters the ability of the animal to partition energy for maintenance and growth and can create downstream effects on carcass quality. Using weather and stocking density driven simulations, Mader (2011) estimated that mud depths ranging from 5 to 10 cm increased animal net energy required for maintenance (NEm) by approximately 10% under moderate winter conditions. Mud depth ranging from 9 to 21 cm increased NEm by approximately 43% under severe cold/wet conditions and reduced energy for gain. For simulated animals under the described mud depth conditions, ADG decreased by 0.1 kg/day and 0.38 kg/day respectively and increased cost of gain by as much as 56% under cold and wet conditions. Dickson et al. (2022) observed that incremental increases in mud depth reduced lying time by 15-20% resulting in higher NEm; reduced resting time compounds with locomotion strain to further increase NEm. Wilkes et al. (2022) demonstrated

an increase in NEm in muddy pen conditions measuring higher leg withdrawal force ( $44 \pm 14.8$  kg force) to move for cattle housed in muddy, unbedded pens compared with clean, bedded pens ( $15 \pm 7.1$  kg force).

Tait et al. (2023) found that adding 15 or 30 cm of woodchip bedding in irrigated pens over a 109-day period significantly increased ADG ( $0.19 - 0.22$  kg), improved gain-to-feed ratio, and increased DMI ( $\sim 0.2 - 0.5$  kg) relative to unbedded controls. A complementary study conducted in winter on finishing cattle showed that straw bedding ( $1.0 - 1.8$  kg/steer/day) improved DMI (5.8%), ADG (21%), and reduced estimated maintenance coefficient ( $\sim 10\%$ ) over unbedded controls ; however, marbling scores were lower in the bedded pens by  $\sim 8\%$  (Smerchek & Smith, 2020). Alternatively, in a study comparing cleaned and uncleaned pens with no bedding application to either cleaning treatment, Neville et al. (2020) found that cleaner/drier pens produced carcasses with  $\sim 9\%$  higher marbling scores compared to uncleaned controls, and no significant differences in ADG (Neville et al., 2020). The study also linked clean/dry pens to  $\sim 8.5\%$  cleaner hide scores compared to uncleaned pens (or infrequently cleaned) pens. In commercial abattoirs, dirtier hide scores consistently showed higher total viable microbial counts on carcass sites (brisket and hock) compared to clean hides (McEvoy et al., 2000). A similar study reported hide aerobic colony counts increased from  $\sim 3.15$  log cfu/cm<sup>2</sup> in clean hides to  $\sim 8.14$  log cfu/cm<sup>2</sup> in severely soiled hides, and that carcasses from dirtier hides have elevated microbial loads—though the slaughter process and hygiene interventions may buffer this effect (Kallem et al., 2022). Hauge et al. (2012) reported that carcasses from dirtier hide score animals prior to slaughter had higher aerobic colony counts than clean animals at the end of the slaughter line.

## **Thermal imaging technologies to detect surface moisture**

Effective pen management requires timely measurements of pen condition, and thermal infrared (TIR) sensing represents a promising tool for monitoring surface wetness patterns that are otherwise difficult to capture using conventional methods. Thermal infrared sensors can be mounted on UAVs to measure upwelling longwave radiance emitted from the surface, which is directly related to surface temperature (Aragon et al., 2020). Variation in longwave radiance produce measurable temperature differentials, and these differentials are indicative of surface wetness because wet materials dissipate heat more slowly and cool more rapidly than dry materials (Verstraeten et al., 2006; Zhang & Zhou, 2016). In bare or sparsely covered surfaces, land-surface temperature gradients and day-night temperature amplitudes, the diurnal surface temperature range, can be mapped to soil/ground moisture using thermal-inertia methods or surface temperature-vegetation index to predict soil moisture (Jia et al., 2023; Verstraeten et al., 2006). Thermal-inertia methods estimate soil moisture by relating the rate and magnitude of surface temperature change to the material's heat capacity and thermal conductivity. Surface temperature-vegetation index methods combine land-surface temperature with vegetation indices to isolate moisture-driven thermal signals from those associated with vegetation cover. Thermal infrared technology in combination with optical indices (vegetation cover, color, and brightness) has improved moisture inferences compared with either alone (Zhang & Zhou, 2016). Wan et al. (2024) found the accuracy of TIR depends on ambient conditions, flight time, flight planning, and in-scene references. Additionally, in-scene references provide TIR-sensor calibration using intentionally placed calibration targets which can reduce bias and more accurately estimate true emissivity of heterogeneous surfaces of land-surface temperature (Aragon et al., 2020). Emissivity is the efficiency with which a surface emits thermal radiation relative to the

blackbody (known standard) at the same temperature and is a key parameter governing the conversion of measured longwave radiance to accurate land-surface temperature.

There are two major methods to inference moisture using TIR-sensors; first, thermal inertia (including apparent thermal inertia), which leverages daily temperature ranges; second, temperature-index methods that relate land surface temperature (normalized with air temperature or vegetation cover) to dryness/available water (Jia et al., 2023; Zhang & Zhou, 2016). The apparent thermal inertia method has been adapted to UAV scale to estimate soil moisture in the field (Jia et al., 2023). This method improves in accuracy when the solar radiation applied to the soil is quantified (and normalized) to better understand surface energy input and reduce variation (Jia et al., 2023). In essence, TIR-sensors measure the temperature differential between objects/surfaces then moisture can be *predicted* or inferred, but to accurately *measure* moisture levels of an object/surface additional in-scene references must be used to calibrate the sensor.

Shi et al. (2024) evaluated the use of UAV-mounted TIR sensors alongside UAV-mounted multispectral cameras in soybeans to predict soil moisture content and found that TIR-sensors alone ( $r^2 = 0.38 - 0.60$ ) directly reflected the crop canopy temperature. This temperature was closely related to the water status of the crop and therefore the soil moisture content. Accuracy is improved ( $r^2 = 0.59 - 0.78$ ) when complementary multispectral camera data is added to the model because it includes data regarding plant growth and structure of the crop canopy. Lacerda et al. (2022) found that TIR-sensors alone mounted on UAVs had reliably tracked cotton crop water status in 2020 ( $r^2 = 0.44 - 0.65$ ) to support irrigation decisions. Integrating thermal imagery with complementary sensing modalities, such as red-green-blue (RGB), TIR, and ground penetrating radar substantially improved moisture estimation accuracy relative to thermal data alone (Vahidi et al., 2025). Vahidi et al., (2025) demonstrated a reduction in prediction error

by approximately 20% and increasing explanatory power ( $\Delta r^2 \sim 0.09 - 0.012$ ) across surface and subsurface depths (10 cm). Reviews of UAV crop water-stress sensing concluded that TIR paired with other indices or artificial intelligence models improves detection sensitivity and generalization across locations (Cho et al., 2024; Dong et al., 2024; Sharma et al., 2025). Although most UAV-mounted TIR research has focused on crops and soils, the same physical principles apply to cattle pen surfaces, where wetter areas show lower daytime land-surface temperatures and reduced diurnal temperature amplitudes (Jia et al., 2023; Zhang & Zhou, 2016). Unmanned aerial vehicle-mounted TIR sensors have the capability to differentiate between wet and dry zones, but a higher level of accuracy is established when combined with other sensors to measure and closely monitor moisture status (Jia et al., 2023). Absolute surface temperature and surface temperature-moisture mapping are sensitive to emissive variability, atmospheric factors, and sensor angle effects which must be corrected to improve accuracy (Elfarkh et al., 2023; Wan et al., 2024). Emissivity values are assumed to be 0.99-0.96 for broad agricultural and environmental settings (Torres-Rua et al., 2019). Differences among soil, manure, and bedding introduce additional variation in emissivity in which studies show that explicitly measuring emissivity in a refined area improves remote sensing predictions. Accuracy of TIR surface temperature measurements paired with ground-measured moisture levels can be used for estimation or prediction of plant health, soil moisture, crop nutrient requirements, and vegetative stage (Olson & Anderson, 2021).

### **Applications of UAV thermal infrared technologies in cattle production**

Unmanned aerial vehicles have been employed for a range of objectives in the cattle industry including bunk reading, animal counting, and production monitoring (Bastos et al., 2024). For counting/identification purposes, RGB imagery has been utilized as it captures

standard red, green, and blue color channels to produce true-color images that closely illustrate what the human eye perceives (Barbedo et al., 2020; Bastos et al., 2024). Combining RGB with TIR sensing extended the counting capabilities across a wider range of environmental conditions, such as low light, dust, or complex backgrounds, where thermal contrast enables animal detection when RGB imagery alone is limited, offering a more efficient alternative to manual counting (Barbedo et al., 2020; Shao et al., 2020; Whitehead & Hugenholtz, 2014). Mufford et al. (2019) quantified cow-calf spacing in a pasture using UAV-RGB video footage and found the measurements provided an excellent indication of inter-individual distancing behavior, with clear value for detecting calving events in pasture/range systems. Dadallage et al. (2024) evaluated UAV-based thermal and RGB video for cattle detection, tracking, and body surface temperature estimation in commercial feedlots and found mean absolute differences between UAV-derived and ground-measured body surface temperature of 2.1°C in spring and 3.3°C in summer, which were considered acceptable for real-time heat stress monitoring. When comparing imaging modalities for animal identification and centroid tracking, RGB imagery outperformed TIR (precision = 0.97 vs 0.56), reflecting higher misclassification rates in thermal-only sensing and highlighting the potential value for multi-modal sensor approaches. Unruh et al. (2017) evaluated UAV-mounted TIR to predict animals that would show heat stress later in the day and concluded that, although TIR sensing can objectively detect heat stress when interpreted with ambient conditions (temperature and relative humidity), morning TIR readings alone did not reliably predict which cattle would develop heat stress later in the day.

### **Transition to the current study**

The reviewed literature demonstrates that pen-surface moisture is a central driver of feedlot microclimate, influencing nutrient distribution, microbial persistence, animal behavior,

performance efficiency, and environmental emissions. While nutrition, stocking density, and bedding practices have been shown to modulate moisture dynamics, pen surfaces remain highly heterogeneous across space and time, making accurate characterization and pen management decisions challenging using conventional visual scoring. Thermal infrared sensing has been widely validated in agriculture systems as a proxy for surface moisture through temperature-based indicators of evaporative cooling and thermal inertia, and emerging livestock studies suggest similar physical relationships apply to feedlot pen surfaces. However, direct application of UAV-mounted thermal imaging for quantifying pen-surface moisture in feedlot environments remains limited, particularly under conditions of dynamic weather. These gaps highlight the need to evaluate UAV-based thermal approaches integrated with ground-truth measurements, as a tool for detecting and monitoring pen-surface moisture patterns, forming the basis for the experimental work described in Chapter 2.

## **Part II – Essential oil supplementation and its effects on beef cattle performance and enteric emissions**

### **Introduction**

Enteric methane (CH<sub>4</sub>) production from ruminant livestock represents both a loss of dietary gross energy and a major contributor to agricultural greenhouse gas emissions (Beauchemin et al., 2020; Johnson & Johnson, 1995). Globally, livestock supply chains account for approximately 14.5% of anthropogenic greenhouse gas emissions, with enteric fermentation representing the largest biological source (Gerber et al., 2013). In the United States, beef and dairy cattle contribute substantially to agricultural CH<sub>4</sub> emissions, and mitigation strategies that reduce CH<sub>4</sub> without compromising animal performance are therefore significant environmental and economic interest (Dillon et al., 2021; Hristov et al., 2014).

A wide range of CH<sub>4</sub> mitigation strategies have been evaluated, including dietary fat supplementation, nitrates, halogenated compounds, ionophores, seaweed-derived bromoforms, and plant secondary compounds (Beauchemin et al., 2020). Many of these approaches effectively suppress CH<sub>4</sub> but are limited by toxicity risk, regulatory constraints, negative impacts on intake or production, or poor long-term efficacy due to microbial adaptation (Beauchemin et al., 2020; Dillon et al., 2021). Essential oils, derived from aromatic plant secondary metabolites, have emerged as a promising class of feed additives due to their antimicrobial properties, potential to modulate rumen fermentation, and their general acceptance as natural or consumer-friendly inputs (Calsamiglia et al., 2007; Cobellis et al., 2016). Essential oil blends have been studied for their ability to modify rumen microbial ecology, alter volatile fatty acid production patterns, reduce proteolysis and methanogenesis, and improve feed efficiency (Belanche et al., 2020; Benchaar et al., 2012; Castro-Montoya et al., 2015). However, responses are variable and depend

on the chemical composition of the essential oil blend, inclusion rate, diet composition, animal physiological state, and adaption period (Beauchemin et al., 2020; Cobellis et al., 2016). This section will summarize the biochemical basis for essential oil function in the rumen, their documented effects on fermentation, performance, and CH<sub>4</sub> emissions. It also focuses specifically on the Agolin (Alltech Inc., Nicholasville, KY) essential oil blend, which has been evaluated across multiple production systems and forms the basis of Chapter 3.

### **Essential oils as modifiers of rumen microbial fermentation**

Essential oils are complex mixtures of volatile, lipophilic secondary plant metabolites primarily composed of terpenes and terpenoids (e.g., thymol, carvacrol, limonene), phenylpropanoids (e.g., eugenol, cinnamaldehyde), and related aromatic compounds (Calsamiglia et al., 2007; Cobellis et al., 2016). Variation in polarity, molecular size, and functional groups among essential oil constituents results in heterogeneous antimicrobial potency and microbial selectivity. The biological activity of essential oils is largely attributed to their hydrophobicity, which allows them to partition into microbial cell membranes and disrupt lipid bilayer organization (Benchaar & Greathead, 2011). This activity disrupts membrane integrity, increases permeability to protons and ions, dissipates the proton motive force, and interferes with membrane-bound enzyme systems responsible for nutrient transport and ATP synthesis (Benchaar & Greathead, 2011; Calsamiglia et al., 2007; Cobellis et al., 2016). Phenolic compounds such as thymol, carvacrol, and eugenol exhibit strong antimicrobial activity because their hydroxyl group allows them to act as protonophores, collapsing transmembrane electrochemical gradients and impairing cellular energy conservation (Benchaar et al., 2007a; Cobellis et al., 2016). Aldehydes such as cinnamaldehyde inhibit key enzymatic reactions involved in microbial growth and amino acid metabolism, while monoterpenes such as limonene

primarily alter membrane fluidity and stress tolerance rather than directly inhibiting fermentation pathways (Calsamiglia et al., 2007; Cobellis et al., 2016).

Gram-positive bacterial populations are generally more susceptible to essential oil compounds compared to gram negative due to the lack of an outer membrane which restricts diffusion of hydrophobic compounds in gram-negative bacteria (Benchaaar et al., 2007a; Cobellis et al., 2016). This differential sensitivity alters competitive interactions among microbial functional groups, shifting the structure and activity of the rumen microbiome rather than simply suppressing fermentation globally. Many gram-positive bacteria are associated with acetate formation and a larger production of hydrogen (Cobellis et al., 2016; Patra & Saxena, 2009). In contrast, several gram-negative propionate-producing bacteria are less sensitive to essential oil compounds, allowing their relative abundance and activity to increase under essential oil supplementation. As a result, essential oil supplementation frequently decreases the acetate-to-propionate ratio and shifts carbon flow toward propionate formation (Benchaaar et al., 2012; Patra & Saxena, 2009). This shift has important energetic and environmental implication because acetate and butyrate formation release metabolic hydrogen, whereas propionate formation consumes hydrogen and functions as a hydrogen sink (Beauchemin et al., 2020; Johnson & Johnson, 1995). Thus, increasing propionate relative to acetate reduces hydrogen availability for methanogenic archaea and lowers CH<sub>4</sub> production potential. Essential oils have in some studies been found to suppress rumen protozoa, which affects methanogenesis through interspecies hydrogen transfer and physical association with methanogens (Patra & Saxena, 2009). Suppression of protozoal populations therefore indirectly limits methanogenic activity even when archaeal abundance is not directly reduced (Jouany & Ushida, 1999).

Several essential oil compounds inhibit proteolytic and hyper-ammonia-producing bacteria, reducing deamination of amino acids and subsequent ruminal ammonia concentrations (Patra & Saxena, 2009; Santos et al., 2010). This can improve the efficiency of microbial protein synthesis by conserving nitrogen within microbial biomass rather than allowing it to accumulate as ammonia can be absorbed and excreted as urea. However, improvements in nitrogen-use efficiency are most likely when fermentable energy supports microbial growth and ruminal ammonia remains above microbial requirements; under nitrogen-limited conditions, further reductions in ammonia can constrain microbial protein synthesis and fiber digestion (Calsamiglia et al., 2007; Cobellis et al., 2016). In several studies, essential oil supplementation reduced ruminal ammonia concentrations and modified digestibility, consistent with suppression of amino acid deamination (Benchaar et al., 2007b; Santos et al., 2010). However, these responses are diet- and dose-dependent, and excessive inhibition of microbial activity can reduce fiber digestion and microbial growth, offsetting potential benefits to nitrogen utilization.

### **Rationale for combining essential oils into blends**

Individual essential oil compounds typically exhibit narrow antimicrobial spectra and steep dose-response relationships, such that effective doses often approach levels that impair intake, fiber digestion, or microbial protein synthesis (Benchaar & Greathead, 2011; Calsamiglia et al., 2007). Combining multiple essential oil compounds into blends allows complementary mechanisms of action across microbial functional groups while reducing the required dose of any single compound (Cardozo et al., 2006). Phenolic compounds primarily suppress gram-positive hydrogen-producing bacteria and protozoa, aldehydes inhibit deaminating and peptidolytic bacteria, and monoterpenes alter microbial stress responses and membrane stability (Cobellis et al., 2016; Patra & Saxena, 2009). This multi-target approach increases robustness of microbial

modulation and reduces the likelihood of rapid microbial adaptation, as resistance would require coordinated physiological changes across multiple pathways rather than adjustment to a single inhibitory mechanism (Castro-Montoya et al., 2015; Cobellis et al., 2016).

Castro-Montoya et al. (2015) concluded that essential oil blends substantially reduce CH<sub>4</sub> production *in vitro*, whereas *in vivo* effects are smaller, more variable, and often transient. Rumen microbial communities adapt through shifts in species composition, membrane lipid profiles, and detoxification capacity, thereby reducing sensitivity to essential oil exposure over time (Cobellis et al., 2016; Patra & Saxena, 2009). Low doses may be insufficient to meaningfully alter fermentation, whereas high doses may suppress fibrolytic bacteria, reduce intake, or impair digestibility (Benchaar et al., 2007a; Calsamiglia et al., 2007). This narrow functional window underscores the importance of formulation strategy, delivery method, and adaptation period in achieving consistent outcomes.

### **Diet context for essential oil supplementation**

The biological effects of essential oils in the rumen are strongly dependent on the composition of the basal diet, ruminal pH, and passage rate (Benchaar & Greathead, 2011). Furthermore, dominant microbial functional groups differ substantially between concentrate-based and forage-based systems (Benchaar & Greathead, 2011; Calsamiglia et al., 2007; Cobellis et al., 2016). Since essential oils act as selective antimicrobials rather than broad-spectrum inhibitors, resulting net effects emerge from interactions with microbial populations supported by the underlying diet (Cobellis et al., 2016; Patra & Saxena, 2009). High-concentrate feedlot diets are characterized by greater starch availability, faster fermentation rates, lower ruminal pH, and a microbial ecosystem favoring amylolytic and propionate-producing bacteria. In contrast, forage-based and grazing diets support higher proportions of fibrolytic bacteria, higher acetate

production, greater ruminal fill, and often slower passage rates (Beauchemin & McGinn, 2006).

These fundamental differences alter both baseline CH<sub>4</sub> production and the scope for essential oil-induced modulation.

### **Essential oils in feedlot and high concentrate diets**

In feedlot systems, essential oil supplementation has most commonly been evaluated under controlled intake conditions, where additive delivery is precise and diet composition is consistent (Benchaar & Greathead, 2011; Cobellis et al., 2016). Under these conditions, essential oils have been shown to modify volatile fatty acid profiles, reduce ruminal ammonia concentrations, and in some cases reduce CH<sub>4</sub> yield (g CH<sub>4</sub>/kg DMI), although responses are variable and dose-dependent (Benchaar et al., 2007b; Castro-Montoya et al., 2015; Patra & Saxena, 2009). Because high-concentrate diets already favor propionate formation, essential oil-induced shifts in hydrogen partitioning may be smaller than in forage systems, and excessive antimicrobial activity can reduce total volatile fatty acid production or impair intake (Calsamiglia et al., 2007; Cobellis et al., 2016). Concurrent use of ionophores or other feed additives in feedlot settings can confound essential oil effects and complicate interpretation (Benchaar & Greathead, 2011). Thus, in feedlot systems, essential oils tend to function as fine-scale modulators of fermentation and nitrogen metabolism rather than as major drivers of CH<sub>4</sub> mitigation, and their efficacy is highly sensitive.

Meyer et al. (2009) evaluated 468 yearling steers fed a typical feedlot diet along with two essential oil blends, monensin, tylosin, and combinations of these additives. They reported that gain:feed tended to improve in the essential oil blend without monensin/tylosin treatment by 4.1% relative to control primarily due to numerically greater ADG and no difference in DMI relative to control. In the same experiment, DMI decreased with the monensin + tylosin versus

control (~0.7 kg/day), whereas the essential oil treatments did not consistently reduce DMI. Benchaar et al. (2006) evaluated dose response to a commercially available essential oil blend (thymol, eugenol, vanillin, limonene) and reported that the blend did not reduce DMI with an increasing dose in a performance trial. Monensin was shown to reduce DMI by approximately 10% compared to control. Average daily gain was similar among treatments, and feed efficiency was not different between control and essential oil or monensin treatments.

In an *in vitro* study using rumen fluid from heifers adapted to a finishing-style substrate (10:90, forage:concentrate), Cardozo et al. (2005) demonstrated that the same plant extracts could behave differently at pH 7.0 versus 5.5, with significant pH x dose interactions across fermentation endpoints. Several extracts (e.g., capsicum, cinnamaldehyde) reduced the acetate:propionate ratio more strongly under the lower pH condition that better mimics high-grain fermentation. Translating these findings in an *in vivo* study, Cardozo et al. (2006) evaluated ruminally cannulated Holstein heifers fed a 90:10 concentrate:straw diet and observed compound-, dose-, and pH-specific responses. Capsicum oil increased DMI and water intake, while a cinnamaldehyde + eugenol mixture reduced acetate proportion relative to propionate and reduced ammonia-nitrogen and lactate, consistent with inhibition of deamination and altered hydrogen use. Anise oil reduced the acetate:propionate ratio, lowered ammonia-nitrogen and protozoal counts, and reduced total VFA concentrations at a high (300 mg/L) dose. These findings align with the broader conclusion that, in concentrate-fed cattle, essential oils may be most relevant as targeted modifiers of nitrogen metabolism (e.g., lower ruminal ammonia via reduced deamination) and volatile fatty acid partitioning, rather than as universally strong CH<sub>4</sub> suppressants. Diet composition alone can influence CH<sub>4</sub> emissions independent of additives. For example, (Beauchemin & McGinn, 2006) compared CH<sub>4</sub> emissions in cattle fed diets differing in

barley silage:barley concentrate ratio and reported no difference in CH<sub>4</sub> emissions, illustrating that baseline diet effects can be variable and may complicate detection of additive responses.

### **Essential oils in forage-based and grazing systems**

In forage-based rations and grazing systems, essential oils are often expected to exert relatively larger shifts in hydrogen partitioning than in feedlot diets because forage fermentation typically favors higher acetate production and greater hydrogen availability for methanogenesis (Beauchemin et al., 2020, 2022; Johnson & Johnson, 1995). However, forage systems introduce two major constraints that shape outcomes: (1) the risk of impairing fiber digestion when essential oil antimicrobial activity is excessive, and (2) greater difficulty achieving a consistent effective dose under grazing conditions (Bowman & Sowell, 1997; Calsamiglia et al., 2007; Cobellis et al., 2016; Hegarty et al., 2021; Kunkle et al., 2000). Controlled forage-based studies demonstrate that essential oil blends can modify rumen fermentation, but CH<sub>4</sub> mitigation is not consistently observed at practical inclusion rates. For example, Tomkins et al. (2015) evaluated an essential oil blend (CRINA Ruminants; dsm-firmenich; Kaiseraugst, Switzerland) and monensin dosed directly into the rumen of steers fed Rhodes grass hay (*Chloris gayana*) ad libitum and reported no effect on CH<sub>4</sub> output, whether expressed as g/d, g/kg DMI, or as a proportion of gross energy intake. Control steers averaged 76.9 g CH<sub>4</sub>/d (14.6 g/kg DMI), whereas essential oil-supplemented cattle at 1 or 2 g/d resulted in 79.8 and 74.5 g CH<sub>4</sub>/d (12.8 and 13.5 g/kg DMI), respectively, with no statistical differences among treatments. Despite the lack of CH<sub>4</sub> reduction, essential oils altered fermentation patterns, increasing butyrate and isovalerate proportions relative to control, and both the essential oil blend and monensin reduced fungal colonization of fiber by an average of 82% compared to control, illustrating that essential

oils can influence fiber-associated microbiota even when whole-animal CH<sub>4</sub> responses are neutral (Tomkins et al., 2015).

Additional forage-based *in vivo* work supports the concept of a narrow effective dose window in which performance can be maintained or modestly improved without depressing digestion. Benchaar et al. (2006) used a growing program based on a 75:25 (grass/legume silage:rolled barley) to compare monensin with increasing doses of an essential oil blend (thymol, eugenol, vanillin, limonene; Vertan®; IDENA; Sautron, France). Essential oil supplementation did not reduce DMI, whereas monensin decreased intake by approximately 10%. Average daily gain was similar among treatments, but feed efficiency responded quadratically, with the greatest efficiency at 2 g essential oil/day rather than at higher inclusion rates. These findings align with mechanistic studies indicating that high essential oil doses can suppress cellulolytic activity and total volatile fatty acid production, whereas moderate doses are more likely to yield targeted shifts in volatile fatty acid partitioning and nitrogen metabolism (Calsamiglia et al., 2007; Cobellis et al., 2016). A similar study evaluated a garlic-cinnamon essential oil extract with or without monensin in grazing beef cattle and reported no differences in body weight gain among treatments (Beck et al., 2017); suggesting that at practical supplementation rates performance effects may be minimal under pasture (5-10% tall fescue, *Festuca arundinacea* Schreb.) conditions. These findings indicate that successful application in grazing systems depends on achieving a reliable effective dose and selecting essential oil compounds that retain ruminal activity within forage driven microbial adaptation dynamics (Beauchemin et al., 2022; Calsamiglia et al., 2007; Cobellis et al., 2016). Additionally, forage-based cattle studies indicate that essential oils can alter ruminal fermentation patterns, including shifts in volatile fatty acid profiles, without consistently reducing CH<sub>4</sub> emissions *in vivo*. For

example, Tomkins et al. (2014) reported changes in specific VFA concentrations with limited effects on CH<sub>4</sub> production, suggesting that responses may depend on dose and ruminal persistence of active compounds. As summarized by Cobellis et al. (2016), variability in CH<sub>4</sub> mitigation outcomes is often attributed to factors such as insufficient effective concentrations and microbial adaptation to essential oil compounds over time.

Because true pasture supplementation trials are few and variable, supporting evidence primarily comes from *in vitro* systems using inoculum from grazing cattle, which better reflect forage adapted ruminal microbiomes. Benetel et al. (2022) used rumen fluid from Nellore cattle grazing tropical pasture to screen ten essential oils at 0, 50, 250, and 500 mg/L. Oregano and white thyme were identified as the most promising oils, reducing net CH<sub>4</sub> production at 250 mg/L inclusion without affecting substrate degradation. At 500 mg/L, CH<sub>4</sub> suppression was substantial where oregano and white thyme produced 0.57 and 0.17 mL CH<sub>4</sub>, respectively, compared with 6.92 mL CH<sub>4</sub> in the control. However, this was accompanied by marked reductions in total gas production, indicating strong antimicrobial action that could be counterproductive *in vivo* if translated directly to cattle dosing (Benetel et al., 2022).

As mentioned previously, the mechanism through which essential oils may influence fermentation in forage-based systems involves their effects on rumen protozoal populations (Beauchemin et al., 2020; Johnson & Johnson, 1995). Rumen protozoa contribute to hydrogen turnover because they are associated with methanogens and release hydrogen during carbohydrate fermentation, thereby supporting methanogenesis. Partial suppression of protozoa can theoretically reduce CH<sub>4</sub> production by decreasing hydrogen as a substrate for methanogens (Jouany & Ushida, 1999). Several essential oil components, particularly phenolic compounds such as thymol and carvacrol, have demonstrated antiprotozoal activity *in vitro*, reducing

protozoal counts or activity without completely eliminating the population (defaunation; Calsamiglia et al., 2007; Cobellis et al., 2016). However, responses in forage-based diets appear highly dose-dependent. Moderate essential oil inclusion may shift protozoal activity enough to alter hydrogen partitioning while maintaining fiber digestion, whereas higher doses risk disrupting the symbiotic balance among protozoa, fungi, and cellulolytic bacteria, potential depressing fiber digestion. Protozoal suppression does not always translate into measurable CH<sub>4</sub> reduction at the animal level; in forage-fed cattle, essential oils may reduce protozoal number or modify their activity while CH<sub>4</sub> emissions remain unchanged due to compensatory shifts in bacterial fermentation pathways or microbial adaptation (Beauchemin et al., 2022; Tomkins et al., 2015). Furthermore, protozoa affect starch sequestration, nutrient recycling, and maintenance of rumen microbial stability; therefore, complete defaunation could have negative effects on nutrient utilization (Eugène et al., 2004; Jouany & Ushida, 1999).

### **Role of nitrogen in essential oil effects across feeding systems**

Across both, high-concentrate and forage-based feeding systems, essential oils consistently reduce ruminal ammonia concentrations by suppressing proteolytic and hyper-ammonia-producing bacteria (Patra & Saxena, 2009; Santos et al., 2010). Whether this effect is beneficial or counterproductive depends on the nitrogen status of the diet. In high-protein, highly degradable diets (e.g., lush spring pasture or high-protein total mixed rations), reduced deamination can improve nitrogen use efficiency and increase the flow of amino acids and microbial protein to the small intestine (Benchaar & Greathead, 2011; Santos et al., 2010). In contrast, under low-protein or mature forage conditions, further suppression of ruminal ammonia can limit microbial growth and fiber digestion, potentially offsetting CH<sub>4</sub> mitigation and reducing performance (Poppi & McLennan, 1995; Sampaio et al., 2009).

## **Previous work associated with Agolin essential oil blend**

### **Development and formulation of Agolin**

Agolin (Alltech Inc., Nicholasville, KY) is a commercially formulated essential oil blend composed primarily of coriander seed oil, eugenol, geranyl acetate, and geraniol, stabilized with organic acid preservatives (Belanche et al., 2020). The product was designed to modulate rumen microbial activity through complementary mechanisms rather than relying on a single bioactive compound, thereby reducing the risk of intake depression or broad inhibition of fiber digestion. Unlike many experimental essential oil products evaluated at pharmacological concentrations, Agolin is marketed at a practical inclusion rate of approximately 1 g/head/day, and this dose has served as the reference point for most controlled trials.

Batley et al. (2024c) evaluated increasing concentrations of Agolin in an *in vitro* rumen fermentation system to assess effects on CH<sub>4</sub> production and fermentation characteristics. Treatments were designed to simulate ruminal exposure equivalent to 622, 933, and 1,244 mg delivered to a 300-kg steer consuming 14 L of water per day. Methane production decreased in a dose-dependent manner, with minimal response observed at lower concentrations, consistent with earlier work (Batley et al., 2024a; Batley et al., 2024b; Belanche et al., 2020). This stepwise dose links observed *in vivo* responses to ruminal exposure levels shown to be effective in controlled fermentations and provides a framework for interpreting grazing applications where intake of the additive is less precise.

### **Impacts of Agolin on dairy cattle performance**

Elcoso et al. (2019) reported increases in energy-corrected milk and improved feed efficiency when cows received Agolin in addition to a conventional total mixed ration. Similarly, Williams et al. (2021) observed higher milk fat and protein yields and a tendency for greater

energy-corrected milk and DMI in high-producing cows supplemented with the blend. These studies demonstrated that Agolin can be fed without depressing intake, a limitation often observed with higher doses of individual essential oils. More recently, Agolin supplementation was shown to modify the rumen microbiome of dairy cows, shifting fermentation toward greater propionate formation and reduced methanogen abundance (Bach et al., 2023). These microbial changes were associated with lower CH<sub>4</sub> production while maintaining milk yield, supporting the concept that Agolin functions as a selective modulator rather than a nonspecific inhibitor of rumen activity. The magnitude of responses depended on the duration of supplementation, with studies suggesting that four weeks of adaptation may be required before stable CH<sub>4</sub> reduction is detected (Batley et al., 2024b; Belanche et al., 2020).

### **Impacts of Agolin in beef and beef x dairy systems**

Availability of evidence in beef cattle is more limited but aligns with dairy findings. Miller et al. (2023) evaluated Agolin in beef x dairy steers fed a concentrate-based diet, with the additive applied as a liquid formulation sprayed onto dark grains included in the diet, and measured enteric emissions using respiration chambers. The average estimated Agolin intake was  $1.1 \pm 0.1$  mL/head/day, comparable to the recommended daily dose. Agolin reduced CH<sub>4</sub> yield at both 46 and 116 days after introduction without affecting ADG or DMI, indicating that mitigation was achieved without compromising performance. The authors noted that differences in chamber intake patterns complicated interpretation of CH<sub>4</sub> yield, but the consistent reduction across time supported a biological effect of the additive.

A series of experiments evaluated the feasibility of delivering the essential oil blend Agolin through drinking water as a CH<sub>4</sub> mitigation strategy, an approach relevant to grazing systems where feed-based inclusion is less practical. In an initial *in vitro* experiment, aqueous

solutions containing Agolin were combined with a non-protein nitrogen (NPN) and mineral supplement and incubated with a medium-quality Rhodes grass (*Chloris gayana*) hay using rumen fluid to simulate fermentation conditions typical of grazing systems (Batley et al., 2024a). Methane production, total gas production, volatile fatty acids, and *in vitro* digestibility were measured to assess both efficacy and potential impacts on fermentation. The *in vitro* treatments included Agolin concentrations corresponding to approximately 622, 933, and 1,244 mg/head/day for a 300-kg steer consuming 14 L of water daily (Batley et al., 2024b). Results from these screening experiments demonstrated that CH<sub>4</sub> reduction was dependent on achieving sufficient aqueous concentration, with minimal responses observed at lower inclusion rates (Batley et al., 2024a; Batley et al., 2024b). This work informed a subsequent 56-day *in vivo* study in which 24 beef steers were fed a basal diet of medium-quality Rhodes grass hay and assigned to drinking water treatments consisting of the NPN supplement alone or the NPN supplement combined with Agolin at two targeted inclusion levels of either 100 or 400 mg/head/day (Batley et al., 2024c). Enteric CH<sub>4</sub> emissions, feed and water intake, and live-weight gain were measured throughout the trial (Batley et al., 2024c). Steers receiving the lower inclusion rate of Agolin exhibited a 16.4% reduction in daily CH<sub>4</sub> emissions over the experimental period, whereas higher inclusion rates were less effective, suggesting that efficacy may be influenced by variability in water intake and potential esophageal groove closure, which could reduce ruminal exposure of the additive (Batley et al., 2024c).

### **Synthesis of Agolin responses**

Across published trials, Agolin supplementation has resulted in 8-15% reductions in CH<sub>4</sub> emissions with neutral or positive effects on performance (Bach et al., 2023; Belanche et al., 2020; Miller et al., 2023). The 2020 meta-analysis concluded that, at the recommended 1 g/day

dose, Agolin improved feed efficiency and milk production while lowering CH<sub>4</sub> without consistent negative impacts on intake (Belanche et al., 2020). However, responses are influenced by: (1) delivery route, feed-based inclusion generally provides more consistent exposure than water or voluntary pellet systems (Batley et al., 2024c; Fouts et al., 2025); (2) adaptation period, reductions are often not evident until several weeks of continuous supplementation (Batley et al., 2024b); (3) basal diet, forage-based diets provide greater scope for hydrogen redirection, whereas high-concentrate systems may show smaller changes in CH<sub>4</sub> intensity (Miller et al., 2023); (4) achieved dose, sub-target intakes can markedly reduce efficacy (Fouts et al., 2025). Agolin represents one of the few essential oil products with multi-study *in vivo* evidence across dairy and beef systems. Nevertheless, variability in achieved dose and diet context remains a central limitation, particularly in grazing cattle, where additive delivery cannot be precisely controlled.

### **Transition to the current study**

Most Agolin research has been conducted in total mixed ration-fed dairy cows or confined beef cattle, with limited evaluation under true pasture conditions (Bach et al., 2023; Elcoso et al., 2019; Miller et al., 2023; Williams et al., 2021). Water-delivery studies demonstrate potential but were performed with medium-quality hay rather than actively growing forage (Batley et al., 2024a; Batley et al., 2024b; Batley et al., 2024c). Consequently, little information exists on how Agolin functions when cattle consume low-yield or nitrogen-variable pastures, conditions where ammonia supply, passage rate, and forage maturity differ substantially from feedlot or dairy systems. Chapter 3 extends Agolin research to grazing beef cattle and evaluates whether CH<sub>4</sub> responses observed in controlled settings translate to pasture environments with fluctuating nutrient supply.

## Literature Cited

- Abdulai, G., Sama, M., & Jackson, J. (2021). A preliminary study of the physiological and behavioral response of beef cattle to unmanned aerial vehicles. *Applied Animal Behaviour Science*, 241. <https://doi.org/10.1016/j.applanim.2021.105355>
- Aragon, B., Johansen, K., Parkes, S., Malbeteau, Y., Al-Mashharawi, S., Al-Amoudi, T., Andrade, C. F., Turner, D., Lucieer, A., & McCabe, M. F. (2020). A calibration procedure for field and UAV-based uncooled thermal infrared instruments. *Sensors*, 20(11). <https://doi.org/10.3390/s20113316>
- Arias, R. A., & Mader, T. L. (2023). Pen surface temperature as a predictor of daily water intake and tympanic temperature in steers finished in feedlots. *Animals*, 13(7). <https://doi.org/10.3390/ani13071150>
- Bach, A., Elcoso, G., Escartín, M., Spengler, K., & Jouve, A. (2023). Modulation of milking performance, methane emissions, and rumen microbiome on dairy cows by dietary supplementation of a blend of essential oils. *Animal*, 17(6). <https://doi.org/10.1016/j.animal.2023.100825>
- Barbedo, J. G. A., Koenigkan, L. V., Santos, P. M., & Ribeiro, A. R. B. (2020). Counting cattle in UAV images—Dealing with clustered animals and animal/background contrast changes. *Sensors*, 20(7). <https://doi.org/10.3390/s20072126>
- Bastos, K. M., Barcelos, J. P., Orioli, G. F., & Nascimento, S. T. (2024). Use of drones for trough reading, animal counting, and production monitoring in feedlot systems. *AgriEngineering*, 6(4). <https://doi.org/10.3390/agriengineering6040253>
- Batley, R. J., Chaves, A. V., Johnson, J. B., Naiker, M., Quigley, S. P., Trotter, M. G., & Costa, D. F. A. (2024a). Rapid screening of methane-reducing compounds for deployment in

- livestock drinking water using in vitro and FTIR-ATR analyses. *Methane*, 3(4).  
<https://doi.org/10.3390/methane3040030>
- Batley, R. J., Romanzini, E. P., Da Silva, K. D., De Souza, W. L., Quigley, S. P., Harper, K. J., Trotter, M. G., Bernardes, P. A., Naiker, M., & Costa, D. F. A. (2024c). The essential oil blend Agolin Ruminant reduces methane production in vitro and in vivo when included in the drinking water of cattle. *Journal of Animal Science*, 102.  
<https://doi.org/10.1093/jas/skae315>
- Batley, R. J., Romanzini, E. P., Johnson, J. B., De Souza, W. L., Naiker, M., Trotter, M. G., Quigley, S. P., De Souza Congio, G. F., & Costa, D. F. A. (2024b). Rapid screening of methane-reducing compounds for deployment via water with a commercial livestock supplement using in vitro and FTIR-ATR analyses. *Methane*, 3(3).  
<https://doi.org/10.3390/methane3030025>
- Beauchemin, K. A., & McGinn, S. M. (2006). Enteric methane emissions from growing beef cattle as affected by diet and level of intake. *Canadian Journal of Animal Science*, 86(3), 401–408. <https://doi.org/10.4141/A06-021>
- Beauchemin, K. A., Ungerfeld, E. M., Abdalla, A. L., Alvarez, C., Arndt, C., Becquet, P., Benchaar, C., Berndt, A., Mauricio, R. M., McAllister, T. A., Oyhantçabal, W., Salami, S. A., Shalloo, L., Sun, Y., Tricarico, J., Uwizeye, A., De Camillis, C., Bernoux, M., Robinson, T., & Kebreab, E. (2022). Invited review: Current enteric methane mitigation options. *Journal of Dairy Science*, 105(12). <https://doi.org/10.3168/jds.2022-22091>
- Beauchemin, K. A., Ungerfeld, E. M., Eckard, R. J., & Wang, M. (2020). Review: Fifty years of research on rumen methanogenesis: Lessons learned and future challenges for mitigation. *Animal*, 14. <https://doi.org/10.1017/S1751731119003100>

- Beck, P. A., Gadberry, M. S., Stewart, C. B., Gray, H. C., Wistuba, T. J., Cravey, M. D., & Gunter, S. A. (2017). Effects of a blended garlic and cinnamon essential oil extract with and without monensin sodium on the performance of grazing steers. *American Registry of Professional Animal Scientists*, 33(2). <https://doi.org/10.15232/pas.2016-01572>
- Belanche, A., Newbold, C., Morgavi, D., Zweifel, B., & Yáñez-Ruiz, D. (2020). A meta-analysis describing the effects of the essential oil blend Agolin Ruminant on performance, rumen fermentation and methane emissions in dairy cows. *Animals*, 10(4). <https://doi.org/10.3390/ani10040620>
- Benchaar, C., Chaves, A. V., Fraser, G. R., Beauchemin, K. A., & McAllister, T. A. (2007a). Effects of essential oils and their components on in vitro rumen microbial fermentation. *Canadian Journal of Animal Science*, 87(3), 413–419. <https://doi.org/10.4141/CJAS07012>
- Benchaar, C., Duynisveld, J. L., & Charmley, E. (2006). Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle. *Canadian Journal of Animal Science*, 86(1), 91–96. <https://doi.org/10.4141/A05-027>
- Benchaar, C., & Greathead, H. (2011). Essential oils and opportunities to mitigate enteric methane emissions from ruminants. *Animal Feed Science and Technology*, 166–167, 338–355. <https://doi.org/10.1016/j.anifeedsci.2011.04.024>
- Benchaar, C., Lettat, A., Hassanat, F., Yang, W. Z., Forster, R. J., Petit, H. V., & Chouinard, P. Y. (2012). Eugenol for dairy cows fed low or high concentrate diets: Effects on digestion, ruminal fermentation characteristics, rumen microbial populations and milk fatty acid

- profile. *Animal Feed Science and Technology*, 178.  
<https://doi.org/10.1016/j.anifeedsci.2012.10.005>
- Benchaar, C., Petit, H. V., Berthiaume, R., Ouellet, D. R., Chiquette, J., & Chouinard, P. Y. (2007b). Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage. *Journal of Dairy Science*, 90(2). [https://doi.org/10.3168/jds.S0022-0302\(07\)71572-2](https://doi.org/10.3168/jds.S0022-0302(07)71572-2)
- Benetel, G., Silva, T. dos S., Fagundes, G. M., Welter, K. C., Melo, F. A., Lobo, A. A. G., Muir, J. P., & Bueno, I. C. S. (2022). Essential oils as in vitro ruminal fermentation manipulators to mitigate methane emission by beef cattle grazing tropical grasses. *Molecules*, 27(7). <https://doi.org/10.3390/molecules27072227>
- Berry, E., & Miller, D. (2005). Cattle feedlot soil moisture and manure content: II. Impact on *Escherichia coli* O157. *Journal of Environmental Quality*, 34, 656–663.  
<https://doi.org/10.2134/jeq2005.0656>
- Bowman, J. G., & Sowell, B. F. (1997). Delivery method and supplement consumption by grazing ruminants: A review. *Journal of Animal Science*, 75(2), 543–550.  
<https://doi.org/10.2527/1997.752543x>
- Calsamiglia, S., Busquet, M., Cardozo, P. W., Castillejos, L., & Ferret, A. (2007). Invited Review: Essential oils as modifiers of rumen microbial fermentation. *Journal of Dairy Science*, 90(6), 2580–2595. <https://doi.org/10.3168/jds.2006-644>
- Cardozo, P. W., Calsamiglia, S., Ferret, A., & Kamel, C. (2005). Screening for the effects of natural plant extracts at different pH on in vitro rumen microbial fermentation of a high-

- concentrate diet for beef cattle. *Journal of Animal Science*, 83(11), 2572–2579.  
<https://doi.org/10.2527/2005.83112572x>
- Cardozo, P. W., Calsamiglia, S., Ferret, A., & Kamel, C. (2006). Effects of alfalfa extract, anise, capsicum, and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high-concentrate diet. *Journal of Animal Science*, 84(10), 2801–2808. <https://doi.org/10.2527/jas.2005-593>
- Castro-Montoya, J., Peiren, N., Cone, J., Zweifel, B., Fievez, V., & Campeneere, S. (2015). In vivo and in vitro effects of a blend of essential oils on rumen methane mitigation. *Livestock Science*, 180, 134–142. <https://doi.org/10.1016/j.livsci.2015.08.010>
- Cho, S. B., Soleh, H. M., Choi, J. W., Hwang, W.-H., Lee, H., Cho, Y.-S., Cho, B.-K., Kim, M. S., Baek, I., & Kim, G. (2024). Recent methods for evaluating crop water stress using AI techniques: A review. *Sensors*, 24, 1–39. <https://doi.org/10.3390/s24196313>
- Cobellis, G., Trbalza-Marinucci, M., & Yu, Z. (2016). Critical evaluation of essential oils as rumen modifiers in ruminant nutrition: A review. *Science of The Total Environment*, 545–546, 556–568. <https://doi.org/10.1016/j.scitotenv.2015.12.103>
- Cole, N. A., Clark, R. N., Todd, R. W., Richardson, C. R., Gueye, A., Greene, L. W., & McBride, K. (2005). Influence of dietary crude protein concentration and source on potential ammonia emissions from beef cattle manure. *Journal of Animal Science*, 83(3), 722–731. <https://doi.org/10.2527/2005.833722x>
- Cortes, J. A., Thomas, A., Hendrick, S., Janzen, E., Pajor, E. A., & Orsel, K. (2021). Risk factors of digital dermatitis in feedlot cattle. *Translational Animal Science*, 5(3), 1–9.  
<https://doi.org/10.1093/tas/txab075>

- Dadallage, K. M., Amogi, B. R., Khot, L. R., & Leal Yepes, F. A. (2024). Mask R-CNN and centroid tracking algorithm to process UAV based thermal-RGB video for drylot cattle heat stress monitoring. *Drones*, 8(9), 1–15. <https://doi.org/10.3390/drones8090491>
- Dickson, E. J., Campbell, D. L. M., Monk, J. E., Lea, J. M., Colditz, I. G., & Lee, C. (2022). Increasing mud levels in a feedlot influences beef cattle behaviours but not preference for feedlot or pasture environments. *Applied Animal Behaviour Science*, 254. <https://doi.org/10.1016/j.applanim.2022.105718>
- Dillon, J. A., Stackhouse-Lawson, K. R., Thoma, G. J., Gunter, S. A., Rotz, C. A., Kebreab, E., Riley, D. G., Tedeschi, L. O., Villalba, J., Mitloehner, F., Hristov, A. N., Archibeque, S. L., Ritten, J. P., & Mueller, N. D. (2021). Current state of enteric methane and the carbon footprint of beef and dairy cattle in the United States. *Animal Frontiers*, 11(4), 57–68. <https://doi.org/10.1093/af/vfab043>
- Dong, H., Dong, J., Sun, S., Bai, T., Zhao, D., Yin, Y., Shen, X., Wang, Y., Zhang, Z., & Wang, Y. (2024). Crop water stress detection based on UAV remote sensing systems. *Agricultural Water Management*, 303, 109059. <https://doi.org/10.1016/j.agwat.2024.109059>
- Driedger, L. J., & Loerch, S. C. (1999). Limit-feeding corn as an alternative to hay reduces manure and nutrient output by Holstein cows. *Journal of Animal Science*, 77(4), 967–972. <https://doi.org/10.2527/1999.774967x>
- Elcoso, G., Zweifel, B., & Bach, A. (2019). Effects of a blend of essential oils on milk yield and feed efficiency of lactating dairy cows. *Applied Animal Science*, 35(3), 304–311. <https://doi.org/10.15232/aas.2018-01825>

- Elfarkh, J., Johansen, K., Angulo, V., Camargo, O. L., & McCabe, M. F. (2023). Quantifying within-flight variation in land surface temperature from a UAV-based thermal infrared camera. *Drones*, 7(10). <https://doi.org/10.3390/drones7100617>
- Emert, A. D., Griffis-Kyle, K., Green, F. B., & Smith, P. N. (2023). Atmospheric transport of particulate matter and particulate-bound agrochemicals from beef cattle feedlots: Human health implications for downwind agricultural communities. *Science of The Total Environment*, 894. <https://doi.org/10.1016/j.scitotenv.2023.164678>
- Erickson, G. E., Klopfenstein, T. J., Milton, C. T., Brink, D., Orth, M. W., & Whittet, K. M. (2002). Phosphorus requirement of finishing feedlot calves. *Journal of Animal Science*, 80(6), 1690–1695. <https://doi.org/10.2527/2002.8061690x>
- Eugène, M., Archimède, H., & Sauvant, D. (2004). Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminants. *Livestock Production Science*, 85(1), 81–97. [https://doi.org/10.1016/S0301-6226\(03\)00117-9](https://doi.org/10.1016/S0301-6226(03)00117-9)
- Fouts, J. Q., Grossi, S., Tricarico, J. M., & Kebreab, E. (2025). Effects of pulse-dosing an essential oil blend to dairy cows on enteric methane emissions and productivity. *Translational Animal Science*, 9. <https://doi.org/10.1093/tas/txaf056>
- Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., & Tempio, G. (2013). *Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities*. Food and Agriculture Organization of the United Nations.
- Gilley, J. E., Berry, E. D., Eigenberg, R. A., Marx, D. B., & Woodbury, B. L. (2008). Spatial variations in nutrient and microbial transport from feedlot surfaces. *Transactions of the ASABE*, 51(2), 675–684. <https://doi.org/10.13031/2013.24380>

- Grandin, T. (2016). Evaluation of the welfare of cattle housed in outdoor feedlot pens. *Veterinary and Animal Science*, 1–2, 23–28. <https://doi.org/10.1016/j.vas.2016.11.001>
- Hagenmaier, J. A., Reinhardt, C. D., Bartle, S. J., & Thomson, D. U. (2016). Effect of shade on animal welfare, growth performance, and carcass characteristics in large pens of beef cattle fed a beta agonist in a commercial feedlot. *Journal of Animal Science*, 94(12), 5064–5076. <https://doi.org/10.2527/jas.2016-0935>
- Hales, K. E., Brown-Brandl, T. M., & Freetly, H. C. (2014). Effects of decreased dietary roughage concentration on energy metabolism and nutrient balance in finishing beef cattle. *Journal of Animal Science*, 92(1), 264–271. <https://doi.org/10.2527/jas.2013-6994>
- Hauge, S. J., Nafstad, O., Røtterud, O.-J., & Nesbakken, T. (2012). The hygienic impact of categorisation of cattle by hide cleanliness in the abattoir. *Food Control*, 27(1), 100–107. <https://doi.org/10.1016/j.foodcont.2012.03.004>
- Hegarty, R. S., Cortez Passetti, R. A., Dittmer, K. M., Wang, Y., Shelton, S., Emmet-Booth, J., Wollenberg, E., McAllister, T., Leahy, S., Beauchemin, K., & Gurwick, N. (2021). *An evaluation of emerging feed additives to reduce methane emissions from livestock. Edition 1. A report coordinated by Climate Change, Agriculture and Food Security (CCAFS) and the New Zealand Agricultural Greenhouse Gas Research Centre (NZAGRC) initiative of the Global Research Alliance (GRA). (Edition 1). Climate Change, Agriculture and Food Security; New Zealand Agricultural Greenhouse Gas Research Centre.*
- Hristov, A. N., Johnson, K. A., & Kebreab, E. (2014). Livestock methane emissions in the United States. *Proceedings of the National Academy of Sciences*, 111(14), E1320. <https://doi.org/10.1073/pnas.1401046111>

- Huhtanen, P., Detmann, E., & Krizsan, S. J. (2016). Prediction of rumen fiber pool in cattle from dietary, fecal, and animal variables. *Journal of Dairy Science*, 99(7), 5345–5357.  
<https://doi.org/10.3168/jds.2015-10842>
- Jaderborg, J. P., Spiehs, M. J., Woodbury, B. L., DiCostanzo, A., & Parker, D. B. (2021). Use of bedding materials in beef bedded manure packs in hot and cool ambient temperatures: Effects on ammonia, hydrogen sulfide, and greenhouse gas emissions. *Transactions of the ASABE*, 64(4), 1197–1209. <https://doi.org/10.13031/trans.14291>
- Jia, R., Liu, J., Zhang, J., Niu, Y., Jiang, Y., Xuan, K., Wang, C., Ji, J., Ma, B., & Li, X. (2023). An improved UAV-based ATI method incorporating solar radiation for farm-scale bare soil moisture measurement. *Remote Sensing*, 15(15). <https://doi.org/10.3390/rs15153769>
- Jiang, B., Tang, W., Cui, L., & Deng, X. (2023). Precision livestock farming research: A global scientometric review. *Animals*, 13(13). <https://doi.org/10.3390/ani13132096>
- Johnson, K. A., & Johnson, D. E. (1995). Methane emissions from cattle. *Journal of Animal Science*, 73(8), 2483–2492. <https://doi.org/10.2527/1995.7382483x>
- Jouany, J. P., & Ushida, K. (1999). *The role of protozoa in feed digestion*. 12(1), 113–128.  
<https://doi.org/10.5713/ajas.1999.113>
- Kallem, B., Kizanlik, P. K., Şahiner, C., & Göksoy, E. Ö. (2022). The impact of cattle hide cleanliness scores on microbial contamination of carcasses during slaughtering. *Macedonian Veterinary Review*, 45(1), 43–51. <https://doi.org/10.2478/macvetrev-2022-0012>
- Kunkle, W., Johns, J., Poore, M., & Herd, D. (2000). Designing supplementation programs for beef cattle fed forage-based diets. *Journal of Animal Science*, 77, 1–12.  
<https://doi.org/10.2527/jas2000.00218812007700ES0012x>

- Lacerda, L. N., Snider, J. L., Cohen, Y., Liakos, V., Gobbo, S., & Vellidis, G. (2022). Using UAV-based thermal imagery to detect crop water status variability in cotton. *Smart Agricultural Technology*, 2, 100029. <https://doi.org/10.1016/j.atech.2021.100029>
- Lee, M., Auvermann, B. W., Tedeschi, L. O., Koziel, J. A., Brandani, C. B., Gouvêa, V. N., Smith, J. K., & Casey, K. D. (2025). Ammonia emissions from beef cattle feedyards: A review. *Frontiers in Animal Science*, 6, 1608387. <https://doi.org/10.3389/fanim.2025.1608387>
- Loerch, S. C. (1990). Effects of feeding growing cattle high-concentrate diets at a restricted intake on feedlot performance. *Journal of Animal Science*, 68(10), 3086. <https://doi.org/10.2527/1990.68103086x>
- Macitelli, F., Braga, J. S., Gellatly, D., & Paranhos Da Costa, M. J. R. (2020). Reduced space in outdoor feedlot impacts beef cattle welfare. *Animal*, 14(12), 2588–2597. <https://doi.org/10.1017/S1751731120001652>
- Mader, T. L. (2011). Mud effects on feedlot cattle. *Nebraska Beef Cattle Reports*, (613), 82–83.
- Mader, T. L., & Colgan, S. L. (2007). Pen density and straw bedding during feedlot finishing. *Nebraska Beef Cattle Reports*, (70), 43–46.
- Mader, T. L., Davis, M. S., & Gaughan, J. B. (2007). Effect of sprinkling on feedlot microclimate and cattle behavior. *International Journal of Biometeorology*, 51(6), 541–551. <https://doi.org/10.1007/s00484-007-0093-8>
- Marques, R. da S., & Cooke, R. F. (2021). Effects of ionophores on ruminal function of beef cattle. *Animals*, 11(10). <https://doi.org/10.3390/ani11102871>
- McDowell, R. W., Sharpley, A. N., Condon, L. M., Haygarth, P. M., & Brookes, P. C. (2000). Processes controlling soil phosphorus release to runoff and implications for agricultural

- management. *Nutrient Cycling in Agroecosystems*, 269–284.  
<https://doi.org/10.1023/A:1014419206761>
- McEvoy, J. M., Doherty, A. M., Finnerty, M., Sheridan, J. J., McGuire, L., Blair, I. S., McDowell, D. A., & Harrington, D. (2000). The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. *Letters in Applied Microbiology*, 30(5), 390–395. <https://doi.org/10.1046/j.1472-765x.2000.00739.x>
- Meyer, N. F., Erickson, G. E., Klopfenstein, T. J., Greenquist, M. A., Luebke, M. K., Williams, P., & Engstrom, M. A. (2009). Effect of essential oils, tylosin, and monensin on finishing steer performance, carcass characteristics, liver abscesses, ruminal fermentation, and digestibility. *Journal of Animal Science*, 87(7), 2346–2354.  
<https://doi.org/10.2527/jas.2008-1493>
- Miller, D., & Berry, E. (2005). Cattle feedlot soil moisture and manure content: I. Impacts on greenhouse gases, odor compounds, nitrogen losses, and dust. *Journal of Environmental Quality*, 34, 644–655. <https://doi.org/10.2134/jeq2005.0644>
- Miller, D. W., Barnes, A. L., Collins, T., Pannier, L., Aleri, J., Maloney, S. K., & Anderson, F. (2024). Welfare and performance benefits of shade provision during summer for feedlot cattle in a temperate climatic zone. *Journal of Animal Science*, 102.  
<https://doi.org/10.1093/jas/skae332>
- Miller, D., & Woodbury, B. (2003). Simple protocols to determine dust potentials from cattle feedlot soil and surface samples. *Journal of Environmental Quality*, 32, 1634–1640.  
<https://doi.org/10.2134/jeq2003.1634>

- Miller, G. A., Bowen, J. M., Dewhurst, R. J., Zweifel, B., Spengler, K., & Duthie, C.-A. (2023). Enteric methane emissions from dairy–beef steers supplemented with the essential oil blend Agolin Ruminant. *Animals*, 13(11). <https://doi.org/10.3390/ani13111826>
- Miller, J., Beasley, B., Yanke, L., Larney, F., Mcallister, T., Olson, B., Selinger, L., Chanasyk, D., & Hasselback, P. (2003). Bedding and seasonal effects on chemical and bacterial properties of feedlot cattle manure. *Journal of Environmental Quality*, 32, 1887–1894. <https://doi.org/10.2134/jeq2003.1887>
- Miller, J., Olson, E., Chanasyk, D., Beasley, B., Yanke, L., Larney, F., Mcallister, T., Olson, B., & Selinger, L. (2006). Bedding and within-pen location effects on feedlot pen runoff quality using a rainfall simulator. *Journal of Environmental Quality*, 35, 505–515. <https://doi.org/10.2134/jeq2005.0205>
- Mufford, J. T., Hill, D. J., Flood, N. J., & Church, J. S. (2019). Use of unmanned aerial vehicles (UAVs) and photogrammetric image analysis to quantify spatial proximity in beef cattle. *Journal of Unmanned Vehicle Systems*, 7(3), 194–206. <https://doi.org/10.1139/juvs-2018-0025>
- Murphy, T. A., & Loerch, S. C. (1994). Effects of restricted feeding of growing steers on performance, carcass characteristics, and composition. *Journal of Animal Science*, 72(9), 2497–2507. <https://doi.org/10.2527/1994.7292497x>
- National Academies of Sciences, Engineering, and Medicine. (2016). *Nutrient Requirements of Beef Cattle* (8th Revised Edition). National Academies Press.
- Nennich, T. D., Harrison, J. H., VanWieringen, L. M., Meyer, D., Heinrichs, A. J., Weiss, W. P., St-Pierre, N. R., Kincaid, R. L., Davidson, D. L., & Block, E. (2005). Prediction of

- manure and nutrient excretion from dairy cattle. *Journal of Dairy Science*, 88(10), 3721–3733. [https://doi.org/10.3168/jds.S0022-0302\(05\)73058-7](https://doi.org/10.3168/jds.S0022-0302(05)73058-7)
- Neville, B. W., Moore, R. L., & Rodehorst, W. J. (2020). Effects of pen cleaning on feedlot performance and carcass characteristics of beef steers fed during the winter in the northern Great Plains. *Livestock Science*, 241. <https://doi.org/10.1016/j.livsci.2020.104204>
- Nutrient management technical note No. 190-nm-1. Effects of diet and feeding management on nutrient content of manure.* (2020). United States Department of Agriculture and Natural Resource Conservation Service.
- Olson, D., & Anderson, J. (2021). Review on unmanned aerial vehicles, remote sensors, imagery processing, and their applications in agriculture. *Agronomy Journal*, 113(2), 971–992. <https://doi.org/10.1002/agj2.20595>
- Olson, E. C. S., Chanasyk, D. S., & Miller, J. J. (2006). Effects of bedding type and within-pen location on feedlot runoff. *Transactions of the ASABE*, 49(4), 905–914. <https://doi.org/10.13031/2013.21736>
- Patra, A. K., & Saxena, J. (2009). Dietary phytochemicals as rumen modifiers: A review of the effects on microbial populations. *Antonie van Leeuwenhoek*, 96(4), 363–375. <https://doi.org/10.1007/s10482-009-9364-1>
- Pickinpaugh, W. J., Neville, B. W., Moore, R. L., & Caton, J. S. (2022). Impacts of added roughage on growth performance, digestibility, ruminal fermentation, and ruminal pH of feedlot steers fed wheat-based feedlot diets containing 30% modified distillers grains with solubles. *Translational Animal Science*, 6(2). <https://doi.org/10.1093/tas/txac051>

- Poppi, D. P., & McLennan, S. R. (1995). Protein and energy utilization by ruminants at pasture. *Journal of Animal Science*, 73(1), 278. <https://doi.org/10.2527/1995.731278x>
- Pratt, C., Redding, M., & Hill, J. (2016). A promising and simple method to quantify soil/manure mixing on beef feedlot pens. *Animal Production Science*, 56(9), 1361–1366. <https://doi.org/10.1071/AN14771>
- Sampaio, C. B., Detmann, E., Lazzarini, I., Souza, M. A. D., Paulino, M. F., & Valadares Filho, S. D. C. (2009). Rumen dynamics of neutral detergent fiber in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. *Revista Brasileira de Zootecnia*, 38(3), 560–569. <https://doi.org/10.1590/S1516-35982009000300023>
- Santos, M. B., Robinson, P. H., Williams, P., & Losa, R. (2010). Effects of addition of an essential oil complex to the diet of lactating dairy cows on whole tract digestion of nutrients and productive performance. *Animal Feed Science and Technology*, 157(1–2), 64–71. <https://doi.org/10.1016/j.anifeedsci.2010.02.001>
- Satter, L. D., Klopfenstein, T. J., & Erickson, G. E. (2002). The role of nutrition in reducing nutrient output from ruminants. *Journal of Animal Science*, 80(E-suppl\_2), E143–E156. [https://doi.org/10.2527/animalsci2002.80E-Suppl\\_2E143x](https://doi.org/10.2527/animalsci2002.80E-Suppl_2E143x)
- Shao, W., Kawakami, R., Yoshihashi, R., You, S., Kawase, H., & Naemura, T. (2020). Cattle detection and counting in UAV images based on convolutional neural networks. *International Journal of Remote Sensing*, 41(1), 31–52. <https://doi.org/10.1080/01431161.2019.1624858>
- Sharma, H., Sidhu, H., & Bhowmik, A. (2025). Remote sensing using unmanned aerial vehicles for water stress detection: A review focusing on specialty crops. *Drones*, 9(4), 241. <https://doi.org/10.3390/drones9040241>

- Shi, H., Liu, Z., Li, S., Jin, M., Tang, Z., Sun, T., Liu, X., Li, Z., Zhang, F., & Xiang, Y. (2024). Monitoring soybean soil moisture content based on UAV multispectral and thermal-infrared remote-sensing information fusion. *Plants*, *13*(17).  
<https://doi.org/10.3390/plants13172417>
- Sip, M. L., & Pritchard, R. H. (1991). Nitrogen utilization by ruminants during restricted intake of high-concentrate diets. *Journal of Animal Science*, *69*(6), 2655–2662.  
<https://doi.org/10.2527/1991.6962655x>
- Smerchek, D. T., & Smith, Z. K. (2020). Bedding application to feedlot steers: Influence on growth performance, estimated maintenance coefficient, carcass characteristics, and circulating metabolites in beef steers. *Animals*, *10*(10), 1766.  
<https://doi.org/10.3390/ani10101766>
- Spiehs, M. J., Woodbury, B. L., Doran, B. E., Eigenberg, R. A., & Kohl, K. D. (2010). Ammonia emissions from feedlot surface of beef deep-bedded monoslope facilities. *International Symposium on Air Quality and Manure Management for Agriculture Conference Proceedings, 13-16 September 2010, Dallas, Texas, 711P0510cd*.  
<https://doi.org/10.13031/2013.32697>
- Tait, L. A., Taylor, P. S., Wilkes, J., McGilchrist, P., Reid, J., & Cowley, F. C. (2023). Effect of woodchip bedding during wet conditions on feedlot cattle productivity, behaviour and relative adrenal gland weight. *Animal*, *17*(12).  
<https://doi.org/10.1016/j.animal.2023.101032>
- Tomkins, N. W., Denman, S. E., Pilajun, R., Wanapat, M., McSweeney, C. S., & Elliott, R. (2015). Manipulating rumen fermentation and methanogenesis using an essential oil and

- monensin in beef cattle fed a tropical grass hay. *Animal Feed Science and Technology*, 200, 25–34. <https://doi.org/10.1016/j.anifeedsci.2014.11.013>
- Torres-Rua, A., Aboutaleb, M., Wright, T., Nassar, A., Guillevic, P., Hipps, L., Gao, F., Jim, K., Alsina, M. M., Coopmans, C., McKee, M., & Kustas, W. (2019). Estimation of surface thermal emissivity in a vineyard for UAV microbolometer thermal cameras using NASA HyTES hyperspectral thermal, Landsat and AggieAir optical data. *Proceedings of SPIE--the International Society for Optical Engineering*, 11008. <https://doi.org/10.1117/12.2518958>
- Unruh, E. M., Theurer, M. E., White, B. J., Larson, R. L., Drouillard, J. S., & Schrag, N. (2017). Evaluation of infrared thermography as a diagnostic tool to predict heat stress events in feedlot cattle. *American Journal of Veterinary Research*, 78(7), 771–777. <https://doi.org/10.2460/ajvr.78.7.771>
- Urso, P. M., Turgeon, A., Ribeiro, F. R. B., Smith, Z. K., & Johnson, B. J. (2021). Review: The effects of dust on feedlot health and production of beef cattle. *Journal of Applied Animal Research*, 49(1), 133–138. <https://doi.org/10.1080/09712119.2021.1903476>
- Vahidi, M., Shafian, S., & Frame, W. H. (2025). Multi-Modal sensing for soil moisture mapping: Integrating drone-based ground penetrating radar and RGB-thermal imaging with deep learning. *Computers and Electronics in Agriculture*, 236. <https://doi.org/10.1016/j.compag.2025.110423>
- Van Donkersgoed, J., Jericho, K. W. F., Grogan, H., & Thorlakson, B. (1997). Preslaughter hide status of cattle and the microbiology of carcasses. *Journal of Food Protection*, 60(12), 1502–1508. <https://doi.org/10.4315/0362-028X-60.12.1502>

- Vasconcelos, J. T., & Galyean, M. L. (2007). Nutritional recommendations of feedlot consulting nutritionists: The 2007 Texas Tech University survey. *Journal of Animal Science*, 85(10), 2772–2781. <https://doi.org/10.2527/jas.2007-0261>
- Verstraeten, W. W., Veroustraete, F., Van Der Sande, C. J., Grootaers, I., & Feyen, J. (2006). Soil moisture retrieval using thermal inertia, determined with visible and thermal spaceborne data, validated for European forests. *Remote Sensing of Environment*, 101(3), 299–314. <https://doi.org/10.1016/j.rse.2005.12.016>
- Wan, Q., Smigaj, M., Brede, B., & Kooistra, L. (2024). Optimizing UAV-based uncooled thermal cameras in field conditions for precision agriculture. *International Journal of Applied Earth Observation and Geoinformation*, 134. <https://doi.org/10.1016/j.jag.2024.104184>
- Whitehead, K., & Hugenholtz, C. H. (2014). Remote sensing of the environment with small unmanned aircraft systems (UASs), part 1: A review of progress and challenges. *Journal of Unmanned Vehicle Systems*, 02(03), 69–85. <https://doi.org/10.1139/juvs-2014-0006>
- Wilkes, J., Tait, A. L., Flavel, R. J., Turnell, J., & Cowley, F. C. (2022). Physical and chemical characteristics of feedlot pen substrate bedded with woodchip under wet climatic conditions. *The Journal of Agricultural Science*, 160(5), 413–422. <https://doi.org/10.1017/S002185962200048X>
- Williams, P., Clark, J., & Bean, K. (2021). Evaluation of Agolin, an essential oil blend, as a feed additive for high producing cows. *Open Journal of Animal Sciences*, 11(02), 231–237. <https://doi.org/10.4236/ojas.2021.112018>
- Wilson, S. C., Morrow-Tesch, J., Straus, D. C., Cooley, J. D., Wong, W. C., Mitlöhner, F. M., & McGlone, J. J. (2002). Airborne microbial flora in a cattle feedlot. *Applied and*

*Environmental Microbiology*, 68(7), 3238–3242.

<https://doi.org/10.1128/AEM.68.7.3238-3242.2002>

Zhang, D., & Zhou, G. (2016). Estimation of soil moisture from optical and thermal remote sensing: A review. *Sensors*, 16(8). <https://doi.org/10.3390/s16081308>

## **Chapter 2 - Use of unmanned aerial vehicle thermal imagery to assess the nutrient landscape of cattle pens**

### **Introduction**

The physical environment of feedlot pens represents an influential yet complex determinant of beef cattle health, welfare, and productivity. Within high-density confined animal systems, surface conditions, characterized by stocking density, moisture balance, manure accumulation, and temperature, directly mediate animal comfort, energy expenditure, and pathogen exposure (Gerber et al., 2013). Variations in pen microclimate can translate into differences in feed efficiency, carcass quality, and disease risk, making accurate and timely assessment of pen conditions a critical component of precision feedlot management (Cortes et al., 2021; Dickson et al., 2022; Mader, 2011). While formal evaluation systems are not widely adopted, feedyards generally rely on traditional visual scoring or manual sampling methods that often lack spatial resolution, temporal consistency, and suffer from objectivity (Bastos et al., 2024). Current methodology limitations can hinder early detection of environmental risk factors and constrain data-driven decision-making.

Recent advances in remote sensing technologies have opened new possibilities for feedlot monitoring with unmanned aerial vehicles (UAVs). Equipped with thermal infrared, multispectral, or high-resolution red, green, blue (RGB) cameras, UAVs can rapidly survey entire pens to capture temperature and surface variability at centimeter-scale precision without disrupting animal behavior (Abdulai et al., 2021; Shao et al., 2020). By integrating aerial imagery with ground-based moisture and nutrient measurements, it becomes possible to model the spatial heterogeneity of pen surfaces, linking the physical environment impacts to animal health and performance. Such an approach aligns with broader framework of precision livestock

farming, which leverages automation, sensing, and analytics to enhance both production efficiency and animal well-being (Jiang et al., 2023).

Environmental stressors within feedlots, including mud, dust, and heat, have been recognized as critical welfare and performance challenges. Muddy or waterlogged pens increase the energy cost of movement, reduce resting time, and increase the risk of digital dermatitis, a contagious skin infection affecting cattle hooves and a major cause of lameness (Cortes et al., 2021; Wilkes et al., 2022). While dry surfaces contribute to dust generation and respiratory irritation (Urso et al., 2021), seasonal variation, bedding management, and nutrient composition of the diet and manure further impact pen microclimate dynamics and contribute to the need for tools capable of capturing both temporal and spatial variation. Unmanned aerial vehicle-based thermal imaging has potential to be a non-invasive means to quantify and detect parameters of high or low extremes that correspond with compromised pen conditions.

Thermal infrared imagery captures the temperature differential based on the surrounding environment (Montanholi et al., 2008). Studies have demonstrated thermal infrared imagery can estimate moisture content in soil and crops with the relationship between evaporative cooling and surface temperature (Verstraeten et al., 2006; Zhang & Zhou, 2016). Wet surfaces absorb and retain more heat, resulting in lower apparent surface temperatures compared to dry substrates. This physical principle allows thermal infrared data to serve as a proxy for surface wetness or moisture status across heterogeneous landscapes. Application of thermal imaging to detect moisture and nutrients within cattle pens enables identification of areas that strongly influence manure decomposition, dust generation, and cattle comfort. The hypothesis of the current study was that UAV-mounted thermal imagery color metrics will be associated with

ground-truth pen-surface condition measurements and can be used to predict pen condition at the pen and sub-pen scale.

The objectives were to:

1. Characterize spatial variation in pen-surface nutrient composition and color metrics derived from UAV thermal imagery within feedlot pens over a single day, comparing pens managed under high-intake versus low-intake feeding programs.
2. Evaluate pen-surface nutrient composition (moisture, organic matter, direct temperature, and color metrics) using a multi-day dataset to assess the consistency of observed relationships under differing environmental conditions.
3. Quantify the association of actual pen-surface nutrient composition with color metrics derived from UAV thermal images to assess the potential of drone thermal imagery as a predictive tool for pen condition.

## **Materials and methods**

### **Animal care and use**

The experiment was conducted at Kansas State University Beef Stocker Unit in Manhattan, KS. All animals involved in this experiment were cared for according to guidelines of the Kansas State University Institutional Animal Care and Use Committee (protocol #IACUC-4650).

### **Animals, experimental design, and feeding programs**

Six outdoor dirt-floor cattle pens (9 x 15 m) were utilized in a completely randomized design. Each pen housed 14 dairy x beef crossbred steers (body weight =  $421.8 \pm 38.6$  kg) and were randomly assigned to one of two feeding programs (n = 3 pens per feeding program; Table 2.1): (1) LOW (low-intake), a limit-fed, low-energy ration formulated to contain 50 Mcal/cwt of

net energy for gain (NEg) and targeted to achieve 0.91 kg/head/day average daily gain, or (2) HIGH (high-intake), an ad libitum, energy-dense ration formulated to contain 64 Mcal/cwt NEg.

### **Sample collection and UAV thermal imaging**

Samples and images were collected on days 111, 112, and 113 of the backgrounding phase. Prior to sample collection, each pen was divided into 15 evenly spaced quadrants (3 m x 3 m; Figure 2.1). Sample collection and UAV thermal imaging were conducted directly following morning feeding (0730 hours) to capture pen-surface images while cattle were occupied at the bunk. Three samples of loose pen surface material were collected per quadrant ( $\approx 0.25$  kg) using 0.26 L disposable plastic cups; a new cup was used for each sample. Samples were placed in individual sealed plastic bags and stored ( $-18^{\circ}\text{C}$ ) until laboratory analysis. Temperature measurements were recorded in triplicate per quadrant during sample collection using a handheld infrared thermometer (Vesogy 9158F). Temperature and surface material sampling locations within each quadrant were selected following a visual assessment of the surface material to capture a representative range of pen surface conditions.

An Autel EVO II UAV (Autel Intelligent Technology Corp., Shenzhen, China) equipped with a thermal sensor (H20T) was flown to capture red, green, blue (RGB) and thermal images of each pen-surface during manure sample collection. Preplanned flights were created for each pen following protocols recommended by the Applied Aviation Research Center at Kansas State University, Salina, KS. Preplanned flight settings included: altitude of 30.5 m, speed of 4 km/h, 90% front overlap, 90% side overlap. Flight boundaries were defined beyond the outer limits of each pen to ensure complete pen coverage. The UAV followed a bidirectional flight pattern oriented east to west, then west to east, repeated across the entire pen area. Each flight produced

a series of overlapping images which were later processed using computer software into composite orthomosaics.

Climatology data, including air temperature, relative humidity, precipitation, wind speed, soil temperature, and solar radiation were collected from the Kansas Mesonet Manhattan, KS weather station located 6.4 km from the research site. Precipitation was consistent on Day 1 and Day 2 (Table 2.3); overnight between Day 2 and Day 3 sampling times, 2.57 cm of rainfall occurred which altered pen moisture levels and identified Day 3 as a precipitation outlier. On Day 3, manure samples were collected and analyzed from one pen per treatment (15 quadrants per pen) due to extreme wet conditions; UAV thermal imaging and pen-surface temperature measurements were recorded from all pens.

### **Sample and thermal image processing**

Pen-surface material samples were dried at 55°C in a conventional drying oven for an initial period of 72 hours and weighed to determine dry matter (DM) according to AOAC method 934.01 (AOAC International, 2019). Each sample was returned to the oven for an additional 2 hours and reweighed. If no further weight loss was observed, the samples were considered to have reached constant weight and were removed from the oven. Total drying duration ranged from 72 to 96 hours depending on initial moisture content. Once dry, samples were ground through a 2-mm screen using a UDY Model 3010-030 Cyclone Mill (UDY Corp., Fort Collins, CO).

Samples were separated by day to use Day 2 for individual nutrient analysis and samples from days 1 and 3 composited by quadrant to evaluate Objectives 2 and 3. Samples collected on days 1 and 3 were analyzed individually for dry matter and organic matter only. Day 2 data were utilized for proof of concept using the full nutrient panel, while days 1 and 3 provided a better

understanding of this technology under various environmental conditions without costing substantial financial and laboratory resources by only analyzing for DM and organic matter (OM).

Samples collected on days 1 and 3 were then analyzed individually for a 100% DM correction factor and OM according to AOAC methods 934.01 and 942.05 (AOAC International, 2019). Corrected DM was analyzed using an additional 104°C oven drying of a 0.5-g subsample for 12 hours. Dry matter used for analysis was calculated by DM x DM-correction and moisture was determined using 1 minus DM. Ash was measured with a 0.5-g subsample weighed into a crucible and placed in a muffle furnace for 8 hours at 550°C. Organic matter was determined using 1 minus ash remaining of sample. Samples collected on Day 2 were analyzed individually for DM and OM, after which 3-g aliquots of ground material from each quadrant sample (n = 3) were composited by quadrant. Quadrant composite samples were subsequently analyzed for DM-correction, neutral detergent fiber (NDF), acid detergent fiber (ADF), and nitrogen content. Neutral detergent fiber and ADF were determined according to Van Soest et al. (1991). Sample NDF was determined using an Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY) with 0.5-g samples in heat sealed F57 fiber filter bags (Ankom Technology, Macedon, NY) and a 75-minute extraction in 100°C neutral detergent solution with additions of sodium sulfite and alpha amylase. After NDF analysis, samples were soaked in 200 mL of acetone, air dried for 2 hours, and placed in a 104°C oven for 3 hours to obtain a final NDF sample weight. The final weight, empty F57 bag weight, and sample weight were used to calculate NDF percent of the sample (see calculations). Samples underwent subsequent ADF analysis in a 60-minute 100°C extraction using acid detergent solution in an Ankom 200 Fiber Analyzer. After ADF analysis, samples were soaked in 200 mL of acetone, air dried for 2 hours, and placed in a 104°C oven for

3 hours to obtain a final ADF sample weight. The final ADF weight along with the final NDF weight were used to calculate ADF percent of the sample (see calculations). Neutral detergent fiber and ADF were calculated on both a DM and as-is basis to determine whether relationships with color metrics differed depending on how fiber concentration was expressed, as moisture content may alter how fiber is represented on the pen-surface. Sample nitrogen was determined via steam distillation Kjeldahl technique using a KjelMaster K-375 with K376 autosampler (Buchi Corp., Flawil, Switzerland) according to method 984.13 (AOAC International, 2019). A 0.5-g sample was weighed into a kjeldahl tube, along with 1 kjeldahl catalyst tablet (4.98 g  $\text{K}_2\text{SO}_4$  0.02  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) and 10 mL  $\text{H}_2\text{SO}_4$ . Tube contents were digested at 410°C for 100-minutes, after which they were steam distilled using 60 mL distilled water, 70 mL NaOH as an alkali addition, 50 mL boric acid as an indicator solution, and 0.1 M HCl as titrant—all doses and titrations done automatically using KjelMaster K-375 (see calculations).

Initial thermal image processing was completed by the Applied Aviation Research Center at Kansas State University-Salina (Salina, KS) using Pix4Dmapper (version 4.8.4; Pix4D SA, Lausanne, Switzerland). Images from each pen were stitched together to create orthomosaic maps which were subsequently visualized in ArcGIS Pro (version 3.3; Esri, Redlands, CA) using the thermal color palette *Ironbow* due to its general-purpose utility for detecting thermal anomalies and illustrating heat distribution. Warmer regions appeared in lighter colors, while cooler regions were represented by darker tones. Individual pen maps were exported as .pdf files to extract color metrics in Adobe Photoshop (version 26.2.0; Adobe Inc., San Jose, CA). In Photoshop, cattle and non-pen-surface objects (waterer lid, fence, and concrete bunk) were manually cropped to remove confounding data while pen-surfaces were kept intact (Figure 2.3). A virtual grid corresponding to the 15 physical sampling quadrants (Figure 2.1) was overlaid on

each pen image. Photoshop was used to extract average color metrics from each quadrant across three color models: RGB (red, green, blue), HSV (hue, saturation, value), HSL (hue, saturation, [HSL\*L] lightness) and Lab (lightness [Lab\*L], red/green [Lab\*a], blue/yellow [Lab\*b]). Both HSV and HSL metrics were evaluated because the brightness components (“value” in HSV and “lightness” in HSL) are mathematically defined differently. “Value” from the HSV color model reflect the maximum channel intensity, whereas HSL “lightness” reflects the average of maximum and minimum channels. Additionally, luminosity was calculated using a weighted RGB equation based on the human eye sensitivity to different color channels and luminance was calculated based on the RGB color model for overall lightness (see calculations). Color parameters were subsequently used in statistical analysis to assess correlations with nutrient sampling data.

## Calculations

Calculations used in this experiment for determination of nutrients and conversion of color metrics.

Neutral detergent fiber (NDF; Van Soest et al., 1991):

$$NDF (\%) = \frac{100 \times (Final\ NDF\ weight - (Bag\ tare\ weight \times Blank\ bag\ correction))}{Sample\ weight}$$

Acid detergent fiber (ADF; Van Soest et al., 1991):

$$ADF (\%) = \frac{100 \times (Final\ ADF\ weight - (Final\ NDF\ weight \times Blank\ bag\ correction))}{Sample\ weight}$$

Total Kjeldahl Nitrogen (TKN; method 984.13; AOAC International, 2019):

$$TKN(\%) = \frac{(Sample\ titration\ volume - Blank\ titration\ volume) \times Normality\ of\ titrant \times 14.007}{Sample\ weight\ (g)}$$

Luminosity (Nguyen & Brown, 2017):

$$Luminosity = 0.2126(R) + 0.7152(G) + 0.0722(B)$$

Luminance (Nguyen & Brown, 2017):

$$Luminance = \frac{\min(RGB) + \max(RGB)}{2}$$

## Statistical Analysis

Data were analyzed as a completely randomized design with pen serving as the experimental unit in R (version 4.4.2, R Foundation for Statistical Computing, Vienna, Austria). Data were analyzed to reflect experimental objectives with data from differing days used based on objective and model used. Pens without manure samples were excluded from statistical analysis. Significance was declared at  $P \leq 0.05$  and trends were noted at  $0.05 < P \leq 0.10$ . Lin's Concordance Correlation Coefficient (CCC) values were declared at:  $> 0.9$  excellent;  $0.8 - 0.9$  good;  $0.6$  to  $0.8$  moderate; below  $0.6$ . Lin's CCC "accuracy" is noted as CCC bias correction factor with values closer to  $1.00$  indicate minimal scale and location bias between observed and predicted values

### Objective 1

Day 2 (single-day) nutrient and color metrics were analyzed to characterize spatial variation in pen-surface nutrient composition and color metrics derived from UAV thermal imagery within feedlot pens and between treatments over a single day. The analysis used linear mixed models with the *lmer* function of the *lme4* package, with fixed effects of feeding program, quadrant, the interaction of feeding program and quadrant, and a random effect of pen. The statistical model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \delta_k + \varepsilon_{ijk}$$

- $Y_{ijk}$  = nutrient and color metric response (e.g., moisture, luminosity) for feeding program  $i$ , quadrant  $j$ , in pen  $k$
- $\mu$  = population mean
- $\alpha_i = i^{th}$  fixed effect of feeding program (HIGH, LOW)
- $\beta_j = j^{th}$  fixed effect of quadrant
- $(\alpha\beta)_{ij}$  = fixed interaction of feeding program and quadrant
- $\delta_k$  = random effect of pen  $k$
- $\varepsilon_{ijk}$  = residual error

## Objective 2

Data from days 1, 2, and 3 of collection were used to evaluate pen-surface nutrient composition using a multi-day dataset to assess the consistency of observed Objective 1 relationships under differing environmental conditions. Quadrant-level estimated marginal means for nutrient and color metrics were calculated using the *emmeans* package in R with Fisher means separation to visualize quadrant differences. Days 1, 2, and 3 combined were analyzed using a linear mixed model with fixed effects of feeding program, quadrant, and feeding program by quadrant interaction and random effects of pen, quadrant nested in pen, and day. The statistical model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \delta_k + \theta_{j(k)} + \nu_m + \varepsilon_{ilkjm}$$

- $Y_{ijk}$  = nutrient and color metric response (e.g., moisture, luminosity) for feeding program  $i$ , quadrant  $j$ , in pen  $k$
- $\mu$  = population mean
- $\alpha_i = i^{th}$  fixed effect of feeding program (HIGH, LOW)
- $\beta_j = j^{th}$  fixed effect of quadrant
- $(\alpha\beta)_{ij}$  = fixed interaction of feeding program and quadrant
- $\delta_k$  = random effect of pen  $k$
- $\theta_{l(k)}$  = random effect of quadrant  $j$  nested in pen  $k$
- $\nu_m$  = random effect of day  $m$
- $\varepsilon_{ijk}$  = residual error

### Objective 3

Multiple days were used to evaluate the association of ground-truth pen-surface nutrient composition with color metrics derived from UAV thermal images to assess the potential of drone thermal imagery as a predictive tool for pen condition. First, a linear mixed model was used to evaluate the relationship between pen-surface nutrient composition (moisture, OM, and moisture:OM ratio) and color metrics, with fixed effects of color metric and feeding program and random effects of pen, quadrant nested in pen, and day. The statistical model used was:

$$Y_{ilkjm} = \mu + \alpha_i + \kappa_l + \delta_k + \theta_{j(k)} + \nu_m + \varepsilon_{ilkjm}$$

- $Y_{ilkjm}$  = response (e.g., moisture) for feeding program  $i$ , color metric  $l$ , pen  $k$ , quadrant  $j$ , day  $m$
- $\mu$  = population mean
- $\alpha_i = i^{th}$  fixed effect of feeding program (HIGH, LOW)
- $\kappa_l = l^{th}$  fixed effect of color metric (e.g., individual metric; R, G, B)
- $\delta_k$  = random effect of pen  $k$
- $\theta_{j(k)}$  = random effect of quadrant  $j$  nested in pen  $k$
- $\nu_m$  = random effect of day  $m$
- $\varepsilon_{ilkjm}$  = residual error

Color metrics with a significant association with pen-surface characteristics were then used to generate pen-surface nutrient composition predictions at the pen-level using a linear model and the *predict* function with color metric as the fixed effect. The *epiR* package was used to evaluate agreement between actual and predicted pen-surface nutrient composition values using Lin's concordance correlation coefficient (CCC), which partitions agreement into a precision component (Pearson's correlation coefficient,  $r$ ) and an accuracy component represented by the bias correction factor ( $C_\beta$ ). The statistical model used was:

$$Y_l = \mu + \kappa_l + \varepsilon_l$$

- $Y_l$  = response (e.g., moisture) for color metric  $l$
- $\mu$  = population mean
- $\kappa_l = l^{th}$  fixed effect of color metric (e.g., R, G, B)
- $\varepsilon_l$  = residual error

## Results and discussion

### Objective 1: Characterize pen-surface nutrient composition and color metrics

Efforts were made to characterize pen spatial heterogeneity of nutrient composition and thermal-derived color metrics across the pen surface and between treatments in a single day. Pen-surface temperature was the only measure that had a significant feeding program by quadrant interaction (Table 2.4), indicating that feeding program effects differed by pen location on Day 2. Because feeding program differences were not spatially consistent, surface temperature was not included in subsequent feeding program-level analyses. For all other nutrient components, no feeding program x quadrant interactions were observed. Therefore, it was assumed that feeding program effects represent overall level shifts rather than changes in the spatial structure of the pen. Quadrant was found to influence ( $P < 0.01$ ) all nutrient and temperature variables (Table 2.4) except ADF on a DM basis, demonstrating substantial spatial heterogeneity across the pen surface on Day 2 based on the quadrant method used (Figure 2.1). Moisture was found to be lower ( $P < 0.05$ ; 26.0-31.0%; Table 2.7) in quadrants located near the rear fenceline (4, 5, 6, 7, and 15) compared to quadrants located near the concrete apron and waterer zone (10, 11, and 12; 55.0-68.0%). Results are consistent with cattle congregation at feeding and watering points and reduced occupancy in the back of the pen, as documented in previous work showing manure and moisture accumulation are typically two to three times greater in wet zones near bunks or waterers compared with drier mound areas (Berry & Miller, 2005; Miller & Woodbury, 2003). Organic matter (OM), fibrous fractions, and nitrogen followed spatial patterns similar to moisture. Organic matter (% of dry matter) was lower ( $P < 0.05$ ; 37.0-44.0%) in quadrants near the back of the pen (4, 5, 6, 7, 13, 14, and 15) compared with bunk- and waterer-adjacent quadrants (1, 9, 10, 11; 52.0-58.0%; Table 2.7). Analysis resulted in lower

NDF (as-is) values ( $P < 0.05$ ; 29.9-38.2%) in rear and mid-pen quadrants (3, 4, 5, 6, 7, 8, 13, 14, 15) relative to areas near the apron (1, 2, 9, and 10; 47.6-58.8%; Table 2.7). Quadrants near the waterer (11 and 12) contained significantly greater ( $P < 0.05$ ) NDF (as-is) content compared to all other quadrants (66.2-81.8%; Table 2.7). A similar pattern was observed for ADF (as-is), with greater values ( $P < 0.05$ ) found in quadrants near the waterer or bunk (1, 11, and 12; 29.6-36.4%) relative to the remainder of the pen (13.4-24.7%; Table 2.7). Nitrogen concentrations followed the same clustering, with quadrants adjacent to the bunk (1, 2, 9, 10, and 11) resulting in greater nitrogen contents ( $P < 0.05$ ; 22.1-23.1%; Table 2.7) compared to the rest of the pen. Spatial groupings indicate statistically distinct nutrient accumulation zones within the pen, and reflect manure deposition patterns driven by use of the pen microenvironment (Gilley et al., 2008). Fisher's unadjusted groupings (Table 2.7) further confirmed these clusters, demonstrating clear and interpretable nutrient "hot spots" and "low spots" across the 15-quadrant layout. Findings agree with established literature describing concentrated nutrient load and manure accumulation in wetter, high-traffic pen zones (Berry & Miller, 2005; Miller et al., 2006), reinforcing that pen-surface nutrient deposition is behaviorally and spatially structured rather than random.

In contrast to nutrients, no UAV thermal-derived color metric differed significantly by quadrant ( $P > 0.05$ ; Table 2.4), although Lab\*a approached significance ( $P = 0.05$ ). The absence of significant quadrant effects for color metrics (Table 2.8) likely reflects (1) limited dynamic range in thermal color channels from a single flight day, (2) environmental and emissivity variability reduced sensitivity of thermal-based color values, or (3) high within-quadrant heterogeneity inherent to pen surfaces. Previous research shows that thermal infrared measurements of heterogeneous ground materials (soil, manure, base substrate) are sensitive to surface emissivity, angle effects, and short-term environmental fluctuations (Elfarkh et al., 2023;

Wan et al., 2024). Because wet-dry contrasts on cattle pen surfaces may require multi-day temperature amplitude data to resolve (Jia et al., 2023; Verstraeten et al., 2006), it is plausible that a single morning flight did not provide enough variation for quadrant-level differentiation in color metrics. Results indicated thermal color metrics did not exhibit significant spatial differentiation at the quadrant scale, subsequent analyses evaluated color variables at the pen-level rather than within-quadrant comparisons.

No quadrant x feeding program (HIGH vs. LOW) interactions were detected, indicating that feeding program effects were consistent across pen locations. Therefore, the main effect of feeding program was used to evaluate nutrient differences between pens assigned to each feeding program. Dry matter intake in HIGH pens was 10.31 kg/head/day compared to 8.18 kg/head/day in LOW pens (Table 2.2). Moisture ( $P = 0.01$ ) and moisture:OM ratio ( $P = 0.02$ ) were greater in HIGH pens (44.0% and 98.0, respectively; Table 2.5) relative to LOW pens (34.0% and 71.0, respectively; Table 2.5), consistent with hypothesized greater manure output and higher overall fecal moisture when cattle consume more feed (Loerch, 1990; Sip & Pritchard, 1991). Pen-surface nitrogen content was greater for LOW pens ( $P < 0.01$ ; 21.5%; Table 2.5) relative to HIGH (18.5%), despite isonitrogenous diets, likely reflecting more concentrated manure nitrogen when fecal mass was reduced under restricted intake. Nitrogen results are supported by findings from Cole et al. (2005) that lower dry matter intake increases nitrogen density of excreta. Conversely, Driedger & Loerch (1999) found that total nitrogen excretion was 22% greater in cows fed a high-forage ration compared to high-corn despite being isonitrogenous diets. Feeding program effects also demonstrated differences ( $P < 0.05$ ) for all thermal-derived color metrics, except for B (Table 2.6). Differences in pen-level thermal-derived color metrics indicated the variables were sensitive to differences in pen condition between feeding programs within a

singular day despite quadrant-level color metrics not displaying differences. This aligns with the hypothesis that UAV-based thermal and RGB indices can reliably detect general pen characteristics at broader spatial scales.

Strong quadrant-level nutrient patterns and clear feeding program-level differences demonstrate that pen surfaces exhibit structured, biologically meaningful heterogeneity driven by cattle behavior, facility design, and manure deposition dynamics. Absence of quadrant-level color differences did not contradict these findings; instead, it suggests that thermal-derived color metrics require either multi-day thermal amplitude data, improved emissivity correction, or integration with additional spectral indices to resolve fine-scale heterogeneity (Jia et al., 2023; Zhang & Zhou, 2016). Additionally, sample size was a limitation of the trial, and a greater sample size could expand knowledge of the sensitivity of the model.

Objective 1 findings support the premise that UAV-derived thermal imaging can capture distinct pen-level differences in pen condition, and nutrient-color relationships should be evaluated at the pen (rather than quadrant) scale. The results provide the foundation for Objective 2, which evaluates temporal repeatability, and Objectives 3, which assess predictive capability of color metrics for nutrient estimation across days.

## **Objective 2: Multi-day pen-surface nutrient components and color metrics**

Evaluation of pen-surface nutrient composition and color metrics using a multi-day dataset was used to assess the consistency of observed relationships in Objective 1 under differing environmental conditions. When days 1, 2, and 3 were modeled together (including precipitation outlier, Day 3), a significant feeding program by quadrant interaction was detected for moisture:OM ratio ( $P = 0.05$ ; Table 2.9), indicating that the spatial distribution of moisture:OM differed by feeding program. The interaction likely reflects feeding program-

induced differences in moisture content superimposed onto a stable spatial nutrient structure. Nutrients (moisture and OM) were highly significant by quadrant ( $P < 0.01$ ; Table 2.12), and estimates demonstrated that the pen region differences observed on Day 2 alone persisted across varied environmental conditions (Table 2.12). Moisture and OM levels were consistently greater for quadrants ( $P < 0.05$ ) located at the concrete apron and waterer (Figure 2.2) compared to the rest of the pen. The observed numeric trends suggest moisture declines from the concrete apron to the rear fenceline (Table 2.12). The consistency of the observed nutrient distribution aligns with established cattle behavior and infrastructure drivers of pen-surface heterogeneity. Cattle spend more time near bunks and waterers, promoting greater moisture accumulation in these regions, while intentional drainage design (pen slope) promote drier conditions toward the mid- and rear- drainage areas (Grandin, 2016; Miller & Woodbury, 2003). The persistence of the nutrient distribution across different days demonstrate that pen-surface nutrient gradients are structural features of cattle behavior and pen design rather than transient environmental fluctuations. However, external moisture events, precipitation, strongly influenced measured pen-surface moisture content. When only one day was included (Objective 1), moisture averaged 44% in HIGH pens and 34% in LOW pens (Table 2.5). In Objective 2, corresponding values were higher at 52.1% and 42.1%, respectively (Table 2.10).

Both single-day and multi-day models consistently detected an approximate 10% difference in moisture between feeding programs. When the multi-day model included the precipitation-affected outlier (Day 3), overall moisture values increased across feeding programs, reflecting the influence of environmental conditions rather than feeding program alone. Moisture remained significant by feeding program ( $P < 0.01$ ; Table 2.10), with HIGH pens averaging 10% higher moisture compared to LOW pens (52.1 vs 42.1%). Results reinforce known nutritional

effects on manure output cattle on restricted-intake, energy-dense diets produce less total manure and excrete lower moisture loads (Loerch, 1990; Sip & Pritchard, 1991). Lower manure volume and reduced water excretion in limit-fed cattle are well documented consequences of improved nutrient digestibility and reduced output (Huhtanen et al., 2016; Murphy & Loerch, 1994). Persistence of feeding program effects across multiple days suggests that feeding strategy consistently influences pen-surface moisture load, providing biological reinforcement for feeding-program-driven differences in moisture.

Evaluation of color metric spatial distribution across Days 1-3 identified several metrics that differed significantly by quadrant (Table 2.9), including R ( $P = 0.02$ ), B ( $P = 0.04$ ), mean RGB ( $P = 0.02$ ), Lab\*L ( $P = 0.04$ ), and Lab\*a ( $P < 0.01$ ), with additional trends observed for luminance ( $P = 0.06$ ) and V ( $P = 0.07$ ). The quadrant-level estimates for the significant color metrics (Table 2.13) exhibited spatial patterns that numerically paralleled moisture gradients (Table 2.12). Moisture differed by quadrant ( $P < 0.01$ ), with the greatest values observed in quadrants 10, 11, and 12 ( $P < 0.05$ ) compared with the rest of the pen, which were simultaneously characterized by numerically lower brightness-related color metrics, including Lab\*L, luminance, and mean RGB, as well as reduced blue channel intensity. Conversely, comparatively drier quadrants (6, 7, 8, and 15) exhibited higher red intensity and greater brightness indices. Consistency with UAV thermal imaging was expected, as UAV thermal imaging show wetter substrates cool through evaporation and exhibit lower apparent surface temperature with altered reflectance characteristics (Verstraeten et al., 2006; Zhang & Zhou, 2016). The stronger pattern detection observed in the multi-day model likely reflects increased statistical power, supporting the viability of the results for spatial inference of pen-surface nutrient heterogeneity.

Feeding program effects for color metrics in the three-day dataset aligned with singular sampling day findings (Table 2.11). Multiple color metrics were significant by feeding program ( $P < 0.01$ ), including luminosity, R, G, mean RGB, luminance, S, V, Lab\*L, Lab\*a, and HSL\*L. Many RGB- and LAB-derived channels exhibited higher values in LOW pens compared to HIGH pens—likely due to drier surface conditions in LOW pens. Drier manure surfaces typically display higher reflectance and lighter color signatures due to reduced water content and crust formation, a phenomenon paralleling soil and residue drying patterns observed in remote sensing literature (Lacerda et al., 2022; Shi et al., 2024).

The combination of (1) quadrant-level nutrient value differences, (2) multiple color metric differences quadrant and treatment, and (3) consistent feeding program effects provides strong evidence that nutrients are unevenly distributed across the pen surface and that thermal-derived color metrics can capture structural, biologically meaningful aspects of pen-surface condition. The findings reinforce that pen-surface heterogeneity is not random, but affected by predictable interactions between cattle behavior, pen layout, drainage patterns, and manure deposition dynamics (Grandin, 2016; Miller & Woodbury, 2003). Introducing multiple days into the dataset also aligns with broader UAV moisture-sensing research, where thermal inertia and reflectance-based methods reliably detect moisture gradients across surfaces even under varying ambient conditions (Jia et al., 2023; Verstraeten et al., 2006). Thermal color metrics more consistently detected differences between treatments relative to quadrant-level variability, indicating a stronger association to pen-level characteristics. The observed stability established a necessary foundation for Objective 3, where pen-level predictive models tested associations of UAV thermal-derived color metrics and nutrient concentrations will be evaluated.

### **Objective 3: Pen-level thermal color metric predictive capabilities**

#### **Association of pen-level thermal color metrics to pen-surface nutrient composition**

To evaluate whether UAV-derived color metrics could detect nutrient variation at the pen level, each color metric was tested in a mixed model with Days 1, 2, and 3. Across all models, nearly all color metrics were strongly associated with pen-level moisture and moisture:OM ratio (color metric  $P < 0.01$ ; Table 2.14), with the exception of hue, which was not different ( $P = 0.13$ ). The observed universal sensitivity to moisture by all color metrics is consistent with fundamental evaporative cooling processes. Moist surfaces exhibit (1) lower daytime surface temperature due to evaporative cooling (2) greater thermal inertia which alters thermal-emissive properties (Verstraeten et al., 2006; Zhang & Zhou, 2016), and (3) darker reflectance signatures in RGB and Lab color space. The stated differences are well documented in UAV-based soil and moisture studies, where thermal infrared and RGB features are among the strongest predictors of surface wetness (Jia et al., 2023; Lacerda et al., 2022; Shi et al., 2024). In the present study, the same principles apply to moisture-based pen surfaces, where moisture affects both visual reflectance and thermal energy dissipation. The fact that color was significant across three sampling days, multiple environmental conditions, and repeated pen-level observations underscores the biological stability of the thermal-color metrics as nutrient predictors. Hue was the only color channel that failed to detect moisture differences; therefore, its lack of significance aligns with prior findings showing that hue is the least useful in moisture-sensitive spectral dimension in both soil and plant canopies (D. Olson & Anderson, 2021; Zhang & Zhou, 2016).

In contrast, saturation was the only color metric that varied significantly ( $P = 0.05$ ; Table 2.14) with changes in organic matter concentration, and a weak trend was observed for the green channel ( $P = 0.10$ ). The lack of associated color metrics is expected because organic matter was

determined as a percentage of OM with a fixed-volume sample, not as a measure of total accumulated manure mass per quadrant/pen. Spectral reflectance and thermal energy signatures respond strongly to surface moisture, texture, bulk material thickness, and temperature gradients, but not to chemical composition when the substrate mass is held constant (Aragon et al., 2020; D. Olson & Anderson, 2021). Although OM is biologically linked to manure deposition, the sampling methodology decoupled OM percentage from the volumetric manure load that UAV imagery could detect. Significance in saturation and a weak trend for the green channel may indicate slight sensitivity to substrate brightness changes associated with OM, but this relationship is minor compared with moisture driven effects. Results indicate that thermal-derived color metrics can capture moisture-driven variability but do not reliably detect OM concentration when OM is measured as a fixed volume chemical fraction.

Across all color models, feeding program effects remained significant for moisture ( $P \leq 0.02$ ; Table 2.14) and moisture:OM ratio ( $P \leq 0.02$ ), but not for OM ( $P > 0.43$ ). The consistency of feeding program effects across all color channels indicates (1) UAV imaging detects pen-level differences created by feeding program and (2) color metrics encode macro-level pen condition changes supporting their suitability for future predictive modeling. The association between pen-surface moisture data and UAV-derived color signatures mirror findings in crop and soil research showing that thermal infrared and RGB data reliably track spatial moisture differences across irregular surfaces (Cho et al., 2024; Vahidi et al., 2025). Although this application is novel in feedlot pens, the same physical mechanisms (evaporative cooling, thermal inertia, and reflectance dynamics) drive image-based detectability regardless of application. Overall, results demonstrate potential for moisture predictions based on thermal-derived color metrics, minimal potential for OM predictions based off the sampling method utilized in the study, and clear

sensitivity to feeding program-level pen condition differences. The findings confirm that color metrics are associated with pen-level models of surface wetness, allowing for generation of nutrient predictions using UAV thermal-derived color metrics.

### **Agreement of actual pen-level nutrient composition to color-metric-predicted values**

After identifying viable color metrics and confirming their association with nutrient patterns at the pen level, linear models were used to evaluate the ability of individual thermal-derived color metrics to predict moisture and moisture:OM ratio. When all three sampling days were included, the model represented a range of environmental conditions, predictive accuracy varied considerably across color metrics. Several RGB-, HSV-, and LAB-based metrics demonstrated moderate predictive capabilities for moisture and moisture:OM ratio, particularly metrics related to brightness (Table 2.15). Across these models, CCC ranged from 0.53 to 0.79 for moisture and moisture:OM ratio (Table 2.15) indicating that thermal color variation provided biologically relevant information about surface-moisture dynamics. Metrics such as luminosity, luminance, mean RGB, Lab\*L, and V captured the differences in nutrients effectively. Lab\*b showed the strongest predictive performance for moisture (CCC = 0.74) and moisture:OM ratio (CCC = 0.85). Lab\*b derived from thermal imagery has not previously been investigated for associations with moisture or nutrient composition. However, as a measure of brightness, Lab\*b may reflect differences in surface reflectance and thermal emissivity that are influenced by moisture content and manure distribution, suggesting utility as an indirect predictor of pen-surface moisture.

Because of limited observations and outlier precipitation effect, models were also tested without Day 3. The removal of Day 3 from the dataset led to substantial improvements in

predictive performance, demonstrating the importance of environmental conditions prior to UAV flight. When the models were tested using only Days 1 and 2, nearly all previous viable color metrics showed marked increases in predictive accuracy and precision (Table 2.16). Moisture prediction improved across color metrics, resulting in Lab\*a, R, V, Lab\*L, luminance, luminosity, and HSL\*L CCC values between 0.90 and 0.97, and accuracy values exceeding 0.99. The greatest correlation for a color metric when predicting moisture was observed for Lab\*a (CCC = 0.97; Table 2.16). Predictions for moisture:OM ratio also improved, with CCC values increasing across all color metrics. Similar to moisture, the metrics with excellent correlation between actual and predicted to moisture:OM were R, V, Lab\*L, Lab\*a, luminosity, luminance, and HSL\*L (CCC > 0.94; Table 2.16). The results indicate that thermal color metrics performed most consistently when environmental conditions remained consistent across sampling periods and that multiple color metrics derived from UAV thermal images can be used to predict moisture and moisture:OM. Lin's CCC values for organic matter predictions based on color metrics ranged from 0.00 to 0.74 when data from Days 1-3 were included, and from 0.08 to 0.69 when Day 3 was excluded. Results indicate that moisture predictions from color metrics derived from UAV thermal imagery were in high agreement (both precision and accuracy) with actual moisture values, whereas organic matter and the moisture:OM ratio were not in strong agreement.

The strong association of brightness- and lightness-associated metrics (luminance, luminosity, V, Lab\*L; Table 2.16) to moisture aligns with physical basis of thermal imaging. As moisture alters surface emissivity and reflectance it creates consistent shifts in pixel intensity detectable by thermal-derived color channels. Prior studies in remote sensing and precision agriculture have reported similar relationships, noting that moisture increases the uniformity of

surface heat signatures and modifies visible-spectrum brightness, thereby improving detectability through RGB and thermal indices (Jia et al., 2023; Shi et al., 2024; Verstraeten et al., 2006; Zhang & Zhou, 2016). Improved performance in the absence of precipitation further corroborates findings from crop and soil imaging applications, where weather-induced variability can disrupt spectral signals and introduce noise into prediction models (Aragon et al., 2020; Miller et al., 2003; Wan et al., 2024). In the context of feedlot pen surfaces, the results suggest that UAV-based thermal imaging is most effective for monitoring nutrient-related moisture conditions when conducted in consistent environments.

## **Implications**

Pen-level moisture in cattle pens may be reliably predicted from thermal-derived color metrics due to the strong agreement between brightness and red color metrics to moisture values, particularly when environmental conditions are consistent and precipitation events are excluded. The strongest moisture-associated color metrics were Lab\*a, Red, Value, Lab\*L, HSL\*L, Luminance, and Luminosity, which captured moisture differences between feeding programs. The consistency of the agreement across multiple days underscores the potential of UAV-based thermal imaging for automated monitoring of pen-surface conditions, offering a scalable tool for feedlot management systems aimed at improving environmental control, reducing labor demands, and supporting timely decision making. However, consistent UAV application in outdoor feedlot environments presents practical challenges, as precipitation and rapidly changing surface moisture conditions can alter thermal signatures independent of underlying pen characteristics. Therefore, implementation of UAV-based pen condition scoring systems will require standardized operational guidelines that account for recent weather patterns and surface drying conditions to ensure reliable interpretation of imagery.

## Tables

**Table 2.1 Nutrient composition (% of DM) of diets fed in high-intake and low-intake feeding programs.**

| Item                                                                                                                                                                          | Feeding Program <sup>a</sup> |       |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|-------|
|                                                                                                                                                                               | LOW                          | HIGH  |
| Nutrient Composition, % of DM                                                                                                                                                 |                              |       |
| Dry matter, % as-is                                                                                                                                                           | 78.00                        | 80.00 |
| Organic matter                                                                                                                                                                | 93.40                        | 95.64 |
| Crude protein                                                                                                                                                                 | 14.94                        | 14.39 |
| Neutral detergent fiber                                                                                                                                                       | 33.24                        | 19.37 |
| Acid detergent fiber                                                                                                                                                          | 17.04                        | 8.12  |
| Calcium                                                                                                                                                                       | 0.88                         | 0.58  |
| Phosphorus                                                                                                                                                                    | 0.62                         | 0.61  |
| Calculated Composition <sup>b</sup>                                                                                                                                           |                              |       |
| NE <sub>m</sub> , Mcal/kg                                                                                                                                                     | 0.91                         | 1.04  |
| NE <sub>g</sub> , Mcal/kg                                                                                                                                                     | 0.58                         | 0.72  |
| <sup>a</sup> HIGH = high-intake, ad libitum, energy-dense (64 Mcal/cwt NE <sub>g</sub> ); LOW = low-intake, limit-fed to 0.91 kg/head/day gain (50 Mcal/cwt NE <sub>g</sub> ) |                              |       |
| <sup>b</sup> Net energy of maintenance (NE <sub>m</sub> ) and net energy of gain (NE <sub>g</sub> ) calculated using NASEM (2016) values of diet ingredients                  |                              |       |

**Table 2.2 Ingredient composition of diets fed in high-intake and low-intake feeding programs.**

| Item                                        | Feeding Program <sup>a</sup> |       |
|---------------------------------------------|------------------------------|-------|
|                                             | LOW                          | HIGH  |
| Dry matter intake, kg/head/day <sup>b</sup> | 8.18                         | 10.31 |
| Ingredient Inclusion, % of DM               |                              |       |
| Wet corn gluten                             | 40.01                        | 30.00 |
| Prairie hay                                 | 30.00                        | 7.00  |
| Dry rolled corn                             | 24.37                        | 58.91 |
| Supplement <sup>c</sup>                     | 5.62                         | 4.09  |

<sup>a</sup> HIGH = high-intake, ad libitum, energy-dense (64 Mcal/cwt NEg); LOW = low-intake, limit-fed to 0.91 kg/head/day gain (50 Mcal/cwt NEg)

<sup>b</sup> Dry matter intake = average DMI per head per day over entire backgrounding period

<sup>c</sup> Supplement formulated to contain (DM-basis): 11.3% crude protein, 20.5%, calcium, 0.6% phosphorus

**Table 2.3 Weather and environmental conditions relevant to experimental sampling period.**

| Day of Trial <sup>a</sup> | Air Temperature |        | Relative Humidity | Rain Amount | Wind Speed |         | Solar Radiation         |
|---------------------------|-----------------|--------|-------------------|-------------|------------|---------|-------------------------|
|                           | Max °C          | Min °C | Avg %             | Total cm    | Avg m/s    | Max m/s | Total MJ/m <sup>2</sup> |
| Day -4                    | 32.6            | 16.5   | 61                | 0           | 1.2        | 5.6     | 25.8                    |
| Day -3                    | 30.8            | 18.5   | 68.7              | 0           | 1.8        | 6.2     | 27                      |
| Day -2                    | 33.5            | 21.6   | 65.8              | 0           | 2.1        | 6.3     | 25.4                    |
| Day -1                    | 35.2            | 25.2   | 53.4              | 0           | 3.4        | 9.9     | 22.8                    |
| Day 1                     | 36.3            | 25.6   | 55                | 0           | 2.3        | 7.8     | 26.2                    |
| Day 2                     | 39.2            | 26.2   | 55                | 0           | 2.6        | 7.3     | 25.8                    |
| Day 3                     | 31.2            | 19.7   | 76.3              | 2.57        | 2.2        | 11.1    | 20.8                    |

<sup>a</sup> Day of Trial includes 4 days prior to first sample collection day

*Source: Kansas Mesonet, Manhattan, KS weather station*

**Table 2.4 Effects of feeding program (HIGH vs. LOW) and pen-surface quadrant on nutrient components and UAV thermal-derived color metrics for a single day (Objective 1).**

| Item                             | Feeding<br>Program <sup>a</sup> | Quadrant        | Feeding Program <sup>a</sup><br>x Quadrant |
|----------------------------------|---------------------------------|-----------------|--------------------------------------------|
|                                  | <i>P</i> - Value                |                 |                                            |
| Nutrient Components              |                                 |                 |                                            |
| Moisture, % as-is                | <b>0.01</b>                     | <b>&lt;0.01</b> | 0.25                                       |
| Organic Matter, % of DM          | 0.34                            | <b>&lt;0.01</b> | 0.97                                       |
| Moisture:Organic Matter          | <b>0.02</b>                     | <b>&lt;0.01</b> | 0.08                                       |
| Neutral Detergent Fiber, % as-is | 0.61                            | <b>&lt;0.01</b> | 0.86                                       |
| Acid Detergent Fiber, % as-is    | 0.14                            | <b>&lt;0.01</b> | 0.54                                       |
| Neutral Detergent Fiber, % of DM | 0.39                            | <b>&lt;0.01</b> | 0.77                                       |
| Acid Detergent Fiber, % of DM    | 0.30                            | 0.14            | 0.62                                       |
| Nitrogen, % of DM                | <b>0.01</b>                     | <b>&lt;0.01</b> | 1.00                                       |
| Direct Temperature, °C           | 0.23                            | <b>&lt;0.01</b> | <b>0.02</b>                                |
| Color Metrics                    |                                 |                 |                                            |
| Red                              | <b>&lt;0.01</b>                 | 0.46            | 1.00                                       |
| Green                            | <b>0.02</b>                     | 0.84            | 0.86                                       |
| Blue                             | 0.25                            | 0.85            | 0.57                                       |
| Hue                              | <b>0.02</b>                     | 0.52            | 0.63                                       |
| Saturation                       | <b>0.01</b>                     | 0.57            | 0.61                                       |
| Value                            | <b>&lt;0.01</b>                 | 0.52            | 1.00                                       |
| Lab*L                            | <b>&lt;0.01</b>                 | 0.69            | 0.99                                       |
| Lab*a                            | <b>&lt;0.01</b>                 | <b>0.05</b>     | 1.00                                       |
| Lab*b                            | <b>&lt;0.01</b>                 | 0.98            | 0.57                                       |
| HSL*L                            | <b>&lt;0.01</b>                 | 0.78            | 0.99                                       |
| Luminance <sup>b</sup>           | <b>&lt;0.01</b>                 | 0.76            | 0.97                                       |
| Luminosity <sup>c</sup>          | <b>&lt;0.01</b>                 | 0.78            | 0.99                                       |
| Mean RGB <sup>d</sup>            | <b>&lt;0.01</b>                 | 0.66            | 0.98                                       |

<sup>a</sup> HIGH = high-intake, ad libitum, energy-dense (64 Mcal/cwt NEg); LOW = low-intake, limited to 0.91 kg/head/day gain (50 Mcal/cwt NEg)

<sup>b</sup> Luminance = (min(RGB) + max(RGB))/2

<sup>c</sup> Luminosity = 0.2126(R) + 0.7152(G) + 0.0722(B)

<sup>d</sup> Mean RGB = (R+G+B)/2

**Table 2.5 Effect of feeding program (HIGH vs. LOW) on nutrient components for a singular day (Objective 1).**

| Item                                                                                                                                                | Feeding Program <sup>a</sup> |       | SE   | P-Value         |
|-----------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|-------|------|-----------------|
|                                                                                                                                                     | HIGH                         | LOW   |      |                 |
| Moisture, % as-is                                                                                                                                   | 44.0                         | 34.0  | 0.03 | <b>0.01</b>     |
| Organic Matter, % of DM                                                                                                                             | 45.0                         | 48.0  | 0.02 | 0.34            |
| Moisture:Organic Matter                                                                                                                             | 98.0                         | 71.0  | 0.08 | <b>0.02</b>     |
| Neutral Detergent Fiber, % as-is                                                                                                                    | 46.20                        | 42.00 | 5.77 | 0.61            |
| Acid Detergent Fiber, % as-is                                                                                                                       | 24.70                        | 15.90 | 4.25 | 0.14            |
| Neutral Detergent Fiber, % of DM                                                                                                                    | 23.30                        | 25.80 | 2.06 | 0.39            |
| Acid Detergent Fiber, % of DM                                                                                                                       | 12.25                        | 9.84  | 1.62 | 0.30            |
| Nitrogen, % of DM                                                                                                                                   | 18.50                        | 21.50 | 0.82 | <b>&lt;0.01</b> |
| Direct Temperature, °C                                                                                                                              | 21.8                         | 23.7  | 1.1  | 0.23            |
| <sup>a</sup> HIGH = high-intake, ad libitum, energy-dense (64 Mcal/cwt NEg); LOW = low-intake, limit-fed to 0.91 kg/head/day gain (50 Mcal/cwt NEg) |                              |       |      |                 |

**Table 2.6 Effect of feeding program (HIGH vs. LOW) on thermal-derived color metrics for a singular day (Objective 1).**

| Item                    | Feeding Program <sup>a</sup> |        | SE    | P-Value         |
|-------------------------|------------------------------|--------|-------|-----------------|
|                         | HIGH                         | LOW    |       |                 |
| Red                     | 56.40                        | 145.30 | 18.10 | <b>&lt;0.01</b> |
| Green                   | 12.90                        | 52.40  | 12.10 | <b>0.02</b>     |
| Blue                    | 82.60                        | 103.80 | 13.20 | 0.25            |
| Hue                     | 275.00                       | 305.00 | 9.18  | <b>0.02</b>     |
| Saturation              | 84.30                        | 69.10  | 4.37  | <b>0.01</b>     |
| Value                   | 32.40                        | 59.10  | 6.04  | <b>&lt;0.01</b> |
| HSL*L                   | 47.70                        | 102.20 | 13.70 | <b>&lt;0.01</b> |
| Lab*L                   | 13.90                        | 37.30  | 5.52  | <b>&lt;0.01</b> |
| Lab*a                   | 29.50                        | 43.00  | 1.86  | <b>&lt;0.01</b> |
| Lab*b                   | -32.81                       | -9.44  | 5.57  | <b>&lt;0.01</b> |
| Luminance <sup>b</sup>  | 33.80                        | 86.00  | 13.60 | <b>&lt;0.01</b> |
| Luminosity <sup>c</sup> | 47.70                        | 101.20 | 13.70 | <b>&lt;0.01</b> |
| Mean RGB <sup>d</sup>   | 50.60                        | 100.50 | 13.60 | <b>&lt;0.01</b> |

<sup>a</sup> HIGH = high-intake, ad libitum, energy-dense (64 Mcal/cwt NEg); LOW = low-intake, limit-fed to 0.91 kg/head/day gain (50 Mcal/cwt NEg)

<sup>b</sup> Luminance = (min(RGB) + max(RGB))/2

<sup>c</sup> Luminosity = 0.2126(R) + 0.7152(G) + 0.0722(B)

<sup>d</sup> Mean RGB = (R+G+B)/2

**Table 2.7 Estimated nutrient components by quadrant for a singular day (Objective 1).**

| Item                    | Quadrant <sup>1</sup> |                     |                     |                     |                     |                     |                     |                     |                      |                    |                    |                     |                     |                     |                     | SE   | P - value       |
|-------------------------|-----------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|----------------------|--------------------|--------------------|---------------------|---------------------|---------------------|---------------------|------|-----------------|
|                         | 1                     | 2                   | 3                   | 4                   | 5                   | 6                   | 7                   | 8                   | 9                    | 10                 | 11                 | 12                  | 13                  | 14                  | 15                  |      |                 |
| Moisture, % as-is       | 44.0 <sup>bc</sup>    | 34.0 <sup>cde</sup> | 33.0 <sup>cde</sup> | 29.0 <sup>de</sup>  | 28.0 <sup>de</sup>  | 26.0 <sup>de</sup>  | 29.0 <sup>de</sup>  | 35.0 <sup>cde</sup> | 41.0 <sup>bcde</sup> | 55.0 <sup>bc</sup> | 60.0 <sup>b</sup>  | 68.0 <sup>a</sup>   | 44.0 <sup>bcd</sup> | 34.0 <sup>cde</sup> | 31.0 <sup>de</sup>  | 4.00 | <b>&lt;0.01</b> |
| Organic Matter, % of DM | 58.0 <sup>a</sup>     | 52.0 <sup>ab</sup>  | 44.0 <sup>c</sup>   | 43.0 <sup>def</sup> | 39.0 <sup>def</sup> | 41.0 <sup>def</sup> | 42.0 <sup>def</sup> | 46.0 <sup>def</sup> | 52.0 <sup>ab</sup>   | 56.0 <sup>ab</sup> | 55.0 <sup>ab</sup> | 51.0 <sup>def</sup> | 41.0 <sup>def</sup> | 37.0 <sup>ef</sup>  | 40.0 <sup>def</sup> | 2.00 | <b>&lt;0.01</b> |
| Moisture:Organic Matter | 0.77 <sup>a</sup>     | 0.67 <sup>a</sup>   | 0.77 <sup>a</sup>   | 0.69 <sup>a</sup>   | 0.74 <sup>a</sup>   | 0.67 <sup>a</sup>   | 0.74 <sup>a</sup>   | 0.77 <sup>a</sup>   | 0.79 <sup>a</sup>    | 0.81 <sup>a</sup>  | 1.08 <sup>b</sup>  | 1.34 <sup>c</sup>   | 1.10 <sup>b</sup>   | 0.92 <sup>d</sup>   | 0.79 <sup>c</sup>   | 0.10 | <b>&lt;0.01</b> |
| NDF, % as-is            | 58.80 <sup>b</sup>    | 47.60 <sup>b</sup>  | 38.20 <sup>c</sup>  | 36.50 <sup>c</sup>  | 29.90 <sup>c</sup>  | 31.80 <sup>c</sup>  | 33.30 <sup>c</sup>  | 33.00 <sup>c</sup>  | 48.50 <sup>b</sup>   | 54.20 <sup>b</sup> | 66.20 <sup>a</sup> | 81.80 <sup>a</sup>  | 37.80 <sup>c</sup>  | 32.20 <sup>c</sup>  | 31.90 <sup>c</sup>  | 6.78 | <b>&lt;0.01</b> |
| ADF, % as-is            | 29.60 <sup>a</sup>    | 21.70 <sup>b</sup>  | 18.60 <sup>b</sup>  | 16.60 <sup>b</sup>  | 14.50 <sup>b</sup>  | 15.00 <sup>b</sup>  | 13.90 <sup>c</sup>  | 13.90 <sup>c</sup>  | 22.30 <sup>b</sup>   | 24.70 <sup>b</sup> | 30.40 <sup>a</sup> | 36.40 <sup>a</sup>  | 18.30 <sup>b</sup>  | 15.40 <sup>b</sup>  | 13.40 <sup>c</sup>  | 4.63 | <b>&lt;0.01</b> |
| NDF, % of DM            | 31.60 <sup>a</sup>    | 28.00 <sup>a</sup>  | 25.20 <sup>b</sup>  | 26.20 <sup>a</sup>  | 21.30 <sup>b</sup>  | 22.10 <sup>b</sup>  | 23.00 <sup>b</sup>  | 21.40 <sup>b</sup>  | 27.80 <sup>a</sup>   | 27.70 <sup>a</sup> | 25.90 <sup>a</sup> | 25.80 <sup>a</sup>  | 20.80 <sup>b</sup>  | 20.20 <sup>b</sup>  | 21.10 <sup>b</sup>  | 2.58 | <b>&lt;0.01</b> |
| ADF, % of DM            | 15.59                 | 12.01               | 12.01               | 11.84               | 10.29               | 10.04               | 9.12                | 8.89                | 12.37                | 12.49              | 11.74              | 11.02               | 9.81                | 9.57                | 8.86                | 1.87 | 0.14            |
| Nitrogen, % of DM       | 23.10 <sup>a</sup>    | 22.10 <sup>a</sup>  | 19.60 <sup>b</sup>  | 18.90 <sup>c</sup>  | 17.70 <sup>d</sup>  | 18.50 <sup>c</sup>  | 18.60 <sup>c</sup>  | 19.90 <sup>b</sup>  | 22.40 <sup>a</sup>   | 23.10 <sup>a</sup> | 23.00 <sup>a</sup> | 20.90 <sup>b</sup>  | 17.80 <sup>c</sup>  | 16.70 <sup>d</sup>  | 17.70 <sup>c</sup>  | 0.95 | <b>&lt;0.01</b> |
| Temperature, °C         | 23.10 <sup>a</sup>    | 23.2 <sup>a</sup>   | 23.5 <sup>a</sup>   | 25.5 <sup>a</sup>   | 23.8 <sup>a</sup>   | 24.3 <sup>a</sup>   | 23.6 <sup>a</sup>   | 23.7 <sup>a</sup>   | 23.4 <sup>a</sup>    | 21.8 <sup>ab</sup> | 20.9 <sup>b</sup>  | 20.8 <sup>b</sup>   | 21.3 <sup>ab</sup>  | 22.4 <sup>ab</sup>  | 22.3 <sup>ab</sup>  | 0.99 | <b>&lt;0.01</b> |

<sup>1</sup> Quadrants are visually defined in Figure 2.1

<sup>abcde</sup> Mean within a row with differing superscripts differ ( $P < 0.05$ )

Values derived from Fisher's least significant difference (LSD) pairwise comparisons with no adjustments for multiple testing

**Table 2.8 Estimated UAV thermal-derived color metrics by quadrant for a singular day (Objective 1).**

| Item                    | Quadrant           |                    |                    |                    |                    |                    |                    |                    |                    |                    |                    |                    |                    |                    |                    | SE    | P - value   |
|-------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|-------|-------------|
|                         | 1                  | 2                  | 3                  | 4                  | 5                  | 6                  | 7                  | 8                  | 9                  | 10                 | 11                 | 12                 | 13                 | 14                 | 15                 |       |             |
| Red                     | 96.70              | 108.50             | 98.70              | 88.00              | 98.80              | 130.20             | 123.70             | 119.20             | 105.80             | 85.20              | 75.80              | 83.00              | 87.70              | 99.80              | 112.20             | 19.90 | 0.46        |
| Green                   | 29.00              | 33.20              | 45.00              | 45.30              | 37.00              | 41.00              | 38.70              | 36.30              | 32.00              | 22.50              | 21.50              | 23.70              | 25.50              | 27.80              | 31.00              | 12.70 | 0.84        |
| Blue                    | 97.20              | 96.80              | 98.70              | 92.50              | 90.70              | 102.80             | 99.80              | 97.50              | 90.00              | 92.70              | 80.30              | 81.00              | 85.00              | 93.70              | 98.80              | 12.50 | 0.85        |
| Hue                     | 295.00             | 302.00             | 297.00             | 289.00             | 297.00             | 313.00             | 309.00             | 247.00             | 242.00             | 289.00             | 286.00             | 288.00             | 292.00             | 297.00             | 304.00             | 21.10 | 0.52        |
| Saturation              | 77.40              | 76.00              | 70.80              | 71.40              | 74.70              | 74.00              | 75.30              | 76.8               | 78.70              | 80.90              | 79.40              | 78.90              | 80.10              | 78.80              | 77.20              | 4.37  | 0.57        |
| Value                   | 44.20              | 47.10              | 44.60              | 41.40              | 44.40              | 54.80              | 53.80              | 52.20              | 48.00              | 41.80              | 36.60              | 39.10              | 42.80              | 46.10              | 48.60              | 6.73  | 0.52        |
| HSL*L                   | 70.90              | 76.70              | 79.30              | 75.50              | 75.20              | 90.30              | 87.90              | 84.20              | 75.90              | 64.60              | 57.40              | 61.70              | 67.30              | 72.80              | 77.50              | 14.40 | 0.78        |
| Lab*L                   | 24.40              | 27.10              | 27.40              | 25.30              | 25.60              | 32.70              | 31.00              | 29.80              | 26.30              | 21.10              | 18.40              | 20.50              | 21.60              | 24.70              | 27.80              | 5.87  | 0.69        |
| Lab*a                   | 36.90 <sup>b</sup> | 38.70 <sup>b</sup> | 30.20 <sup>b</sup> | 24.20 <sup>a</sup> | 32.50 <sup>b</sup> | 43.30 <sup>c</sup> | 42.20 <sup>c</sup> | 41.80 <sup>c</sup> | 38.90 <sup>b</sup> | 36.20 <sup>b</sup> | 31.40 <sup>b</sup> | 32.20 <sup>b</sup> | 34.90 <sup>b</sup> | 38.70 <sup>b</sup> | 41.70 <sup>b</sup> | 4.20  | <b>0.05</b> |
| Lab*b                   | -25.50             | -21.00             | -21.40             | -20.10             | -19.30             | -16.30             | -17.10             | -17.80             | -18.60             | -28.00             | -24.00             | -20.80             | -22.30             | -23.10             | -21.60             | 6.40  | 0.98        |
| Luminance <sup>2</sup>  | 57.00              | 62.90              | 67.20              | 63.50              | 61.60              | 74.70              | 71.10              | 68.10              | 60.70              | 49.20              | 44.50              | 47.90              | 50.90              | 56.90              | 63.00              | 13.90 | 0.76        |
| Luminosity <sup>3</sup> | 70.90              | 76.70              | 79.30              | 75.50              | 75.20              | 90.30              | 87.90              | 84.20              | 75.90              | 64.60              | 57.40              | 61.70              | 67.30              | 72.80              | 77.50              | 14.40 | 0.78        |
| Mean RGB <sup>4</sup>   | 74.30              | 79.50              | 80.80              | 75.30              | 75.50              | 91.30              | 87.40              | 84.30              | 75.90              | 66.80              | 59.20              | 62.60              | 66.10              | 73.80              | 80.70              | 13.70 | 0.66        |

<sup>1</sup> Quadrants are visually defined in Figure 2.1

<sup>2</sup> Luminance = (min(RGB) + max(RGB))/2

<sup>3</sup> Luminosity = 0.2126(R) + 0.7152(G) + 0.0722(B)

<sup>4</sup> Mean RGB = (R+G+B)/2

<sup>abc</sup> Mean within a row with differing superscripts differ ( $P < 0.05$ ).

*Values derived from Fisher's least significant difference (LSD) pairwise comparisons with no adjustments for multiple testing*

**Table 2.9 Effects of feeding program (HIGH vs. LOW) and quadrant on nutrient components and UAV thermal-derived color metrics using data from all collection days (Objective 2).**

| Item                    | Feeding Program <sup>a</sup> x Quadrant |                 |             |
|-------------------------|-----------------------------------------|-----------------|-------------|
|                         | Feeding Program <sup>a</sup>            | Quadrant        | Quadrant    |
|                         | <i>P</i> - Value                        |                 |             |
| Moisture, % as-is       | <b>&lt;0.01</b>                         | <b>&lt;0.01</b> | 0.15        |
| Organic Matter, % of DM | 0.42                                    | <b>&lt;0.01</b> | 0.83        |
| Moisture:Organic Matter | <b>&lt;0.01</b>                         | <b>&lt;0.01</b> | <b>0.05</b> |
| Temperature, °C         | 0.06                                    | <b>&lt;0.01</b> | 0.53        |
| Red                     | <b>&lt;0.01</b>                         | <b>0.02</b>     | 1.00        |
| Green                   | <b>&lt;0.01</b>                         | 0.45            | 0.98        |
| Blue                    | 0.52                                    | <b>0.04</b>     | 0.81        |
| Hue                     | 0.52                                    | 0.59            | 0.62        |
| Saturation              | <b>&lt;0.01</b>                         | 0.38            | 0.92        |
| Value                   | <b>&lt;0.01</b>                         | 0.07            | 1.00        |
| HSL*L                   | <b>&lt;0.01</b>                         | 0.15            | 1.00        |
| Lab*L                   | <b>&lt;0.01</b>                         | <b>0.04</b>     | 1.00        |
| Lab*a                   | <b>&lt;0.01</b>                         | <b>&lt;0.01</b> | 1.00        |
| Lab*b                   | 0.11                                    | 0.85            | 0.85        |
| Luminance <sup>b</sup>  | <b>&lt;0.01</b>                         | 0.06            | 1.00        |
| Luminosity <sup>c</sup> | <b>&lt;0.01</b>                         | 0.15            | 1.00        |
| Mean RGB <sup>d</sup>   | <b>&lt;0.01</b>                         | <b>0.02</b>     | 1.00        |

<sup>a</sup> HIGH = high-intake, ad libitum, energy-dense (64 Mcal/cwt NEg); LOW = low-intake, limit-fed to 0.91 kg/head/day gain (50 Mcal/cwt NEg)

<sup>b</sup> Luminance = (min(RGB) + max(RGB))/2

<sup>c</sup> Luminosity = 0.2126(R) + 0.7152(G) + 0.0722(B)

<sup>d</sup> Mean RGB = (R+G+B)/2

**Table 2.10 Effect of feeding program (HIGH vs. LOW) on nutrient components using data from all collection days (Objective 2).**

| Item                                                                                                                                                | Feeding Program <sup>a</sup> |      | SE   | <i>P</i> -Value |
|-----------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|------|------|-----------------|
|                                                                                                                                                     | HIGH                         | LOW  |      |                 |
| Moisture, % as-is                                                                                                                                   | 52.1                         | 42.1 | 8.31 | <b>&lt;0.01</b> |
| Organic Matter, % of DM                                                                                                                             | 44.9                         | 47.2 | 2.2  | 0.42            |
| Moisture:Organic Matter                                                                                                                             | 1.17                         | 0.91 | 0.20 | <b>&lt;0.01</b> |
| Temperature, °C                                                                                                                                     | 24.0                         | 26.6 | 0.97 | 0.06            |
| <sup>a</sup> HIGH = high-intake, ad libitum, energy-dense (64 Mcal/cwt NEg); LOW = low-intake, limit-fed to 0.91 kg/head/day gain (50 Mcal/cwt NEg) |                              |      |      |                 |

**Table 2.11 Effect of feeding program (HIGH vs. LOW) on thermal-derived color metrics using data from all collection days (Objective 2).**

| Item                    | Feeding Program <sup>a</sup> |       | SE   | P-Value         |
|-------------------------|------------------------------|-------|------|-----------------|
|                         | HIGH                         | LOW   |      |                 |
| Red                     | 74.7                         | 124.9 | 11.7 | <b>&lt;0.01</b> |
| Green                   | 14.7                         | 37.9  | 7.7  | <b>0.01</b>     |
| Blue                    | 90.7                         | 98.1  | 8.1  | 0.52            |
| Hue                     | 284                          | 291   | 7.5  | 0.52            |
| Saturation              | 83.4                         | 74.9  | 2.8  | <b>&lt;0.01</b> |
| Value                   | 37                           | 53.6  | 3.9  | <b>&lt;0.01</b> |
| HSL*L                   | 55.1                         | 87.4  | 8.0  | <b>&lt;0.01</b> |
| Lab*L                   | 18.4                         | 31.6  | 2.9  | <b>&lt;0.01</b> |
| Lab*a                   | 34.8                         | 42.1  | 1.8  | <b>&lt;0.01</b> |
| Lab*b                   | -32.9                        | -16.9 | 7.6  | 0.11            |
| Luminance <sup>b</sup>  | 41.6                         | 71.1  | 7.4  | <b>&lt;0.01</b> |
| Luminosity <sup>c</sup> | 55.1                         | 87.4  | 8.0  | <b>&lt;0.01</b> |
| Mean RGB <sup>d</sup>   | 60.9                         | 87.9  | 5.8  | <b>&lt;0.01</b> |

<sup>a</sup> HIGH = high-intake, ad libitum, energy-dense (64 Mcal/cwt NEg); LOW = low-intake, limit-fed to 0.91 kg/head/day gain (50 Mcal/cwt NEg)

<sup>b</sup> Luminance = (min(RGB) + max(RGB))/2

<sup>c</sup> Luminosity = 0.2126(R) + 0.7152(G) + 0.0722(B)

<sup>d</sup> Mean RGB = (R+G+B)/2

**Table 2.12 Estimated nutrient components by quadrant using data from all collection days (Objective 2).**

| Item              | Quadrant <sup>1</sup> |                   |                   |                   |                   |                   |                   |                   |                    |                     |                   |                   |                   |                   |                   | SE   | P-value         |
|-------------------|-----------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|---------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|-----------------|
|                   | 1                     | 2                 | 3                 | 4                 | 5                 | 6                 | 7                 | 8                 | 9                  | 10                  | 11                | 12                | 13                | 14                | 15                |      |                 |
| Moisture, % as-is | 0.50 <sup>c</sup>     | 46.9 <sup>c</sup> | 39.9 <sup>c</sup> | 38.7 <sup>c</sup> | 37.8 <sup>c</sup> | 36.3 <sup>c</sup> | 42.4 <sup>c</sup> | 46.7 <sup>c</sup> | 47.7 <sup>c</sup>  | 52.9 <sup>abc</sup> | 63.9 <sup>a</sup> | 69.5 <sup>a</sup> | 50.5 <sup>c</sup> | 42.2 <sup>c</sup> | 40.9 <sup>c</sup> | 8.60 | <b>&lt;0.01</b> |
| OM, % of DM       | 56.6 <sup>ab</sup>    | 50.3 <sup>b</sup> | 43.2 <sup>c</sup> | 42.0 <sup>c</sup> | 40.4 <sup>c</sup> | 40.6 <sup>c</sup> | 42.4 <sup>c</sup> | 46.4 <sup>b</sup> | 52.0 <sup>ab</sup> | 55.0 <sup>a</sup>   | 54.8 <sup>a</sup> | 51.0 <sup>a</sup> | 38.4 <sup>c</sup> | 37.7 <sup>c</sup> | 39.5 <sup>c</sup> | 2.41 | <b>&lt;0.01</b> |
| Moisture:OM       | 0.50 <sup>b</sup>     | 0.47 <sup>b</sup> | 0.40 <sup>b</sup> | 0.39 <sup>b</sup> | 0.38 <sup>b</sup> | 0.36 <sup>c</sup> | 0.42 <sup>b</sup> | 0.47 <sup>b</sup> | 0.48 <sup>b</sup>  | 0.53 <sup>b</sup>   | 0.64 <sup>a</sup> | 0.70 <sup>a</sup> | 0.51 <sup>a</sup> | 0.42 <sup>b</sup> | 0.41 <sup>b</sup> | 0.09 | <b>&lt;0.01</b> |
| Temperature, °C   | 25.5                  | 26.3              | 26.1              | 26.8              | 26.4              | 27.2              | 26.5              | 25.9              | 25.6               | 23.6                | 22.8              | 22.8              | 23.9              | 25.4              | 25.4              | 1.29 | 0.08            |

<sup>1</sup> Quadrants are visually defined in Figure 2.1

<sup>abc</sup> Mean within a row with differing superscripts differ ( $P < 0.05$ )

Values derived from Fisher's least significant difference (LSD) pairwise comparisons with no adjustments for multiple testing

**Table 2.13 Estimated UAV thermal-derived color metrics by quadrant using data from all collection days (Objective 2).**

| Item                    | Quadrant <sup>1</sup> |                    |                   |                    |                    |                    |                    |                    |                     |                     |                    |                   |                     |                    |                    | SE   | P-value         |
|-------------------------|-----------------------|--------------------|-------------------|--------------------|--------------------|--------------------|--------------------|--------------------|---------------------|---------------------|--------------------|-------------------|---------------------|--------------------|--------------------|------|-----------------|
|                         | 1                     | 2                  | 3                 | 4                  | 5                  | 6                  | 7                  | 8                  | 9                   | 10                  | 11                 | 12                | 13                  | 14                 | 15                 |      |                 |
| Red                     | 91.8 <sup>a</sup>     | 106.7 <sup>b</sup> | 98.6 <sup>a</sup> | 92.1 <sup>a</sup>  | 101.9 <sup>a</sup> | 131.2 <sup>b</sup> | 123.8 <sup>b</sup> | 118.2 <sup>b</sup> | 105.5 <sup>b</sup>  | 79.8 <sup>a</sup>   | 69.4 <sup>a</sup>  | 78.2 <sup>a</sup> | 84.0 <sup>a</sup>   | 100.9 <sup>a</sup> | 114.2 <sup>b</sup> | 14.8 | <b>0.02</b>     |
| Green                   | 22.6                  | 27.4               | 31.1              | 30.3               | 29.0               | 36.0               | 33.2               | 30.8               | 27.9                | 18.1                | 16.7               | 19.1              | 20.2                | 24.0               | 28.3               | 8.42 | 0.45            |
| Blue                    | 95.9 <sup>c</sup>     | 97.2 <sup>c</sup>  | 98.5 <sup>c</sup> | 95.2 <sup>bc</sup> | 95.3 <sup>bc</sup> | 103.0 <sup>c</sup> | 100.0 <sup>c</sup> | 99.5 <sup>c</sup>  | 92.7 <sup>abc</sup> | 91.7 <sup>abc</sup> | 80.7 <sup>ab</sup> | 78.8 <sup>a</sup> | 88.7 <sup>abc</sup> | 97.6 <sup>c</sup>  | 101.4 <sup>c</sup> | 7.7  | <b>0.04</b>     |
| Hue                     | 293                   | 302                | 297               | 291                | 297                | 313                | 281                | 248                | 243                 | 287                 | 283                | 287               | 290                 | 298                | 304                | 20.4 | 0.59            |
| Saturation              | 80.0                  | 78.2               | 76.1              | 77.5               | 78.1               | 75.7               | 77.5               | 78.2               | 80.6                | 82.7                | 81.9               | 80.9              | 82.0                | 79.9               | 78.2               | 3.11 | 0.38            |
| Value                   | 43.1                  | 46.7               | 43.8              | 42.3               | 45.1               | 54.3               | 53.1               | 50.7               | 47.7                | 41.1                | 36.3               | 37.8              | 41.8                | 45.7               | 49.2               | 4.83 | 0.07            |
| HSL*L                   | 66.8                  | 73.7               | 72.0              | 69.7               | 72.7               | 87.8               | 84.4               | 80.1               | 73.4                | 61.9                | 55.3               | 58.4              | 64.1                | 70.9               | 77.5               | 9.45 | 0.15            |
| Lab*L                   | 22.9 <sup>a</sup>     | 26.5 <sup>a</sup>  | 25.7 <sup>a</sup> | 24.2 <sup>a</sup>  | 25.7 <sup>a</sup>  | 32.6 <sup>b</sup>  | 30.7 <sup>b</sup>  | 29.4 <sup>b</sup>  | 26.3 <sup>a</sup>   | 19.9 <sup>a</sup>   | 17.1 <sup>a</sup>  | 19.2 <sup>a</sup> | 20.9 <sup>a</sup>   | 25.0 <sup>a</sup>  | 28.3 <sup>b</sup>  | 3.81 | <b>0.04</b>     |
| Lab*a                   | 37.8 <sup>a</sup>     | 40.5 <sup>a</sup>  | 36.2 <sup>a</sup> | 33.4 <sup>a</sup>  | 37.7 <sup>a</sup>  | 45.2 <sup>c</sup>  | 43.9 <sup>b</sup>  | 43.2 <sup>b</sup>  | 40.2 <sup>a</sup>   | 36.0 <sup>a</sup>   | 31.3 <sup>a</sup>  | 31.6 <sup>a</sup> | 35.9 <sup>a</sup>   | 40.3 <sup>a</sup>  | 43.0 <sup>b</sup>  | 3.13 | <b>&lt;0.01</b> |
| Lab*b                   | -29.1                 | -24.3              | -26.5             | -26.7              | -25.2              | -18.6              | -19.2              | -21.3              | -21.8               | -31.3               | -28.1              | -22.8             | -27.5               | -26.7              | -24.1              | 7.27 | 0.85            |
| Luminance <sup>2</sup>  | 51.9                  | 59.3               | 59.3              | 56.6               | 58.8               | 72.4               | 68.2               | 65.1               | 58.7                | 45.2                | 40.0               | 43.9              | 47.4                | 55.7               | 62.6               | 8.91 | 0.06            |
| Luminosity <sup>3</sup> | 66.8                  | 73.7               | 72.0              | 69.7               | 72.7               | 87.8               | 84.4               | 80.1               | 73.4                | 61.9                | 55.3               | 58.4              | 64.1                | 70.9               | 77.5               | 9.45 | 0.15            |
| Mean RGB <sup>4</sup>   | 70.9 <sup>a</sup>     | 77.9 <sup>a</sup>  | 77.1 <sup>a</sup> | 73.7 <sup>a</sup>  | 76.4 <sup>a</sup>  | 91.0 <sup>d</sup>  | 86.5 <sup>d</sup>  | 83.7 <sup>a</sup>  | 76.1 <sup>a</sup>   | 63.9 <sup>b</sup>   | 56.4 <sup>c</sup>  | 59.6 <sup>b</sup> | 65.2 <sup>a</sup>   | 75.1 <sup>a</sup>  | 82.2 <sup>c</sup>  | 7.93 | <b>0.02</b>     |

<sup>1</sup> Quadrants are visually defined in Figure 2.1

<sup>2</sup> Luminance = (min(RGB) + max(RGB))/2

<sup>3</sup> Luminosity = 0.2126(R) + 0.7152(G) + 0.0722(B)

<sup>4</sup> Mean RGB = (R+G+B)/2

<sup>abc</sup> Mean within a row with differing superscripts differ ( $P < 0.05$ ).

*Values derived from Fisher's least significant difference (LSD) pairwise comparisons with no adjustments for multiple testing*

**Table 2.14 Association of pen-level nutrient components to UAV thermal imagery-derived color metrics and estimated nutrient levels from all collection days (Objective 3).**

| Item                 | Color Metric            | Feeding Program <sup>a</sup> |      | SE   | Feeding Program <sup>a</sup> | Color Metric    |
|----------------------|-------------------------|------------------------------|------|------|------------------------------|-----------------|
|                      |                         | HIGH                         | LOW  |      | <i>P</i> -value              |                 |
| Moisture, %<br>as-is | Red                     | 50.0                         | 43.7 | 7.92 | <b>&lt;0.01</b>              | <b>&lt;0.01</b> |
|                      | Green                   | 50.1                         | 43.2 | 7.69 | <b>&lt;0.01</b>              | <b>&lt;0.01</b> |
|                      | Blue                    | 51.7                         | 42.3 | 8.51 | <b>&lt;0.01</b>              | <b>&lt;0.01</b> |
|                      | Hue                     | 52.0                         | 42.1 | 8.28 | <b>&lt;0.01</b>              | 0.14            |
|                      | Saturation              | 50.3                         | 43.0 | 7.71 | <b>&lt;0.01</b>              | <b>&lt;0.01</b> |
|                      | Value                   | 49.8                         | 44.1 | 8.11 | <b>0.02</b>                  | <b>&lt;0.01</b> |
|                      | HSL*L                   | 49.8                         | 43.8 | 7.91 | <b>0.01</b>                  | <b>&lt;0.01</b> |
|                      | Lab*L                   | 50.0                         | 43.6 | 7.91 | <b>&lt;0.01</b>              | <b>&lt;0.01</b> |
|                      | Lab*a                   | 51.3                         | 43.2 | 8.54 | <b>&lt;0.01</b>              | <b>&lt;0.01</b> |
|                      | Lab*b                   | 50.0                         | 43.5 | 7.56 | <b>&lt;0.01</b>              | <b>&lt;0.01</b> |
|                      | Luminance <sup>b</sup>  | 50.0                         | 43.5 | 7.82 | <b>0.01</b>                  | <b>&lt;0.01</b> |
|                      | Luminosity <sup>c</sup> | 49.8                         | 43.8 | 7.91 | <b>0.01</b>                  | <b>&lt;0.01</b> |
|                      | Mean RGB <sup>d</sup>   | 50.2                         | 43.4 | 7.98 | <b>0.01</b>                  | <b>&lt;0.01</b> |
| OM, % of DM          | Red                     | 45.1                         | 47.0 | 2.09 | 0.50                         | 0.15            |
|                      | Green                   | 45.2                         | 47.0 | 2.05 | 0.50                         | 0.10            |
|                      | Blue                    | 44.9                         | 47.1 | 2.19 | 0.45                         | 0.19            |
|                      | Hue                     | 44.9                         | 47.2 | 2.19 | 0.44                         | 0.14            |
|                      | Saturation              | 45.3                         | 47.0 | 2.04 | 0.52                         | <b>0.05</b>     |
|                      | Value                   | 45.1                         | 47.0 | 2.11 | 0.50                         | 0.27            |
|                      | HSL*L                   | 45.2                         | 47.0 | 2.08 | 0.51                         | 0.16            |
|                      | Lab*L                   | 45.2                         | 47.0 | 2.08 | 0.44                         | 0.50            |
|                      | Lab*a                   | 44.9                         | 47.1 | 2.18 | 0.45                         | 0.55            |
|                      | Lab*b                   | 45.1                         | 47.0 | 2.09 | 0.49                         | 0.28            |
|                      | Luminance <sup>b</sup>  | 45.2                         | 47.0 | 2.07 | 0.51                         | 0.11            |
|                      | Luminosity <sup>c</sup> | 45.2                         | 47.0 | 2.08 | 0.51                         | 0.16            |
|                      | Mean RGB <sup>d</sup>   | 45.1                         | 47.0 | 2.09 | 0.50                         | 0.11            |
| Moisture:OM          | Red                     | 1.12                         | 0.94 | 0.19 | <b>0.02</b>                  | <b>&lt;0.01</b> |
|                      | Green                   | 1.12                         | 0.93 | 0.18 | <b>0.01</b>                  | <b>&lt;0.01</b> |
|                      | Blue                    | 1.16                         | 0.92 | 0.20 | <b>0.02</b>                  | <b>&lt;0.01</b> |
|                      | Hue                     | 1.17                         | 0.91 | 0.20 | <b>&lt;0.01</b>              | 0.13            |
|                      | Saturation              | 1.12                         | 0.93 | 0.18 | <b>0.02</b>                  | <b>&lt;0.01</b> |
|                      | Value                   | 1.12                         | 0.95 | 0.19 | <b>0.02</b>                  | <b>&lt;0.01</b> |
|                      | HSL*L                   | 1.12                         | 0.95 | 0.18 | <b>0.02</b>                  | <b>&lt;0.01</b> |
|                      | Lab*L                   | 1.12                         | 0.94 | 0.19 | <b>0.02</b>                  | <b>&lt;0.01</b> |

|                         |      |      |      |                 |                 |
|-------------------------|------|------|------|-----------------|-----------------|
| Lab*a                   | 1.15 | 0.93 | 0.20 | <b>0.01</b>     | <b>&lt;0.01</b> |
| Lab*b                   | 1.12 | 0.94 | 0.18 | <b>&lt;0.01</b> | <b>&lt;0.01</b> |
| Luminance <sup>b</sup>  | 1.12 | 0.94 | 0.18 | <b>0.02</b>     | <b>&lt;0.01</b> |
| Luminosity <sup>c</sup> | 1.12 | 0.95 | 0.18 | <b>0.02</b>     | <b>&lt;0.01</b> |
| Mean RGB <sup>d</sup>   | 1.12 | 0.94 | 0.19 | <b>0.02</b>     | <b>&lt;0.01</b> |

---

<sup>a</sup> HIGH = high-intake, ad libitum, energy-dense (64 Mcal/cwt NEg); LOW = low-intake, limited to 0.91 kg/head/day gain (50 Mcal/cwt NEg)

<sup>b</sup> Luminance = (min(RGB) + max(RGB))/2

<sup>c</sup> Luminosity = 0.2126(R) + 0.7152(G) + 0.0722(B)

<sup>d</sup> Mean RGB = (R+G+B)/2

---

**Table 2.15 Pen-level nutrient predictions using UAV thermal-derived color metrics from all collection days (Objective 3).**

| Item        | Color Metric            | <i>P</i> -value | CCC <sup>a</sup> | CCC Lower | CCC Upper | Accuracy <sup>c</sup> |
|-------------|-------------------------|-----------------|------------------|-----------|-----------|-----------------------|
| Moisture    | Red                     | 0.15            | 0.61             | -0.14     | 0.92      | 0.92                  |
|             | Green                   | 0.22            | 0.51             | -0.24     | 0.88      | 0.87                  |
|             | Blue                    | 0.54            | 0.19             | -0.35     | 0.63      | 0.58                  |
|             | Hue                     | 0.58            | 0.15             | -0.34     | 0.57      | 0.53                  |
|             | Saturation              | 0.22            | 0.51             | -0.24     | 0.88      | 0.87                  |
|             | Value                   | 0.19            | 0.55             | -0.21     | 0.89      | 0.89                  |
|             | HSL*L                   | 0.21            | 0.53             | -0.23     | 0.89      | 0.88                  |
|             | Lab*L                   | 0.19            | 0.56             | -0.20     | 0.90      | 0.90                  |
|             | Lab*a                   | 0.18            | 0.57             | -0.18     | 0.90      | 0.90                  |
|             | Lab*b                   | 0.08            | 0.74             | 0.04      | 0.95      | 0.96                  |
|             | Luminance <sup>b</sup>  | 0.21            | 0.53             | -0.22     | 0.89      | 0.88                  |
|             | Luminosity <sup>c</sup> | 0.21            | 0.53             | -0.23     | 0.89      | 0.88                  |
|             | Mean RGB <sup>d</sup>   | 0.29            | 0.43             | -0.29     | 0.84      | 0.82                  |
|             |                         |                 |                  |           |           |                       |
| OM          | Red                     | 0.09            | 0.71             | 0.00      | 0.95      | 0.96                  |
|             | Green                   | 0.08            | 0.74             | 0.04      | 0.95      | 0.96                  |
|             | Blue                    | 0.77            | 0.05             | -0.24     | 0.33      | 0.31                  |
|             | Hue                     | 0.93            | 0.00             | -0.08     | 0.09      | 0.09                  |
|             | Saturation              | 0.09            | 0.72             | 0.01      | 0.95      | 0.96                  |
|             | Value                   | 0.08            | 0.73             | 0.03      | 0.95      | 0.96                  |
|             | HSL*L                   | 0.08            | 0.73             | 0.03      | 0.95      | 0.96                  |
|             | Lab*L                   | 0.08            | 0.73             | 0.03      | 0.95      | 0.96                  |
|             | Lab*a                   | 0.14            | 0.62             | -0.13     | 0.92      | 0.93                  |
|             | Lab*b                   | 0.19            | 0.56             | -0.20     | 0.90      | 0.90                  |
|             | Luminance <sup>b</sup>  | 0.08            | 0.73             | 0.03      | 0.95      | 0.96                  |
|             | Luminosity <sup>c</sup> | 0.08            | 0.73             | 0.03      | 0.95      | 0.96                  |
|             | Mean RGB <sup>d</sup>   | 0.09            | 0.71             | 0.00      | 0.95      | 0.96                  |
|             |                         |                 |                  |           |           |                       |
| Moisture:OM | Red                     | 0.05            | 0.79             | 0.16      | 0.96      | 0.98                  |
|             | Green                   | 0.09            | 0.71             | 0.00      | 0.95      | 0.96                  |
|             | Blue                    | 0.65            | 0.11             | -0.31     | 0.49      | 0.45                  |
|             | Hue                     | 0.62            | 0.13             | -0.33     | 0.53      | 0.49                  |
|             | Saturation              | 0.09            | 0.71             | -0.01     | 0.94      | 0.96                  |
|             | Value                   | 0.07            | 0.74             | 0.05      | 0.95      | 0.97                  |
|             | HSL*L                   | 0.08            | 0.73             | 0.02      | 0.95      | 0.96                  |
|             | Lab*L                   | 0.07            | 0.75             | 0.07      | 0.95      | 0.97                  |
|             | Lab*a                   | 0.08            | 0.74             | 0.04      | 0.95      | 0.97                  |
|             | Lab*b                   | <b>0.03</b>     | 0.85             | 0.32      | 0.98      | 0.99                  |
|             | Luminance <sup>b</sup>  | 0.08            | 0.73             | 0.02      | 0.95      | 0.96                  |

|                         |      |      |       |      |      |
|-------------------------|------|------|-------|------|------|
| Luminosity <sup>c</sup> | 0.08 | 0.73 | 0.02  | 0.95 | 0.96 |
| Mean RGB <sup>d</sup>   | 0.13 | 0.64 | -0.11 | 0.92 | 0.93 |

---

<sup>a</sup> CCC = Lin's Concordance Correlation Coefficient where > 0.9 excellent; 0.8 - 0.9 good; 0.6 to 0.8 moderate; below 0.6.

<sup>b</sup> Luminance = (min(RGB) + max(RGB))/2

<sup>c</sup> Luminosity = 0.2126(R) + 0.7152(G) + 0.0722(B)

<sup>d</sup> Mean RGB = (R+G+B)/2

<sup>e</sup> Accuracy = CCC bias correction factor, values closer to 1.00 indicate minimal scale and location bias between observed and predicted values

---

**Table 2.16 Pen-level nutrient predictions using UAV thermal-derived color metrics from data with precipitation outlier day removed (Objective 3).**

| Nutrient    | Color Metric            | <i>P</i> -value | CCC <sup>a</sup> | CCC Lower | CCC Upper | Accuracy <sup>c</sup> |
|-------------|-------------------------|-----------------|------------------|-----------|-----------|-----------------------|
| Moisture    | Red                     | <b>&lt;0.01</b> | 0.95             | 0.69      | 0.99      | 1.00                  |
|             | Green                   | <b>0.02</b>     | 0.88             | 0.41      | 0.98      | 0.99                  |
|             | Blue                    | 0.54            | 0.18             | -0.35     | 0.62      | 0.58                  |
|             | Hue                     | 0.41            | 0.29             | -0.34     | 0.75      | 0.71                  |
|             | Saturation              | <b>0.02</b>     | 0.88             | 0.39      | 0.98      | 0.99                  |
|             | Value                   | <b>0.01</b>     | 0.93             | 0.60      | 0.99      | 1.00                  |
|             | HSL*L                   | <b>0.01</b>     | 0.91             | 0.52      | 0.99      | 1.00                  |
|             | Lab*L                   | <b>&lt;0.01</b> | 0.93             | 0.59      | 0.99      | 1.00                  |
|             | Lab*a                   | <b>&lt;0.01</b> | 0.97             | 0.78      | 1.00      | 1.00                  |
|             | Lab*b                   | <b>0.02</b>     | 0.88             | 0.41      | 0.98      | 0.99                  |
|             | Luminance <sup>b</sup>  | <b>0.01</b>     | 0.91             | 0.51      | 0.99      | 1.00                  |
|             | Luminosity <sup>c</sup> | <b>0.01</b>     | 0.91             | 0.52      | 0.99      | 1.00                  |
|             | Mean RGB <sup>d</sup>   | <b>0.02</b>     | 0.88             | 0.41      | 0.98      | 0.99                  |
|             |                         |                 |                  |           |           |                       |
| OM          | Red                     | 0.13            | 0.64             | -0.10     | 0.93      | 0.93                  |
|             | Green                   | 0.10            | 0.69             | -0.04     | 0.94      | 0.95                  |
|             | Blue                    | 0.59            | 0.14             | -0.33     | 0.56      | 0.52                  |
|             | Hue                     | 0.70            | 0.08             | -0.29     | 0.42      | 0.38                  |
|             | Saturation              | 0.12            | 0.65             | -0.09     | 0.93      | 0.94                  |
|             | Value                   | 0.12            | 0.67             | -0.07     | 0.93      | 0.94                  |
|             | HSL*L                   | 0.11            | 0.68             | -0.06     | 0.94      | 0.95                  |
|             | Lab*L                   | 0.12            | 0.65             | -0.09     | 0.93      | 0.94                  |
|             | Lab*a                   | 0.24            | 0.49             | -0.26     | 0.87      | 0.86                  |
|             | Lab*b                   | 0.17            | 0.59             | -0.17     | 0.91      | 0.91                  |
|             | Luminance <sup>b</sup>  | 0.12            | 0.66             | -0.08     | 0.93      | 0.94                  |
|             | Luminosity <sup>c</sup> | 0.11            | 0.68             | -0.06     | 0.94      | 0.95                  |
|             | Mean RGB <sup>d</sup>   | 0.14            | 0.63             | -0.12     | 0.92      | 0.93                  |
|             |                         |                 |                  |           |           |                       |
| Moisture:OM | Red                     | <b>&lt;0.01</b> | 0.97             | 0.79      | 1.00      | 1.00                  |
|             | Green                   | <b>&lt;0.01</b> | 0.93             | 0.59      | 0.99      | 1.00                  |
|             | Blue                    | 0.52            | 0.20             | -0.35     | 0.64      | 0.60                  |
|             | Hue                     | 0.45            | 0.26             | -0.35     | 0.72      | 0.68                  |
|             | Saturation              | <b>0.01</b>     | 0.91             | 0.53      | 0.99      | 1.00                  |
|             | Value                   | <b>&lt;0.01</b> | 0.96             | 0.74      | 0.99      | 1.00                  |
|             | HSL*L                   | <b>&lt;0.01</b> | 0.95             | 0.68      | 0.99      | 1.00                  |
|             | Lab*L                   | <b>&lt;0.01</b> | 0.95             | 0.72      | 0.99      | 1.00                  |
|             | Lab*a                   | <b>&lt;0.01</b> | 0.95             | 0.68      | 0.99      | 1.00                  |
|             | Lab*b                   | <b>0.01</b>     | 0.90             | 0.50      | 0.99      | 1.00                  |
|             | Luminance <sup>b</sup>  | <b>&lt;0.01</b> | 0.94             | 0.65      | 0.99      | 1.00                  |

|                         |                 |      |      |      |      |
|-------------------------|-----------------|------|------|------|------|
| Luminosity <sup>c</sup> | <b>&lt;0.01</b> | 0.95 | 0.68 | 0.99 | 1.00 |
| Mean RGB <sup>d</sup>   | <b>0.01</b>     | 0.91 | 0.52 | 0.99 | 1.00 |

---

<sup>a</sup> CCC = Lin's Concordance Correlation Coefficient where > 0.9 excellent; 0.8 - 0.9 good; 0.6 to 0.8 moderate; below 0.6.

<sup>b</sup> Luminance = (min(RGB) + max(RGB))/2

<sup>c</sup> Luminosity = 0.2126(R) + 0.7152(G) + 0.0722(B)

<sup>d</sup> Mean RGB = (R+G+B)/2

<sup>e</sup> Accuracy = CCC bias correction factor, values closer to 1.00 indicate minimal scale and location bias between observed and predicted values

---

## Figures

**Figure 2.1 Virtual map of pen quadrants (n = 15) used for manure and UAV thermal-derived color metric sampling.**



**Figure 2.2 Visual depiction of terms used to describe relative pen orientation labeled with general pen infrastructure landmarks.**

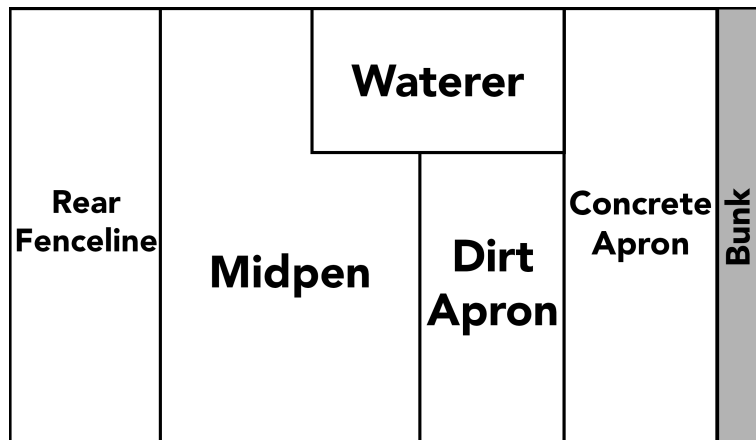
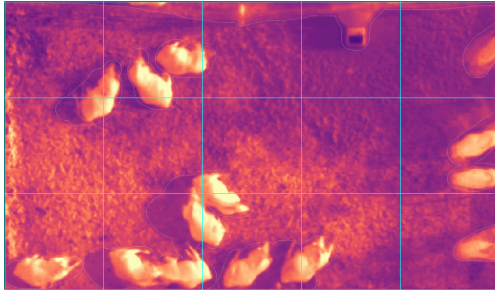
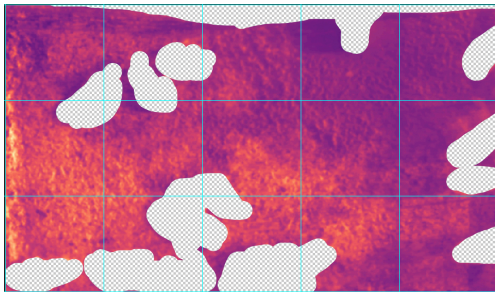


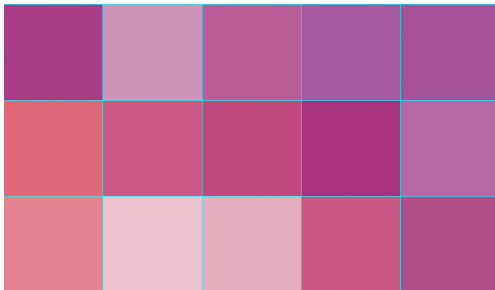
**Figure 2.3 (a, b, c) Visual representation of the process of confounding object removal and color metric extraction from Adobe Photoshop using example pen thermal orthomosaic.**



a) Full pen orthomosaic with 15-quadrant virtual grid superimposed.



b) Pen orthomosaic with cattle and confounding objects removed.



c) Pen gradient creation using average color from each quadrant to thermal-derived extract color metrics.

## Literature Cited

- Abdulai, G., Sama, M., & Jackson, J. (2021). A preliminary study of the physiological and behavioral response of beef cattle to unmanned aerial vehicles. *Applied Animal Behaviour Science*, 241. <https://doi.org/10.1016/j.applanim.2021.105355>
- AOAC International. (2019). *Official methods of analysis* (21st ed.). AOAC International.
- Aragon, B., Johansen, K., Parkes, S., Malbeteau, Y., Al-Mashharawi, S., Al-Amoudi, T., Andrade, C. F., Turner, D., Lucieer, A., & McCabe, M. F. (2020). A calibration procedure for field and UAV-based uncooled thermal infrared instruments. *Sensors*, 20(11). <https://doi.org/10.3390/s20113316>
- Bastos, K. M., Barcelos, J. P., Orioli, G. F., & Nascimento, S. T. (2024). Use of drones for trough reading, animal counting, and production monitoring in feedlot systems. *AgriEngineering*, 6(4). <https://doi.org/10.3390/agriengineering6040253>
- Berry, E., & Miller, D. (2005). Cattle feedlot soil moisture and manure content: II. Impact on *Escherichia coli* O157. *Journal of Environmental Quality*, 34, 656–663. <https://doi.org/10.2134/jeq2005.0656>
- Cho, S. B., Soleh, H. M., Choi, J. W., Hwang, W.-H., Lee, H., Cho, Y.-S., Cho, B.-K., Kim, M. S., Baek, I., & Kim, G. (2024). Recent methods for evaluating crop water stress using AI techniques: A review. *Sensors*, 24, 1–39. <https://doi.org/10.3390/s24196313>
- Cole, N. A., Clark, R. N., Todd, R. W., Richardson, C. R., Gueye, A., Greene, L. W., & McBride, K. (2005). Influence of dietary crude protein concentration and source on potential ammonia emissions from beef cattle manure. *Journal of Animal Science*, 83(3), 722–731. <https://doi.org/10.2527/2005.833722x>

- Cortes, J. A., Thomas, A., Hendrick, S., Janzen, E., Pajor, E. A., & Orsel, K. (2021). Risk factors of digital dermatitis in feedlot cattle. *Translational Animal Science*, 5(3), 1–9.  
<https://doi.org/10.1093/tas/txab075>
- Dickson, E. J., Campbell, D. L. M., Monk, J. E., Lea, J. M., Colditz, I. G., & Lee, C. (2022). Increasing mud levels in a feedlot influences beef cattle behaviours but not preference for feedlot or pasture environments. *Applied Animal Behaviour Science*, 254.  
<https://doi.org/10.1016/j.applanim.2022.105718>
- Driedger, L. J., & Loerch, S. C. (1999). Limit-feeding corn as an alternative to hay reduces manure and nutrient output by Holstein cows. *Journal of Animal Science*, 77(4), 967–972. <https://doi.org/10.2527/1999.774967x>
- Elfarkh, J., Johansen, K., Angulo, V., Camargo, O. L., & McCabe, M. F. (2023). Quantifying within-flight variation in land surface temperature from a UAV-based thermal infrared camera. *Drones*, 7(10). <https://doi.org/10.3390/drones7100617>
- Gerber, P. J. & Food and Agriculture Organization of the United Nations (Eds.). (2013). *Tackling climate change through livestock: A global assessment of emissions and mitigation opportunities*. Food and Agriculture Organization of the United Nations.
- Gilley, J. E., Berry, E. D., Eigenberg, R. A., Marx, D. B., & Woodbury, B. L. (2008). Spatial variations in nutrient and microbial transport from feedlot surfaces. *Transactions of the ASABE*, 51(2), 675–684. <https://doi.org/10.13031/2013.24380>
- Grandin, T. (2016). Evaluation of the welfare of cattle housed in outdoor feedlot pens. *Veterinary and Animal Science*, 1–2, 23–28. <https://doi.org/10.1016/j.vas.2016.11.001>

- Huhtanen, P., Detmann, E., & Krizsan, S. J. (2016). Prediction of rumen fiber pool in cattle from dietary, fecal, and animal variables. *Journal of Dairy Science*, 99(7), 5345–5357.  
<https://doi.org/10.3168/jds.2015-10842>
- Jia, R., Liu, J., Zhang, J., Niu, Y., Jiang, Y., Xuan, K., Wang, C., Ji, J., Ma, B., & Li, X. (2023). An improved UAV-based ATI method incorporating solar radiation for farm-scale bare soil moisture measurement. *Remote Sensing*, 15(15). <https://doi.org/10.3390/rs15153769>
- Jiang, B., Tang, W., Cui, L., & Deng, X. (2023). Precision livestock farming research: A global scientometric review. *Animals*, 13(13). <https://doi.org/10.3390/ani13132096>
- Lacerda, L. N., Snider, J. L., Cohen, Y., Liakos, V., Gobbo, S., & Vellidis, G. (2022). Using UAV-based thermal imagery to detect crop water status variability in cotton. *Smart Agricultural Technology*, 2, 100029. <https://doi.org/10.1016/j.atech.2021.100029>
- Loerch, S. C. (1990). Effects of feeding growing cattle high-concentrate diets at a restricted intake on feedlot performance. *Journal of Animal Science*, 68(10), 3086.  
<https://doi.org/10.2527/1990.68103086x>
- Mader, T. L. (2011). Mud effects on feedlot cattle. *Nebraska Beef Cattle Reports*, (613), 82–83.
- Miller, D., & Woodbury, B. (2003). Simple protocols to determine dust potentials from cattle feedlot soil and surface samples. *Journal of Environmental Quality*, 32, 1634–1640.  
<https://doi.org/10.2134/jeq2003.1634>
- Miller, J., Beasley, B., Yanke, L., Larney, F., Mcallister, T., Olson, B., Selinger, L., Chanasyk, D., & Hasselback, P. (2003). Bedding and seasonal effects on chemical and bacterial properties of feedlot cattle manure. *Journal of Environmental Quality*, 32, 1887–1894.  
<https://doi.org/10.2134/jeq2003.1887>

- Miller, J., Olson, E., Chanasyk, D., Beasley, B., Yanke, L., Larney, F., Mcallister, T., Olson, B., & Selinger, L. (2006). Bedding and within-pen location effects on feedlot pen runoff quality using a rainfall simulator. *Journal of Environmental Quality*, 35, 505–515.  
<https://doi.org/10.2134/jeq2005.0205>
- Montanholi, Y. R., Odongo, N. E., Swanson, K. C., Schenkel, F. S., McBride, B. W., & Miller, S. P. (2008). Application of infrared thermography as an indicator of heat and methane production and its use in the study of skin temperature in response to physiological events in dairy cattle. *Journal of Thermal Biology*, 33(8), 468–475.  
<https://doi.org/10.1016/j.jtherbio.2008.09.001>
- Murphy, T. A., & Loerch, S. C. (1994). Effects of restricted feeding of growing steers on performance, carcass characteristics, and composition. *Journal of Animal Science*, 72(9), 2497–2507. <https://doi.org/10.2527/1994.7292497x>
- Nguyen, R. M. H., & Brown, M. S. (2017). Why you should forget luminance conversion and do something better. *2017 IEEE Conference on Computer Vision and Pattern Recognition (CVPR)*, 6750–6758. <https://doi.org/10.1109/CVPR.2017.627>
- Olson, D., & Anderson, J. (2021). Review on unmanned aerial vehicles, remote sensors, imagery processing, and their applications in agriculture. *Agronomy Journal*, 113(2), 971–992.  
<https://doi.org/10.1002/agj2.20595>
- Shao, W., Kawakami, R., Yoshihashi, R., You, S., Kawase, H., & Naemura, T. (2020). Cattle detection and counting in UAV images based on convolutional neural networks. *International Journal of Remote Sensing*, 41(1), 31–52.  
<https://doi.org/10.1080/01431161.2019.1624858>

- Shi, H., Liu, Z., Li, S., Jin, M., Tang, Z., Sun, T., Liu, X., Li, Z., Zhang, F., & Xiang, Y. (2024). Monitoring soybean soil moisture content based on UAV multispectral and thermal-infrared remote-sensing information fusion. *Plants*, 13(17).  
<https://doi.org/10.3390/plants13172417>
- Sip, M. L., & Pritchard, R. H. (1991). Nitrogen utilization by ruminants during restricted intake of high-concentrate diets. *Journal of Animal Science*, 69(6), 2655–2662.  
<https://doi.org/10.2527/1991.6962655x>
- Urso, P. M., Turgeon, A., Ribeiro, F. R. B., Smith, Z. K., & Johnson, B. J. (2021). Review: The effects of dust on feedlot health and production of beef cattle. *Journal of Applied Animal Research*, 49(1), 133–138. <https://doi.org/10.1080/09712119.2021.1903476>
- Vahidi, M., Shafian, S., & Frame, W. H. (2025). Multi-Modal sensing for soil moisture mapping: Integrating drone-based ground penetrating radar and RGB-thermal imaging with deep learning. *Computers and Electronics in Agriculture*, 236.  
<https://doi.org/10.1016/j.compag.2025.110423>
- Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy Science*, 74(10), 3583–3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Verstraeten, W. W., Veroustraete, F., Van Der Sande, C. J., Grootaers, I., & Feyen, J. (2006). Soil moisture retrieval using thermal inertia, determined with visible and thermal spaceborne data, validated for European forests. *Remote Sensing of Environment*, 101(3), 299–314. <https://doi.org/10.1016/j.rse.2005.12.016>

Wan, Q., Smigaj, M., Brede, B., & Kooistra, L. (2024). Optimizing UAV-based uncooled thermal cameras in field conditions for precision agriculture. *International Journal of Applied Earth Observation and Geoinformation*, 134.

<https://doi.org/10.1016/j.jag.2024.104184>

Wilkes, J., Tait, A. L., Flavel, R. J., Turnell, J., & Cowley, F. C. (2022). Physical and chemical characteristics of feedlot pen substrate bedded with woodchip under wet climatic conditions. *The Journal of Agricultural Science*, 160(5), 413–422.

<https://doi.org/10.1017/S002185962200048X>

Zhang, D., & Zhou, G. (2016). Estimation of soil moisture from optical and thermal remote sensing: A review. *Sensors*, 16(8). <https://doi.org/10.3390/s16081308>

## **Chapter 3 - Growth performance and enteric gas emissions in grazing beef heifers fed Agolin**

### **Introduction**

Enteric methane (CH<sub>4</sub>) production from ruminant livestock represents both a loss of dietary gross energy and a significant contributor to agricultural greenhouse gas emissions (Beauchemin et al., 2020; Johnson & Johnson, 1995). In beef systems, mitigating CH<sub>4</sub> without compromising performance is challenging, as effective strategies can be constrained by numerous factors, including toxicity risk, regulatory limitations, inconsistent efficacy over time, or negative impacts on intake and digestion (Beauchemin et al., 2020; Dillon et al., 2021). Plant secondary compounds, including essential oils, have therefore received interest as CH<sub>4</sub> mitigation tools because of their ability to modify rumen microbial activity through selective antimicrobial effects while maintaining consumer acceptance as “natural” feed additives (Calsamiglia et al., 2007; Cobellis et al., 2016).

Agolin® (Alltech Inc.; Nicholasville, KY) is a commercially formulated essential oil blend developed to modulate rumen fermentation to improve efficiency and reduce enteric CH<sub>4</sub>. The blend includes bioactive components such as coriander seed oil, eugenol, geranyl acetate, and geraniol, stabilized with organic acid preservatives for delivery in livestock systems (Belanche et al., 2020). Agolin has been evaluated at a targeted inclusion rate of ~1 g/head/day across multiple studies and production environments. Across the available literature, modest reductions in CH<sub>4</sub> emissions and neutral to favorable production responses have been demonstrated for cattle fed Agolin, though outcomes have depended on dose consistency, adaptation period, animal type, and basal diet nutrient composition (Batley et al., 2024; Belanche et al., 2020; Miller et al., 2023).

Controlled feeding studies in lactating dairy cows have provided much of the early evidence base for Agolin. Production responses have ranged from neutral to positive, with improvements in milk production and feed efficiency, suggesting that the essential oil blend can be included without the intake depression occasionally observed with other antimicrobials (Elcoso et al., 2019; Williams et al., 2021). Recent work has linked Agolin supplementation to shifts in rumen microbiome composition and fermentation end products, consistent with CH<sub>4</sub> mitigation mechanisms, including increases in propionate and reductions in methanogen abundance in some contexts (Bach et al., 2023). Another determinant of Agolin response is delivery strategy; for grazing applications, daily delivery of a supplement is not always feasible. Therefore, product pulse-dosing approaches intended to deliver targeted daily doses can fall short when intake is voluntary or variable, which may weaken CH<sub>4</sub> responses even when production endpoints remain favorable or unchanged (Bowman & Sowell, 1997; Fouts et al., 2025; Kunkle et al., 2000; Williams et al., 2018). Additionally, the biological response of essential oils appears to be influenced by ruminal nitrogen dynamics and may depend on the balance between rumen-degradable protein supply and fermentable energy availability (Calsamiglia et al., 2007; Cobellis et al., 2016). Essential oils can inhibit certain rumen microbial populations, including hyper-ammonia-producing bacteria responsible for rapid amino acid deamination, thereby reducing ruminal ammonia formation. In systems where rumen ammonia concentrations exceed microbial requirements, suppressing deamination may enhance nitrogen-use efficiency by allowing greater microbial capture of dietary nitrogen. However, in environments with limited nitrogen supply, further reductions in ruminal ammonia could constrain microbial growth. These dynamics may be particularly relevant in grazing systems, where forage maturity, intake patterns, and nutrient composition fluctuate over time.

Previous work has evaluated Agolin supplementation in dairy x beef steers fed high-concentrate diets under controlled conditions and demonstrated reduced CH<sub>4</sub> yield without adverse effects on production metrics or dry matter intake, supporting the concept that CH<sub>4</sub> mitigation can be achieved without sacrificing performance (Miller et al., 2023). Despite this encouraging evidence in confined systems, a key knowledge gap is that the majority of research with this compound has been conducted in total mixed ration-fed dairy cattle or feedlot-style beef diets, where intake of the additive can be tightly controlled in relatively consistent diets. Typically, higher CH<sub>4</sub> intensity is observed for forage-based diets due to greater acetate production resulting in more hydrogen available for methanogenesis (Beauchemin et al., 2022; Johnson & Johnson, 1995). At the same time, the microbial ecosystem in forage-fed cattle relies heavily on fibrolytic microbial communities. If antimicrobial activity from an essential oil product excessively inhibits fibrolytic populations, fiber digestion may be impaired (Calsamiglia et al., 2007; Cobellis et al., 2016). Reduced fiber degradation can slow ruminal passage rate and increase rumen fill, which may ultimately limit voluntary intake in forage-based environments.

Based on previous work, it was hypothesized that grazing beef heifers supplemented with Agolin would produce lower enteric CH<sub>4</sub> emissions without affecting performance when compared to grazing beef heifers provided a supplement without Agolin. The objective of the study was to evaluate enteric CH<sub>4</sub> and carbon dioxide (CO<sub>2</sub>) emissions, emission intensity (g CH<sub>4</sub>/kg average daily gain), and performance in beef heifers supplemented with Agolin and managed under conditions representative of commercial grazing operations.

## Materials and methods

### Animal care and use

The experiment was conducted on triticale pasture located near Minneola, KS. All animals involved in this experiment were cared for according to guidelines of the Kansas State University Institutional Animal Care and Use Committee (IACUC; protocol #IACUC-5111).

### Animals, treatments, and experimental design

Sixty-three crossbred beef heifers ( $n = 63$ ; initial body weight =  $274 \pm 42$  kg) grazed a 24-hectare triticale pasture (*Triticosecale* spp.) in southwest Kansas for 70 days beginning in mid-winter. Prior to trial initiation, each heifer was weighed, visually identified using existing ear tags, and fitted with a radio-frequency identification (RFID) tag in the left ear to enable automated monitoring of supplement intake and emissions. Heifers underwent a 20-day pre-trial acclimation period to ensure familiarity with an Automated Head Chamber System (AHCS; C-Lock Inc., Rapid City, SD) and a SmartFeed Pro automated feeder (C-Lock Inc., Rapid City, SD) and to characterize individual visit behavior. Following acclimation, heifers were assigned to supplementation treatments based on initial body weight, AHCS, and automated feeder visit frequency. All heifers were comingled within a single pasture throughout the experimental period to ensure identical grazing conditions. Treatments (Table 3.1) consisted of:

1. **CON:** 0.41 kg DM/day of dry distillers grains without Agolin inclusion ( $n = 31$ )
2. **AGO:** 0.41 kg DM/day of dry distillers grains mixed to include an Agolin (Alltech Inc., Nicholasville, KY) dose of 725.8 mg/head/day or 200 mg/100 kg body weight targeted to a final body weight of 362.9 kg ( $n = 32$ )

## **Supplement delivery and intake monitoring**

Supplement delivery and intake were controlled using two SmartFeed Pro automated feeders (C-Lock Inc., Rapid City, SD), which use RFID technology to regulate individual animal access, record supplement intake, and visit frequency and duration. Each automated feeder was assigned to contain either CON or AGO supplement. Heifers were then assigned to feeders depending on treatment allocation starting on Day 1. Automated feeders were located near the water source and remained under roof to limit moisture and wind as variables that could affect weight readings and feed integrity. The units were fitted with wooden risers (approximately 16 cm in height) within the feeder to ensure complete accessibility of the supplement for smaller-framed heifers, and feeder weigh bars were calibrated prior to the start of the trial, following procedures according to the equipment operations manual. Supplements were hand-delivered into the feeders once daily to ensure access to fresh supplement and essential oil in order to appropriately evaluate Agolin efficacy without potential stability concerns. Maximum supplement intake was limited to 0.41 kg DM/heifer to ensure consistent delivery of the intended Agolin dose. Data from the SmartFeed Pro units were retrieved from the C-Lock Inc. website weekly and stored for analysis. Any residual supplement remaining in each feeder was removed and discarded weekly.

## **Forage and body weight data collection**

Forage availability was unrestricted throughout the study, and pasture conditions were typical of late-winter, early-spring triticale growth in southwest Kansas for the majority of the trial. Forage samples were collected on Day 0 and Day 52 at 10 random sampling locations using a 0.25 m<sup>2</sup> quadrat and placed in an airtight plastic bag. Samples were dried at 55°C in a conventional drying oven for an initial period of 72 hours and weighed to determine dry matter at

Kansas State University-Olathe (Olathe, KS) according to AOAC method 934.01 (AOAC International, 2019). Each sample was returned to the oven for an additional 2 hours and reweighed. If no further weight loss was observed, the samples were considered to have reached constant weight and were removed from the oven. Once dry, samples were ground through a 2-mm screen using a UDY Model 3010-030 Cyclone Mill (UDY Corp., Fort Collins, CO). Samples were placed in airtight plastic bags and shipped to a commercial laboratory for compositional analysis (ServiTech Inc., Dodge City, KS; Table 3.2). In addition to treatment supplement, all cattle received free-choice access to a commercial mineral supplement (Stockmaster Wheat Pasture Mineral; Hubbard Feeds, Mankato, MN) formulated for grazing beef cattle with a targeted intake of approximately 56.7 g/head/day (Table 3.3). During the later portion of the trial, the producer partner provided dry hay to supplement the triticale pasture as forage availability declined relative to the beginning of the grazing period (Table 3.2). Hay samples were collected; however, nutrient composition was not determined due to logistical constraints.

Individual body weights were collected at the beginning, middle, and end of the experimental period to determine animal performance. Heifers were moved to a holding pen in the afternoon on Days -1, 28, and 70 and were withheld from feed while provided ad libitum access to water to obtain individual 16-hour shrunk body weights to minimize variation associated with gut fill. Weights were collected using the feedyard processing facility adjacent to the triticale pasture (W-W Livestock Systems, Thomas, OK; Tru-Test by Datamars, Lamone, Switzerland). Average daily gain (ADG) was calculated for each heifer using linear regression of body weight across the three measurement timepoints.

## **Enteric gas emissions measurement**

Enteric emissions (CH<sub>4</sub> and CO<sub>2</sub>) were quantified using an AHCS located approximately 30-m from the automated feeders and water access point. The AHCS measured enteric gas emissions during voluntary animal visits by spot sampling respired gases while delivering approximately 35 g bait feed (alfalfa pellets; Gunter & Beck, 2018; Hristov et al., 2015). The unit was fitted with alley panels to limit multiple heifers from accessing the AHCS at one time. Visits were scheduled for a maximum of six pellet drops per visit, with 30-second intervals between drops and a minimum of four hours between visits. The AHCS gas analyzer was calibrated weekly, and the air flux sensor was calibrated every 14 days by releasing a predetermined quantity of CO<sub>2</sub> into the system using a 90-g prefilled CO<sub>2</sub> cylinder for three minutes, repeated six times (Hristov et al., 2015). The released quantity of CO<sub>2</sub> was compared to the measured quantity of CO<sub>2</sub> to ensure recovery results were  $100 \pm 5\%$ . To maintain a consistent airflow rate in the AHCS collection tube between 27 and 40 L/second (Alemu et al., 2021), the air filter was cleaned every 14 days. Automated Head Chamber System data were retrieved from the C-Lock Inc. website weekly and stored for analysis. Only visits greater than two minutes in duration were used in statistical analysis (Beck et al., 2024). Arithmetic means were calculated for each animal's CH<sub>4</sub> and CO<sub>2</sub> measurements (g/day) from all visits over the duration of the trial (Beck et al., 2025).

## **Data preparation**

To ensure adequate treatment exposure and enteric emissions measurements, only heifers classified as both consistent supplement and AHCS users were included in statistical analyses. Consistent supplement users were defined as heifers that consumed  $\geq 50\%$  of the allotted supplement ( $\geq 0.25$  kg/day) *and* demonstrated regular feeder visitation ( $\geq 41\%$ ) over the duration

of the experimental period ( $\geq 29$  visits). Consistent AHCS users were defined as heifers with  $\geq 11$  visits ( $\geq 2$  minutes) over the duration of the experimental period (Alemu et al., 2021). After removing heifers that did not meet both visitation requirements, 28 animals remained for statistical analyses.

## Statistical analysis

Data were analyzed using linear models in R (version 4.5.2; R Foundation for Statistical Computing, Vienna, Austria) with the `lm` function. Models with a fixed effect of treatment (AGO vs CON) were developed to evaluate the following dependent variables: ADG, CH<sub>4</sub> emissions, CO<sub>2</sub> emissions, and emission intensity (EI). Individual animal served as the experimental unit. Model assumptions were evaluated through residual analysis, and influential observations were identified using Cook's Distance. Animals with confirmed body weight measurement abnormalities were excluded from analysis prior to final model fitting. Following body weight outlier removal, the final sample size was  $n = 28$  ( $n = 16$  AGO,  $n = 12$  CON) for emissions analysis and  $n = 26$  ( $n = 16$  AGO,  $n = 12$  CON) for performance and emission intensity analyses. Initial BW for the treatment groups used for statistical analysis was approximately 275 for AGO and 294 kg for CON. Significance was declared at  $P \leq 0.05$ , and tendencies were noted at  $0.05 < P \leq 0.1$ . Statistical model used:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

- $Y_{ij}$  = observed response for the  $j^{th}$  animal receiving the  $i^{th}$  treatment (e.g., ADG, CH<sub>4</sub> emissions, CO<sub>2</sub> emissions, or EI)
- $\mu$  = overall mean
- $\alpha_i$  =  $i^{th}$  fixed effect of treatment (AGO, CON)
- $\varepsilon_{ij}$  = residual error associated with animal  $j$  in treatment  $i$

## **Results and discussion**

### **Supplement intake and treatment exposure**

Supplement allocation was limited to 0.41 kg DM/head/day; however, actual intake was variable, likely due, in part, to voluntary consumption patterns characteristic of grazing cattle (Bowman & Sowell, 1997; Kunkle et al., 2000; Williams et al., 2018) as well as intermittent equipment disruptions. Periods of zero intake occurred throughout the trial and were associated with documented SmartFeed Pro malfunctions, temporary feeder inaccessibility due to mechanical breakage, and cold weather conditions that impaired equipment and altered cattle behavior.

The occurrence of zero-intake days, the average number of days per treatment when animals did not consume supplement, reduced average daily supplement intake relative to the 0.41 kg DM/head/day target and consequently reduced Agolin exposure below the formulated prescribed dose (Table 3.5). Heifers were dosed assuming a projected final body weight of 362.9 kg, resulting in a prescribed rate of 200 mg Agolin per 100 kg body weight and a formulated delivery of 725.8 mg/head/day at target supplement intake. However, mean supplement intake for AGO heifers was 0.27 kg DM/head/day, representing 66.3% of the intended supplement delivery, resulting in an actual Agolin intake of 481.5 mg/head/day (66.3% of the prescribed dose) if averaged across all days. Mean supplement intake for CON heifers was 0.19 kg DM/head/day when averaging across days, representing 46.5% of intended supplement delivery. When calculated only for days on which supplement was consumed, average intake was 0.44 kg DM/head/day for AGO heifers and 0.43 kg DM/head/day for CON heifers. Corresponding Agolin intake was 776.8 mg/head/day for AGO heifers and represented 106.8% and 105.3% of the intended supplement intake for AGO and CON treatments, respectively. The resulting Agolin

delivery was 106.8% of the intended dose. These calculations reflect voluntary supplementation patterns typical of grazing systems, in which cattle do not necessarily visit supplement feeders each day, and indicate that when heifers consumed supplement, intake met or exceeded the targeted delivery rate. While supplementation frequency was lower than targeted, the observed intake pattern is consistent with previous studies evaluating energy supplementation in cattle grazing similar winter forages (Thompson et al., 2019). Variation in visitation behavior is common in grazing systems where self-fed supplementation results in cattle consuming supplement intermittently. For example, Williams et al. (2018) reported pasture-based, self-fed supplement intake ranged from 0 to 1.21 kg/head/day with a supplement intake coefficient of variation of 51%. In the present study, the primary factor contributing to lower-than-targeted supplement intake was likely equipment malfunctions that temporarily limited feeder access. Agolin heifers consumed supplement for approximately 17 additional days per animal compared to CON heifers across the 70-day grazing period.

### **Performance recordings**

Average daily gain did not differ between treatments ( $P = 0.18$ ; Table 3.4). Heifers supplemented with Agolin gained 0.69 kg/day on average compared with CON heifers gaining 0.62 kg/day (Table 3.4). The absence of negative performance effects is biologically meaningful. Grazing cattle consuming high-forage diets rely on fibrolytic microbial populations, and at certain inclusion levels, essential oils can exert antimicrobial activity and inhibit fibrolytic bacteria. In turn, this reduces neutral detergent fiber digestion and heightens the theoretical risk to performance if fiber fermentation is impaired (Calsamiglia et al., 2007; Cobellis et al., 2016). However, ADG was not different between treatments, indicating that Agolin inclusion did not disrupt productive fermentation under the dietary conditions of this study.

The triticale forage contained on average 25.4% crude protein (Table 3.2) over the duration of the trial, suggesting that rumen degradable nitrogen was unlikely to be limited. Under high-protein forage conditions, hydrogen capture via propionate formation rather than methanogenesis may improve energetic efficiency without constraining microbial growth (Beauchemin et al., 2022; Johnson & Johnson, 1995). This mechanism is consistent with observations from dairy systems where Agolin supplementation maintained milk yield (Elcoso et al., 2019; Williams et al., 2021).

### **Enteric emissions**

Heifers supplemented with Agolin had approximately 8.96% lower enteric CH<sub>4</sub> emissions (162.6 g/d) than CON (178.6 g/day;  $P = 0.05$ ). An 8.96% reduction under forage-based grazing conditions is notable, as CH<sub>4</sub> production is typically greater in forage-fed cattle due to higher acetate production and increased hydrogen availability (Beauchemin et al., 2022; Johnson & Johnson, 1995). The magnitude of reduction observed in the current study aligns with a meta-analysis that found an 8.8% mean reduction in CH<sub>4</sub> production (g CH<sub>4</sub>/day) for lactating dairy cows supplemented with Agolin for more than four weeks (Belanche et al., 2020). A separate study observed a 10% numeric decrease in CH<sub>4</sub> yield from four Belgian Blue beef heifers fed a combination of silage and concentrate after a 46-day period of Agolin inclusion in the diet (Castro-Montoya et al., 2015).

Carbon dioxide emissions did not differ between treatments, with AGO producing 6,120.02 g/d and CON producing 6,507.44 ( $P = 0.15$ ). Carbon dioxide emissions generally reflect the largest portion of fermentative activity and metabolic heat production and are closely correlated to intake (Arthur et al., 2018; Huhtanen et al., 2021; Wolin, 1960). The absence of differences suggests that total fermentation was not reduced for AGO heifers compared to CON.

Results demonstrated a decrease in CH<sub>4</sub> production while CO<sub>2</sub> production was not different between treatments, which supports the theory of altered hydrogen disposal rather than depressed fermentation (Beauchemin et al., 2020; Janssen, 2010).

Emission intensity (EI; g CH<sub>4</sub>/kg ADG) integrates performance and CH<sub>4</sub> emissions into a single value (Beauchemin et al., 2022). Emission intensity was found to be lower in AGO heifers compared to CON (242.81 and 293.50, respectively;  $P = 0.03$ ; Table 3.4). The observed findings represent an approximate 17.27% reduction in EI. The reduction reflects both a decrease in CH<sub>4</sub> production and a numeric increase in ADG for AGO heifers compared to CON heifers.

Forage dry matter availability declined substantially over the course of the trial during the late growing season (~1613 to <100 kg/ha; Table 3.2). During the latter portion of the grazing period, dry hay was supplemented to ensure heifers met their nutritional requirements. While offering a supplemental forage has the potential to influence triticale intake behavior, fermentation patterns, and CH<sub>4</sub> production, this practice is standard for the region and represents real-world management conditions. Variation in fiber digestibility, protein content, and pasture biomass availability can alter rumen microbial dynamics and CH<sub>4</sub> yield (Arndt et al., 2022; Beauchemin et al., 2022). Further research is needed to better understand the implications of seasonal shifts on essential oil efficacy. Regardless of seasonal shifts in forage mass and nutrient composition in the current study, heifers supplemented with Agolin demonstrated a decrease in CH<sub>4</sub> production and improved EI. The findings suggest that the impacts of supplementing grazing cattle with a dry distillers grains based supplement containing Agolin were sustained over the experimental period despite fluctuating pasture conditions.

## **Implications**

Supplementing grazing heifers with distillers grains containing Agolin resulted a 8.96% decrease in CH<sub>4</sub> emissions and a 17.27% improvement in EI compared to heifers supplemented with distillers grains without Agolin. Results indicated that ADG was not significantly different between the treatment groups; however, a numeric increase in ADG was observed for heifers supplemented with Agolin. The ability to observe these treatment effects under real-world grazing conditions strengthens the practical validity of the findings. The hypothesis that Agolin supplementation would reduce CH<sub>4</sub> without negatively affecting performance was supported. The present findings extend previous confinement-based evaluations of Agolin through observations of CH<sub>4</sub> mitigation under commercial grazing conditions. However, further research is warranted to better define dose consistency, long-term adaptation, and responses under diverse forage environments.

## Tables

**Table 3.1 Formulated ingredient inclusion and nutrient composition of the Agolin and control supplements.**

| Item                           | AGO Supplement <sup>a</sup> | CON Supplement <sup>a</sup> |
|--------------------------------|-----------------------------|-----------------------------|
| Ingredient Inclusion, % as-fed |                             |                             |
| Dried distillers grains        | 92.00                       | 92.00                       |
| Calcium carbonate (limestone)  | 5.84                        | 6.00                        |
| Mineral oil                    | 2.00                        | 2.00                        |
| Agolin Ruminant                | 0.16                        | —                           |
| Nutrient Composition, % of DM  |                             |                             |
| Dry matter                     | 89.80                       | 89.80                       |
| Crude protein                  | 27.05                       | 27.05                       |
| Fat                            | 7.53                        | 7.53                        |
| Neutral detergent fiber        | 25.76                       | 25.76                       |
| Acid detergent fiber           | 10.12                       | 10.12                       |
| Ash                            | 10.03                       | 10.19                       |
| Calcium                        | 2.31                        | 2.37                        |
| Phosphorus                     | 0.69                        | 0.69                        |
| Potassium                      | 0.83                        | 0.83                        |
| Magnesium                      | 0.28                        | 0.28                        |
| Sulfur                         | 0.49                        | 0.49                        |
| Sodium                         | 0.23                        | 0.23                        |
| Copper, mg/kg                  | 48.58                       | 48.58                       |
| Iron, mg/kg                    | 234.64                      | 235.12                      |
| Zinc, mg/kg                    | 73.60                       | 73.60                       |
| Selenium, mg/kg                | 0.32                        | 0.32                        |
| Vitamin E, IU/kg               | 35.98                       | 35.98                       |

<sup>a</sup> AGO: supplement mixed to contain Agolin (Alltech Inc., Nicholasville, KY) at dose of 725.8 mg/head/day or 200 mg/100 kg body weight with projected final body weight of 362.9 kg; CON: supplement mixed without containing Agolin

**Table 3.2 Nutrient composition of triticale forage during early and late periods of the grazing trial.**

| Item                                                                                                                                                         | Early Season <sup>a</sup> | Late Season <sup>b</sup> |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|--------------------------|
| Nutrient Composition, % of DM                                                                                                                                |                           |                          |
| Dry matter, % as-is                                                                                                                                          | 95.60                     | 94.40                    |
| Crude protein                                                                                                                                                | 22.30                     | 28.50                    |
| Neutral detergent fiber                                                                                                                                      | 45.0                      | 43.4                     |
| Acid detergent fiber                                                                                                                                         | 26.90                     | 25.00                    |
| Calculated Composition <sup>c</sup>                                                                                                                          |                           |                          |
| NE <sub>m</sub> , Mcal/kg                                                                                                                                    | 1.72                      | 1.79                     |
| NE <sub>g</sub> , Mcal/kg                                                                                                                                    | 1.10                      | 1.17                     |
| Total digestible nutrients, % of DM                                                                                                                          | 72.30                     | 74.50                    |
| Forage dry matter availability (kg/ha) <sup>d</sup>                                                                                                          | ~1,613                    | <100                     |
| <sup>a</sup> Early season = Day 0 forage samples                                                                                                             |                           |                          |
| <sup>b</sup> Late season = Day 52 forage samples                                                                                                             |                           |                          |
| <sup>c</sup> Net energy of maintenance (NE <sub>m</sub> ) and net energy of gain (NE <sub>g</sub> ) calculated using NASEM (2016) values of diet ingredients |                           |                          |
| <sup>d</sup> Forage dry matter availability (kg/ha) = oven-dried quadrat weight (g; 0.25 <sup>2</sup> ) * 40                                                 |                           |                          |

**Table 3.3 Manufacturer-guaranteed nutrient composition of pasture mineral supplement formulated for a target intake of 56.7 g/head/day.**

| Item                                                                                                                                                                                          | Minimum <sup>a</sup> | Maximum <sup>a</sup> |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|----------------------|
| Guaranteed Analysis, as-fed <sup>b</sup>                                                                                                                                                      |                      |                      |
| Calcium, %                                                                                                                                                                                    | 16.4                 | 19.6                 |
| Phosphorus, %                                                                                                                                                                                 | 2.0                  | —                    |
| Salt, %                                                                                                                                                                                       | 16.4                 | 19.6                 |
| Magnesium, %                                                                                                                                                                                  | 5.0                  | —                    |
| Potassium, %                                                                                                                                                                                  | 0.1                  | —                    |
| Copper, PPM <sup>c</sup>                                                                                                                                                                      | 1,000.0              | —                    |
| Selenium, PPM <sup>c</sup>                                                                                                                                                                    | 20.0                 | —                    |
| Zinc, PPM <sup>c</sup>                                                                                                                                                                        | 4,000.0              | —                    |
| Vitamin A, IU/kg <sup>d</sup>                                                                                                                                                                 | 110,231.0            | —                    |
| Vitamin D, IU/kg <sup>d</sup>                                                                                                                                                                 | 11,023.0             | —                    |
| Vitamin E, IU/kg <sup>d</sup>                                                                                                                                                                 | 110.0                | —                    |
| <sup>a</sup> Values represent manufacturer-guaranteed minimum and/or maximum concentrations as listed on the product label for Stockmaster Wheat Pasture Mineral (Hubbard Feeds; Mankato, MN) |                      |                      |
| <sup>b</sup> All concentrations are expressed on an as-fed basis                                                                                                                              |                      |                      |
| <sup>c</sup> PPM = parts per million = mg/kg                                                                                                                                                  |                      |                      |
| <sup>d</sup> IU = international units                                                                                                                                                         |                      |                      |

**Table 3.4 Performance and enteric emissions of grazing beef heifers supplemented with dried distillers grains with or without Agolin.**

| Item                                    | AGO <sup>a</sup> | CON <sup>a</sup> | SEM <sup>b</sup> | P-value     |
|-----------------------------------------|------------------|------------------|------------------|-------------|
| Average daily gain, kg/day <sup>c</sup> | 0.69             | 0.62             | 0.04             | 0.18        |
| Enteric methane, g/day                  | 162.60           | 178.60           | 5.05             | <b>0.05</b> |
| Enteric carbon dioxide, g/day           | 6,120.02         | 6,507.44         | 171.92           | 0.15        |
| Emission intensity <sup>d</sup>         | 242.81           | 293.50           | 14.17            | <b>0.03</b> |

<sup>a</sup> AGO = Agolin supplement; CON = control supplement

<sup>b</sup> SEM = standard error of the mean

<sup>c</sup> Average daily gain calculated using slope of initial, intermediate, and final body weight

<sup>d</sup> Emission intensity = g enteric methane/kg average daily body weight gain

**Table 3.5 Target and average supplement intake on a dry matter basis, zero-intake frequency, and resulting Agolin dose calculations during the 70-day grazing trial.**

| Item                                                         | AGO <sup>a</sup> | CON <sup>a</sup> | SEM   | <i>P</i> - Value |
|--------------------------------------------------------------|------------------|------------------|-------|------------------|
| Number of zero-intake days, days <sup>b</sup>                | 26.8             | 39.2             | 2.11  | <0.01            |
| Percent of days consuming supplement, % <sup>c</sup>         | 61.7             | 44.0             | 3.01  | <0.01            |
| Target supplement intake, kg DM/head/day                     | 0.41             | 0.41             | —     | —                |
| Actual supplement intake, kg DM/head/day <sup>c</sup>        | 0.27             | 0.19             | 0.02  | <0.01            |
| Percent of target supplement intake achieved, % <sup>f</sup> | 66.3             | 46.5             | 3.86  | <0.01            |
| Target Agolin intake, mg/head/day <sup>d</sup>               | 725.80           | —                |       |                  |
| Actual Agolin intake, mg/head/day                            | 481.5            | —                |       |                  |
| Percent of target Agolin intake achieved, % <sup>f</sup>     | 66.3             | —                |       |                  |
| Calculated to exclude zero-intake days                       |                  |                  |       |                  |
| Supplement intake, kg DM/head/day                            | 0.44             | 0.43             | <0.01 | 0.42             |
| Percent of target supplement intake, % <sup>f</sup>          | 106.8            | 105.3            | 2.11  | 0.42             |
| Agolin intake, mg/head/day                                   | 776.8            | —                |       |                  |
| Percent of target Agolin intake, % <sup>f</sup>              | 106.8            | —                |       |                  |

<sup>a</sup> AGO = Agolin supplement; CON = control supplement

<sup>b</sup> Zero-intake days = average number of days on which supplement intake was recorded as 0 kg by the SmartFeed Pro system

<sup>c</sup> Percent of days consuming supplement = (days with non-zero intake divided by total trial days)\*100

<sup>d</sup> Target Agolin intake from supplement containing Agolin (Alltech Inc., Nicholasville, KY) at dose of 725.8 mg/head/day or 200 mg/100 kg body weight with projected final body weight of 362.9 kg

<sup>e</sup> Actual supplement intake values include zero-intake days

<sup>f</sup> Percent of target values calculated as actual intake divided by formulated target intake

## Literature Cited

- Alemu, A. W., Pekrul, L. K. D., Shreck, A. L., Booker, C. W., McGinn, S. M., Kindermann, M., & Beauchemin, K. A. (2021). 3-nitrooxypropanol decreased enteric methane production from growing beef cattle in a commercial feedlot: Implications for sustainable beef cattle production. *Frontiers in Animal Science*, 2. <https://doi.org/10.3389/fanim.2021.641590>
- AOAC International. (2019). *Official methods of analysis* (21st ed.). AOAC International.
- Arndt, C., Hristov, A. N., Price, W. J., McClelland, S. C., Pelaez, A. M., Cueva, S. F., Oh, J., Dijkstra, J., Bannink, A., Bayat, A. R., Crompton, L. A., Eugène, M. A., Enahoro, D., Kebreab, E., Kreuzer, M., McGee, M., Martin, C., Newbold, C. J., Reynolds, C. K., ... Yu, Z. (2022). Full adoption of the most effective strategies to mitigate methane emissions by ruminants can help meet the 1.5 °C target by 2030 but not 2050. *Proceedings of the National Academy of Sciences*, 119(20). <https://doi.org/10.1073/pnas.2111294119>
- Arthur, P. F., Bird-Gardiner, T., Barchia, I. M., Donoghue, K. A., & Herd, R. M. (2018). Relationships among carbon dioxide, feed intake, and feed efficiency traits in ad libitum fed beef cattle. *Journal of Animal Science*, 96(11). <https://doi.org/10.1093/jas/sky308>
- Bach, A., Elcoso, G., Escartín, M., Spengler, K., & Jouve, A. (2023). Modulation of milking performance, methane emissions, and rumen microbiome on dairy cows by dietary supplementation of a blend of essential oils. *Animal*, 17(6). <https://doi.org/10.1016/j.animal.2023.100825>
- Batley, R. J., Romanzini, E. P., Da Silva, K. D., De Souza, W. L., Quigley, S. P., Harper, K. J., Trotter, M. G., Bernardes, P. A., Naiker, M., & Costa, D. F. A. (2024). The essential oil blend Agolin Ruminant reduces methane production in vitro and in vivo when included

in the drinking water of cattle. *Journal of Animal Science*, 102.

<https://doi.org/10.1093/jas/skae315>

Beauchemin, K. A., Ungerfeld, E. M., Abdalla, A. L., Alvarez, C., Arndt, C., Becquet, P.,

Benchaa, C., Berndt, A., Mauricio, R. M., McAllister, T. A., Oyhantçabal, W., Salami,

S. A., Shalloo, L., Sun, Y., Tricarico, J., Uwizeye, A., De Camillis, C., Bernoux, M.,

Robinson, T., & Kebreab, E. (2022). Invited review: Current enteric methane mitigation

options. *Journal of Dairy Science*, 105(12). <https://doi.org/10.3168/jds.2022-22091>

Beauchemin, K. A., Ungerfeld, E. M., Eckard, R. J., & Wang, M. (2020). Review: Fifty years of research on rumen methanogenesis: Lessons learned and future challenges for mitigation.

*Animal*, 14. <https://doi.org/10.1017/S1751731119003100>

Beck, M. R., Thompson, L. R., Moffet, C. A., Reuter, R. R., & Gunter, S. A. (2025). Method:

Comparing averaging methods for gas flux data generated by automated head chamber systems. *Animal - Open Space*, 4. <https://doi.org/10.1016/j.anopes.2025.100106>

Beck, M. R., Thompson, L. R., Proctor, J. A., Reuter, R. R., & Gunter, S. A. (2024).

Recommendations on visit duration and sample number requirements for an automated head chamber system. *Journal of Animal Science*, 102.

<https://doi.org/10.1093/jas/skae158>

Belanche, A., Newbold, C., Morgavi, D., Zweifel, B., & Yáñez-Ruiz, D. (2020). A meta-analysis describing the effects of the essential oil blend Agolin Ruminant on performance, rumen

fermentation and methane emissions in dairy cows. *Animals*, 10(4).

<https://doi.org/10.3390/ani10040620>

- Bowman, J. G., & Sowell, B. F. (1997). Delivery method and supplement consumption by grazing ruminants: A review. *Journal of Animal Science*, 75(2), 543–550.  
<https://doi.org/10.2527/1997.752543x>
- Calsamiglia, S., Busquet, M., Cardozo, P. W., Castillejos, L., & Ferret, A. (2007). Invited Review: Essential oils as modifiers of rumen microbial fermentation. *Journal of Dairy Science*, 90(6), 2580–2595. <https://doi.org/10.3168/jds.2006-644>
- Castro-Montoya, J., Peiren, N., Cone, J., Zweifel, B., Fievez, V., & Campeneere, S. (2015). In vivo and in vitro effects of a blend of essential oils on rumen methane mitigation. *Livestock Science*, 180, 134–142. <https://doi.org/10.1016/j.livsci.2015.08.010>
- Cobellis, G., Trabalza-Marinucci, M., & Yu, Z. (2016). Critical evaluation of essential oils as rumen modifiers in ruminant nutrition: A review. *Science of The Total Environment*, 545–546, 556–568. <https://doi.org/10.1016/j.scitotenv.2015.12.103>
- Dillon, J. A., Stackhouse-Lawson, K. R., Thoma, G. J., Gunter, S. A., Rotz, C. A., Kebreab, E., Riley, D. G., Tedeschi, L. O., Villalba, J., Mitloehner, F., Hristov, A. N., Archibeque, S. L., Ritten, J. P., & Mueller, N. D. (2021). Current state of enteric methane and the carbon footprint of beef and dairy cattle in the United States. *Animal Frontiers*, 11(4), 57–68.  
<https://doi.org/10.1093/af/vfab043>
- Elcoso, G., Zweifel, B., & Bach, A. (2019). Effects of a blend of essential oils on milk yield and feed efficiency of lactating dairy cows. *Applied Animal Science*, 35(3), 304–311.  
<https://doi.org/10.15232/aas.2018-01825>
- Fouts, J. Q., Grossi, S., Tricarico, J. M., & Kebreab, E. (2025). Effects of pulse-dosing an essential oil blend to dairy cows on enteric methane emissions and productivity. *Translational Animal Science*, 9. <https://doi.org/10.1093/tas/txaf056>

- Gunter, S. A., & Beck, M. R. (2018). Measuring the respiratory gas exchange by grazing cattle using an automated, open-circuit gas quantification system. *Translational Animal Science*, 2(1), 11–18. <https://doi.org/10.1093/tas/txx009>
- Hristov, A. N., Oh, J., Giallongo, F., Frederick, T., Weeks, H., Zimmerman, P. R., Harper, M. T., Hristova, R. A., Zimmerman, R. S., & Branco, A. F. (2015). The use of an automated system (GreenFeed) to monitor enteric methane and carbon dioxide emissions from ruminant animals. *Journal of Visualized Experiments*, (103). <https://doi.org/10.3791/52904>
- Huhtanen, P., Bayat, A., Lund, P., & Guinguina, A. (2021). Residual carbon dioxide as an index of feed efficiency in lactating dairy cows. *Journal of Dairy Science*, 104(5), 5332–5344. <https://doi.org/10.3168/jds.2020-19370>
- Janssen, P. H. (2010). Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. *Animal Feed Science and Technology*, 160(1–2), 1–22. <https://doi.org/10.1016/j.anifeedsci.2010.07.002>
- Johnson, K. A., & Johnson, D. E. (1995). Methane emissions from cattle. *Journal of Animal Science*, 73(8), 2483–2492. <https://doi.org/10.2527/1995.7382483x>
- Kunkle, W., Johns, J., Poore, M., & Herd, D. (2000). Designing supplementation programs for beef cattle fed forage-based diets. *Journal of Animal Science*, 77, 1–12. <https://doi.org/10.2527/jas2000.00218812007700ES0012x>
- Miller, G. A., Bowen, J. M., Dewhurst, R. J., Zweifel, B., Spengler, K., & Duthie, C.-A. (2023). Enteric methane emissions from dairy–beef steers supplemented with the essential oil blend Agolin Ruminant. *Animals*, 13(11). <https://doi.org/10.3390/ani13111826>

- Thompson, L. R., Beck, M. R., Gunter, S. A., Williams, G. D., Place, S. E., & Reuter, R. R. (2019). An energy and monensin supplement reduces methane emission intensity of stocker cattle grazing winter wheat. *Applied Animal Science*, 35(4), 433–440. <https://doi.org/10.15232/aas.2018-01841>
- Williams, G. D., Beck, M. R., Thompson, L. R., Horn, G. W., & Reuter, R. R. (2018). Variability in supplement intake affects performance of beef steers grazing dormant tallgrass prairie. *The Professional Animal Scientist*, 34(4), 364–371. <https://doi.org/10.15232/pas.2017-01720>
- Williams, P., Clark, J., & Bean, K. (2021). Evaluation of Agolin, an essential oil blend, as a feed additive for high producing cows. *Open Journal of Animal Sciences*, 11(02), 231–237. <https://doi.org/10.4236/ojas.2021.112018>
- Wolin, M. J. (1960). A theoretical rumen fermentation balance. *Journal of Dairy Science*, 43(10), 1452–1459. [https://doi.org/10.3168/jds.S0022-0302\(60\)90348-9](https://doi.org/10.3168/jds.S0022-0302(60)90348-9)