

Performance, rumen fermentation, and enteric emissions measurement methodologies in
commercial feedlot cattle fed ionophore-containing diets

by

Madison Leeann Bemisderfer

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:

Major Professor
Dr. Haley Larson

Copyright

© Madison Bemisderfer 2026.

Abstract

Ionophores are widely used in beef cattle diets to improve feed efficiency through alterations in ruminal fermentation. Most evaluations of ionophore responses and enteric methane (CH₄) emissions have been conducted under controlled research conditions rather than commercial feedlot environments, creating a need for additional evidence from large-pen production settings as well as methodological approaches capable of accurately characterizing ruminal fermentation and CH₄ production under commercial conditions. The objectives of this thesis were to evaluate (1) the effects of rotational feeding of monensin and laidlomycin propionate on cattle performance and ruminal fermentation and (2) methodological approaches to estimate enteric CH₄ production under commercial feedlot conditions. Experiment 1 evaluated growth performance of 584 crossbred beef heifers (initial BW = 290 ± 26 kg) assigned to receive either consistent dietary inclusion of monensin (MON; 200 mg/heifer/d) or rotational ionophore exposure (ROT), alternating every 28 d between dietary inclusion of monensin (200 mg/heifer/d) and laidlomycin propionate (75 mg/heifer/d) during a 112-d growing period. Heifers were limited to a common diet containing 1.26 Mcal/kg NE_g and 14.3% crude protein (DM basis). Across the feeding period, ROT had increased average daily gain (1.54 vs. 1.41 kg/d; $P \leq 0.01$) and improved gain efficiency (G:F; 0.171 vs. 0.162; $P \leq 0.01$) compared to MON, while dry matter intake did not differ ($P > 0.10$) by design. Experiment 2 evaluated ruminal fermentation responses using *ex vivo* batch culture fermentation with rumen inoculum collected from ruminally cannulated steers exposed to either consistent dietary inclusion of monensin (200 mg/steer/d) or a rotational program of dietary ionophore inclusion of 56 d monensin, 28 d laidlomycin propionate (75 mg/steer/d), and a final 28 d monensin. Following the 28-d laidlomycin propionate feeding period (d 84), ROT tended to produce greater CH₄ during *ex vivo*

fermentation (19.47 vs. 15.30 mL; $P = 0.06$) and exhibited a lower carbon dioxide (CO₂) to CH₄ ratio (6.14 vs. 8.75; $P = 0.03$) compared to MON. To understand the reliability of the *ex vivo* batch culture techniques, an additional experiment was conducted where CH₄ production estimated from *ex vivo* batch culture fermentation was compared with *in vivo* measurements obtained using automated head chamber systems (AHCS; GreenFeed; C-Lock Inc., Rapid City, SD) in beef × dairy steers, including ruminally cannulated steers co-housed with commercial steers under commercial feedlot conditions. Methane estimates from *ex vivo* fermentation scaled using substrate intake (145.8 g CH₄/steer/d) or rumen fill (149.5 g CH₄/steer/d) were similar to pen-level AHCS estimates (139.2–143.9 g CH₄/steer/d; $P > 0.10$), whereas CH₄ values measured from ruminally cannulated steers using AHCS were substantially lower (39.3 g CH₄/steer/d; $P < 0.05$). Collectively, these findings demonstrate that rotational feeding of monensin and laidlomycin propionate can improve growth performance in growing cattle under commercial feedlot conditions and that batch culture fermentation-based approaches can generate CH₄ estimates comparable to pen-level *in vivo* measurements when appropriate biological scaling assumptions are applied.

Keywords

Ionophores, commercial feedlot production, rumen fermentation, methane emissions

Table of Contents

List of Figures	viii
List of Tables	ix
Acknowledgements	x
Dedication	xi
Chapter 1 - Literature review of relevant topics	1
Introduction.....	1
Ionophores in beef cattle nutrition	2
Effects on rumen fermentation	6
Ionophores and methane emissions	10
Effects on cattle performance	12
Rotational ionophore feeding.....	14
Challenges in evaluating rumen modifiers under commercial conditions	17
Knowledge gaps and justification for thesis	20
References.....	24
Chapter 2 - Effects of rotations of monensin and laidlomycin propionate on commercial cattle	
performance and <i>ex vivo</i> fermentation during the growing phase	28
Introduction.....	28
Materials and methods	30
Study overview	30
Experiment 1: heifer performance	30
Study animals and facility.....	30
Dietary treatments and ration.....	31
Arrival processing and health management	32
Body weight collection	32
Experiment 2: <i>ex vivo</i> gas production	33
Study animals and facility.....	33
Dietary treatments and ration.....	34
Arrival processing and health management.....	34
<i>Ex vivo</i> batch culture fermentation	35

Co-housed commercial beef × dairy steers	37
Laboratory sample analysis for all experiments	37
Statistical analysis for all experiments.....	38
Experiment 1: heifer performance	38
Experiment 2: <i>ex vivo</i> gas production	39
Results.....	41
Experiment 1: heifer performance	41
Experiment 2: <i>ex vivo</i> gas production	42
Discussion	43
Implications	46
Tables.....	47
References.....	55
Chapter 3 - Comparing <i>ex vivo</i> batch culture and <i>in vivo</i> automated head chamber system	
methods for daily methane production estimates in commercial beef × dairy steers	57
Introduction.....	57
Materials and methods	60
Study overview	60
Animals and facility	60
Arrival processing and health management	60
Ration and feeding	61
Laboratory sample analysis.....	62
Methane quantification methods	63
<i>In vivo</i> methane measurement using Automated Head Chamber Systems	63
Rumen fluid collection and <i>ex vivo</i> batch culture fermentation.....	65
Scaling <i>ex vivo</i> daily gas production to estimated daily methane output.....	66
Rumen fill–based scaling method	66
Ration intake–based scaling method.....	67
<i>Ex vivo</i> methane scaling calculations.....	68
Statistical analysis	68
Results.....	69
Discussion.....	70

Implications	74
Tables	75
Figures	78
References.....	79

List of Figures

Figure 3.1 Daily enteric methane emissions (g CH ₄ /hd/d) estimates by AHCS and scaled <i>ex vivo</i> quantification methods.	78
---	----

List of Tables

Table 2.1 Outline of strategic rotations indicating ionophore inclusion in ration by day for Experiment 1 treatment groups.	47
Table 2.2 Ingredient and nutrient composition of commercial growing ration (dry matter basis) in Experiment 1 & Experiment 2.	48
Table 2.3 Outline of strategic rotations indicating ionophore inclusion in ration by day for Experiment 2 treatment groups.	49
Table 2.4 Performance within 28-d periods of study comparing cattle receiving consistent monensin (MON) to cattle receiving 28-d rotations between monensin and laidlomycin propionate (ROT) in Experiment 1.	50
Table 2.5 Cumulative performance across multiple 28-d periods of study comparing cattle receiving consistent monensin (MON) to cattle receiving 28-d rotations between monensin and laidlomycin propionate (ROT) in Experiment 1.	51
Table 2.6 <i>Ex vivo</i> modeled gas production kinetics by treatment and sampling period comparing cattle receiving consistent monensin (MON) to cattle receiving a rotational ionophore program of 56 d monensin, followed by 28 d laidlomycin propionate and a final 28 d monensin (ROT) from cannulated steer donor fluid collected in Experiment 2.	52
Table 2.7 <i>Ex vivo</i> gas composition by treatment and sampling period comparing cattle receiving consistent monensin (MON) to cattle receiving a rotational ionophore program of 56 d monensin, followed by 28 d laidlomycin propionate and a final 28 d monensin (ROT) from cannulated steer donor fluid collected in Experiment 2.	53
Table 2.8 Average daily gain (kg/d) of commercial beef × dairy steers cohoused with cannulated donor steers comparing cattle receiving consistent monensin (MON) to cattle receiving a rotational ionophore program of 56 d monensin, followed by 28 d laidlomycin propionate and a final 28 d monensin (ROT) in Experiment 2.	54
Table 3.1 Ingredient and nutrient composition of limit-fed growing ration (dry matter basis).	75
Table 3.2 Methane quantification methods utilized to estimate daily enteric methane emissions (g CH ₄ /hd/d) of commercial beef × dairy growing steers.	76
Table 3.3 Daily enteric methane emissions (g CH ₄ /hd/d) estimate by AHCS and scaled <i>ex vivo</i> quantification methods.	77

Acknowledgements

First and foremost, I would like to thank my labmates, Logan Diller and Ashlee Gibbons, for their involvement in every aspect of the on-farm trials. From weigh days and sample collection to animal handling and long days at the feedyard, their dedication and willingness to help made this research possible. The many hours spent traveling to the feedyard, working through the demands of field research, and supporting each other along the way made this experience both productive and memorable.

I am sincerely grateful to my advisor, Dr. Haley Larson, for her guidance and mentorship throughout my graduate program. I am especially thankful for the opportunities her program provided to work directly with commercial feedlot systems and to network with industry professionals and collaborating companies.

I would also like to extend my appreciation to Matt Shelor, manager of Clark County Feeders (Minneola, KS), as well as the entire cowboy and maintenance crew for their assistance and support during the trial. Their cooperation, provision of facilities, and management of the cattle made this research possible. I am also grateful to Matt's family, including Marsha and Maiya, for their hospitality while we were working at the feedyard. Maiya's help with sample collection was greatly appreciated.

Finally, I would like to thank my committee members, Dr. Logan Thompson and Dr. T. G. Nagaraja, for their guidance throughout this project. Dr. Thompson's help in understanding and interpreting GreenFeed data was invaluable, and Dr. Nagaraja's rumen metabolism course was one of the most impactful classes I took during my graduate program.

Dedication

This thesis is dedicated to my family, especially my parents, whose love and support have shaped my entire life and guided me through every step of my education. Their encouragement has always pushed me to keep going, even when things felt difficult. I am also thankful for my grandparents, who have always expressed their pride and unwavering support in everything I pursue.

Finally, I dedicate this work to my late grandfather-in-law, Eddie Hessong. Our time together was far too short, but he always made my husband and me feel that the work we do in agriculture is meaningful and important. He welcomed me as one of his own, and his encouragement and belief in us will always stay with me.

Chapter 1 - Literature review of relevant topics

Introduction

Feed efficiency is one of the major factors influencing profitability in cattle finishing operations (Langemeier et al., 1992). Ionophores, a class of feed additives introduced to cattle production in the 1970s, have been widely adopted because of their demonstrated abilities to alter ruminal fermentation, leveraging mechanisms that improve feed utilization and regulate voluntary feed intake (Bergen & Bates, 1984; Russell & Strobel, 1989). National survey data indicate that 90–97% of feedlots use ionophores in receiving and finishing diets, reflecting their status as one of the most common feed technologies in U.S. beef production (Samuelson et al., 2016).

Ionophores are regulated as animal drugs in the United States. While this group of drugs is classified as antibiotics due to their action against bacteria, they are not used in human medicine and are categorized as not medically important antimicrobials (CEAH, 2024). Through shifting prevalence of certain microbial species in the rumen, ionophores can shift volatile fatty acid (VFA) production toward propionate. The change in VFA profile is hypothesized to reduce the availability of dihydrogen (H_2) for methanogenesis and thereby influence enteric methane (CH_4) emissions, although *in vivo* responses have been variable (Bergen & Bates, 1984; Chen & Wolin, 1979; Russell & Strobel, 1989).

Despite their widespread use and long history in beef production, much of the research on ionophores has been conducted under controlled experimental conditions, and relatively little work has been carried out in commercial feedlot settings (Cernicchiaro et al., 2016; Duffield et al., 2012; Golder & Lean, 2016). The lack of commercial-scale research limits the ability to

evaluate how fermentation responses, animal performance, and environmental outcomes associated with ionophore use manifest under applied conditions.

The following review will examine ionophores used in beef cattle systems, with an emphasis on their mechanisms of action in the rumen, regulatory status, and effects on rumen fermentation and animal performance. It will also consider why rotational use of ionophores has been proposed as a strategy to sustain biological responses during a feeding period. In addition, this review will discuss why commercial-scale research on rumen modifiers is relatively scarce, and will explore the benefits, limitations, and practical considerations of experimental and monitoring approaches that can be used to study ionophores under applied conditions.

Ionophores in beef cattle nutrition

Ionophores are polyether antibiotics that form lipid-soluble complexes with cations and mediate their transport across microbial membranes, thereby perturbing ion gradients essential for microbial metabolism (Nagaraja, 1995). Ionophores act at the site of the microbial membrane, thus they are most effective against Gram-positive bacteria. In contrast, Gram-negative species are largely protected by their outer membrane. Selective inhibition of Gram-positive bacteria alters ruminal microbial populations and contributes to shifts in fermentation end products (Callaway et al., 2003). The net result of this selective inhibition is characterized by increased propionate production and reduced acetate, lactate, and metabolic H₂. This shift energetically improves efficiency of energy capture from feed (Russell & Strobel, 1989).

All commercially approved ionophores for U.S. cattle production are carboxylic polyether antibiotics derived from *Streptomyces* species through microbial fermentation (Bergen & Bates, 1984; Nagaraja, 1995). Monensin sodium is produced by *Streptomyces cinnamonensis*, lasalocid sodium by *Streptomyces lasaliensis*, and laidlomycin propionate by *Streptoverticillium*

eurocidicum. Other ionophores, including salinomycin (*Streptomyces albus*) and narasin (*Streptomyces aureofaciens*), are also products of microbial fermentation but are only approved for use in poultry and swine in the United States (U.S. Food & Drug Administration, 2025).

Ionophores were first isolated in the 1950s, and the chemical structure of monensin was first described in 1967 (Agtarap et al., 1967). Relevance of ionophores to the livestock industry emerged in 1967, when monensin and related compounds were shown to be effective coccidiostats in poultry (Shumard & Callender, 1967). Monensin (Rumensin®, NADA 095-735) was subsequently approved by the U.S. Food and Drug Administration on December 16, 1975, for use in feedlot cattle with a labeled claim of improving feed efficiency. Lasalocid (Bovatec®, NADA 096-298) followed with approval for beef cattle on August 6, 1982. The most recent cattle approval was laidlomycin propionate (Cattlyst®, NADA 141-025), which received FDA approval on March 4, 1994, for improving rate of weight gain and feed efficiency in feedlot cattle (U.S. Food & Drug Administration, 2025).

Other ionophores have been approved in the United States, but only for non-ruminant species. Salinomycin (NADA 128-686) was approved in 1983 for use in broiler chickens, and narasin (NADA 118-980) was approved in 1986 for use in broilers and has since gained approval for use in swine as well. To date, neither compound has ever been approved for use in cattle in the United States (U.S. Food & Drug Administration, 2025).

Ionophores are regulated in the United States as approved animal drugs under the New Animal Drug Application (NADA) process (CEAH, 2024). Each NADA specifies the approved cattle class, dosage range, and product indications. Approved indications vary by product but include improved feed efficiency, improved rate of weight gain, and/or prevention or control of coccidiosis (U.S. Food & Drug Administration, 2025). FDA label language also defines the

conditions under which ionophores may be fed. According to the FDA, ionophores must be fed continuously, or for 14 or more consecutive days (Code of Federal Regulations, 2025). This regulatory framework allows for long-term administration in cattle diets, with coccidiosis prevention or control claims tied to specified durations of continuous use (U.S. Food & Drug Administration, 2025). In addition, several ionophores are cleared for use in combination with other medicated feed additives, provided compatibility is established and claims are consistent with those on the approved labels (U.S. Food & Drug Administration, 2025). Examples include combinations of monensin with melengestrol acetate or tylosin, and lasalocid with decoquinate.

Feeding rates and label claims for ionophores are defined on a product-specific basis and vary by molecule, cattle class, and production stage (U.S. Food & Drug Administration, 2025). In feedlot cattle, monensin is approved for use between 5.5 to 44 mg/kg of complete feed (DM basis), whereas laidlomycin propionate is approved between 5.5 to 11 mg/kg and lasalocid between 11 to 33 mg/kg of complete feed (DM basis) (U.S. Food & Drug Administration, 2025). Labeled inclusion rates reflect regulatory approvals and observed biological responses, but they do not provide a standardized basis for comparing intrinsic antimicrobial potency across compounds. Consequently, differences among ionophores are typically evaluated using mechanistic, microbial, or functional response data rather than direct potency comparisons (Nagaraja, 1995).

Ionophores also differ in the specific microbial functional groups they inhibit (Bergen & Bates, 1984; Russell & Strobel, 1989). Although all commercially used ionophores preferentially inhibit Gram-positive bacteria, studies have reported differences in the extent to which individual compounds affect lactate-producing bacteria, hyper-ammonia-producing bacteria, and other Gram-positive taxa (Nagaraja, 1995; Russell & Houlihan, 2003; Russell & Strobel, 1989).

Differences in microbial targeting are associated with variation in fermentation end products, ruminal pH dynamics, and nitrogen metabolism.

Ionophores further differ in their ion transport specificity (Gräfe et al., 1989). Monensin is commonly described as preferentially transporting sodium ions (Na^+) in exchange for protons, whereas lasalocid and laidlomycin propionate exhibit differing affinities for potassium ions (K^+) and other cations (Gräfe et al., 1989; Russell & Houlihan, 2003; Russell & Strobel, 1989).

Variation in cation selectivity affects intracellular ion balance and microbial susceptibility. In sensitive bacteria, ionophores disrupt transmembrane ion gradients, increasing the energetic cost of maintaining intracellular homeostasis and ultimately reducing microbial growth and activity. Resulting changes in microbial activity contribute to differences in ruminal fermentation responses among ionophores.

Reported functional differences among ionophores are consistent with these mechanistic distinctions. Monensin has been associated with regulation of dry matter intake and increases in propionate production, along with improvements in feed efficiency (Duffield et al., 2012; Russell & Houlihan, 2003; Van Maanen et al., 1978). Lasalocid has been reported to produce effects that are similar to monensin but differ in magnitude across diets and inclusion rates (Golder & Lean, 2016). Laidlomycin propionate has been described as having relatively strong potassium transport selectivity, but comparatively fewer studies have evaluated its effects across production systems (Gräfe et al., 1989). Direct head-to-head comparisons among ionophores are limited, particularly under commercial feedlot conditions (Cernicchiaro et al., 2016; Marques & Cooke, 2021). The lack of head-to-head product comparisons limits the ability to attribute observed differences in fermentation or performance responses to specific mechanistic properties of individual compounds. The scarcity of direct comparative studies may be influenced by practical

and economic barriers, including the cost and logistical complexity of large-scale feedlot trials, feed manufacturing constraints when incorporating multiple ionophores, and limited industry incentive to directly compare competing products.

Effects on rumen fermentation

Functional distinctions among ionophores ultimately manifest through alterations in rumen microbial activity and fermentation end products (Nagaraja, 1995). Ionophores act primarily on Gram-positive bacteria and disrupt transmembrane ion gradients, therefore their effects are expressed not only as changes in microbial community structure but also as shifts in VFA production, nitrogen metabolism, and gas formation (Bergen & Bates, 1984; Russell & Strobel, 1989). Fermentation responses represent the primary biological mechanism through which ionophores influence animal performance and greenhouse gas emissions.

Ionophore supplementation has been consistently associated with shifts in VFA profiles across a wide range of diets and is often characterized by increased propionate production and a reduced acetate:propionate ratio (Bergen & Bates, 1984; Russell & Strobel, 1989; Van Maanen et al., 1978). The shift reflects selective inhibition of Gram-positive, acetate- and butyrate-producing bacteria and a relative enrichment of Gram-negative, propionate-producing populations. Propionate serves as a primary gluconeogenic precursor in ruminants, so increased propionate production improves the energetic efficiency of fermentation by increasing the proportion of metabolizable energy captured as glucose equivalents rather than as heat or CH₄. Diet composition, particularly the proportion of rapidly fermentable carbohydrate, as well as ionophore dose and duration of exposure influence the magnitude of changes in ruminal VFA profiles.

Rumen environment can be influenced by more than VFA profiles. Ionophores have shown influence on ruminal pH dynamics by modifying lactate production and utilization (Nagaraja, 1995; Russell & Strobel, 1989). Ionophores broadly suppress the growth of Gram-positive, lactate-producing bacteria such as *Streptococcus bovis* and *Lactobacillus spp.*, which indirectly favors lactate-utilizing populations. Microbial shifts influenced by ionophores can reduce the accumulation of lactic acid during rapid carbohydrate fermentation and promote greater pH stability under high-concentrate feeding conditions. The extent of the effects is highly dependent on diet composition, feeding management, and inclusion rate. For these reasons, ionophores are not considered a primary strategy for preventing ruminal acidosis. Instead, their influence on pH reflects secondary effects of altered microbial competition and fermentation patterns rather than direct buffering capacity (Bergen & Bates, 1984).

Ionophore supplementation influences ruminal nitrogen metabolism through selective effects on microbial populations involved in amino acid deamination and ammonia production (Chen & Wolin, 1979; Poos et al., 1979; Russell & Strobel, 1989). Through selective inhibition of Gram-positive bacteria, including organisms associated with high rates of amino acid deamination, ionophores reduce amino acid deamination in the rumen. Suppression of ammonia-producing bacteria activity can decrease the conversion of dietary and microbial amino acids to ammonia, thereby altering ruminal ammonia concentrations and the balance between nitrogen capture in microbial protein versus nitrogen loss through absorption and excretion.

By moderating excessive deamination, ionophores may improve the efficiency with which ruminal microbes incorporate nitrogen into microbial protein, particularly under conditions where adequate fermentable energy is available to support microbial growth (Poos et al., 1979; Russell & Strobel, 1989). The magnitude of the effects is influenced by dietary protein

degradability, fermentable carbohydrate supply, and overall rumen microbial dynamics, and responses may vary across feeding systems. Nitrogen metabolism shifts represent an additional pathway, beyond VFA profile changes, through which ionophores influence the efficiency of ruminal fermentation.

Changes in VFA production induced by ionophores are closely linked to alterations in H_2 balance within the rumen (Chen & Wolin, 1979; Russell & Strobel, 1989). Different fermentation pathways vary in their generation and utilization of reducing equivalents, including H_2 . Pathways leading to acetate and butyrate formation are associated with greater net production of reducing equivalents, whereas propionate formation serves as a H_2 -utilizing pathway. Consequently, shifts in VFA profiles toward greater propionate and reduced acetate proportions alter the pool of reducing equivalents available within the rumen environment.

Methanogenesis is one of the primary routes by which excess reducing equivalents are removed from the rumen ecosystem. Methanogenic archaea use H_2 to reduce carbon dioxide (CO_2) to CH_4 (Russell & Strobel, 1989). By promoting fermentation patterns that favor propionate formation, ionophores can indirectly influence the balance between H_2 production and utilization, thereby affecting gas formation patterns associated with ruminal fermentation (Chen & Wolin, 1979; Russell & Strobel, 1989). The effects arise from changes in microbial competition and fermentation pathways rather than direct inhibition of methanogens in most cases.

Although ionophores produce consistent short-term shifts in ruminal fermentation, the magnitude and persistence of these responses over extended feeding periods have been reported to vary (Guan et al., 2006; Appuhamy et al., 2013; Russell & Strobel, 1989). Several studies have suggested that rumen microbial communities may adapt to ionophore exposure over time,

potentially attenuating some fermentation responses, including changes in VFA profiles or other metabolic parameters (Chen & Wolin, 1979; Russell & Strobel, 1989). Microbial adaptation to ionophore exposure may involve shifts in microbial community composition, changes in relative abundance of ionophore-sensitive versus more tolerant populations, or altered expression of metabolic pathways.

Importantly, changes in VFA profiles, CH₄ production, and nitrogen metabolism do not appear to occur at the same rate or magnitude over time (Guan et al., 2006; Russell & Strobel, 1989). Some parameters may remain relatively stable, whereas others show diminished magnitude following prolonged exposure. Dietary composition, feeding management, and ionophore dose can influence the extent to which fermentation responses are maintained (Poos et al., 1979; Russell & Strobel, 1989). Variability in persistence of response is central to questions surrounding long-term ionophore effectiveness and provides biological rationale for considering strategies that may influence microbial exposure patterns (Chen & Wolin, 1979; Russell & Strobel, 1989).

The combined effects of ionophores on VFA production, nitrogen metabolism, H₂ balance, and the persistence of microbial responses indicate that these compounds influence multiple, interconnected components of ruminal fermentation. Partitioning of dietary substrates among fermentation end products, microbial biomass synthesis, and gaseous byproducts governs ruminal energy capture and microbial growth. As a result, ionophore-induced shifts in microbial activity influence multiple components of rumen function simultaneously. The persistence of individual fermentation responses over time varies, reflecting functional adaptation of the ruminal microbial ecosystem to continued ionophore exposure (Chen & Wolin, 1979; Guan et al., 2006; Russell & Strobel, 1989). This complexity highlights that ionophore effects cannot be

fully described by single parameters but rather reflect integrated changes across fermentation pathways.

Ionophores and methane emissions

Enteric CH₄ production is a natural consequence of anaerobic fermentation in the rumen. During carbohydrate fermentation, reducing equivalents are generated and must be removed to maintain redox balance within the microbial ecosystem, ultimately contributing to the formation of H₂. Methanogenic archaea utilize H₂ to reduce CO₂ to CH₄, providing a major pathway for the disposal of excess reducing equivalents (Bergen & Bates, 1984; Chen & Wolin, 1979; Russell & Strobel, 1989). As a result, factors that alter H₂ production or utilization during fermentation can influence CH₄ formation.

Evidence for ionophore effects on CH₄ production has been reported in both *in vitro* and *in vivo* systems. *In vitro* batch and continuous culture studies commonly demonstrate reductions in CH₄ production when ionophores are included in fermentation substrates (Chen & Wolin, 1979; Domesick & Martin, 1999; Russell & Martin, 1984). Reductions observed *in vitro* are often substantial, commonly ranging from approximately 40 to 80% depending on substrate type and microbial adaptation status, with larger effects observed in unadapted systems and fermentations of rapidly fermentable substrates (Domesick & Martin, 1999; Russell & Martin, 1984). The magnitude of reduction observed *in vitro* likely reflects the use of controlled systems with relatively high ionophore concentrations, absence of passage rate, and limited microbial adaptation, conditions that differ substantially from those encountered *in vivo*. Controlled *in vitro* systems allow clear observation of fermentation shifts but may not fully capture the complexity of *in vivo* rumen dynamics, including host interactions, passage rate, and feeding behavior (Johnson, 1966). *In vivo* studies in cattle have produced more variable results, with some

reporting consistent reductions in CH₄ yield (Appuhamy et al., 2013; Thornton & Owens, 1981) and others observing diminished or transient responses over time, suggesting microbial adaptation to continued ionophore exposure (Guan et al., 2006; Rumpler et al., 1986). Reported reductions in CH₄ yield with monensin supplementation are generally modest, typically ranging from approximately 2–10%, with the greatest effects often observed during initial periods of feeding (Appuhamy et al., 2013). In addition, responses in CH₄ production and CH₄ yield are not always aligned, as changes in dry matter intake and fermentation efficiency can influence total emissions independently of emissions per unit of intake. Variation among studies likely reflects differences in diet composition, ionophore inclusion rate, duration of feeding, and measurement methodology.

Reports comparing different ionophores suggest that the magnitude of CH₄ response may differ among compounds, although findings are not consistent across studies (Domescik & Martin, 1999; Russell & Martin, 1984). In mixed ruminal microorganism fermentations, monensin generally reduced CH₄ production by approximately 20–40% compared to laidlomycin propionate, depending on substrate and microbial adaptation status (Domescik & Martin, 1999). In contrast, monensin produced only modestly greater reductions in CH₄ than lasalocid in amino acid-based fermentation systems, with reductions typically differing by approximately 5–10% (Russell & Martin, 1984). These findings suggest that differences among ionophores may depend on substrate type and experimental conditions, limiting direct comparison across studies. Much of the available literature focuses on monensin, with comparatively fewer data available for other ionophores (Appuhamy et al., 2013; Thornton & Owens, 1981).

Taken together, these findings suggest that CH₄ responses to ionophores are governed by the same fermentation shifts that influence energy partitioning in the rumen, but the stability of

these responses over time may depend on how microbial populations reorganize under continued exposure (Chen & Wolin, 1979; Guan et al., 2006; Russell & Strobel, 1989). Methane production reflects the integrated outcome of multiple fermentation pathways rather than a single microbial process. Variability in diet composition, feeding management, and microbial adaptation may explain the inconsistent magnitude of *in vivo* responses reported across studies.

Effects on cattle performance

The effects of ionophores on cattle performance arise from the cumulative influence of altered ruminal fermentation, nutrient partitioning, and feed intake patterns (Bergen & Bates, 1984; Goodrich et al., 1984; Poos et al., 1979). By modifying microbial populations and fermentation end products, ionophores can change the efficiency with which dietary energy and nitrogen are converted into animal growth even when total feed intake is not increased.

Monensin supplementation is consistently associated with reductions in dry matter intake (DMI) of approximately 3–6% in feedlot cattle (Duffield et al., 2012; Goodrich et al., 1984). Early summaries reported a 6.4% reduction in feed intake with monensin (Goodrich et al., 1984), while more recent meta-analysis estimates intake reductions closer to 3% (Duffield et al., 2012), reflecting continued but modest intake suppression under modern feeding conditions. In contrast, intake responses differ among ionophores. Lasalocid supplementation is not typically associated with altered DMI responses, indicating that improved performance can occur without changes in feed consumption (Golder & Lean, 2016). Compared with monensin, laidlomycin propionate increases DMI by approximately 2–3%, demonstrating compound-specific differences in intake regulation among ionophores (Cernicchiaro et al., 2016).

Despite differences in intake response, ionophore supplementation consistently improves average daily gain (ADG), although the magnitude of response is modest (Cernicchiaro et al.,

2016; Duffield et al., 2012; Golder & Lean, 2016). Monensin supplementation increases ADG by approximately 2.5% in growing and finishing beef cattle fed high-concentrate, feedlot-type diets relative to cattle not provided monensin, demonstrating improved growth performance despite reduced feed intake (Duffield et al., 2012). Similarly, lasalocid supplementation increases ADG by approximately 2–3% without affecting DMI, indicating improved utilization of consumed nutrients (Golder & Lean, 2016). Compared with monensin, laidlomycin propionate increases ADG by approximately 3–4%, although this response is accompanied by increased DMI rather than reduced intake (Cernicchiaro et al., 2016). Overall, ionophores consistently improve growth rate, but the specific mechanism differs among compounds depending on whether performance improvements occur with reduced intake, unchanged intake, or increased intake.

Across ionophore compounds, improvements in feed efficiency represent the most consistent and economically important response to supplementation (Cernicchiaro et al., 2016; Duffield et al., 2012; Golder & Lean, 2016). Monensin improves feed efficiency by approximately 2.5–3.0% in modern production settings, primarily through reduced feed intake while maintaining or increasing ADG (Duffield et al., 2012). Lasalocid supplementation similarly improves feed efficiency despite no change in intake (Golder & Lean, 2016). In contrast, laidlomycin propionate produces feed efficiency comparable to monensin but achieves this through proportional increases in both intake and gain rather than reduced intake (Cernicchiaro et al., 2016). Improved feed efficiency is therefore a consistent outcome of ionophore supplementation, regardless of differences in intake response among compounds.

Effects of ionophore supplementation on carcass characteristics are generally less pronounced than effects on intake, growth, and feed efficiency (Cernicchiaro et al., 2016; Duffield et al., 2012; Golder & Lean, 2016). Observed differences in carcass outcomes among

ionophores appear to reflect differences in growth performance rather than direct effects on carcass composition. For example, improvements in carcass-adjusted ADG with laidlomycin propionate relative to monensin are attributable to increased growth rate rather than changes in carcass traits (Cernicchiaro et al., 2016). Collectively, findings indicate that carcass responses are secondary to improvements in growth rate and efficiency rather than primary outcomes of ionophore supplementation.

Collectively, performance responses to ionophores appear to reflect the integrated consequences of altered ruminal fermentation, energy partitioning, and intake regulation rather than uniform increases in growth rate (Bergen & Bates, 1984). Improvements in feed efficiency are often more consistent than changes in ADG and intake responses vary across studies, suggesting that ionophores influence multiple physiological control points that may respond differently depending on diet composition, management, and production stage (Bergen & Bates, 1984; Cernicchiaro et al., 2016; Duffield et al., 2012; Golder & Lean, 2016). The majority of performance data are derived from monensin-focused studies, therefore comparatively less is known about how alternative ionophores may influence the balance between intake regulation and efficiency under commercial conditions. The results indicate that performance responses are context-dependent and may not be fully characterized by single-ionophore evaluations alone.

Rotational ionophore feeding

Ionophores differ in cation transport selectivity, microbial inhibition patterns, and resulting fermentation responses, which can influence intake regulation and animal performance (Bergen & Bates, 1984; Gräfe et al., 1989; Russell & Strobel, 1989). For example, monensin preferentially transports sodium ions and is commonly associated with reduced feed intake and improved feed efficiency, whereas lasalocid and laidlomycin propionate exhibit differing

affinities for potassium and other cations and have been reported to produce distinct intake and growth responses (Cernicchiaro et al., 2016; Golder & Lean, 2016; Gräfe et al., 1989; Russell & Strobel, 1989). The compound-specific differences in microbial targeting and ion transport suggest that ruminal microbial populations may be exposed to distinct selective pressures depending on the ionophore administered. Consequently, continuous exposure to a single ionophore may favor microbial populations with reduced susceptibility to its mode of action, whereas altering ionophore exposure during a feeding period could modify microbial selection dynamics (Guan et al., 2006; Russell & Strobel, 1989). The concept of ionophore rotation is therefore based on the hypothesis that sequential exposure to ionophores with differing biological properties may influence ruminal fermentation, feed intake patterns, and performance responses differently than continuous use of a single compound (Bergen & Bates, 1984; Newbold et al., 1993).

Biological rationale for rotational feeding is based on microbial adaptation and compound-specific differences in antimicrobial activity (Bergen & Bates, 1984; Newbold et al., 1993). Ionophores exert selective inhibition primarily against Gram-positive bacteria, and prolonged exposure to a single ionophore can alter ruminal microbial populations and fermentation responses over time (Chen & Wolin, 1979; Russell & Strobel, 1989). *In vitro* adaptation studies conducted using ruminal fluid from cattle previously exposed to monensin or lasalocid demonstrated altered VFA production patterns and microbial fermentation responses compared with un-adapted microbial populations, confirming that microbial susceptibility changes following ionophore exposure (Duff et al., 1995). Similarly, fermentation studies evaluating laidlomycin propionate and monensin have shown compound-specific differences in microbial fermentation characteristics following ionophore exposure, indicating that individual

ionophores exert distinct selective pressures on ruminal microbial communities (Domescik & Martin, 1999). The mechanistic differences suggest that altering ionophore exposure could modify microbial competition and fermentation pathways relative to continuous exposure to a single compound.

Experimental rotational studies have primarily focused on combinations of monensin and lasalocid, with no available published literature incorporating laidlomycin propionate in rotational programs (Duff et al., 1995; McKinnon et al., 1992; Morris et al., 1990; Shreck et al., 2016). The absence of rotational studies incorporating laidlomycin propionate likely reflects both the later approval of laidlomycin propionate in 1994 and its more limited adoption relative to monensin and lasalocid in commercial feeding programs (Samuelson et al., 2016; U.S. Food & Drug Administration, 2025). Existing studies demonstrate that rotational feeding can influence performance under certain conditions, but responses are inconsistent and appear to depend on ionophore combination, diet composition, and microbial adaptation state (McKinnon et al., 1992; Morris et al., 1990; Shreck et al., 2016). Furthermore, most rotational studies have focused primarily on growth performance and feed efficiency, with fewer evaluations simultaneously measuring fermentation characteristics, microbial adaptation, or gas production responses (Duff et al., 1995; Morris et al., 1990). Collectively, available studies demonstrate that rotational ionophore feeding can influence performance outcomes; however, responses are inconsistent across experiments. Positive responses have primarily been observed in high-concentrate feedlot systems, whereas other experiments report no benefit relative to continuous ionophore feeding, indicating that effectiveness depends on ionophore combination, diet composition, and microbial adaptation state.

Despite biological rationale and previous research indicating potential beneficial outcomes, practical implementation of rotational ionophore feeding is constrained by regulatory approval and label requirements in the United States. FDA regulations stipulate that ionophores must be fed “continuously,” which, as defined previously, is defined as ≥ 14 consecutive days (21 C.F.R. § 558.3, 2025). Regulatory requirements therefore limit the feasibility of short-interval rotational strategies, such as daily or weekly rotation, under commercial production conditions in the US. Additionally, only three ionophores—monensin, lasalocid, and laidlomycin propionate—are approved for use in beef cattle in the United States, further restricting the range of rotational combinations available in American production systems (U.S. Food & Drug Administration, 2025).

Collectively, available evidence indicates that rotational ionophore feeding is biologically plausible based on differences in antimicrobial activity and microbial adaptation processes, but its practical implementation and effectiveness remain incompletely defined under commercial production conditions. Regulatory constraints requiring continuous feeding and inconsistent performance responses across studies contribute to uncertainty regarding the practical utility of rotational ionophore programs in beef cattle production. Existing knowledge gaps highlight the need for integrated evaluation of rotational ionophore feeding strategies using commercially approved compounds, with simultaneous assessment of fermentation responses, microbial adaptation, and animal performance under applied feedlot conditions.

Challenges in evaluating rumen modifiers under commercial conditions

Despite decades of research on ionophores and other rumen modifiers, much of the foundational work has been conducted under controlled experimental conditions that differ substantially from modern commercial feedlot environments (Bergen & Bates, 1984; Chen &

Wolin, 1979; Russell & Strobel, 1989). Studies have provided critical mechanistic insights into microbial inhibition, fermentation pathways, and nutrient metabolism, but controlled experimental settings do not fully capture the management, behavioral, and environmental variability present in commercial feedlots. In commercial systems, cattle are managed and fed in groups, and feed delivery and intake are typically measured at the pen level rather than the individual animal level (Samuelson et al., 2016). As a result, the pen becomes the experimental unit for performance and intake measures, reducing the ability to directly associate ruminal microbial responses with individual animal performance outcomes. Variation in feeding behavior, bunk access, and animal interactions within pens further contributes to variability in intake and performance responses to feed additives.

Direct measurement of ruminal fermentation in commercial cattle is limited by challenges associated with obtaining representative rumen samples (Johnson, 1966; Russell & Strobel, 1989). Ruminally cannulated animals provide controlled access to rumen contents and have been widely used to evaluate microbial populations, fermentation end products, and feed additive responses. However, cannulated animals are typically managed under experimental conditions that differ from commercial feedlot systems, including individual feeding and controlled diet delivery. These differences limit the ability to directly extrapolate fermentation responses observed in cannulated animals to commercial cattle populations. Alternative sampling approaches, including oro-ruminal or esophageal sampling, have been used to obtain rumen fluid from non-cannulated cattle but each introduces additional variability related to sampling location and saliva contamination (Lodge-Ivey et al., 2009).

In vitro and *ex vivo* fermentation systems provide an alternative approach for evaluating ruminal microbial responses under controlled conditions (Johnson, 1966). Batch culture and

continuous culture systems allow direct evaluation of fermentation characteristics, gas production, and microbial responses to dietary substrates and feed additives (Johnson, 1966; Russell & Martin, 1984; Tedeschi et al., 2022). The systems provide valuable mechanistic insight while minimizing variation associated with animal behavior and feed intake. However, *in vitro* and *ex vivo* systems do not replicate animal-level processes such as feed intake regulation, rumen motility, digesta passage rate, and host absorption of fermentation products. As a result, fermentation responses observed *in vitro* may not fully reflect responses observed *in vivo*.

Measurement of enteric CH₄ production under commercial conditions presents additional challenges. *In vitro* and *ex vivo* batch culture fermentation systems can provide insights into gas production kinetics and composition through controlled fermentations conducted in sealed vessels (Tedeschi et al., 2022). *In vitro* systems rely on defined substrates and microbial inoculum, and *ex vivo* systems utilize rumen fluid collected from donor animals to maintain more biologically representative fermentation conditions. However, translating results from these systems to whole-animal CH₄ emissions remains uncertain.

Open- and closed-circuit respiration chambers quantify total gas exchange by continuously measuring airflow entering and leaving a sealed chamber housing an individual animal (Tedeschi et al., 2022). Methane production is calculated from the difference in CH₄ concentration between inlet and outlet air streams, adjusted for airflow rate and chamber volume. Respiration chambers provide highly controlled and precise measurements of daily CH₄ output and are widely regarded as the reference method for CH₄ quantification in ruminants. However, respiration chamber systems require specialized animal confinement, dedicated facilities, and substantial financial investment, limiting their feasibility for large-scale feedlot applications.

Automated head chamber systems (AHCS), such as GreenFeed-type systems, allow CH₄ measurement in commercial settings (Jonker et al., 2016; Tedeschi et al., 2022). The system attracts cattle to a baited feeding station, where airflow is actively drawn across the muzzle during voluntary visits. Methane concentration is measured in real time using infrared gas analyzers, and gas flux is calculated from the product of CH₄ concentration and measured airflow rate. Daily CH₄ production is subsequently estimated by integrating emissions across multiple short-duration visits and extrapolating to a 24-hour period based on visit frequency and duration. However, AHCS rely on consistent voluntary animal visits and are therefore influenced by animal behavior, feeding patterns, and visit frequency. Behavioral variation introduces uncertainty that must be considered when interpreting CH₄ production data in commercial populations. Variation in visit behavior can also arise from differences among animals in feed intake, social dominance, body weight, and feeding motivation, which can influence access to the system and contribute to between-animal variation in estimated CH₄ production.

Taken together, the challenges highlight the difficulty of evaluating rumen modifiers under commercial feedlot conditions, where experimental control is inherently limited. Mechanistic studies provide important insight into microbial responses and fermentation pathways, but translating research findings to commercial production systems remains complex. This methodological gap contributes to the limited availability of integrated commercial-scale data linking ruminal fermentation, CH₄ production, and animal performance responses to ionophore supplementation.

Knowledge gaps and justification for thesis

Although ionophores have been extensively studied for their effects on ruminal fermentation and cattle performance, several important gaps remain in the literature. The

majority of mechanistic and performance research has focused on monensin, leaving comparatively less information available for other approved ionophores used in beef cattle, specifically laidlomycin propionate (Cernicchiaro et al., 2016; Duffield et al., 2012; Golder & Lean, 2016). Direct head-to-head comparisons among ionophores under similar dietary and management conditions are limited in large commercial feedlot settings, making it difficult to determine how molecule-specific differences in microbial inhibition and fermentation responses translate to applied production outcomes.

In addition, most published studies evaluate continuous feeding of a single ionophore throughout a production phase (Cernicchiaro et al., 2016; Duffield et al., 2012; Golder & Lean, 2016). Although evidence suggests that fermentation responses may shift with prolonged exposure to ionophores (Appuhamy et al., 2013; Russell & Strobel, 1989), relatively few investigations have examined how variation in ionophore exposure during a feeding period influences integrated outcomes including ruminal fermentation dynamics, gas production, and animal performance responses (Duff et al., 1995; McKinnon et al., 1992; Morris et al., 1990; Shreck et al., 2016). Consequently, the biological and practical implications of alternative ionophore programs, including sequential or rotational use, remain incompletely characterized under commercial conditions.

A further limitation in the literature is that performance, fermentation, and CH₄ emission outcomes are often evaluated independently rather than within integrated experimental designs. Studies that measure ruminal fermentation characteristics are often conducted under controlled experimental conditions using cannulated animals or *in vitro* systems (Domesick & Martin, 1999; Russell & Martin, 1984), whereas large-scale feedlot performance trials often lack concurrent measurements of ruminal fermentation or CH₄ emissions (Shreck et al., 2016).

Likewise, CH₄-focused investigations frequently emphasize measurement methodology or controlled experimental systems without simultaneous integration of performance and fermentation data (Rumpler et al., 1986; Thornton & Owens, 1981). This separation limits the ability to directly connect microbial-level mechanisms with whole-animal productivity and gas production responses in applied feedlot systems.

Methodological and logistical constraints further contribute to knowledge gaps. Obtaining representative rumen samples in commercial feedlot cattle remains challenging, particularly in large pens where routine handling is limited and the use of ruminally cannulated animals is uncommon. While cannulated steers provide controlled access to rumen contents, there is limited information on how such animals integrate into large commercial pens and maintaining them under commercial conditions introduces additional management and welfare considerations. Measurement of enteric CH₄ under feedlot conditions presents similar obstacles. Equipment capable of quantifying emissions, including AHCS, requires substantial infrastructure, financial investment, and consistent animal participation. Ensuring that cattle voluntarily and repeatedly use the systems and determining whether collected data accurately represent whole-pen emissions introduces further uncertainty. In addition, implementing multiple dietary treatments across large commercial pens requires substantial space, feed manufacturing capacity, and coordination, particularly when feed additives must be precisely incorporated and monitored over time. Integrating performance, fermentation, and CH₄ data generated under these conditions into a cohesive and interpretable framework therefore presents both logistical and analytical complexity. Simultaneous evaluation of animal performance, ruminal fermentation characteristics, and CH₄ production under practical feedlot conditions is necessary to improve

understanding of how ionophore programs influence both productivity and fermentation-derived outcomes in applied systems.

The present study aimed to address system limitations by integrating a commercial-scale rotational ionophore feeding trial with complementary *ex vivo* batch culture fermentation evaluation. The design allowed simultaneous assessment of cattle performance, enteric CH₄ emissions, and ruminal fermentation responses under practical feedlot conditions while providing mechanistic validation of observed *in vivo* outcomes.

References

- Agtarap, A., Chamberlin, J. W., Pinkerton, M., & Steinrauf, L. K. (1967). Structure of monensic acid, a new biologically active compound. *Journal of the American Chemical Society*, 89(22), 5737–5739.
- Appuhamy, J. A. D., Strathe, A. B., Jayasundara, S., Wagner-Riddle, C., Dijkstra, J., France, J., & Kebreab, E. (2013). Anti-methanogenic effects of monensin in dairy and beef cattle: A meta-analysis. *Journal of Dairy Science*, 96(8), 5161–5173.
<https://doi.org/10.3168/jds.2012-5923>
- Bergen, W. G., & Bates, D. B. (1984). Ionophores: Their Effect on Production Efficiency and Mode of Action. *Journal of Animal Science*, 58(6), 1465–1483.
<https://doi.org/10.2527/jas1984.5861465x>
- Callaway, T. R., Edrington, T. S., Rychlik, J. L., Genovese, K. J., Poole, T. L., Jung, Y. S., Bischoff, K. M., Anderson, R. C., & Nisbet, D. J. (2003). *Ionophores: Their Use as Ruminant Growth-Promotants and Impact on Food Safety*. 4, 43–51.
- Center for Epidemiology and Animal Health. (2024). *Health management on U.S. feedlots, 2021*. U.S. Department of Agriculture, Animal and Plant Health Inspection Service.
<https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/monitoring-and-surveillance/nahms>
- Cernicchiaro, N., Corbin, M., Quinn, M., Prouty, F., Branine, M., & Renter, D. G. (2016). Meta-analysis of the effects of laidlomycin propionate, fed alone or in combination with chlortetracycline, compared with monensin sodium, fed alone or in combination with tylosin, on growth performance, health, and carcass outcomes in finishing steers in North America¹. *Journal of Animal Science*, 94(4), 1662–1676.
<https://doi.org/10.2527/jas.2015-0086>
- Chen, M., & Wolin, M. J. (1979). Effect of Monensin and Lasalocid-Sodium on the Growth of Methanogenic and Rumen Saccharolytic Bacteria. *Applied and Environmental Microbiology*, 38(1), 72–77. <https://doi.org/10.1128/aem.38.1.72-77.1979>
- Code of Federal Regulations. (2025). Animal drugs for use in animal feeds—Definitions and general considerations (21 C.F.R. § 558.3). *Title 21: Food and Drugs*. U.S. Government Publishing Office. <https://www.ecfr.gov/current/title-21/chapter-I/subchapter-E/part-558>
- Domesick, E. J., & Martin, S. A. (1999). Effects of laidlomycin propionate and monensin on the in vitro mixed ruminal microorganism fermentation. *Journal of Animal Science*, 77(8), 2305. <https://doi.org/10.2527/1999.7782305x>
- Duff, G. C., Galyean, M. L., & Branine, M. E. (1995). Effects of adaptation to lasalocid, monensin or a daily rotation of lasalocid and monensin on in vitro fermentation of a 90% concentrate diet. *Canadian Journal of Animal Science*, 75(1), 129–134.
<https://doi.org/10.4141/cjas95-017>

- Duffield, T. F., Merrill, J. K., & Bagg, R. N. (2012). Meta-analysis of the effects of monensin in beef cattle on feed efficiency, body weight gain, and dry matter intake. *Journal of Animal Science*, 90(12), 4583–4592. <https://doi.org/10.2527/jas.2011-5018>
- Golder, H. M., & Lean, I. J. (2016). A meta-analysis of lasalocid effects on rumen measures, beef and dairy performance, and carcass traits in cattle. *Journal of Animal Science*, 94(1), 306–326. <https://doi.org/10.2527/jas.2015-9694>
- Goodrich, R. D., Garrett, J. E., Gast, D. R., Kirick, M. A., Larson, D. A., & Meiske, J. C. (1984). Influence of Monensin on the Performance of Cattle. *Journal of Animal Science*, 58(6), 1484–1498. <https://doi.org/10.2527/jas1984.5861484x>
- Gräfe, U., Reinhardt, G., & Miosga, N. (1989). Monovalent cation specificity of passive transport mediated by laidlomycin and 26-deoxylaidlomycin. *Journal of Basic Microbiology*, 29(6), 391–394. <https://doi.org/10.1002/jobm.3620290620>
- Guan, H., Wittenberg, K. M., Ominski, K. H., & Krause, D. O. (2006). Efficacy of ionophores in cattle diets for mitigation of enteric methane. *Journal of Animal Science*, 84(7), 1896–1906. <https://doi.org/10.2527/jas.2005-652>
- Johnson, R. R. (1966). Techniques and Procedures for In Vitro and In Vivo Rumen Studies. *Journal of Animal Science*, 25(3), 855–875. <https://doi.org/10.2527/jas1966.253855x>
- Jonker, A., Molano, G., Antwi, C., & Waghorn, G. C. (2016). Measured using respiration chambers, the sulfur hexafluoride. *Journal of Animal Science*, 94(10), 4326–4337. <https://doi.org/10.2527/jas2016-0646>
- Langemeier, M., Schroeder, T., & Mintert, J. (1992). Determinants of cattle finishing profitability. *Journal of Agricultural and Applied Economics*, 24(2), 41–47. <https://doi.org/10.1017/S0081305200018367>
- Lodge-Ivey, S. L., Browne-Silva, J., & Horvath, M. B. (2009). Technical note: Bacterial diversity and fermentation end products in rumen fluid samples collected via oral lavage or rumen cannula. *Journal of Animal Science*, 87(7), 2333–2337. <https://doi.org/10.2527/jas.2008-1472>
- Marques, R. D. S., & Cooke, R. F. (2021). Effects of Ionophores on Ruminal Function of Beef Cattle. *Animals*, 11(10), 2871. <https://doi.org/10.3390/ani11102871>
- McKinnon, J. J., Cohen, R. D. H., Kowalenko, W. S., & Janzen, E. D. (1992). The effects of feeding monensin and lasalocid together in the same diet or in a daily rotation program on performance and carcass characteristics of feedlot cattle. *Canadian Journal of Animal Science*, 72(2), 273–278. <https://doi.org/10.4141/cjas92-034>
- Morris, F. E., Branine, M. E., Galyean, M. L., Hubbert, M. E., Freeman, A. S., & Lofgreen, G. P. (1990). Effect of rotating monensin plus tylosin and lasalocid on performance, ruminal fermentation, and site and extent of digestion in feedlot cattle. *Journal of Animal Science*, 68(10), 3069. <https://doi.org/10.2527/1990.68103069x>

- Nagaraja, T. G. (1995). Ionophores and antibiotics in ruminants. In A. Chesson & R. J. Wallace (Eds.), *Biotechnology in animal feeds and animal feeding* (pp. 173–204). VCH.
<https://doi.org/10.1002/9783527615353>
- Newbold, C. J., Wallace, R. J., & Walker, N. D. (1993). The effect of tetrone and monensin on fermentation, microbial numbers and the development of ionophore-resistant bacteria in the rumen. *Journal of Applied Bacteriology*, 75(2), 129–134.
<https://doi.org/10.1111/j.1365-2672.1993.tb02757.x>
- Poos, M. I., Hanson, T. L., & Klopfenstein, T. J. (1979). Monensin Effects on Diet Digestibility, Ruminant Protein Bypass and Microbial Protein Synthesis. *Journal of Animal Science*, 48(6), 1516–1524. <https://doi.org/10.2527/jas1979.4861516x>
- Rumpler, W. V., Johnson, D. E., & Bates, D. B. (1986). The Effect of High Dietary Cation Concentration on Methanogenesis by Steers Fed Diets with and without Ionophores. *Journal of Animal Science*, 62(6), 1737–1741. <https://doi.org/10.2527/jas1986.6261737x>
- Russell, J. B., & Houlihan, A. J. (2003). Ionophore resistance of ruminal bacteria and its potential impact on human health. *FEMS Microbiology Reviews*, 27(1), 65–74.
[https://doi.org/10.1016/S0168-6445\(03\)00019-6](https://doi.org/10.1016/S0168-6445(03)00019-6)
- Russell, J. B., & Martin, S. A. (1984). Effects of Various Methane Inhibitors on the Fermentation of Amino Acids by Mixed Rumen Microorganisms in Vitro. *Journal of Animal Science*, 59(5), 1329–1338. <https://doi.org/10.2527/jas1984.5951329x>
- Russell, J. B., & Strobel, H. J. (1989). Effect of ionophores on ruminal fermentation. *Applied and Environmental Microbiology*, 55(1), 1–6. <https://doi.org/10.1128/aem.55.1.1-6.1989>
- Samuelson, K. L., Hubbert, M. E., Galyean, M. L., & Löest, C. A. (2016). Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico State and Texas Tech University survey1. *Journal of Animal Science*, 94(6), 2648–2663.
<https://doi.org/10.2527/jas.2016-0282>
- Shreck, A. L., Behlke, E. J., Paddock, Z. D., Burciaga-Robles, L. O., Parr, S. L., Booker, C. W., Hannon, S. J., Raaphorst, H. S., Byers, L., Rademacher, R. D., Quinn, M. J., & May, M. L. (2016). Effect of daily rotational feeding of lasalocid and monensin to yearling feedlot steers fed in western Canada. *The Professional Animal Scientist*, 32(5), 561–569.
<https://doi.org/10.15232/pas.2015-01489>
- Shumard, R. F., & Callender, M. E. (1967). Monensin, a new biologically active compound. VI. Anticoccidial activity. *Antimicrobial Agents and Chemotherapy*, 7, 369–377.
<https://pubmed.ncbi.nlm.nih.gov/5596162/>
- Tedeschi, L. O., Abdalla, A. L., Álvarez, C., Anuga, S. W., Arango, J., Beauchemin, K. A., Becquet, P., Berndt, A., Burns, R., De Camillis, C., Chará, J., Echazarreta, J. M., Hassouna, M., Kenny, D., Mathot, M., Mauricio, R. M., McClelland, S. C., Niu, M., Onyango, A. A., ... Kebreab, E. (2022). Quantification of methane emitted by ruminants:

- A review of methods. *Journal of Animal Science*, 100(7), skac197.
<https://doi.org/10.1093/jas/skac197>
- Thornton, J. H., & Owens, F. N. (1981). Monensin supplementation and in vivo methane production by steers. *Journal of Animal Science*, 52(3), 628–634.
<https://doi.org/10.2527/jas1981.523628x>
- U.S. Food and Drug Administration. (2025). *Approved animal drug products (Green Book)*.
<https://www.fda.gov/animal-veterinary/products/approved-animal-drug-products-green-book>
- Van Maanen, R. W., Herbein, J. H., McGilliard, A. D., & Young, J. W. (1978). Effects of Monensin on In Vivo Rumen Propionate Production and Blood Glucose Kinetics in Cattle. *The Journal of Nutrition*, 108(6), 1002–1007.
<https://doi.org/10.1093/jn/108.6.1002>

Chapter 2 - Effects of rotations of monensin and laidlomycin propionate on commercial cattle performance and *ex vivo* fermentation during the growing phase

Introduction

Ionophores are commonly included in feedlot diets to improve feed efficiency and performance by altering ruminal fermentation (Bergen & Bates, 1984; Russell & Strobel, 1989). Industry survey data indicate that monensin and lasalocid are the two primary ionophores used in U.S. feedlot diets, with monensin overwhelmingly predominant in finishing cattle (Samuelson et al., 2016).

Ionophores disrupt ion transport in ruminal microbes, preferentially acting on Gram-positive populations. The change in microbial population often causes a shift in volatile fatty acid (VFA) production toward propionate (Bergen & Bates, 1984; Russell & Strobel, 1989). Monensin appears to alter ruminal fermentation by disrupting cation gradients, particularly sodium (Na^+), across the cell membranes of Gram-positive bacteria (Russell & Strobel, 1989). Laidlomycin propionate, although similar in structure and function to monensin, exhibits greater affinity for transporting potassium (K^+) than sodium (Na^+) (Gräfe et al., 1989). Because ionophores differ in their ionic selectivity and resulting effects on ruminal microbes, it has been proposed that management strategies combining their mechanistic distinctions could enhance cattle performance and alter ruminal fermentation (Owens, 2021).

Currently, combination clearances involving feeding two ionophores are not approved for use in U.S. beef cattle diets according to U.S. Food and Drug Administration (FDA) Type A medicated article listings in the *Green Book* (U.S. Food and Drug Administration, 2025). Instead,

ionophore rotation has been suggested as a management strategy to capitalize on two ionophores' mechanistic distinctions during the same feeding period. Ionophore rotation in beef cattle diets has been investigated since the 1980s, with some studies reporting improvements in feed conversion of up to 6% (Morris et al., 1990; Shreck et al., 2016). Existing rotational studies have primarily focused on monensin and lasalocid rotations on both daily and weekly intervals. Research utilizing laidlomycin propionate is comparatively limited, with most studies conducted during the 1980s and 1990s (Domescik & Martin, 1999; Galyean et al., 1992; Gräfe et al., 1989; Russell & Strobel, 1989) and minimal evaluation of the specifics of rotational feeding systems.

In the United States, FDA-approved ionophores are regulated for “continuous feeding” (21 C.F.R. § 558.3, 2025). Continuous feeding is defined as an uninterrupted administration of feed for a minimum of 14 d. Short-term rotation studies with daily or weekly intervals have not demonstrated consistent performance benefits (Casey et al., 1994; Guan et al., 2006; Morris et al., 1990). Experimental evidence indicates that the ruminal microbial and fermentation responses to monensin peak approximately 14–42 days after initiation (Crossland et al., 2017; Guan et al., 2006). Accordingly, a 28-d rotation interval was selected to coincide with the monensin response. Additionally, a 28-d interval would allow diet changes to be made on the same day of the week each month, which would ease implementation of a rotational strategy by feedyard management.

The objectives of this research were to (1) evaluate repeated 28-d rotations of monensin (Rumensin 90; Elanco, Indianapolis, IN) and laidlomycin propionate (Cattlyst 50G; Phibro Animal Health Corporation, Teaneck, NJ) for effects on growth performance of limit-fed feedlot heifers, with an emphasis on gain, average daily gain (ADG), and gain-to-feed ratio (G:F), and

(2) characterize rumen fermentation responses to rotational feeding of monensin and laidlomycin propionate using *ex vivo* batch culture techniques.

Materials and methods

All procedures involving animals were approved by the Kansas State University Institutional Animal Use and Care Act (IACUC, protocol 5006) and complied with the *Guide for the Care and Use of Agricultural Animals in Research and Teaching*.

Study overview

Two experiments were conducted concurrently at Clark County Feeders (Minneola, KS) to evaluate rotational feeding of monensin and laidlomycin propionate under both applied and mechanistic conditions. Experiment 1 was a large-scale commercial heifer trial designed to evaluate growth performance responses to 28-d ionophore rotation during the growing period. Experiment 2 used ruminally cannulated donor steers housed under the same commercial conditions to characterize rumen fermentation dynamics associated with rotational ionophore feeding. Both experiments used the same basal growing diet, feed manufacturing practices, and feedyard management system. However, ionophore rotation schedules differed between experiments due to differences in experimental objectives and sampling timelines.

Experiment 1: heifer performance

Study animals and facility

Cattle were received on a rolling basis, resulting in three trial start dates across placement groups. Heifers were housed in drylot pens that averaged 29.9 ± 1.2 m²/head of pen space and included 25.5–30.0 m of bunkline, resulting in 0.545 ± 0.025 m/head of bunk space. Each pen was equipped with automatic waterers to provide animals ad libitum access to water.

A total of 584 crossbred beef heifers, consisting of various combinations of British breed crosses, were used. Initial body weight (BW) averaged 290 ± 26 kg. Upon arrival, heifers were blocked by arrival date and allocated to pens ($n = 10$) within each block according to processing order, resulting in an average of 59 ± 15 animals per pen.

Dietary treatments and ration

Within each placement time block, pens were randomly assigned to one of two dietary treatments. The monensin ionophore treatment (MON; initial BW = 296 ± 29 kg) consisted of a basal diet providing a monensin dose of 200 mg/heifer/d (Rumensin 90; Elanco Animal Health, Greenfield, IN). The rotational ionophore treatment (ROT; initial BW = 284 ± 27 kg) consisted of the same basal diet, except the ionophore source alternated every 28 d between a monensin dose of 200 mg/heifer/d (Rumensin 90; Elanco Animal Health, Greenfield, IN) and a laidlomycin propionate dose of 75 mg/heifer/d (Cattlyst 50G; Phibro Animal Health Corporation, Teaneck, NJ). Rotation occurred twice, resulting in two complete 28-d rotations and a total feeding period of 112 d (Table 2.1). Ionophore inclusion rates were achieved through premix incorporation during ration manufacturing using Accu-Trac Micromachine System (Micro Technologies, Amarillo, TX).

Cattle were limit-fed to achieve a targeted ADG of approximately 1.36 kg per animal per d and were fed twice daily. Rations were formulated according to feed mill and consulting nutritionist records (Table 2.2). The basal growing diet was formulated on a DM basis to contain 43.5% steam-flaked corn, 32.4% mixed alfalfa hay, 18.4% wet corn distillers grains, 4.9% pelleted protein blend, and 0.8% corn oil. The complete ration provided 1.26 Mcal/kg net energy for gain (NEg) and 14.3% crude protein (DM basis) (Table 2.2). Diet composition was consistent across treatments except for the ionophore source. Diets were manufactured on-site and

delivered to each pen using commercial-grade horizontal rotary feed mixer trucks (Roto-Mix 620-16XD; Roto-Mix LLC, Dodge City, KS). Feed bunks were managed daily using standard feedyard bunk-reading procedures to ensure consistent intake.

Arrival processing and health management

To minimize prior exposure to ionophores, heifers were sourced primarily from grazing systems to ensure, to the greatest extent possible, that all animals were monensin-naïve at the start of the study. Upon arrival to the feedyard, heifers were placed on the facility's standard starter ration which was modified to be devoid of ionophores and instead contained decoquinatate (Deccox; Phibro Animal Health Corporation, Teaneck, NJ) for 28 d. After the starter period, cattle were transitioned to the experimental growing diet containing the assigned ionophore treatments. Upon arrival, cattle were processed according to the yard's standard protocol for the facility. Under this protocol, each animal received oral fenbendazole (Safe-Guard; Merck Animal Health, Rahway, NJ), injectable ivermectin (Ivermax; Aspen Veterinary Resources, Loveland, CO), a modified-live viral respiratory vaccine (VISTA 5; Merck Animal Health, Rahway, NJ), a 7-way clostridial bacterin-toxoid (VISION 7; Merck Animal Health, Rahway, NJ), and a trenbolone acetate/estradiol benzoate implant (Synovex Choice; Zoetis, Parsippany, NJ).

Animal health was managed by feedyard personnel according to standard protocols for the facility, including pulling and treating sick cattle as needed and removing mortalities when present. Detailed morbidity and mortality data were not collected. However, no significant changes in headcount were recorded for cattle enrolled on the trial.

Body weight collection

On d 0 and d 112, both individual animal and pen-level BW were recorded prior to morning feeding. Individual BW were recorded using a digital livestock scale indicator (TW-3; Gallagher Group Ltd., Hamilton, New Zealand) connected to electronic chute weigh bars (G06300; Gallagher Group Ltd., Hamilton, New Zealand). Pen-level BW were recorded using a platform pen scale equipped with a digital scale indicator (Model 560; GSE Scale Systems, Novi, MI). Interim pen-level BW were collected in the afternoon at 28-d intervals using a platform pen scale. Interim pen-level BW were adjusted to 96% of the recorded value to approximate shrunk weights and account for gut fill. Dry matter intake (DMI) was determined from daily feed delivery records maintained by the AHI management platform (Animal Health International, Greeley, CO).

Experiment 2: *ex vivo* gas production

Study animals and facility

Experiment 2 was conducted concurrently with the commercial heifer trial described in Experiment 1 to characterize gas production and gas composition responses by rumen microbes to rotational ionophore feeding. Ruminally cannulated donor steers were co-housed with commercial beef × dairy steers at Clark County Feeders (Minneola, KS) and exposed to the same basal growing diet and feedyard management system, with ionophore source differing by treatment.

A total of five ruminally cannulated Holstein × Angus crossbred steers (initial BW = 443 ± 15 kg) served as rumen fluid donors. Six cannulated steers were initially enrolled in the study; however, one animal died due to an unrelated health condition prior to the sampling period. Donor steers were co-housed within one of two commercial drylot pens containing 34 or 37 commercial beef × dairy crossbred steers (initial BW = 269 ± 23 kg). To minimize potential

location effects while maintaining treatment assignment, a physical pen switch was conducted on d 56. Pens were dirt-surfaced drylot pens with 0.833 ± 0.025 m/head of bunk space and equipped with automatic waterers providing ad libitum access to water.

Dietary treatments and ration

Pens were randomly assigned to receive one of two dietary treatments. The monensin ionophore treatment (MON; n = 3 cannulated steers; n = 34 commercial steers) consisted of a basal diet providing a monensin dose of 200 mg/steer/d (Rumensin 90; Elanco Animal Health, Greenfield, IN). The rotational ionophore treatment (ROT; n = 2 cannulated steers; n = 37 commercial steers) consisted of the same basal diet, except the ionophore source varied, providing a monensin dose of 200 mg/steer/d (Rumensin 90; Elanco Animal Health, Greenfield, IN) from d 0 to 56 and d 85 to 112 and a laidlomycin propionate dose of 75 mg/steer/d (Cattlyst 50G; Phibro Animal Health Corporation, Teaneck, NJ) from d 57 to 84 (Table 2.3). All steers in Experiment 2 received the same basal limit-fed growing diet as cattle in Experiment 1 (Table 2.2). Ionophore inclusion rates were achieved through premix incorporation during ration manufacturing using Accu-Trac Micromachine System (Micro Technologies, Amarillo, TX). Feed bunks were managed daily using standard feedyard bunk-reading procedures to ensure consistent intake.

Arrival processing and health management

Upon arrival, commercial beef $\times$ dairy steers received oral fenbendazole (Safe-Guard; Merck Animal Health), injectable ivermectin (Ivermax; Aspen Veterinary Resources, Loveland, CO), a modified-live viral respiratory vaccine (VISTA 5; Merck Animal Health, Rahway, NJ), a 7-way clostridial bacterin-toxoid (VISION 7; Merck Animal Health, Rahway, NJ), and a 5-way leptospirosis bacterin (Leptoform-5; Zoetis, Parsippany, NJ). Commercial steers were also

administered a trenbolone acetate/estradiol benzoate extended-release implant (Synovex ONE Feedlot; Zoetis, Parsippany, NJ) according to arrival protocol for the facility. Cannulated donor steers were maintained as research animals and managed for cannula integrity and general health under daily observation. Routine husbandry procedures were performed as needed to ensure animal well-being throughout the study as previously described for Experiment 1.

Prior to initiation of the experimental ionophore feeding schedule (d 0), cannulated donor steers and co-housed commercial beef $\times$ dairy steers were managed under the standard receiving and adaptation protocol for the facility. Upon arrival, steers were placed on a high-roughage starter ration devoid of ionophores that instead contained decoquinate (Deccox; Phibro Animal Health Corporation, Teaneck, NJ), for approximately 28 d before the start of the experimental feeding period. Throughout this adaptation phase and the experimental feeding period, steers were managed under the same feed manufacturing, feeding frequency, bunk management, and daily animal observation procedures described for Experiment 1.

***Ex vivo* batch culture fermentation**

Rumen fluid was collected from all cannulated donor steers on experimental d 56, 84, and 112, corresponding to points of adequate rumen adaptation following key ionophore exposure periods. On each sampling day, rumen contents were collected four hours after morning feeding. Approximately equal volumes of ruminal liquid and solid digesta were manually withdrawn from the ventral rumen sac via the cannula and immediately strained through four layers of cheesecloth into pre-warmed, insulated containers. Rumen fluid from each donor steer was kept separate and not pooled to preserve steer as the observational unit. Fluid temperature was maintained near 39°C during transport to the laboratory, which occurred within 30 minutes of

collection. Upon arrival to the on-site lab, rumen fluid bottles were continuously purged with carbon dioxide (CO₂) to preserve anaerobic conditions.

Ex vivo batch culture fermentation technique was conducted using a vented ANKOM gas production system (ANKOM Technology, Macedon, NY) following anaerobic fermentation procedures adapted from Van Soest et al. (1991) and Pell & Schofield (1993). Fermentation vessels received 0.50 g (as-fed basis) of the appropriate total mixed ration (TMR) corresponding to the donor steer's dietary ionophore treatment at the time of collection. Samples of TMR were collected directly from pen feed bunks prior to rumen fluid collection and processed to a consistent particle size using a coffee grinder. Each fermentation vessel then received 40 mL of bicarbonate-buffered fermentation medium containing macro- and micromineral components prepared according to Goering & Van Soest (1970), followed by 10 mL of the corresponding rumen fluid inoculum within each sampling period. For each donor steer, two treatment vessels and two blank vessels were prepared. Blank vessels contained buffer solution and rumen fluid only (no substrate) and were used to correct for endogenous gas production not associated with TMR digestion.

All vessels were flushed with CO₂, sealed using ANKOM RF screwcaps, and incubated at 39°C for 48 h using a benchtop orbital shaker (Solaris 4000 R; Thermo Fisher Scientific, Waltham, MA). The outflow port of the ANKOM RF system for each sample was fitted with an adaptor to direct all produced gas into a foil Tedlar gas sampling bag (Supel-Inert Gas Sampling Bag; Supelco, Bellefonte, PA) for continuous collection over the incubation period. Total gas production and gas production kinetics were determined from pressure measurements recorded by the ANKOM RF system and converted to cumulative gas volume using estimates of headspace volume, venting pressure threshold, gas constant, and measured pressure. Cumulative

gas production data from each vessel were fitted to a nonlinear gas production model as described by Groot et al. (1996) to estimate fermentation kinetic parameters, including maximum gas production (A ; mL), fractional gas production rate (K ; h^{-1}), and lag phase time (λ ; h). Model fitting was conducted using pressure-derived gas production curves recorded by the ANKOM RF system. At 48 h, samples of the cumulative gas production were collected from each Tedlar bag using a 15-mL plastic syringe fitted with a Luer-lock stopcock valve to prevent gas loss during storage. Samples were sent to Colorado State University (Fort Collins, CO) for analysis of CO₂-to-methane (CH₄) ratio (CO₂:CH₄) using gas chromatography. Gas composition was determined using a gas chromatograph (Shimadzu GC-14A; Shimadzu Corp., Kyoto, Japan) equipped with a thermal conductivity detector maintained at 100°C following procedures described by (Reyes et al., 2023). Total CO₂ and CH₄ production were calculated by multiplying total cumulative gas production by the measured fractional concentration of CO₂ and CH₄ in the gas sample, assuming CH₄ and CO₂ represented the predominant fermentation gases.

Co-housed commercial beef × dairy steers

To relate *ex vivo* fermentation responses to *in vivo* growth performance of co-housed steers, individual body weights were obtained from all steers within Experiment 2 pens on d 0, 28, 56, 84, and 112. Average daily gain was calculated for both cannulated donor steers and non-cannulated beef × dairy steers co-housed with donors.

Laboratory sample analysis for all experiments

Ration ingredient samples were collected every 28 d, beginning at trial initiation and analyzed for nutrient composition to verify diet formulation (Table 2.2). Ingredient samples were dried at 55°C in a conventional drying oven for 72 h to determine dry matter according to AOAC method 934.01 (AOAC International, 2019). Samples were then ground through a 2-mm screen

using a cyclone sample mill (UDY Corporation, Fort Collins, CO). Samples were subsequently analyzed for nutrient composition. Corrected dry matter (DM) for each ingredient was determined by oven drying a 0.5 g subsample at 104°C for 3 h (AOAC method 934.01) (AOAC International, 2019), and ash was determined by combusting a 0.5 g subsample of each sample in a muffle furnace at 550°C for 8 h (AOAC method 934.05) (AOAC International, 2019). Nitrogen concentration was determined via steam distillation Kjeldahl technique using a KjelMaster K-375 with K376 autosampler (Buchi Corp., Flawil, Switzerland) according to AOAC method 984.13 (AOAC International, 2019). A 0.5 g subsample was weighed into a kjeldahl tube and combined with 1 kjeldahl catalyst tablet (4.98 g K₂SO₄ 0.02 CuSO₄*5H₂O) and 10 mL H₂SO₄. Tube contents were digested at 410°C for 100 min, 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 were done automatically using KjelMaster K-375. Crude protein was calculated as N × 6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to (Van Soest et al., 1991). Samples were analyzed for NDF and ADF using an ANKOM 200 Fiber Analyzer (ANKOM Technology, Macedon, NY). Approximately 0.5 g subsamples were sealed in F57 filter bags (ANKOM Technology, Macedon, NY) and analyzed using neutral detergent solution with sodium sulfite and α-amylase for NDF determination. Following NDF extraction, samples were rinsed with acetone, dried, and weighed. Acid detergent fiber was subsequently determined using acid detergent solution in the same analyzer, with final weights used to calculate ADF concentration of the sample. Net energy for gain was estimated during diet formulation (NASEM, 2016).

Statistical analysis for all experiments

Experiment 1: heifer performance

All data were analyzed using R statistical software (version 4.4.1; R Core Team, 2024) utilizing the lme4, car, and emmeans packages. Data were analyzed as a randomized block design with pen as the experimental unit. Pens were blocked by arrival time to the feedyard. For each 28-d period, ADG, DMI, G:F, and total gain over the feeding period were modeled using linear mixed models with a fixed effect of treatment and a random intercept for placement block. For DMI and G:F models, initial BW was additionally included as a covariate to account for baseline differences among pens. The same model structures were used for cumulative intervals (0–56, 0–84, 0–112 d). Prespecified pen-level covariates (bunk space, headcount) were screened as fixed effects and were not retained in the model ($P > 0.10$). Treatment means $\pm$ SE were reported as estimated marginal means. Statistical significance was declared when $P \leq 0.05$, with tendencies when $0.05 < P \leq 0.10$.

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

- Y_{ij} = response (ADG, total gain) for treatment i in placement block j
- μ = population mean
- α_i = fixed effect of treatment i (MON, ROT)
- γ_j = random effect of placement time block j
- ε_{ij} = residual error

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

- Y_{ij} = response (DMI, G:F) for treatment i in placement block j
- μ = population mean
- α_i = fixed effect of treatment i (MON, ROT)
- γ_j = random effect of placement time block j
- δ_{ij} = initial BW as covariate
- ε_{ij} = residual error

Experiment 2: *ex vivo* gas production

All data were analyzed using R statistical software (version 4.4.1; R Core Team, 2024) utilizing the lme4, car, and emmeans packages. Generalized linear models were used to evaluate the effect of treatment on *ex vivo* gas production responses. *Ex vivo* gas production responses analyzed included both fermentation kinetic parameters (maximum gas production [A], fractional gas production rate [K], and lag phase time [λ]) and gas composition variables (cumulative total gas production, total CH₄ production, total CO₂ production, and the CO₂:CH₄ ratio). *Ex vivo* variables were analyzed separately by sampling period (d 56, 84, and 112). Treatment was included as the fixed effect. Least squares means were generated using the emmeans package, and treatment differences were declared significant at $P \leq 0.05$, with tendencies discussed at $0.05 < P \leq 0.10$.

$$Y_i = \mu + \alpha_i + \varepsilon_i$$

- Y_i = response (A, K, λ , cumulative gas, CH₄, CO₂, or CO₂:CH₄ ratio) for treatment i
- μ = population mean
- α_i = fixed effect of treatment i (MON, ROT)
- ε_i = residual error

To further understand *ex vivo* results in comparison to commercial performance results, linear models were used to explore the effects of treatment on ADG for both the cannulated donor steers and commercial beef $\times$ dairy steers. Initial BW was included as a covariate to account for baseline differences among steers. Treatment was included as a fixed effect. Least squares means were generated for treatment comparisons. Treatment differences were declared significant at $P \leq 0.05$, with tendencies discussed at $0.05 < P \leq 0.10$

$$Y_i = \mu + \alpha_i + \gamma_i + \varepsilon_i$$

- Y_i = response (ADG) for treatment i
- μ = population mean
- α_i = fixed effect of treatment i (MON, ROT)

- γ_i = initial BW as covariate
- ε_i = residual error

Results

Experiment 1: heifer performance

Least squares means ($\pm$ pooled SEM) for commercial heifer performance are presented in Table 2.4 separated by 28-d periods and in Table 2.5 demonstrating performance over cumulative intervals.

From d 0–28, when no ionophore differences between treatments were introduced, no treatment differences were observed for ADG, DMI, or G:F ($P > 0.10$; Table 2.4). From d 29–56, following the first 28-d rotation to include laidlomycin propionate in ROT, feed efficiency tended to be greater ($P = 0.07$) for ROT as compared with MON (0.180 vs 0.149 ± 0.013 , respectively), whereas ADG and DMI did not differ ($P > 0.10$) between treatments. During d 57–84, when both treatments were again receiving monensin, no treatment differences were detected ($P > 0.10$) for ADG, DMI, or G:F. In the final period (d 85–112), following the second 28-d rotation to include laidlomycin propionate in ROT, ADG was greater ($P = 0.01$) for ROT (1.53 ± 0.10) compared with MON (1.20 ± 0.10). Similarly, G:F was greater ($P = 0.01$) for ROT (0.128 ± 0.003) than MON (0.101 ± 0.003). By design, DMI did not differ ($P > 0.10$) between treatments.

Cumulatively across d 0–56, during which MON cattle received consistent monensin and ROT cattle completed their first 28-d inclusion of laidlomycin propionate following 28 d of monensin, G:F tended to be greater ($P = 0.06$) for ROT compared with MON (0.188 vs 0.170 ± 0.008 , respectively), whereas ADG was only numerically greater for ROT (1.71 vs 1.56 ± 0.08 , respectively; $P = 0.16$; Table 2.5). Across d 0–84, when MON cattle had consumed 84 d of

consistent monensin and ROT cattle were exposed to 56 total d monensin interrupted by 28 d laidlomycin propionate, G:F again tended to be greater ($P = 0.06$) for ROT, while ADG remained similar ($P > 0.10$) between treatments. Over the entire 112-d feeding period, when comparing consistent monensin exposure for MON to two full 28-d rotations alternating between monensin and laidlomycin propionate for ROT, ADG was greater ($P \leq 0.01$) for ROT (1.54 ± 0.04) than MON (1.41 ± 0.04). G:F was also greater ($P = 0.01$) for ROT (0.152 ± 0.003) compared with MON (0.140 ± 0.003), whereas DMI did not differ ($P > 0.10$) between treatments.

Experiment 2: *ex vivo* gas production

Least squares means ($\pm$ SEM) for *ex vivo* gas production kinetics are presented in Table 2.6, gas composition variables in Table 2.7, and average daily gain of commercial beef $\times$ dairy steers co-housed with cannulated donors in Table 2.8.

On d 56, when both treatments were receiving monensin in their ration, no treatment differences were detected ($P \geq 0.10$) in modeled gas production kinetics, including maximum gas production, fractional gas production rate, or lag phase (Table 2.6). Likewise, total gas production, total CO₂ production, total CH₄ production, and the CO₂:CH₄ ratio did not differ ($P \geq 0.10$) between treatments (Table 2.7).

On d 84, following the 28-d period in which ROT steers received laidlomycin propionate while MON steers continued monensin, no treatment differences were observed ($P > 0.10$) in modeled gas production kinetics (Table 2.6), and total gas production and CO₂ production did not differ ($P > 0.10$) between treatments (Table 2.7). Total CH₄ production tended to be greater ($P = 0.06$) in ROT than MON (19.47 vs 15.30 ± 1.43 mL, respectively), and the CO₂:CH₄ ratio was lower ($P = 0.03$) in ROT than MON (6.14 vs 8.75 ± 0.69 , respectively).

On d 112, after both treatments had returned to monensin for the final 28-d period, no treatment differences were observed ($P > 0.10$) in modeled gas production kinetics (Table 2.6). Similarly, total gas production, CO₂ production, CH₄ production, and the CO₂:CH₄ ratio did not differ ($P > 0.10$) between treatments (Table 2.7).

As expected, ADG of commercial beef $\times$ dairy steers co-housed with cannulated donors did not differ ($P > 0.10$) between treatments during d 1–56, when both treatment groups received consistent monensin (Table 2.8). Across d 1–84, following the 28-d rotation to laidlomycin propionate in ROT, ADG was greater ($P = 0.03$) for ROT (1.67 ± 0.06) compared with MON (1.52 ± 0.06). No statistical difference in ADG ($P > 0.10$) was observed across the full 112-d period, following ROT transitioning back to monensin for the final 28 d (Table 2.8).

Discussion

Over the 112-d growing period, rotating monensin and laidlomycin propionate every 28 d improved ADG and feed efficiency of commercial beef heifers compared with consistent monensin feeding, while dry matter intake, by design, remained unaffected. Consequently, over the entire 112-d feeding period, ROT heifers gained approximately 8.7% more than MON heifers. These outcomes support the concept that alternating ionophores at a 28-d interval can enhance animal performance in commercial feedlot settings, consistent with earlier reports of rotational benefits. Shreck et al. (2016) observed an approximate 4.8% increase in ADG and improved feed efficiency with monensin and lasalocid rotation. Similarly, Morris et al. (1990) reported improved feed efficiency without differences in intake when rotating monensin and lasalocid. In Experiment 2, although there was not a statistically significant difference in ADG over the entirety of the experimental feeding period for the commercial beef $\times$ dairy steers co-housed with the cannulated donor steers, there was a numeric improvement in total gain for ROT

steers of approximately 8.3% over the 112-d feeding period as compared to MON steers, aligning directionally with the performance outcomes observed in Experiment 1. It is important to note that Experiment 2 included only two pens of cattle and was designed primarily to support rumen fermentation measurements, thus performance responses should be viewed as supportive of the findings from Experiment 1 rather than a definitive performance comparison.

Ex vivo fermentation responses observed in Experiment 2 provide insight into the biological basis for the performance responses in Experiment 1 and Experiment 2 co-housed cattle. Fermentation kinetics did not differ between treatments at any sampling period, indicating that ionophore rotation did not alter the rate or extent of substrate digestion. Instead, treatment differences were limited to gas composition and occurred only during specific exposure windows. Immediately following the laidlomycin propionate feeding period in the ROT treatment (d 84), CH₄ production tended to be greater and CO₂:CH₄ was lower relative to MON. These shifts resolved once cattle returned to monensin feeding, indicating that fermentation responses reflected differences in ionophore mechanism of action rather than persistent adaptations in digestive capacity.

Importantly, the CH₄-related shift observed at d 84 of Experiment 2 corresponded with the period during which improved ADG of beef × dairy ROT cattle was detected (d 57–84). This temporal alignment suggests that rotational ionophore feeding enhanced efficiency through short-term modulation of fermentation end-product pathways rather than through sustained suppression of methanogenesis. Ionophores exert selective pressure primarily against Gram-positive bacteria, and adaptation to monensin has been reported within 2–6 weeks of continuous feeding, with initial reductions in CH₄ production and protozoal populations diminishing over time (Crossland et al., 2017; Guan et al., 2006). Alternating ionophores at 28-d intervals may

disrupt this adaptation process, exposing microbial communities to changing antimicrobial spectra before stable populations re-establish. These microbial perturbations may alter H₂ utilization patterns and VFA production profiles in ways that improve energetic efficiency, even if CH₄ production itself is not persistently reduced. Thus, alternating ionophores on a 28-d schedule in Experiment 1 may have reintroduced selective pressure before full adaptation could occur, allowing performance advantages to accumulate over time. Possible explanations include enhanced responses in less efficient cattle later in the growing phase and shifts in rumen microbial populations due to repeated exposure to alternating ionophores (Bergen & Bates, 1984; Gräfe et al., 1989; Russell & Strobel, 1989). Future research comparing these two treatments to a negative control could help in understanding these dynamics.

Importantly, by design intake did not differ between treatments, suggesting that improvements in total gain and feed efficiency reflected differences in nutrient utilization rather than changes in consumption. This distinction is particularly relevant under limit-feeding conditions, where intake is managed to target specific rates of gain. The absence of differences in fermentation kinetics alongside shifts in CH₄-related variables across ionophore exposures further supports the interpretation that ionophore rotation influenced fermentation end-product profiles rather than digestion rate.

Overall, these findings provide novel evidence that ionophore rotation can be a practical tool for improving cattle performance under commercial conditions. Because FDA defines continuous feeding as 14 d or longer, the 28-d intervals used in this study represent a label-compliant approach to alternating monensin and laidlomycin propionate. Together, performance and *ex vivo* fermentation data suggest that rotational ionophore strategies modify ruminal fermentation patterns by leveraging products with differing mechanisms of action. Differences in

CH₄-related responses were observed with varying ionophore exposure, highlighting their value as performance-enhancing tools under the conditions of this study. Additional research is warranted to further elucidate the biological mechanisms underlying these responses and to evaluate the consistency of these effects across production phases.

Implications

Rotating monensin and laidlomycin propionate every 28 d during the growing phase improved gain and feed efficiency of limit-fed beef heifers compared with consistent monensin feeding, while intake was unaffected. Performance improvements demonstrating an 8.7% increase in gain over a 112-d period for cattle rotated between ionophores compared to consistent monensin feeding indicate that this label-compliant strategy can enhance energetic efficiency without increasing feed consumption. *Ex vivo* fermentation results suggest that these responses were associated with differences in ionophore mechanisms of action that modulate rumen fermentation, supporting the use of rotational ionophore programs as performance-enhancing tools rather than CH₄ mitigation strategies under similar feeding conditions. This approach may offer economic value to feedlot producers and warrants further evaluation across diets, cattle types, and production phases.

Tables

Table 2.1 Outline of strategic rotations indicating ionophore inclusion in ration by day for Experiment 1 treatment groups.

Days on Feed (DOF)	MON	ROT
1-28	Monensin ¹	Monensin ¹
29-56	Monensin ¹	Laidlomycin propionate ²
57-84	Monensin ¹	Monensin ¹
85-112	Monensin ¹	Laidlomycin propionate ²
¹ Monensin included at dose of 200 mg/head/d in form of Rumensin 90 (Elanco Animal Health, Greenfield, IN) ² Laidlomycin propionate included at dose of 75 mg/head/d in form of Cattlyst 50G (Phibro Animal Health Corp, Teaneck, NJ)		

Table 2.2 Ingredient and nutrient composition of commercial growing ration (dry matter basis) in Experiment 1 & Experiment 2.

Ingredient	% of DM	
	MON	ROT
Steam-flaked corn ¹	43.5	43.5
Ground hay ²	25.7	25.7
Wet corn distillers grains	18.4	18.4
Alfalfa hay	6.7	6.7
Pelleted protein blend	4.9	4.9
Corn oil	0.8	0.8
Monensin ³ , mg/kg DM	20.7	20.7 ^a
Laidlomycin propionate ⁴ , mg/kg DM	--	8.2 ^b
Nutrient		% of DM
Dry matter, % as-fed		72.2
Protein		14.3
NPN		1.8
Fat		4.8
Calcium		0.9
Phosphorus		0.4
Magnesium		0.2
Salt		0.3
NEm, Mcal/kg		1.92
NEg, Mcal/kg		1.26
¹ Corn steam-flaked to a targeted flake density of 26.5 lb/bu (0.34 kg/L)		
² Blend of sorghum hay and wheat straw		
³ Monensin in form of Rumensin 90 (Elanco Animal Health, Greenfield, IN)		
⁴ Laidlomycin propionate in form of Cattlyst 50G (Phibro Animal Health Corp, Teaneck, NJ)		
^a Experiment 1: d 1-28, 57-84; Experiment 2: d 1-56, 85-112		
^b Experiment 1: d 29-56, 85-112; Experiment 2: d 57-84		

Table 2.3 Outline of strategic rotations indicating ionophore inclusion in ration by day for Experiment 2 treatment groups.

Days on Feed (DOF)	MON	ROT
1-28	Monensin ¹	Monensin ¹
29-56	Monensin ¹	Monensin ¹
57-84	Monensin ¹	Laidlomycin propionate ²
85-112	Monensin ¹	Monensin ¹
¹ Monensin included at dose of 200 mg/head/d in form of Rumensin 90 (Elanco Animal Health, Greenfield, IN) ² Laidlomycin propionate included at dose of 75 mg/head/d in form of Cattlyst 50G (Phibro Animal Health Corp, Teaneck, NJ)		

Table 2.4 Performance within 28-d periods of study comparing cattle receiving consistent monensin (MON) to cattle receiving 28-d rotations between monensin and laidlomycin propionate (ROT) in Experiment 1.

DOF	Item ¹	MON	ROT	SEM	<i>P</i> -value ²
1-28^a	ADG, kg/d	1.60	1.65	0.12	0.71
	DMI, kg/d	8.39	8.48	0.32	0.84
	G:F	0.190	0.196	0.009	0.61
29-56^b	ADG, kg/d	1.52	1.77	0.14	0.18
	DMI, kg/d	10.11	10.21	0.37	0.52
	G:F	0.149	0.180	0.013	0.07
57-84^c	ADG, kg/d	1.30	1.17	0.13	0.44
	DMI, kg/d	11.20	11.02	0.34	0.38
	G:F	0.120	0.106	0.012	0.41
85-112^d	ADG, kg/d	1.20	1.53	0.10	0.01
	DMI, kg/d	12.02	12.11	0.41	0.68
	G:F	0.101	0.128	0.008	0.01

¹ ADG = average daily gain, DMI = dry matter intake, G:F = gain-to-feed ratio
² Significance declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$
^a from d 1–28, MON and ROT both received monensin for 28 d
^b from d 29–56, MON received monensin; ROT received laidlomycin propionate
^c from d 57–84, MON received monensin; ROT received monensin
^d from d 85–112, MON received monensin; ROT received laidlomycin propionate

Table 2.5 Cumulative performance across multiple 28-d periods of study comparing cattle receiving consistent monensin (MON) to cattle receiving 28-d rotations between monensin and laidlomycin propionate (ROT) in Experiment 1.

DOF	Item ¹	MON	ROT	SEM	<i>P</i> -value ²
1-56^a	ADG, kg/d	1.56	1.71	0.08	0.16
	DMI, kg/d	9.25	9.34	0.31	0.64
	G:F	0.170	0.188	0.007	0.06
1-84^b	ADG, kg/d	1.48	1.54	0.04	0.20
	DMI, kg/d	9.88	9.93	0.31	0.94
	G:F	0.153	0.161	0.003	0.06
1-112^c	ADG, kg/d	1.41	1.54	0.04	<0.01
	DMI, kg/d	10.43	10.48	0.32	0.78
	G:F	0.14	0.152	0.003	<0.01

¹ ADG = average daily gain, DMI = dry matter intake, G:F = gain-to-feed ratio
² Significance declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$
^a from d 1–56, MON received monensin for 56 d; ROT received monensin for 28 d followed by laidlomycin propionate for 28 d
^b from d 1–84, MON received monensin for 84 d; ROT received 56 total d of monensin with an interim 28 d laidlomycin propionate
^c from d 1–112, MON received monensin for 112 d; ROT received monensin for a total of 56 d and laidlomycin propionate for a total of 56 d alternating ionophore inclusion every 28 d

Table 2.6 *Ex vivo* modeled gas production kinetics by treatment and sampling period comparing cattle receiving consistent monensin (MON) to cattle receiving a rotational ionophore program of 56 d monensin, followed by 28 d laidlomycin propionate and a final 28 d monensin (ROT) from cannulated steer donor fluid collected in Experiment 2.

Sampling Day	Item ¹	MON	ROT	SEM	<i>P</i> -value ²
56^a	A, mL	123.78	102.07	13.25	0.14
	K, h ⁻¹	0.16	0.19	0.02	0.15
	λ , h	0.03	0.16	0.20	0.55
84^b	A, mL	146.50	136.44	9.39	0.32
	K, h ⁻¹	0.18	0.17	0.01	0.36
	λ , h	0.16	-0.01	0.11	0.16
112^c	A, mL	126.07	111.50	19.49	0.48
	K, h ⁻¹	0.24	0.29	0.03	0.15
	λ , h	-1.01	-0.94	0.24	0.80

¹ A = maximum gas production, K = fractional gas production rate, λ = lag phase time; obtained from nonlinear regression of cumulative gas production curves based on ANKOM pressure data

² Significance declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$

^a on d 56, MON and ROT had received monensin for 56 d

^b on d 84, MON had received monensin for 84 d; ROT had received 56 total d of monensin followed by 28 d laidlomycin propionate

^c on d 112, MON had received monensin for 112 d; ROT had received monensin for a total of 84 d and laidlomycin propionate for an interim 28 d

Table 2.7 *Ex vivo* gas composition by treatment and sampling period comparing cattle receiving consistent monensin (MON) to cattle receiving a rotational ionophore program of 56 d monensin, followed by 28 d laidlomycin propionate and a final 28 d monensin (ROT) from cannulated steer donor fluid collected in Experiment 2.

Sampling day	Item	MON	ROT	SEM	<i>P</i> -value ¹
56^a	Total gas production, mL	125.79	104.22	19.8	0.36
	Total CO ₂ , mL	105.16	90.11	21.78	0.54
	Total CH ₄ , mL	20.62	14.10	3.25	0.14
	CO ₂ :CH ₄ ratio	5.38	6.39	1.59	0.57
84^b	Total gas production, mL	148.0	139.0	6.7	0.27
	Total CO ₂ , mL	132.71	119.50	5.91	0.11
	Total CH ₄ , mL	15.30	19.47	1.43	0.06
	CO ₂ :CH ₄ ratio	8.75	6.14	0.69	0.03
112^c	Total gas production, mL	129.3	116.4	29.2	0.69
	Total CO ₂ , mL	109.20	98.94	24.16	0.70
	Total CH ₄ , mL	20.10	17.44	5.20	0.64
	CO ₂ :CH ₄ ratio	5.51	5.66	0.45	0.76
¹ Significance declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$ ^a on d 56, MON and ROT had received monensin for 56 d ^b on d 84, MON had received monensin for 84 d; ROT had received 56 total d of monensin followed by 28 d laidlomycin propionate ^c on d 112, MON had received monensin for 112 d; ROT had received monensin for a total of 84 d and laidlomycin propionate for an interim 28 d					

Table 2.8 Average daily gain (kg/d) of commercial beef × dairy steers cohoused with cannulated donor steers comparing cattle receiving consistent monensin (MON) to cattle receiving a rotational ionophore program of 56 d monensin, followed by 28 d laidlomycin propionate and a final 28 d monensin (ROT) in Experiment 2.

DOF	MON	ROT	SEM	<i>P</i> -value ²
1-56^a	1.49	1.47	0.07	0.78
1-84^b	1.52	1.67	0.06	0.03
1-112^c	1.57	1.62	0.04	0.31
³ Significance declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$ ^a from d 1–56, MON and ROT received monensin for 56 d ^b from d 1–84, MON received monensin for 84 d; ROT received 56 total d of monensin followed by 28 d laidlomycin propionate ^c from d 1–112, MON received monensin for 112 d; ROT received monensin for a total of 84 d and laidlomycin propionate for an interim 28 d				

References

- AOAC International. (2019). *Official methods of analysis* (21st ed.). AOAC International.
- Bergen, W. G., & Bates, D. B. (1984). Ionophores: Their Effect on Production Efficiency and Mode of Action. *Journal of Animal Science*, 58(6), 1465–1483.
<https://doi.org/10.2527/jas1984.5861465x>
- Casey, N. H., Wessels, R. H., & Meissner, H. H. (1994). Feedlot growth performance of steers on salinomycin, monensin and a daily rotation between the two. *Journal of the South African Veterinary Association*, 65(4), 160–163.
https://hdl.handle.net/10520/AJA00382809_1505
- Code of Federal Regulations. (2025). Animal drugs for use in animal feeds—Definitions and general considerations (21 C.F.R. § 558.3). *Title 21: Food and Drugs*. U.S. Government Publishing Office. <https://www.ecfr.gov/current/title-21/chapter-I/subchapter-E/part-558>
- Crossland, W. L., Tedeschi, L. O., Callaway, T. R., Miller, M. D., Smith, W. B., & Cravey, M. (2017). Effects of rotating antibiotic and ionophore feed additives on volatile fatty acid production, potential for methane production, and microbial populations of steers consuming a moderate-forage diet. *Journal of Animal Science*, 95(10), 4554–4567.
<https://doi.org/10.2527/jas2017.1665>
- Domesick, E. J., & Martin, S. A. (1999). Effects of laidlomycin propionate and monensin on the in vitro mixed ruminal microorganism fermentation. *Journal of Animal Science*, 77(8), 2305. <https://doi.org/10.2527/1999.7782305x>
- Galyean, M. L., Malcolm, K. J., & Duff, G. C. (1992). Performance of feedlot steers fed diets containing laidlomycin propionate or monensin plus tylosin, and effects of laidlomycin propionate concentration on intake patterns and ruminal fermentation in beef steers during adaptation to a high-concentrate diet. *Journal of Animal Science*, 70(10), 2950–2958. <https://doi.org/10.2527/1992.70102950x>
- Goering, H. K., & Van Soest, P. J. (1970). *Forage fiber analyses (apparatus, reagents, procedures, and some applications)* (Vol. 387). US Agricultural Research Service.
- Gräfe, U., Reinhardt, G., & Miosga, N. (1989). Monovalent cation specificity of passive transport mediated by laidlomycin and 26-deoxylaidlomycin. *Journal of Basic Microbiology*, 29(6), 391–394. <https://doi.org/10.1002/jobm.3620290620>
- Groot, J. C. J., Cone, J. W., Williams, B. A., Debersaques, F. M. A., & Lantinga, E. A. (1996). Multiphasic analysis of gas production kinetics for in vitro fermentation of ruminant feeds. *Animal Feed Science and Technology*, 64(1), 77–89.
[https://doi.org/10.1016/S0377-8401\(96\)01012-7](https://doi.org/10.1016/S0377-8401(96)01012-7)

- Guan, H., Wittenberg, K. M., Ominski, K. H., & Krause, D. O. (2006). Efficacy of ionophores in cattle diets for mitigation of enteric methane. *Journal of Animal Science*, 84(7), 1896–1906. <https://doi.org/10.2527/jas.2005-652>
- Morris, F. E., Branine, M. E., Galyean, M. L., Hubbert, M. E., Freeman, A. S., & Lofgreen, G. P. (1990). Effect of rotating monensin plus tylosin and lasalocid on performance, ruminal fermentation, and site and extent of digestion in feedlot cattle. *Journal of Animal Science*, 68(10), 3069. <https://doi.org/10.2527/1990.68103069x>
- National Academies of Sciences, Engineering, and Medicine. (2016). *Nutrient Requirements of Beef Cattle: Eighth Revised Edition*. The National Academies Press.
- Owens, F. (2021). *Ionophores for growing and finishing beef cattle: History, benefits, challenges, and global warming*. Minnesota Nutrition Conference, St. Paul, MN.
- Pell, A. N., & Schofield, P. (1993). Computerized monitoring of gas production to measure forage digestion in vitro. *Journal of Dairy Science*, 76(4), 1063–1073. [https://doi.org/10.3168/jds.S0022-0302\(93\)77435-4](https://doi.org/10.3168/jds.S0022-0302(93)77435-4)
- Reyes, A., Loh, H. Y., Thorndyke, M. P., Guimaraes, O., Goodall, R., Delmore, R. J., Belk, K. E., & Engle, T. E. (2023). The Influence of Tannins, Saponins, and Direct Fed Microbials on In Vitro Rumen Fermentation Characteristics and Microbiota. *Corpus Journal of Dairy and Veterinary Science (CJDVS)*, 4(3), 1–7. <https://doi.org/10.54026/CJDVS1060>
- Russell, J. B., & Strobel, H. J. (1989). Effect of ionophores on ruminal fermentation. *Applied and Environmental Microbiology*, 55(1), 1–6. <https://doi.org/10.1128/aem.55.1.1-6.1989>
- Samuelson, K. L., Hubbert, M. E., Galyean, M. L., & Löest, C. A. (2016). Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico State and Texas Tech University survey1. *Journal of Animal Science*, 94(6), 2648–2663. <https://doi.org/10.2527/jas.2016-0282>
- Shreck, A. L., Behlke, E. J., Paddock, Z. D., Burciaga-Robles, L. O., Parr, S. L., Booker, C. W., Hannon, S. J., Raaphorst, H. S., Byers, L., Rademacher, R. D., Quinn, M. J., & May, M. L. (2016). Effect of daily rotational feeding of lasalocid and monensin to yearling feedlot steers fed in western Canada. *The Professional Animal Scientist*, 32(5), 561–569. <https://doi.org/10.15232/pas.2015-01489>
- U.S. Food and Drug Administration. (2025). *Approved animal drug products (Green Book)*. <https://www.fda.gov/animal-veterinary/products/approved-animal-drug-products-green-book>
- 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)

Chapter 3 - Comparing *ex vivo* batch culture and *in vivo* automated head chamber system methods for daily methane production estimates in commercial beef × dairy steers

Introduction

Enteric methane (CH₄) production is an inherent consequence of anaerobic ruminal fermentation and represents both an energetic loss to the animal and a source of greenhouse gas emissions from ruminant production systems (Hristov et al., 2013; Tedeschi et al., 2022). During carbohydrate fermentation, reducing equivalents (dihydrogen; H₂) are generated and must be removed to maintain redox balance within the rumen microbial ecosystem. Methanogenic archaea use H₂ to reduce carbon dioxide (CO₂) to CH₄, thereby serving as a principal hydrogen sink (Chen & Wolin, 1979; Russell & Strobel, 1989). Because CH₄ production reflects the integrated outcome of volatile fatty acid (VFA) formation, H₂ utilization, microbial competition, and substrate availability, accurate quantification of CH₄ production is necessary for interpreting ruminal fermentation efficiency and evaluating mitigation strategies. Given increasing interest in how dietary interventions, such as ionophores, influence both fermentation efficiency and greenhouse gas emissions, robust and field-applicable methods for quantifying CH₄ production are necessary to interpret treatment responses under commercial feedlot conditions.

Several techniques have been developed to quantify CH₄ emissions in ruminants, each differing in precision, infrastructure requirements, and applicability to commercial production systems. Open- and closed-circuit respiration chambers quantify total gas exchange by continuously measuring airflow into and out of a sealed chamber housing an individual animal (Tedeschi et al., 2022). Methane production is calculated from the difference in CH₄

concentration between inlet and outlet air, adjusted for airflow rate and chamber volume.

Respiration chambers provide highly controlled and precise measurements of daily CH₄ output and are widely regarded as the reference method for CH₄ quantification in ruminants. However, they require specialized confinement of animals, specialized facilities, and substantial financial investment, limiting their feasibility for large-scale feedlot applications (Tedeschi et al., 2022).

Automated head chamber systems (AHCS; GreenFeed-type systems) provide a practical alternative for estimating CH₄ emissions under commercial conditions (Jonker et al., 2016; Tedeschi et al., 2022). The system attracts cattle to a baited feeding station, where airflow is actively drawn across the muzzle during voluntary visits. Methane concentration is measured in real time using infrared gas analyzers, and gas flux is calculated from the product of CH₄ concentration and measured airflow rate. Daily CH₄ production is subsequently estimated by integrating emissions across multiple short-duration visits and extrapolating to a 24-h period based on visit frequency and duration. Although AHCS platforms enable CH₄ measurement in group-housed cattle without confinement, estimates are influenced by animal behavior, visit frequency, bait consumption, and environmental airflow conditions. Furthermore, physical factors affecting air capture efficiency, particularly in ruminally cannulated animals, may influence measured emissions at the individual level.

Ex vivo fermentation systems provide a laboratory-based batch culture approach for evaluating ruminal gas production under controlled conditions while maintaining rumen microbial adaptation conditions within live ruminant systems (Johnson, 1966; Russell & Martin, 1984; Tedeschi et al., 2022). A common application of *ex vivo* fermentation techniques in CH₄ research involves administering dietary treatments to donor animals for an adaptation period prior to rumen fluid collection, allowing rumen microbial populations to respond to the treatment

before inoculating batch culture systems. In the batch culture system, rumen fluid collected from donor animals is incubated anaerobically with a defined substrate at physiological temperature. Gas production is quantified over a fixed incubation period using pressure transducers or gas collection devices, and gas composition is determined by chromatographic analysis of collected gas samples. These *in vitro* systems allow estimation of total gas production and CH₄ proportion while minimizing variability associated with animal behavior and feed intake patterns. However, because *in vitro* systems measure gas production over a finite incubation interval using a defined quantity of substrate or rumen fluid, data scaling is required to estimate daily animal-level CH₄ production. Scaling approaches may scale CH₄ production by ration intake of the animal or by estimated ruminal fill of the animal of interest. The extent to which *ex vivo* CH₄ estimates derived from these scaling methods correspond to *in vivo* AHCS measurements under commercial feedlot conditions remains unclear.

Accordingly, the objective of this study was to compare estimated daily CH₄ production derived from *ex vivo* batch culture using the ANKOM RF Gas Production System (ANKOM Technology, Macedon, NY), scaled using rumen fill or ration intake corrections, with *in vivo* automated head chamber system (GreenFeed, C-Lock Inc., Rapid City, SD) measurements obtained from commercial beef × dairy steers and co-housed ruminally cannulated beef × dairy steers during the growing phase. Comparisons included evaluation of cleaned and uncleaned pen-level AHCS datasets as well as CH₄ measurements obtained directly from cannulated steers. We hypothesized that *ex vivo* extrapolation approaches would yield CH₄ estimates comparable to pen-level AHCS measurements, whereas CH₄ measured from cannulated steers using AHCS would differ from pen-level estimates due to technical constraints affecting air capture efficiency.

Materials and methods

Study overview

All procedures involving animals were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC; protocol 5006) and complied with the Guide for the Care and Use of Agricultural Animals in Research and Teaching.

This experiment was conducted at Clark County Feeders (Minneola, KS) to compare CH₄ estimation approaches in beef × dairy steers in a commercial feedyard setting. Cattle, housing conditions, and feeding management were identical to those described in Experiment 2 of Chapter 2. However, the present analysis only used 56 d of the experimental feeding period when consistent dietary treatment was applied across pens. During this period, all cattle received the same basal growing ration containing monensin, and no ionophore rotation occurred.

Animals and facility

A total of 72 beef × dairy crossbred steers were included in this study, consisting of 67 non-cannulated commercial steers (initial body weight (BW) = 272 ± 24 kg) and 5 ruminally cannulated Holstein × Angus crossbred steers (initial BW = 443 ± 15 kg). Cannulated steers were co-housed with the commercial beef × dairy steers across two dirt-surfaced drylot pens. Pens provided 0.833 ± 0.025 m/head of bunk space and were equipped with automatic waterers to allow ad libitum access to water.

Arrival processing and health management

Upon arrival, commercial beef × dairy steers were processed according to the feedyard's standard protocol and received oral fenbendazole (Safe-Guard; Merck Animal Health), injectable ivermectin (Ivermax; Aspen Veterinary Resources, Loveland, CO), a modified-live viral respiratory vaccine (VISTA 5; Merck Animal Health, Rahway, NJ), a 7-way clostridial bacterin-

toxoid (VISION 7; Merck Animal Health, Rahway, NJ), and a 5-way leptospirosis bacterin (Leptoform-5; Zoetis, Parsippany, NJ). Commercial steers were also administered a trenbolone acetate/estradiol benzoate extended-release implant (Synovex ONE Feedlot; Zoetis, Parsippany, NJ) according to yard protocol. All steers were fitted with a radio frequency identification (RFID) ear tag in the left ear to enable individual animal identification and integration with the AHCS data acquisition system. Cannulated steers were managed as research animals and monitored daily for cannula integrity and general health.

Prior to initiation of the 56-d evaluation period, steers were managed under the feedyard's standard receiving and adaptation protocol. Cattle were placed on a high-roughage starter ration containing decoquinate (Deccox; Phibro Animal Health Corporation, Teaneck, NJ) and no ionophore for approximately 28 d before transitioning to a growing ration.

Ration and feeding

During the 56-d evaluation period, all steers were limit-fed (targeting an average daily gain of 1.36 kg per animal per d) a common total mixed ration (TMR) delivered twice daily using horizontal rotary mixer trucks (Roto-Mix 620-16XD; Roto-Mix LLC, Dodge City, KS) (Table 3.1). Rations were formulated according to feed mill and consulting nutritionist records.

The growing ration was formulated to provide 1.26 Mcal/kg net energy for gain (NEg) and 14.3% crude protein (dry matter (DM) basis) (Table 3.1). On a DM basis, the ration contained 43.5% steam-flaked corn, 32.4% mixed alfalfa hay, 18.4% wet corn distillers grains, 4.9% pelleted protein blend, and 0.8% corn oil. Monensin (Rumensin 90; Elanco Animal Health, Greenfield, IN) was included continuously at 200 mg/steer/d for the entirety of the 56-d evaluation window. Feed bunks were managed daily using standard commercial bunk-reading procedures to maintain consistent limited intake to the targeted average daily gain. Pen-level

intake was determined from daily feed delivery records maintained by the AHI management platform (Animal Health International, Greeley, CO).

Laboratory sample analysis

Ration ingredient samples were collected every 28 d beginning at trial initiation and analyzed for nutrient composition to verify diet formulation (Table 3.1). Ingredient samples were dried at 55°C in a conventional drying oven for 72 h to determine dry matter according to AOAC method 934.01 (AOAC International, 2019). Samples were then ground through a 2-mm screen using a cyclone sample mill (UDY Corporation, Fort Collins, CO). Samples were subsequently analyzed for nutrient composition. Corrected DM for each ingredient was determined by oven drying a 0.5 g subsample at 104°C for 3 h according to AOAC method 934.01 (AOAC International, 2019), and ash was determined by combusting a 0.5 g subsample of each sample in a muffle furnace at 550°C for 8 h according to AOAC method 934.05 (AOAC International, 2019). Nitrogen concentration was determined via steam distillation Kjeldahl technique using a KjelMaster K-375 with K376 autosampler (Buchi Corp., Flawil, Switzerland) according to AOAC method 984.13 (AOAC International, 2019). A 0.5 g subsample was weighed into a kjeldahl tube and combined with 1 kjeldahl catalyst tablet (4.98 g K₂SO₄ 0.02 CuSO₄·5H₂O) and 10 mL H₂SO₄. Tube contents were digested at 410°C for 100 min, 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 were done automatically using KjelMaster K-375. Crude protein was calculated as N × 6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to (Van Soest et al., 1991). Samples were analyzed for NDF and ADF using an ANKOM 200 Fiber Analyzer (ANKOM Technology, Macedon, NY). Approximately 0.5 g subsamples were sealed in F57 filter bags

(ANKOM Technology, Macedon, NY) and analyzed using neutral detergent solution with sodium sulfite and α -amylase for NDF determination. Following NDF extraction, samples were rinsed with acetone, dried, and weighed. Acid detergent fiber was subsequently determined using acid detergent solution in the same analyzer, with final weights used to calculate ADF concentration of the sample. Net energy for gain was estimated during diet formulation (NASEM, 2016).

Methane quantification methods

To compare CH₄ estimation approaches under commercial feedlot conditions, five CH₄ quantification methods were evaluated (Table 3.2). Three estimates derived from *in vivo* AHCS measurements were evaluated: (1) AHCS—cleaned commercial steer data, (2) AHCS—uncleaned commercial steer data, and (3) AHCS—ruminally cannulated steers. Additionally, two estimates derived from *ex vivo* batch culture fermentation scaled to the animal level were evaluated: (4) *ex vivo*—ration intake-scaled and (5) *ex vivo*—rumen fill-scaled. These methods were subsequently compared to assess differences among CH₄ estimation approaches.

***In vivo* methane measurement using Automated Head Chamber Systems**

Enteric CH₄ emissions were quantified throughout the 56-d period using AHCS (GreenFeed, C-Lock Inc., Rapid City, SD), with one unit placed in each pen (2 units total). Prior to the start of the 56-d trial period, steers received RFID tags in their left ear and underwent a 21-d acclimation period to ensure familiarity with the AHCS. The AHCS estimates enteric gas production during voluntary animal visits by continuously sampling exhaled and eructated gases while delivering a controlled quantity of bait to encourage animal participation. Alfalfa pellets were used as bait and were dispensed in discrete allotments during each visit. Each visit allowed a maximum of six pellet drops, with approximately 30 s between each drop, resulting in

approximately 35 g of alfalfa pellets per visit. A minimum interval of 4 h was required between individual animal visits to limit repeated sampling within short timeframes (Hristov et al., 2015). Steers were allowed access to the AHCS units for the duration of the experimental period. To prevent more than one animal from accessing the unit simultaneously, each system was equipped with a panel alley that restricted entry to a single animal.

During each AHCS visit, airflow was actively drawn across the muzzle of the animal, and CH₄ and CO₂ concentrations were measured using infrared gas analyzers. Gas flux was calculated from measured gas concentration and airflow rate within the collection tube. The air flux sensor was calibrated every 28 d by releasing a known quantity of CO₂ from a 90 g prefilled CO₂ cylinder into the system for 3 min. This process was repeated six times during calibration, and the measured CO₂ recovery was compared to the known release amount (Hristov et al., 2015). Airflow rate within the collection tube was maintained between 27 and 40 L/s, and the air filter was cleaned every 28 d to maintain consistent flow conditions.

Gas emissions data (CH₄ and CO₂) were retrieved from the C-Lock Inc. data management platform prior to analysis. Data selection procedures outlined by Beck et al. (2025) were utilized. Only visits exceeding 2 min in duration were considered. From this common dataset, three AHCS-derived CH₄ estimation approaches were generated for comparison. To generate the AHCS—cleaned dataset, only visits from commercial beef × dairy steers with ≥ 10 valid visits across the 56-d evaluation period were retained (Alemu et al., 2021). To generate the AHCS—uncleaned dataset, all commercial beef × dairy steer visits exceeding 2 min in duration were retained regardless of animal visit frequency. To generate the AHCS—cannulated steers dataset, all visits exceeding 2 min in duration from ruminally cannulated steers were retained (all cannulated steers exceeded the ≥ 10 valid visit threshold during the evaluation period). Diurnal

variation in CH₄ emissions estimates for each dataset was considered and corrected for using least squares means data cleaning procedure as described by Beck et al. (2025). Least squares means generated CH₄ estimates for individual animals were then averaged within pen to generate a CH₄ estimate per pen (g CH₄/steer/d). Pen was considered the experimental unit for statistical analyses.

Rumen fluid collection and *ex vivo* batch culture fermentation

At the end of the trial on d 56, rumen contents were collected from each cannulated steer (n = 5) four hours after morning feeding. Equal proportions of ruminal liquid and solid digesta were manually withdrawn from the ventral rumen sac via the cannula. Rumen contents were immediately strained through four layers of cheesecloth into pre-warmed, insulated containers. Rumen fluid from each donor steer was maintained separately and transported to the laboratory within 30 minutes of collection. Temperature was maintained near 39°C during transport, and rumen fluid was continuously purged with CO₂ upon arrival to preserve anaerobic conditions.

Ex vivo batch culture fermentation technique was conducted using a vented ANKOM RF gas production system (ANKOM Technology, Macedon, NY) following anaerobic fermentation procedures adapted from Van Soest et al. (1991) and Pell & Schofield (1993). Fermentation vessels received 0.50 g (as-fed basis) of the appropriate TMR. Samples of TMR were collected directly from pen feed bunks prior to rumen fluid collection and processed to a consistent particle size using a coffee grinder. Each fermentation vessel then received 40 mL of bicarbonate-buffered fermentation medium containing macro- and micromineral components prepared according to Goering & Van Soest (1970), followed by 10 mL of the corresponding rumen fluid inoculum within each sampling period. For each donor steer, two treatment vessels and two blank vessels were prepared. Blank vessels contained buffer solution and rumen fluid

only (no substrate) and were used to correct for endogenous gas production not associated with TMR digestion.

Vessels were flushed with CO₂, sealed using ANKOM RF screw caps, and incubated anaerobically at 39°C for 48 h in a benchtop orbital shaker (Solaris 4000 R; Thermo Fisher Scientific, Waltham, MA). Gas pressure was recorded continuously using ANKOM RF modules and converted to cumulative gas volume using estimates of headspace volume, venting pressure threshold, gas constant, and measured pressure. The outflow port of each ANKOM RF module was fitted with an adaptor which directed produced gas into a foil Tedlar gas sampling bag (Supel-Inert Gas Sampling Bag; Supelco, Bellefonte, PA) for cumulative collection over the incubation period. Following the 48-h fermentation, gas was withdrawn from each Tedlar bag using a 15-mL plastic syringe fitted with a Luer-lock stopcock valve to prevent gas loss during storage. Samples were sent to Colorado State University (Fort Collins, CO) for analysis of CO₂:CH₄ using gas chromatography. Gas composition was determined using a gas chromatograph (Shimadzu GC-14A; Shimadzu Corp., Kyoto, Japan) equipped with a thermal conductivity detector maintained at 100°C following procedures described by Reyes et al., (2023). Total CH₄ production was calculated by multiplying cumulative gas production over the first 24 h of the batch culture fermentation by the measured fractional concentration of CH₄ in the gas sample.

Scaling *ex vivo* daily gas production to estimated daily methane output

Ex vivo CH₄ production measured over the 48-h incubation period was scaled to estimated daily animal-level CH₄ output using two independent approaches: rumen fill-based and ration intake-based.

Rumen fill-based scaling method

On d 56 after rumen fluid collection was complete and approximately 4 h after the afternoon feeding, rumen evacuations were conducted on two ruminally cannulated steers (one from each pen). Solid and liquid rumen contents were manually removed through the cannula and transferred into a large pre-weighed collection vessel. The filled vessel was weighed to determine total rumen digesta mass (wet basis), and all contents were returned to the rumen within 30 min of the start of evacuation. The proportion of body weight represented by rumen contents was calculated for each of the two evacuated steers and averaged. The average proportion was then applied to the body weight of the remaining cannulated steers to estimate the mass of rumen fill for each steer. Because rumen contents were measured at a single postprandial time point and extrapolated proportionally to additional animals, this value is referred to as rumen fill rather than rumen capacity.

For the rumen fill-based scaling method, CH₄ production per jar was first standardized to inoculum volume by dividing total CH₄ production per fermentation vessel (g CH₄/jar) by the 10 mL rumen fluid inoculum added to each jar, resulting in g CH₄/mL rumen fluid. Methane production expressed per mL of rumen fluid was multiplied by the estimated total rumen fill volume (mL/steer) to estimate g CH₄ per rumen fill. Estimated g CH₄ per rumen fill was multiplied by 2 to account for twice-daily feeding and associated postprandial rumen fill patterns. Final rumen-fill-based CH₄ production was expressed as g CH₄ per steer per d (see calculations below).

Ration intake-based scaling method

For the ration intake-based scaling method, CH₄ produced per jar was first standardized to vessel substrate mass by dividing total CH₄ production per fermentation vessel (g CH₄/jar) by the 0.50 g (as-fed basis) of TMR incubated in each jar, resulting in g CH₄/g substrate (as-fed).

Methane production per g substrate was multiplied by average daily intake per commercial beef × dairy steer within each pen to estimate daily CH₄ production. Average daily intake was calculated based on daily feed delivery records maintained by the AHI management platform (Animal Health International, Greeley, CO). Substrate-based CH₄ production was expressed as g CH₄ per steer per d (see calculations below).

***Ex vivo* methane scaling calculations**

For both scaling approaches, CH₄ estimates were first generated for each cannulated steer and subsequently averaged by pen to generate a single *ex vivo* batch culture CH₄ estimate (g/steer/d) per method to represent the animals in each pen prior to statistical analysis. Pen was considered the experimental unit for comparison with AHCS-derived CH₄ estimates.

Calculations used in this experiment to scale *ex vivo* gas production to daily CH₄ estimates per steer were:

Ration Intake-Scaled:

Average intake during the 56-d evaluation was 7.2 kg/hd/d (as-fed basis).

$$\text{CH}_4(\text{g})/\text{hd}/\text{d} = \frac{\text{Vessel Total CH}_4(\text{g})}{\text{Vessel TMR (0.50 g)}} \times \text{Intake (g)}/\text{hd}/\text{d}$$

Rumen Fill-Scaled:

$$\text{CH}_4(\text{g})/\text{hd}/\text{d} = \frac{\text{Vessel Total CH}_4(\text{g})}{\text{Vessel inoculum (10 mL)}} \times \text{Rumen Contents (mL)}/\text{hd} \times 2 \text{ Feedings}/\text{d}$$

Statistical analysis

All statistical analyses were conducted in R (version 4.4.2; R Core Team) using the lme4 and emmeans packages. Methane estimates derived from each quantification approach were averaged within pen prior to analysis, and pen was considered the experimental unit. Linear

models were used to evaluate differences among CH₄ quantification methods. The five methods compared were: (1) AHCS—cleaned (animals with ≥ 10 valid visits), (2) AHCS—uncleaned, (3) AHCS—cannulated steers, (4) *ex vivo*—ration intake-scaled, and (5) *ex vivo*—rumen fill-scaled (Table 3.2). Least squares means were generated using the emmeans package, and pairwise comparisons among methods were conducted using Tukey-adjusted contrasts to control for multiple comparisons. Statistical significance was declared at $P \leq 0.05$, and tendencies were discussed at $0.05 < P \leq 0.10$.

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

- Y_i = estimated daily CH₄ production (g CH₄/steer/d) for quantification method i
- μ = population mean
- α_i = fixed effect of CH₄ quantification method i
- ε_i = residual error

Results

Mean daily CH₄ production (g CH₄/hd/d) differed among quantification methods ($P < 0.05$; Table 3.3; Figure 3.1). Methane estimates derived from AHCS—cleaned data (143.9 g CH₄/hd/d), AHCS—uncleaned data (139.2 g CH₄/hd/d), *ex vivo*—ration intake-scaled estimates (145.8 g CH₄/hd/d), and *ex vivo*—rumen fill-scaled estimates (149.5 g CH₄/hd/d) did not differ from one another ($P > 0.10$). In contrast, CH₄ production measurements from AHCS—cannulated steers (39.3 g CH₄/hd/d) were lower ($P < 0.05$) than all other quantification approaches, representing a reduction of approximately 72–74% relative to the other methods. No differences were detected ($P > 0.10$) between cleaned and uncleaned AHCS pen-level estimates, and CH₄ estimates derived from the two *ex vivo* scaling approaches also did not differ from each other ($P > 0.10$).

Discussion

Four of the five quantification approaches—cleaned pen-level AHCS data, uncleaned AHCS data, *ex vivo* ration intake-scaled estimates, and *ex vivo* rumen-fill-scaled estimates—produced no observed differences in CH₄ values. In contrast, CH₄ measured from ruminally cannulated steers using the AHCS was substantially lower than all other methods. These findings demonstrate agreement between pen-level *in vivo* CH₄ measurements and *ex vivo* extrapolation approaches under the conditions of this study. However, previous work has emphasized that extrapolation of fermentation-based measurements to whole-animal CH₄ production is inherently limited due to the separation of rumen microbial activity from host-animal metabolism, as *in vitro* systems isolate microbial processes from the animal while whole-animal methods require assumptions that can increase uncertainty when scaling across conditions (Johnson, 1966; Tedeschi et al., 2022).

The similarity between cleaned and uncleaned AHCS pen-level datasets indicates that imposing a ≥ 10 -visit inclusion threshold did not materially alter CH₄ estimates in this limit-fed system when CH₄ production was summarized using least squares means. Although the cleaned dataset excluded animals with fewer qualifying visits, pen-level CH₄ estimates remained statistically comparable. The lack of differences suggests that visitation behavior was sufficiently consistent to generate similar CH₄ estimates when summarized at the pen level. GreenFeed-based estimates can be influenced by visit frequency and the temporal distribution of visits, as accurate estimation requires repeated sampling across the diurnal cycle and sufficient visits per animal over multiple days to represent daily emission patterns (Jonker et al., 2016; Tedeschi et al., 2022). Consequently, inclusion or exclusion of animals with fewer AHCS visits did not meaningfully influence pen-level CH₄ estimates in this system. Whether similar consistency

would be observed under ad libitum feeding conditions, where visitation patterns may be more heterogeneous, warrants further investigation, particularly because GreenFeed-derived estimates are sensitive to visitation frequency and diurnal sampling distribution (Jonker et al., 2016; Tedeschi et al., 2022).

Methane production measured directly from ruminally cannulated steers using the AHCS was markedly lower than all other quantification methods. Given that cannulated steers were co-housed within the same pens and consumed the same diet as commercial steers, true biological differences in fermentation are unlikely to explain this discrepancy. One possible explanation is incomplete sealing at the cannula interface during AHCS measurements. Accurate AHCS quantification depends on efficient capture of respired and eructated gases and consistent airflow measurement during each visit, as flux is calculated from gas concentration and airflow within the system (Tedeschi et al., 2022). Disruptions in gas capture, including potential air leakage near the cannula site or altered head positioning during visitation, could reduce CH₄ estimations. Leakage of ruminal gases through the cannula has been shown to reduce gas recovery at the head, with as little as 6 to 19% of emitted gas captured in poorly sealed cannulas, thereby contributing to reduced or more variable CH₄ estimates (Beauchemin et al., 2012). More broadly, differences among CH₄ quantification methods are expected due to inherent methodological assumptions and limitations (Tedeschi et al., 2022). This discrepancy does not undermine the primary comparisons of the study. In practical feedlot applications, CH₄ quantification would typically rely either on pen-level *in vivo* measurement or on *ex vivo* fermentation systems independently. The reduced CH₄ recovery observed in cannulated steers represents a methodological artifact rather than a biologically meaningful divergence in CH₄ production.

Ex vivo batch culture-derived CH₄ estimates scaled by either ration intake or rumen fill were statistically indistinguishable from commercial steer pen-level AHCS measurements. Ration intake-based scaling assumed proportionality between CH₄ production and daily feed intake, whereas rumen-fill-based scaling assumes proportionality between CH₄ production and the mass of fermentable substrate present within the rumen at a defined postprandial time point. Despite differing assumptions, both approaches produced CH₄ estimates closely aligned with commercial pen-level AHCS measurements. Agreement suggests that CH₄ production quantified under controlled 48-hour batch culture conditions, when scaled using biologically grounded assumptions, can approximate *in vivo* CH₄ output in commercial growing cattle under limit-fed conditions. Although *ex vivo* systems isolate microbial fermentation from host-animal processes, previous work has noted that such approaches can still provide meaningful insight when interpreted within the context of microbial processes isolated from host-animal influences, where fermentation activity may not fully reflect whole-animal digestion and metabolism (Johnson, 1966; Tedeschi et al., 2022).

Several methodological considerations warrant acknowledgement. Rumen fill was directly measured in two steers and extrapolated to additional cannulated steers using the proportional relationship between rumen mass and body weight. This approach assumes linear scaling of rumen contents with body size and does not account for individual animal variation in rumen volume, digesta turnover, or feeding behavior. However, substantial biological variation exists in estimating rumen fill at any single postprandial time point, and rumen capacity is not a fixed physiological constant. Variation in rumen mass occurs due to differences in body size, intake level, diet composition, and time relative to feeding. Given this inherent variability and the consistency observed between rumen-fill-scaled and substrate-scaled CH₄ estimates,

proportional scaling based on steer body size is unlikely to have compromised the overall validity of the findings.

Cannulated steers used in this study were larger than the commercial beef $\times$ dairy steers housed within the same pens. Although differences in body size may influence absolute rumen volume, the use of larger cannulated animals was necessary to minimize risk of injury and cannula damage in group-housed feedlot environments. Smaller cannulated animals are more susceptible to mounting and social pressure from pen mates, increasing the likelihood of cannula displacement or tissue trauma. Selection of larger cannulated steers prioritized animal welfare while still providing rumen inoculum representative of the shared pen environment. Because rumen contents were measured in only two cannulated steers, rumen fill was expressed as a proportion of body weight and averaged before being applied to the remaining cannulated steers used for *ex vivo* scaling rather than relying on absolute rumen fill mass from individual animals. These rumen fill estimates were then used as a physiological approximation when scaling *ex vivo* CH₄ production to estimate CH₄ output of the commercial steers within each pen.

For ration intake-based scaling, CH₄ production per g of substrate was scaled using average pen-level intake rather than intake specific to cannulated steers. Although cannulated steers likely consumed greater absolute quantities of feed due to larger body weight, the objective of this scaling approach was to estimate CH₄ production representative of the commercial pen population. Use of average pen-level intake therefore aligns the *ex vivo* estimate with the CH₄ output of interest at the population level rather than with the physiology of individual donor steers. In addition, pen-level intake can be readily obtained from feed delivery records routinely maintained in commercial feedyards, making intake-based scaling a simple and practical approach for estimating CH₄ production under commercial conditions.

It is important to recognize that cattle in this study were limit-fed a fixed quantity of feed daily. This controlled intake structure reduced day-to-day intake variability and facilitated biologically meaningful scaling of *ex vivo* batch culture CH₄ production to daily intake. The close agreement among commercial pen-level AHCS measurements and *ex vivo* scaling approaches observed here may partially reflect the stability inherent to limit-feeding systems. Application of these scaling methods to cattle fed *ad libitum*, where intake fluctuates more substantially among days and animals, requires further evaluation.

Implications

The lack of statistical differences between pen-level automated head chamber system measurements and scaled *ex vivo* CH₄ estimates demonstrates that controlled batch culture fermentation, when scaled using biologically justified assumptions, can approximate daily CH₄ production under commercial growing conditions. This finding provides a practical framework for CH₄ estimation in feedlot systems where large-scale *in vivo* gas quantification may be cost-prohibitive or logistically impractical. Validated *ex vivo* approaches may offer a feasible tool for screening dietary interventions, evaluating feed additives, or generating emission estimates within commercial research programs without requiring continuous *in vivo* gas monitoring. By integrating fermentation-based CH₄ quantification with commercial production systems, researchers and industry stakeholders may be better equipped to assess the environmental impact of nutritional strategies and make informed management decisions in response to increasing pressure to quantify and mitigate greenhouse gas emissions. Further evaluation under *ad libitum* finishing conditions will help determine the robustness of these scaling approaches across production phases. Nonetheless, results support the use of *ex vivo* fermentation as a complementary and scalable method for CH₄ estimation in commercial feedlot cattle.

Tables

Table 3.1 Ingredient and nutrient composition of limit-fed growing ration (dry matter basis).

Ingredient	% of DM
Steam-flaked corn ¹	43.5
Ground hay ²	25.7
Wet corn distillers grains	18.4
Alfalfa hay	6.7
Pelleted protein blend	4.9
Corn oil	0.8
Monensin ³ , mg/kg DM	20.7
Nutrient	% of DM
Dry matter, % as-fed	72.2
Protein	14.3
NPN	1.8
Fat	4.8
Calcium	0.9
Phosphorus	0.4
Magnesium	0.2
Salt	0.3
NEm, Mcal/kg	1.92
NEg, Mcal/kg	1.26
¹ Corn steam-flaked to a targeted flake density of 26.5 lb/bu (0.34 kg/L)	
² Blend of sorghum hay and wheat straw	
³ Monensin in form of Rumensin 90 (Elanco Animal Health, Greenfield, IN)	

Table 3.2 Methane quantification methods utilized to estimate daily enteric methane emissions (g CH₄/hd/d) of commercial beef × dairy growing steers.

Daily CH ₄ quantification method	CH ₄ capture method ¹	Sample population
1. AHCS—cleaned	<i>In vivo</i> GreenFeed	Cleaned commercial beef × dairy (n = 36)
2. AHCS—uncleaned	<i>In vivo</i> GreenFeed	Commercial beef × dairy (n = 67)
3. AHCS—cannulated steers	<i>In vivo</i> GreenFeed	Cannulated steers (n = 5)
4. <i>Ex vivo</i> —substrate-scaled	<i>Ex vivo</i> ANKOM	Rumen fluid—cannulated steers (n = 5)
5. <i>Ex vivo</i> —rumen fill-scaled	<i>Ex vivo</i> ANKOM	Rumen fluid—cannulated steers (n = 5)
¹ <i>In vivo</i> measurements obtained using GreenFeed AHCS (C-Lock Inc., Rapid City, SD). <i>Ex vivo</i> measurements obtained using ANKOM RF Gas Production System (ANKOM Technology, Macedon, NY).		

Table 3.3 Daily enteric methane emissions (g CH₄/hd/d) estimate by AHCS and scaled *ex vivo* quantification methods.

CH₄ quantification method	CH₄ estimate, g/d $\pm$ SEM¹
AHCS—cleaned	143.9 $\pm$ 14.7 ^a
AHCS—uncleaned	139.2 $\pm$ 14.7 ^a
AHCS—cannulated steers	39.3 $\pm$ 14.7 ^b
<i>Ex vivo</i> —substrate-scaled	145.8 $\pm$ 14.7 ^a
<i>Ex vivo</i> —rumen fill-scaled	149.5 $\pm$ 14.7 ^a
¹ Values without common superscripts differ ($P < 0.05$).	

Figures

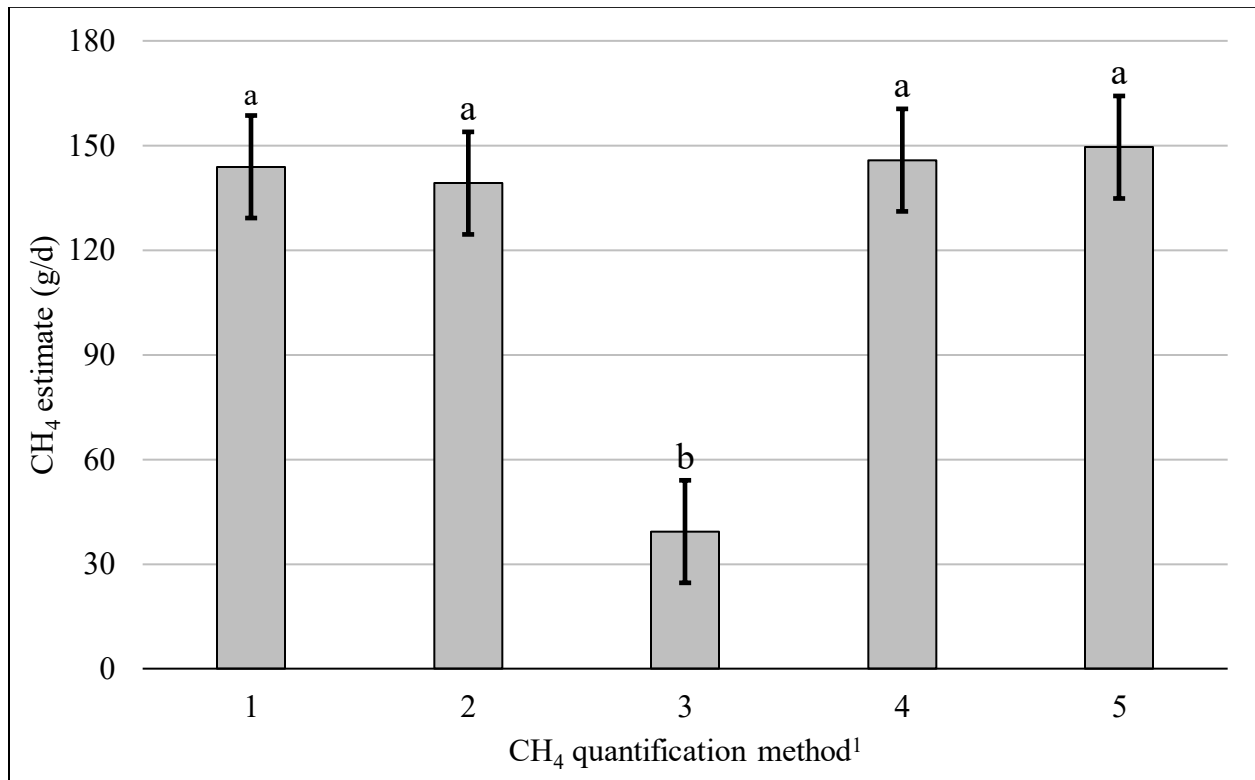


Figure 3.1 Daily enteric methane emissions (g CH₄/hd/d) estimates by AHCS and scaled *ex vivo* quantification methods.

¹ Quantification methods: 1. AHCS—cleaned, 2. AHCS—uncleaned, 3. AHCS—cannulated steers, 4. *ex vivo*—substrate scaled, 5. *ex vivo*—rumen fill scaled. Bars without common superscripts differ ($P < 0.05$).

References

- 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, 641590. <https://doi.org/10.3389/fanim.2021.641590>
- AOAC International. (2019). *Official methods of analysis* (21st ed.). AOAC International.
- Beauchemin, K. A., Coates, T., Farr, B., & McGinn, S. M. (2012). Technical Note: Can the sulfur hexafluoride tracer gas technique be used to accurately measure enteric methane production from ruminally cannulated cattle? *Journal of Animal Science*, 90(8), 2727–2732. <https://doi.org/10.2527/jas.2011-4681>
- 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, 100106. <https://doi.org/10.1016/j.anopes.2025.100106>
- Bergen, W. G., & Bates, D. B. (1984). Ionophores: Their Effect on Production Efficiency and Mode of Action. *Journal of Animal Science*, 58(6), 1465–1483. <https://doi.org/10.2527/jas1984.5861465x>
- Chen, M., & Wolin, M. J. (1979). Effect of Monensin and Lasalocid-Sodium on the Growth of Methanogenic and Rumen Saccharolytic Bacteria. *Applied and Environmental Microbiology*, 38(1), 72–77. <https://doi.org/10.1128/aem.38.1.72-77.1979>
- Goering, H. K., & Van Soest, P. J. (1970). *Forage fiber analyses (apparatus, reagents, procedures, and some applications)* (Vol. 387). US Agricultural Research Service.
- Hristov, A. N., Oh, J., Firkins, J. L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H. P. S., Adesogan, A. T., Yang, W., Lee, C., Gerber, P. J., Henderson, B., & Tricarico, J. M. (2013). SPECIAL TOPICS — Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options1. *Journal of Animal Science*, 91(11), 5045–5069. <https://doi.org/10.2527/jas.2013-6583>
- 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), 52904. <https://doi.org/10.3791/52904>
- Johnson, R. R. (1966). Techniques and Procedures for In Vitro and In Vivo Rumen Studies. *Journal of Animal Science*, 25(3), 855–875. <https://doi.org/10.2527/jas1966.253855x>

- Jonker, A., Molano, G., Antwi, C., & Waghorn, G. C. (2016). Measured using respiration chambers, the sulfur hexafluoride. *Journal of Animal Science*, 94(10), 4326–4337. <https://doi.org/10.2527/jas2016-0646>
- National Academies of Sciences, Engineering, and Medicine. (2016). *Nutrient Requirements of Beef Cattle: Eighth Revised Edition*. The National Academies Press.
- Pell, A. N., & Schofield, P. (1993). Computerized monitoring of gas production to measure forage digestion in vitro. *Journal of Dairy Science*, 76(4), 1063–1073. [https://doi.org/10.3168/jds.S0022-0302\(93\)77435-4](https://doi.org/10.3168/jds.S0022-0302(93)77435-4)
- Reyes, A., Loh, H. Y., Thorndyke, M. P., Guimaraes, O., Goodall, R., Delmore, R. J., Belk, K. E., & Engle, T. E. (2023). The Influence of Tannins, Saponins, and Direct Fed Microbials on In Vitro Rumen Fermentation Characteristics and Microbiota. *Corpus Journal of Dairy and Veterinary Science (CJDVS)*, 4(3), 1–7. <https://doi.org/10.54026/CJDVS1060>
- Rumpler, W. V., Johnson, D. E., & Bates, D. B. (1986). The Effect of High Dietary Cation Concentration on Methanogenesis by Steers Fed Diets with and without Ionophores. *Journal of Animal Science*, 62(6), 1737–1741. <https://doi.org/10.2527/jas1986.6261737x>
- Russell, J. B., & Martin, S. A. (1984). Effects of Various Methane Inhibitors on the Fermentation of Amino Acids by Mixed Rumen Microorganisms in Vitro. *Journal of Animal Science*, 59(5), 1329–1338. <https://doi.org/10.2527/jas1984.5951329x>
- Russell, J. B., & Strobel, H. J. (1989). Effect of ionophores on ruminal fermentation. *Applied and Environmental Microbiology*, 55(1), 1–6. <https://doi.org/10.1128/aem.55.1.1-6.1989>
- Tedeschi, L. O., Abdalla, A. L., Álvarez, C., Anuga, S. W., Arango, J., Beauchemin, K. A., Becquet, P., Berndt, A., Burns, R., De Camillis, C., Chará, J., Echazarreta, J. M., Hassouna, M., Kenny, D., Mathot, M., Mauricio, R. M., McClelland, S. C., Niu, M., Onyango, A. A., ... Kebreab, E. (2022). Quantification of methane emitted by ruminants: A review of methods. *Journal of Animal Science*, 100(7), skac197. <https://doi.org/10.1093/jas/skac197>
- 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)